In Vitro Inhibitory Effect of the Extract Powder of Rosemary (Rosmarinus Officinalis), Oleander (Nerium Oleander), Grenadier (Punica Granatum) on the Growth of Fusarium Oxysporum Fs Albidinis and in Vivo Test Antagonist Fungi on the Incidence and the control of Vascular wilt Disease of Date Palm in Palm Grove in Fiquig South of Morocco

Redouane Benabbes, Iliass Lahmass, Faiza Souna, Mohammed El Youbi, Ennouamane Saalaoui, Abdellaker Hakko, Mohammed Bouakkak

Laboratory of Biochemistry, Department of Biology, Faculty of Science University Mohamed Ist, Box.524,60000 Oujda, Morocco

**ABSTRACT**

**Objective:** The aim of the present work was to study the effect of three natural extracts of powder Rosemary (Rosmarinus officinalis), oleander (Nerium Oleander), pomegranate (Punica granatum), are used locally as a phytosanitary treatment against the Bayoud were evaluated in vitro for their fungicidal on Fusarium oxysporum fs albidinis causal agent of vascular wilt disease of date palm. **Results:** radial growth and the recovery of the mycelial pellet was almost completely inhibited from the concentration of 3 g/l to 97.08 %, 85%, and 70% for Pomegranate extract, Rosemary, and Laurier respectively through three repetitions; the minimum inhibitory concentration was evaluated at 1.55 g/l; 1.76 g/l and 2.14 g/l respectively for the three samples. Five fungi were selected after in vitro tests (direct confrontation) among 12 drawn from the microflora of the Figuig oasis located in the west of Morocco, as antagonist foa; in vivo tests on vitro plants inoculated with the pathogen and treated by the antagonists was conducted to assess the impact of the disease, the plants inoculated with foa and treated with antagonists showed no mortality, with vegetative growth pretty good but still slightly lower than the negative control. **Conclusion:** In conclusion, Extracts of natural powder leaves and fruits of pomegranate, rosemary and oleander showed significantly effective inhibition rate on the radial mycelial in vitro growth of Foa.

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**INTRODUCTION**

The date palm (*Phoenix dactylifera* L.) is a monocot dioecious perennial, belongs to *Arecaceae* family [6]. This tree has a major socioeconomic importance of Saharan populations of the south and south east of Morocco; However, these last years, the date production known a significant decrease mainly due to vascular fusariosis, known locally as the Bayoud caused by a soil fungus *Fusarium oxysporum* *fs albidinis* [5,8].

Many efforts have been deployed to fight against this disease, which generally are limited as is the case for all parasitic vascular diseases. And remain in their preventive whole; saw the inefficiencies of control measures being considered, and the lack of truly resistant genotype added to the high potentionnality conservation of the pathogen in the soil, this disease has become an epidemic character which requires the consideration of other alternatives, including those based on the exploitation of the antagonist to a share of microorganisms (bacteria and fungi) and other parts of some medicinal plant extracts.

The objective of our study was to test in vitro inhibitor extracts of medicinal 3 plants of Rosemary (R), bay leaf (L) and Grenadier (G) effect on the radial mycelial growth of *foa* and the control of the incidence of the disease by testing in vivo 5 selected as antagonists derived from the floor of the oasis of Fiquig in the South Eastern Morocco referring to negative control fungi [15].

**Keywords:** Rosemary (*Rosmarinus officinalis*), oleander (*Nerium Oleander*), Grenadier (*Punica granatum*) extract powder, *Fusarium oxysporum* *fs albidinis* (*foa*), Bayoud antagonist fungi, date palm (*Phoenix dactylifera*).
MATERIALS AND METHODS

1/ isolation of the pathogen:

The Fusarium oxysporum f.sp albedinis (Foa) used in this study was isolated from Bouffaggous Gharas palm rachis infected by the vascular fusariosis according to the method of (Locke T., 1974). Fragments of 0.5cm³ are disinfected with ethanol followed by a flombage. These fragments are then deposited on PDA and incubated for one week at 27 ° C. and then 2 to 4 days under natural light.

