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Research Article

Antifungal activity of *Punica granatum* root extracts and their potential role to trigger date palm defense reaction against bayoud disease

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Abstract: *Fusarium oxysporum* f. sp. *albedinis* (Foa) is a cosmopolitan soil-borne fungus responsible for the most destructive disease of the date palm tree *Phoenix dactylifera* L. in Morocco. In the present study, we used aqueous and methanolic root extracts from *Punica granatum* to evaluate their ability to induce date palm defense against Foa and their antifungal activity. The *in vitro* treatment of Foa by these extracts showed different inhibitory effects depending on the nature of the extract. The methanolic extract showed significant inhibition of both mycelial growth (51%) and biomass production (86.3%), while the aqueous extract inhibited the sporulation (99.3%) and the spore germination (75.9%) of the pathogen. Moreover, treatment of date palms with methanolic extract has shown a significant increase in phenolic content and peroxidase activity known to be involved in date palm defense against Foa. These preliminary results open a promising field to control date palm Fusarium wilt.

Keywords: antifungal activity, date palm, defense reaction, *Fusarium oxysporum* f. sp. *albedinis*, pomegranate root extract

Introduction

The date palm crop is the mainstay of oasis agriculture in the desert. In addition to its ecological and social roles, it contributes to 60% of farmers' agricultural income in the oasis. It offers dates and other by-products for domestic, artisanal, and industrial uses. In Morocco, the current average cultivated area is about 51000 hectares, with nearly 6.9 million date palm trees (Meziani, 2019). Unfortunately, date palm cultivation faces several constraints, but the most destructive one is the "bayoud" disease caused by a vascular pathogen, *Fusarium oxysporum* f. sp. *albedinis* (Foa) (Jaiti *et al*., 2009). Several research programs have come up to combat this Fusarium wilt. However, the results haven't given rise to any effective treatment.

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Chemical treatments are inefficient, and the prophylactic methods are not of interest due to the contamination of several palm plantations and their non-durable impact. Therefore, planting resistant cultivars remains the only efficient and economical method to control this disease (Dihazi *et al*., 2012).

Currently, natural products are increasingly sought for sustainable agriculture. Several studies have shown that plant extracts and their essential oils have insecticidal (Skalský *et al*., 2020; Abbasi *et al*., 2012); bactericidal (Wang *et al*., 2019; Alcaráz *et al*.,2015) or fungicidal activities (Rabillu *et al*., 2021; Kalleli *et al*., 2020; Mekam *et al*., 2019; Moutassem *et al*., 2019). In addition, many recent kinds of research have highlighted the activities of plant compounds in stimulating plant defense mechanisms (Belgacem *et al*., 2021). This has opened new horizons regarding plant disease management using new industrial chemical and biological processes (Regnault-Roger and Philogene, 2008). The use of plant extracts represents a new approach to controlling Foa, and has been initiated by a few researchers. Boulenouar *et al*. (2012) showed that some aromatic and medicinal plants from Algeria (*Nerium oleander, Pergularia tomentosa, Citrillus colocynhis, Fredolia aretioides, Launea arborescens*) can inhibit Foa. Recently, Bouhlali *et al*. (2020) reported antifungal activity of five plant extracts (*Acacia cyanophylla, Cupressus atlantica, Eucalyptus torquata, Nerium oleander,* and *Scinus molle*) against Foa.

In this work, we first evaluated the effect of *Punica granatum* root extracts on *Fusarium oxysporum* f. sp *albedinis* growth and virulence. Then we assessed the effectiveness of these extracts in inducing date palm defense and protecting it against bayoud disease. We investigated the peroxidase activity and the phenolic content known for their involvement in date palm defense reactions (Jaiti *et al*., 2008; 2009; Dihazi *et al*., 2012; El Hasni *et al*., 2004).

Materials and Methods

Plant materials and crude extracts preparation

Date palm: seedlings of the cultivar "Boufegous" (BFG, susceptible) were cultivated in plastic containers filled with a mixture of sterile sand and peat (2:1) in a greenhouse and under 16 h light regime, 60-70% relative humidity and 25 °C ± 2.

Pomegranate tree is a fruit tree belonging to the Punicaceae family. It is a small tree with a shrub habit of the Mediterranean regions. The roots of *Punica granatum* were harvested from the oasis of Erfoud (South East of Morocco) in January 2019. This species was chosen based on farmers 'observations who believe that the co-cultivation of date palms with pomegranate mitigates bayoud disease.

The roots were dried at room temperature and ground. Six g of the powder was macerated in 40 ml of distilled water for the aqueous extract and 40 ml of methanol (80%) for the methanolic extract. The preparations were then homogenized for 24 hours at room temperature using a magnetic stirrer P-SELECTA. The resulting homogenate was filtered using 1 mm WHATMAN paper. The filtrate was evaporated in a WTB-BINDER oven at 40 °C. The powder obtained constitutes the total aqueous or methanolic extracts. These powders were then solubilized in distilled water at a rate of 20 g/L for the subsequent tests.

Fungal strain and technique of inoculation

The aggressive strain of Foa was isolated from the rachis of infected palms with typical symptoms of "bayoud" disease in the region of Erfoud. Fungal culture was routinely conducted in darkness on Potato Dextrose Agar (PDA) medium or liquid Czapeck medium at 25 ± 2 °C. Date palm seedlings were inoculated at the 2-3 leaf stage (3-4 months old) by micro-injecting into roots of 10µL of Foa conidial suspension (10^6 spores/ml) (Fig. 1).

