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# Occurrence of an antifungal principle in the root extract of a Bayoud – resistant date palm cultivar

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**Abstract**

An hexane extract from roots of Black Boo Stammi, a cultivar of date palm, resistant to *Fusarium oxysporum* f. sp. *albedinis*, contained substances which inhibited the spore germination and the growth of the germ tubes of the three isolates of the pathogen that were tested. Extract from roots of Jihel, a susceptible cultivar, lacked these substances.

*Additional keywords:* biochemical resistance, *Phoenix dactilifera* L., *Fusarium oxysporum* f. sp. *albedinis.*

## Introduction

Bayoud is the most important vascular wilt disease of date palm *(Phoenix dactilifera* L.,). This name Bayoud comes from the arabic word 'abiadh' meaning white which refers to the whitening of the fronds of diseased trees. The causal agent is *F. oxysporum* Schl. f. sp. *albedinis (Killian & Maire) Gordon. The pathogen is soil-borne. The destructive 'tracheomycosis' has destroyed about two third (*$10^7$ trees) of the Moroccan palm groves (Pereau-Leroy, 1958; Saaidi, 1979). In Algeria, the disease has already destroyed the western and central groves ($3.10^6$ trees). Bayoud has not only caused the loss of the world's most reknown cultivars and particularly those which have been the most productive, but has also accelerated the desertification. The effective means of controlling Bayoud lies in continued research into resistant cultivars and clones among natural populations of date palm or through a hybridization programme (Saaidi, 1979).

Recently, Zaid and Tisserat (1983, 1984) have developed a tissue culture technique to rapidly propagate high quality-resistant date palm cultivars.

Oihabi (1984) found no anatomical differences between Black Boo Stammi and Jihel, respectively resistant and susceptible cultivars. The same author had shown the lack of any mechanical barrier in the resistant cultivar and he suggested that the resistance should be explained only on a physiological basis.

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The aim of this study was to elucidate the mechanism of resistance.

In many cases, resistance of plants to fungus diseases has been reported to be due to the presence of chemical substances in plants. Generally, two different mechanisms are involved. In the first one, toxic compounds are already present in the host-plant before penetration of the pathogen (Angell et al., 1930; Link and Walker, 1933). In the second one, the plants produce antifungal substances (phytoalexins) only after the infection by the pathogen and penetration of the host-cells (Cruickshank, 1963; Bailey and Mansfield, 1982). Phytoalexins are formed as the result of an interaction between the host and the parasite.

This paper reports the first results of experiments on the chemical resistance of the date palm towards F. *oxys porum* f. sp. *albedinis*. Germination of microconidia and germ tube growth were studied in root extracts of two cultivars, viz. the highly resistant cultivar Black Boo Stammi and the very susceptible cultivar Jihel.

## Materials and methods

*Culture of the fungus.* Three isolates of the pathogen were tested: Boo Feggos from Ouhmidi, Medjool from Tafilalet and Sair from Talat Bani. The isolates were treated in the following way.

*Medjool isolate.* The fungus was isolated from diseased rachides and maintained in sterilized soil. About 50 mg of this soil was placed in a petri dish containing potato dextrose agar (PDA) and incubated in the dark for 15 days at 28 °C. After this period, the culture was exposed to artificial light. F. *oxysporum* f. sp. *albedinis* forms a fine, clear and curly mycelium in which small orange-pink sporodochia are produced. At this stage, a 3-mm plug of fungal mycelia was taken from the leading edge of the culture and placed in the center of a PDA-containing petri dish and incubated in the dark at 28 °C for one week. From this subculture, standard microconidia suspensions in sterile distilled water were prepared. The samples contained ca. $10^5$ spores $ml^{-1}$.

*Boo Feggos and Sair isolates.* The procedure was the same as for the Medjool isolate, except that the inoculum for the PDA plates was not taken from soil cultures but directly from diseased rachides.

*Extraction procedure.* Disinfected healthy roots were finely ground and Soxhlet-extracted with 95% ethanol for 10 hours. After cooling and filtration, the ethanolic solution was evaporated to dryness in vacuo. The oily product left was extracted at room temperature with hexane for 24 hours. The hexane-insoluble fraction was treated analogously with diethyl ether. Finally, the ether-insoluble fraction was extracted with ethyl acetate. In all cases, the solutions were allowed to stand overnight over dry sodium sulfate. The solvent was evaporated in vacuo.

*Biological assays.* Aliquots of the three extracts were dissolved in a minimal volume of ethanol and sterile distilled water was added to give a concentration that corresponded with the amount present in 1.0 g fresh weight of roots per ml solution (containing 3% ethanol). In control experiments, aqueous alcoholic mixture did not show an inhibitory effect.

