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In-vitro Study of Anti-Fusarium Effect of Thymol, Carvacrol, Eugenol and Menthol

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Abstract

This study aims at shedding light on the anti-fusarium action of some Major Compounds (MCs) of Essential oils (EOs). To this end, *Fusarium oxysporum f. sp. dianthi* (Fod) was used as fungal model. A screening of four MCs was conducted in order to identify the most active one with the highest anti-*Fusarium* effect, using both agar and broth dilution methods. The obtained data revealed that thymol, with its minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values was the most effective, with concentrations between 0.25 mg ml^{-1} and 1 mg ml^{-1}. Among the four compounds tested, thymol and carvacrol showed anti-*Fusarium* activity by inhibiting the germination and destroying Fod conidia. As a preliminary test, we have also tested the applicability of thymol on soil disinfection, with very promising results. The antifungal activity of the four MCs used in this study involves inhibition of germination and destruction of Fod conidia. Thymol, the most effective MC, has shown great effect on soil disinfection. This work is a preliminary contribution aiming at developing an alternative product, environmental friendly, safe for both the workers and consumers, with high anti-Fusarium activity.

Keywords: Major compounds; Essential oils; *Fusarium oxysporum f. sp. dianthi*; Fungicidal action; Soil disinfection

Introduction

Fusarium wilt in carnations is a vascular disease caused by the fungus *Fusarium oxysporum f. sp. dianthi* (Fod). It is the most common and serious fungal disease that attacks carnations worldwide [1]. The fungus can cause big financial losses to the floriculture industry. To control the *Fusarium* wilt, growers use systemic fungicides like methyl bromide [1], chloropicrin and benomyl [2]. Beside the fact that these chemicals are toxic to humans and the environment [3], they can also cause selection for resistant mutated pathogen [4].

Severe epidemics of *Fusarium* wilt in the 1980s and 1990s have caused a strong reduction in the acreage devoted to the carnation industry in some areas of Southern Europe, such as Italy, France, and Spain [5]. *Fusarium* has obliged the industry players to leave Europe and the United States towards other countries such as Colombia and Morocco [5]. Despite the availability of cultivars that are relatively resistant to some strains of Fod, *Fusarium* wilt remains a threatening disease for this crop wherever it is grown [5].

Several publications from our laboratory have previously reported *in vitro* and *in vivo* antifungal activity of some EOs and their MCs, especially the phenolic compounds such as thymol, carvacrol and eugenol [6-8].

The major aim of the present work was to elucidate the *in vitro* antifungal activity of some MCs of EOs on the growth of Fod in order to develop an alternative to chemical fungicides.

Materials and Methods

Fungal strain

The Fod strain used in this study was isolated in our laboratory from a diseased carnation plant collected from a greenhouse of Prim'Rose Company (Azemmour, Morocco).

The Fod strain identification was done using a conventional method based on the colony's macroscopic characteristics and the microscopic appearance of conidia [9].

Culture media

Potato dextrose agar (PDA) (Biokar, France) was used for fungus identification [9]. Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB) (Biokar, France) and Malt extract broth (MEB) were used for fungus culture and antifungal test [10].

Preparation of conidial suspension

Conidia of fod were harvested from 7-day-old cultures in PDA by pouring a sterile 0.9% aqueous solution of NaCl onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia cells was adjusted to 10^6 ml^{-1} and was determined by transferring 10 µl of the sample suspension of Fod conidia to a Malassez chamber for microscopic examination and counting. Fod conidia were counted in 10 different fields utilizing standard techniques [11].

Anti-fungal agents

Four MCs were used in this study: Thymol; Carvacrol; Eugenol and Menthol, all were obtained from Sigma-Aldrich (Steinheim, Germany). Each MC was dispersed in a suspension containing 0.2% agar in sterile distillated water in order to maintain viscosity to disperse MCs without adding solvent or detergent [12]. A stock suspension of 100 mg ml^{-1} for each MC was then prepared.

