REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.13.e1767
ISSN 2007-3380

Original article /Artículo original

Chemical characterization of *Tagetes linifolia* essential oil: *in vitro* and *in vivo* tests on *Fragaria × ananassa* fruits

Caracterización química de aceite esencial de *Tagetes linifolia*: pruebas *in vitro* e *in vivo* en frutos de *Fragaria × ananassa*

Ruíz González, M. A.[1*], *Serrato Cruz, M. A.*[1*], *Valadez Moctezuma, E.*[1], *Solano Vidal, R.*[3], *González Tepale, R. M.*[2]

[1]*Dpto. de Fitotecnia. Universidad Autónoma Chapingo. Carr. Federal México-Texcoco, km 38.5. C.P. 56230, Texcoco, Estado de México.*
[2]*Laboratorio de Naturales. Preparatoria agrícola Chapingo. Carr. Federal México-Texcoco, km 38.5. C.P. 56230, Texcoco, Estado de México.*
[3]*Parasitologia Agrícola. Universidad Autónoma Chapingo. Carr. Federal México-Texcoco, km 38.5. C.P. 56230, Texcoco, Estado de México.*

ABSTRACT

Essential oils are effective in controlling pathogens and are recognized as safe for humans and the environment. The objective was to characterize the chemical compounds in the essential oil of *Tagetes linifolia* and to evaluate the *in vitro* and *in vivo* effects against *Botrytis cinerea*. The essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS). *In vitro* assays included six concentrations of oil (1, 0.5, 0.1, 0.01, 0.001, and 0.0001 %), a control, and the fungicide Cabrio® C against *B. cinerea*. Probit analysis was used to estimate the lethal concentration LC_{90} and LC_{50}. Subsequently, LC_{90}, LC_{50}, Tween 80 0.01 % and a control were evaluated on conidial germination *in vitro*. LC_{90} and LC_{50} were also evaluated on strawberry fruits. Twenty-five chemical compounds were identified in the essential oil. The 1 and 0.5 % concentrations inhibited *B. cinerea* with no statistical difference compared to the commercial fungicide. Tween 80 promoted conidial germination (89 %) while the lethal concentration LC_{90} (1.6 %) completely inhibited germination. Fruit treated with LC_{90} showed lower incidence and severity. The essential oil exhibited fungistatic effect against *B. cinerea* and a protective effect on strawberry fruits.

KEY WORDS: Incidence, gray mold, severity, *Tagetes linifolia*.

Please cite this article as/Como citar este artículo: Ruíz González, M. A., Serrato Cruz, M. A., Valadez Moctezuma, E., Solano Vidal, R., González Tepale, R. M. (2025). Chemical characterization of Tagetes linifolia essential oil: *in vitro* and *in vivo* tests on Fragaria × ananassa fruits. *Revista Bio Ciencias*, 13, e1767. https://doi.org/10.15741/revbio.13.e1767

Article Info/Información del artículo
Received/Recibido: September 30, 2024.
Accepted/Aceptado: November 12, 2025.
Available online/Publicado: December 15, 2025.

***Corresponding Author:**

Miguel Angel Ruíz-González. Dpto. de Fitotecnia. Universidad Autónoma Chapingo. Carr. Federal México-Texcoco, 38.5. C.P. 56230 Texcoco, México. Teléfono: (786) 129 4571. E-mail: miguelruiz93@live.com.mx

Revista Bio ciencias
ISSN 2007-3380

RESUMEN

Los aceites esenciales son eficaces para controlar patógenos, además, son reconocidos como seguros para el humano y el ambiente. El objetivo fue caracterizar los compuestos químicos en el aceite esencial de *Tagetes linifolia* y evaluar su efecto *in vitro* e *in vivo* contra *Botrytis cinerea*. El aceite esencial se analizó mediante cromatografía de gases-masas (GC-MS). Se evaluaron *in vitro* seis concentraciones de aceite (1, 0.5, 0.1, 0.01, 0.001 y 0.0001 %), un testigo y fungicida Cabrio C contra *B. cinerea*. Mediante análisis Probit se estimó la concentración letal LC_{90} y LC_{50}. Posteriormente, LC_{90}, LC_{50}, Tween 80 al 0.01 % y un testigo se evaluaron en la germinación *in vitro* de conidios. LC_{90} y LC_{50} fueron retomados para su evaluación en frutos de fresa. En el aceite esencial se identificaron veinticinco compuestos químicos. Las concentraciones de 1 y 0.5 % inhibieron a *B. cinerea* sin diferencias estadísticas con respecto al fungicida comercial. Tween 80 promovió la germinación de conidios (89 %) y con la concentración letal LC_{90} (1.6 %) no hubo germinación. Los frutos tratados con LC_{90} mostraron menor incidencia y severidad. El aceite esencial tuvo efecto fungistático contra *B. cinerea* y efecto protector en los frutos de fresa.

PALABRAS CLAVE: Incidencia, moho gris, severidad, *Tagetes linifolia*.

Introduction

Strawberry (*Fragaria × ananassa*) is a crop of global importance, in 2023, approximately 434,977 ha were cultivated worldwide, with a production of 10,485,454.06 t (FAOSTAT, 2025). Mexico is one of the leading producers, in 2024, the national planted area was 15,936.46 ha, with a yield of 696,112.63 t (SIAP, 2024). Its fruits are a source of vitamins and various bioactive compounds (Padmanabhan *et al*., 2016). However, they are highly susceptible to mechanical damage, dehydration, field rots, storage, transportation, and commercialization (Petrasch *et al*., 2019). Losses between 25 % and 55 % have been reported during pre-harvest and up to 89 % during post-harvest (Vanti *et al*., 2021). *Botrytis cinerea* causes gray mold disease, with a high incidence on strawberry fruits. This pathogen affects approximately 1,400 plant species, resulting in economic losses (Elad *et al*., 2016).

Current control strategies against this and other pathogens include synthetic fungicides such as carbendazim, dicarboximides, anilinopyrimidines, phenylpyrroles, quinone outside inhibitors, and succinate dehydrogenase inhibitors (Shao *et al*., 2020). For example, Cabrio C® (boscalid and pyraclostrobin) is an effective fungicide, as it inhibits mitochondrial respiration in *Botrytis cinerea* (Gül *et al*., 2024). However, several strains of *B. cinerea* have already developed resistance to Boscalid and pyraclostrobin (Gül *et al*., 2024). Also, synthetic fungicides have been

Revista Bio ciencias
ISSN 2007-3380

shown to be highly toxic to a wide range of beneficial soil microorganisms, contaminate water and food, and pose a risk to aquatic biota (Zubrod *et al*., 2019; Edward, 2021). Moreover, cases of fungicide poisoning in agricultural regions are a significant consideration in regulating their continued use, as they have been linked to neurotoxicity, genetic mutations, the development of cancerous tumors, congenital malformations, and endocrine disruption (Ahmad *et al*., 2024).

