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Research Article

Thymus fontanesii Bois and Reut Essential Oils Effect on *Fusarium oxysporum* f.sp. *albidinis* Date Palm *Phoenix datylifera* Parasite

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ABSTRACT

Fusarium oxysporum f sp *albedinis* is among the most aggressive soil fungi causing death of date palm. As a biological control mean tool, we evaluated the antifungal essential oil (EO) activity extracted by hydro distillation from *Thymus fontanesii* leaves harvested from Tafraoui region in Oran south (Algeria), on the pathogen *Fusarium oxysporum* f sp *albedinis Fusarium*. EO which were tested *in vitro* at different concentrations (1000, 400, 200, 100, 50, 33.3 and 20 ppm) in order to define the fungal growth inhibition rate (MIC). Inhibition is obtained from 100 ppm. *Thymus fontanesii* essential oil *in vitro* test on leaves inoculated with *Fusarium oxysporum* f sp *albedinis* shows an 100 % inhibitory activity on disease development.

Key words: Antifungal activity; Bayud; *in vitro, in vivo;* disease

INTRODUCTION

Date palm is an essential plant in Saharan regions oasis ecosystem, known for its tolerance to harsh climatic conditions. A lot of Palm trees are strongly damaged by fungal diseases essentially the Bayud due to *Fusarium oxysporum* f.sp. *albedinis* a serious threat to the date palm (Abass, 2013). This vascular fusariosis is the most destructive and aggressive date palm disease in North Africa (Hakkou *et al.*, 2012).

Use of chemical biopesticides to fight although they are effective, are dangerous for man and the environment and whose use is more restricted due to their high toxicity (Villaverde *et al*., 2016.

To overcome this, it is becoming increasingy essential to replace them with products derived from plants (example essential oils) which are more environmentally friendly (Schultz and Nicholas, 2000).

Thymus is a genus that covers a wide range of biological activities, namely antiseptic, antibacterial, antifungal (Faleiro *et al.*, 2003). *Thymus fontanesii* species activity is carried out in this investigation.

Thymus leaves are harvested and used to extract essential oils (EO), and their antifungal effect is assessed *in vitro* on *Fusarium oxysporum* f.sp *Fusarium*. *albedinis* growth and on Palm leaves. Biological control using plant extracts containing several secondary metabolites remains rare, (Belboukhari and Cheriti, 2009) and *Thymus fontanesii* could contribute in reducing Bayud damage.

MATERIALS AND METHODS

Thymus fontanesii essential oils extraction

Leaves of thymus were used for essential oils extraction. They were harvested on 01/03/2016 in the Sidi Ghanem located south of Oran in Algeria (Latitude: 35.4831, Longitude: 0.527143 35 ° 28′ 59″ North, 0 ° 31′ West 38″). It is characterized by a semi-arid climate dry and cold. The leaves were dried for two weeks in a dry place, then crushed for extraction of essential oils (EO) by hydro distillation.

Inhibition test on radial mycelial growth (IR) *Fusarium*

oxysporum f. SP. *albedinis* (Foa), strain was kept at 4°C on the P.S.A (Potato, Sucrose, Agar) medium, which promoted rapid growth and abundant fungal sporulation. Under laminar flow hood, oils was first homogenized with water agar at 2% to facilitate its dissemination in the culture medium and then mixed in the PSA medium distributed in Petri dishes. The inhibitory effect of several concentrations of thymus essential oils on *Fusarium* oxysporum f. sp. *albedinis* (Foa) growth was tested (1000, 400, 200, 100, 50,

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33.3 and 20 ppm). The different Petri dishes were then inoculated with 5 mm diameter mycelial disk taken from a 7 days *Fusarium oxysporum* f. sp. old *albedinis* colony. For control, the essential oils were substituted by distilled water.

Each experiment was ran in triplicate. Petri dishes were incubated in the dark at 25 ± 2 ° C. Mycelial growth was measured daily until this mycelium in control reach dish edge. The inhibition rate (IR) of each is calculated as reported by Hmouni *et al*. (1996):

IR (%) =100 x (dC-dE)/dC
IR(%) = Inhibition rate in percentage.
dC = Colony diameter in control.
dE = Colony diameter in treated.