Determination of anti-*Fusarium* effect of MCs in agar medium

The MCs were added to the medium at the concentrations of (0;

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0.125; 0.25; 0.5; 1; 2 and 4 mg ml^{-1}) from a stock suspension and poured in sterile Petri dishes (90 × 16 mm). The determination of anti-*Fusarium* effect of each MC in SDA medium was carried out by the inoculation of 10 µl calibrated drop (10^4 conidia) in agar plates containing increasing concentrations of MCs. Three Petri dishes were considered for each concentration, each petri dish contained three calibrated drops of Fod and the petri dish were incubated for 5 days at 27°C.

The diameter of mycelial colony of Fod was measured in centimeters for each concentration of all the MCs used and was compared to the control agar plate.

The minimal inhibitory concentration (MIC) was determined as the lowest antifungal agent concentration at which no mycelial growth was visually observed.

The anti-fusarium effect of MCs was expressed by the percentage of growth inhibition of Fod and calculated using the following formula [13]:

$$I(\%) = (D_c - D_i)/D_c \times 100$$

Where D_c and D_i represent respectively the mycelial growth diameters in control and in treated petri dishes.

Determination of anti-*Fusarium* effect of MCs in liquid medium

The determination of anti-*fusarium* effect of MCs in liquid medium (MEB) was conducted in triplicate using sterile 96 well microplates by adding 20 µl of conidial suspension of Fod (2×10^4 conidia ml^{-1}) and 70 µl of increasing concentrations of MCs (0; 0.0625; 0.125; 0.25; 0.5; 1; 2 and 4 mg ml^{-1}) to 160 µl of MEB medium in order to have a final volume of 250 µl in each well. After 5 days of incubation at 27°C, the mycelial growth was assessed by measuring optical density (OD) at 600 nm using spectrophotometer (J.P. Selecta).

The anti-*Fusarium* effect of MCs was expressed by the percentage of inhibition of Fod and calculated using the following formula:

$$I(\%) = (OD_c - OD_i)/OD_c \times 100$$

Where OD_c and OD_i represent respectively the optical density of mycelial growth in control and in treated wells.

The MIC was defined as the lowest MC concentration at which no mycelia growth was visually observed after 5 days of incubation at 27°C.

In order to determine the minimal fungicidal concentration (MFC), 20 µl from the wells showing no growth were aseptically transferred into sterile tubes containing 980 µl of sterile MEB medium. The MFC was defined as the lowest antifungal agent concentration at which no mycelial growth was visually observed after 5 days of incubation at 27°C.

The Fod conidial germination assay

To study the effect of MCs on conidial germination, 100 µl of freshly prepared conidia suspension (10^6 ml^{-1}) was added to 900 µl of MCs solutions at different concentrations (0; 0.0625; 0.125; 0.25; 0.5 and 1 mg ml^{-1}), in sterile SDB. The control tube contained 100 µl of 10^6 ml^{-1} freshly prepared conidia suspension in 900 µl of sterile SDB.

After 20 h at 27°C, 40 µl of Conidia suspensions were deposited between a slide and a coverslip and the conidia was observed at magnification 100x using an optical microscope.

The proportion of germinated conidia was counted as the ratio of germinated conidia among 100 observed conidia.

The Fod conidial destruction assay

Each MC was tested in increasing concentrations i.e., 0; 0.0625; 0.125; 0.25; 0.5 and 1 mg ml^{-1}.

100 µl of freshly prepared conidia suspension (10^6 ml^{-1}) was put in direct contact with 900 µl of MC at various concentrations in sterile 0.9% aqueous solution of NaCl containing 0.2% agar.

The *Fusarium* conidia destruction assay was evaluated after an incubation at 27°C for 24 h.

As control tube, we used a 900 µl aqueous solution of sterile 0.9% of NaCl containing 0.2% agar and 100 µl of 10^6 ml^{-1} freshly prepared conidia suspension.

