International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:7, No:9, 2013

The Effect *in vitro* of Flavonoid Aglycones Extracts from Roots of Date Palm Cultivars on *Fusarium oxysporum* F. Sp. *albedinis*

T. Azouaoui – Ait Kettout, R. Gaceb – Terrak, F. Rahmania

***Abstract*—**Date production in North Africa is facing a worrying slowdown and a decline because of *Fusarium* wilt or bayoud date palm (*Phoenix dactylifera* L., caused by *Fusarium oxysporum* f. sp. *albedinis* (*F. o. a*). The objective of this work is to study the *in vitro* effect of flavonoid aglycones extracted from the roots of two cultivars of date palm (one sensitive to bayoud (Deglet Nour) and the other resistant (Takerboucht)) on the growth and production fusaric acid of the pathogen. Results show that during the first week of development of *F.o.a* on potato dextrose liquid medium, the flavonoid aglycones extracts of the susceptible cultivar roots stimulates mycelial growth as well as conidiogenesis of *F. o. a*, nevertheless it has no effect on the synthesis of fusaric acid. However, the flavonoid aglycones extract of resistant cultivar roots stimulates mycelial growth and decreases both the number of conidia production and fusaric acid. It therefore appears possible that the resistant cultivar aglycones have two types of action: they either inhibit the synthesis of fusaric acid, or they metabolize this toxin into hydrosoluble product, this is called detoxification.

***Keywords*—**Flavonoid Aglycones, date palm, fusaric acid, *Fusarium oxysporum* f. sp. *albedinis*.

I. Introduction

Fusarium oxysporum f. sp. *albedinis*, a pathogenic fungus responsible of date palm fusariosis or bayoud, is difficult to remove from soil. It is the most important soilborne pathogen limiting dates production in many areas of the North Africa.

More than 50% of isolates of the known *Fusarium* species are toxigenic and produce deleterious secondary metabolites [1].

It is known that roots exudates and decaying residues (decomposing litter) are phytotoxic due to the presence of allelochemicals identified as organic acids, especially phenolic acids, such as cinnamic, vanillic, coumaric, and ferulic acid [2]-[4].

The mycelial growth of *F. oxysporum* f. sp. *albedinis* is inhibited by the cell wall-bound phenolics in date palm roots [5]. However, little information is available on the influence of roots flavonoids on *F. o. a*.

This study is undertaken the aim of tested the flavonoid aglycones extracted from the roots of date palm on the growth of *F. o. a*, to determine if there may be some relationship between root extracts and the virulence factors of *Fusarium oxysporum* f. sp. *albedinis*.

T. Azouaoui – Ait Kettout is with the Laboratory of Research on Arid Zones, University of Science and Technology Houari Boumediene, BP 32 El-Alia, 16111 Bab-Ezzouar, Algiers, Algeria (corresponding author; e-mail: tazouaoui@usthb.dz, t_aitkettout@yahoo.fr).

R. Gaceb – Terrak is with the Laboratory of Research on Arid Zones, University of Science and Technology Houari Boumediene, BP 32 El-Alia, 16111 Bab-Ezzouar, Algiers, Algeria (e-mail: rgaceb@usthb.dz).

F. Rahmania is with the Laboratory of Research on Arid Zones, University of Science and Technology Houari Boumediene, BP 32 El-Alia, 16111 Bab-Ezzouar, Algiers, Algeria (e-mail: frahmania@usthb.dz).

II. Material and Methods

A. Plant Material

Our work is focused on the roots of level 1 and 2, [6] of two cultivars of date palm *Phoenix dactylifera* L.; Takerboucht (TK) from the INRA experimental station of Adrar and Deglet Nour (DN) a palm grove of Ghardaia (southern Algeria).

DN is a very susceptible cultivar to *Fusarium oxysporum* f. sp. *albedinis*, the causative agent of bayoud and Takerboucht is the only Algerian cultivar resistant to this disease.

B. Pathogen Strains

The virulent strain of *Fusarium oxysporum* f. sp. *albedinis*, is isolated from rachis of a male palm affected by fusariosis. It is stored on PDA medium.

C. Flavonoid Aglycones Extraction

The extraction protocol used is determined by Bate-Smith [7] then taken over by Lebreton et al. [8].

