# TUNISIAN THYME ESSENTIAL OIL FOR CONTROLLING ENNIATINS PRODUCING FUSARIUM SPECIES

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## Abstract

*Enniatins are emerging mycotoxins produced by Fusarium species. They are frequently found as contaminants in cereal grains in recent years. The synthesis of these secondary metabolites is often a response to biotic and abiotic stresses. Therefore, the introduction of fungicides into the environment of toxigenic fungi can lead to an increase in mycotoxin accumulation. The search for molecules with both antifungal and antimycotoxigenic activities is a challenge to preserve food safety. Essential oils (EOs) have a promising potential as natural fungicides and should be used both to control fungi and/or their mycotoxin production. In the present work, we extracted the essential oil of Tunisian* Thymus capitatus *and tested its effect in vitro on two enniatin-producing* Fusarium *strains when placed in contact with the mycelium in solid medium or used as a fumigant. In agar medium, contact tests measured with two different sets of EOs for a strain of* Fusarium avenaceum *provided an* $IC_{50}$ *of 0.05 µL EO* $mL^{-1}$*. For another strain of* Fusarium *sp. it was found an* $IC_{50}$ *of 0.08 µL EO* $mL^{-1}$*. Fungistatic activity induced no overproduction of enniatins and in contrast, decreases of 55% were found in some samples. Volatile components diffusing in a sealed container also had fungistatic activity that was both dose (5 to 25 µL 1.5* $L^{-1}$*) and time (6 to 24 days) dependent. No fungal acclimation to the EOs and no persistent effect of the EOs were observed, but the decrease in protective effect with increasing incubation time was probably due to EOs alterations.*

**Keywords:** *Fusarium head blight, mycotoxins, biocontrol, cereals*

## 1. INTRODUCTION

Fusarium head blight (FHB), caused by more than 20 different *Fusarium* species, is one of the most widespread worldwide and devastating fungal diseases that results in yield and quality losses of wheat and other small grain cereals [1]. In addition to grain yield and quality reductions, some FHB fungi are also responsible for the contamination of grains with a variety of mycotoxins. Zearalenone (ZEA) and type B trichothecenes, including deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives are mainly produced by *Fusarium graminearum* and *Fusarium culmorum*. In the European Union (EU), ZEA and DON are regulated mycotoxins with maximum levels set in a variety of foodstuffs [2]. Other mycotoxins that are neither routinely monitored nor subjected to a regulation are called emerging mycotoxins [3]. Enniatins (ENNs) are a main group of *Fusarium* emerging mycotoxins produced by various fungal species. Among the ENNs producing *Fusarium* species,, *F. avenaceum* and *F. tricinctum* are the most common ones isolated from small grain cereals in Europe and were highlighted as the major contributors of grain contamination with these emerging mycotoxins [4]. ENNs have various biological activities with antibacterial, antifungal, herbicidal and insecticidal [5] which might contribute to their virulence in potato tubers [6]. In the case of wheat, exogenously applied enniatins can cause tissue necrosis, but these mycotoxins did not appear to be a key disease factor in an experiment with genetically modified *F. avenaceum* inactivated in its production of ENNs, but they may enhance disease pressure during interactions inside pathogen complexes [7].

ENNs alter the cellular membrane integrity of mammalian cells and interact synergistically with other mycotoxins, leading to toxicity increases. ENNs are the most widespread emerging mycotoxins, and European cereal crops are frequently contaminated, sometimes at high levels, regardless of the geographical latitude and the associated climatic conditions of their growing area [6]. In the Mediterranean regions of North Africa, populations can be exposed to very high doses of ENNs, with contamination levels in some foodstuffs reaching ten or even hundreds of milligrams per kilogram, and a specific risk in infant foods [8-10]. Therefore, strategies to control the levels of this group of mycotoxins in food and feed have to be developped [4].