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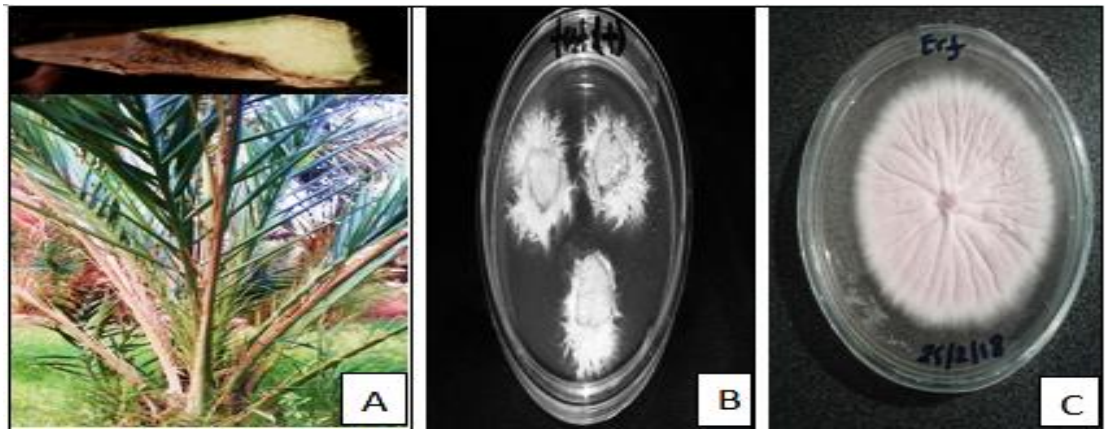

Figure 1 Visual diagnosis and isolation of the causal agent of Bayoud disease of date palm, internal and external symptoms of the infected date palm rachis (A), Isolation of the pathogen from infected rachis on PDA medium (B), purified pathogen culture (C).

***In vitro* effect of plant-extracts on the Foa mycelial growth, sporulation, spore germination and biomass production**

Effect of plant extracts on radial growth.

Agar discs (ø 5mm) from the young cultures of Foa were placed into the center of sterile Petri dishes containing the solid culture medium (PDA) alone for the control or supplemented with different concentrations of the extracts. The extracts were added to the previously sterilized medium before distribution in a Petri dish using a sterile Millipore filter (Sterivex-HV Pressure Filter Unit, 0.45 µm pore size, PVDF membrane, gamma irradiated, sterile). Precise volumes of these extracts were used to obtain different concentrations (1; 2; 4, and 8%). Ten repetitions were performed for each of the concentrations. The radial growth of the colonies was noted every 48 hours during 10 incubation days at 25 °C in the dark.

The evaluation of the inhibition exerted by extracts on the mycelial growth was estimated by the percentage of inhibition according to the following formula:

$$Ic\% = \frac{d_c - d_t}{d_c} \times 100$$

Where: dc, dt are mycelial growth diameters in control and treated Petri plates, respectively.

Effect of plant extracts on spore production.

The assessment of the sporulation was carried out according to the method of Kanoun et al. (2014). Cultures in liquid medium were performed by adding 1ml of a suspension of Foa of 2.10^6 spores/ml, prepared from mycelial cultures on 7-day old PDA medium, in Erlenmeyer flasks containing 40ml of Czapeck medium (Sucrose 30 g, $NaNO_3$ 2 g, K_2HPO_4 1 g, KCl 0.5 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g) alone for the control or supplemented with different concentrations of the extracts. The extracts were added to the previously sterilized medium using a sterile Millipore filter. Precise volumes of these extracts were used to obtain different concentrations (1; 2; 4, and 8%).

To calculate the number of spores produced, samples from the Czapeck medium were diluted, and the number of spores was counted with a MALASSEZ cell under a light microscope.

The evaluation of the inhibition exerted by the extracts on sporulation was estimated by calculating the percentage of inhibition according to the following formula:

$$Is\% = \frac{N_t - N_c}{N_t} \times 100$$

Where: Nc, Nt are the number of the spores estimated for the control and in the presence of the plant extracts, respectively

Effect of plant extracts on the conidial germination.

To follow the conidial germination, we spread 0.2 ml of 10^6/ml conidia suspension from 7-day-old

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culture on the surface of Petri plates containing the extracts at different concentrations and water alone for the control. The germination of the conidia was observed after 24 hours of incubation at 25°C in the dark. Germination was effective if the germ tube length was greater than the smallest diameter of the conidia. The germinated spore count was performed from 300 conidia. Five Petri plates were used for each test, and the experiment was repeated three times. The percentage of inhibition was calculated by the following formula:

$$\mathrm{Ig\%} = \frac{\mathrm{Gc} - \mathrm{Gt}}{\mathrm{Gc}} \times 100$$

Where: Gc, Gt are number of germinated spores estimated for the control and in the presence of the plant extracts, respectively.

Effect of plant extracts on biomass production.
To measure the biomass production, we filtered Foa cultures in Czapeck medium through a filter paper WHATMAN 1mm, and the mycelium obtained was weighed in the fresh state and then after being dried in an oven at 74 °C for 3 h.

Effect of plant extracts on the Foa's hydrolase activities
The enzymatic activity of hydrolases was measured in the culture filtrate according to the protocols described by El Modafar and El Boustani (2000). The specific activity of both enzymes was expressed as unit/mg proteins.

For proteases, the reaction medium contains 1 ml of culture filtrate, 1 ml of casein solution at 2% as substrate, and 2 ml of buffer phosphate 50 mM pH 5.8. After 2 h of incubation at 37 °C, the enzymatic reaction was stopped by 0.5 ml of trichloracetic acid at 50%, and the optical density was read at 405 nm.

As for cellulases, the reaction medium contains 1 ml of culture filtrate, 2.5 ml of 500 mM citrate phosphate buffer pH 4.5, and 1 ml of 0.12% carboxymethylcellulose as substrate. After 2 hours of incubation at 37 °C, the enzymatic reaction was stopped by adding 2 ml of the 1% dinitrosalicylic acid reagent, and the absorbance was read at 540 nm.

Effect of root extracts on the induction of date palm defense mechanisms
The assessment of the *P. granatum* extracts effect on date palm was conducted by cultivating 2-3-month-old seedlings (80 plants) in hydroponic media containing methanolic extracts (160 mg/L) or in distilled water for the control (80 plants). After 2 weeks, 40 plants from each group were inoculated with Foa, while the other 40 plants were kept as uninoculated healthy controls. The seedlings were then incubated under a 16 h light regime and 25 °C ± 2.

