

Bioactivity of a methanolic extract of *Peganum harmala* L. seeds on the inflorescence rot agent (*Mauginiella scaettae*) and the fusarium rot agent (*Fusarium oxysporum* fsp *albedinis*) of date palm

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Abstract. The antifungal activity of the methanolic extract of *Peganum harmala* L. seeds harvested in Algerian Sahara was assessed on the mycelial growth of *Mauginiella scaettae* the causal agent of inflorescence rot and *Fusarium oxysporum* f.sp *albedinis* the causal agent of vascular fusariosis of date palm (Bayoud). The phytochemical analyses revealed the absence of tannins, flavonoids, steroids and coumarins, and among others, alkaloids, saponosides and terpenoids. The antifungal tests of different concentrations prove a strong inhibitory activity of the seed extract towards *Mauginiella scaettae* with an average inhibition rate of 100% after 72 h from 20% concentration to 100% (v/v). For *Fusarium oxysporum* f. sp *albedinis*, the inhibition rate increased with the extract concentration and the mycelial growth in the treated plates showed a low growth rate compared to the growth of this fungus in the control. The ANOVA test reveals that the extract of *Peganum harmala* seeds is very effective against *Mauginiella scaettae* and *Fusarium oxysporum* f.sp *albedinis*, it appears to have antifungal and mycelial growth inhibitory activity.

Key words: *Peganum harmala* L, *Mauginiella scaettae*, *Fusarium oxysporum* f. sp *albedinis*.

INTRODUCTION

The date palm culture is a specificity of arid and semi-arid regions in Algeria, totaling 20 million date palms on an area of 170,500 hectares and a production of

1,151,909 tons of dates (FAO, 2020). In the desert, this heritage plays essential ecological, economic and social roles. Nevertheless, this heritage witnessed significant production losses caused by a variety of pests. Among these, *Mauginiella scaettae* and *Fusarium oxysporum* f.sp *albedinis* (*Foa*) are responsible for inflorescence rot and fatal vascular fusariosis of date palms, with a direct impact on date production and the longevity of the tree (Djerbi, 1983) in addition, many plant species synthesize substances (phenols, sesquiterpenes and glucosides) that have antifungal properties and may be of interest for crop protection, (De Corato et al., 2007). Among these species *Peganum harmala* L. has been used for a long time in traditional medicine (Chehma, 2006; Zougagh et al., 2019), and is known for its antibacterial, antifungal, antiviral and insecticidal properties (Jinous & Fereshteh, 2012) *Peganum harmala* L. (Zygophyllaceae), a spontaneous perennial plant from the Algerian northern Sahara (Ozenda, 1977).

The aim of this research is to assess the anti-fungal activity of the extract of the seeds of *Peganum harmala* L against *M. scaettae* and *F. oxysporum*.

MATERIALS AND METHODS

Plant material: the extracts were obtained from *Peganum harmala* L. seeds collected in the region of Ouedlabyad [32°32'49.4 "N; 3°36'44.2 "E], province of Daya Ben Dahoua, state of Ghardaia. The seeds were successively rinsed with water, dried in the shade (20 days) at room temperature (20 to 25 °C) and finally crushed before extraction.

Fungal material: a strain of *M. scaettae* was isolated from small pieces of contaminated spathe, soaked in bleach (60%) for 5 minutes and rinsed with sterile distilled water. Once dried, they were cultured in Potato Dextrose Agar (PDA) medium and incubated for 7 days at 21 °C. To obtain pure isolates, successive transplants were made by transferring mycelial fragments into a new PDA medium. The pure strain of *M. scaettae* was obtained after isolation and purification in the laboratory of the university of Ghardaia and identified with Mr. Bensaci Messaoud Bachagha. The *Foa* strain was isolated, transplanted and identified at the laboratory of the regional plant protection station in Ghardaia.

Preparation of the methanolic extracts: they were obtained by solubilization in a mixture of distilled water and methanol (1/3–2/3) (v/v), through extraction by reflux. The extraction device consists of a flask (1,000 mL) heated with a heating mantle and topped with a refrigerating mechanism. A quantity of 100 g of seeds powder of *Peganum harmala* is deposited in the flask containing 600 mL of water-methanol solution, the mixture was boiled at 45 °C for six hours; then the mixture was filtered. To remove the methanol, the filtrate was evaporated under vacuum in a rotary evaporator set at 50 °C and a rotation speed of 80 rpm (Tonk et al., 2006).