Essential oils (EOs) are complex mixtures of volatile compounds biosynthesized by plants, characterized by low molecular weights and bioactivities in the vapors phase that are not remanant and they are generally recognized as safe (GRAS). They are a promising source of active compounds with antifungal and antimycotoxic activities that could replace synthetic fungicides used as protective agents during crop cultuvation or storage. EOs of various plants collected in Tunisia were evaluated *in vitro* for their antifungal activity on several phytopathogenic fungi including *F. avenaceum*. The Tunisian plants tested were: *Mentha pulegium, Pinus alpensis*, *Pinus pinea*, *Pistacia lentiscus*, *Pistacia terebintus*, *Pistacia vera, Rosmarinus officinalis, Thymus capitatus* [11-15]. The latter showed the highest extraction yield (1.9 to 3.15 %) of EO and the strongest mycelial growth inhibition effect [13]. However, to the best of our knowledge, antifungal activity of volatile compounds with no contact of the EOs with the mycelium and the effect of EOs on ENNs production by *Fusarium* species have not been studied.

In this work, the antifungal and antimycotoxigenic potential of EOs from aerial parts of *T. capitatus* on two ENNs producing *Fusarium* spp. was evaluated by a) contact in the culture medium and b) fumigation using two different EO batches.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

#### *2.1.1. Plant material and isolation of essential oils*

Two batches of EO were obtained from plants of different origins. *Thymus capitatus* 1 was collected in March 2016 from the region of Kbouch (36° 10′ 56″ North, 8° 42′ 53″ East) situated in the North-Ouest of Tunisia, and air dried [16]; *Thymus capitatus* 2 was bought in a medicinal plant market in Tunisia. The EOs were extracted by hydrodistillation using a classic Clevenger-type apparatus. Anhydrous sodium sulphate was added to the extracted oils to remove residual water. The EOs were stored in the dark at 4°C until their chemical analysis and their use in biological tests. The extraction yield was calculated as the percentage of the weight of oil collected (mg) to the weight of the dry plant processed (g).

#### *2.1.2. Fungal material*

A strain of *F. avenaceum* (I496) isolated from maize in France in 2007 was selected from the fungal collection of the French National Research Institute for Agriculture, Food and Environment (INRAE/MycSA collection). A stock culture maintained at 4°C on potato dextrose agar (PDA) slants under mineral oil was cultivated on PDA medium and kept at 4°C until the run of the different tests. A second strain of *Fusarium* sp (33T) was isolated as a monospore culture from wheat grains harvested in Tunisia in 2018.

All the cultures for the measurements of mycelial growth rates and production of ENNs were realized on FDM-agar, a synthetic medium promoting the production of ENNs by *F. avenaceum* [17]. For the inoculation of the FDM-agar, a preculture was performed on PDA plates at 25 °C in the dark for 4 to 6 days, and the inoculation of the FDM-agar was done either as spore suspensions prepared as described by Gautier *et al* [18] placed at the center of the plate, or plugs of 0.5 cm diameter that were cut and transplanted.

*2.2. Methods*

*2.2.1.* In vitro *contact assay of the effect of* T. captitatus *EO*

Essential oils were diluted in ethanol and added in the FDM-agar culture medium cooled to 50-55°C (still liquid) after autoclaving, stirred for 5 min and 10 mL were poured into 9 cm diameter Petri dishes. Controls with diluting solvent were prepared. Then, the plates were inoculated at the centre, either with plugs or spore suspensions (10 µL of $10^6$ spores $ml^{-1}$). The inoculated plates were incubated at 25°C for 10 days. Photos of the colonised plates were taken and the area covered by the mycelium on the agar medium was mesured using Image J software package (National Institutes of Health, Bethesda, MD, USA). At the end of the culture, the agar medium with the mycelium of each plate was collected and stored at -20°C for further ENNs analysis.