Determination of bayoud disease severity
The disease severity was estimated by calculating the percentage of dead plants after 105 days of culture.

Proteins extraction and peroxidase activity assay
Seedling roots (200 mg F.W.) were homogenized in 1 ml of Tris– maleate buffer pH 6.5 (0.1 M) containing Triton X-100 (0.1 g/l). After centrifugation at 10 000 g for 15 min, the supernatant was used to determine peroxidase activity by measuring the oxidation of guaiacol at 470 nm. Briefly, twenty microliters of the extracted protein were added to 2 ml of reaction mixture consisting of a solution of 0.1 M Tris–maleate buffer (pH 6.5), 25mM guaiacol, and 25 mM H_2O_2. The enzymatic activity was expressed as enzymatic unit g^{-1} FW.

Phenolic compounds determination
The soluble phenolic compounds were extracted from the root tissues as described by El Hadrami *et al*. (1997). Their concentration was estimated at 760 nm using the Folin Ciocalteu method (Macheix *et al*., 1990). The content of phenolic compounds was expressed as mg equivalent of gualic acid per g of fresh weight (FW).

Statistical analysis
Statistical analyses were performed using SPSS software version 25 and presented as means ± standard error (SE). One-way analysis of variance test (ANOVA) and Bonferroni tests with $p < 0.05$ as the significance level were used

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to determine the significance of the factors "extract", "concentration", and "incubation time" on growth and development parameters and enzymatic activities in vitro of *Fusarium oxysporum* f.sp *albedinis*.

Results

In vitro effect of the plant extracts on Foa mycelial growth, sporulation, spore germination, and biomass production

Effect of the plant extracts on Foa growth.

The results presented in Fig. 2 show an inhibitory effect of the extracts on pathogen growth. The concentration of both extracts had a significant effect on the inhibition of mycelial growth (one-way ANOVA, $p < 0.01$), while time had no significant effect. The concentration of 8% exhibited the most significant inhibitory effect on the 8th day of Foa culture (51% for methanolic extract and 33.5% for aqueous one).

Effect of plant extracts on Foa spore production.

The results presented in Fig. 3 show that the two extracts of the pomegranate inhibited the fungal spore production. The concentration of both extract types significantly affected the inhibition of fungal sporulation (one-way ANOVA, $p < 0.01$) with no significant effect on incubation time. The most inhibitory effect was obtained after three days of culture at 8% for the two extracts, 99.3% for the aqueous extract, and 88.3% for the methanolic extract.

Effect of plant extracts on Foa conidial germination

The Fig. 4 shows that the treatment with the two extract types significantly inhibit Foa spore germination (one-way ANOVA, $p < 0.01$) with no significant differences in concentration for any extract. The higher inhibition (75.9%) was obtained for the aqueous extract at 8%.

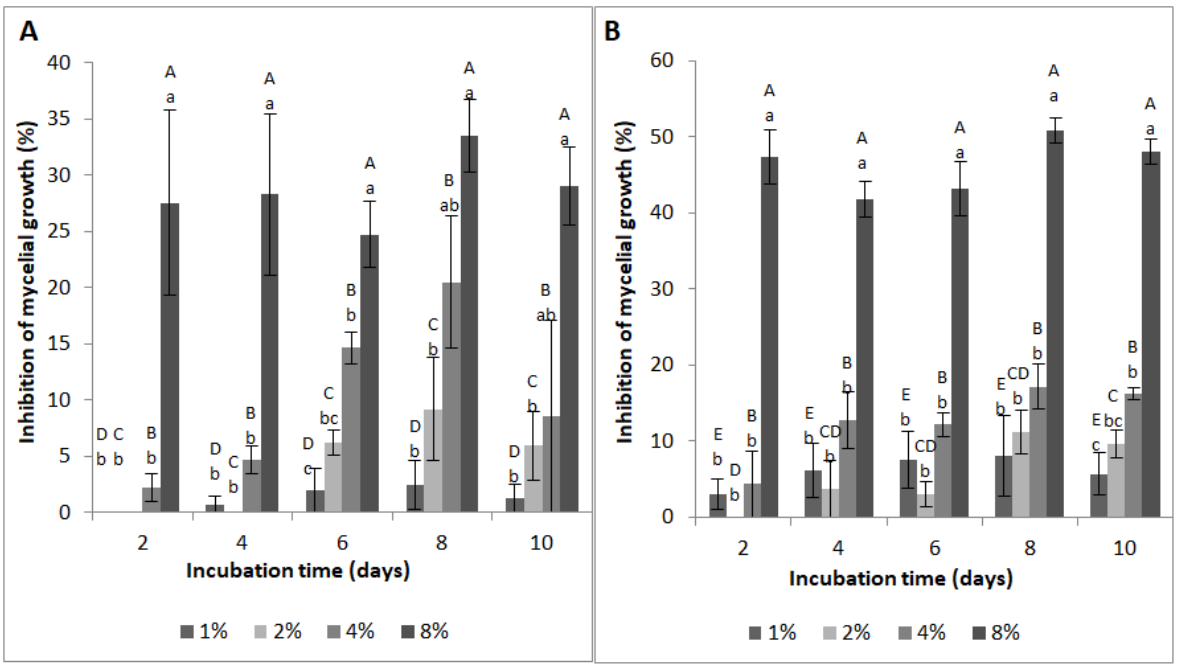

Figure 2 Effect of different concentrations of aqueous (A) and methanolic (B) root extracts of *Punica granatum* on mycelial growth inhibition of *Fusarium oxysporum* f.sp. *albedinis* on PDA medium. Each bar represents the mean of ten repetitions. Lowercase letters indicate significant differences among incubation time (Bonferroni tests, $p<0.05$) and the uppercase letters indicate significant differences among extract concentrations (Bonferroni tests, $p<0.05$). Error bars represent standard errors.