Antifungal trials: they were performed according to the technique of Grover & Moore. (1962): dilutions were prepared to obtain final extract concentrations of 5%, 10%, 20%, 30%, 40%, 50% and 100%. In sterile Petri plates of 9 cm diameter, 15 mL of PDA and 1 mL of extract were placed with circular shaking to distribute the extract uniformly. The plates with and without extract (controls) were inoculated as follows: a mycelial disc of the phytopathogenic fungus with a diameter of 5 mm taken from the periphery of a 7-day-old culture is aseptically placed in the centre. The procedure is repeated three

times for each concentration of extract. Petri plates were incubated at 21 ± 2 °C for seven days. The diameters of the fungal colonies were measured daily (Pandey et al., 1982).

Phytochemical analyses: the phytochemical screening was performed according to the methods described by several authors (Harborne, 1973; Trease & Evans, 1989; Diallo, 2000; Dohou, 2003; Mibindzou, 2004 and Koffi, 2015).

HPLC Analysis: HPLC analyses were performed on a Waters liquid chromatograph equipped with a model pump LC-20ADXR and PDA detector using C18 reversed-phase packing column (GraceSmart RP18, 4.6×150 mm, $5 \mu\text{m}$; Grace, Deerfield, IL, USA) for the separation, the column was thermostated at 30 °C using a CTO-20A column oven and Empower v.2 Software (Waters Spa, Milford, MA, USA) was used for the acquisition of data. Isocratic elution was performed using the mobile phase, which consisted of ultrapure water methanol (40: 60, v/v) and injected directly on-line degassed by using Degasex, mod. DG-4400 (Phenomenex, Torrance, CA, USA) at a flow rate of 1 mL min^{-1} . The standard solutions ($100 \mu\text{ mL}^{-1}$) were diluted in the mobile phase, then all the prepared sample solutions of extract and the standard were centrifuged and $10 \mu\text{L}$ of the supernatant was injected into HPLC and achieved at wavelength of 435 nm with a bandwidth of 4 nm, the time of HPLC experience is 75 mn.

Statistical analysis: A two way ANOVA was performed to verify whether the observed effect is growth dependent and/or concentration dependent/time dependent. The data obtained was subjected to a randomized repeated measures analysis of variance using *Statistica* 10 software. The results were also subjected to a multiple comparison of means using the Tukey HSD (Honest Significance Test).

RESULTS AND DISCUSSION

Extracts composition: the phytochemical tests confirmed the presence of secondary metabolites in the extracts of *P. harmala* L. seed, with an extraction yield of 20.01%. Phytochemical analyses were carried out following classical technics (observation of reactions) to highlight the presence of chemical groups known for their antifungal effects. In Table 1 of phytochemical screening of *P. harmala* L seed extracts revealed the absence of tannins, flavonoids, and reducing compounds, as well as coumarins and steroids. Terpenoids are moderately present, while free quinones and alkaloids are abundant. These results are similar to those of Bouabedelli et al. (2016) and Babaousmail et al. (2014). Based on those results reported in the literature, the presence of alkaloids in our *P. harmala* L. seeds extract was elucidated using HPLC chromatography method. The quantitative analysis was carried out to detect the presence of five known alkaloids, namely Harmol, harmine, harmaline, and peganine (vasicine), in methanol seeds extracts

Table 1. Phytochemical screening results of *Peganum harmala* L. seeds extracts

Chemical compounds	Seeds
Tannins	-
Catechism	-
Gallic	-
Flavonoids	-
Catechism	-
Gallic	-
Anthocyanins	-
Catechols	-
Leucoanthocyanins	-
Coumarins	-
Free quinones	+++
Alkaloids	+++
Terpenoids	++
Saponosides	+
Steroids	-

(-) – Absence; (+) – low concentration; (++) – medium concentration; (+++) – high concentration.

of *P. harmala* L. which were confirmed by comparing the retention times on HPLC chromatogram spectra of the extract with the reference samples as shown in Fig. 2 by comparison with the same time as the external standard substance, we concluded that the first peak in HPLC chromatogram eluting at 18.435 min with 10.983% of the average contents was in accordance with Harmol, the second peak eluting at 30.831 min was tentatively identified as Harmaline with an average contents of 8.414%. The retention times for harmine were observed to be at 43.604 minutes, with average contents of 54.815. We observed that the *P. harmala* seeds produced peaks in HPLC chromatogram and the peak corresponding to vasicine was observed which revealed that this bioactive molecule was also identified and quantified in the dry seeds (Herraiz et al., 2017; Abbas et al., 2021). According to these results, harmol, harmine, and harmaline were the main alkaloids in extracts, with a total average content of 74.212% and harmine was noted as the major β -carboline alkaloids compounds in this plant as shown in Table 2.