2/ Isolation and purification of antagonistic fungi isolates:

The density of the microflora is estimated by the dilution method of soil suspension [4] and spread on culture media. 10g of soil are crushed before being aseptically suspended in 100 ml of sterile distilled water. From this solution, a dilution series of the suspension is carried tenth, three replicates for each dilution are provided, the isolation of microorganisms is through the use of two selective media, the culture medium supplemented with antibiotic PDA for fungi and Muller Hinton peptone medium for bacteria after incubation of one week in an oven at 27 °C, AF named fungi are isolated, purified, multiply and keep at 4 °C in steril sand. After testing direct confrontation with foa on PDA, 5 fungi were selected and named: AF2, AF3, AF4, AF6 and AF7.

3/ Preparation of fungal inoculum and antagonists:

The flask containing the Czapeck medium sterile was sown by foa and antagonists strain already prepared (Chakroune K. & Hakkou, 2005). These preparations were then placed in a water bath (27 ± 2 ° C) with stirring (60 rev / min) for 15 days. Cultures are then filtered to remove mycelia and the filtrate obtained was then centrifuged at 3000 g at 4 ° C for 15 minutes. The pellets were washed 2 to 3 times and recovered in 2 liters of distilled sterile water suspensions. Concentrations were estimated by counting foa spores on Malassez cell and counting foa colonies on solid media PDA and Komada.

4/ Preparation of the seedlings and substrates:

Vitro plants used in this test are those varieties Ennajda multiplied in the royal domain of Meknes at the age of 24 months. The substrate used in this test consists of a mixture of peat and sand after sieving through a sieve mile 2000μm and vermiculite (2V peat / sand 1V / vermiculite1V), the assembly is sterilized at 120 ° C.

5/ culturing of vitro-plants:

The substrate mixture already prepared is introduced into pots of 3000 ml, those pots are divided into 4 substrates:
Substrate 1: 8 pots not inoculated with the pathogen and not treated with antagonists (negative Control-)
Substrate 2: 8 pots inoculated only by foa at 104 CFU / ml of substrate (Positive control+)
Substrate 3: 40 pots were treated by the antagonists only (AF2, AF3, AF4, AF6 and AF7), 8 pots for each antagonist at 105 CFU / ml of substrate.
Substrate 4: 40 pots were inoculated with foa (104 CFU / ml) and treated with antagonists (105 CFU / ml). 8 pots for each antagonist.

The vitro-plants are then released from their original substrate and planted in pots already prepared and placed in greenhouse. Irrigation is done regularly and according to the 10 months. After this, a comparison of leaf and root biomass of each batch of pots relative to the negative control is established.

6/ preparation of powder extracts for 3 plants used:

leaves of rosemary, oleander and the envelope of the pomegranate fruit are dried in the shade and at room temperature for one week, then crushed and made into powder in a Moulinex type mill, 15 g of each powder is suspended betting in 100ml of sterile distilled water stirring (60 tr/min) for 72 hours. Then the suspension is filtered. In view of determining the minimum inhibitory concentration (IC50) [13], 4 final concentrations (taking into account the dilutions) were selected (0, 5, 1, 2, 3.4 g / l) in this study. Each extract was emulsified in the medium culture just before pouring into Petri dishes of 80 mm diameter. Mycelial disc of 5mm diameter were taken from the peripheral growth of the pathogen is placed in the center of the new Petri dish containing 20ml PDA. 3 Petri dishes kneaded were planted by concentration and incubated at 25 ± 2 ° C. The radial mycelial growth was measured in millimeters on two perpendicular diameters plotted on the bottom of the Petri dish. This measurement is made just before the filaments reach the edge of the Petri dish in the control batches 7 Day this experience is repeated 3 times [16].

When mycelial growth was not observed for a given concentration, the boxes are opened and knead the mycelial disc is transplanted into a new petri dish containing PDA medium. The inhibition rate of each extract was evaluated against mycelial growth in the control using the modified formula of :

\[
\% \text{ Inhibition} = \left( \frac{D_o - D_x}{D_o} \right) \times 100
\]
RESULTS AND DISCUSSION

The increase in the percentage of root and leaf biomass produced with the application of antagonists on vitro plants vary with antagonist used and with AF3 and AF7 there is the highest percentage increase with 188.48% and 188 , followed by 26% respectively AF2, AF4 and AF6 with 176.36, 169.24 and 156.17, respectively, compared to the original biomass vitro plant for each antagonist used, but this increase is lower compared to non-inoculated vitro plants by the pathogen and treated only by antagonists, which remains at its lower turn to uninoculated and untreated vitro plant (negative control), and it should be noted a mortality percentage of 62.5% in vitro plants of the positive control (vitro plant inoculated with the pathogen and not treated with the antagonists) that despite the variety used and resistant (Annajda). These results are in perfect agreement with those of in vitro tests by direct confrontation.

