Antimicrobial Activity of Fenchol-based Eutectic Solvents

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ABSTRACT

Eutectic solvents (ES) have several interesting characteristics, such as eco-friendliness, biodegradability, and potential for various technological applications, including antimicrobial activity. Therefore, this study aimed to study the physical and chemical characteristics of terpene-based solvents and their fungal inhibition activities. To this end, different ESs were produced from fenchol and carboxylic acids of different chain sizes. For ES characterization, the phase diagram and inhibitory activity of *Fusarium Graminearum* were analyzed. According to the results of the phase diagram, 3 of the eight solvents showed behavior close to ideal. Moreover, for antifungal activity, solvents FC8 and FC10 showed 100% inhibition of fungal growth with an initial concentration of 100 mg L^{-1}, and FC12 was able to inhibit fungal growth by 100% with an initial concentration of 200 mg L^{-1}. The other solvents also showed some inhibition for different concentrations. Therefore, the ESs in this study may be a promising alternative for sustainable and safe pest control.

Keywords: *Fusarium graminearum*. Eutectic solvents. Biocontrol. Antifungal activity.

1 INTRODUCTION

In the last two decades, eutectic solvents (ES) have been intensively studied as potential replacements for organic solvents. This new class of solvents, ES, has the advantages of low volatility, thermal stability and adjustable physicochemical properties, low toxicity and biodegradability (1). ESs are obtained from a eutectic mixture that forms a homogeneous liquid; this formation occurs through hydrogen bonds between its components, resulting in a lower melting point(2).

Of the various classes of ES studied, terpene-based solvents have gained prominence due to their low viscosity and low toxicity. Terpenes come from natural sources such as fungi, bacteria, algae, and plants (3). Essential oils are rich in terpenes, whose properties are vast, such as antimicrobial, anti-inflammatory, antioxidant, antimutagenic, spasmolytic, analgesic, and sedative properties (4,5).

Due to the properties of ES added to the characteristics of terpenes, different solvents were formed based on alpha-terpineol with different carbon chains. Their physicochemical characteristics and antifungal activity against the fungus *Fusarium graminearum* were studied.

The fungus *Fusarium graminearum* is responsible for the highly destructive *Fusarium* head blight disease that affects wheat crops globally (6). The application of fungicide is an alternative to control and prevent the appearance of phytopathogenic funds. Several fungicides were used, such as tebuconazole, prothioconazole, and metconazole, fenamacril, pidiflumetofen, dehydrogenase, pyraclostrobin, azoxystrobin, and cresoxin-methyl. However, extensive long-term application of these fungicides has caused fungicide resistance in crops (7). With this arises the need for new chemical compounds with low antifungal activity, toxicity for plants, and low persistence, which are biodegradable.

2 MATERIAL & METHODS

A series of eutectic mixtures were prepared by mixing fenchol and each one of the long-chain carboxylic acids by weighing the appropriate amounts of each component into a sealed glass vial. All the mixtures were heated up to 323 K and kept under magnetic stirring until clear, transparent, and homogeneous solutions were obtained.

For the Solid-liquid (T, x) Phase Equilibria diagram, the Mixtures of fenchol and each one of the different fatty acids were prepared in the whole composition range, allowing the determination of the solid-liquid phase equilibria diagrams of these new DESs. The (T, x) phase diagrams were measured in a glass flask using a visual method at atmospheric pressure under constant stirring. They were heated in an oil bath under stirring using a heating plate until the complete melting of the mixtures was observed. After this first heating cycle, the temperature was turned off, and during the cooling cycle, temperatures corresponding to the first crystal appearance were recorded.

The fungal inhibition activity was carried out in a solid medium containing 15 g L^{-1} malt extract, 0.75 g L^{-1} peptone, and 18 g L^{-1} agar with pH adjusted to approximately 5.5. Different concentrations of fenchol-based DESs (100, 200, 400, and 600 mg L^{-1}) diluted in DMSO in a 1:5 ratio were added to this sterile solid medium. Negative controls of solid media containing DMSO were performed. The fungus *F. graminearum* was inoculated on the seventh day of growth in the media containing the solvents, incubated, and maintained at a temperature of 28°C. For seven days, growth was measured with a ruler.

