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In-vitro Antifungal Activity of Eco-friendly Essential Oils against Pathogenic Seed Borne Fungi

Ghada A. Youssef [(1)#], Asmaa S. Mohamed [(2)]
[(1)]Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria 2143, Egypt; [(2)]Institute of Graduate Studies and Research, Environmental Studies, Alexandria University, Alexandria, Egypt.

ESSENTIAL oils (EOs) as volatile products of plant secondary metabolism, possess significant antimicrobial activity, and have wide applications in the food industry and medicinal field. The purpose of this study focused on evaluating the antifungal properties of two commercial EOs, Chamomile (*Chamomilla recutita* L.) and Ginger (*Zingiber officinale* Roscoe). EOs were analyzed using gas chromatography-mass spectrometry (GC-MS). The most common compound identified in chamomile essential oil was α-bisabolol oxide A (49.09%) as a dominant compound, followed by En-yn-dicycloether (8.12%). The major compound of ginger essential oil was identified as isopulegol acetate (53.92%). The antimycotic potentiality of essential oils was detected *in vitro* by using agar disc diffusion method, microdilution assay for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) against three pathogenic fungal strains, *Penicillium chrysogenum, Aspergillus niger* and *Aspergillus flavus*. The pathogenic strains were isolated from a stored seed-borne pathogen such as bean, popcorn, and rice. The results indicated that chamomile EO was the most effective extract and showed a potent antifungal activity against all the selected fungi when compared to ginger EO with maximum inhibition zone (5.15±0.07cm) against *Penicillium chrysogenum*. The minimum inhibitory concentration and minimum fungicidal concentration of the chamomile EO on the test fungi were in the range of 1.25-2.5µg/ml and 2.5–5.0µg/ml, respectively. Chamomile EO extract would be a suitable candidate for further research to validate its role in pharmaceutical applications and agricultural purposes for safe and eco-friendly seed-treatments.

Keywords: Essential oils, Seed-borne pathogen, Antifungal activity, Minimum inhibitory concentration, Minimum fungicidal concentration.

Introduction

Deterioration of varied types of harvested and stored products viz. legumes, cereals, spices, fruits, vegetables, etc. is a chronic and highly sever problem due to the presence of ubiquitous molds of tropical climate with hot and high relative humidity. The good quality seeds play a major role for the production of healthy crops. These seed-borne fungi that are growing on stored seeds are one of the most significant and critical agent in the deterioration of seed quality. Several fungi are serious pathogens which might cause severe infection for seeds under field and storing conditions. Fungi like *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, etc. that are able to produce toxic secondary metabolites called mycotoxins (Sweeney & Dobson, 1998; Marin et al., 2013). The mycotoxins can be mutagenic, teratogenic, nephrotoxic, immunosuppressive, carcinogenic, and causing feed refusal (Alshannaq & Yu, 2017). These species are often classified as field fungi, usually pose a high mycotoxicological and phytopathological risk before or after harvest level in processed food and feed products (Logrieco et al., 2003).

Several strategies have been evidenced for

#Corresponding author email: aminghada66@gmail.com Tel.: (020100) 407-3123, Fax: 20 203 3911794
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preventing aflatoxin contamination of susceptible plants and crops (Cleveland & Bhatnagar, 1992). The application of chemical and physical approaches has led to serious environmental hazard impacts and health problems due to the toxic residues, hormonal imbalance, carcinogenicity, and spermatotoxicity (Pandey, 2003; Kumar et al., 2007). In recent years, there is an increasing demand to design an eco-friendly safe methods of reducing infection by aflatoxigenic Aspergilli and to inhibit aflatoxin biosynthesis for the development of resistance by micro-organisms (Tolouee et al., 2010). Plants contain a wide variety of secondary valuable metabolites, are considered a rich source of bioactive compounds such as tannins, terpenoids, alkaloids, and flavonoids which have free radical scavenging activity, antioxidant capacity, and a wide array of antifungal properties (Arif et al., 2009). Essential oils primarily have the ability to penetrate and disrupt the fungal cell wall, which disturb the permeabilization process. Interest has been focused on the potential applications of essential oils as alternative conventional synthetic and chemical additives. They are environmentally friendly, biodegradable, and do not remain toxic residues to contaminate the environment (Abdel-Kader et al., 2011; Yooussef et al., 2016).