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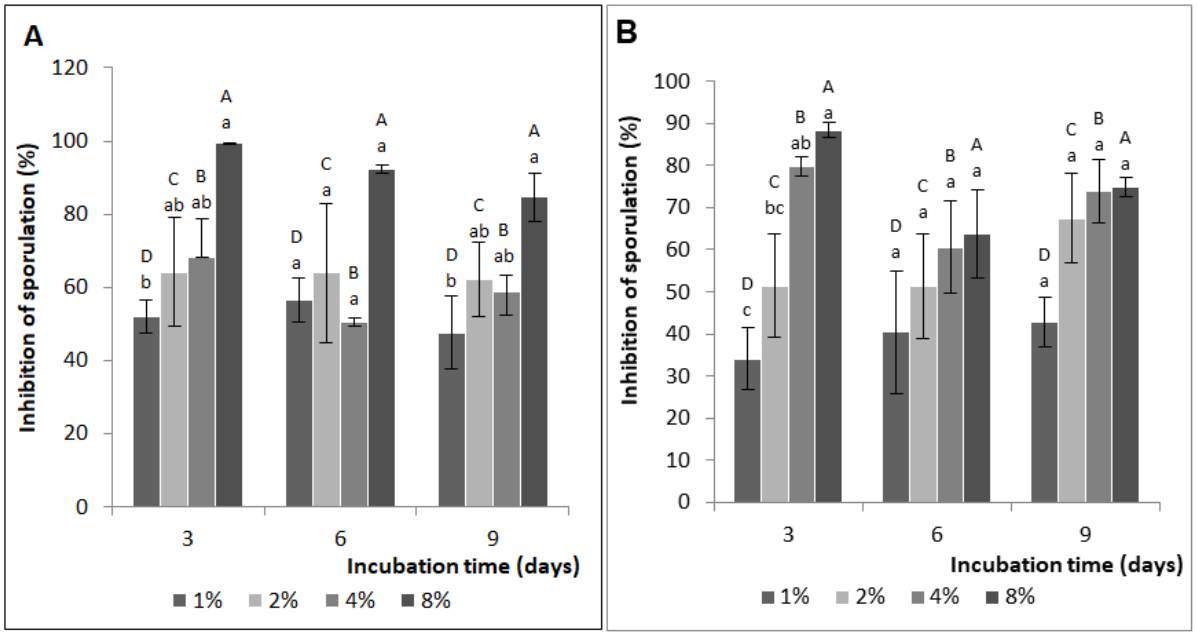

Figure 3 Fungal sporulation inhibition under the effect of different concentrations of aqueous (A) and methanolic (B) root extracts of *Punica granatum*. Each bar represents the mean of three repetitions. Lowercase letters indicate significant differences among extract concentrations (Bonferroni tests, $p < 0.05$), and the uppercase letters indicate significant differences among incubation time (Bonferroni tests, $p < 0.05$). Error bars represent standard errors.

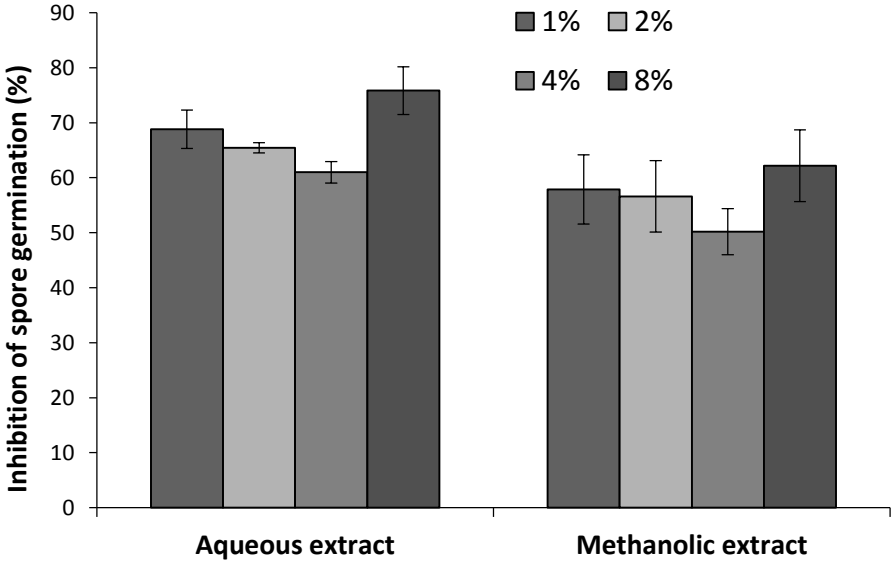

Figure 4 Effect of different concentrations of aqueous and methanolic root extracts of *Punica granatum* on spore germination inhibition of *Fusarium oxysporum* f.sp. *albedinis*. Each bar represents the mean of three repetitions per concentration. Error bars represent standard errors.

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Effect of plant extracts on Foa biomass production

The Table 1 illustrates the inhibitory effect of aqueous and methanolic pomegranate extracts on the fresh and dry mass of Foa cultures. The concentration of both extract types had a significant effect on the inhibition of mass production (one-way ANOVA, $p < 0.01$) and a significant effect on incubation time for the methanolic and aqueous extracts. The greatest inhibition of biomass production was obtained for the methanolic extract at 8%. It was 71% and 86.26% for the fresh and dry mass, respectively, which was noted at 3 days of culture.

Effect of the plant extracts on Foa hydrolase activities

The results showed that pomegranate root extracts didn't significantly affect Foa hydrolase activities.

Effect of root extracts on the induction of date palm defense mechanisms

Disease severity

All date palm seedlings inoculated with Foa developed several disease symptoms expressed as root browning, leaf rolling and withering, and finally, plants death (Fig. 5). In the absence of pomegranate root extracts treatment, 85% of date palm seedlings died after 105 days of Foa inoculation.

Table 1 Fresh and dry mass production inhibition of Foa under the effect of different concentrations of aqueous and methanolic root extracts of *Punica granatum*.

