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ANTIFUNGAL ACTIVITY OF THE *PISTACIA ATLANTICA* TAR AGAINST *FUSARIUM OXYSPORUM F. SP. ALBEDINIS,* THE CAUSE OF THE BAYOUD OF THE DATE PALM IN SOUTHWEST ALGERIA

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Abstract. This study aims to estimate the antifungal activity of the *Pistacia atlantica* tar against *fusarium oxysporum f. sp. albedinis*, the cause of the bayoud of the date palm in southwest Algeria, isolated Fusarium oxysporum f. sp. albedinis was from oases in southwest Algeria and identified by PCR technique analysis then the extraction tar of wood the *Pistacia atlantica* was carried out by the distillation per descensum technique and density of 0.90, a refractive index of 1.5155, a pH of 5.5, and a dry water ratio of 33.34% were revealed by the results of the physicochemical examination.The result antifungal activity dose of 2.78 g/ml, Pistacia Atlantica tar efficiently suppressed Foa growth in vitro. These results are highly encouraging for applying this extract to infected date palms in the field to reduce the damage brought on by this fungus.

Keywords: *antifungal activity, Bayoud, Fusarium oxysporum f. sp. albedinis, Pistacia Atlantica tar.*

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1. Introduction

The date palm, or *Phoenix dactylifera*, is a blessing in scorching desert areas, for millions of people living in dry areas, dates are a crucial source of food security (Muhammad Siddiq, 2013).

The Bayoud is an Algerian term for phytopathogenic fungi that infect a variety of plants, primarily the date palm (Dihazi *et al*., 2012).

Is a soil-borne fungus that brings tracheal fungal illness, the date palm eventually withers and dies as a result of parasites attacking palm plants through the roots and colonizing the entire vascular system (Bouhlali *et al*., 2020).

Chemical therapy is one of the many control measures that have been thought of, but it is still ineffective and challenging to implement. Selecting and cultivating cultivars resistant to *Fusarium oxysporum f. sp. albedinis* is the most efficient strategy to lower the prevalence of this disease. Unfortunately, Bayoud disease-resistant date palm lines frequently provide subpar fruit (Dihazi *et al.*, 2012).

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Given that, several agrochemicals, notable fungicides, have been demonstrated to be mutagenic, carcinogenic, or teratogenic in the past, their continued and indiscriminate use poses a serious threat to both human health and the existing ecogeographical conditions of people. Recent trends encourage the employment of biological control methods as an alternative to lowering the heavy use of pesticides to prevent environmental contamination. (Isaac & Abu-Tahon, 2014).

The hunt for novel, potent antifungal medications is one of the most significant concerns that humanity is now confronting. It's a highly challenging task that requires a deep understanding of science as well as a lot of tenacity. These inspire scientists to find, and create new powerful, active, and less harmful chemicals for systemic activities (Boussalah *et al*., 2013; Abdel-monaim *et al*., 2011).

To increase the interest in plant extracts as antibacterial and antiparasitic agents, greater attention must be dedicated to this issue. The ideal region for screening plants for biological activity is southern Algeria, with its abundant floral resources and ethnobotanical heritage.

Many plants and plant-based products are efficient antimicrobials against fungi that cause food and grain storage, foliar diseases, soilborne pathogens, and the vascular wilt that affects many different cultures (Cherifa *et al*., 2022).

The Anacardiaceae family includes the fast-growing pistachio of Atlas (*Pistacia atlantica*). It might be 20 meters tall. This plant can survive abiotic conditions like dryness and is grown in a variety of soil types (Boudy, 1948; El Zerey-Belaskri *et al*., 2018) *Pistacia atlantica* has a wide geographic range that includes North Africa, and the Middle. Afghanistan, Iran, and the East (Zohary, 2013).

The edible drupes, which are also rich in oil, are consumed frequently as food by the locals and are used in traditional medicine to cure several illnesses. The oil has several uses, including in food, cosmetics, and medicine (Chapagain *et al*., 2008; Obidah *et al*., 2009).

The objective of this work is the isolation of *Fusarium oxysporum f. sp. albedinis*, from different oases in southwestern Algeria and strains are identified by PCR method analysis and evaluate the activity and determination of the minimum inhibitory concentration of the Pistacia atlantica tar against these fungal.