Therefore, alternative options have been sought, and essential oils from plants have been proposed as a way to minimize the use of these synthetic products. These generate less concern for human health and environmental pollution (Zulu *et a*l., 2023). Several essential oils have demonstrated antifungal activity both *in vitro* and *in vivo* against different species of phytopathogenic fungi (Abdi-Moghadam *et al*., 2023). Due to their high volatility, ephemeral nature, and biodegradability (Falleh *et al*., 2020), they have been granted GRAS (Generally Recognized as Safe) status by the Food and Drug Administration (FDA) (Jackson-Davis *et al*., 2023). Therefore, they are used as fruit preservatives, as they slow down or inhibit fungal growth (Freche *et al*., 2022). For example, *Echinophora platyloba* oil (0.5 %), *Citrus*×*limon* oil (0.01 %), and *Cinnamomum cassia* oil (0.01 %) showed potential for strawberry fruit preservation (Freche *et al*., 2023; Kebriti *et al*., 2025).

The *Tagetes* genus is considered endemic to the American continent (Serrato, 2014[a]). Therefore, it has been studied for various purposes. The *in vitro* fungicidal and fungistatic effects against *B. cinerea* have already been documented with the essential oils from *T. lucida* and *T. remotiflora* (Ruíz-González *et al*., 2025[a]; Ruíz-González *et al*., 2025[b]). Their abundant chemical compounds, primarily monoterpenes, sesquiterpenes, and phenylpropanoids (Salehi et al., 2018; Serrato, 2014[a]), are responsible for inhibiting mycelial growth and sporulation in certain fungi (Achimón *et al*., 2021).

Although information is available on the uses of various *Tagetes* species in the cosmetics and food industries, in traditional medicine, and as bactericides and pesticides (Salehi *et al*., 2018; Serrato *et al*., 2014[b]), the biological properties of some species remain unknown, such as *Tagetes linifolia* Seaton, which is regionally distributed in Mexico (Turner, 1996). It is a perennial shrub, 20-40 cm tall (Turner, 1996), and, to date, no information is available on its chemical composition, biological effects, or potential uses (Serrato *et al*., 2014[b]). Due to the destruction and alteration of natural habitats, the study and understanding of endemic species represent an option for their use and conservation (Coelho *et al*., 2020). Moreover, the cultivation of *T. linifolia* offers advantages for its use, as it is a shrub species with the ability to resprout. The objective was to characterize the chemical compounds in the essential oil of *T. linifolia* and evaluate its effect *in vitro* and *in vivo* against *B. cinerea*.

Revista Bio ciencias
ISSN 2007-3380

Materials and Methods

Essential oil extraction and chemical analysis

In Tezontepec, Puebla, Mexico (19.505131, -97.503948), flowering plants of *T. linifolia* were collected in October 2022 (Figure 1). The fresh tissue consisting of flowers, stems, and leaves was ground, and a 4 kg sample was subjected to hydrodistillation using an Italian-type distiller, a yield of 0.87 mL kg^{-1} was obtained. The essential oil was transferred to amber glass bottles and stored under refrigeration (4 °C). Three herbarium specimens were deposited in the "Herbario-Hortorio JES" of the Universidad Autónoma Chapingo (voucher 35885).

Figure 1. ***Tagetes linifolia*** **flowering at Tezontepec, Puebla, Mexico.**

Source: Miguel Angel Ruíz González.

The chemical analysis of the essential oil was performed at the Laboratorio de Productos Naturales of the Preparatoria Agrícola Chapingo using the Solid-Phase Microextraction (SPME) technique (Xu & Ouyang, 2018). The fiber was exposed to the vapor phase of the sample (1 µL) and injected into a gas chromatograph coupled to an ion trap mass spectrometer (GC-MS Agilent Technologies 7890 Agilent 240, Agilent Technologies). A VF-5ms capillary GC column, 30 × 0.25 (0.25), was used. The injector temperature was set at 250 °C, with a split/splitless injection mode (1:20). A VF-5 MS column (30 m × 250 µm × 0.25 µm) was used with helium as the carrier gas and a flow rate of 0.5 mL min^{-1} and a pressure of 0.5 atm. The oven was programmed with a heating ramp from 70 °C to 120 °C at 2 °C min^{-1}, then from 120 °C to 230 °C at 15 °C min^{-1}. The sample was analyzed in duplicate.

The chemical compounds were identified by comparing the retention times and mass spectra with the National Institute of Standards and Technology (NIST) mass spectral library and

Revista Bio ciencias
ISSN 2007-3380

confirmed by calculating the Kovats index, as compared to the literature (NIST: https://webbook.nist.gov/chemistry/). n-Alkanes (C8-C20) were used to calculate this index, and a variation of ± 40 units was used to identify the compounds. The relative abundance of each compound was estimated based on peak area.

In vitro evaluation

The experiment was conducted at the Laboratorio de Resistencia Genética at the Universidad Autónoma Chapingo, Texcoco, Mexico. *B. cinerea* (GenBank accession number: PP401673.1) was reactivated in Potato Dextrose Agar (PDA) culture medium (BD Bioxon®) and incubated in a culture oven (Ecoshel, 91210) under dark conditions at 18 ± 2 °C for five days.

Through dilutions, emulsions were prepared using 0.01 % Tween 80 (Sigma Aldrich Company, Darmstadt, Germany), double-distilled water, and essential oil at six concentrations (1, 0.5, 0.1, 0.01, 0.001, and 0.0001 %), the control was PDA medium sterilized in autoclave (AESA, CV 300) at 120 °C for 15 min. 1 g L^{-1} of the fungicide Cabrio C (boscalid and pyraclostrobin) was used as a reference. Antifungal activity was evaluated using the poisoned medium technique (Erhonyota et al., 2023). A four-day-old colony was used to obtain a 3.5 mm disc containing mycelium with a sterile dissection needle, which was placed in the center of 90 mm diameter Petri dishes. The incubation conditions remained the same. Over a period of five days, and every 24 hours, mycelial growth was measured using a digital vernier (Truper, 14388). Mycelial growth inhibition was estimated from the data obtained (Fokkema, 1973). At the end of the *in vitro* evaluation, the lethal concentrations LC_{50} and LC_{90} were determined using Probit analysis in the SAS academic software (Castillo, 2007).