The number of conidia was determined in a Malassez chamber (as previously described) after 1 h, 3 h, 6 h and 24 h of incubation at 27°C and the percentage of conidial destruction was calculated using the following equation [14]:

$$I(\%) = (N_c - Ni)/N_c \times 100$$

Where N_c and N_i represent respectively the number of conidia in the control and the treated tubes.

Fusarium density in the soil

18 aliquots of 100 g sterilized soil were placed in sterile 400 ml plastic boxes. Fod was incorporated into sterilized soil (previously sifted through a 2-mm sieve, and then was autoclaved 30 min at 121°C) at an inoculum density of 10^6 spores per gram of soil.

Experimental treatments were: (1) uninfested and untreated soil, only water added as a check for residual populations of Fod (not included in analysis); (2) infested and untreated soil, only water added; (3) infested and treated soil with thymol. Four concentrations of thymol were used for treatments (0.25; 0.5; 1 and 2 mg g^{-1} of soil) and each concentration was calculated per gram of soil. Population densities of Fod were determined using the serial dilution technique at day 0 (before soil contamination), 1, 3, 7, 14, and 21 days after soil contamination.

One gram of soil from each batch was placed in a sterile tube containing 9 ml of 0.9% NaCl. From this stock solution, series of dilutions were carried out (10^{-2}, 10^{-3}... 10^{-6}). 100 µl from each dilution were deposited on the surface of three Petri dishes containing SDA medium. Plates were then incubated at 27°C for 5 days. The number of Fod colonies was defined from countable plates.

Data analysis

The mycelial growth data was subjected to ANOVA statistical analysis. Mean and standard error of data were calculated using IBM SPSS Statistics software, version 20. The separation of means was done using the Least Significant Difference (LSD) test at $p<0.05$.

Results

Determination of anti-*fusarium* effect of MCs in agar medium

The effect of MCs on the growth of Fod in agar medium is shown in Tables 1 and 2. MCs exhibited varying levels of antifungal activity against Fod. The MIC values of MCs (Table 1) ranged between 0.25 mg ml^{-1} and 2 mg ml^{-1}. The most effective inhibitors were thymol and carvacrol with a MIC value of 0.25 mg ml^{-1} followed by eugenol with a MIC value of 0.5 mg ml^{-1} while menthol had a MIC value of 2 mg ml^{-1}.

The inhibitory effect of MCs on the growth of Fod is depicted on Table 2. The mycelial growth of Fod was significantly reduced in

Major compound	MIC (mg mL^{-1})
Thymol	0.25
Carvacrol	0.25
Eugenol	0.5
Menthol	2

Table 1: MIC values of the tested MCs on Fod in agar medium.

Major Compounds	Mycelial growth inhibition (%)						
	Control	0.125 mg ml^{-1}	0.25 mg ml^{-1}	0.5 mg ml^{-1}	1 mg ml^{-1}	2 mg ml^{-1}	4 mg ml^{-1}
Thymol	0.00 ± 0.00^{a}	88.9 ± 0.11^{b}	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Carvacrol	0.00 ± 0.00^{a}	54.5 ± 0.11^{c}	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Eugenol	0.00 ± 0.00^{a}	31.6 ± 0.07^{d}	51.8 ± 0.12^{c}	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Menthol	0.00 ± 0.00^{a}	10.8 ± 0.12^{e}	24.3 ± 0.09^{f}	39.8 ± 0.11^{g}	86.7 ± 0.11^{b}	100.0 ± 0.00	100.0 ± 0.00
Data are means (n=9) ± SD, different letters indicate significant differences (p<0.05) by LSD test.							

Table 2: Effect of MCs on mycelial growth of Fod after 5 days of incubation at 27°C.

Major compound	MIC (mg ml^{-1})	MFC (mg ml^{-1})
Thymol	0.5	1
Carvacrol	0.5	1
Eugenol	1	2
Menthol	4	>10

Table 3: MIC and MFC of the tested MCs on Fod in liquid medium.

