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ORIGINAL RESEARCH PAPER

EVALUATION OF ANTIFUNGAL ACTIVITY OF FREE AND ENCAPSULATED CLOVE OIL IN β-CYCLODEXTRIN AGAINST AN ALGERIAN ISOLATE OF *FUSARIUM OXYSPORUM* F. SP *RADICIS LYCOPERSICI*

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ABSTRACT. Essential oils play an important role in the fight against plant pathogens. Clove essential oils are widely used as antifungal, antibacterial, insecticidal, and antioxidant agents. To increase its solubility and reduce its volatility, clove essential oil was encapsulated in β cyclodextrin, using the freeze-drying method at a weight ratio of 16:84. The composition of clove essential oil was evaluated using gas chromatography-mass spectrometry (GC-SM). Gas chromatography-mass spectrometry analysis revealed the presence of 11 components, representing 98.7% of the essential oils. The major component was eugenol (79.7%), followed by eugenyl acetate (16.3%). The antifungal activity of clove essential oil and its inclusion complex with β cyclodextrin was tested against an Algerian isolate of *Fusarium oxysporum f.sp radicis lycopersici* (FORL) and a reference strain, *Fusarium oxysporum f.sp lycopersici* race 2 (FOL) in liquid medium. The obtained results demonstrated that the antifungal efficiency of clove essential oil against FORL and FOL enhanced after encapsulation in β-cyclodextrin. A significant difference between the inhibition rates obtained with free clove essential oil and those obtained with the inclusion complex (β-cyclodextrin-clove oil) was observed for the concentrations studied.

Keywords: Clove essential oil, β-cyclodextrin, inclusion complex, antifungal activity, *Fusarium oxysporum* f. sp *radicis lycopersici*, tomato

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Introduction

Plants are threatened by various pathogens including fungi. Fungal diseases cause significant economic losses, lower crop yields, and lead to deterioration of food quality, as some of these fungi produce toxic compounds with consequent effects on human health (Oliveira *et al*., 2021; Palfi *et al*., 2019).

Fungi belonging to the *Fusarium* genus are among the most important pathogens that have important negative effects on plant production in a wide range of plant hosts (Zabka and Pavela, 2018). *Fusarium* can contaminate agricultural products, both in the field and during storage (Maery *et al*., 2018). These fungi affect wheat and other vascular plants including tomatoes (Kasprowicz *et al*. 2010).

Tomato (*Solanum lycopersicum* L.) is a vegetable consumed worldwide, either as an unprocessed fruit or as a processed product, and is considered a vital food (Srinivas *et al*., 2019; Gonçalves *et al*., 2020) with important nutritional value. However, this plant faces different threats, including pathogens such as *Fusarium oxysporum* (Thabet and Khalifa, 2018).

The *Fusarium oxysporum* species complex is phylogenetically diverse and present worldwide in various environments (indoor environments, soil, and water) (Rahman *et al*., 2021; Lamo and Takken, 2020). This complex includes more than 100 host-specific strains (Gordon, 2017).

Particularly, *Fusarium oxysporum* is responsible for causing wilt, a plant disease that affects many horticultural crops. This disease is transmitted via the soil (Park *et al*., 2017).

To reduce the impact of fungal diseases, synthetic products have long been widely used as fungicides. They are potent (Abdul Aziz, 2012) antimicrobials and are easy to use (Cárdenas-Laverde *et al*., 2021). Although efficient, their use poses several risks (Chacón *et al*., 2021).

However, excessive use of these chemicals is a threat to the ecological balance, affecting both agricultural products and human health, particularly because they are carcinogenic (Bashir *et al*., 2020). In addition, the resistance of fungi to chemical treatments is continuously increasing, and the efficiency of fungicides is decreasing considerably (Tabassum and Vidyasagar, 2013).

In recent years, essential oils have emerged as an interesting alternative to fight fungal pathogens because of their active molecules and nontoxic effects on the environment (Kalagatur *et al*., 2018). Essential oils are secondary metabolites present in different plant parts (flowers, buds, leaves, seeds, twigs, bark, wood, fruit, and roots) (Dhifi *et al*., 2016). Essential oils contain several terpenes (monoterpenes, sesquiterpenes, and their oxygenated derivatives, like alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides), phenolic compounds, and phenylpropanoid compounds derived from acetate-mevalonic acid and shikimic acid (Basak and Guha, 2018).

Syzygium aromaticum (clove) belongs to the *Myrtaceae* family and is native to the Maluku Islands in Indonesia. Several studies have reported anticarcinogenic, antiviral, antibacterial, and antifungal activities of clove essential oils (Batiha *et al*., 2020). They are also used in the production of perfumes. This oil is composed

essentially of eugenol, phenylpropanoid, eugenyl acetate, monoterpenic ester, and β-caryophyllene, a sesquiterpene (Hasheminejad *et al*., 2019).

However, essential oils are easily degraded in the environment because of their sensitivity to light, air, and temperature (Cetin Babaoglu *et al*., 2016). It should also be noted that essential oils are insoluble in water because of their lipophilic nature (Hill *et al*., 2013).