Evaluation of Probit concentrations in conidia

The LC_{50} and LC_{90} concentrations were evaluated on *B. cinerea* conidia. In a 250 mL Erlenmeyer flask, 100 mL of sterile double-distilled water and 10 µL of Tween 80 were added. Conidia of *B. cinerea* were obtained from a 12-day-old colony with sporulation by scraping. The Erlenmeyer flask was vortexed (Cole-Parmer, Illinois, USA) for 10 s and the conidia count was performed in triplicate in a Neubauer chamber (Marienfeld, CNLB01). A conidia suspension of $5×10^5$ was obtained.

In PDA medium combined with essential oil, the following treatments were prepared: a) PDA+LC_{50}, b) PDA+LC_{90}, c) PDA+Tween 80 at 0.01 %, and d) control with only PDA. Prior to emptying the PDA medium, 2 µL of the suspension (100 conidia) was added to the center of each Petri dish, and then the PDA medium was emptied at room temperature. The Petri dishes were homogenized by circular movements to distribute the conidia in the culture medium and incubated under the previously described conditions. Every 24 h, the germinated conidia were counted until 96 h.

Revista Biociencias ISSN 2007-3380

Evaluation of Probit concentrations on fruits

Commercially ripe fruits from strawberry var. "FESTIVAL" plants were harvested and disinfected with sodium hypochlorite (Clorox®) at 50 ppm for 3 min. The fruits were rinsed with sterile double-distilled water and placed on absorbent paper. Treatments with inoculated and uninoculated fruits were evaluated. The fruits were immersed for 15 seconds in the essential oil emulsion of LC_{50} and LC_{90}, prepared with Tween 80 (0.01 %) and double-distilled water.

Five fruits were placed in transparent hinged polystyrene containers and, after 6 hours, only the fruits in the inoculation treatment were inoculated by spraying with 10 mL of a suspension of conidia (1×10^6) of *B. cinerea*. The containers were refrigerated at 4 °C, and the development of fruit rot was evaluated using an incidence analysis (Equation 1) at five, ten, and fifteen days post-inoculation. Fifteen days post-inoculation, surface mycelium growth was recorded to estimate severity (Equation 2) (McKinney, 1923). The scale developed by Romanazzi *et al*. (2013) was used: 0) healthy fruit; 1) 1-20 % of the fruit surface infected; 2) 21-40 % of the fruit surface infected; 3) 41-60 % of the fruit surface infected; 4) 61-80 % of the fruit surface infected; 5) more than 80 % of the fruit surface infected. Uninoculated fruit and inoculated fruit without the application of essential oils were used as controls.

Equation 1: estimate of incidence on fruit

$$\text{Incidence} = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} * 100$$

Equation 2: severity estimation in fruits on a scale of 0-5

$$\text{Severity} = \frac{\sum(\text{nixi})}{\text{NZ}} * 100$$

xi: severity scale score

ni: number of fruits with scale xi

N: total number of fruits observed

Z: highest score

Data analysis

A completely randomized design was used in the experiments. The *in vitro* treatments with PDA medium had five replicates. For the fruit evaluation, three replicates were performed with five fruits in each replicate. The variables inhibition, germination percentage, incidence, and severity were analyzed using the SAS academic software through analysis of variance and Tukey's multiple comparison tests ($p < 0.05$).

Results and Discussion

Chemical compounds in essential oil

Twenty-five chemical compounds were identified in the essential oil of *T. linifolia*, including 40 % terpenoids, 32 % monoterpenes, 12 % sesquiterpenes, and some esters (8 %), ketones (4 %), and alcohols (4 %) (Table 1), which is consistent with Salehi *et al*. (2018), who mention that *Tagetes* essential oils are rich in monoterpenes and low in sesquiterpenes. The major compounds were: 1,5-heptadien-4-ol, 3,3,6-trimethyl- (34.99 %), (E)-Tagetone (13.47 %), and β-Pinene (3.24 %). The first major compound is reported for the first time in *Tagetes*, while the other compounds are common in the genus (Salehi *et al*., 2018). The compound 1,5-heptadien-4-ol, 3,3,6-trimethyl- was also reported as the primary compound (7.36 %) in the essential oil of *Artemisia austro-yunnanensis* (Chen-Xing *et al*., 2014), while (E)-Tagetone (6.24 %) is found in *Tagetes minuta* (De Oliveira *et al*., 2018). The compounds present in *T. linifolia* oil may have significant effects and diverse applications.

In vitro evaluation

The 0.5 % and 1 % concentrations inhibited mycelial growth by 83 and 86 %, respectively, with no statistical differences compared to the commercial fungicide (Figure 2). Even at low dilutions (0.01 and 0.001 %), inhibition was 19 % and 28 %, with statistical differences compared to the control (Figure 2). The inhibitory effect on *B. cinerea* has already been observed in other *Tagetes* species (Ruíz-González *et al*., 2025[a]; Ruíz-González *et al*., 2025[b]) and its effect is mainly attributed to monoterpenes in a concentration-dependent manner (Pedroso *et al*., 2024). The high inhibition could also be attributed to the amount of terpenes present in the essential oil, as these have been shown to decrease mitochondrial content (Haque *et al*., 2016).

Revista Bio ciencias
ISSN 2007-3380

Table 1. Chemical compounds and relative abundance (RA) in *Tagetes linifolia* essential oil.

	Compound	RA	KIC		Compound	RA	KIC
*	1,5-Heptadien-4-ol, 3,3,6-trimethyl	34.99	1068 ± 12.8	-	3-Carene	0.15	1005 ± 3.2
*	(E)-Tagetone	13.47	1136 ±11.8	*	β-Linalool	0.12	1081 ± 14.3
-	β-Pinene	3.24	970 ± 12.4	=	6-Methyl-5-hepten-2-one	0.09	958 ± 17
-	trans-β-Ocimene	2.55	1034 ± 0.1	*	2-Isopropylidene-5-methylhex-4-enal	0.09	1151 ± 9.5
=	1-Pentanol, 5-cyclopropylidene-	2.54	1085 ± 22.1	*	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1α,2α,5α)-	0.08	1151 ± 36.7
-	α-Thujene	1.54	968 ± 24.9	/	Cariofileno	0.07	1424 ± 1.8
-	Camphene	0.70	943 ± 4.6	*	Elsholtzia ketone	0.06	1167 ± 23.9
-	o-Cimene	0.60	1025 ± 0.1	/	α-Bergamotene	0.05	1407 ± 15.6
*	Verbenone, (L)-	0.56	1191 ± 34.7	-	α-Pinene	0.05	931 ± 4.9
-	Limonene	0.52	1014 ± 10.8	+	cis-3-Hexenyl acetate	0.04	983 ± 15
-	β-Sabinene	0.31	964 ± 2.7	*	endo-Borneol	0.04	1148 ± 18.2
+	Sorbic acid vinyl ester	0.30	990 ± 33.4	/	(-)-β-Bourbonene	0.01	1408 ± 12.2
*	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	0.20	1178 ± 31.4				

KIC: Kovats index calculated ± standard deviation, C10H16 (-): monoterpenes, C15H24 (/): sesquiterpenes, (=): ketones, (+): esters, terpenoids (*): terpenes that undergo biochemical modifications through enzymes that add oxygen molecules and move or remove methyl groups.