Major Compound	Mycelial growth inhibition (%)						
	Control	0.125 mg/ml	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml
Thymol	0.00 ± 0.00^{a}	78,7 ± 0.54^{b}	95.9 ± 3.17^{e}	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Carvacrol	0.00 ± 0.00^{a}	61.5 ± 3.46^{b}	90.5 ± 0.51^{h}	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Eugenol	0.00 ± 0.00^{a}	59.8 ± 3.86^{c}	85.5 ± 3.05^{d}	89.7 ± 1.16^{e}	92.2 ± 1.11^{e}	100.0 ± 0.00	100.0 ± 0.00
Menthol	0.00 ± 0.00^{a}	39.3 ± 3.40^{f}	65.9 ± 1.99^{c}	78.0 ± 4.30^{h}	86.7 ± 0.11^{d}	97.5 ± 0.26^{e}	100.0 ± 0.00
Data are Means (n=3) ± SD, different letters indicate significant differences (p<0.05) by LSD test.							

Table 4: Inhibitory effect of MCs on the growth of Fod in liquid medium.

Major compounds	Conidia germination (%)					
	Control	0.0625 mg ml^{-1}	0.125 mg ml^{-1}	0.25 mg ml^{-1}	0.5 mg ml^{-1}	1 mg ml^{-1}
Thymol	100 ± 0.00^{a}	66.6 ± 4.44^{b}	19.6 ± 2.44^{c}	0 ± 0.00	0 ± 0.00	0 ± 0.00
Carvacrol	100 ± 0.00^{a}	100 ± 0.00^{a}	78.3 ± 3.50^{b}	0 ± 0.00	0 ± 0.00	0 ± 0.00
Eugenol	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	68 ± 2.60^{b}	0 ± 0.00	0 ± 0.00
Menthol	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	70.3 ± 5.10^{b}
Values are means (n=3) ± SD, different letters indicate significant differences (p<0.05) by LSD test.						

Table 5: Percentage of germinated conidia after 20 h of incubation at 27°C.

response to very low concentrations of thymol and carvacrol (0.125 mg ml^{-1}), while eugenol only halved the growth potential at a concentration of 0.25 mg ml^{-1}. Menthol was the least active inhibitor tested in the present study, since at the concentration of 0.5 mg ml^{-1} the reduction of growth was only 39.8%.

Table 2 also shows the partial inhibitory concentrations (PIC) of MCs. Thymol, the most active compound, inhibited 90% of growth with half of its MIC value (0.125 mg ml^{-1}).

Determination of anti-fusarium effect of MCs in liquid medium

The effect of MCs on the growth of Fod in liquid medium is shown in Tables 3 and 4. MCs exhibited varying levels of antifungal activity against Fod. Table 3 shows that the MIC values of MCs in liquid medium ranged between 0.5 mg ml^{-1} and 4 mg ml^{-1}, with a potent effect of thymol and carvacrol (0.5 mg ml^{-1}) followed by eugenol (1mg ml^{-1}). Menthol showed the least antifungal activity with a MIC value of 4 mg ml^{-1}. The MFC values of MCs ranged between 1 mg ml^{-1} and more than 10 mg ml^{-1}. Thymol and carvacrol had the lowest MFC value (1 mg ml^{-1}) followed by eugenol (2 mg ml^{-1}). Menthol did not show any fungicidal effect even with the highest concentration (10 mg ml^{-1}).

The results depicted on Table 4 show that thymol and carvacrol were the most efficient MCs with an inhibition reaching 95.3% and 90.5%, respectively, at low concentration (0.25 mg ml^{-1}) followed by eugenol with a percentage of inhibition of 92.2% at 0.5 mg ml^{-1}. Menthol was the less active MC with a percentage of inhibition of 97.5% at relatively high concentration (2 mg ml^{-1}).