Incubation time (days)	Concentration (%)	Fresh mass		Dry mass	
		Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanolic Extract
3	1	26.4 ± 10.9 ^{ef}	20.97 ± 7.9 ^{ef}	43.09 ± 7.93 ^{bcdef}	15.67 ± 3.22 ^{ef}
	2	13.44 ± 10.88 ^{f}	38.57 ± 8.33 ^{cdef}	32.22 ± 9.01 ^{def}	27.80 ± 4.91 ^{ef}
	4	43.22 ± 9.81 ^{bcdef}	70.52 ± 3.42 ^{abc}	51.28 ± 4.54 ^{abcde}	76.57 ± 2.8 ^{ab}
	8	68.6 ± 3.56 ^{abcd}	71.12 ± 5.13 ^{abc}	86.2 ± 2.11 ^{a}	86.26 ± 0.4 ^{a}
6	1	19.1 ± 7.4 ^{cd}	16.9 ± 4.82 ^{d}	25.1 ± 4.53 ^{bcd}	25.41 ± 1.51 ^{bcd}
	2	16.76 ± 7.59 ^{d}	35.6 ± 8.41 ^{abcd}	16.37 ± 3.3 ^{d}	29.17 ± 3.53 ^{bcd}
	4	12.36 ± 3.02 ^{d}	36.91 ± 12.37 ^{abcd}	23.92 ± 2.31 ^{bcd}	50.02 ± 2.66 ^{abc}
	8	34.7 ± 3.96 ^{abcd}	41.66 ± 5.46 ^{abcd}	54.74 ± 4.8 ^{ab}	62.81 ± 6.1 ^{a}
9	1	25.13 ± 7.03 ^{b}	10.84 ± 5.41 ^{b}	12.3 ± 4.51 ^{b}	20.99 ± 2.4 ^{b}
	2	27.16 ± 6.75 ^{b}	14.94 ± 7.19 ^{b}	19.51 ± 5.73 ^{b}	34.48 ± 4.4 ^{ab}
	4	25.95 ± 10.33 ^{b}	30.18 ±10.98 ^{ab}	25.95 ± 4.06 ^{b}	47.78 ± 9.4 ^{ab}
	8	35.18 ± 9.46 ^{ab}	36.85 ± 7.95 ^{ab}	37.86 ± 4.3 ^{ab}	67.67 ± 7.9 ^{a}

Values in average (n = 3) ± SE. Averages, in the same period, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.05$).

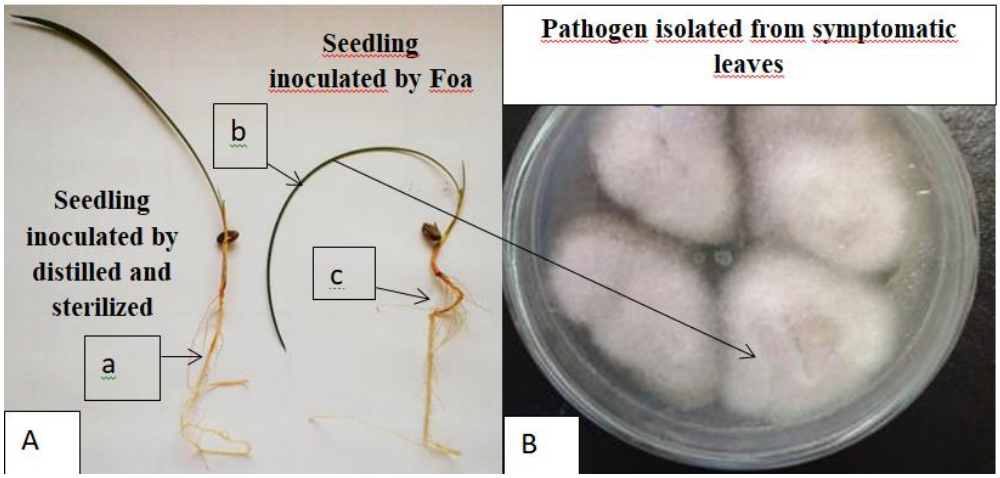

Figure 5 Pathogenicity test on seedlings confirmed the virulence of *Fusarium oxysporum* f.sp. *albedinis* (A), localized browning due to mechanical stress (a), leaves rolling and winding (b), Progressive root wet necrosis (c), re-isolation of pathogen from symptomatic leaves (B).

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Pre-treated date palm with root extract two weeks before Foa infection reduced the plant mortality by 25%. Treated date palm seedlings with pomegranate methanolic extracts at 160 mg/L without Foa inoculation led to plant mortality of 15% (Table 2).

Table 2 Effect of pomegranate **root** methanolic extract on plant mortality of date palm seedlings 105 days after inoculation with *Fusarium oxysporum* f. sp. *albedinis.*

Treatments	Plant mortality (%)
Untreated Uninfected (Control)	10
Untreated Infected	85
Treated Infected	25
Treated Uninfected	15

Enzymes activity and phenolic content

The Figure 6 shows that Foa infection increased POX activity.

This activity was 5 and 3 folds higher than the control respectively, in treated inoculated plants and untreated inoculated ones after 105 days of inoculation. The same observation was noticed for the phenolic content.

Discussion

The current study is part of the search for strategies to control plant pathogens through plant extracts known for their richness in bioactive molecules with anti-microbial action. The use of this strategy in the case of bayoud disease is very important especially because of the severity of this disease and the absence of effective chemical control. Moreover, this strategy is eco-friendly and provides a safe method to control Foa and to ensure the sustainability of the oasian ecosystem.

The effect of the extracts on Foa strain revealed an inhibitory activity on the mycelial growth, biomass production, sporulation, and spore germination. This inhibitory effect became greater when the extract concentration increased. Indeed, the higher the volume of these extracts in the culture medium, the higher the rate of inhibition of the mycelial growth. The maximum inhibition was recorded at 8% concentration. In addition, microscopic observations showed that the spore germination was strongly inhibited at the earliest incubation times in the presence of extracts compared to controls. In fact, in the absence of extracts (controls), Foa spores exhibited a normal development reflected by normal germination of the spores after 24 hours of incubation at 25 °C. The germination of spores was reported as a key process in the pathogenesis of fungi. Foa spores germinate upon contact with the roots and multiply on their surface. They form a mycelial tangle which, in places, gives rise to appressoria corresponding to the points of penetration of the fungus (Oihabi, 1991). Thus, the inhibition of the germination of spores exerted by the pomegranate root extracts constitutes a promising means to control date palm Fusarium wilt. Similar results were obtained in previous studies using pomegranates peel extracts against several plant fungal pathogens such as *Botrytis cinerea*, *Penicillium digitatum*, *Alternaria alternata*, *Stemphylium botryosum*, *Colletotrichun acutatum senso strico*, *Fusarium oxysporum*, *Aspergillus parasiticus*, *Monilinia lax* and *Monilinia fructigena* (Belgacem *et al*., 2021). On the other hand, Bouhlali *et al*. (2020) demonstrated that mycelium growth, sporulation, and spore germination of Foa were inhibited by polyphenol-rich extracts of *Cupressus atlantica* and *Eucalyptus torquata* and that this inhibition is dose-dependent.