3g of dry plant material are hydrolyzed in hot waterbath during 40min by 240mL of HCl (2N). An insufflation of oxygen (every 10min) is necessary to allow oxidation of proanthocyanidins into anthocyanidin. After cooling of the solution, we carried out the separation of flavonoids using two diethyl ether baths (90 and 60mL). The organic ethereal higher phase, containing flavones and flavonols from the O-glycosides, is evaporated to dryness at ambient air. Dry residues are recovered by 10mL of ethanol 96° which are destined for differential dosing and thin layer chromatography of flavonoid aglycones (flavones and flavonols) and by 10mL of sterile distilled water for bioassays *in vitro* on *F. oxysporum* f. sp. *albedinis*.

From the dry residue taken up in ether ethanol 95°, first, we perform dilution with ethanol 95° (reference curve) and secondly with a solution of aluminum chloride ($AlCl_3$) to 1% in ethanol 96°, after reacting with $AlCl_3$ during 15min, the

reading is done at 420nm

D.Measurement of F. o. a. Colony Growth and Sporulation

A 5-mm agar plug taken from a 7-day-old PDA (potato dextrose agar) culture with and without flavonoid aglycones (1ml) is inoculated into the center of the plate and is incubated at 28°C for 10 days (d). Colony diameter is measured in four directions on each plate after incubation for 2 and 10 d. For each extract, three plates are used with two replicates.

For the sporulation, 5-mm agar plug taken from a 7-day-old PDA culture is inoculated in a liquid culture with and without flavonoid aglycones (1ml) and incubated at 28°C. The broth is filtered to collect conidia. The number of conidia is counted the 7th days. Three flasks are used and three replicates are prepared for each cultivar.

E. Extraction and Determination of Fusaric Acid

Fusaric acid production is determined from the growth in potato dextrose broth. The broth is filtered using Whatman n°1 in order to remove the conidia. FA is sequentially extracted three times with 30mL of ethyl acetate in a separating funnel (3min of extraction each time). The ethyl acetate extracts firstly are pooled and then evaporated by a rotator evaporator at 40°C until dryness. The residue is dissolved in 3mL of 96° ethanol (PA grade).

After elution the contents of FA in each sample are determined spectrophotometrically (Shimadzu, Japan) at the absorbance λ =270nm.

The quantification of FA is made according to the calibration curve for standard fusaric acid (0.16mg.mL^{-1} of distilled water).

III. Results and Discussion

A. Flavonoid aglycones Content

Flavonoid aglycones are at very low concentrations, varying between 0.03 to 0.04mg.g^{-1}. The rate is substantially most important in the susceptible cultivar compared to the resistant cultivar (Fig. 1).

The works of Gaceb Terrak [9] have shown that these extracts contain in addition to flavonoid aglycones (flavones and flavonols), phenolic acids, lipids and aliphatic and aromatic hydrocarbons.

The compounds of soluble phenolic of the root are mainly composed of acidic caféoylshikimiques, ferulic, sinapic, para-coumaric and parahydroxybenzoic Acids [9], [10].

B. Effect of Extract Roots on the Mycelial Colony Growth

Fig. 2 shows that the colony growth of *F.o.a.* on PDA is stimulated by the roots extracts of takerboucht and Deglat Nour, compared with the control. This rate is respectively of 14 and 6% to the 10th day of growth *F.o.a.*

However, El Modafar and El Boustani [5] show that the mycelial growth of *F. o. a.* was inhibited by cell wall-bound phenolics in resistant cultivars of date palm roots.

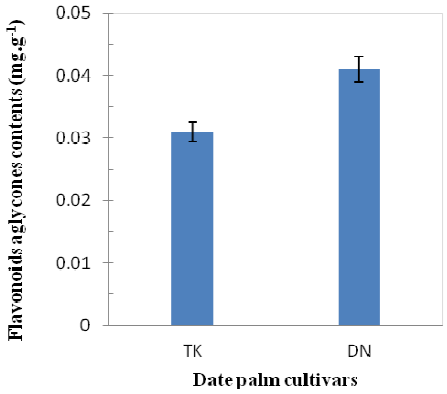

Fig. 1 Flavonoid aglycones contents in the roots of two cultivars of date palm studied. Tk: Takerboucht, DN: Deglet Nour

Wu et al. [11], [12] show that p-hydroxybenzoic and sinapic acids inhibit the colony growth of the *Fusarium oxysporum* f. sp. *niveum* on PDA.