Molecular encapsulation seems to be an interesting alternative for preventing the volatilization effect and subsequent degradation. Several encapsulation processes have been used and encapsulation with cyclodextrins is an effective solubilizing agent (Cetin Babaoglu *et al*., 2016).

Cyclodextrin can be the product of the enzymatic degradation of starch or the metabolism of certain bacteria**.** Cyclodextrins are cyclic oligosaccharides, consisting of 6 (α-cyclodextrin), 7 (β-cyclodextrin), 8 (γ-cyclodextrin) or several glucopyranose units linked by α (1,4) glycosidic bonds. They are cone-shaped, the outer part is hydrophilic, and the inner cavities are hydrophobic, allowing the formation of inclusion complexes (Abarca *et al*., 2015).

The food industry is constantly conducting research and developing molecules with positive health effects. Cyclodextrins are interesting products because of the properties they possess (Matencio *et al.*, 2020). Several studies have reported the use of β-cyclodextrin inclusion complexes and essential oils in food. These inclusion complexes are used in food and food packaging materials to improve the microbiological quality of food during storage, thereby ensuring good preservation (Marques, 2010).

Thus, the aim of the present study is to investigate the antifungal activity of clove essential oil encapsuled in β-cyclodextrin. This effect was tested using an Algerian isolate of *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) (Debbi *et al.*, 2018). This strain is present in most Mediterranean countries (Edel-Hermann *et al.*, 2012) including Algeria *(Debbi et al.*, 2018) causing important economic losses.

Materials and methods

Fungal strains

In this study, two fungal strains were used: *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) (an Algerian isolate) (Debbi *et al*., 2018) and *Fusarium oxysporum* f. sp. *lycopersici* race 2 (FOL) (reference strain 4287). These strains were provided by the Biotechnology Research Center in Constantine, East Algeria, Brazil.

Plant and chemicals

Clove buds came from India and were purchased from a local herbalist in Bejaia, northeastern Algeria. β-cyclodextrin was provided by Sigma Aldrich (Steinheim, Germany).

Essential oil extraction

Clove buds underwent hydrodistillation for 3-4 hours, using a Clevenger-type apparatus, as described by the European Pharmacopoeia (European Pharmacopoeia Commission, European Pharmacopoeia 5th Ed. Council of Europe: Strasbourg

Cedex, France, 2004). After the hydrodistillation process, oil was harvested using a glass flasks, and dried with anhydrous sodium. The essential oil was stored in the refrigerator at 4 °C until further use.

The percentage oil yield was calculated (Kalagatur *et al*., 2018) using the following equation:

$$\text{Oil yield } (\%) = \frac{\text{w (EO)}}{\text{w (P)}} \times 100$$

where w (EO) is the weight of the essential oil and w (P) is the weight of the plant material.

Preparation of inclusion complex (β-cyclodextrin-clove essential oil)

The inclusion complex (β-cyclodextrin -clove essential oil) was obtained using the freeze-drying method described by Santos *et al*. (2014) with certain modifications.

For a weight ratio of 16:84, 840 mg of β-cyclodextrin was dissolved in 75 ml of previously heated distilled water. Once the β-cyclodextrin dissolved in distilled water, 160 mg of clove essential oil was added to the solution and the mixture was stirred continuously for 24 hours, away from light, at room temperature. The resulting mixture was frozen at -80°C and then freeze-dried for 48 h (Freeze Dryer, Martin Christ, Alpha 1-4, Germany). The obtained inclusion complex was stored in the refrigerator at 4°C until further use.

Gas chromatography-mass spectrometry (GC–MS) analysis conditions

Chemical analysis of clove oil was performed by gas-chromatography coupled with mass-spectrometry using Agilent technologies apparatus (GC system 7890A/ 5975C inert XL EI/CI MSD, Palo Alto, CA, USA) equipped with an apolar column HP-5MS (30 m x 0.25 mm x 0.25µm). The oven temperature was programmed at 60°C for 8 min, then increased by 4°C/min to 250°C for 25 min. The carrier gas used was He, which was at a flow of 1.5 mL/min. The injector operating at split mode had an injection volume of 0.2 µL, detector's temperature was adjusted at 250°C (Belazougui *et al*., 2023).

The components were identified by comparing their retention indices relative to n-alkanes (C7–C28) with those reported in the literature (Adams, 2007), and by matching their mass spectra to those of commercial mass spectra libraries (Wiley 7N and NIST 2011). Semi-quantitative analysis was performed using electronically obtained data from the FID area without using correction factors.

Antifungal activity of clove essential oil and inclusion complex (β-cyclodextrin-clove essential oil)

The study of the antifungal activity of clove essential oil and inclusion complex (β-cyclodextrin-clove essential oil) was performed in liquid medium by determining the rate of the mycelium inhibition (Elhouiti *et al*., 2017).

Preparation of spore suspension

Fusarium oxysporum f.sp *radicis lycopersici* (FORL) and *Fusarium oxysporum* f.sp *lycopersici* (FOL) were grown in potato dextrose agar (PDA) medium for 7 days at 25°C in the dark.