The compounds β-pinene, (-)-β-bourbonene, and trans-β-ocimene were also identified in the essential oil of *T. remotiflora* and *T. lucida*, and their inhibition of *B. cinerea* was outstanding (Ruíz-González *et al*., 2025[a]; Ruíz-González *et al*., 2025[b]). Therefore, the inhibitory effect observed with *T. linifolia* is also attributed to these compounds (Table 1) and to the ability of essential oils to be absorbed into the lipophilic surface of the mycelium (Kıran *et al*., 2016), as well as to the

Revista Bio ciencias ISSN 2007-3380

synergistic effect of all compounds, as indicated by Hleba *et al*. (2024). It has been demonstrated that β-pinene serves as the primary compound for the development of new fungicides (Zhang *et al.,* 2025), as it acts by interfering with the cell wall (Andrade *et al*., 2019). This compound had a relative abundance of 3.24 % in *T. linifolia* (Table 1). Also, the compound 1,5-heptadien-4-ol, 3,3,6-trimethyl- previously identified in *Artemisia austro-yunnanensis* exhibits antioxidant activity (Chen-Xing *et al*., 2014), and (E)-Tagetone present in *T. minuta*, together with other characteristic compounds of the species, has antifungal, antibacterial, insecticidal, nematicidal, antiviral, and antimicrobial activity (Bandana *et al*., 2018). Therefore, the inhibition results of *T. linifolia* against *B. cinerea* open the possibility of exploring its effects on other pathogens.

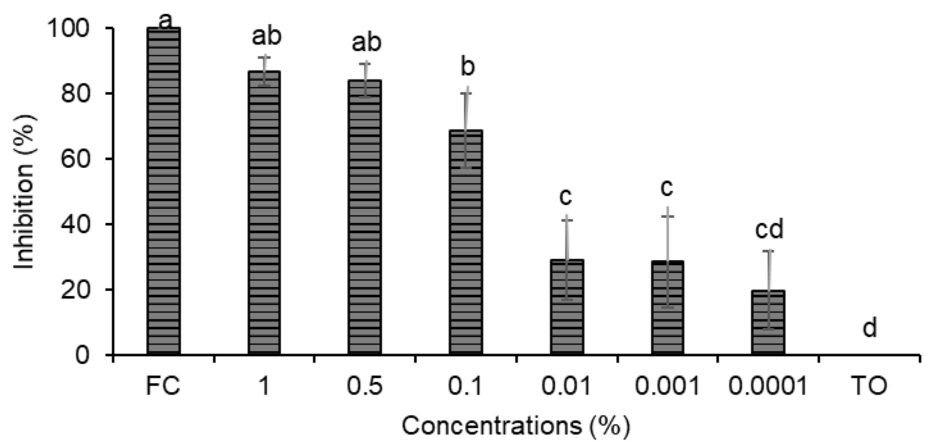

Figure 2. Inhibition of *Botrytis cinerea* mycelial growth with *Tagetes linifolia* essential oil.

Means with the same letters are not statistically different (Tukey ≤ 0.05). TO: control, FC: commercial fungicide Cabrio C. 1, 0.5, 0.1, 0.01, 0.001, 0.0001: concentration of essential oil used (v/v).

Probit analysis

Probit analysis was used to determine the lethal concentrations of 50 % (LC_{50}) and 90 % (LC_{90}), LC_{50} at 0.04 % (v/v) and LC_{90} at 1.6 % (v/v) (Table 2). These concentrations were subsequently used to evaluate conidia germination on fruits.

Revista Bio ciencias
ISSN 2007-3380

Table 2. Lethal concentrations LC_{50} and LC_{90} of *Tagetes linifolia* essential oil obtained using Probit analysis.

Essential oil (v/v)	LC_{50}	LC_{90}	*p*-value
Tagetes linifolia	0.04%	1.6%	<0.0001

LC_{50}: lethal concentration causing 50% mortality, LC_{90}: lethal concentration causing 90% mortality, *p*-value: represents heterogeneity in the analyzed population.

Evaluation of Probit concentrations in conidia

At LC_{90} (1.6 %) conidia germination was inhibited entirely with statistical differences compared to the control, while at LC_{50} (0.04 %) resulted in 17 % germination at 96 h (Figure 3). In other studies, essential oils from *Origanum vulgare* and *Thymus vulgaris* (0.08 %) have been evaluated, which partially inhibited the germination of conidia of *Penicillium expansum* (83.14-98.34 %) and *Botrytis cinerea* (51.73-71.57 %), and at 0.1 % there was total inhibition (Fincheira *et al*., 2023). The essential oils of *Curcuma longa*, *Pimenta dioica*, *Rosmarinus officinalis*, and *Syzygium aromaticum* (0.1 %) contained similar compounds (α-pinene, endo-borneol, camphene, β-pinene, limonene, β-linalool, verbenone, and caryophyllene) to those found in *T. linifolia* (Table 1). They inhibited the germination of *Fusarium verticillioides* conidia by 24.2 to 87.2 % (Achimón *et al*., 2021). This supports the fact that essential oils have a synergistic effect and act in a concentration-dependent manner (Hleba *et al*., 2024; Pedroso *et al*., 2024).

On the other hand, the surfactant Tween 80 promoted conidia germination (89 %) even more than the control (79 %). However, when used to prepare the emulsion with the essential oil (LC_{50} and LC_{90}), it seemed to have no effect, as potent inhibition of germination was observed (Figure 3). Although surfactants (Tween 20 and Tween 80) are used to release conidia from the conidiophores, they can interfere with the germination of some fungi. In *Pyrenophora triticirepentis*, Tween 20 at 0.02 % delayed germination, however, within two hours, all conidia had germinated (Jacques *et al*., 2021). Mwamburi *et al*. (2015) observed that Tween 80 inhibits the germination of *Beauveria bassiana* at concentrations of 0.5 and 1 %, but stimulates germination at concentrations of 0.1 %. Tu *et al*. (2014) observed that *Aureobasidium pullulans* cells appeared more dispersed and larger after application of 0.02 % Tween 80. Due to the effects observed with Tween 80, it is suggested to standardize concentrations and evaluate its use with beneficial fungi, as its ability to promote conidial germination could offer advantages.