Fod conidial germination assay

The effect of MCs on conidia germination of Fod is shown in Table 5. The results in Table 5 show that the most effective inhibitors on Fod conidial germination were thymol and carvacrol (with an inhibition value of 100% at 0.25 mg ml^{-1}). For eugenol, a complete inhibition of the conidial germination was obtained at 0.5 mg ml^{-1}, while the effect of menthol was the lowest.

The conidia destruction assay

The effect of different concentrations of MCs on Fod conidia

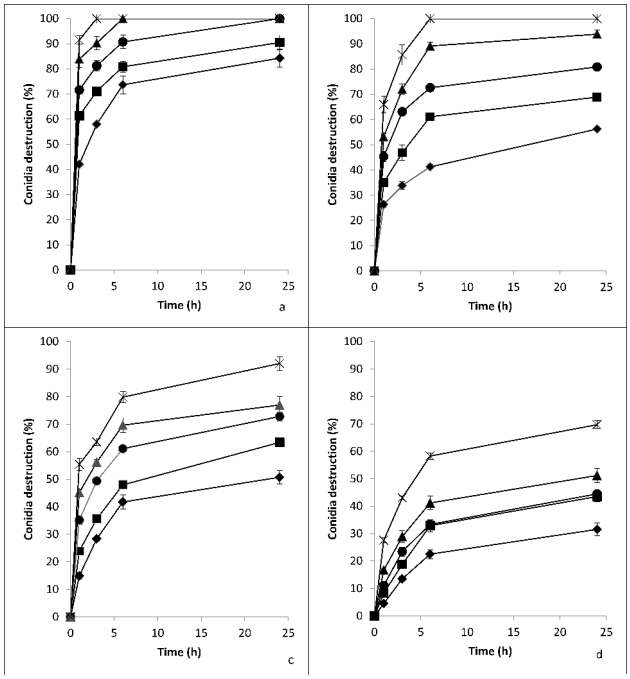

Figure 1: Time course of percentage of conidia destruction treated by different concentrations of MC. a: Thymol, b: Carvacrol, c: Eugenol, d: Menthol. (Values are means (n=3) ± SD). Concentrations of MC: (◆): 0.0625 mg ml^{-1}, (■): 0.125 mg ml^{-1}, (●): 0.25 mg ml^{-1}, (①): 0.5 mg ml^{-1}, (×): 1 mg ml^{-1}.

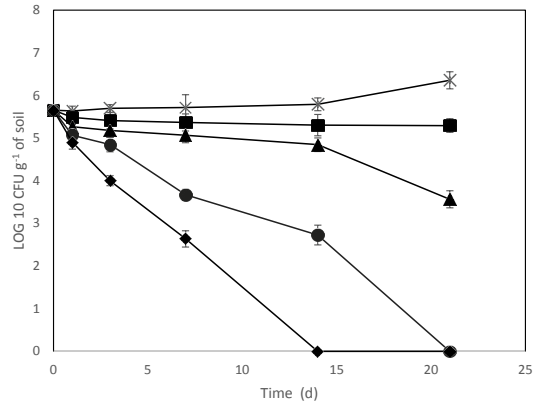

Figure 2: Soil densities of Fod over time as affected by soil treatment with different concentrations of thymol. **(**Values are means LOG 10 transformation of CFU cm^{-3} of soil. Data represent average of 3 trials of experiment with three replications per treatment per trial). Concentrations of thymol: (×): 0 mg ml^{-1}, (■): 0.25 mg ml^{-1}, (①): 0.5 mg ml^{-1}, (●): 1 mg ml^{-1}, (◆): 2 mg ml^{-1}).

destruction is shown in Figure 1. Thymol had the strongest destruction effect on conidia. Indeed, at the concentration of 1 mg ml^{-1} and after only 3 h of incubation the conidia were completely destroyed (Figure 1a). At the same concentration, carvacrol needed 6 h to have the same effect obtained with thymol (Figure 1b). On the other hand, eugenol and menthol needed 24 h to destroy respectively 92% and 69% of conidia (Figures 1c and 1d).