Table 2. HPLC chromatogram data for alkaloids detected in *P. harmala* L methanol seeds extracts

2	Ret. time	Substance	Area, %
1	18.435	Harmol	10.983
2	30.831	Harmaline	8.414
3	43.604	Harmine	54.815

Inhibition rate

According to Doumbouya et al. (2012), the inhibition rate (IR) of mycelial growth compared to the control is calculated according to the formula:

$$IR (\%) = 100 \times (dC - dE) / dC$$

dC – Diameter of the control colony; dE – Diameter of the colony treated with the extract.

Table 3. Growth inhibition of *Mauginiella scaettae* in the presence of *P. harmala* seed extracts

Inhibition rate	72 h	96 h	120 h	144 h	168 h	Average IR
5%	21.96 ± 0.06	31.04 ± 0.06	11.21 ± 0.13	10.93 ± 0.12	4.52 ± 0.19	15.93
10%	86.68 ± 0.16	77.98 ± 0.21	66.32 ± 0.28	65.21 ± 0.30	61.69 ± 0.44	71.57
20%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
30%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
40%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
50%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
100%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100

The antifungal tests according to the method of Grover & Moore (1962) showed that in the presence of the *Peganum harmala* extract we noted that there is great effectiveness in inhibiting of mycelial discs. The concentrations of extracts of *Peganum harmala* seeds have an inhibitory action on the mycelial growth of the strains of *Foa*, the inhibition also appeared at the lowest extract concentration (5%) with 15.93% inhibition rate and increased to a maximum (nearly 84%) with the pure extract (Tables 3 and 4). On *M. scaettae*, inhibition is significant (71.57%) from 10% extract, becoming total from 20% extract concentration. There is no mycelial growth in Petri plates treated with 20%, 30%, 40%, 50%, 100% extract concentration.

Table 4. Growth inhibitions of *Fusarium oxysporum* f.sp *albedinis* in the presence of *P. harmala* L. seed extract

Inhibition rate	72 h	96 h	120h	144 h	168 h	Average IR
5%	24.33 ± 1.15	20.59 ± 0.71	17.96 ± 0.07	27.84 ± 0.14	24.99 ± 0.28	23.14
10%	35.12 ± 1.73	36.29 ± 0.76	35.16 ± 0.08	40.52 ± 0.21	32.39 ± 0.13	35.90
20%	54.10 ± 1.53	39.24 ± 0.58	40.65 ± 0.12	45.58 ± 0.15	40.91 ± 0.23	44.09
30%	66.26 ± 0.29	56.88 ± 0.58	56.26 ± 0.06	58.22 ± 0.10	54.55 ± 0.06	58.44
40%	62.21 ± 0.29	60.82 ± 0.58	59.40 ± 0.06	62.67 ± 0.03	61.37 ± 0.06	61.29
50%	81.10 ± 0.76	74.53 ± 1.53	70.32 ± 0.06	70.91 ± 0.12	67.61 ± 0.07	72.89
100%	83.78 ± 0.06	85.29 ± 0.58	82.84 ± 0.12	84.81 ± 0.17	82.95 ± 0.20	83.94

Determination of the minimum inhibitory concentration (MIC)

The MIC corresponds to the lowest concentration of extract for which no growth of the treated fungus colony is observed, visible to the naked eye; therefore, it has a fungistatic effect and does not provide any information on the situation of the fungus population; particularly it does not allow to specify whether it has been partially or totally killed or whether it has simply stopped the growth (Berezin & Dellamonica, 1999). The fungi treated with concentrations that showed a total absence of mycelial growth were transferred to plates containing PDA medium to confirm the minimum inhibitory concentrations (MIC). We noted that a dose of 20% is the MIC for *Peganum harmala* L seed extract against *M. scaettae* with zero mycelial growth and even after a second mycelial disc subculture.

