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Research Article

Antimicrobial Activity of Essential Oils from Two Ornamental Plants

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Abstract

This study evaluated the antimicrobial activity of essential oils from sweet basil and thyme plants. Plants subjected to different treatments during cultivation. The content of plants essential oils was examined by GC-MS and 21 different components were identified in sweet basil and 16 components were recognized in thyme essential oils. The major components of sweet basil essential oil were camphor and 2-propenoic acid while 1,8-cineole and borneol were the major components of thyme essential oil. The antimicrobial activity of sweet basil and thyme essential oil was assessed against *Erwinia amylovora, Aspergillus flavus, Aspergills oryzae and Fusarium oxysporum* using agar plates method. The results showed that the sweet basil essential oil obtained from (magnetic water treatment+ magnetic iron at a rate of 150 mg/Kg) gave the most potent inhibitory effect on the growth of *Erwinia amylovora, Aspergillus flavus and Fusarium oxysporum*. The most potent inhibitory effect was observed from the thyme essential oil obtained from (saltwater +magnetic iron at a rate of 150 mg/Kg). These results obtained the importance of essential oil impacts on inhibit growth of toxic microbes.

Keywords: *Antimicrobial activity, essential oils, sweet basil, thyme.*

Introduction

Combating diseases and pests caused by fungi and bacteria, whether in the medical or agricultural fields, is an important goal to protect humans, animals, and plants. Microbes that cause these diseases lead to huge losses in crops and great damage to plants, and consequently a great economic loss for farmers. They are also very harmful to human health because they release toxins in human bodies (El Soud *et al*., 2015).

Fungal and bacterial growth causes numerous health and economic problems including: plant diseases with serious effects on the agriculture sector, food toxicity and deterioration of animals and human's health. Fungal and bacterial growth also affect grains in their soils and vegetables and fruits in their storage facilities. Synthetic antimicrobial agents are usually sprayed in food storage facilities to reduced bacterial and fungal growth with leads to food contamination and microbial resistant (Jakowienko *et al*., 2011).

One of the most common and dangerous microorganisms affecting grains is Aspergillus flavus that produces aflatoxins. This fungus produces at least 16 types of toxins, among them four major aflatoxins including B1- B2- G1- G2, which cause serious human diseases (El-Soud *et al*., 2015). Erwinia amylovora is a bacterium known as fire blight

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that causes plant cells death and distortion of plant cell walls. It infects plants including apple and pear trees, resulting in complete destruction of these crops and sever economic loss (Laala *et al*., 2012).

This bacterium was first reported in1960 in Japan and New Zealand followed by a widespread in Europe and Mediterranean countries (Zhang & Geider, 1997). The serious effects of fungi and bacteria on plants, humans and animal's health urged scientists to search for safe and environmentally friendly antimicrobials. Mother Nature provided and will continue to provide humanity with many antimicrobial agents with high potency and safety index (Chouhan *et al*., 2017)

The use of medicinal and aromatic plants like sweet basil and thyme leaves is common in cooking as a spice in a variety of dishes worldwide. The leaves and flowers of sweet basil and thyme were used by ancient Egyptians in their medicinal recipes to treat several diseases (El-Soud *et al*., 2015). They are also used in folk medicine in many continents and for the preparation of many drugs including toothpastes, antimicrobial topical preparations. They are used in cosmetic products for the preparation of perfumes and creams. The essential oils of sweet basil and thyme showed also potent biological effects including antimicrobial activity (Jakowienko *et al*., 2011). Many studies reported the potent antimicrobial effect of essential oils against bacteria and fungi highlighting their ability to act as bacteriostatic and fungistatic as well as bactericidal and fungicidal agents(Karami-Osboo *et al*., 2010).

The phytochemical content as well as the biological activity of herbal plants extracts, and essential oils differ with species (Lemrhari *et al*., 2015). In the current study we evaluated the antimicrobial effects of sweet basil and thyme essential oils that were obtained from plants subjected to different cultivation conditions. The objective of this study was to investigate the effect of electromagnetic water, potassium silicate (foliar application) and magnetic iron (as a soil conditioner) on essential oils antimicrobial activity obtained from sweet basil and thyme plants under saline irrigation treatment.

