Effect of salinity levels on antifungal activity of essential oil from *Thymus* against *Fusarium oxysporum*

Efecto de los niveles de salinidad sobre la actividad antifúngica del aceite esencial de *Thymus* contra *Fusarium oxysporum*

Efeito dos níveis de salinidade na atividade antifúngica do óleo essencial de *Thymus* contra *Fusarium oxysporum*

Boualem Boumaaza[1,2]*
M'hamed Benada[3]
Sofiane Boudalia[4]
Ibrahim E. Benzohra[5]
Abdelhamid Gacemi[6]
Omar Khaladi[4]
Mohamed Benkhelifa[1]

[1]Department of Agronomy, University of Abdelhamid Ibn Badis, BP 300, 27000, Mostaganem, Algeria.
[2]University of Ibn khaldounTiaret. BP 78 zaâroura 14000, Tiaret, Algéria.
[3]University of Ahmed Zabana. BP 48000 Bormadia Relizane.
[4]Laboratory of Biology, Water and Environment (LBEE), University of 8th May, 1945 Guelma, BP 401 24000 Guelma, Algeria.
[5]Scientific and Technical Research Center on Arid Regions (CRSTRA) BP. Box 1682 RP 07000, Biskra, Algeria. Conservation Laboratory Wetlands.
[6]INRA Station de Recherche Hmadna. BP 48017 Relizane.

Rev. Fac. Agron. (LUZ). 2022, 39(3): e223941
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v39.n3.07

Crop Production
Associate editor: Dra. Lilia Urdaneta
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

Received: 01-06-2022
Accepted: 02-08-2022
Published: 22-082022

Keywords:
Antimicrobial activity
Biocontrol
Plant extracts
Medicinal plant
Sodium chloride

Abstract

Thyme (*Thymus* sp.), a medicinal plant of the family *Lamiaceae*, is used in traditional medicine, contains a wide array of medicinally active components, in their great majority of a rather complex mixture of thymol, ρ-Cymene, γ-Terpinene, β-Caryophyllen, etc. This study aimed to evaluate the efficacy of *Thymus vulgaris* extract against *Fusarium oxysporum* f. sp. *radicis-lycopersici* strain under saline conditions, assuming soil with high salt content of the arid regions. Essential oil was extracted by hydrodistillation technique using a Clevenger apparatus. The essential oil compounds were identified by GC-MS analysis. Antifungal activity of essential oil against *Fusarium oxysporum* f. sp. *radicis-lycopersici* was investigated by agar dilution method. The main constituents of thyme essential oil were thymol (76.96 %), ρ-cymene (9.89 %) and γ-Terpinene (1.92 %). Essential oil from *Thymus* resented high *in vitro* activity, in controlling conidial germination and mycelial growth. However, the oil was significantly not active against the spore production under a salinity medium. The results showed that mycelial growth was stimulated in concentrations with 0.6-1.5 %. In contrast, it was significantly reduced at a higher concentration (2 %). The application of NaCl caused a significant increase in the conidia production at various concentrations tested. NaCl has a minor inhibitory effect on conidial germination only when the concentration was 2 %. The results of this study indicate that salinity decreases the efficacy of essential oil against the pathogen.

*Corresponding author: agroboum@hotmail.fr

Resumen

El tomillo (*Thymus* sp.), una planta de la familia *Lamiaceae*, se utiliza en la medicina tradicional, contiene una amplia gama de componentes medicinalmente activos, en su gran mayoría de una mezcla bastante compleja de timol, ρ-cimeno, γ-terpineno y β-Cariofileno, entre otros. El objetivo del presente estudio fue evaluar la eficacia del extracto de *Thymus vulgaris* contra *Fusarium oxysporum* f. sp. *radicis-lycopersici* bajo condiciones salinas, simulando suelos con altos contenidos de sales de las regiones áridas. El aceite esencial se extrajo mediante hidrodestilación utilizando el aparato Clevenger. Los compuestos del aceite esencial se identificaron mediante el análisis GC-MS. La actividad antifúngica del aceite se evaluó mediante el método de dilución en agar. Los principales componentes del aceite esencial del tomillo fueron timol (76,96 %), ρ-cimeno (9,89 %) y γ-terpineno (1,92 %). El aceite presentó alta actividad *in vitro*, en el control de la germinación de los conidios y del crecimiento micelial. Sin embargo, el aceite no inhibió la esporulación en el medio salino. El crecimiento micelial fue estimulado al 0,6-1,5 %, pero se redujo significativamente a la concentración más alta (2 %). La aplicación de NaCl provocó un aumento significativo en la producción de conidios en todas las concentraciones. La germinación conidial fue inhibida ligeramente por el NaCl solo al 2 %. Los resultados indican que la salinidad disminuye el potencial antifúngico del aceite esencial de *Thymus* contra el patógeno.

Palabras clave: Actividad antimicrobiana, biocontrol, extractos de plantas, planta medicinal, cloruro de sodio.