Fusarium density in the soil

Treatment of soil with aqueous emulsions of Thymol is shown in Figure 2.

Figure 2 shows significant differences in soil densities of Fod. For the untreated lot, soil density of Fod increased after 21 days from 5.63 Log_{10} (CFU cm^{-3}) to 6.35 Log_{10} (CFU cm^{-3}) whereas the treated lot with 0.5 mg ml^{-1} of thymol has shown a decrease after 21 days from 5.26 Log_{10} (CFU cm^{-3}) to 3.56 Log_{10} (CFU cm^{-3}). For the treated lot with 2 mg ml^{-1} of thymol, we noticed a total disappearance of Fod after 14 days.

Discussion

Choice of anti-fungal agents

Essential oils are a complex mixture of many components, and are known for having an anti-fungal activity Daferera, Barrera-Necha, Ahmad Khan, et al. [15-17]. However, the determination of the active molecules was challenging. Therefore, we have chosen to use pure MC such as thymol, carvacrol, eugenol and menthol.

Determination of MIC in agar medium and MIC and MFC in liquid medium

The Results in this part of the current study show a MIC value of 0.25 mg ml^{-1} for thymol and carvacrol, and 0.5 mg ml^{-1} for eugenol. These MIC values are much lower than the values described by Nguefack, Dambolena, Menniti et al. [18-21] with other fusarium species. The difference in values is most likely due to the use of different techniques; more precisely the size of conidia inoculum [22], and the use of detergents or solvents that influence the antifungal activity [23].

We have also determined the MIC and MFC in liquid medium. The results obtained from this liquid medium show that the MIC (99.9% inhibition of growth) of the thymol is 0.5 mg ml^{-1}. Although this value is twice the MIC obtained in agar medium, such difference was expected due to the use of different experimental methods. In fact, other laboratories have observed similar differences [24,25].

We opted for this method that relies on both the MIC (99.9% inhibition) and the concentrations that give partial inhibitions (PICs) because it allows better comparison between the agents used. Additionally, this method allowed us to come up with certain conclusions. In fact, while thymol and carvacrol have the same MIC value in agar medium (0.25 mg ml^{-1}), thymol showed a drastic growth inhibition (89%), whereas carvacrol only showed 54% growth inhibition at the ½ MIC value (0.125 mg ml^{-1}). We therefore conclude that thymol would be more effective than carvacrol.

Within field applications, the aim is not to completely eradicate the mold, but rather to diminish the fungal burden and growth [26].

Calculated MFC values were twice the MIC value, except for menthol, where the maximum concentration used (10 mg ml^{-1}) was found to be incapable of exerting a fungicidal action.

This difference between MIC and MFC has been reported by several authors on different Fusarium species [27-29] and other mold species [15,17].

The results of the MIC and MFC obtained in agar and liquid media thus show that thymol and carvacrol possess an anti-*Fusarium oxysporum* dose-dependent activity with values much lower than those described by the other laboratories [30].

Effect of MC on germination of Fod conidia

Since we used an inoculum of conidia in our work, we asked how MCs act on conidia. We therefore decided to evaluate the action of 4 MCs on the germination of conidia.

We worked on a liquid medium as described by Remmal et al.

[23], we observed the behavior of conidia under microscope in a time-dependent manner. Additionally, other laboratories used the same strategy to evaluate germination [21].

The results obtained show an important inhibition of germination at concentrations below the MIC in liquid medium, especially for thymol, followed by carvacrol and eugenol. Results demonstrated this inhibition through a continued reduction in the number of conidia until their eventual disappearance at concentrations above the MIC. These results also show a delayed germination effect at 20 h as indexed by the MC and concentration used. Using thymol as an example, ¼ of MIC caused 80% germinative inhibition and ½ of MIC caused 100% germinative inhibition.