*2.2.2.* In vitro *fumigation assay of the effect of* T. captitatus *EO*

Hermetically sealed jars of 1.5 L were used as fumigation chambers. Three Petri dishes containing 10 mL of FDM-agar medium and inoculated at the centre with $10^4$ spores in 10 µL of distilled water were placed uncovered in each jar. Treatments with EO volatiles were obtained by placing 1 cm² of sterile filter paper soaked with 0, 5, 10, 15 or 25 µL of EO. The jars were icubated at 25°C for 6, 10, 17, 24 days. A second series of experiments was performed using 0 or 25 µL of EO and incubation for 28 days. Further, either spores or 0.5 cm diameter plugs were collected from the 28 day old cultures and used to inoculate FDM-agar Petri dishes placed back into control jars or into jars contraining 25 µL of EO. The colonisation of the Petri dishes was followed as mentionned above.

*2.2.3. Extraction and analysis of enniatins*

Ethyl acetate was used as a solvent for ENNs extraction from the entire content of an agar plate. The sliced agar was mixed with 35 mL of the solvent and stirred at 250 rpm for 15 min, filtered through filter paper and 5 mL was dried at 45°C under a nitrogen flow. Quantification of enniatin A (ENN-A), enniatin A1 (ENN-A1), enniatin B (ENN-B) and enniatin B1 (ENN-B1) was performed by LC/DAD as described in [18].

*2.2.4. Gas Chromatography analysis of* T. capitatus *essential oil*

EO composition was investigated by Gas chromatography mass spectrometry (GC-MS) analysis conducted on Agilent 7890 gas chromatograph, coupled to an Agilent 5975C mass spectrometer with electron impact ionization (70 eV). HP-5 column (30 m × 0.25 mm, film thickness 0.25 µm) was used. The operating conditions were as follows: the detector temperature, 280 °C; the injector temperature, 210 °C; the oven temperature program varied from 180 °C (1 min) to 200 °C (15 min) then from 220 °C (3 min) to 300 °C (10 min). Carrier gas was H2 (0.9 mL/min); split ratio of 1:100 and a linear velocity of 36.45 cm/s was used as carrier gas and mass spectral range was recorded from m/z 50-550 amu. The injected volume was 1 µL of 1% EO solution in Hexane (Purity ≥ 97%). The identities of the EO components were established by comparison of their MS spectra with those reported in literature and by computer matching with the Wiely mass spectra library (Eleventh Edition/ NIST 2009).

*2.2.5. Statistical analysis*

IC50 was calculated using linear regression method. Mycelial growth and ENNs concentrations were analysed by ANOVA and Duncan's multiple range tests using SAS Software (Statistical Analysis System, version 9, Cary, NC, USA).

## 3. RESULTS

*3.1. Extraction yield of EO from* T. capitatus *aerial part and composition.*

It was obtained 1.36 g of EO from 100 g of dried aerial part of *T. capitatus* from batch 1 (TC1) and 2.45 g per 100 g of dried aerial part from batch 2 (TC2). The table 1 shows the diffrent compounds found in TC1 and TC2. The chemical profiles of the two batches of EO showed a similair proportion between the different terpene's chemical families. The oxygenated monoterpenes were the major

compounds of both EOs followed by monoterpenes hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Furthermore, it was found that both EOs were carvacrol chemotype. Amounts of 83.88% carvacrol were detected in the first batch of TC1, and 82.1% in the second batch. Moreover, p-Cymene was the second major compound present at 6.08% in the first batch and 5.69% in the second batch.