Resumo

O tomilho (*Thymus* sp.), planta medicinal da família *Lamiaceae*, utilizada na medicina tradicional, contém uma grande variedade de componentes medicinais ativos, em sua grande maioria uma mistura bastante complexa de Timol, ρ-Cymene, γ-Terpinene, β-Caryophyllen, etc. O presente estudo foi avaliar a eficácia do extrato de *Thymus vulgaris* contra *Fusarium oxysporum* f. sp. *radicis-lycopersici* sob condições salinas, assumindo solos com alto teor de sal das regiões áridas. O óleo essencial por extraído pe la técnica de hidrodestilação utilizando aparelho de Clevenger. O composto de óleo essencial por identificado por análise GC-MS. Atividade antifúngica do óleo esencial contra *Fusarium oxysporum* f. sp. *radicis-lycopersici* por investigado pelo método de diluição em agar. O principais constituintes do óleo esencial de tomilho foram timol (76.96 %), ρ-cimeno (9.89 %) e γ-Terpineno (1.92 %). O óleo esencial de Timo apresentou alta atividade *in vitro*, no control da germinação de conídios e do crescimento micelial. No entanto, o óleo não por significativamente ativo contra produção de esporos meio de salinidade. Os resultados sugerem que o crescimento micelial por estimulado em concentrações de 0,6-1.5 %. Pelo contrário, reduziu significativamente na concentração mais alta (2 %). A aplicação de NaCl causou um aumento significativo na produção de conidiogênese em várias concentrações testadas. A germinação dos conídios for levemente inibida pelo cloreto de sódio somente quando a concentração for de 2 %. Os resultados este estudo indicaque a salinidade diminui a eficácia do óleo esencialcontra o patógeno.

Palavras-chave: Actividad antimicrobiana, biocontrole, extratos de plantas, planta medicinal, cloreto de sódio.

Introduction

Fusarium oxysporum is an ubiquitous fungus, widely distributed among soil, plants, plant debris and other organic substrates. This fungus is one of the most important phytopathogenic and toxigenic fungi, affecting the health and survivability of plants in more than 100 different crops (Al-Hatmi *et al*., 2016). Additionally, *Fusarium oxysporum* has numerous specialized forms (f. sp.) that infect a range of host plants causing diseases such as vascular wilt, corm rot, and root rot (Edel-Hermann and Lecomte, 2019). Another important characteristic of the species of this genus is their ability to produce a various of mycotoxins that can play an important role in the pathogenesis (Lombard *et al*., 2019). Preventive measures, such as chemical pesticides have controlled the disease to some extent. However, excessive application of fungicides has led to severe damage to soil microbial communities and fertility and may leave behind toxic residues in treated products (Meena *et al*., 2020). Additionally, intensive use of fungicides can result in the development of pathogens acquired resistance to the fungicide (Corkley *et al*., 2021).

Due to the problems caused by chemical substances, the development of alternative control measures is of great importance. Biological control has been considered as a desirable and realistic alternative. Numerous studies have demonstrated the ability of several essential oils to possess antibacterial, antifungal, antiviral, antioxidant activities and play an important role in the protection of the plants against plant pathogens both *in vitro* and *in vivo* (Mutlu-Ingok *et al*., 2020; Marín-Tinoco *et al*., 2021). Note that essential oils can be an effective solution, as their toxicity is much lower, better specificity of action, biodegradable and environmentally friendly (Campos *et al*., 2019). Being volatile, they can act as fumigants, repellents, and contact insecticides or as reproduction inhibitors (Gao *et al*., 2020; Silva-Marrufo and Marín-Tinoco, 2021). Essential oils interact with the odor receptors of insect pests (*Ceratitis capitata*), triggering various behaviors: flight, attraction, oviposition, etc. (Benelli *et al*., 2012). The studies published on the activity of essential oils as herbicides are numerous and generally cover seed germination inhibition tests (Yilar *et al.,* 2020). Those that appear to be the most active are essential oils containing phenols (thymol, carvacrol), ketones (carvone, pulegone) or etheroxides (eucalyptol or 1,8-cineol).

Plants of the genus *Thymus* are widely used in food flavouring, as well as aroma additives, in perfumery, in folk medicine and in pharmacological sector as natural antioxidants and antimicrobial agents (Gema, 2020). In North Africa, most of the thyme produced is in the form of dried herbs. With mean annual rainfalls of 150-400 mm.years^{-1}, the yield of *Thymus vulgaris as* fresh herbs may be 5 to 6 t.ha^{-1} with 2 t.ha^{-1} for the production of dried herbs. With an irrigated system, in the presence of fertilization, the yield can be 9.77 t.ha^{-1}, with an oil recovery yield of 43.14 to 48.9 kg.ha^{-1} (Kozera *et al*., 2015).