3 RESULTS & DISCUSSION

According to the phase equilibrium diagram (Fig.1), the solvents FC12OH, FC10, and FC14 showed behavior close to ideal in which the hydrogen bond networks (HB) obtained by mixtures of the solvent components are similar to their components (8). The other solvents present a certain deviation from ideality, suggesting the existence of a greater interaction of LH between the components of the eutectic system.

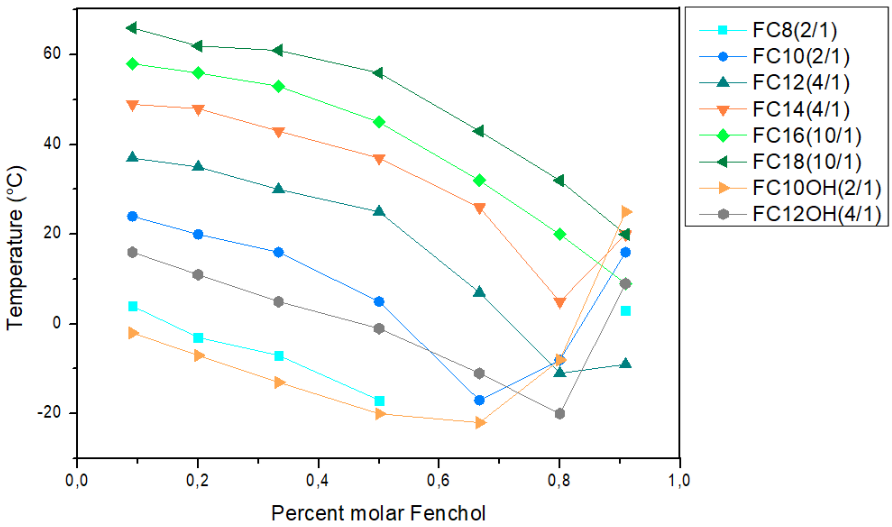
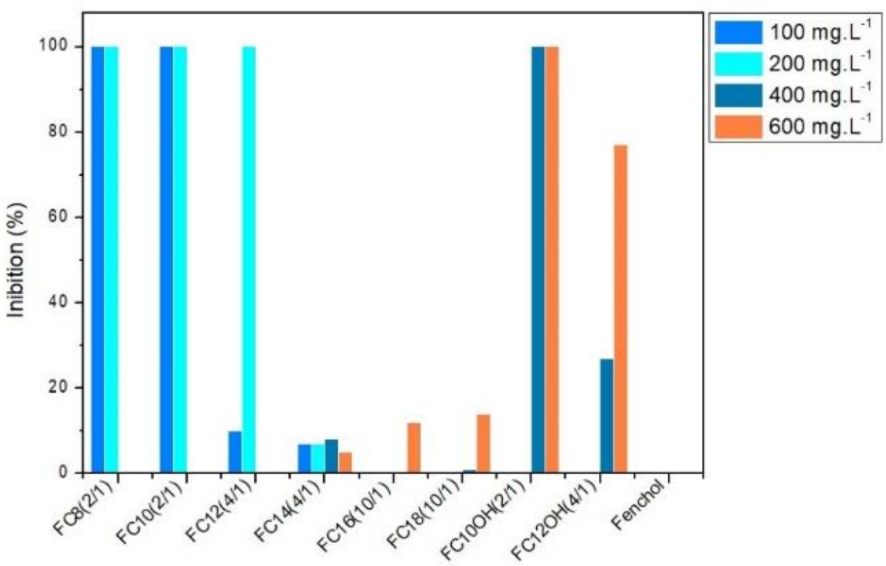


Figure 1 Solid-liquid phase diagrams of mixtures composed of fenchol and each of the different fatty acids in various molar ratios.