Mycelial growth rate (MG)

According to Cahagnier & Richard-Molard. (1998), the rate of mycelial growth at each concentration is determined by the formula:

$$VC = [D1/Te1] + [(D2-D1)/Te2] + [(D3-D2)/Te3] + \dots + [(Dn-Dn-1)/Ten]$$

D – Diameter of the growth area of each day (mm); Te – Incubation time (day).

P. harmala L seed extract affects the mycelial growth rate of both *M. scaettae* and *Foa*, the average daily growth rate of which is low compared to the controls in all treated boxes (Fig. 1).

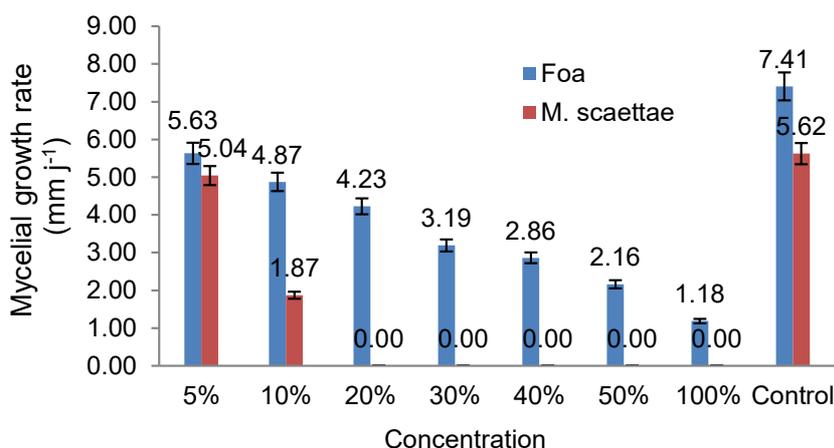


Figure 1. Mycelial growth rate of *M. scaettae* in the presence of different concentrations of *P. harmala* L. seed extract.

Statistical analysis

ANOVA reveals that all the concentrations of *P. harmala* L seed extract show a significant difference as an inhibitory extract of both phytopathogenic fungi (Table 5), *Fusarium oxysporum* f.sp *albedinis*, *Mauginiella scaettae* ($F = 102.173$; $P = 0$) ($F = 129.9257$; $P = 0$). The same was true for the ‘time’ factor ($F = 302.490$; $P = 0$) ($F = 334.0321$; $P = 0$). The interaction between ‘factor’, ‘concentration’ and ‘time’ (both factors) is significant. The antifungal action of *P. harmala* L seed extracts is highly significant on *M. scaettae* and significant on *Foa* (Fig. 1).

Table 5. Analysis of variance (ANOVA)

Effect	Pests	SS	Degree freedom	MS	F	P
Concentration	<i>Foa</i>	8,286.15	7	469.45	102.173	0.00000
	<i>M. scaettae</i>	4,804.140	7	686.306	129.9257	0.00000
Time	<i>Foa</i>	1,230.84	4	307.71	302.490	0.00000
	<i>M. scaettae</i>	444,158	4	111.04	334.0321	0.00000
Concentration x Time	<i>Foa</i>	245.38	28	8.76	8.76	0.00000
	<i>M. scaettae</i>	851.017	28	30.393	30.393	0.00000

The mycelial growth in the presence of the methanolic extract was evaluated during seven days of incubation, at the optimal growth temperature of *M. scaettae* (21 °C) and the optimal growth temperature of *Foa* (25 °C). The antifungal activity was estimated by comparing the mycelial growth of the boxes treated with the extracts at different concentrations with that of the control.