It has been reported that essential oils possess various actions on microorganisms, such as disruption of the cytoplasmic membrane, disruption of the proton driving force, electron leakage and coagulation of the protein content of cells, acidification from inside the cell, blocking the production of cellular energy and the synthesis of structural components. Thymol and carvacrol have antimicrobial activity, which is related to inhibition of membrane permeability, resulting in ions and ATP leakage, inhibition ergosterol biosynthesis and cell death (Alizadeh *et al*., 2018).

Antimicrobial activity of essential oils is strongly affected by many factors such as high temperature, poor water solubility, light, and oxygen (de Souza *et al*., 2008; Turek and Stintzing, 2012). This study aimed to assess the antifungal activity of the essential oil from *Thymus* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* in growth media amended with various levels of sodium chloride.

Materials and methods

Inoculum preparation

The tested *Fusarium oxysporum* f. sp. *radicis-lycopersici* strain M27b27245 was preserved at the Plant Protection Laboratory of the Agronomy Science Department, University of Abdelhamid Ibn Badis, Mostaganem, Algeria. *Fusarium oxysporum* readily produced conidia after 14 days on potato dextrose agar (PDA; potato dextrose agar. SPA CRAPC Algeria) plate at 25 ˚C in the dark. Conidial suspension was obtained by flooding plates with distilled water and rubbing gently with a glass rod, then filtering through sterile cheesecloth. The conidia concentration present in the initial suspension (adjusted to the concentration $1x10^5$ conidia.mL^{-1}) was quantified using the hemocytometer.

Analysis of the essential oil of *Thymus vulgaris*

Thymus vulgaris was supplied for the study by the National Agronomic Institute of Tunisia (INAT), and the experiment was conducted from December 2017 to January 2018. Hydro-distillationwascarried out using a Clevenger-type device according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 2016), where 500 g of vegetable matter is introduced with 3 L water into a 5 L flask. After installation and closing of the assembly, the flask heater is started up with an optimum adjustment of the heating to allow stability of the extraction at a constant and well-controlled speed. The vapor charged with essential oil arrives in the condenser. The total duration of the extraction was estimated at 3 h. The essential oil differs from the hydrosol by its difference in density and color. It is separated from the by decantation. It is then dried with anhydrous sodium sulfate (Na_2SO_4) then recovered and stored in a cool place (4 °C).

Oil extracted was analyzed by GC/MS using a gas chromatograph (HP 5890-Serie II) coupled to a mass spectrometer (HP-MSD 5972 A) with an HP-5MS capillary column (30 m x 0. 25 mm, film thickness, 0.25 µm). The temperature of the column was programmed at 50 ˚C for 1 min, then 7 ˚C / min at 250 ˚C, and finally left at 250 ˚C for 5 min. The helium injection (1 mL.min^{-1}). The temperature of the injector port was 240 ˚C, while that of the detector set at 250 ˚C. The ionizing energy was 70 eV, the full-scan mass spectra: from 40 to 500 amu. The identification of the components was carried out on the basis of chromatographic retention indices and by comparison of the mass spectra recorded with the calculated spectral library (Al-Asmari *et al*., 2017).

***In vitro* antifungal assay**

Effects on mycelial growth

The antifungal activity of the essential oil against to *Fusarium oxysporum* f. sp. *radicis-lycopersici* was investigated by agar dilution method (Souza *et al*., 2002). In sterile Petri dishes with 9 cm diameter containing 20 mL potato dextrose agar (PDA) mixed with five levels of NaCl to obtain final concentrations 0.3, 0.6, 1, 1.5 and 2 %. Then, 100 µL of the essential oil was evenly spread on the surface of Petri dishes containing PDA + NaCl. Essential oil was previously diluted, to then adjust the concentration to 1 %. 30 µL of the fungi suspension 105 conidia.mL^{-1} was placed at the center of each Petri plate. In this assay, an amount of essential oil was added at minimum inhibitory concentration (MIC) (1 %). Cultures were incubated at 25 °C in the dark. Colony diameters were measured in two perpendicular directions, after 24 h and again after 96 h. Each treatment had five replications. In the control group, sterile distilled water was used in place of the oil, and no additional NaCl was added.

Effects on sporulation

Conidia suspension of *Fusarium oxysporum* f. sp. *radicis-lycopersici* was obtained from two weeks-old culture incubated at 25 ˚C in the dark. Plates were flooded with sterile-distilled water using 0.5 % (v/v) Tween 80 solution and conidia were gently dislodged from the mycelium using a sterile magnetic stir bar placed on the agar and set stirring for 5 min to loosen the conidia. The suspension was filtered through a glass filter to eliminate mycelium. Finally, the conidia concentration was quantified using the hemocytometer.

Effects on the germination of conidia

To determine the influence of NaCl upon the antifungal activity of the essential oil on spore germination of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, a drop containing 100 conidia was transferred on to water agar plates enriched with NaCl and essential oil. The plates were incubated at 25 ˚C in the dark for 24 h. The results were expressed as the percentage of germinated conidia observed under the optical microscope at 400x magnification. The conidium was considered as germinated if the germ tube length was at least twice the length of the conidium.