| Compounds | Ret Time | Ret index | TC1 | TC2 |
|---|---|---|---|---|
| α-thujene | 5.687 | 921 | 0.35 | 0.45 |
| α-pinene | 5.857 | 926 | 0.42 | 0.46 |
| Camphene | 6.202 | 938 | 0.16 | 0.14 |
| β-myrcene | 7.115 | 975 | 0.66 | 0.84 |
| α-phellandrene | 7.494 | 995 | 0.08 | 0.17 |
| 3-Carene | 7.651 | 1000 | ---- | 0.06 |
| α-terpinene | 7.800 | 1017 | 0.66 | 1.07 |
| **p-Cymene** | 8.004 | 1026 | **6.08** | **5.69** |
| d-Limonene | 8.116 | 1029 | 0.28 | 0.37 |
| γ-terpinene | 8.893 | 1035 | 1.90 | 3.33 |
| Trans-Sabinene hydrate | 9.142 | 1037 | 0.09 | 0.07 |
| α-Terpinolene | 9.695 | 1064 | 0.11 | 0.14 |
| Linalool | 9.972 | 1074 | 0.89 | 0.87 |
| Borneol | 11.862 | 1134 | 0.71 | 0.52 |
| Terpinen-4-ol | 12.167 | 1148 | 0.72 | 0.70 |
| α-terpineol | 12.527 | 1159 | 0.13 | 0.14 |
| Thymol (isomer) | 15.081 | 1291 | 0.13 | 0.10 |
| Thymol | 15.255 | 1301 | 0.32 | 0.42 |
| **Carvacrol** | 15.585 | 1307 | **83.88** | **82.10** |
| Caryophyllene | 18.669 | 1421 | 1.11 | 1.51 |
| Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- | 20.889 | 1500 | 0.13 | 0.10 |
| Caryophyllene oxide | 22.667 | 1561 | 0.84 | 0.41 |
| **Total identified compounds** | | | 99.65 | 99.65 |
| **Monoterpenehydrocarbons** | | | 10.79 | 12.78 |
| **Oxygenated monoterpene** | | | 86.78 | 84.84 |
| **Sesquiterpenehydrocarbons** | | | 1.11 | 1.51 |
| **Oxygenated sesquiterpene** | | | 0.97 | 0.52 |
| **Extraction yield %(w/w)** | | | 1.36 | 2.45 |

**Table 1.** Chemical composition (%) of essential oils extracted from two batches of *T. capitatus* (TC1 and TC2) using GC-MS

### *3.2. Inhibition of mycelial growth by EO of* T. capitatus *in the agar medium and effect on the accumulation of ENNs*

The EO isolated from aerial parts of *T. capitatus* 1 significantly inhibited (86% inhibition at 10 days) the mycelial growth of *F. avenaceum* I496 in the *in vitro* contact test performed on agar medium containing 0.1 µL EO $mL^{-1}$ medium, regardless of inoculation (plugs or spore suspensions) (Figure 1). When the experiment was repeated with different EO concentrations in the medium, for TC1, it was observed up to 95% inhibition after 7 days using 0.1 µL EO $mL^{-1}$ and 100% (no growth) using 0.2 µL EO $mL^{-1}$, with the $IC_{50}$ estimated to be 0.05 µL EO $mL^{-1}$. While with TC2, it was observed 95 and 99 % inhibition achieved at 0.04 and 0.08 µL EO $mL^{-1}$, respectively (Figure 2).

For the strain *Fusarium sp* 33T, the inhibition rate reached 79% after 10 days with 0.1 µl EO $mL^{-1}$ (Figure 1) using TC1 and $IC_{50}$ was 0.08 µL EO $mL^{-1}$. With TC2, the inhibition rate exhibited by 0.02 and 0.04 µL EO $mL^{-1}$ was 70 and 98 %, rescpectively.