The spore suspension was obtained by flooding the fungal cultures with 3 mL of sterile physiological water and scraping the surfaces of the cultures to release conidia. The obtained suspension was then stirred using a vortex, and the concentration was adjusted to 10^6 conidia/cm^3, through enumeration in a hemocytometer chamber.

The antifungal activity in liquid medium

A 96-well microplate was used, in each well 100 µL of the potato dextrose broth (PDB) medium was added and the wells were inoculated with 100 µL of the spore suspension with a concentration of 10^6 conidia/mL. After seeding, clove essential oil was added to the wells in order to obtain three concentrations: **200**, **300,** and **400** ppm. The same protocol and the same concentrations were used for the inclusion complex (β-cyclodextrin-clove essential oil). The experiments were performed in triplicates for each fungus. A spectrophotometer (BioTek ELx800 Inc. USA) was used to read the absorbance at a wavelength of 630 nm on D_0 (first day of optical density assessment before incubation) and D_3 (day four of density assessment) after incubation of the microplates at 25°C.

The growth inhibition was determined (Khan *et al*., 2018) using the following equation:

$$\text{Growth inhibition} = ((\Delta C - \Delta t) \div \Delta c) \times 100$$

where **Δc** is the absorbance of the control fungal culture at 630 nm on D_3, minus the measured absorbance of the control fungal culture on D_0.

Δt: Absorbance of fungal culture with clove essential oil at 630 nm in D_3 minus measured absorbance of fungal culture with clove essential oil in D_0.

The same formula was used to calculate the inhibition rate with the inclusion complex.

Statistical analysis

Statistical analyses were performed using the SPSS software (version 26) (IBM SPSS, Armonk, USA). The normality of the samples was verified using the Shapiro-Wilk test. Antifungal activity of free clove essential oil and inclusion complex (β-cyclodextrin-clove essential oil) on *Fusarium oxysporum* f.sp *radicis lycopersici* (FORL) and on *Fusarium oxysporum* f.sp *lycopersici* (FOL) were performed using Student's t-test. Results were considered significant at $P < 0.05$.

Results and discussion

Extraction of clove essential oil

The extracted clove essential oil was transparent, light yellowish in color, and fragrant. The obtained yield was **9**%, a value that is higher than that reported by Hamini-Kadar et al. (2014) with hydrodistillation and by Aguilar-Gonzalez et al. (2015), with only 2.7% when using microwave assisted hydrodistillation. Higher values were reported by Wenqiang et al. (2007), with 11.5% and 19.6% extracted using hydrodistillation and supercritical CO_2 extraction, respectively.

Ben Hassine et al. (2021) reported an extraction yield of 7.1 ± 0.8% for ungrounded clove buds and an extraction yield of 14.3 ± 0.6% for buds that were grounded. Decreasing the particle size increases the extraction rate and yield. The yield of essential oils obtained by hydrodistillation depends on the distillation conditions (time, temperature, pressure, and quality of the plant) (Aguilar-gonz *et al*., 2015). A previous study (Alfikri *et al*., 2020) reported that buds from young trees had higher essential oil yields than those from mature trees. Other factors, such as the environment, geographical location, and method of extraction of the essential oil, also affects the extraction yield (Mutlu-Ingok *et al*., 2020).

Gas chromatography-mass spectrometry (GC–MS) and analysis conditions

The chemical composition of clove essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS). Eleven compounds were identified, representing 98.7% of the total active molecules (Table 1).

Table 1. Chemicals compounds of clove essential oil by Gas chromatography-mass spectrometry GC-MS

N°	Compound	RT	CRI	LRI	%	Identification
1	Methyl salicylate	17.26	1191	1190	0.1	RI, MS
2	Chavicol	20.08	1249	1247	0.3	RI, MS
3	**Eugenol**	24.18	1357	1357	**79.7**	RI, MS, Sd
4	β-Caryophyllene	25.47	1419	1417	1.4	RI, MS, Sd
5	α-Humulene	25.51	1452	1452	0.2	RI, MS
6	Germacrene D	27.37	1482	1484	0.1	RI, MS
7	δ-Cadinene	28.68	1521	1522	0.1	RI, MS
8	**Eugenyl acetate**	29.17	1524	1524	**16.3**	RI, MS
9	Caryophyllene oxide	30.44	1582	1582	0.5	RI, MS
10	Caryophylla-4[12], 8[13]-dien-5.beta-ol	31.96	1640	1639	0.1	RI, MS
11	Benzyl benzoate	35.51	1759	1759	0.1	RI, MS
				Total (%)	**98.7**	

Compounds are listed in order of elution relative to the HP MS column; RT: retention time; CRI : calculated retention index; LRI : literature Retention Index; Identification : RI = by comparison of the retention index of the compound with those reported in literature (ADAMS, 2007), MS : comparison of the mass spectrum of the compound with those of the literature (Adams, 2007) and those of the computerized database (Wiley 7N). Sd: retention times compared to a pure compound injected under the same conditions as the essential oil.