All results are presented as mean ± standard deviation. Results were analyzed using at test and analysis of variance (ANOVA). Significance was considered at $P< 0.05$ using StatBox®, Version 6.0.4 (Grimmer Soft, Fr.) (2009). The tests were performed in triplicate.

Results and discussion

Essential oil chemical composition

Seventeen compounds of the essential oil of *Thymus vulgaris* representing 99.24 % of the total detected constituents were identified (table 1). Among the mentioned phenolic compounds, thymol was present in the largest amounts (76.69 %) followed by ρ-cymene (9.89 %), γ-terpinene (1.92 %); caryophyllene oxide (1.69 %); β-linalool (1.51 %) and β-caryophyllen (1.32 %). A similar result was previously described by Kolsum *et al.* (2017) that showed 32 compounds from *Thymus vulgaris* with dominance of thymol (32.67 %) and p-cymene (16.68 %).The major components of the oil were thymol (36.81 %) and *p*-cymene (30.9 %) (Moghaddam and Mehdizadeh, 2020).

Activity of essential oil on mycelial growth

The results of antifungal activity assays showed that the 1 % (v/v) thyme essential oil significantly inhibited ($P<0.01$) the mycelium growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* after 24 h of incubation at 25 ˚C compared to untreated (figure 1a).

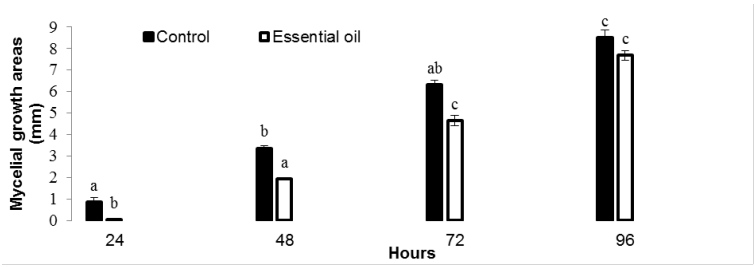
The essential oil reached an inhibition rate of 43 % for *Fusarium oxysporum* f. sp. *radicis-lycopersici* after culturing for 48 h. In the research carried out by Galovičová *et al.* (2021), amounts of free thyme oil between 62.5 and 500 µL.mL^{-1} were necessary to inhibit 46-87 % the growth of *Serratia marcescens*. *Thymus vulgaris* essential oil completely suppressed the colony growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Kumar *et al*., 2007; Aksit *et al*., 2022). After 96 h, the mycelium growth of the strain on the essential oil-treated groups became larger, and the inhibition rate declined (10.86 %), which may be because the oil had evaporated.

Table 1. Chemical constituents of *Thymus vulgaris* leaves essential oil using gas chromatography-mass spectrometry (GC/MS).

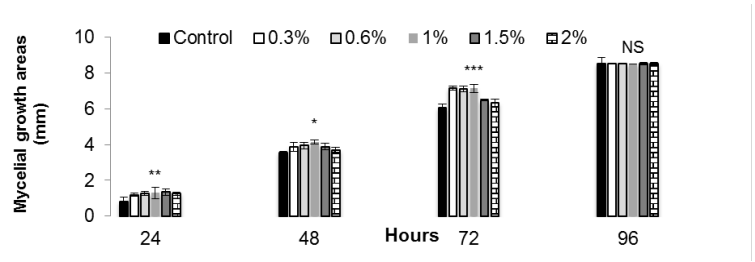
Compounds	RT.min-1	RI	Content (%)
Thujene	10.24	1140	0.71
α-Pinene	10.32	1165	0.49
cis-Sabinene hydrate	10.66	777	0.46
Camphene	10.76	846	0.14
β-Myrcene	11.74	889	0.71
α-Terpinene	11.88	916	0.58
β- Phelladrene	12.18	1012	0.37
γ-Terpinene	12.55	965	1.92
ρ-Cymene	12.80	1024	9.89
Carvacryl Acetate	13.72	1303	1.19
β-Linalool	15.36	998	1.51
4-Terpineol	16.07	1107	0.65
β-Caryophyllen	16.12	1319	1.32
Borneol	17.19	1012	0.49
Caryophyllene oxide	20.18	1479	1.69
Thymol	21.99	1196	76.96
Eugenol	21.61	1300	0.16
Total identified (%)			99.24

The opposite result was observed for essential oil from *Thymus.* In which conidia production of *Fusarium oxysporum* f. sp. *radicis-lycopersici* was stimulated (figure 2). The average number of conidia on unamended (control) PDA media plates after 14 days of incubation at 25 ˚C were $5.65x10^5$ conidia.mL^{-1} while grown colonies amended with *Thymus* essential oil, production of conidia was significantly increased than the control, and up to $13.7x10^5$ conidia.mL^{-1}. Thymol and *p*-cymene are the major compounds found in *Thymus vulgaris* and have shown potent fungicidal and/ or fungistatic activities against various phytopathogenic fungi such as *Fusarium* spp., *Aspergillu*s spp., *Cladosporium* spp., *Mucor* spp. and *Rhizopus* spp. (Kumar *et al*., 2007). Thymol and *p*-cymene provide irreversible damage to the cell wall, cytoplasm membrane and nuclear membrane of filamentous fungi (Rasooliand Owlia, 2005).