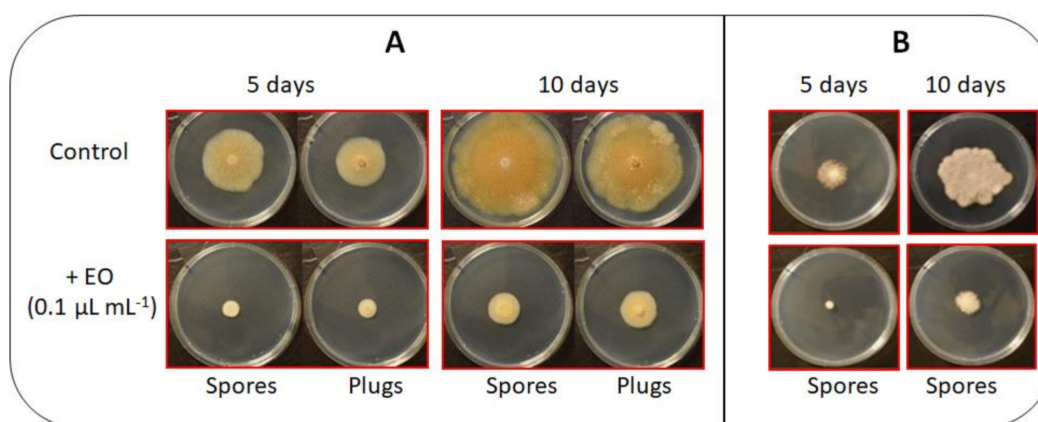


**Fig. 1.** Inhibition of the mycelial growth of *F. avenaceum* by EO of *T. capitatus,* batch 1. A-Photographs of Petri dishes, 5 and 10 days after inoculation in the centre with plugs or spore suspensions of *F. avenaceum* I496; B- Photographs of Petri dishes 5 and 10 days after inoculation by spore suspension of *F. sp.* 33T.

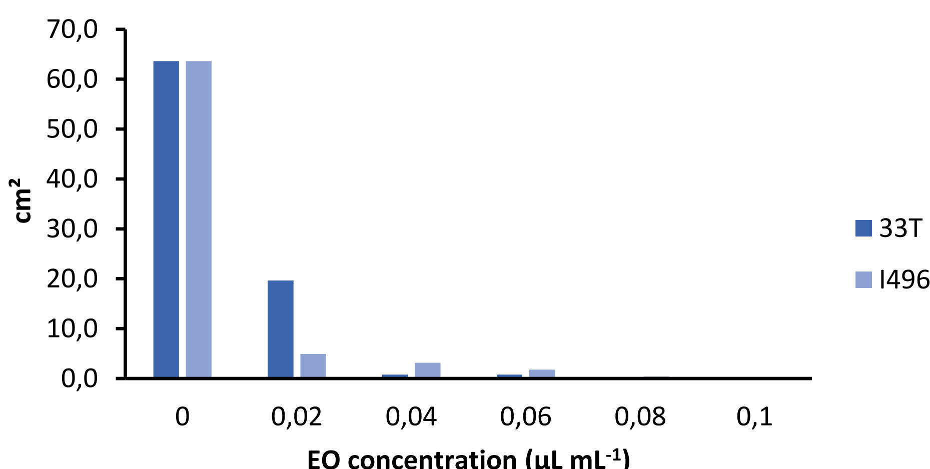


**Fig. 2.** Surface of mycelial colonies of two *Fusarium* spp strains on FDM-agar media supplemented with *T. capitatus*, batch 2, measured after incubation for 10 d at 25°C. Means of 3 replicates.33T = *Fusarium* sp., I496 = *F. avenaceum*

The ENN-A, ENN-A1, ENN-B, ENN-B1 levels were measured in the agar medium collected at 10 days (inoculation with spores). With TC1 at 0.1 µL mL$^{-1}$, the accumulation of total ENNs per biomass unit (surface area) of *F. avenaceum* I496 was significantly inhibited by 55% (Table 2) and the final concentration in the Petri dish decreased by 84 %. While no ENN-A nor ENN-A1 were found, the EO affected the distribution between ENN-B and ENN-B1 by increasing the concentration in ENN-B1 (Table 2). It was observed an inhibition rate of 65%, in a replicate in which the level of total ENNs in the control was higher: 2.058 g cm$^{-2}$. With TC2 at the concentration of 0.04 µL EO mL$^{-1}$ affecting the growth rate by 95%, the accumulation of total ENNs was not significantly inhibited (Table 2). It was also observed that *Fusarium* sp. 33T was a better producer of ENNs *in vitro* than *F. avenaceum* I496. This strain produced ENN-A1 in addition to ENN-B and ENN-B1 but no ENN-A, and accumulation of ENNs was also inhibited to 55 % by TC1. However, the effect was not significant and the distribution between ENN-B, ENN-B1 and ENN-A1 was not affected by the treatment. With TC2 at a concentration of 0.04µL EO mL$^{-1}$, the growth rate was affected by 99%. Overall, ENNs accumulation per ml of the agar medium was inhibited significantly by 96% compared to the control. However, the results calculated per cm² of the biomass showed no inhibition of the ENNs accumulation.