A chromatogram of the clove essential oil is shown in Figure 1. The major compound was eugenol (79.7%) (Figure 2) followed by eugenyl acetate, which was 16.3% (Figure 3). The clove essential oil contains other components at low percentages including: Methyl salicylate (0.1%), Chavicol (0.3%), β-Caryophyllene (1.4%), α-

Humulene (0.2%), Germacrene D (0.1%), δ-Cadinene (0.1%), Caryophyllene oxide (0.5%), Caryophylla-4[12], 8[13]-dien-5.beta-ol (0.1%), and benzyl benzoate (0.1%). These results are similar to those reported by Omidbeygi *et al*. (2007), showing that the main component is eugenol, though at a lower percentage (63.37%). The results of the present study are also consistent with those of Sattary *et al*. (2020) reporting 77.61% of eugenol and with those reported by Sharma *et al*. (2017) with 75.41%.

Variations in essential oil composition depends on several external and endogenous factors. External factors are related to the environment, such as light and precipitation, and endogenous factors are related to the plant, such as the age and the part of the plant used (Barra, 2009).

Several studies have reported that the drying method affects the quantitative and qualitative composition of essential oils (Moghaddam and Mehdizadeh, 2017).

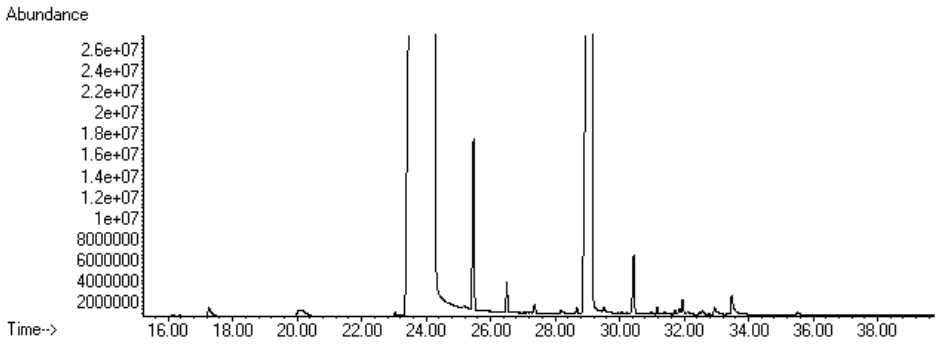

Figure 1. Clove essential oil chromatogram obtained using gas chromatography-mass spectrometry (GC/MS).

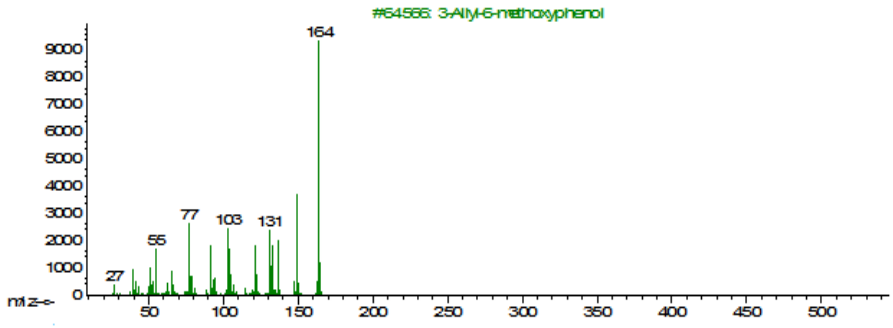

Figure 2. Eugenol mass spectra

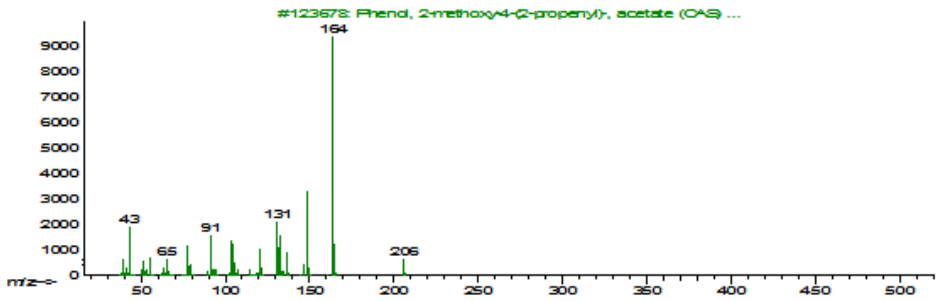

Figure 3. Acetate eugenyl mass spectra

Antifungal activity of clove essential oil and inclusion complex (β-cyclodextrin-clove essential oil) in a liquid medium

The antifungal activity of pure clove essential oil and the inclusion complex with cyclodextrin was evaluated by determining the inhibition of fungal growth in liquid medium (Figure 4 and Figure 5). The antifungal activity was proportional to the concentration used for both fungus *Fusarium oxysporum f.sp radicis lycopersici* and *Fusarium oxysporum f.sp lycopersici*.

The inhibition rates of clove essential oil by *Fusarium oxysporum f.sp lycopersici* (FOL) was 5.18% at 200 ppm, 5.92% at 300 ppm, and 6.31% at 400 ppm (Figure 4). This inhibition was significantly lower than what was observed in the Algerian isolate (*Fusarium oxysporum f.sp radicis lycopersici)* FORL, with 10.39% at 200 ppm, 14.09% at 300 ppm and 15.18% at 400 ppm (Figure 5).