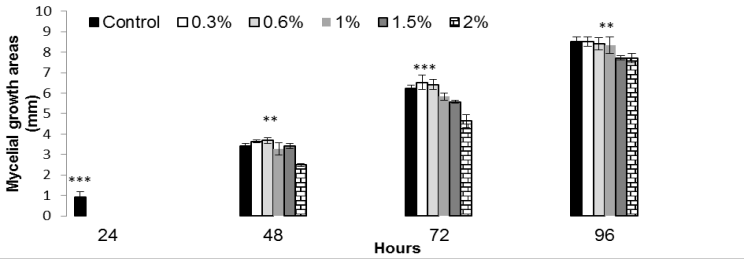
The effects of essential oil on the spore germination of *Fusarium oxysporum* f. sp. *radicis-lycopersici*are shown in figure 3. In the absence of NaCl, thyme oil was found to be the most effective inhibition (86 %) of spore germination. The essential oil may inhibit the cell growth and proliferation by interrupting ergosterol biosynthesis (Gao *et al*., 2016). Essential oil enables us to integrate into the lipids of the cell membrane, increasing permeability occur as a due of loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool, which eventually lead to leaking of intracellular constituents, coagulation of cell contents, lysis and cell death (Turgis *et al*., 2012). Antimicrobial activity was due to disturbance of the permeability of the membrane and the release of some inhibition enzymes such as ATPase, histidine decarboxylase, and amylase from the cellular content (Scollard *et al*., 2016). Carvacroland thymol disrupted the ergosterol biosynthesis and membrane integrity (Ahmad *et al*., 2011). Evaluation of *Thymus vulgaris* essential oil against fungi has shown a decrease from 4.04 to 6.27 fold of Tri4 gene expression by the use of qRT-PCRassay (Kolsum *et al*., 2017).



(a) Effect of fungal load of essential oil



(b) Effect of salinity levels (0.3, 0.6, 1, 1.5 and 2 %)



(c) Combined effect of salinity levels and essential oil.

Figure 1. Effect of salinity levels and essential oil on mycelial growth of *Fusarium oxysporum* at 24, 48, 72 and 96 h. (1a) Effect of fungal load of essential oil. (1b) Effect of salinity on mycelial growth. (1c) Effect of salinity and essential oil on mycelial growth. ns, *, ** and *** non-significant, significant at 0.05, 0.01 and 0.001 probability level, respectively.

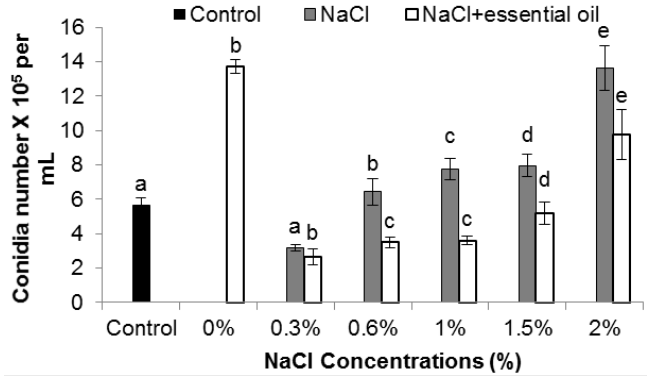


Figure 2. Effect of salinity and essential oil on conidia production by *Fusarium oxysporum*. Values with different letters show significant difference (P≤0.05).

Effects of NaCl on mycelial growth, germination and conidia production

After 24 h of incubation, the mycelial growth eached a diameter of 0.82 mm in the control. Whereas under gradual concentrations of NaCl, colony diameters were 1.2 mm at 0.3 % NaCl, 1.27 mm at 0.6 %, 1.3 mm at 1 %, 1.35 mm at 1.5 % and 1.2 mm at 2 % respectively (figure 1b). The average mycelial growth was significantly in the saline medium over the five levels of NaCl compared to the control. Figure 1b shows that the saline medium has a significant influence on the stimulation of mycelial growth compared to the control. Similar supporting results have been reported (Boumaaza *et al.*, 2015).

Figure 2 shows that the application of sodium salt caused a significant increase in the conidia production at various concentrations tested compared with control ($P< 0.001$). By adding 2 % of NaCl to the culture medium, an increase in spore production by $13.6x10^5$ conidia.mL^{-1} can be obtained compared to the control $5.65x10^5$ conidia.mL^{-1}. Ahigh concentration of sodium chloride showed to be the most favorable to the sporulation.