Essential oils are aromatic oily liquids also called volatile odoriferous oils. They are extracted from various plant organs (seeds, bark, twigs, buds, stems, leaves, flowers, fruits, wood, and even the roots). Essential oils (EOs) are complex mixtures of natural volatile compounds synthesized naturally during the secondary metabolic activity, in which mono– and sesquitrpene constituents predominate, and their oxygen-substituted derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides) which are the combination of C, H, and O, and responsible for the biological activity (Bahraminejad et al., 2016). They can be obtained by fermentation, extraction, or expression (cold pressing), but the steam distillation is the most commonly method used for commercial scale production from most aromatic plants. Efficacy of EOs has been reported in several studies against pathogens and food contaminants that can be used as antibacterial additives (Djenane et al., 2012 ; El-Shouny et al., 2017; Othman & Abd El-Mongy, 2016; Stefanakis et al., 2013). EOs have been shown to possess significant antibacterial, antifungal, antiviral, insecticidal, anti-parasitic and antioxidant proprieties (Abd El-Zaher et al., 2019; Benjilali et al., 1986; Burt, 2004; Kordali et al., 2005; Kaloustian et al., 2008; Said et al., 2016). Although essential oils are known to be good antimicrobial agents, some microorganisms are stimulated by them and use EOs as a carbon energy source (Vokou et al., 2002). Currently, about 300 EOs are commercially significant, for the pharmacological attributes, alternative medicine, herbal therapies, food preservation, sanitary, agronomic, cosmetic, and perfume industries (Burt, 2004; Delamare et al., 2007). Recently, EO is respectable by public and green food consumers and widely used as food flavors with low concentrations in food processing to keep it safe and extend its shelf- life (Burt, 2004). The most interesting area of application is incorporated into packaging system, coated onto polymer surface, and provide multifunctions termed “active or smart packaging (Appendini & Hotchkiss, 2002).

Ginger is the rhizome of *Zingiber officinale* Roscoe, a herbaceous plant belonging to Zingiberaceae family (Salmon et al., 2012). The rhizome (ginger) is one of the popular spices in the world used in the conventional medicine for its health advantages (Salmon et al., 2012; Varakumar et al., 2017; Yeh et al., 2014). Ginger has been used to relieve dizziness, vomiting, nausea, headache, rheumatic diseases, and utilized in the treatment of gastrointestinal disturbances (Singh et al., 2016). It has anti-inflammatory (Dugasani et al., 2010), antioxidant (Singh et al., 2008), antipyretic (Ueki et al., 2008), anticancer (Jeong et al., 2009), antimicrobial and antifungal effects (Ali et al., 2008; Park et al., 2008).

Chamomile (*Matricaria chamomilla* L.) is an annual herbaceous plant which is cultivated commercially as a medicinal herb in Egypt. The flowers of *M. chamomilla* contain the blue essential oil due to the presence of terpenoid chamazulene which has been generally used for the anti-inflammatory, antimicrobial, antioxidant, antiplatelet, and even cancer-suppressive properties (Bhaskaran et al., 2010; McKay & Blumberg, 2006). Mitoshi et al. (2012) demonstrate the immunomodulatory and antioxidant activities of chamomile oil. *M. chamomilla* EO extract exhibited significant inhibitory action against a wide range of microbial strains (Cvetanović et al., 2015; Shikov et al., 2008; Franke & Schilcher, 2005).

The efficiency and potentiality of essential oils (EOs) has been detected in several studies against pathogens and food contaminants (Djenane et al., 2012), suggesting their applications in the food industry and the prolongation of their shelf- life (Benjilali et al., 1986; Burt, 2004; Fratianni et al., 2010; Dobre et al., 2011). EOs might play a significant role in future biotechnological approaches (Moradi et al., 2017; Arraiza et al., 2018). The present investigation deals with the evaluation of antifungal properties of two commercial EOs, ginger (*Zingiber officinale* Roscoe) and chamomile (*Chamomilla recutita* L.) against seed-borne pathogenic fungal strains isolated from some post harvested grains.