Material and Methods

Field experiment was conducted on a sandy loamy soil at private farm, 80-kilometer Cairo-Alexandria desert road, Egypt, on sweet basil (Ocimum basilicum) and thyme (Thymus vulgaris) latitude, 30.35°N, long 32.16° 45.83° E Cairo governorate, Egypt during the period from 2018-2019.

Experiment layout

All soil preparation for sweet basil and Thyme planting was done. The experimental area was consisted of 36 plots with area 16 m^2 (2 x 8 cm) for each one, the distance between each plant 30 cm. The experiment was laid-out in split-split plot design with three replicates. The treatments were as the following:

- Main plot applied at two kinds of water (magnetic water and Ground water).
- Sub plot applied with two sources of fertilizers (potassium silicate as foliar spray) and (magnetic iron as soil conditioner).
- Sub-Sub plot was applied as rates of fertilizers (0-100-150 mg/L for potassium silicate) and (0-100-150 mg/Kg for magnetic iron).

Essential oil extract

Essential oils were extracted from the leaf's samples of each treatment by distillation according to the method of **British Pharmacopoeia.(1963)** and the oil percentages were recorded. Samples of the extracted essential oils were subjected to GC/ MS for the compositional analysis of volatile oil.

GC-MS analysis of the essential oil

The GC-Ms analysis of the essential oil was carried out using gas chromatography-mass spectrometry at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; rising at 3.0 °C /min to 240 °C and held for 1 min. The injector and detector were held at 240 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Antimicrobial Activity of Essential Oils

Antimicrobial activity of **essential oils** was evaluated against the plant pathogenic bacterial strain *Erwinia amylovora and five plant pathogenic fungal strains (Aspergillus flavus* and *Fusarium oxysporum*), in addition to non-plant pathogens *Aspergillus oryzae*. These plant pathogenic cultures were obtained from the Mycology Laboratory, Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. For the preparation of the seed cultures of these microorganisms, a loopful of two-day- or seven-day-old culture for bacteria and fungi, respectively, was inoculated in conical flasks of 100 mL, each containing 20 mL of tryptic soy broth and potato dextrose broth media (Merck, Darmstadt, Germany), respectively. The inoculated flasks were incubated for 24 and 48 h at 37 °C and 28 °C for bacteria and fungi, respectively. The antimicrobial activity of essential oils was determined by the agar well diffusion method according to (Pulit-Prociak *et al*. 2021). Muller Hinton agar and potato dextrose agar (Merck, Darmstadt, Germany) plates were inoculated with 100 µL of the prepared bacterial (10^6 CFU/mL) and fungal (1.5–5.0 × 10^7 CFU/mL) seed cultures respectively and distributed with a sterile cotton swab on the surface of the prepared media. With a sterilized cork borer, agar cups (8 mm in diameter) were cut from the pre-inoculated plates. One hundred microliters essential oils at a concentration of 1000 µg/mL were transferred to the agar cup. One hundred microliters of the base fluid was also transferred into another cup to be used as vehicle control. In addition, the antibiotic control used for the bacteria was trimethoprim/sulfamethoxazole (25 µg/mL) and the antifungal control used for the fungi was fluconazole (25 µg/mL) (Bioanalyse, Ankara, Turkey). All plates were kept at 4 °C for 2 h to allow the investigated compounds to diffuse. The plates were then incubated for 24 h at 37 °C for bacteria and 72–96 h at 28 °C for fungi. After incubation, the diameters of the zones of inhibition were measured and recorded (CLSI., 2021) and (Perez *et al*., 1990).

Statistical Methods

The results were statistically analysed using Co-stat computer package to calculate F ratio according to (Snedecor & Cochran.1980) Least significant differences method (L.S.D) was used to differentiate means at the 0.05 level of probability

Results and Discussion

Chemical composition of essential oils for sweet basil plant on some microbes.

The recent changes in climate affected countries all over the world, resulting in significant rise in temperature and an increase in plant pests and diseases. The climate change led to a severe reduction in the quality and quantity of fresh water. Many countries are trying to find replacement to fresh water to be used in irrigation.

In this study, saline water, magnetized water, magnetic iron as a soil conditioner and potassium silicate as a foliar spray were used to help the plant withstand salt stress. Salt stress is a major biological stress that negatively affects plant growth and components including essential oils (Elhindi *et al*., 2016).