The germination of conidia that were affected by higher levels of NaCl is shown in figure 3. An inhibition of 2.66 % in germination was observed at 2 % salinity. However, the lower levels of NaCl (0.3, 0.6 and 1 %) were significantly not active against *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

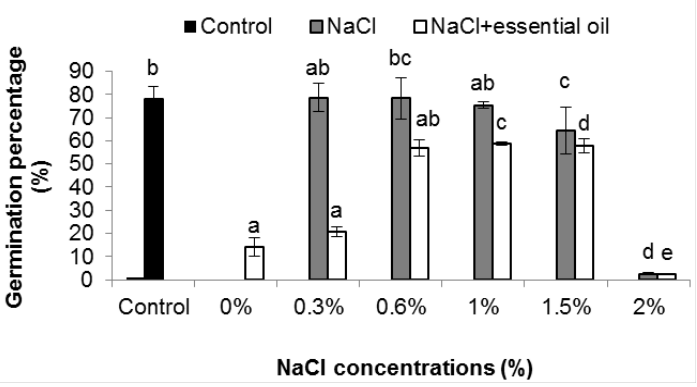


Figure 3. Effect of salinity and essential oil on conidia germination by *Fusarium oxysporum.* Values with different letters show significant differences ($P\leq0.05$).

Combined effects of salinity and essential oil on *Fusarium oxysporum*

The combined effects of salinity levels and essential oil on *Fusarium oxysporum* are shown in figure 1c. After 48h of incubation, the growth of the mycelium treated with essential oil decreased to higher salinity levels. Compared to untreated salinity (3.4 mm), the mycelium growth achieved 3.63, 3.68, 3.26, 3.41 and 2.48 mm was obtained by the concentrations of 0.3, 0.6, 1, 1.5 and 2 % NaCl, respectively.

The results shown in figure 2 revealed that the combined effects of salinity levels and essential oil caused a significant increase in spore production at various levels tested compared with control. However, this combination increase the antifungal activity of essential oil compared to oil alone ($13.7x10^5$ conidia.mL^{-1}), the conidia production achieved 2.66, 3.5, 3.6, 5.2 and $9.76x10^5$ conidia.mL^{-1} were obtained by with concentrations of NaCl 0.3, 0.6, 1, 1.5 and 2 %, respectively.

As seen from figure 3, combined effects of salinity levels and essential oil caused a significant decrease in the antifungal activity of essential oil compared to oil alone (14 %). The spore germination achieved of 20.66, 57, 58.66 and 58 % was obtained by the concentrations of 0.3, 0.6, 1 and 1.5 % respectively. The results of this study indicated that salinity decreases the efficacy of essential oils against the pathogen.

According to Perumal *et al*. (2016), the efficacy of essential oils against the microorganisms mainly depend on the characteristics of their components and influenced by many factors such as high temperature, low water activity, low nutrient conditions and UV light for effective establishment and disease control. Several studies have investigated the control efficacy of essential oils against *Fusarium* sp. (Xing *et al.,* 2014). To date, there have been limited studies on the use essential oils in mycology under salinity conditions. In accordance with Biswas *et al*. (2011), essential oil contents are also affected by environmental factors such as salinity, water stress and soil pollution (Stancheva *et al*., 2014). The degradation of essential oils depends on some climatic and edaphic factors that influence the course of antifungal activity. The constituents of essential oils are particularly prone to oxidative damage; like light and heat, salts, especially copper and ferrous ions, are thought to promote auto-oxidation (Choe and Min, 2006).

Conclusion

According to these results, the essential oil of thyme showed significant antifungal activity on the strain studied. Also, it can be said that the salinity of the culture medium leads to an *in vitro* stimulation of mycelial growth, there by reducing the antifungal activity of this oil against this pathogen. In the absence of NaCl, thyme oil was found to be the most effective inhibition of spore germination.

Literature cited

Ahmad, A., Khan, A., Kumar, P., Bhatt, R. P., & Manzoor, N. (2011). Antifungal activity of *Coriaria nepalensis* essential oil by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. *Yeast*, *28*(8), 611-617. https://doi.org/10.1002/yea.1890

Aksit, H., Bayar, Y., Simsek, S., & Ulutas, Y. (2022). Chemical composition and antifungal activities of the essential oils of *Thymus* species (*Thymus pectinatus, Thymus convolutus, Thymus vulgaris*) against plant pathogens. *Journal of Essential Oil Bearing Plants*, *25*(1), 200-207. DOI: 10.1080/0972060X.2022.2043189

Al-Asmari, A. K., Athar, M. T., Al-Faraidy, A. A., & Almuhaiza, M. S. (2017). Chemical composition of essential oil of *Thymus vulgaris* collected from Saudi Arabian market. *Asian Pacific Journal of Tropical Biomedicine*, *7*,147-50. https://doi.org/10.1016/j.apjtb.2016.11.023