In addition, the antifungal activity varied according to the extract type. Indeed, the root methanolic extract was more effective against Foa than the aqueous one for inhibiting mycelial growth, biomass, and the spore production. It has been reported that ethanolic pomegranate peel extract completely inhibited the germination of *B. cinerea* and *C. acutatum* conidia, while it was less effective against *P. digitatum* and *P. expansum* (Pangallo *et al*., 2017; Li Destri Nicosia *et al*., 2016). Similarly, Aoki *et al*. (2019) demonstrated that the methanolic extract of *Piper betle* leaves exhibited a high inhibitory effect on the hyphal growth of *P. viticola*.

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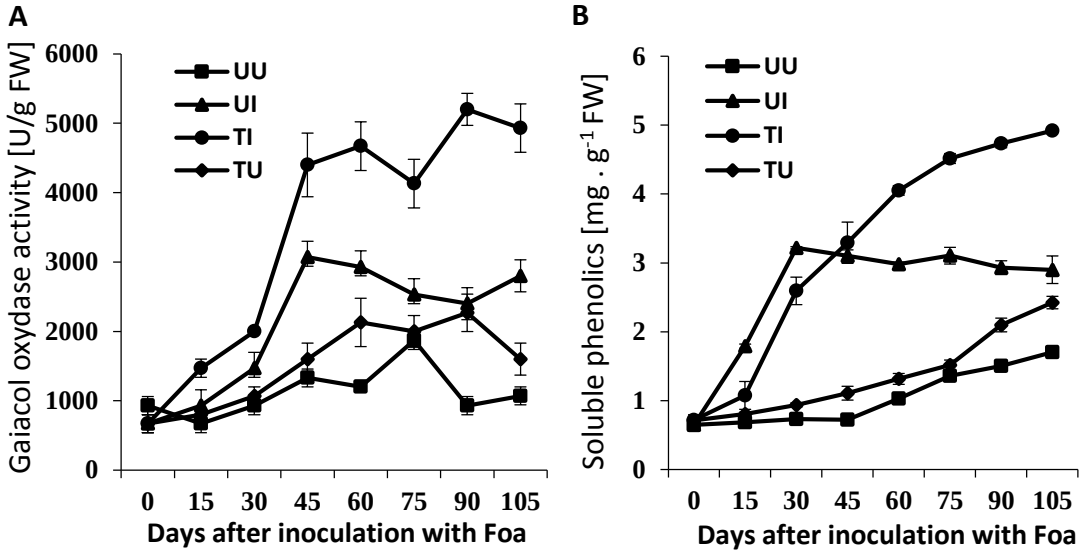

Figure 6 Variation on guaiacol oxidase activities in root of untreated uninfected seedlings (UU), untreated infected seedlings (UI), treated infected seedlings (TI), treated uninfected seedlings (TU) (A) and their soluble phenolics content after pathogen inoculation (B).

The inhibition of Foa hydrolytic enzymes by pomegranate root extracts was moderate and insignificant. This could be related to the insufficiency of the doses used in this study. In previous results, we showed that the aqueous oleander extract was the most effective in reducing the enzymatic activities of cellulases and proteases (unpublished results).

Besides the direct antifungal activity, pomegranate root extracts activated date palm defense responses regarding the increase of the peroxidase activity and the phenolic content, two biochemical markers of date palm resistance to Fusarium. In the Foa/date palm pathosystem, the enhancement of date palm resistance by jasmonic acid, salicylic acid, chitosan, hypoaggressive Foa isolate, and arbuscular mycorrhizal fungi treatments were correlated with an increase in POX activity (Jaiti *et al*., 2009; Dihazi *et al*., 2011). Their role in protecting plants against diseases is often associated with their capability to cross-link the cell wall compounds to strengthen the cell wall (formation of lignin, extension cross-link, dityrosine bonds) and also to create a highly toxic environment by massively producing reactive oxygen species (Passardi *et al*., 2005). These conditions are likely to stop the pathogen spread. On the other hand, the phenolic compounds were reported to be involved in date palm response to Foa infection (El Hadrami *et al*., 1997; El Modafar, 2010; Jaiti *et al*., 2008; Dihazi *et al*., 2012; Meddich *et al*., 2015). El Hassni *et al*. (2021) showed that hydroxycinnamic acids especially those found in reaction of date palm to Foa inoculation; mainly caffeic acid, p-coumaric acid, ferulic acid and sinapic acid (El Hassni *et al*., 2004; Jaiti *et al*., 2008), exhibit a highest inhibition of mycelial growth and sporulation of Foa. These phenolic acids also decreased the activities of Foa hydrolytic enzymes, cellulases, and pectin methylesterase.

Conclusion

The results presented in this work highlighted the antifungal activity of the pomegranate root extracts against Foa. In addition, the capability of the pomegranate extracts to trigger date palm defense response seems to involve phenolic metabolism and peroxidase activity. The

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mechanisms of action of the pomegranate extract on Foa, their specificity of action, and their stability in the environment need to be studied. Given that this natural substance has never been tested against Foa and that there are few means to control this pathogen, an in-depth study should be considered.