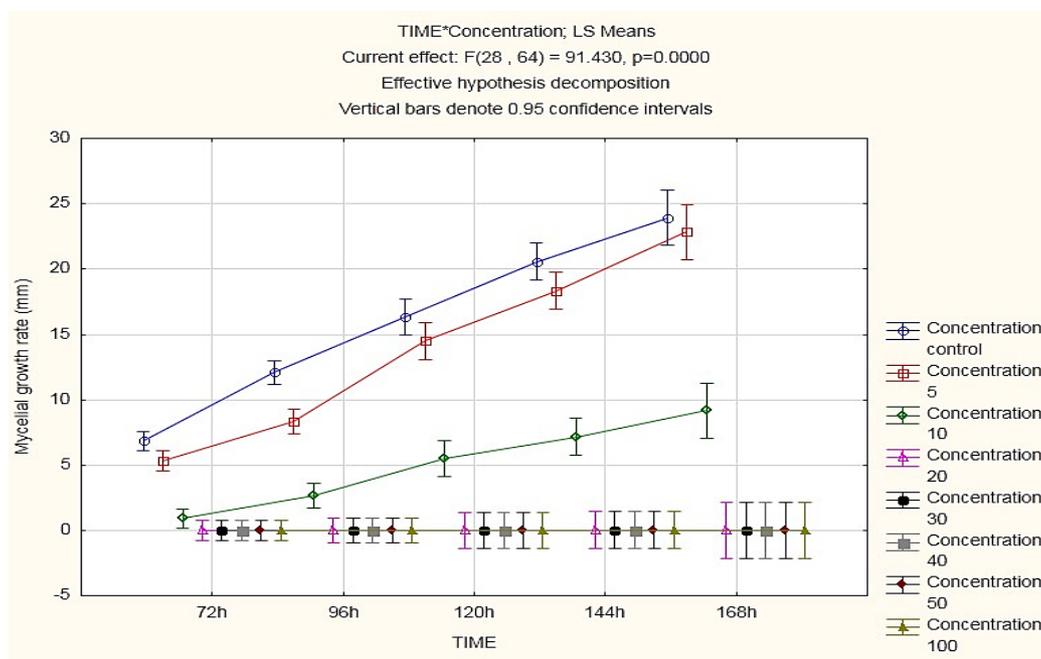


Figure 2. Effect of methanolic extract of *Peganum harmala* L. on the growth of *Mauginiella scaettae*.

The seed extract was more active on *M. scaettae* showing a fungistatic effect with slowed mycelial growth at 5% concentration and a fungicidal effect at 20% concentration at which mycelial growth becomes null (Fig. 2). The concentrations of extracts of *Peganum harmala* seeds have an inhibitory action on the mycelial growth of the strains of *Foa* (Fig. 3).

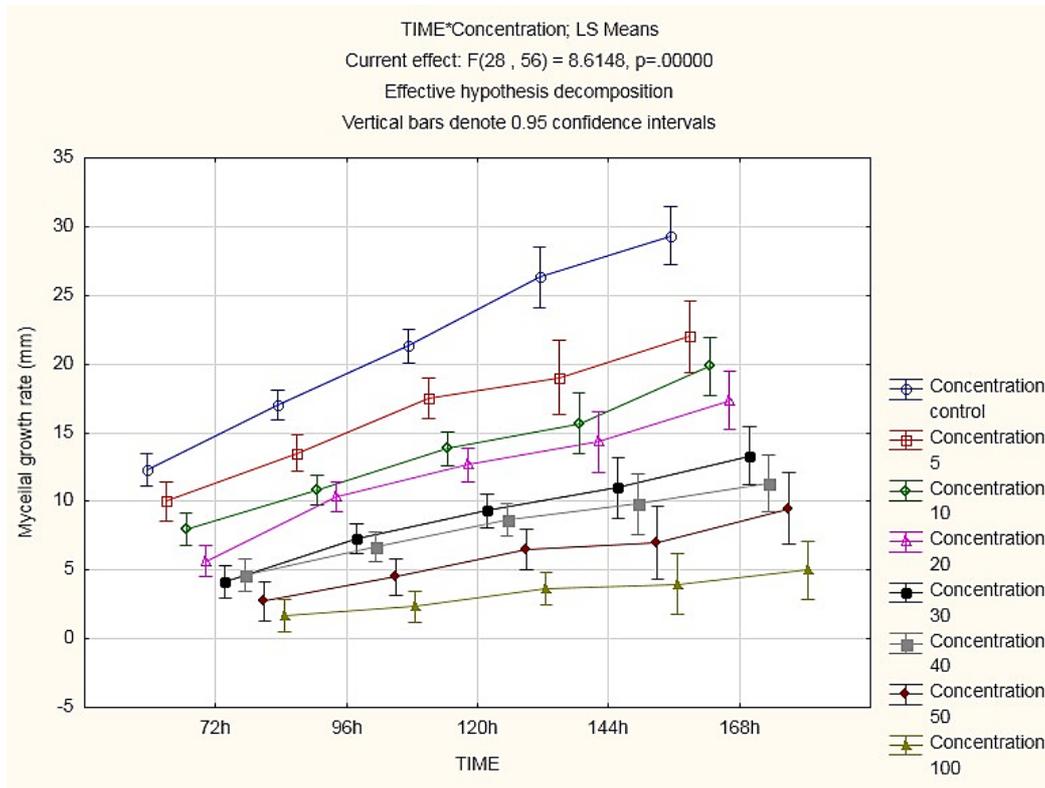


Figure 3. Effect of methanolic extract of *Peganum harmala* L. on the growth of *Fusarium oxysporum* sp. *albedinis*.