Al-Hatmi, A. M., Meis, J. F & de Hoog, G. S. (2016). *Fusarium*: molecular diversity and intrinsic drug resistance. *Plant Pathology*, *12*(4), e1005464. https://doi.org/10.1371/journal.ppat.1005464

Alizadeh, B. B., Tabatabaei, Y. F., Vasiee, A., & Mortazavi, S. A. (2018). *Oliveria decumbens* essential oil: Chemical compositions and antimicrobial activity against the growth of some clinical and standard strains causing infection. *Microbial Pathogenesis, 114*, 449-452. doi: 10.1016/j.micpath.2017.12.033

Benelli,G.,Flamini, G., Canale, A., & Conti, B. (2012). Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitis capitata*(Wiedemann)(*Diptera Tephritidae*). *Crop Protection*, *42*, 223-229. https://doi.org/10.1016/j.cropro.2012.05.024

Biswas, S., Koul, M., and Bhatnagar, A. K. (2011). Effect of salt, drought and metal stress on essential oil yield and quality in plants. *Natural Product Communications*, *6*(10), 1559-1564. https://doi.org/10.1177/1934578X1100601036

Boumaaza, B., Benkhelifa, M., & Belkhoudja, M. (2015). Effects of two salts compounds on mycelial growth, sporulation, and spore germination of six isolates of *Botrytis cinerea* in the western north of Algeria. *International Journal of Microbiology*, *2015*. ID 572626. https://doi.org/10.1155/2015/572626

British Pharmacopoeia Commission. *British Pharmacopoeia.* London: TSO (2016). https://www.tsoshop.co.uk/?Action=Book&ProductId=9780113230006

Campos, E. V., Proença, P. L., Oliveira, J. L., Bakshi, M., Abhilash, P. C., & Fraceto, L. F. (2019). Use of botanical insecticides for sustainable agriculture: future perspectives. *Ecological Indicators*, *105*, 483-495. https://doi.org/10.1016/j.ecolind.2018.04.038

Choe, E., and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 169-186. https://doi10.1111/1541-4337.12006

Corkley, I., Fraaije, B., & Hawkins, N. (2021). Fungicide resistance management: Maximizing the effective life of plant protection products. *Plant Pathology*, *71*(1), 150-169.https://doi.org/10.1111/ppa.13467

Edel-Hermann, V., and Lecomte, C. (2019). Current status of *Fusarium oxysporum* formae speciales and races. *Phytopathology*, *109*, 512-530. https://doi.org/10.1094/PHYTO-08-18-0320-RVW

de Souza, E. L., Montenegro Stamford, T. L., Lima, EdeO., Barbosa Filho, J. M., & Mayo Marques, M. O. (2008). Interference of heating on the antimicrobial activity and chemical composition of *Origanum vulgare* L. (*Lamiaceae*) essential oil. *Ciência Tecnologia de Alimentos Campinas*, *28*(2), 418-422. https://www.scielo.br/j/cta/a/kNxCJnWT6G98YrrbjZxmB7K/?format=pdf&lang=en

Galovičová, L., Borotová, P., Valková, V., Vukovic, N., Vukic, M., Štefániková, J., Ďúranová, H., & Kowalczewski, P. (2021). *Thymus vulgaris* essential oil and its biological activity. *Plants*, *10*(9), 1959. https://doi.org/10.3390/plants10091959

Gao, S., Kunpeng, Z., Luting, W., Guanyun, W., Wenfeng, X., Yaoyao, L., Yonglei, Z., Aoxiang, G., & Bin, L. (2020). Insecticidal activity of *Artemisia vulgaris* essential oil and transcriptome analysis of *Tribolium castaneum* in response to oil exposure. *Frontiers in Genetics, 25*(11), 589. doi: 10.3389/fgene.2020.00589

Gao, T., Zhou, H., Zhou, W., Hu, L., & Chen, J. (2016). The fungicidal activity of thymol against *Fusarium graminearum* via inducing lipid peroxidation and disrupting ergosterol biosynthesis. *Molecules*, *21*(6), 770. https://doi.org/10.3390/molecules21060770

Gema, N. (2020). A review on applications and uses of *Thymus* in the food industry. *Plants* (Basel), *9*(8), 961. doi: 10.3390/plants9080961

Kolsum, D., Hojjatollah, S., & Ali Reza, K. (2017). Down-regulatory effect of *Thymus vulgaris* L. on growth and Tri4 gene expression in *Fusarium oxysporum* strain. *Microbial Pathogenesis*, *104*, 1-5. https://doi.org/10.1016/j.micpath.2017.01.011

Kozera, W., Majcherczak, E., Barczak, B., Knapowski, T., Wszelaczyńska, E., & Pobereżny, J. (2015). Response of the yield and mineral composition of garden thyme (*Thymus vulgaris* L.) herbage to various NPK proportions. *Journal of Elementology*, *20*(4), 921-931. https://doi.org/10.5601/jelem.2015.20.1.802