Statement of Conflicting Interests

The Authors state that there is no conflict of interest.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Brahim Rabach and Laila Lbekri. The first draft of the manuscript was written by Brahim Rabach, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

Abbasi, H., Akhtar A. and Sharf, R. 2012. Effect of bark extract of different plant species on the hatching of Meloidogyne incognita. Archives of Phytopathology and Plant Protection, 45(10): 1201-1203. DOI: doi.org/10.1080/03235408.2012.659553.

Alcaráz, L. E., Fusco, M. R., Mattana, C. M., Satorres, S. E. and Laciar, A. L. 2015. Antibacterial activity of methanolic and aqueous extracts of *Ligaria cuneifolia* and *Tripodhantus flagellaris*. Emirates Journal of Food and Agriculture, 27(11): 831-836. DOI: doi.org/10.9755/ejfa.2015-04-124.

Aoki, Y., Van Trung, N. and Suzuki, S. 2019. Impact of Piper betle leaf extract on grape downy mildew, effects of combining 4-allylpyrocatechol with eugenol, α-pinene or β-pinene. Plant Protection Science, 55(1): 23-30. DOI: doi.org/10.17221/53/2018-PPS.

Belgacem, I., Li, M., Destri Nicosia, G., Pangallo, S., Abdelfattah, A., Benuzzi, M., Agosteo, G. E. and Schena, L. 2021. Pomegranate Peel Extracts as Safe Natural Treatments to Control Plant Diseases and Increase the Shelf-Life and Safety of Fresh Fruits and Vegetables. Plants, 10: 453. DOI; doi.org/10.3390/plants10030453.

Bouhlali, E. D. T., Derouich, M., Ben-Amar, H., Meziani, R. and Essarioui, A. 2020. Exploring the potential of using bioactive plant products in the management of *Fusarium oxysporum* f.sp. *albedinis*: the causal agent of Bayoud disease on date palm (*Phoenix dactylifera* L.). Beni-Suef University Journal of Basic and Applied Sciences, 9: 46. DOI: doi.org/10.1186/s43088-020-00071-x.

Boulenouar, N., Marouf, A., Cheriti, A. and Belboukhari, N. 2012. Medicinal Plants Extracts as Source of Antifungal Agents against *Fusarium oxysporum* f. sp. *albedinis*. Journal of Agricultural Science and Technology, 14: 659-669. jast.modares.ac.ir/article-23-3393-en.html.

Dihazi A., Serghini, M. A., Jaiti, F., Daayf, F., Driouich, A., Dihazi, H. and El Hadrami, I. 2011. Structural and biochemical changes in salicylic-acid-treated date palm roots challenged with *Fusarium oxysporum* f. sp. *albedinis*. Journal of Pathogens, Volume 2011, Article ID 280481, 9 pages. DOI: doi.org/10.4061/2011/280481.

Dihazi, A., Jaiti, F., Wafataktak Kilani, O., Jaoua, S., Driouich, A., Baaziz, M., Daayf, F. and Serghini, M. 2012. Use of two bacteria for biological control of bayoud disease caused by *Fusarium oxysporum* in date palm (*Phoenix dactylifera* L) seedlings. Plant Physiology and Biochemistry, 55: 7-15. DOI: doi.org/10.1016/j.plaphy.2012.03.003.

El Hadrami, I., T. Ramos, M. El Bellaj, A. El Idrissi-Tourane and J.J. Macheix., 1997. A sinapic derivative as an induced defence compound of date palm against *Fusarium oxysporum* f. sp. *albedinis*, the agent causing

[DOR: 20.1001.1.22519041.2022.11.4.5.2]

bayoud disease. Journal of Phytopathology, 145 (8-9): 329-333. DOI: doi.org/10. 1111/j.1439-0434.1997.tb00409.x.

El Hassni, M., Laadouzaa, H., El Hadrami, A., Dihazi, A., Rakibi, Y., Lemjiber, N. and Naamani, K. 2021. An *in vitro* evaluation of the effect of hydroxycinnamic acids on the growth and hydrolytic enzyme production in *Fusarium oxysporum* f. sp. *albedinis*. Archives of Phytopathology and Plant Protection, DOI: doi.org/10.1080 /03235408.2021.1920311.

El Hassni, M., Jaiti, F., Dihazi, A., Ait Barka, E., Daayf, F. and El Hadrami, I. 2004. Enhancement of defence responses against Bayoud disease by treatment of date palm seedlings with an hypoaggressive *Fusarium oxysporum* isolate. Journal of Phytopathology, 152 (3): 182-189. DOI: doi.org/10.1111/j.1439-0434.2004.00824.x.

El Modafar, C. and El Boustani, E. 2000. Relationship between cell wall susceptibility to cellulases and pectinases of *Fusarium oxysporum* and susceptibility of date palm cultivars. Biologia Plantarum, 43 (4): 571-576.

El Modafar, C. 2010. Mechanisms of date palm resistance to Bayoud disease: current state of knowledge and research prospects. Physiological and Molecular Plant Pathology, 74 (5-6): 287-294. DOI: doi.org/10.1016/j.pmpp.2010.06.008.

Jaiti, F., Kassami, M., Meddich, A. and El Hadrami, I. 2008. Effect of arbuscular mycorrhization on the accumulation of hydroxycinnamic acid derivatives in date palm seedlings challenged with *Fusarium oxysporum* fsp. *albedinis*. Journal of Phytopathology, 156: 641-646. DOI: doi.org/10.1111/j.1439-0434.2008.01411.x.

Jaiti, F., Verdeil, J. L. and El Hadrami, I. 2009. Effect of jasmonic acid on the induction of polyphenoloxidase and peroxidase activities in relation to date palm resistance against *Fusarium oxysporum* f. sp. *albedinis*. Physiological and Molecular Plant Pathology, 74 (1): 84-90. DOI: doi.org/10.1016/j.pmpp.2009.09.005.

Kalleli, F., Abid, G., Salem, I. B., Boughalleb-M'hamdi, N. and M'hamdi, M. 2020. Essential oil from fennel seeds (*Foeniculum vulgare*) reduces Fusarium wilt of tomato (*Solanum lycopersicon*). Phytopathologia Mediterranea, 59 (1): 63-76. DOI: doi.org/10.14601/Phyto-11143.