Revista Bio ciencias
ISSN 2007-3380

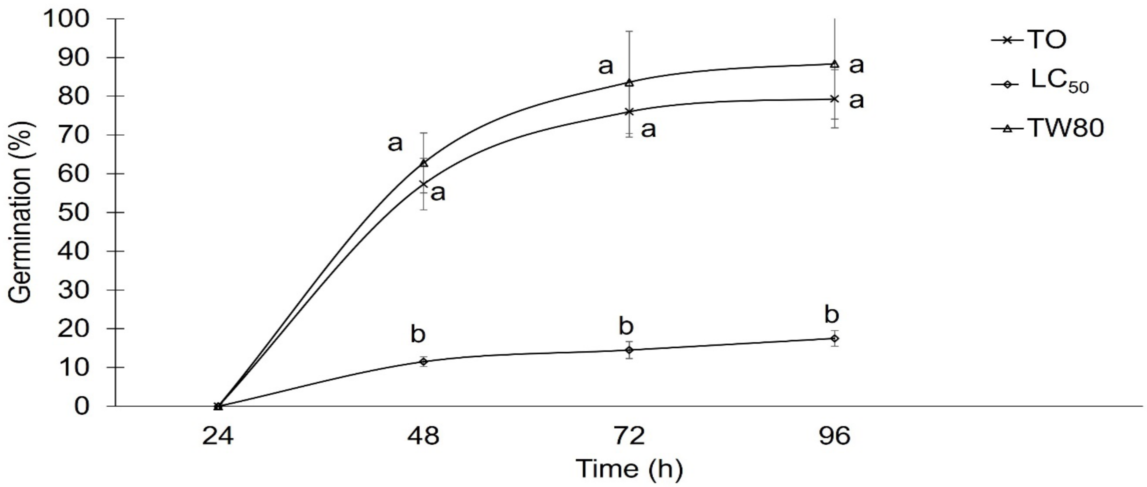

Figure 3. Conidia germination of *Botrytis cinerea* in PDA medium with lethal concentration LC_{50}, Tween 80 (TW80), and control (TO).

Treatment with lethal concentration LC_{90} is not included as there was no conidia germination. Means with the same letters are not statistically different (Tukey ≤ 0.05).

Evaluation of Probit concentrations on fruits

The incidence of gray mold caused by *B. cinerea* was lower in uninoculated fruits treated with the lethal concentration LC_{90}, while in inoculated fruits the incidence was higher (Table 3). During five days of refrigeration, fruits treated with LC_{90} showed no pathogen incidence, after ten days, the incidence was 6.6 %, and after fifteen days, the incidence was 50 % (Table 3). With the application of LC_{90}, the surface infection was 1-20 % (Figure 4), unlike the inoculated and uninoculated control treatments, which exhibited the highest incidence from day five to day fifteen (80-90 %). Although, strawberry fruits are susceptible to physical, physiological, or pathological damage (Qaderi *et al*., 2022). Israfi *et al*. (2022) and Da Costa *et al*. (2023) mention that essential oils can slow down the progression of fungal and bacterial diseases in fruits. Moreover, they demonstrated that *Melaleuca alternifolia* essential oil (0.05 %) reduced the severity of *Colletotrichum musae* in bananas by up to 98 % (Da Costa *et al*., 2023).

Severity was higher (Table 3) in the inoculated and uninoculated control treatments (4.8 and 3.8) as the infected fruit surface ranged from 61 to 81 % (Figure 4), a condition that renders the fruit unsuitable for consumption. Fruits treated with the median lethal concentration (LC_{50} and LC_{50}) exhibited lower incidence than the control after five days, but the severity renders them ineffective as strawberry fruit preservatives (Table 3 and Figure 4). The protective effect against *B. cinerea* in strawberry fruits has already been demonstrated with essential oils from *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba*, *Ocimum americanum*, *Citrus×limon*, and *Cinnamomum*

Revista Bio ciencias
ISSN 2007-3380

cassia (Fontana *et al*., 2021; Freche *et al*., 2023). On the other hand, Ji *et al*. (2024) demonstrated that *Origanum vulgare* oil (0.006 %) reduced the incidence of *Botrytis cinerea* and *Alternaria alternata*, and also induced defense-related enzymes such as phenylalanine ammonium-lyase, polyphenol oxidase, peroxidase, chitinase, and β-1,3-glucanase in blueberry fruits (*Vaccinium corymbosum* L. "O'Neal"). The application of essential oils is promising in food preservation, and the results of this study provide an important precedent for the use of *T. linifolia* essential oil as a potential product to counteract the negative effects caused by *B. cinerea*.

Table 3. Incidence and severity of *Botrytis cinerea* in strawberry fruits treated with LC_{90} and LC_{50} essential oils and refrigerated for 15 days.

Treatments	Incidence			Severity
	5 d	10 d	15 d	15 d
TO uninoculated	13.3 ± 11.5 [ab]	46.6 ± 11.5 [ab]	80 ± 20 [a]	3.8 ± 0.8 [a]
TO inoculated	40 ± 20 [a]	53.3 ± 11.5 [a]	90 ± 11.5 [a]	4.8 ± 0.4 [a]
LC_{90}	0 ± 0 [b]	6.6 ± 11.5 [c]	50 ± 20 [a]	1 ± 0.7 [c]
LC_{90}I	6.6 ± 11.5 [b]	13.3 ± 11.5 [bc]	73.3 ± 23 [a]	1.8 ± 0.4 [bc]
LC_{50}	6.6 ± 11.5 [b]	40 ± 20 [abc]	73.3 ± 11.5 [a]	3 ± 1.8 [ab]
LC_{50}I	0 ± 0 [b]	46.6 ± 11.5 [ab]	93.3 ± 11.5 [a]	4.8 ± 0.4 [a]
p-value	0.0509	0.0040	0.1762	<.0001
DMS	31.6	36	51.2	1.9

TO: control, LC: lethal concentration, I: inoculated, severity: damage scale 0-5. Means with the same letters are not statistically different (Tukey ≤ 0.05).

In this study, the LC_{90} treatment of *T. linifolia* was effective in controlling the disease in post-harvest up to five days (Table 3 and Figure 4), an important precedent to explore its application in vapor phase (Tančinová *et al*., 2022) or prepare active biofilms based on polysaccharides with essential oils for food preservation (Khan *et al*., 2023). Optimal concentrations still need to be standardized, as essential oils can have organoleptic effects and alter multiple physicochemical properties related to fruit quality (Freche *et al*., 2022).