| | | ***F. avenaceum* I496** | | | ***Fusarium* sp. 33T** | | | |
|---|---|---|---|---|---|---|---|---|
| **Batch of EO** | **Treatment** | **ENN-B** | **ENN-B1** | **Total ENNs** | **ENN-B** | **ENN-B1** | **ENN-A1** | **Total ENNs** |
| **Batch 1** | **Control** | 0.676 | 0.026 | 0.702 (0.052) | 4.912 | 2.518 | 0.544 | 7.974 (3.508) |
| | **0.1 µL EO mL$^{-1}$** | 0.185 | 0.130 | 0.315 (0.081) | 2.492 | 1.149 | 0.164 | 3.805 (2.657) |
| **Batch 2** | **Control** | 0.028 | 0 | 0.028 (0.015) | 0.111 | 0.0869 | 0.041 | 0.239 (0.123) |
| | **0.04 µL EO mL$^{-1}$** | 0.029 | 0.009 | 0.039 (0.020) | 0.480 | 0.196 | 0 | 0.676 (0.956) |

**Table 2.** Concentrations of enniatins (ENNs) released in the medium (µg per units of mycelial colonies (cm$^{-2}$)), means of triplicates and (standard deviations).

### *3.3. Inhibition of mycelial growth of* F. avenaceum *by volatile components of* T. capitatus *EO*

The volatile components of TC2 had a significant inhibitory effect on the mycelial growth of *F. avenaceum* that was dose and time dependant (Figure 3). After 10 days, when the control reached the physical limit of the plate, only the lowest concentration (5 µL EO difusing in 1.5 L$^{-1}$ air) showed a low myclial growth, reaching the limit of the plate 14 days later. The presence of volatiles compounds from the EO increased the lag phase and slowed down the mycelial growth rate. This is also illustrated with the increasingly higher concentratations. In this experiment, plates were collected at d 10 for the measurement of concentrations of total ENNs. The concentration was 1.34 µg cm$^{-2}$ and 1.02 µg cm$^{-2}$ in the control and the treatment, respectively. The difference corresponding to 24 % of inhibition was significant.

The mycelial growth experiment was replicated with 25 µL EO in 1.5 L jars and an incubation for 28 days. The means of the mycelial surface was 11 (+/- 10) cm². Spores and plugs were collected from these cultures and used to inoculate FDM-agar plates which were placed either in control jars or in jars with 25 µL EO in 1.5 L. After 10 days of cultures in jars without EO, the mycelia reached the border of the dishes in both treatment and control, regardless of the inoculation method. There was no consequence of previous contacts with EO volatiles. When placed again in presence of the same

concentration of EO, both plugs and spores produced only a very low quantity of mycelium after 28 days (<2.5 cm²). The difference with the first exposure to EO volatiles was not significant.