Kanoun, K., Abbouni, B. Bénine, M. L., Benmahdi, F. Z. and Marouf, B. 2014. Study of the efficacy of ethanolic extract of *Punica granatum* linn bark on two phytopathogenic strains: *Ascocyhta rabiei* (pass.) labr and *Fusarium oxysporum* f. sp. *radicis lycopersici*. European Scientific Journal, 10 (12): 1857-7881 (in Frensh).

Li Destri Nicosia, M. Pangallo, G., G. Raphael, S., Romeo, F. V., Strano, M. C., Rapisarda, P., Droby, S. and Schena, L. 2016. Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract. Postharvest. Biology and Technology, 114: 54-61. DOI: doi.org/ 10.1016/j.postharvbio.2015.11.012.

Macheix, J. J., Fleuriet, A. and Billot, J. 1990. Phenolic Composition of individual Fruits. Fruit Phenolics. Boca Raton, FL, CRC Press.

Meddich, A., Oihabi, A., Jaiti, F., Bourzik, W. and Hafidi, M. 2015. Role of arbuscular mycorrhizal fungi in the tolerance of date palm (*Phoenix dactylifera*) to vascular fusariosis and water deficit. Botany, 93 (6): 369-377 (in Frensh). DOI: doi.org/10.1139/cjb-2014-0249.

Mekam, P. N., Martini, S., Nguefack, J., Tagliazucchi, D., Mangoumou, G. N., Stefani E. 2019. Activity of extracts from three tropical plants towards fungi pathogenic to tomato (*Solanum lycopersicum*). Phytopathologia Mediterranea, 58 (3): 573-586. DOI: doi.org/10.13128/Phyto-10891.

Meziani, R. 2019. Optimization of a micropropagation scheme by organogenesis of the Majhoul date palm variety. (Doctoral dissertation), Faculty of Sciences and Techniques, Moulay Ismail University, Errachidia, Morocco.

Moutassem, D., Belabid, L. Bellik, Y., Ziouche, S. and Baali, F. 2019. Efficacy of

[DOR: 20.1001.1.22519041.2022.11.4.5.2]

essential oils of various aromatic plants in the biocontrol of Fusarium wilt and inducing systemic resistance in chickpea seedlings. Plant Protection Science, 55 (3): 202-217.

Oihabi, A. 1991. Etude de l'influence des mycorhizes à vésicules et arbuscules sur le bayoud et la nutrition du palmier dattier. (Doctoral dissertation), Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco.

Pangallo, S., Nicosia, M. G. L. D., Agosteo, G. E., Abdelfattah, A., Romeo, F. V., Cacciola, S. O., Rapisarda, P. and Schena, L. 2017. Evaluation of a pomegranate peel extract as an alternative means to control olive anthracnose. Phytopathology, 107 (12): 1462-1467. DOI: doi.org/10.1094/PHYTO-04-17-0133-R.

Passardi, F., Cosio, C. Penel, C. and Dunand, C. 2005. Peroxidases have more functions than a Swiss army Knife. Plant Cell Reports, 24: 255-265. DOI: 10.1007/s00299-005-0972-6.

Rabilu, S. A., Agyemang, E. A. and Farkas, B. 2021. Antifungal activity of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts against *Botrytis cinerea*. Journal of Central European Agriculture, 22(2), p. 420-428. DOI: 10.5513/JCEA01/22.2.3104.

Regnault-Roger, C. and Philogene, B. 2008. Past and Current Prospects for the Use of Botanicals and Plant Allelochemicals in Integrated Pest Management. Pharmaceutical Biology, 46: 41-52. DOI: doi.org/10.1080/13880200701729794.

Skalskỳ, M., Ouředníčková, J., Niedobová, J. and Hortová, B. 2020. Efficacy of agrochemicals against *Phyllobius oblongus*. Plant Protection Science, 56 (2): 116-122.

Wang, L.Y., How, W. C., Shen, T. A., Di, R. and Luo, Y. 2019. Chemical composition, antioxidant and bioactivities of essential oils from *Melaleuca bracteata* leaves. Plant Protection Science, 56 (1): 18-29. DOI: doi.org/10.17221/38/2019-PPS.

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فعالیت ضدقارچی عصاره ریشه *Punica granatum* و نقش بالقوه آن در تحریک واکنش دفاعی درخت خرما در برابر بیماری پژمردگی فوزاریومی نخل خرما

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چکیده: *Fusarium oxysporum* f. sp. *albedinis* (Foa) یک قارچ خاک برد است که مسئول مخرب‌ترین بیماری درخت خرما *Phoenix dactylifera* L. در مراکش به‌شمار می‌رود. در مطالعه حاضر، از عصاره‌های آبی و متانولی ریشه انار *Punica granatum* برای ارزیابی توانایی آن‌ها در القای دفاع نخل خرما در برابر Foa و فعالیت ضدقارچی آن‌ها استفاده شد. در آزمایش‌های درون شیشه‌ای، این عصاره‌ها بسته به ماهیت عصاره، اثرات بازدارندگی متفاوتی را نشان داد. عصاره متانولی مهار قابل‌توجهی از رشد میسلیوم (۵۱ درصد) و تولید زیستتوده (۸۶/۳ درصد) را نشان داد. درحالی‌که عصاره آبی مانع از اسپورزایی (۹۹/۳ درصد) و جوانه‌زنی اسپور پاتوژن (۷۵/۹ درصد) شد. علاوه براین، تیمار خرما با عصاره متانولی افزایش قابل‌توجهی در محتوای فنلی و فعالیت پراکسیداز نشان داده است که در دفاع نخل خرما در برابر بیماری نقش دارد. این نتایج اولیه زمینه امیدوارکننده‌ای را برای کنترل پژمردگی فوزاریومی خرما باز می‌کند.

واژگان کلیدی: فعالیت ضدقارچی، خرما، واکنش دفاعی، *Fusarium oxysporum* f. sp. *albedinis*، عصاره ریشه انار

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