Moreover, the minimum inhibitory concentration (MIC), which is the smallest concentration in essential oils for which no development is visible to the naked eye. It is determined from the smaller values (at least at 25%) of fungal mycelial growth. The inhibitory concentration 50% (IC50) was also calculated.

Thyme *essential oils In vitro* effect on *Phoenix dactylifera* L. leaves

Thyme essential oils bioassay on date palm infected by *Fusarium oxysporum* f. sp *albidinis* is undertaken on date palms (*Phoenix dactilyfera*) leaves collected *in natura*. Under laminar flow hood, these are cut into 10-12 cm fragments, then washed with tap water and disinfected with alcohol, rinsed with sterile distilled water then put in tubes containing a sterile Knop solution. To evaluate thyme essential oils effect, two lots of 10 tubes each (Fig. 1) were prepared and inoculated with 5µl of 10^6 spores/ml suspension, prepared from a 7 days old *Fusarium oxysporum* sp *albidinis* culture.

Thyme solution was prepared by adding 250 ml of essential oils emulsified in 0.6 ml of ethanol to 20 ml of sterile distilled water. After a week from the appearance of necrotic spots, 5 µl of the prepared solution is applied *in vitro* (three consecutive days) on 10 inoculated explants so considered as inoculated/Treated lot. Fusariosis evolution is followed and evaluated on explants inoculated with and without treatment and compared to a control (without inoculation).

Disease severity evaluation

Disease the severity of the is calculated according to the equation reported by Maitlo *et al*. (2013) Disease Severity (DS) % = (number of infected leaves /total number) × 100

After the application of essential oil, the evolution of the disease is evaluated according to the equation:

Disease Incidence (DI) % = (Disease severity before EO application- DS after EO application)× 100

Statistical analysis

For Thyme essential oil Inhibitory effect mycelial growth, results are subject to statistical treatment by one way ANOVA. The difference between means is assessed by the Duncan test α=0.05.
Disease incidence data is analyzed with student T test α=0.05.

RESULTS

Thyme essential oil Inhibitory effect on *Fusarium oxysporum* f.sp. *albedinis* mycelial growth

Fusarium oxysporum f.sp. *albedinis* mycelial growth of in presence of different concentrations of *Thymus fontanesii* essential oisl is evaluated by measuring colony diameter (Fig. 2). Results showed that mycelium growth evolution varies depending on EO concentration added to medium compared to negative control (medium PSA only) and positive one where the essential oils are replaced by sterile distilled water.

Analysis of variance ANOVA (Table 1) showed that the variability in the colonies diameters was influenced by incubation time and thyme essential oils concentrations added to culture media.

Fig. 1: *In vivo* bioassay on *Phoenix dactylifera* L. leaves

Table 1: Analysis of *Fusarium oxysporum* f.sp. *albedinis* colonies diameters variance obtained after 8 days on PSA culture medium added with *Thymus fontanesii* essential oils (EO)

Source	ddl	F	Sig.
EO concentration	8	125,152	0,000**
Time	3	88,595	0,000**
EO concentration* Time	24	16,490	0,000**

Colonies diameters averages comparison with Duncan test (Table 2) revealed that 1000, 400, 200 and 100 ppm of thyme essential oils inhibit totally and significantly the growth of the *Fusarium oxysporum* f.sp. *albedinis* compared to control and 50, 33.3 and 20 ppm.

Essential oils effect at 20 ppm is statistically similar to negative (5.79 cm) and positive controls (5.76 cm). However, it is significantly higher than 33.3 ppm (1.34 cm) and 50 ppm (1.3 cm) effects. Thus, fungus growth is entirely inhibited from 100 ppm (Table 3). This suggests that the *Thymus fontanesii* essential oils minimum inhibitory concentration (MIC) on *Fusarium oxysporum* f.sp. *albedinis* growth is equivalent to 100 ppm.

Thyme essential oil application effect on the of the disease incidence evolution (DI) on leaves: Inoculated leaves show characteristic symptoms of Fusariosis Fig. 3.

Disease incidence (Fig.5) was significantly lower in leaves inoculated and then treated (35%) compared to the untreated leaves (56.66%). This suggests that the application of the essential oil of thyme is effective to reduce the progression of the disease.