The current results corroborate with those of a previous study (Thabet and Khalifa, 2018) reporting an inhibition of the growth diameter of *Fusarium oxysporum* with a total inhibition of 4% for clove oil.

Palfi *et al*. (2019) and Singh Rana *et al*. (2011) also reported on the antifungal activity of clove on *Fusarium oxysporum* with only 9 µL/10 mL PDA and 10 µl/ mL, respectively.

The same inhibitory effect was reported by Hamini-kadar *et al*. (2014) against three pathogenic fungi namely *Fusarium oxysporum* f.sp *radicis lycopercisi, F. commune* and *F. redolen*; they reported complete inhibition of *Fusarium oxysporum* f. sp *radicis lycopercisi* at 1 µL/ml.

Sharma *et al*. (2017) reported the effectiveness of other essential oils, including mint, lemongrass, and eucalyptus, against *Fusarium oxysporum* f. sp. *Lycopersici*-1322. Using clove oil, the authors reported antifungal activity with an MIC of 31.25 ppm. The MIC values of lemongrass, mint, and eucalyptus essential oils ranged from 62.5 to 500 ppm.

The antifungal activity of essential oils depends on their chemical composition and concentration. Eugenol is the main component of clove essential oil and is responsible for its antifungal activity. Essential oils affect fungi by disrupting the fungal cell membrane and its permeability, which leads to leakage of materials and

ions into the extracellular medium and unbalanced osmotic conditions, thus causing the death of the fungal cell (Sattary *et al*., 2020).

The antifungal mechanism of eugenol can be explained by the acidic nature of its hydroxyl group, which forms hydrogen bonds with its active enzymatic core (Muñoz Castellanos *et al*., 2020).

Moreover, the antifungal efficiency of clove essential oil against FORL and FOL enhanced after encapsulation in β-cyclodextrin. Moreover, the results showed a significant difference between the inhibition rates with free essential oil compared to the inclusion complex (β-cyclodextrin-clove essential oil). The inclusion complex inhibited the growth of *Fusarium oxysporum* f.sp *lycopersici* by 21.24%, 56.32%, and 70.13% at 200 ppm, 300 ppm and 400 ppm, respectively (Figure 4).

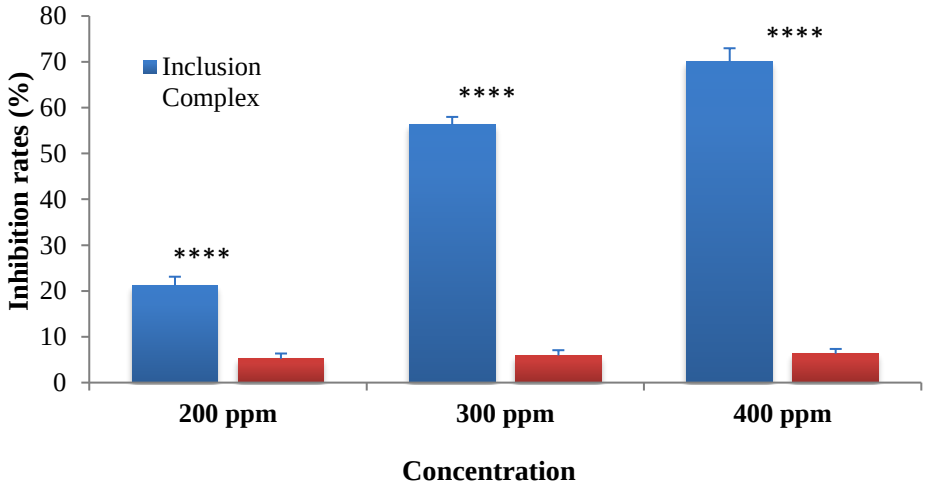

Figure 4. Antifungal activity of free clove essential oil and inclusion complex (β-cyclodextrin-clove essential oil) on *Fusarium oxysporum* f.sp *lycopersici* (FOL) (**** $p \leq 0.0001$ Highly significant)

The Algerian isolate (FORL) showed greater sensitivity ($P < 0.001$) than FOL. At 200 ppm, 300 ppm, and 400 ppm, the inhibition rates were 33.20%, 65.83%, and 87.47%, respectively (Figure 5).

These results can be explained by the fact that encapsulation in β-cyclodextrin enhanced the solubility of clove essential oil and increased its dispersibility in liquid medium. Eugenol, the main component of clove essential oil, is highly volatile, insoluble in water, and has poor oxidative stability (Shao *et al*., 2018), limiting its optimal fungicidal effect. The results obtained with the inclusion complex can be explained by the fact that β-cyclodextrin significantly improved the therapeutic effect of eugenol.

This holds true when working in a liquid medium, where molecular solubility can be a limiting factor in expressing antimicrobial activity. As the final use of antifungal agents in the field is exclusively in liquid form, the use of solubilizing systems such as cyclodextrins is of high importance.