2. Materials and methods

Tar extraction

In October 2021, the wood of *Pistacia atlantica* was gathered in the Bechar area of southwest Algeria. the extraction was carried out by the distillation per descensum technique. Small chunks of wood were chopped into bits and put in a specific dispositive for five hours. Until analysis, the distilled tar was kept at room temperature. (Benlarbi, 2019).

Pistacia Atlantica tar physical and chemical characteristics

The AFNOR methods were used to calculate the tar's specific density at 20°C (AFNOR NFT60-214), refractive index (RI-AFNOR NFT 60-212), acid index (AFNOR NFT 60-204), and pH (AFNOR NFT 04-408).

Fungal isolate

Fusarium oxysporum f. sp. albedinis strains were isolated in date palm rachis from southwest Algeria that had Bayoud symptoms. The rachis was cut into small pieces, cleaned with 50% ethanol for one minute, and then repeatedly rinsed with sterile distilled water. Three or four pieces were placed on each potato dextrose agar (PDA) plate, and the plates were incubated at 25 °C for 5-7 days in full darkness (Bahriz and Bouras, 2020). *Fusarium oxysporum f. sp. albedinis* isolates were discovered in the first step based on microscopic and macroscopic characteristics as well as the microculture method.

Fusarium oxysporum f. sp. albedinis PCR analysis

We used Nunes *et al*. (2011) CTAB methodology as our reference method for DNA extraction, with a few modifications, in the Universal approach, a single protocol that is applied to all types of biological materials, including plants, algae, blood, bacteria, and fungi, will be more demanding. A combination of 100 mM Tris-HCl, pH 8.0, 25 Mm EDTA, 1.5 M NaCl, 2% CTAB, β-mercaptoethanol, and PVP were utilized as the extraction buffer in this instance (in the case of the plant sample). The procedure uses the traditional steps of homogenization with liquid nitrogen, incubation in a water bath at 65°C, and deproteinization with chloroform-isoamyl alcohol, followed by ethanol precipitation and washing. As an alternative to the aforementioned, the "nuclei method" and "protoplast method," which are universal techniques, may be utilized successfully to create high-quality, megabase-sized DNA. When proteinase K (RNasThe RNA was removed when proteinase K (RNase; 10mg/ml) was added;10mg/ml) was added, the RNA was removed. To prepare the DNA for PCR reactions, it was dissolved in 200 µl of TE buffer (10 mM Tris-HCl, pH 6.0, 1 mM EDTA, pH 8.0), measured, and diluted to a concentration of about 5 ng/l.

Two primer pairs were used as PCR primers to amplify *Fusarium oxysporum f. sp. albedinis*: FOA1 (CAGTTTATTAGAAATGCCGCC) coupled with BIO3 (GGCGATCTTGATTGTATTTGGTG) and FOA28 (ATCCCCGTAAAGCCCTGAAGC) coupled with TL3 (GGTCGT CCGCAGAGTATACCGGC).

25 µl were used for the PCR reactions, which contained 1 µl of genomic DNA (about 100 ng), 1.5 mM MgCl2, 25 mM dNTP, 0.5 U of Taq DNA polymerase (Biomatic), 2.5 µl of 10 reaction buffer, 1 µl of DMSO, 17 µl of ultra-pure water, and 0.5 M of each primer. A GTC96S Thermal Cycler was used for the amplification (Cleaver) (Fernandez *et al*., 1998).

The amplification program included one cycle for 4 min at 95°C followed by 30 cycles for 30 s at 92°C, 30 s at 60°C, and 30 s at 72°C for the BIO3-FOA1 primer pair and 30 cycles for 30 s at 92°C, 30 s at 62°C, and 45 s at 72°C for the TL3-FOA28 primer pair. One cycle for 15 min at 72°C was conducted after the 30 cycles. After amplification, 8 µl of the PCR amplification products were electrophoresed in 2.0% agarose gels in TBE buffer at 80 V for 2 h. The gels were then stained with ethidium bromide and photographed under UV light (Fernandez et al., 1998; Rosado-Álvarez *et al*., 2014).

In vitro antifungal activity and determination of the minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by the method of contact direct assay as described by Bhutani *et al*. (2018) with slight modifications.

The technique consists in adding selected volumes of tar on sterile tubes containing 15ml of PDA medium, to obtain the following Concentrations (0.30, 0.60, 1.21, 1.52, 1.83, 2.15, 2.46, 2.78, and 3.10 µg/ml) respectively, then, the mixtures are stirred till the homogenization of the extract with the medium.