This difference is probably caused the low concentration of flavonoid aglycones of cultivars tested

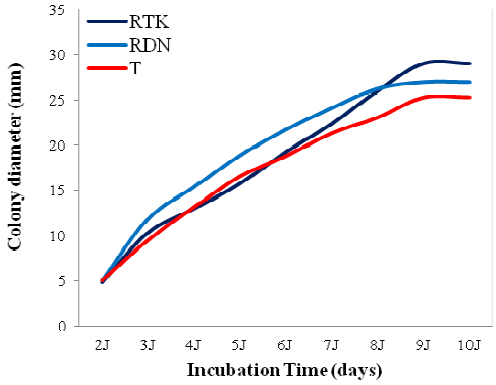

Fig. 2 *In vitro* effect of roots extracts of date palm cultivars on the mycelial growth of *F.o.a.* T: Control, Tk: Takerboucht, DN: Deglet Nour

C. Effect of Roots Extracts on the Sporulation

The sporulation in liquid culture 7 days after incubation is severely reduced (57 %) by roots extracts of resistant cultivar (takerboucht). However, the susceptible cultivar (Deglet Nour) severely stimulates the number of conidia compared to control (Fig. 3).

Hao et al [13] attest that root exudates from watermelon could significantly stimulate the growth of *Fusarium oxysporum*. Nevertheless, Wu et al. [11], [12] have found that sporulation of *Fusarium oxysporum* f. sp. *niveum* was decreased at high concentrations of p-hydroxybenzoic and sinapic acids in a liquid culture.

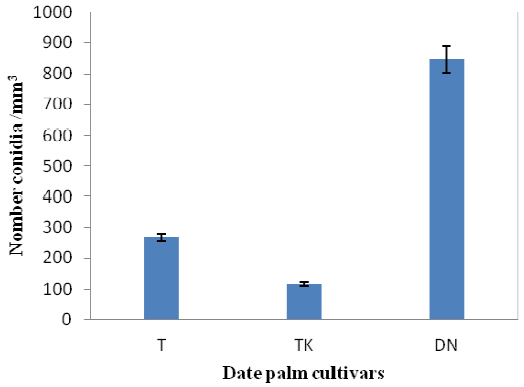

Fig. 3 Effect of roots extracts of date palm cultivar on sporulation of *Fusarium oxysporum*. f. sp *albedinis* in a potato dextrose liquid culture at 7 days. T: Control, Tk: Takerboucht, DN: Deglet Nour

D.Mycotoxin Production

Production of fusaric acid by *Fusarium oxysporum* f. sp. *albedinis* in liquid culture is inhibited to 51% by the roots extracts of resistant date-palm (TK) compared with the control, while no effect on mycotoxin production is observed in the presence of the cultivar extract of the sensitive date-palm (DN) (Fig. 4).

However, Wu et al. [11], [12] have found that fusaric acid production of *F. o.* f. sp. *niveum* in a liquid culture is stimulated by p-hydroxybenzoic and sinapic acids depending on the concentration.

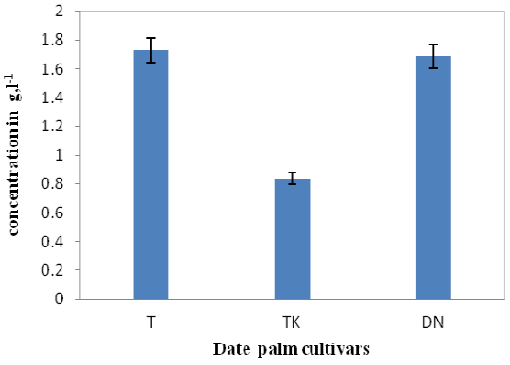

Fig. 4 Different effect of roots extracts of date palm cultivars on mycotoxin production on *Fusarium oxysporum* f. sp. *albedinis*. T: Control, Tk: Takerboucht, DN: Deglet Nour

IV. Conclusion

The flavonoids pathway produces a diverse array of plant compounds with functions in UV protection, as antioxidants, pigments, auxin transport regulators, defence compounds against pathogens and during signalling in symbiosis

The addition of root extracts (flavonoid aglycones) of date palm cultivar sensitive promotes the mycelia growth of pathogens (*Fusarium oxysporum* f. sp. *albedinis*), but the extent of stimulation was inferior to its effect on the sporulation. However, they have no effect on the production of virulence factor (AF).