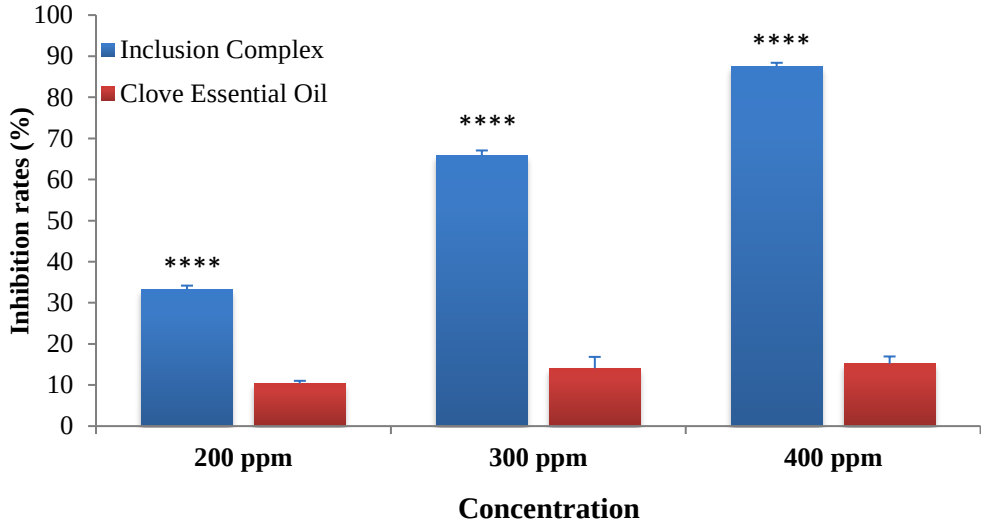

Figure 5. Antifungal activity of free clove essential oil and inclusion complex (β-cyclodextrin clove oil) on *Fusarium oxysporum f.sp radicis lycopersici* (FORL) (**** $p \leq 0.0001$ Highly significant)

Conclusion

In the present work, the inclusion complex (β-cyclodextrin-clove essential oil) was prepared using the freeze-drying method. This complex proved to be efficient in inhibiting the growth of *Fusarium oxysporum* f. sp. *lycopersici*, but inhibited the Algerian isolate (*Fusarium oxysporum* f. sp. *radicis lycopersici*) to a greater extent. Encapsulation in β-cyclodextrin appears to significantly enhance the solubility of clove essential oil, which was shown by the highest antifungal activity. The inclusion complex β-cyclodextrin clove essential oil appears to be a good candidate for safe and effective control of fungal pathogens.

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References

Abarca, R.L., Rodríguez, F.J., Guarda, A., Galotto, M.J., Bruna, J. E. 2015. Characterization of beta-cyclodextrin inclusion complexes containing an essential oil component. *Food Chemistry*, **196**, 968–975.

Abdul Aziz, A. 2012. In vitro antifungal activity of three Saudi plant extracts against some Phytopathogenic fungi. *Plant Protection Research*, **3**(8), 277-284

Adams, R. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL, USA.

Aguilar-gonz, A.E., Palou, E., Aurelio, L. 2015. Antifungal activity of essential oils of clove (*Syzygium aromaticum*) and/or mustard (*Brassica nigra*) in vapor phase against grey mold (*Botrytis cinerea*) in strawberries. *Innovative Food Science and Emerging Technologie*, **32**, 1-27.

Alfikri, F.N., Pujiarti, R., Wibisono, M. G., Hardiyanto, E.B. 2020. Yield, quality, and antioxidant activity of clove (*Syzygium aromaticum* L.) bud oil at the different phenological stages in young and mature trees. *Scientifica*, **2020**(4), 1-8.

Barra, A. 2009. Factors affecting chemical variability of essential oils: A review of recent developments. *Natural Product Communications*, **4**(8), 1147-1154.

Basak, S., Guha, P. 2018. A review on antifungal activity and mode of action of essential oils and their delivery as nano-sized oil droplets in food system. *Journal of Food Science and Technology*, **55**(12), 4701-4710.

Bashir, L.U., Abdulkadir, A., Shah, M.M., Hamisu, A., Sharif, U., Kamalu, A.A. 2020. Phytochemical screening and antifungal potentials of *Citrus limon* peels against *Fusarium oxysporum* and *Rhizopus stolonifer* causing rots in water melon (*Citrullus lanatus* L .). *Experimental Sciences*, **11**, 1-5.

Batiha, G.E.S., Alkazmi, L.M., Wasef, L.G., Beshbishy, A.M., Nadwa, E.H., Rashwan, E.K. 2020. S*yzygium aromaticum* l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, **10**(2), 1-17.

Belazougui, K., Mesrouk, S., Mohammedi, H., Akcha, S., Aïnouz, L., Mecherara-Idjeri, S.F. 2023. Phytochemical analysis, mineral composition, Assessment of antioxidant properties and cytotoxic potential of *Ephedra alata*. subsp. Alenda secondary metabolites. *Food Bioscience*, **53**, 102657.