Sweet basil is an annual herbaceous plant belonging to Lamiaceae family, it is about 1 meter high and has white or purple flowers. Sweet basil leaves contain essential oil, and their leaves are used as culinary herbs in cooking. There are more than 30 varieties of sweet basil in the tropical and sub-tropical regions, and it is considered native to India and the African continent (Arshad Ullah, 2019). Sweet Basil is known for its economic and medicinal importance, as it is used in the manufacture of perfumes, soaps, insecticides and fungicides (Sharafzadeh, 2011).

The results from the quantitative and qualitative analysis of sweet basil essential oils indicated that the average yield of the essential oils was (0.37 %). More than 20 compounds were identified. The known compounds were grouped into three groups including major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace compounds (less than 1%). Based on this classification, (Camphor and 2-propenoic acid) were major compounds, (Comphene, D-limonene, 1-8 cineole, linalool, caryophyllene, α-cadinol, and 3-cyclohexen) were minor components (Sabinene, α-pinene, α-myrcene, cis-ocimene, γ-terpinene, α-terpinolene, endo-borneol, humulene, germacrene-D, bicyclogermacrene, naphthalene, and cubenol) were considered as traces compounds.

The inhibitory effect of sweet basil extract oil against some economically important plant pathogenic microbes including *Erwinia amylovora, Aspergillus flavus, Aspergillus oryzae and Fusarium oxysporum* is shown in table (1). The essential oils obtained from sweet basil under all treatment showed certain degree of antimicrobial effect but the most significant effect was obtained with the essential oils from plant under (magnetic water treatment+ magnetic iron at rate of 150 mg/Kg) The antimicrobial effect may be attributed to the major compounds including camphor and 2-propenoic acid. Comphor is the main component of Comphor trees essential oils and it showed several biological activities including (anti-inflammatory, analgesic, insect repellant in various topical skin preparations) (Chenni *et al*., 2016). Propionic acid is a colorless liquid oil (that also showed anti- fungal activity and is used as an anti-microbial agent for food preservation) (Chen *et al*., 2013).

Numerous studies demonstrated that camphor is the major component of some essential oils that exhibited anti-bacterial and anti-fungal activity. Camphor isolated from (Rosemary Officinalis) exhibited antimicrobial activity against pathogens that cause in meat spoilage (Chen *et al*., 2013). Previous studies showed that sweet basil essential oil exhibited activity on *A.fluvus* fungi (Jakowienko *et al*., 2011). The same oil showed inhibitory effect on *Fusarium oxysporum* growth that infects tomato plants (Amani & Mohamed, 2015). Another study proved the antifungal potential of the sweet basil essential oil on Fusarium oxysporum, by inhibiting the growth of investigated Fusarium sp. and reducing growth of aerial mycelium in all tested species (Kocić-Tanackov *et al*., 2011).

Table (1): effect of sweet basil essential oil extract against microbial activity

TREATMENTS			**Mean diameter of inhibition zone of microbes (mm)**			
Water type	**Applications**	**Concentration**	**E.amylovora**	**A.oryzae**	**A.flavus**	**F.oxysporum**
Magnetic water (MW)	**Fe_3O_4 (mg/kg)**	**0.0**	16.83	10.99	0.00	11.99
		100	19.9	16.00	34.99	13.99
		150	27.00	13.99	37.99	25.01
	K_2SiO_3 (mg/L)	**0.0**	16.83	10.99	0.00	11.99
		100	24.03	0.00	0.00	0.00
		150	24.99	15.01	23.01	24.00
Ground water (GW)	**Fe_3O_4 (mg/kg)**	**0.0**	22.04	13.00	16.99	19.99
		100	15.99	9.99	0.00	0.00
		150	20.99	22.01	34.99	23.00
	K_2SiO_3	**0.0**	15.99	11.99	16.99	19.99

	(mg/L)	**100**	23.04	10.00	18.01	20.00
		150	21.00	0.00	0.00	0.00
LSD. at 0.05			**LSD. at 0.05**			
Water type			0.188	0.027	0.005	0.009
Applications			0.192	0.012	0.006	0.004
Concentration			0.901	0.01	0.008	0.005
Water type× concentration			0.231	0.014	0.012	0.007
Applications× Concentration			0.231	0.014	0.012	0.007
Water type ×Applications			0.189	0.012	0.009	0.006
Water type ×Applications × concentration			0.127	0.203	0.016	0.010

Chemical composition of essential oil for Thyme plant on some microbes

Thyme is a perennial shrub with an average height of about 20-40 cm. It belongs to the family ***Lamiaceae***. There are more than 50 different types of thyme, but the type of thyme "Thymus vulgaris" is the most popular and widespread species. The origin of this herb goes back to the Mediterranean region and is currently cultivated in many countries around the world such as Egypt, Spain, Italy, Morocco, and south Africa due to its suitability to different climatic conditions. Thyme is cultivated for its aromatic leaves, rich in essential oils and is used in the cooking and pharmaceutical industries.