Flavonoid aglycones of roots date palm resistant (Takerboucht) inhibit the conidiogenesis and the production of mycotoxins (FA).

From these results, we can deduce that flavonoids may be responsible for the resistance of the host against the pathogen during biotic stress.

References

[1] W.F.O. Marasas, "Toxigenic *Fusarium* species" in The Pennsylvania State University Press. University Park, PA, 1984, p. 328.

[2] S. K. Ohno, S. Tomita-Yokotani, M. Kosemura, Node, T. Suzuki, M. Amano, K. Yasui, T. Goto, S. Yamamura and K. A. Hasegawa, "Species-selective allelopathic substance from germinating sunflower (*Helianthus annuus* L.) seeds". *Phytochem.*, vol 56, pp. 577-581, 2001.

[3] .G. Lee, B.Y. Lee and H.J. Lee, "Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.)". *Sci. Horticul*., vol 110, pp. 119-128, 2006.

[4] Z.P. Hao, Q. Wang, P. Christiea and X.L. Li, "Allelopathic potential of watermelon tissues and root exudates". *Sci. Horti*. DOI: 10.1016/ *J.scientia*, 2006.

[5] C. El Modafar, E. El Boustani," Cell wall-bound phenolic acid and lignin contents in date palm as related to its resistance to *Fusarium oxysporum*". *Biol. Plant*., vol 44, pp. 125-130, 2001.

[6] P. Munier, 'Le palmier dattier. Techniques agricoles et productions tropicales. Éd. Maisonneuve et Larose, Paris, 1973, 221 p.

[7] E.C. Bate-Smith," Leuco-anthocyanins: 1- Detection and identification of anthocyanidins formed from leuco-anthocyanins in plant tissues", *Biochem. J.,*vol 58, pp. 122-125,1954.

[8] Ph. Lebreton, M. Jay, B. Voirin, M.P. Bouchez, « Sur l'analyse qualitative et quantitative des flavonoïdes », *Chim. Anal*., vol 49 n°7, pp. 375-383, 1967.

[9] R. Gaceb Terrak, Contribution à la connaissance des interactions palmier dattier (*Phoenix dactylifera* L.) - agent causal du bayoud (*Fusarium oxysporum* f. sp. *albedinis*) par analyses phytochimiques des lipides et des phénylpropanoïdes. Thèse de Doctorat d'Etat 2010, USTHB 198p.

[10] A. Ziouti, C. El-Modafar, A. Fleuriet, S. El-Boustani , J.J. Macheix, "Phenolic compounds in date palm cultivars sensitive and resistant to *Fusarium oxysporum*". *Biol. Plant*.,vol 38, n° 3, 451-457, 1996.

[11] H.S. Wu, S.H.Shen, J.M. Han, Y.D. Liu, S.D. Liu, "The effect *in vitro* of exogenously applied p-hydroxybenzoic acid on *Fusarium oxysporum* f. sp. *niveum*". *Phytopathol. Mediterr*., vol 48, pp. 439–446, 2009.

[12] H.S. Wu, Y. Wang,W. Bao, D. Y. Liu, W. Raza, Q.W. Huang, Z.S. Mao, Q.R. Shen," Responses of *Fusarium oxysporum* f. sp. *niveum* to exogenously added sinapic acid *in vitro*. *Biol Fertil Soils* . vol 45, pp. 443–447, 2009.

[13] W.Y. Hao, L.X. Ren, W. Ran, Q.R. Shen, " Allelopathic effects of root exudates from watermelon and rice plants on *Fusarium oxysporum* f. sp. *niveum*". *Plant Soil*., vol 336, pp. 485–497, 2010.