Ben Hassine, D., Kammoun El Euch, S., Rahmani, R., Ghazouani, N., Kane, R., Abderrabba, M., Bouajila, J. 2021. Clove buds essential oil: The impact of grinding on the chemical composition and its biological activities involved in consumer's health security. *BioMed Research International*, **2021**(4), 1-11.

Cárdenas-Laverde, D., Barbosa-cornelio, R., Coy-Barrera, E. 2021. Antifungal activity against *Fusarium oxysporum* of botanical end-products: An integration of chemical composition and antifungal activity datasets to identify antifungal bioactives. *Plants*, **10**(12), 2563.

Cetin Babaoglu, H., Bayrak, A., Ozdemir, N., Ozgun, N. 2016. Encapsulation of clove essential oil in hydroxypropyl beta-cyclodextrin for characterization, controlled release, and antioxidant activity. *Journal of Food Processing and Preservation*, **41**(5), 1-8.

Chacón, C., Bojórquez-Quintal, E., Caamal-Chan, G., Ruíz-Valdiviezo, V.M., Montes-Molina, J.A., Garrido-Ramírez, E.R., Rojas-Abarca, L.M., Ruiz-Lau, N. 2021. In vitro antifungal activity and chemical composition of piper auritum kunth essential oil against *Fusarium oxysporum* and *Fusarium equiseti*. *Agronomy*, **11**(6), 1-13.

De Lamo, F.J., Takken, F.L. W. 2020. Biocontrol by *Fusarium oxysporum* using endophyte-mediated resistance. *Frontiers in Plant Science*, **11**(37), 1-15.

Debbi, A., Boureghda, H., Monte, E., Hermosa, R. 2018. Distribution and genetic variability of fusarium oxysporum associated with tomato diseases in Algeria and a Biocontrol strategy with indigenous *Trichoderma* spp. *Frontiers in Microbiology*, **9**, 1-11.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., Mnif, W. 2016. Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, **3**(4), 1-16.

Edel-Hermann, V., Gautheron, N., Steinberg, C. 2012. Genetic diversity of Fusarium oxysporum and related species pathogenic on tomato in Algeria and other Mediterranean countries. *Plant Pathology*, **61**(4), 787-800.

Elhouiti, F., Tahri, D., Takhi, D., Ouinten, M., Barreau, C. 2017. Variability of composition and effects of essential oils from *Rhanterium adpressum* Coss. & Durieu against mycotoxinogenic *Fusarium strains*. *Archives of Microbiology*, **199**(11), 1345-1356.

European Pharmacopoeia Commission. 2004. European Pharmacopoeia 5th Ed. Council of Europe: Strasbourg Cedex, France.

Gonçalves, D.C., Queiroz, V.T. De, Costa, A.V., Lima, W.P., Belan, L.L., Moraes, W.B., Lopes, N., Póvoa, P., Cardoso, H., Póvoa, C. 2020. Reduction of Fusarium wilt symptoms in tomato seedlings following seed treatment with *Origanum vulgare* L. essential oil and carvacrol. *Crop Protection*, **141**, 1-34.

Gordon, T.R. 2017. Fusarium oxysporum and the Fusarium Wilt Syndrome. *Annual Review of Phytopathology*, **55**(1), 23-39.

Hamini-kadar, N., Mebrouk, K. 2014. Antifungal activity of clove (*Syzygium aromaticum* L.) essential oil against phytopathogenic fungi of tomato (*Solanum lycopersicum* L) in Algeria. *Journal of Experimental Biology and Agricultural Sciences*, **2**(5), 447-454.

Hasheminejad, N., Khodaiyan, F., Safari, M. 2019. Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles. *Food Chemistry*, **275**, 113-122.

Hill, L., Gomes, C., Taylor, T. 2013. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. *LWT - Food Science and Technology*, **51**(1), 86-93.

Inder Singh, R., Aarti Singh, R., Ram Charan, R. 2011. Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol. *Brazilian Journal of Microbiology*, **42**(4), 1269-1277.

Kalagatur, N., Nirmal Ghosh, O., Sundararaj, N., Mudili, V. 2018. Antifungal activity of chitosan nanoparticles encapsulated with cymbopogon martinii essential oil on plant pathogenic fungi *Fusarium graminearum*. *Frontiers in Pharmacology*, **9**, 1-13.

Kasprowicz, M. J., Kozioł, M., Gorczyca, A. 2010. The effect of silver nanoparticles on phytopathogenic spores of Fusarium culmorum. *Canadian Journal of Microbiology*, **56**(3), 247-253.

Khan, S., Shinwari, M. I., Haq, A., Badshah, M. 2018. Fourier-transform infrared spectroscopy analysis and antifungal activity of methanolic extracts of *Medicago parviflora*, *Solanum nigrum*, *Melilotus alba* and *Melilotus indicus* on soil-borne phytopathogenic fungi. *Pakistan Journal of Botany*, **50**(4), 1591-1598.

Maery, F., Ferreira, D., Hirooka, E.Y., Ferreira, F.D., Silva, V., Aparecida, S., Mossini, G.,

Machinski Jr. M. 2018. Effect of *Zingiber officinale* Roscoe essential oil in fungus control and deoxynivalenol production of *Fusarium graminearum* Schwabe in vitro. *Food Additives & Contaminants: Part A*, **35**(11), 1-7.