Although the application of essential oils is an effective and safe alternative for humans (Taghavi *et al*., 2018), it is important to consider that 1149.43 kg of fresh tissue would be required to obtain 1 L of T. *linifolia* essential oil. Alternative methods, such as microwave-assisted extraction, which offers higher yields and shorter extraction times, should be explored (Souiy, 2023). Agronomic management of *T. linifolia* could serve as an alternative approach to increase the essential oil content, as suggested by Georgieva *et al*. (2022). Likewise, future studies will continue to research other fruits and experiment with combinations of essential oils to observe their effects. The endemic nature, aromatic profile, abundance and striking flowering of *T. linifolia* make it a valuable phytogenetic resource for future research. It even has ornamental potential, an important aspect that will contribute significantly to its conservation.

Revista Bio ciencias
ISSN 2007-3380

Figure 4. Protective effect of *Tagetes linifolia* essential oil fifteen days after incubation in inoculated and uninoculated fruits.

LC_{50} and LC_{90}: Probit concentrations, TO: control, I: inoculated fruits.

Conclusions

The endemic nature of *T. linifolia* makes its study and conservation important. The chemical characterization of *T. linifolia* essential oil allowed the identification of a new major compound (1,5-heptadien-4-ol, 3,3,6-trimethyl-) for the *Tagetes* genus. Previous reports on this compound indicate its antioxidant activity, and the relative abundance of this compound in conjunction with the monoterpenes, terpenoids, and sesquiterpenes of *T. linifolia* showed biological activity against *B. cinerea in vitro* and *in vivo*. The inhibitory effect on conidia germination stands out. The reduction in incidence and severity on strawberry fruits is one of the evaluated properties, however, *T. linifolia* essential oil could also be applied to other fruits affected by *B. cinerea* or tested for its effect on other microorganisms. More detailed analyses of biological activity are necessary, and future studies should incorporate additional factors, such as different temperature ranges, which play a crucial role in fruit preservation.

Revista Bio ciencias
ISSN 2007-3380

Authors contribution

MARG and MASC designed, conducted the research, and wrote the paper; EVM and RSV provided access to laboratory materials and plant material, and assisted with the experiments and methodology; RMGT and MARG analyzed the samples using chromatography equipment and identified chemical compounds.

All authors reviewed and approved the paper.

Funding

This research was funded by the authors' own funds.

Acknowledgements

We appreciate the support of the Laboratorio de Productos Naturales of the Preparatoria Agrícola Chapingo.

Conflict of interest

The authors declare that they have no conflict of interest.

Referencias

Abdi-Moghadam, Z., Mazaheri, Y., Rezagholizade-Shirvan, A., Mahmoudzadeh, M., Sarafraz, M., Mohtashami, M., Shokri, S., Ghasemi, A., Nickfar, F., Darroudi, M., Hossieni, H., Hadian, Z., Shamloo, E., & Rezaei, Z. (2023). The significance of essential oils and their antifungal properties in the food industry: A systematic review. *Heliyon*, 9(11), Article e21386. https://doi.org/10.1016/j.heliyon.2023.e21386

Achimón, F., Brito, V. D., Pizzolitto, R. P., Sánchez, A. R., Gómez, E. A., & Zygadlo, J. A. (2021). Chemical composition and antifungal properties of commercial essential oils against the maize phytopathogenic fungus *Fusarium verticillioides*. *Revista Argentina de Microbiología*, 53(4), 292-303. https://doi.org/10.1016/j.ram.2020.12.001

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil, K., Saati, A. A., Wahab, S., Elbendary, E. Y., Kambal, N., Abdelrahman, M. H., & Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. *Heliyon*, 10(7), Article e29128. https://doi.org/10.1016/j.heliyon.2024.e29128

Andrade, A. C., Rosalen, P. L., Freires, I. A., Scotti, M. T., De Aquino, S. G., & De Castro,

R. D. (2019). Antifungal activity, mode of action, docking prediction and anti-biofilm effects of (+)-β-pinene enantiomers against *Candida* spp. *Current Topics in Medicinal Chemistry*, 18(29), 2481-2490. https://doi.org/10.2174/1568026618666181115103104

Bandana, K., Raina, R., Kumari, M., & Rani, J. (2018). *Tagetes minuta*: An overview. *International Journal of Chemical Studies*, 6(2), 3711-3717. https://www.chemijournal.com/archives/2018/vol6issue2/PartAZ/6-2-452-241.pdf

Castillo Márquez. L. E. (2007). Introducción al SAS para Windows. Universidad Autónoma Chapingo. 3 edición. 295 p.

Chen-Xing, Z., Mi, Z., Jing, H., Ya-Fang, D., & Bao-Cai, L. (2014). Chemical composition and antioxidant activity of the essential oil from the flowers of *Artemisia austro-yunnanensis*. *Journal of Chemical and Pharmaceutical Research*, 6(7),1583-1587. https://jocpr.com/vol6-iss7-2014/JCPR-2014-6-7-1583-1587.pdf

Coelho, N., Gonçalves, S., & Romano, A. (2020). Endemic plant species conservation: Biotechnological approaches. *Plants*, 9(3), 345. https://doi.org/10.3390/plants9030345

Da Costa, D. G., Ribeiro, W. R., Gonçalves, D. C., Dian, V. S., Da Silva Xavier, A., De Oliveira, Á. A., Menini, L., & Costa, H. (2023). Use of *Melaleuca alternifolia* essential oil as an efficient strategy to extend the shelf life of banana fruits. *Biochemical Systematics and Ecology*, 108, Article 104641. https://doi.org/10.1016/j.bse.2023.104641

De Oliveira, D. H., Abib, P. B., Giacomini, R. X., Lenardão, E. J., Schiedeck, G., Wilhelm, E. A., Luchese, C., Savegnago, L., & Jacob, R. G. (2018). Antioxidant and antifungal activities of the flowers' essential oil of *Tagetes minuta*, (Z)-tagetone and thiotagetone. *Journal of Essential Oil Research*, 31(2), 160-169. https://doi.org/10.1080/10412905.2018.1519465

Edward, D. (2021). Impact of fungicide pesticides on human and environment. *Journal of Agricultural Science and Food Research*, 12(3), 1-2. https://www.longdom.org/open-access/impact-of-fungicide-pesticides-on-human-and-environment.pdf

Elad, Y., Pertot, I., Cotes Prado, A. M., & Stewart, A. (2016). Plant hosts of *Botrytis* spp. In: *Botrytis* the fungus, the pathogen and its management in agricultural systems. Fillinger, S., & Elad, Y. (pp. 413-486). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-23371-0_20