Materials and Methods

Plant material and extraction of essential oil

Fresh rhizomes of the Ginger (*Zingiber officinale* Roscoe) and flower head of Chamomile (*Chamomilla recutita* L.) were purchased from a specialized local market in Alexandria, Egypt. The obtained fresh plant samples were washed to remove dirt from the surface of the samples. One hundred grams of fresh flower-head of *Chamomilla recutita* L. and the rhizomes of *Zingiber officinale* Roscoe were cut and placed separately into the necked round extraction flask and soaked with water and allowed to boil for 4hrs. The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus (Chand et al., 2016). Essential oils (EOs) were collected, dried over anhydrous sodium sulfate and stored in dark glass bottles at 4°C until used for both phytochemical, antifungal, and safety evaluation studies.

Gas chromatography-mass spectrometry (GC/MS) analysis of essential oils (EOs)

GC/MS analysis of chamomile and ginger essential oils was done at the Faculty of Agriculture, Alexandria University. One ml of hexane was added to each EO sample in ratio 1:10ml and mixed well. Then, one µL was directly injected to GC/MS forthe analysis of chemical components of EOs, GC Ultra ISQ apparatus with automatic sampler 7683B series-injector and split/split less injection system. The GC was fitted with MS capillary column (TG-1MS, 30m, 0.32mm I.D., 0.25µm film thickness). The temperature of program was as follows: Temperature of injector 250°C, Pressure 146.99 Kilopascal (kPa), temperature of MS detector 280°C, temperature of oven 40°C for 4min, then gradient 4°C /min to 260°C, 4min hold time, 63min was the finaltime and transfer line temperature was 200°C. Helium was used as carrier gas at kPa pressure with flow 1ml/min, linear velocity 30cm/s.The mass spectrometer had a vacuum compensation on, solvent delay time 4min to avoid the solvent peak, split ratio 1:10 and electronic pressure control on. Scan time was 29-650 m/z. Ionization energy was set at 70eV. The components of the EOs were identified by matching their mass spectral fragmentation patterns with those reported in computerized MS- data bank spectral libraries NIST98 and WlLEY 138 (Sparkman, 2012).

Isolation of pathogenic fungi

The antifungal potency of each EO was evaluated using pathogenic fungal strains. The pathogens were isolated from different types of stored grains such as wheat, rice, popcorn, peanuts, andbean. The experiment was carried out on potato dextrose agar (PDA) medium. Three replicates were taken at a rate of 10 grains of each type/ petri plate. The seeds were placed on sterilized glass petri plates containing 20ml potato dextrose agar medium. The plates were incubated at 25±2°C for 3-4 days. At the end of the incubation period, Subculture (purification) of fungi growing out of seeds was prepared on suitable agar slants for further examination. The most obvious fungal strains were identified in the Mycology Center at Faculty of Science, Assiut University, Egypt.

Preparation of fungal suspensions

The fungal spores were harvested after seven days for proper sporulation by pouring mixture of sterile glycerol and distilled water to the surface of the plate and scraping the spores with sterile glass rod. The spores were later standardized before use. Inoculum of each culture was suspended into sterile normal saline solution (0.90% w/v of NaCl) and the suspension was gently homogenized. The turbidity of the fungal spore suspension was compared to a 0.5 McFarland standard. Sterile normal saline solution or inoculum of the culture was added until the fungal suspension matched the McFarland standard (Ivanova et al., 2013).

Antifungal properties of essential oils

The antimicrobial activities of EOs were tested against three fungal pathogenic isolates (*Penicillium chrysogenum, Aspergillus flavus,* and *Aspergillus niger*). Their efficacy was qualitatively and quantitatively assessed by the

presence or absence of inhibition zones by agar disc diffusion method, Minimum inhibitory concentrations (MICs), and Minimum fungicidal concentrations (MFCs) assay.