The antifungal activity of plant extracts is due to their biologically active compounds. The antifungal activity of this extract against *Mauginiella scaettae* is attributed mainly to the terpenoids found in high concentrations (+++) in the seed extract, which have an antibacterial effect and were also reported by Shane et al. (1999).

Saadabi (2006) reports for several plant species and different solvents that compared to aqueous or chloroformic extraction, methanolic extracts have a more anti-fungal effect, an effect antimicrobien often associated among others with richness in alkaloids.

Many studies have noted that the alkaloids of *P. harmala* L. seeds have antimicrobial activity namely antifungal, antibacterial (Prashanth & John, 1999; Saadabi, 2006; Nenaah, 2010) and insecticidal (Rharrabe et al., 2007). This is demonstrated by the results related to the rates of inhibition of mycelial growth of *M. scaettae* and *Foa* (Tables 3 and 4).

The saponins are relatively abundant in the extracts of seeds. According to the work of Viollon & Chaumont (1994), saponins have an inhibitory effect on fungal growth. While terpenoids present in the seeds in good quantity are recognized for their antimicrobial activities and alkaloids have antibacterial activity (Kuc, 1985).

This result was accordance with these findings in previous reports by Herraiz et al., (2010); Aziz et al. (2017), who detected an amount of harmol, harmaline, and harmine in the *Peganum harmala* L seed methanol extract. On the other hand, Bukhari et al., 2008 and Iranshahy et al., 2019) indicated that the *P. harmala* methanol extract seeds contained harman alkaloids (harmine and harmaline) and the same substance was identified by the HPLC analysis of *P. harmala* collected from the mountain of Saint Katherine (Sinai, Egypt).

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According to the literature, the presence of alkaloids in our *P. harmala* L. seeds extract was elucidated using HPLC chromatography method. The quantitative analysis was carried out to detect the presence of four known alkaloids, namely harmol, harmine, harmaline, and peganine (Vasicine), in methanol seeds extracts of *P. harmala* L. which were confirmed by comparing the retention times on HPLC chromatogram spectra of the extract with the reference samples as shown in Fig. 2. By comparison with the same time as the external standard substance, we concluded that the first peak in HPLC chromatogram eluting at 18.435 min with 10.983% of average contents was in accordance with harmol, the second peak eluting at 30.831 min was tentatively identified as harmaline with an average content of 8.414%. The retention times for harmine were observed at 43.604 minutes with average contents of 54.815. We observed that the *P. harmala* seeds were produced four peaks in HPLC chromatogram and the peak corresponding to vasicine was observed which revealed that this bioactive molecule was also identified and quantified in the dry seeds (Herraiz et al., 2017; Abbas et al., 2021). According to these results, harmol, harmine, harmaline were the main alkaloids in extracts with a total average content of 74.212% and harmine was noted as the major β -carboline alkaloids compounds in this plant as shown in Fig. 3 and Table 1 this result was in accordance with the findings of Herraiz et al. (2010); Aziz et al. (2017) where they were detected amount of harmol, harmaline, and harmine in the *Peganum harmala* L seed methanol extract. They detected amount of harmol, harmaline, and harmine in the

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CONCLUSIONS

The Saharian plants have different uses in traditional pharmacopoeia due to their active materials derived from secondary metabolites contained in their different organs, *Peganum harmala* L. has been reported as an insecticidal toxic plant in some tests. The different doses used were selected after several preliminary tests and meet the purpose of the work, which is the detection of the minimum inhibitory concentration of mycelial growth. The results of the tests showed that the seed extract of *Peganum harmala* L. has inhibitory activity on *Mauginiella scaettae* from 20% (v/v) concentration and therefore can be considered an effective antifungal agent to treat the inflorescence rot disease of date palm. This work can be deepened to determine the chemical compounds responsible for this antifungal bioactivity. Also, the results obtained on these two date palm pathogenic fungi are still preliminary and they have to be extended to other plants in the Algerian Sahara, as well as the execution of anti-microbial effect tests combined to their mixtures and the extension of these tests to other pathogens.

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