Marques, H.M. C. 2010. A review on cyclodextrin encapsulation of essential oils and volatiles. *Flavour and Fragrance Journal*, **25** (5), 313-326.

Matencio, A., Navarro-Orcajada, S., García-Carmona, F., López-Nicolás, J.M. 2020. Applications of cyclodextrins in food science. A review. *Trends in Food Science and Technology*, **104**(4), 132-143.

Moghaddam, M., Mehdizadeh, L. 2017. Chemistry of essential oils and factors influencing their constituents. *Soft Chemistry and Food Fermentation*, 379-419.

Muñoz Castellanos, L., Amaya Olivas, N., Ayala-Soto, J., De La O Contreras, C.M., Zermeño Ortega, M., Sandoval Salas, F., Hernández-Ochoa, L. 2020. In vitro and in vivo antifungal activity of clove (*Eugenia caryophyllata)* and pepper (*Piper nigrum L.*) essential oils and functional extracts against Fusarium oxysporum and Aspergillus niger in tomato (Solanum lycopersicum L.). *International Journal of Microbiology*, **2020**(5), 1-8.

Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D.N., Karbancioglu-Guler, F., Capanoglu, E. 2020. Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: an updated review. *Molecules*, **25**(20), 1-49.

Oliveira, T.N.S., Silva-Filho, C.M.S., Malveira, E.A., Aguiar, T.K.B., Santos, H.S., Albuquerque, C.C., Morais, M.B., Teixeira, E.H., Vasconcelos, M.A. 2021. Antifungal and antibiofilm activities of the essential oil of leaves from *Lippia gracilis* Schauer against phytopathogenic fungi. *Journal of Applied Microbiology*, **130**(4), 1117-1147.

Omidbeygi, M., Barzegar, M., Hamidi, Z., Naghdibadi, H. 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus Xavus* in liquid medium and tomato paste. *Food Control*, **18**(12), 1518-1523.

Palfi, M., Konjevoda, P., Vrandečić, K., Ćosić, J. 2019. Antifungal activity of essential oils on mycelial growth of *Fusarium oxysporum* and *Bortytis cinerea*. *Emirates Journal of Food and Agriculture*, **31**(7), 544-554.

Park, J.Y., Kim, S.H., Kim, N.H., Lee, S.W., Jeun, Y.C., Hong, J.K. 2017. Differential inhibitory activities of four plant essential oils on *in vitro* growth of F*usarium oxysporum* f. sp. *fragariae* causing Fusarium wilt in strawberry plants. *Plant Pathology Journal*, **33**(6), 582-588.

Rahman, M.Z., Ahmad, K., Kutawa, A.B., Siddiqui, Y., Saad, N., Geok Hun, T., Hata, E. M., Hossain, M. I. 2021. Biology, diversity, detection and management of *Fusarium oxysporum* f. Sp. *niveum* causing vascular wilt disease of watermelon (*Citrullus lanatus*): A review. *Agronomy*, **11**(7), 1-24.

Sattary, M., Amini, J., Hallaj, R. 2020. Antifungal activity of the lemongrass and clove oil encapsulated in mesoporous silica nanoparticles against wheat's take-all disease. *Pesticide Biochemistry and Physiology*, **170**(4), 104696.

Shao, Y., Wu, C., Wu, T., Li, Y., Chen, S., Yuan, C., Hu, Y. 2018. Eugenol-chitosan nanoemulsions by ultrasound-mediated emulsification: Formulation, characterization and antimicrobial activity. *Carbohydrate Polymers*, **193**, 144-152.

Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., Kundu, B. 2017. Antifungal activities of selected essential oils against *Fusarium oxysporum* f. sp. *lycopersici* 1322, with emphasis on Syzygium aromaticum essential oil. *Journal of Bioscience and*

Bioengineering, **123**(3), 308-313.

Srinivas, C., Devi, D.N., Murthy, K.N., Dhananjaya, C., Lakshmeesha, T.R., Singh, B., Kumar, N., Niranjana, S.R., Hashem, A., Alqarawi, A.A., Tabassum, B., Fathi, E., Allah, A., Nayaka, S.C. 2019. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity– A review. *Saudi Journal of Biological Sciences*, **26**(7), 1315-1324.

Tabassum, N., Vidyasagar, G. 2013. Antifungal investigations on plant essential oils. a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5** (2), 19-28.

Thabet, M., Khalifa, W. 2018. Antifungal activities of clove oil against root rot and wilt pathogens of tomato plants. *American-Eurasian Journal of agriculture & Environmental Sciences*, **18**(3), 105-114.

Wenqiang, G., Shufen, L., Ruixiang, Y., Shaokun, T., Can, Q. 2007. Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food Chemistry*, **101**(4), 1558–1564.

Zabka, M., Pavela, R. 2018. Review Chapter : Fusarium genus and essential oils. *Natural Antimicrobial Agents, Sustainab*, 95-120.