Table 2: *Fusarium oxysporum* f.sp. *albedinis* colonies diameter means (cm) under *Thymus fontanesii* essential oils effect at différent concentrations

Treatement (ppm)	Control	Control/ water	1000	400	200	100	50	33.3	20
Colonies diameter (cm)	5,79± 0.3ᵃ	5.76±0.1 ᵃ	0ᶜ	0ᶜ	0ᶜ	0ᶜ	1.3 ±0.1ᶜ	1.34± 0.1ᵈ	5.79± 0.2ᵃ

Means followed by same letters arene sont pas statistiquement différentes.

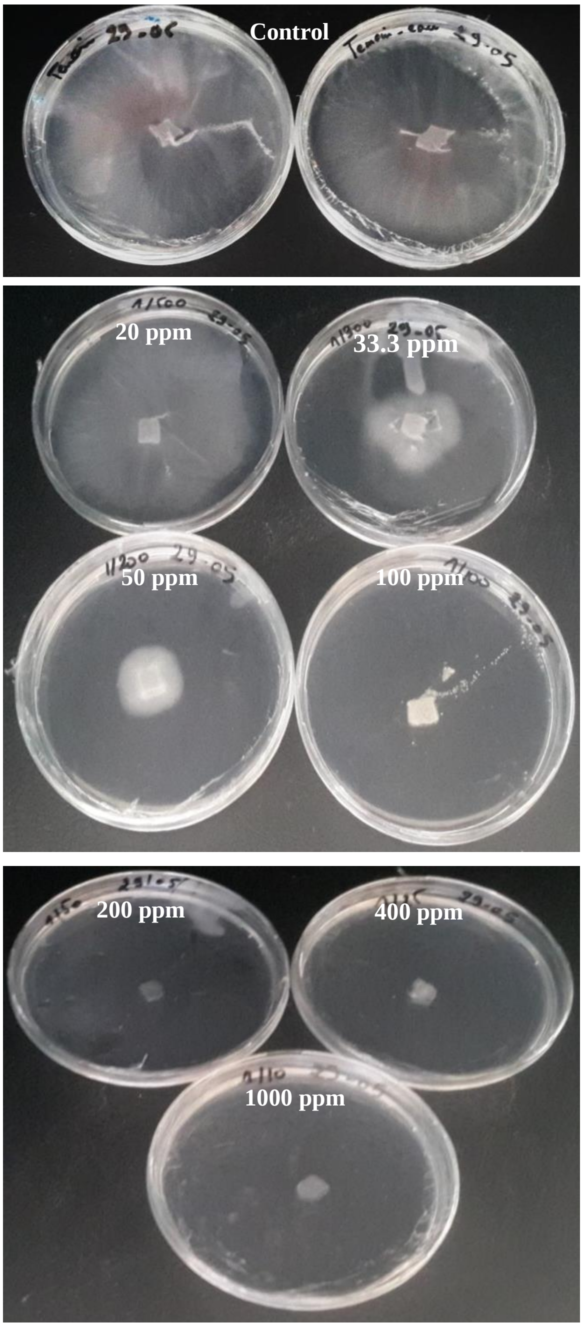

Fig. 2: Different *Thymus fontanesii* EO concentrations effect on *Fusarium oxysporum* f.sp. *albedinis* growth

Table 3: Thyme essential oils inhibition rate (IR) on *Fusarium oxysporum* f.sp. *albedinis*

Treatment ppm	IR (%)
0	0
20	0
33.3	62.28
50	68.42
100	100
200	100
400	100
1000	100

DISCUSSION

Aromatic plants are an unlimited source of natural bioactive substances and compounds such as essential oils (Singh, 2014). Lamiaceae regrouping over 400 species known for their antimicrobial activities (Borugă *et al*, 2014) among them, *Thymus fontanesii* essential oils possesses significant antifungal activity given its chemical composition (Ghorab *et al*., 2014). Their effects are tested on date palm Fusariosis causal agent *Fusarium oxysporum* f.sp. *albedinis*.