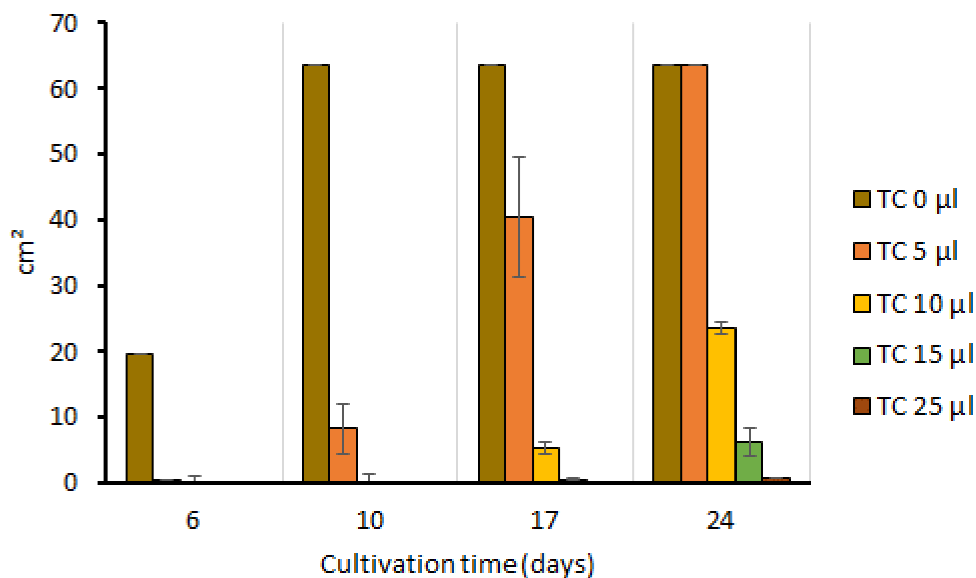


**Fig. 3.** Kinetics of the mycelial growth of *F. avenaceum* on FDM-agar in the presence of volatile components of *T. capitatus* EO (TC2) at different concentrations (0 to 25 µL of EO difusing in 1.5 L jars). Means +/- standard deviations, 3 replicates.

## 4. DISCUSSION

*Thymus capitatus* (L.) Hoffmanns*,* a Lamiaceae endemic to the Mediterranean region, is used in traditional medicine to treat numerous diseases. With extraction yields above 2 mL per 100 g of dried aerial part obtained in the present work and previous studies [13, 15] *T. capitatus* is an excellent source of EO. Previous works on the effect of *T. capitatus* EO on *F. avenaceum* used *in vitro* contact assays on PDA, inoculation with mycelium plugs and incubation for 7 days at 24°C [13, 15]. Antifungal activities of essential oils of four thymes collected from different localities in Tunisia, ranged for 3 to 52 % inhibition of mycelial growth, with 0.1 µL EO $mL^{-1}$ and for 50 to 93 % with 0.4 µL EO $mL^{-1}$ [13]. In the present work, 95 to 99 % of inhibition was achieved with two batches of EO at 0.1 µL $mL^{-1}$. This higher effect could be due to differences in the susceptibility of the *F. avenaceum* strain used and in the quality of the EOs. With the other *Fusarium* strain, it was observed lower inhibition rates, but they still were higher than in [13]. Qualitative and quantitative differences with consequences on their biological activity are currently observed between EOs from the same species, depending on the batch of plants used, as observed in the present work.

The EOs of *T. capitatus* from the Maghreb region (Morocco, Algeria, Tunisia, and Libya) are mainly composed of carvacrol, α-pinene, γ-terpinene, thymol, and p-cymene [19]. *Thymus capitatus* is generally a carvacrol chemotype, even if thymol chemotypes were sometime reported [20]. As expected, the *T. capitatus* EO obtained in the present work was carvacrol chemotype in agreement with previous reports on Tunisian *T. capitatus* EO in which 69.15 to 83.86 % of EO components were carvacrol [13, 15].

The antifungal activity of EOs and extracts of *T. capitatus* against many fungal strains has been reviewed [19]. Carvacrol is believed to be responsible for a large part of this activity. The monoterpene reduced by 50 % the incidence of *Fusarium oxysporum* wilt in tomato plants after seed treatment, without causing phytotoxic action, and was shown to have a fungistatic but not fungicidal effect *in vitro* [21]. Carvacrol has been proposed as an environmentally friendly agent to fight seeds' damping-off diseases and as a food preservative. Metabolomic profiling data of *Penicillium digitatum*

revealed that carvacrol-induced oxidative stress and disturbed energy production and amino acid metabolism [22]. It can also induce damage to the RNA molecules and affect the ROS production [23]. However, the antifungal potential depends not only on the main components and their concentrations, but also on compounds present at lower concentrations and their synergistic actions. Considering the antifungal activity observed in the present sutdy, *T. capitatus* EOs of carvacrol chemotype and also containing other compounds (p-cymene, γ-terpinene…) has a great potential for being used to control *F. avenaceum*, a generalist pathogen responsible for diseases in numerous crop species.