![Fig. 1: leaf biomass of vitro plants inoculated by foa, treated and untreated by antagonists.](image1)

For powder extracts of medicinal plants, there is the highest percentage for the pomegranate followed by rosemary and oleander with inhibition (97.08%, 85% and 70%) respectively this inhibition increases with the concentration; IC50s were determined to 1.55 g / l for the pomegranate; 1.76 g / l for the rosemary and 2.14 g / l for the oleander. fungal strain *Foa* therefore has a significant sensitivity vis-à-vis extracts of these three plants.

![Fig. 2: Root biomass vitro plants inoculated by foa, treated and untreated by antagonists.](image2)

![Fig. 3: Inhibition of Rosemary extracts powder.](image3)
Fig. 4: Percentage of inhibition of the pomegranate extract powder.

Fig. 5: Percentage of inhibition of the oleander extract powder.

Fig. 6: Summary of inhibition rate of 3 Extracts.

1) Discussion:

The in vitro inhibitory effect of the extract powder of 3 plants was effective on telluric fungal strain of *Fusarium oxysporum f.sp albidinis*. Indeed, the mycelial growth of the fungus was inhibited by the three extracts \[7,11\]. The positive correlation between the inhibition rates and different concentrations for each sample demonstrates the inhibitory potency of the extracts on the fungus. The minimum inhibitory concentration is different according to the extract \[20\]. According to some authors, fungus do not react in the same way vis-à-vis bio pesticides \[3\] which could explain the behavior of pathogenic agent vis-à-vis the three extracts. Moreover, fruit and flowers would therefore have lost a part of their aroma during powder processing; which could justify this difference in efficacy between these 3 powders. According to some authors \[17,19,1\], composition constituents the inhibitory character Depond largely on the state of plant extract (fresh or dried).

Treatment of *Fusarium* wilt of date palm in the greenhouse with antagonistic fungi and with a substrate (sand, peat and vermiculite) receptive vis-à-vis *Foa* because they allow him to exercise without difficulty pathogenicity, showed the effectiveness of these antagonists especially AF3et AF7 on the control and the incidence of disease. Indeed, foliar and root biomass occurred following treatment with antagonists in vitro plants inoculated by *Foa*, is significant although it is slightly less than the negative control (not inoculated and untreated vitro plants). Reverse against, no mortality was observed in vitro plant treated. Therefore these results confirm the presence of the antifungal substances secreted during development of these microorganisms. The
intensity of the inhibitory action is a function of the antagonistic microorganism [9]. This difference in activity against this pathogen could be explained by the quality and / or quantity of excreted toxic substances. The suppressive effect exerted by these antagonistic fungi on Foa could be attributed on the one hand, the intense action of fungistasis and antibiosis that act directly on the activities of the parasite, and secondly, at the onset of induced resistance in date palm seedlings.

VI) Conclusion:

Extracts of natural powder leaves and fruits of pomegranate, rosemary and oleander showed significantly effective inhibition rate on the radial mycelial in vitro growth of Foa, and the pomegranate has a more effective antifungal activity against Rosemary and oleander. This in vitro study will be the basis for determining adequate and effective infected concentrations to apply on the date palm seen in the use of these medicinal plants as biopesticides against this telluric fungus. Like this, the use of fungal antagonists gave a significant result with a good evolution of the leaf and root fresh biomass with total absence of mortality, so it appears in the final powder extracts of Pomegranate, Rosemary, and oleander antagonistic fungi have good behavior vis-à-vis the Foa, which can lead to a good contribution to the national strategy for the fight against Foa.

Picture 1: Pomegranate powder extract.

Picture 2: Rosmary powder extract.

Picture 3: Oleander powder extract.

Picture 4: The control with Foa.
**Picture 5:** Negative control (inoculated and not treated with Foa antagonists vitroplants).

**Picture 6:** Final state of a plant-vitro foa inoculated and not treated with the antagonists.

**Picture 7:** Comparison of vitro-plant inoculated and treated with a vitro-plant inoculated and untreated

**REFERENCES**