In the fungal growth inhibition tests for F. graminearum, as well as in the viscosity and density tests, it is possible to notice an inhibition trend according to the size of the DES carboxylic acid chains, Figure 2. The solvents FC8 and FC10 showed 100% inhibition for the lowest concentration tested, 100 mg L^{-1}, followed by the solvent FC12, which inhibited 100% of fungal growth with a concentration of 200 mg L^{-1}. The precursor of DES, fenchol did not show inhibitory activity for any of the concentrations tested, while all eutectic mixtures showed a certain degree of inhibition. Regarding the inhibition trend, it is possible to note a high inhibitory capacity for DES with shorter chains and a lower inhibition for DES with longer chains. The antimicrobial activity of DES has been investigated, the purpose of which is to promote the fight against agricultural pests that are safer, eco-friendly, and safe for health (9).

Figure 1 Inhibition of *F. Graminearum* growth as a function of ES concentration.



Synthetic fungicides are widely used to combat pests in plantations. However, the vast majority of them present serious health problems in addition to being persistent in the environment, contaminating soil and water (10). Therefore, studies on the alternative use of other biodegradable compounds with less risk to health, such as DES, are a promising alternative for ecological and sustainable pest control.

4 CONCLUSION

According to the results obtained, the eutectic solvents FC8, FC10, and FC12 were effective in fungal inhibition. Toxicological and biodegradation studies can be carried out in order to guarantee the control of agricultural pests without any harmful effects on human health or the environment. Other technological applications can be used for other solvents once their eutectic mixture characteristics have been observed.

REFERENCES

1. KAOUI, S. CHEBLI, B. ZAIDOUNI, S. BASAID, K. MIR, Y. Deep eutectic solvents as sustainable extraction media for plants and food samples: A review. Sustainable Chemistry and Pharmacy. 2023 Apr;31:100937.

2. ZHANG, X. MI, T. GAO, W. WU, Z. YUAN, C. CUI, B. et al. Ultrasonication effects on physicochemical properties of starch–lipid complex. Food Chemistry. 2022 Sep 15;388:133054.

3. GONZÁLEZ DE CASTILLA, A. BITTNER, J.P. MÜLLER, S. JAKOBTORWEIHEN, S. SMIRNOVA, I. Thermodynamic and transport properties modeling of deep eutectic solvents: A review on gE-models, equations of state, and molecular dynamics. Journal of chemical & engineering data. 2019;65(3):943–67.

4. ABBASZADEH, S. SHARIFZADEH, A. SHOKRI, H. KHOSRAVI, A. ABBASZADEH, A. Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. Journal de mycologie medicale. 2014;24(2):e51–6.

5. SALEHI, H.S. RAMDIN, M. MOULTOS, O.A. VLUGT, T.J.H. Computing solubility parameters of deep eutectic solvents from Molecular Dynamics simulations. Fluid Phase Equilibria. 2019 Oct;497:10–8.

6. MCMULLEN, M. BERGSTROM, G. DE WOLF, E. DILL-MACKY, R. HERSHMAN, D. SHANER, G. et al. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant disease. 2012;96(12):1712–28.

7. MIAO, J. LI, Y. HU, S. LI, G. GAO, X. DAI, T. et al. Risco de resistência, mecanismo de resistência e efeito na produção de DON de um novo fungicida SDHI ciclobutrifluram em *Fusarium graminearum*. Pesticide Biochemistry and Physiology. 2024 Feb 1;199:105795.

8. MARTINS, M.A.R. CRESPO, E.A. PONTES, P.V.A. SILVA, L.P. BÜLOW, M. MAXIMO, G.J. et al. Tunable Hydrophobic Eutectic Solvents Based on Terpenes and Monocarboxylic Acids. ACS Sustainable Chem Eng. 2018 Jul 2;6(7):8836–46.

9. GIDADO, M.J. GUNNY, A.A.N. GOPINATH, S.C.B. WONGS-AREE, C. MAKHTAR, M.M.Z. SHUKOR, H. Formulação de nanoemulsão eutética profunda hidrofóbica seletiva de óleo em água como fungicidas verdes para mitigação do fungo antracnose *Colletotrichum gloeosporioides*. Process Biochemistry. 2023 Dec 1;135:40–9.

10. TAO, H. BAO, Z. JIN, C. MIAO, W. FU, Z. JIN, Y. Toxic effects and mechanisms of three commonly used fungicides on the human colon adenocarcinoma cell line Caco-2. Environmental Pollution. 2020;263:114660.