PDA plates containing various concentrations of the tar extract were inoculated with the spore suspensions $10^6$of each strain and incubated at 25°C for 7 days. (El Hassni *et al*., 2004) The inhibition rate was calculated using the formula % Inhibition = (DT – D)/ DT × 100 in which DT = diameter of the FOA on the control plate and D = diameter of the FOA on the plate containing the extract. This technique is inspired by the opposed culture technique, recommended by Patel and Brown. (1969).

Additionally, a control assay was employed, and three copies of each test were run. The minimum inhibitory concentration (MIC) of the tar extract was the lowest concentration that completely inhibited the visible growth of the FOA (Verma *et al*., 2011).

Statistical analyses

All the measurements were made in triplicate and the results obtained were expressed as the mean±standard deviation (SD). One-way ANOVA was carried out to test for any significant difference. Differences between means at $P \leq 0.05$ level were considered significant.

3. Results and discussion

Pistacia atlantica tar is Very slightly soluble in water, soluble in chloroform, ether, and ethyl acetate, partially and soluble in petroleum ether. The results of the physical and chemical of vegetable tar are shown in Table 1. Our results are very close to those reported by (Benlarbi, 2019).

Table 1. Physicochemical characterization of vegetable tar of Pistacia Atlantica

Rate of dry water	Specific density at 20 °C	Refractive index	PH
33.34	0.90	1.5155	5.5

Isolation and morphology of Fusarium oxysporum f. sp. albedinis

The *Fusarium oxysporum f. sp. albedinis* strains were isolated from the rachis of contaminated cultivar palms in the southwest Algeria palm grove. The *Fusarium oxysporum f. sp. albedinis* isolates revealed minor macroscopic morphological variability, such as the color of the mycelium as observed by (Benabbes *et al*., 2015). The macroscopic nature of the culture is characterized by white, cottony mycelium (Figure 1).

To identify *Fusarium oxysporum f. sp. albedinis* pathogenic isolates, specific PCR assay primer pairs TL3-FOA28 and FOA1-BIO3 were used. Using this specific PCR assay, 400 bp and 204 bp fragments were detected, and the strains we isolated from

infected date palms were identified as the *Fusarium oxysporum f. sp. albedinis* Bayoud pathogen (Figure 1).

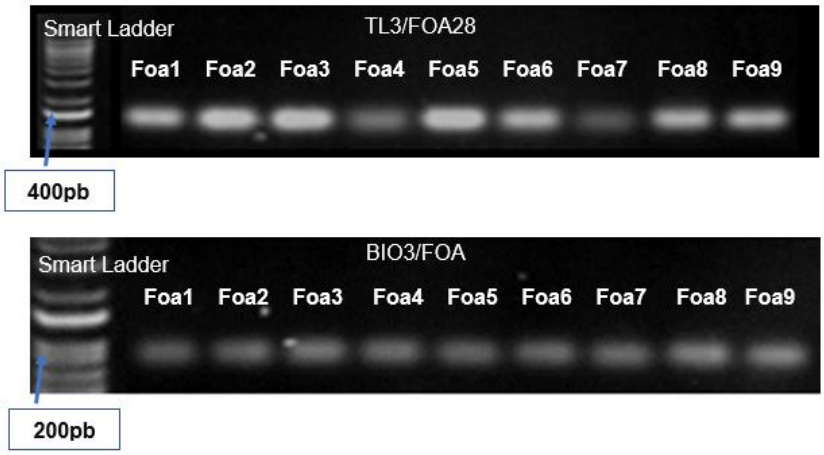

Fig 1. PCR assays for specific identification of *Fusarium oxysporum f. sp. albedinis* BIO3/FOA1 instead of BOI3/FOA
(Original Source, 2021)

Antifungal activity and MIC determination

The antifungal activity recorded in the present study by the inhibition of radial growth on a solid medium revealed that *Pistacia atlantica* tar exhibited significantly high antifungal activity against *Fusarium oxysporum f. sp. albedinis* (Table 2).