*Fusarium avenaceum* is not only a plant pathogen but also a producer of mycotoxins, mainly ENNs. The production of ENNs as well as other mycotoxins, may play a key role in the adaptive response of the fungus to stresses [4]. Therefore, it is important to ensure that any antifungal compound used to control *F. avenaceum* had no significant enhancing effect on ENNs production when applied at doses below the lowest concentration that inhibits 100% of mycelial growth (MIC). In the present work, *in vitro* observations showed that *T. capitatus* EO tended to decrease the level of ENNs accumulation in the culture medium per unit of mycelial biomass (estimated as the surface of the mycelial colony) for two *Fusarium* strains, with different abilities to produce ENNs. Overall, *T. capitatus* EO had a great potential to significantly limit the risk of contamination of crops by ENNs, by acting both as an antifungal and an antimycotoxinogenic treatment.

One of the characteristics of EOs is that they are composed of lipophilic and highly volatile plant secondary metabolites. *Fusarium* species and other phytopathogenic fungi generally grow on plant tissues and not in liquid media. They are permanently in contact with volatile compounds present in their environment and it is worthwhile to check the activity of an EO when used as a fumigant, without a direct contact of the source of volatile components with the mycelium or conidia. The fungistatic activity of volatile components of *T. capitatus* EO was confirmed over a long period of 24 days. The duration of the lag phase before the onset of mycelial growth and the kinetics of mycelial growth depended on the amount of EO. Acclimation of the fungus to the EO and degradation of EO over time were the two possible explainations regarding the data reported in Figure 3. After transplanting of the spores or plugs in new containers with or without fresh EO, there were no differences with the initial culture. The fungus was no longer affected by its previous contact with EO volatiles and continued to be very susceptible to the volatile compounds from the fresh EO. Therefore, it was not the acclimation but the decrease in active components concentrations due to EO degradation that was responsible for the loss of inhibition activity over time. EOs are known to be sensitive to conversion and degradation reactions modulated by temperature, light, and oxygen availability [23]. The protection of EOs against these physico-chemical factors and a gradual release of effective amounts of EO are two main constraints for their use in a biocontrol process. Nanoencapsulation of EOs, with a large surface area per unit volume, would represent a valid and efficient strategy to improve the concentration of EO volatiles in plant areas where the fungi are preferentially located, i.e. in liquid-solid interfaces. Nanoencapsulation of *T. capitatus* EO in chitosan nanoparticules increased its bactericidal activity against foodborne pathogens [24]. The inhibitory concentrations of the biodegradable and biocompatible poly(ε-caprolactone) (PCL) polymer-based nanocapsules were two to four times lower than those of pure essential oils on a panel of fourteen different fungi belonging to the phyla Ascomycota and Basidiomycota [25]. Another challenge in the use of thyme EOs is their potential cytotoxic nature. However, *T. capitatus* EO was reported to be not cytotoxic[26].

## 5. CONCLUSIONS

The present study showed that *T. capitatus* is a natural source of bioactive substances against *F. avenaceum* and it proved to be an excellent candidate for the development of an alternative solution to synthetic fungicides for the control of the ENNs accumulation in crops. Further researches are needed to assess the *in-planta* activity of *T. capitatus* EO and its potential effect on a broad fungal spectrum. A nanoencapsulation system of the *T. capitatus* EO that could provide a solution to a practical use of this oil as a biofungicide is under study.

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