Table 1. Effects of root extracts of a susceptible and a resistant cultivar of date palm on the germination and the germ tubes growth of three isolates of *Fusarium oxysporum* f sp. *albedinis*.

| Extracts[1] | Boo Feggos | | Medjool | | Sair | |
|---|---|---|---|---|---|---|
| | Germination[2] (mean ± S.E.) | Germ tubes growth[3] in µm (mean ± S.E.) | Germination (mean ± S.E.) | Germ tubes growth in µm (mean ± S.E.) | Germination (mean ± S.E.) | Germ tubes growth in µm (mean ± S.E.) |
| BBS[4], hexane | 9.0 ± 1.0 a | 4.3 ± 0.3 a[5] | 1.0 ± 0.6 a[5] | 3.4 ± 0.3 a[5] | 0.0 ± 0.0 a[5] | 0.0 ± 0.0 a[5] |
| JHL[4], hexane | 64.3 ± 2.3 b | 32.7 ± 1.6 b | 60.0 ± 5.3 b | 50.1 ± 2.4 bc | 86.0 ± 2.5 b | 68.2 ± 2.9 b |
| BBS, diethyl ether | 62.3 ± 1.8 b | 32.2 ± 1.8 b | 74.7 ± 5.6 bc | 47.7 ± 2.3 bc | 93.7 ± 1.5 c | 29.6 ± 1.5 c |
| JHL, diethyl ether | 67.3 ± 1.8 bc | 33.1 ± 1.8 b | 80.0 ± 1.0 c | 52.4 ± 1.7 b | 96.7 ± 0.7 c | 50.5 ± 2.2 d |
| BBS, ethyl acetate | 72.7 ± 2.9 c | 55.6 ± 3.3 c | 84.0 ± 3.1 cd | 50.4 ± 2.4 bc | 89.7 ± 2.0 bc | 40.1 ± 1.8 e |
| JHL, ethyl acetate | 85.3 ± 2.4 d | 58.8 ± 3.2 c | 90.3 ± 3.8 d | 62.7 ± 2.8 d | 93.3 ± 1.8 c | 78.4 ± 4.4 f |
| Water containing 3% ethanol | 84.0 ± 0.6 d | 25.6 ± 2.0 d | 69.7 ± 3.7 b | 41.6 ± 1.9 e | 78.7 ± 4.9 b | 10.2 ± 0.5 g |

[1] An aliquot of the extract in ethanol was diluted with sterile distilled water to give a concentration that corresponded with the amount present in 1.0g of fresh wt. root $ml^{-1}$ (containing 3% ethanol).
[2] Mean of one hundred microconidia per slide in three replicates.
[3] Mean length of thirty germ tubes per slide in three replicates.
[4] BBS, Black Boo Stammi, a resistant cultivar of date palm; JHL, Jihel, a susceptible cultivar.
[5] Values followed by different letters differ significantly on each column (P = 0,05).
S.E. = Standard Error.

The biological activity of the extracts was estimated according to Biehn et al. (1968). Twenty $\mu$l of the extract was placed on each of three slides and mixed with 20 $\mu$l spore suspension in water containing $10^5$ spores $ml^{-1}$. These slides were placed over moist filter paper in a petri dish and incubated for 16 hours in the dark at 28 °C, the optimal conditions for spore germination. The germination was then stopped with formaldehyde vapor. A drop of trypan blue in lactophenol was added to the suspension. The percentage of spore germination was determined and the length of germ tubes measured. All assays were repeated three times with each of the three isolates.

*Statistical analysis.* A Student-Fisher t-test (Heller, 1974) using a program adapted by J.Y. Le Gallo for microcomputer DAI.PC has been used for analysis of the data for germ tube growth as well as for percentage germination. The values obtained have been compared with $t_{0.05}$ values given by Student's table.

**Results and discussion**

The effects of various extracts on spore germination and on the growth of the germ tubes are summarized in Table 1. The most relevant results are the following ones.
(1) In the control, spore germination of the Boo Feggos isolate was 84.0% and the mean of germ tube length 25.6 $\mu$m. In the root extract of Black Boo Stammi made in hexane, the corresponding values were 9.0% and 4.3 $\mu$m. This shows a definite inhibition of spore germination in the Black Boo Stammi extract. The extract of roots of Jihel, the sensitive cultivar, did not show such an inhibitory effect.
(2) The inhibitory substances were not extracted or were extracted only in minute amounts, from the roots when using diethyl ether or ethyl acetate. The fact that the growth of the germ tubes in these extracts from the resistant cultivar was less than growth in those from the sensitive one may be attributed either to small amounts of the same product present in the hexane extract or to other antifungal substances.
(3) Growth of the germ tubes of the Boo Feggos isolate was significantly better in an extract made in ethyl acetate than in the other extracts and water (Table 1). This implies that these extracts contained stimulatory compounds. Similar effects have been reported by Purkayastha and Deverall (1965) in a study on effects of extracts of bean leaves on germ tubes growth of *Botrytis fabae.*

The response of the three isolates to the root extracts of the resistant and the sensitive cultivars showed the same pattern.
(4) The Sair-isolate showed a greater difference in response to the hexane root extracts of the two cultivars than the other isolates showed. Germination and germ tubes growth was completely inhibited in the extract of the resistant cultivar.

This work shows that the extract of roots of the resistant cultivar, Black Boo Stammi, made in hexane, contains one or more antifungal compounds highly active against F. *oxysporum* f. sp. *albedinis.* On the other hand, stimulatory materials occur in extract of the resistant and the susceptible cultivar, made in diethyl ether and ethyl acetate. Investigation will be done to elucidate the chemical structure of the active principle.

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## Samenvatting

*Een schimmelremmend principe in het wortelextract van een dadelpalmcultivar die resistent is tegen de Bayoudziekte*

In een hexaanextract van wortels van de dadelpalm, cv. Black Boo Stammi die resistent is tegen de Bayoudziekte, bevonden zich stoffen die de sporekieming en de groei van kiembuizen onderdrukken. Dit gold voor alle drie isolaten van *Fusarium oxysporum* f. sp. *albedinis*, die werden getoetst. In extracten van de vatbare cultivar Jihel werden de kieming en de groei niet geremd.

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