Table 2. Antifungal activity of *Pistacia atlantica* tar against *Fusarium oxysporum f. sp. albedinis* using radial growth contact direct method

	Concentration (µg/ml)									
	Control	**0.30**	**0.60**	**1.21**	**1.52**	**1.83**	**2.15**	**2.46**	**2.78**	**3.10**
Foa 1	60.33±0.35	49.13±0.32	37.03±0.05	27.97±0.25	22.30±0.51	15.03±0.25	7.13±0.15	3.03±0.05	1.03±0.15	**0**
Foa2	57.43±0.51	45.26±0.30	29.9±0.26	25.2±0.52	19.73±0.46	19.06±0.11	14±0	10.23±0.25	0.1±0.1	**0**
Foa3	60.73±0.64	57.5±0.5	40.3±0.3	32.5±1.32	20.36±0.55	18.3±0.26	13.23±0.25	10.23±0.25	3±0.1	**0**
Foa4	58.83±0.28	44.5±0.5	39.66±0.57	26.96±0.15	22.16±0.20	17.16±0.28	10.8±0.26	4.9±0.1	1.93±0.05	**0**
Foa5	59.03±0.15	50.13±0.32	41.33±0.41	29.36±1.18	23.1±0.65	17.03±0.05	10.1±0.17	4.1±0.1	0.1±0.17	**0**
Foa6	54.76±0.68	40.3±0.43	34.96±0.15	25±0.1	23.16±0.28	18.06±0.05	10.83±0.76	5.06±0.11	**0**	**0**
Foa 7	57.7±0.60	39.26±0.25	40.26±0.25	35.26±0.30	30.46±0.45	22±0	17.36±0.32	11.5±0.5	4.5±0.5	**0**
Foa 8	61.76±0.40	54.73±0.64	50.2±0.34	40.33±0.57	31.23±0.25	22.3±0.3	12.3±0.3	8.66±0.57	6.33±0.41	**0**
Foa 9	75.63±0.55	55.83±	34.33±0.28	28.16±0.57	19.26±0.28	13.46±0.25	13.46±0.50	10.2±0.2	8.13±0.32	4.7±0.36

Data are presented as means ± SDM (n=3), P≤0.05.

Table 3. Differences between groups (ANOVA)

	F	P-value	F-CRIT
Foa 1	19799.7154	1.7326E-37	2.39281411
Foa 2	10096.7829	1.4549E-34	2.39281411
Foa 3	4592.39491	3.8262E-31	2.39281411
Foa 4	13047.5045	1.1212E-35	2.39281411
Foa 5	6216.47072	1.8555E-32	2.39281411
Foa 6	7240.40687	4.0416E-33	2.39281411
Foa 7	6990.47998	5.7418E-33	2.39281411
Foa 8	8258.94798	1.0843E-33	2.39281411
Foa 9	12722.6411	1.4427E-35	2.39281411

The vegetable tar induced a great inhibition over the mycelial growth of the most investigated strains of Fusarium oxysporum f. sp. albedinis, from the concentration of 2.78µg/ml to 3.10µg/ml. The highest percentage is about 99% against the strain of FOA6 in the concentration of 2.78µg/ml, compared to the other strains of *Fusarium oxysporum f. sp. albedinis*. However, showed the same sensitivity for all strains against the vegetable tar with the same concentration of 3.10 µg/ml. We found that our *Pistacia atlantica* tar extract inhibited *Fusarium oxysporum f. sp. albedinis* growth on the PDA medium indicating the presence of potent antifungal compounds in our extract.

Similar antifungal activity has been observed for *Olea Europea* and *Juniperus oxycedrus* tars by (Bendjima *et al*., 2020) and (Terfaya *et al*., 2019), and these studies confirm the results of (Benlarbi *et al*., 2014) who's reported that tar had antifungal activities against studied fungi: Aspergillus niger, A. flavus, Penicillium purpurogenum, *Fusarium oxysporum f. sp. Albedinis* (Gumgumjee, 2020). P-value< 0.05 (significance level) so there is a significant difference between the groups (09 strains) that is to say there is an influence of the concentration on the champions (Table 3).

According to Akrout et al. (2007), it may be inferred that vegetable tar has fungistatic or fungicidal effects on the strains tested at specific doses. This may be because it prevents the growth of mold toxins and mycelial elongation, sporulation, and spores.

4. Conclusion

The results of Antifungal activity have been determined to have an effect against *Fusarium oxysporum f. sp. albedinis*, with a high recorded activity of tar Pistacia Atlantica.

These results are very promising for practicing this extract in the field on diseased date palms to limit the damage caused by this fungus, by in vivo tests carried out on the spot and controlled, aiming at elaborating a biopesticide, is necessary, on one hand, to confirm the results we have obtained and, on another hand, to study their potential efficacy under different

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