Thyme is considering a valuable natural resource that is economically promising with increasing demand for it by the pharmaceutical, cosmetic and food industries. Thyme is a highly adaptable species to a variety of environmental conditions (Llorens-Molina & Vacas, 2016). Sixteen components of thyme essential oil components were identified:

including 1,8-cineole, camphene, α-terpinene, p-cymene, camphor, borneol, 3-cyclohexen-1-ol, α-terpinolene, cyclocolorenone, germacrene-D, hydroxymethyl, 6-trimethyl-3-methylbuta-3-oxatricyclooctane, 4-bromo-naphthalen amine, caryophyllene, longiverbenone, caryophyllene oxide.

The most potent antimicrobial effect of thyme essential oil compounds came from saltwater treatment. 1,8-cineole was the major compound (12.61%) in thyme essential oil obtained under (saltwater + Fe_3O_4 at rate 100mg/Kg) treatment followed by (saltwater + Fe_3O_4 at rate 150mg/Kg) treatment were (12.09%) and the result were in agreement with previous reports(Llorens-Molina & Vacas, 2016). The highest values of camphene and comphor were (1.968 % and 5.566%) observed under (saltwater + Fe_3O_4 at rate 100mg/Kg), treatment. The changes in essential oils constituents with different plants treatment may be attributed to saltwater which affects plant morphological characters such as plant height, cell division, cell elongation and photosynthesis. The plant in response to stress and these changes change in the production of secondary metabolites including essential oils as self-defense components against stress conditions. Stress conditions caused by the use of salt lead to an acceleration of the biosynthesis of the essential oil (Ezz El-Din *et al*., 2009).

The inhibitory activity of thyme essential oil against some economically important plant pathogenic microbes *Erwinia amylovora, Aspergillus flavus, Aspergillus oryzae and Fusarium oxysporum* are shown in table (2). Essential oils obtained from plants under different treatments showed antimicrobial effect. The most potent effect was produced by essential oil from plant under (salt water treatment+ magnetic Iron at rate of 150 mg/Kg) The antimicrobial effect of thyme essential oils was attributed to the presence of phenolic or aromatic compounds (Raveau *et al*., 2020). Previous reports indicated that the Aspergillus sp. was sensitive to essential oils from lemongrass, clove, oregano, and thyme(Střelková *et al*., 2021). Thyme essential oils showed potent antifungal effect against F. oxysporum (Palfi *et al*., 2019).

Table (2): effect of thyme essential oil extract against microbial activity

TREATMENTS			Mean diameter of inhibition zone of microbes (mm)			
Water type	**Applications**	**Concentration**	**E.amylovora**	**A.oryzae**	**A.flavus**	**F.oxysporum**
Magnetic water (MW)	**Fe_3O_4 (mg/kg)**	**0.0**	16.99	0.00	0.00	11.99
		100	24.99	0.00	0.00	15.01
		150	35.00	0.00	0.00	15.01
	K_2SiO_3 (mg/L)	**0.0**	16.99	0.00	0.00	11.99
		100	27.00	0.00	0.00	0.00
		150	26.99	0.00	0.00	0.00
Ground water (GW)	**Fe_3O_4 (mg/kg)**	**0.0**	31.00	0.00	0.00	17.99
		100	27.99	17.99	0.00	18.00
		150	30.00	32.00	0.00	17.99
	K_2SiO_3 (mg/L)	**0.0**	31.00	0.00	0.00	17.98
		100	21.99	25.99	0.00	20.01
		150	23.66	0.00	0.00	0.00
LSD. at 0.05			**LSD. at 0.05**			
Water type			0.004	0.010	0.00	0.004
Applications			0.006	0.003	0.00	0.006
Concentration			0.007	0.005	0.00	0.007
Water type× concentration			0.011	0.007	0.00	0.011
Applications× Concentration			0.011	0.007	0.00	0.011
Water type ×Applications			0.009	0.006	0.00	0.009
Water type ×Applications × concentration			0.015	0.010	0.00	0.015