Erhonyota, C., Edo, G. I., & Onoharigho, F. O. (2023). Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. *Acta Ecológica Sinica*, 43(4), 684-689. https://doi.org/10.1016/j.chnaes.2022.08.006

Falleh, H., Jemaa, M. B., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. *Food Chemistry*, 330, Article 127268. https://doi.org/10.1016/j.foodchem.2020.127268

Food and Agriculture Organization Statistical database [FAOSTAT]. (2025, 30 de julio). Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL/visualize

Fincheira, P., Jofré, I., Espinoza, J., Levío-Raimán, M., Tortella, G., Oliveira, H. C., Diez, M. C., Quiroz, A., & Rubilar, O. (2023). The efficient activity of plant essential oils for inhibiting *Botrytis cinerea* and *Penicillium expansum*: Mechanistic insights into antifungal activity. *Microbiological Research*, 277, Article 127486. https://doi.org/10.1016/j.micres.2023.127486

Fokkema, N. J. (1973). The role of saprophytic fungi in antagonism against *Dreschlera sorokiniana* (*Helminthosporium sativum*) on agar plates and rye leaves with pollen. *Physiological Plant Pathology*, 3(2),195-202. https://doi.org/10.1016/0048-4059(73)90082-9

Fontana, D. C., Neto, D. D., Pretto, M. M., Mariotto, A. B., Caron, B. O., Kulczynski, S. M., & Schmidt, D. (2021). Using essential oils to control diseases in strawberries and peaches. *International Journal of Food Microbiology*, 338, Article 108980. https://doi.org/10.1016/j.ijfoodmicro.2020.108980

Freche, E., Gieng, J., Pignotti, G. A., Ibrahim, S. A., & Feng, X. (2022). Applications of lemon or *Cinnamon* essential oils in strawberry fruit preservation: A review. *Journal of Food Processing and Preservation*, 46(9). https://doi.org/10.1111/jfpp.16526

Freche, E., Gieng, J., Pignotti, G. A., Ibrahim, S. A., Tran, H. T., Ahn, D. U., & Feng, X. (2023). Effects of lemon or cinnamon essential oil vapor on physicochemical properties of strawberries during storage. *Han-guksikpumjeojang-yutonghakoeji*, 30(4), 549-561. https://doi.org/10.11002/kjfp.2023.30.4.549

Georgieva, R., Delibaltova, V., & Chavdarov, P. (2022). Change in agronomic characteristics and essential oil composition of coriander after application of foliar fertilizers and biostimulators. *Industrial Crops and Products*, 181, Article 114819. https://doi.org/10.1016/j.indcrop.2022.114819

Gül, E., Karakaya, A., & Ergül, A. (2024). Mutations associated with boscalid and pyraclostrobin resistance of *Botrytis cinerea* from vegetable fields in Turkey. *Tropical Plant Pathology*, 49(3), 429-435. https://doi.org/10.1007/s40858-024-00646-4

Haque, E., Irfan, S., Kamil, M., Sheikh, S., Hasan, A., Ahmad, A., Lakshmi, V., Nazir, A., & Mir, S. S. (2016). Terpenoids with antifungal activity trigger mitochondrial dysfunction in *Saccharomyces cerevisiae*. *Microbiology*, 85(4), 436-443. https://doi.org/10.1134/s0026261716040093

Hleba, L., Hlebová, M., & Charousová, I. (2024). *In vitro* evaluation of synergistic essential oils combination for enhanced antifungal activity against *Candida* spp. *Life*, 14(6), 693. https://doi.org/10.3390/life14060693

Israfi, N. A. M., Ali, M. I. A. M., Manickam, S., Sun, X., Goh, B. H., Tang, S. Y., Ismail, N., Razis, A. F. A., Ch'ng, S. E., & Chan, K. W. (2022). Essential oils and plant extracts for tropical fruits protection: From farm to table. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.999270

Jackson-Davis, A., White, S., Kassama, L. S., Coleman, S., Shaw, A., Mendonça, A. F., Cooper, B., Thomas-Popo, E., Gordon, K. W., & London, L. (2023). A review of regulatory standards and advances in essential oils as antimicrobials in foods. *Journal of Food Protection*, 86(2), Article 100025. https://doi.org/10.1016/j.jfp.2022.100025

Jacques, S., Lenzo, L., Stevens, K., Stevens, Lawrence, J., & Tan, K. C. (2021). An optimized sporulation method for the wheat fungal pathogen *Pyrenophora tritici-repentis*. *Plant Methods*, 17(52), 4-12. https://doi.org/10.1186/s13007-021-00751-4

Ji, Y., Hu, W., Guan, Y., & Saren, G. (2024). Effects of plant essential oil treatment on the growth of pathogenic fungi and the activity of defense-related enzymes of fungi-inoculated blueberry. *Horticulturae*, 10(4), 318. https://doi.org/10.3390/horticulturae10040318

Kebriti, I., Solgi, M., & Velashjerdi, M. (2025). Improving quality of strawberry by novel essential oil nanoemulsions of *Echinophora platyloba* combined with *Aloe vera* gel and gum arabic. *Scientific Reports*, 15(1). https://doi.org/10.1038/s41598-025-86259-6

Khan, S., Abdo, A. A. A., Shu, Y., Zhang, Z., & Liang, T. (2023). The extraction and impact of essential oils on bioactive films and food preservation, with emphasis on antioxidant and antibacterial activities a review. *Foods*, 12(22), Article 4169. https://doi.org/10.3390/

foods12224169
Kıran, S., Kujur, A., & Prakash, B. (2016). Assessment of preservative potential of *Cinnamomum zeylanicum* Blume essential oil against food borne molds, aflatoxin B1 synthesis, its functional properties and mode of action. *Innovative Food Science and Emerging Technologies*, 37, 184-191. https://doi.org/10.1016/j.ifset.2016.08.018

McKinney, H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. *Journal Agricultural Research*, 26, 195-217. https://eurekamag.com/research/013/404/013404640.php?srsltid=AfmBOoro6bjNi4Y4u-fwwzS0faGgRaBlzcP-EFreUWaqKnKBee2VGS6I

Mwamburi, L. A., Laing, M., & Miller, R. (2015). Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*. *Brazilian Journal of Microbiology*, 46(1), 67-74. https://doi.org/10.1590/s1517-838246120131077

Padmanabhan, P., Mizran, A., Sullivan, J. A., & Paliyath, G. (2016). Strawberries. En Elsevier eBooks (pp. 193-198). https://doi.org/10.1016/b978-0-12-384947-2.00667-x