Kumar, A. R., Shukla, P., Singh, C. S., Prasad, N. K., & Dubey. (2007). Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungi infestation of food commodities. *Innovative Food Science and Emerging Technologies*, *9*(4), 575-580. https://doi.org/10.1016/j.ifset.2007.12.005

Lombard, L., Sandoval-Denis, M., Lamprecht, S. C., & Crous, P. W. (2019). Epitypification of *Fusarium oxysporum*: Clearing the taxonomic chaos. *Persoonia, 43*, 1-47. https://doi.org/10.3767/persoonia.2019.43.01

Marín-Tinoco, R. I., Camacho-Luis, A., Silva-Marrufo, O., Diaz-Diaz, M., & Ortega-Ramirez, A. T. (2021). Inhibition of *Candida albicans* by oregano (*Lippia* spp.) essential oil from municipality of Rodeo, Durango, Mexico. *Journal of Microbiology & Health Education*, *3*(1), 70-76. http://journalmhe.org/ojs3/index.php/jmhe/article/view/14

Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., Sharma, M. P., Yadav, G. S., Jhariya, M. K., Jangir, C. K., Pathan, S. I., Dokulilova, T., Pecina, V., & Marfo, T. D. (2020). Impact of agrochemicals on soil microbiota and management: AReview. *Land*, *9*(2), 34. https://doi.org/10.3390/land9020034

Moghaddam, M. and Mehdizadeh, L. (2020). Chemical composition and antifungal activity of essential oil of *Thymus vulgaris* grown in Iran against some plant pathogenic fungi. *Journal of Essential Oil Bearing Plants*, *23*(5), 1072-1083. doi.org/10.1080/0972060X.2020.1843547

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), 4711. https://doi.org/10.3390/molecules25204711

Perumal, A. B., Sellamuthu, P. S., Nambiar, R. B., & Sadiku, E. R. (2016). Antifungal activity of five different essential oils in vapour phase for the control of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae in vitro* and on mango. *International Journal of Food Science and Technology*, *51*(2), 411-418. https://doi.org/10.1111/ijfs.12991

Rasooli, I. and Owlia, P. (2005). Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. *Phytochemistry*, *66*(24), 2851-2856. https://doi.org/10.1016/j.phytochem.2005.09.029

Scollard, J., McManamon, O., & Schmalenberger, A. (2016). Inhibition of *Listeria monocytogenes* growth on fresh-cut produce with thyme essential oil and essential oil compound verbenone. *Postharvest Biology and Technology*, *120*, 61-68. https://doi.org/10.1016/j.postharvbio.2016.05.005

Silva-Marrufo, O. and Marín-Tinoco. R. I. (2021). Substitute of synthetic chemical fungicides using oregano essential oil for controlling *Fusarium oxysporum*. *Gestión y Ambiente*, *24*(3), 73-80. https://doi.org/10.15446/ga.v24nSupl3.95526

Souza, L. K. H., Oliveira, C. M. A., Ferri, P. H., Santos, S. C., Oliveira Júnior, J. G., Miranda, A. T. B., Lião, L. M., & Silva, M. R. R. (2002). Antifungal properties of brazilian cerrado plants. *Brazilian Journal of Microbiology*, *33*(3), 247-249. https://doi.org/10.1590/S1517-83822002000300012

Stancheva, I., Geneva, M., Markovska, Y., Tzvetkova, & Mitova, N. (2014). A comparative study on plant morphology, gas exchange parameters, and antioxidant response of *Ocimum basilicum* L. and *Origanum vulgare* L. grown on industrially polluted soil. *Turkish Journal of Biology*, *38*(1), 89-102. https://doi.org/10.3906/biy-1304-94

StatBox®, Version 6.0.4 (Grimmer Soft, Fr). (2009). Company in France, Europe.89 Governor General Street Eboué 92136 Issy-les-Moulineaux France.

Turek, C., and Stintzing, F. C. (2012). Impact of different storage conditions on the quality of selected essential oils. *Food Research International*, *46*, 341-353. https://doi.org/10.1016/j.foodres.2011.12.028

Turgis, M., Vu K. D., Dupont, C., & Lacroix, M. (2012).Combined antimicrobial effect of essential oils and bacteriocins against foodborne pathogens and food spoilage bacteria. *Food Research International*, *48*(2), 696-702. https://doi.org/10.1016/j.foodres.2012.06.016

Xing, F., Hua, H., Selvaraj, J. N., Zhao, Y., & Zhou, L. (2014).Growth inhibition and morphological alterations of *Fusarium verticillioides* by cinnamon oil and cinnamaldehyde. *Food Control*, *46*, 343-350. https://doi.org/10.1016/j.foodcont.2014.04.037

Yilar, M., Bayar, Y., & Abaci, B. A. A. (2020). Allelopathic and antifungal potentials of endemic *Salvia absconditiflora* Greuter & Burdet collected from different locations in Turkey. *Allelopathy Journal, 49*(2), 243-25. https://doi.0rg/10.26651/allelo.j/2020-49-2-1268