These results are in agreement with results obtained by Dambolena et al. and Remmal et al. [21,31] using other *Fusarium* species, however, the concentrations inhibiting the germination are superior. This difference in concentrations between our lab and theirs is most likely due to methodological reasons cited above. These results led us to ask the following question: Do MCs act inside the fungal cells by modifying the cellular metabolism as previously described by Isman, Chaillot et al. and Divband et al. [32-34] or they act through modifying the structural integrity of the cell as described by Gao et al. [35].

In an attempt to answer this mechanistic question, we perceived three important phenomena through microscopic observation of conidia germination:

1. The decrease in number of conidia corresponded to the concentration of MC.

2. The presence of broken conidia cells, which have lost their intracellular content.

3. The presence of swollen and deformed conidia.

Based on these observations, we believe that the effect of MCs acts through modifying the structural integrity of the cell [35], previously obtained similar observations.

We decided to evaluate the ability of MC to destroy conidia by performing a microscopic count on the Malassez Counting chamber of conidia treated in a non-nutrient medium.

Effect of MC on the destruction of Fod conidia

The results obtained show a dose-dependent and time-dependent decrease in the number of conidia. The results also show that thymol at a concentration equal to MIC in liquid medium (0.5 mg ml^{-1}) causes 100% destruction of the conidia after 6 h, whereas carvacrol at the same concentration causes 100% destruction after 24 h.

As for thymol at the concentration equal to the MFC, a total destruction of the conidia was obtained after 3 h, with 93% destruction of conidia obtained after just 1 h. This rapidity of destructive action would be in favor of thymol for the direct action on the envelope of conidia. This hypothesis is borne out by the work of Bennis and Chami et al. [6,7] which shows a similar action of oregano EO and carvacrol on *Saccharomyces* using a scanning electron microscope (SEM). Moreover, the work of Xing et al. [29] shows the action of cinnamaldehyde on hyphae and conidia of *Fusarium verticillioides* using an SEM and TEM (transmission electron microscope).

On a fundamental level, advanced studies should be able to establish the relationship between the direct action of MC on the envelope of conidia and the changes observed intracellularly on the expression of certain genes [33].

On an applied level, we thought about the possibility of utilizing these results in the agricultural field to fight Fusarium wilt.

Fusarium oxysporum attacks plants through the roots to ascend into the vessels [36]. One of the established methods to fight *Fusarium* is soil decontamination using fungicidal agents before planting [37]. We therefore decided to test soil decontamination using thymol, since it is the MC which proved to be the most effective.

Decontamination of the soil by thymol

We artificially infested soil samples (previously sterilized) with 10^6 conidia g^{-1} of soil, treating these samples with different concentrations of thymol and closely monitoring the Fusarium load in each soil sample.

Our results show that 21 days after treatment, the number of conidia increased 10 times in the untreated batch, thereby demonstrating that under our experimental conditions, Fusarium was physiologically capable of surviving and multiplying.

The results also show that at half the MIC (0.25 mg ml^{-1}) the curve remained almost constant whereas a concentration equal to the MIC (0.5 mg ml^{-1}) caused a decrease of 95% from day 14. The concentration equal to MFC caused a very large decrease from day 14 to day 21, where there was no *Fusarium* observed.

These results encouraged us to pre-test the action of thymol on carnations planted in Fod artificially infested soil. The results obtained show a significant reduction of the Fusarium load in the soil compared to the untreated control. This reduction was reflected in the health status of treated plants, which produced much larger roots and stem than plants planted in untreated infested soil (results not shown).

These results are quite promising for the development of a Fusarium treatment using thymol, a natural product safe for farmers, the environment, and consumers. This thymol-based treatment would be an excellent alternative to currently used fungicides such as methyl bromide, chloropicrin, and benomyl which are toxic to the farmers, the consumers and the environment [2].

Conclusion

This work is a fundamental contribution toward showing the anti-fusarium action of MCs, with the possible development of natural alternatives capable of replacing chemical fungicides currently used in agriculture, specifically floral agriculture.

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Conflict of Interest

No conflict of interest declared.

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