Pedroso, M. B., Scariot, F. J., Rocha, R. K. M., Echeverrigaray, S., & Delamare, A. P. L. (2024). Antifungal activity and mechanism of action of monoterpenes against *Botrytis cinerea*. *Ciência e Agrotecnologia*, 48. https://doi.org/10.1590/1413-7054202448018823

Petrasch, S., Knapp, S. J., Van Kan, J., & Blanco-Ulate, B. (2019). Gray mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. *Molecular Plant Pathology*, 20(6), 877-892. https://doi.org/10.1111/mpp.12794

Qaderi, R., Mezzetti, B., Capocasa, F., & Mazzoni, L. (2022). Stability of strawberry fruit (*Fragaria x ananassa* Duch.) nutritional quality at different storage conditions. *Applied Sciences*, 13(1), 313. https://doi.org/10.3390/app13010313

Romanazzi, G., Feliziani, E., Santini, M., & Landi, L. (2013). Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. *Postharvest Biology and Technology*, 75, 24-27. https://doi.org/10.1016/j.postharvbio.2012.07.007

Ruíz-González, M. Á., Solano-Vidal, R., Valadez-Moctezuma, E., & Serrato-Cruz, M. Á. (2025[a]). Compuestos químicos del aceite esencial de *Tagetes lucida* y efecto contra *Botrytis cinerea*. *Revista Mexicana de Ciencias Agrícolas*, 16(3), Article e3613. https://doi.org/10.29312/remexca.v16i3.3613

Ruíz-González, M. Á., Solano-Vidal, R., Valadez-Moctezuma, E., & Serrato-Cruz, M. Á. (2025[b]). Destilados y aceite homeopático de *Tagetes remotiflora*: efecto en *Botrytis cinerea*. *Revista Mexicana de Ciencias Agrícolas*, 16(2), Article e3494. https://doi.org/10.29312/remexca.v16i2.3494

Salehi, B., Valussi, M., Morais-Braga, M. F. B., Carneiro, J. N. P., Leal, A. L. A. B., Coutinho, H. D. M., Vitalini, S., Kręgiel, D., Antolak, H., Sharifi-Rad, M., Silva, N. C. C., Yousaf, Z., Martorell, M., Iriti, M., Carradori, S., & Sharifi-Rad, J. (2018). *Tagetes* spp. essential oils and other extracts: chemical characterization and biological activity. *Molecules*, 23(11), Article 2847. https://doi.org/10.3390/molecules23112847

Serrato, C. M. A. (2014[a]). El recurso genético cempoalxóchitl (*Tagetes* spp.) de México (Diagnóstico). Chapingo, Estado de México. https://www.gob.mx/cms/uploads/attachment/file/225091/El_recurso_gen_tico_del_cempoalxochitl__tagetes_spp__de_mexico__diagnostico_.pdf

Serrato, C. M. A., Bautista, R. F., & Monroy, S. A. S. (2014[b]). Guía para conocer el Germoplasma Mexicano de Cempoalxóchitl (*Tagetes* spp.). México. https://www.gob.mx/cms/uploads/attachment/file/225099/Guia_para_conocer_el_germoplasma_mexicano_de_cempoalxochitl__tagetes_spp_.pdf

Shao, W., Zhao, Y., & Ma, Z. (2020). Advances in understanding fungicide resistance in *Botrytis cinerea* in China. *Phytopathology*, 111(3), 455-463. https://doi.org/10.1094/phyto-07-20-0313-ia

Servicio de Información Agroalimentaria y Pesquera [SIAP]. (2024, 30 de julio). Anuario Estadístico de la Producción Agrícola. https://nube.agricultura.gob.mx/cierre_agricola/

Souiy, Z. (2023). Essential oil extraction process. En Biochemistry. https://doi.org/10.5772/intechopen.113311

Taghavi, T., Kim, C., & Rahemi, A. (2018). Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. *Microorganisms*, 6(4), Article 104. https://doi.org/10.3390/microorganisms6040104

Tančinová, D., Mašková, Z., Mendelová, A., Foltinová, D., Barboráková, Z., & Medo, J. (2022). Antifungal activities of essential oils in vapor phase against *Botrytis cinerea* and their potential to control postharvest strawberry gray mold. *Foods*, 11(19), Article 2945. https://doi.org/10.3390/foods11192945

Tu, G., Wang, Y., Ji, Y., & Zou, X. (2014). The effect of Tween 80 on the polymalic acid and pullulan production by *Aureobasidium pullulans* CCTCC M2012223. *World Journal of Microbiology & Biotechnology*, 31(1), 219-226. https://doi.org/10.1007/s11274-014-1779-9

Turner, B. L. (1996). Tageteae and Anthemideae. In Turner B. L. The comps of Mexico - A systematic account of the family Asteraceae (pp. 73-86). *Plant Resources Center, Department of Botany*, the University of Texas. https://books.google.com.mx/books/about/The_Comps_of_Mexico_Tageteae_and_Anthemi.html?id=6gZHAAAAYAAJ&redir_esc=y

Vanti, G. L., Leshem, Y., & Masaphy S. (2021). Resistance response enhancement and reduction of *Botrytis cinerea* infection in strawberry fruit by *Morchella conica* mycelial extract. *Postharvest Biology and Technology*, 175. https://doi.org/10.1016/j.postharvbio.2021.111470

Xu, J., & Ouyang, G. (2018). Extraction: Solid-Phase microextraction. En *Elsevier eBooks*. https://doi.org/10.1016/b978-0-12-409547-2.14552-4

Zhang, L., Huang, Y., Xiao, Z., Feng, X., Zhang, J., Chen, S., He, H., Liao, S., Wang, Z., & Si, H. (2025). Antifungal action of β-pinene derivatives containing quaternary ammonium scaffold against *Rhizoctonia solani* with damaging cell membranes and affecting energy metabolism. *Pesticide Biochemistry and Physiology*, Article 106586. https://doi.org/10.1016/j.pestbp.2025.106586

Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J. R., Scharmüller, A., Smalling, K. L., Stehle, S., Schulz, R., & Schäfer, R. B. (2019). Fungicides: an overlooked pesticide class?. *Environmental Science & Technology*, 53(7), 3347-3365. https://doi.org/10.1021/acs.est.8b04392

Zulu, L., Gao, H., Zhu, Y., Wu, H., Xie, Y., Liu, X., Yao, H., & Rao, Q. (2023). Antifungal effects of seven plant essential oils against *Penicillium digitatum*. *Chemical and Biological Technologies in Agriculture*, 10(1). https://doi.org/10.1186/s40538-023-00434-3