Different concentrations of *Thymus fantanesii* EO are tested *in vitro* on *Fusarium oxysporum* f.sp. *albedinis* growth. The results show a significant inhibitory activity and antifungal activity rises significantly with concentrations increasing.

Thymus fontanesii essential oils effect on various pathogens is often testified. Haddouchi *et al*. (2011) reported an antibacterial activity (bacteria gram + and gram) and antifungal against *Fusarium oxysporum* sp.. They are also tested on other fungi (Cruz *et al.*, 1989; Reddy *et al.* 1998; Marino *et al.*, 1999; Daferera *et al.*, 2000). This effectiveness can be explained by extracted oils chemical characteristics and active molecules presence that inhibit the fungus growth. Indeed, several authors show that essential oils rich in phenolic derivatives (carvacrol and thymol) have strong antimicrobial and antifungal activity (Trombetta *et al.,* 2002). Pathogen growth inhibition would be due to natural organic compounds with recognized antimicrobial activities and is reported in other plants presence of (Singh, 2014).

Essential oils effect on other phytopathogenic agents is also reported. This is the case of peppermint, geranium, rose and lemon EO on *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. (El-Mougy *et al*., 2007). This phenomenon is widely reported by many researchers indicating the efficiency of essential oils as antifungal inhibitors for a large number of pathogens (Akgül and Kivanc, 1988; Kumar and Tripathi, 1991; Singh *et al*, 1992; Singh *et al*., 1994).

Moreover, leaves inoculated with *Fusarium oxysporum* f.sp. *albedinis* spore suspension showed Fusariosis characteristic symptoms, namely necrotic spots. Results showed that disease impact on leaves is significantly lower among those treated compared with untreated leaves. This suggested that the application of the essential oil of thyme might reduce disease progression.

For *Phoenix dactilyfera*, various plant extracts (*Limonia strumfeei*, *Loneae arboresens*, *Acacia radiana* and *Asteriscus graveolens*) is recommended in the fight against *Fusarium oxysporum* f.sp. *albedinis* (Boulenouar *et al*., 2012.

Thymus fontanesii essential oils reveal an antifungal effect on *Fusarium oxysporum* f.sp. *albedinis in vitro* where mycelial growth decreased significantly with oils concentrations increase in and total growth inhibition observed from 100 ppm. In the same way, disease severity

Fig. 3: *Thymus fantanasii* EO effect on date palm leaves inoculated with *Fusarium oxysporum* f.sp. *albedinis* spore suspension (fusariosis causal agent).

Fig. 4: Symptoms on date palm leaves inoculated with *Fusarium oxysporum* f.sp. *albedinis* spore suspension (necrotic spots - chlorosist –leaves winding)

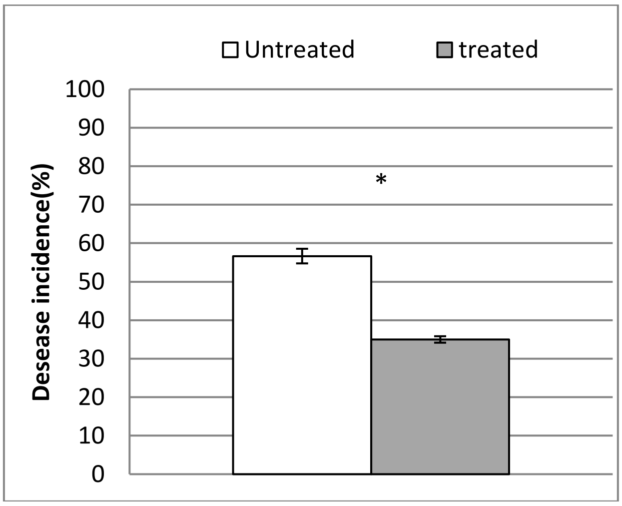

Fig. 5: Disease incidence on *Phoenix dactylifera* L leaves inoculated with *Fusarium oxysporum* f.sp. *albedinis* spores and treated by thyme essential oils; *****Means significantly different with Student T test.

the treated leaves with the *Thymus fontanesii* EO is significantly lower than that observed in non-treated. These results suggest the possibility of exploiting *Thymus fontanesii* EO for their antifungal properties against the Bayud agent.

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