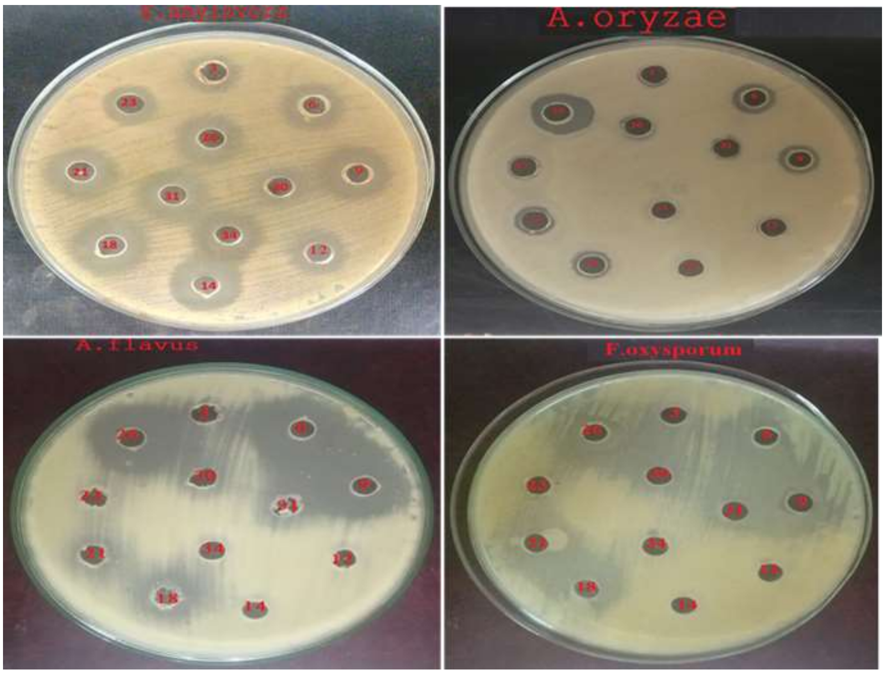

Figure (1): Antimicrobial activity of Sweet basil oil against E.amylovora, *A.oryzae, A.flavus* and *F.oxysporum.*

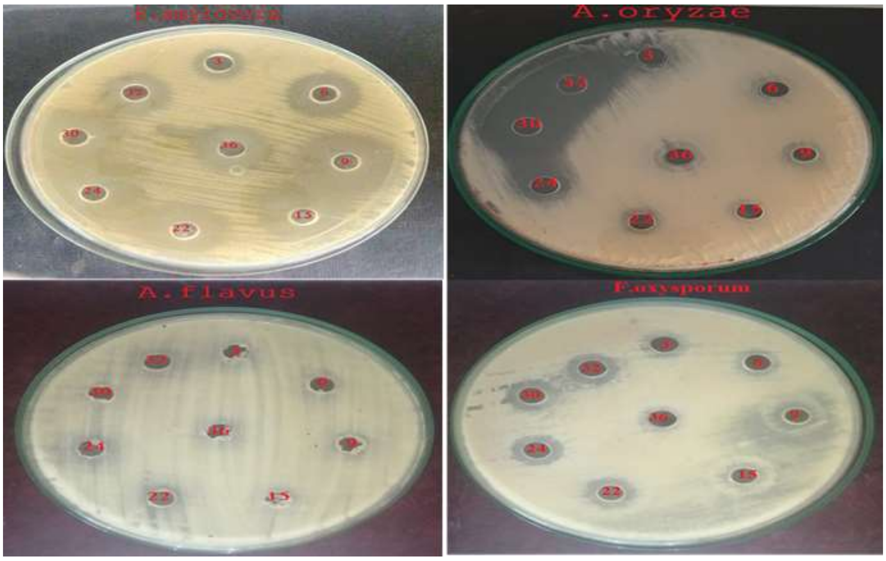

Figure (2): Antimicrobial activity of Thyme oil against *E.amylovora, A.oryzae, A.flavus* and *F.oxysporum.*

Conclusion

The study proved that Camphor and Camphane are the major component were isolated from Sweet Basil and Thyme essential oils that exhibited anti- bacterial and anti-fungal activity against *E.amylovora, A.oryzae, A.flavus* and *F.oxysporum.*

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