Agar disc diffusion method

Chamomile and ginger EOs were dissolved in DMSO (Dimethyl sulfoxide) 1:1 (v/v) to give stock solution after which they were mixed for total solubilization at 180 rpm for 10min. In screening of selected essential oils for antifungal activity by the disc diffusion method by Bauer et al. (1966), the appropriate solidified medium PDA was inoculated with 100µl of spore suspension (10^5 spores/ml) and spread over the plates using a sterile rod display. Sterile filter discs (Whatman no. 1, England, 6cm diameter) were saturated with 10µl of stock solutions of EOs and placed on the agar surface using forceps dipped in ethanol and flamed. Filter disc moistened with DMSO solution was placed on the seeded petri dish as a negative control. All petri dishes were sealed with sterile laboratory parafilm to avoid evaporation of the essential oils. The plates were left for 30min at room temperature to allow the oil diffusion, and then were incubated at 28°C, for 48-120hr. The mean diameter of inhibition zones was measured in centimeters, for each disc and evaluated for susceptibility or resistance using the comparative standard method (Dobre et al., 2011). Each test was performed in three replicates.

Minimal inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)

MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after overnight incubation. The assay was achieved by using a Microtiter plate-based assay, sterile 96-well plate by Ivanova et al. The fungal growth was measured as an increase in absorbance at 600nm using a spectrophotometer (Ivanova et al., 2013). MFC was detected at the lowest concentration of the oil at which no growth occurred on the plates after subculturing (Euloge et al., 2012). The MFCs of oils were measured by inoculation of the inhibited fungal discs of the oil-treated plates into freshly prepared PDA petri plates. The growth was observed after 72hrs of incubation at 28°C.

Statistical analysis

Data were expressed as Mean ± SE and tested for normal distribution then subjected to students' *t*-tests analysis for comparing each treatment with control. A value of $P<0.05$ was considered statistically significant. Data were processed with excel software at Microsoft Office 2010.

Results and Discussion

GC/MS analysis of essential oils

The results obtained by GC–MS analysis of chamomile essential oil are tabulated in Table 1. Twenty four compounds were identified, which accounting for 86.5% of total oil composition. Chamomile oil is known to be variable in composition and often contains α-bisabolol oxide A (49.09%) as a dominant component. En-yn-dicycloether (8.12%), α-bisabolol oxide B (3.46%), dendrolasin (2.49%), and chamzulene (2.33%), were observed as the major compounds. Other significant constituents were also detected. The blue color of the EO is due to the presence of chamazulene compound (Ciko et al., 2016). Various studies (Orav et al., 2001; Šalamon, 2004; Gupta et al., 2010; Khattab et al., 2010; Nurzyñska-Wierdak, 2011; Ghasemi et al., 2013; Siddiqui, 2014; Amiri & Sharafzadeh, 2014; Hajaji et al., 2017; Acimovic et al., 2018; Alireza, 2012) have shown that a valuable sesquiterpene called α-bisabolol oxide A was the main component, and this was in agreement with our results. Likewise, (Orav et al., 2001; Amiri & Sharafzadeh, 2014; Gawde et al., 2014; Stanojevic et al., 2016) reported the estimation of the presence of spiro-ether such as En-yn-dicycloether in chamomile essential oil.

The chemical composition of *Zingiber officinale* Roscoe EO is presented in Table 2. In this essential oil, twenty compounds were identified among which isopulegol acetate (53.92%) was the major component followed by β-sesquiphallendrene (10.26%), gramine (9.19%), 5-Heptadecene,1-bromo (4.86), zingiberene (3.5%), dilaurylthiodipropionate (3.01%) and palmitic acid (2.24%). Other significant constituents were also estimated but less than one percent. The wide variations in the chemical composition of essential oils were detected not only for the existence of different varieties of the plant but also might be attributed to the varied climatic conditions, seasonal factors, regions, stage of maturity and flowering, harvesting time of plants (El-Baroty et al., 2010; Nampoothiri et al., 2012; Hamad et al., 2016; Dai et al., 2013; Sultan et al., 2005).

TABLE 1. Quantitative chemical composition of *Chamomilla recutita* L. essential oil.

P.N	$t_{R\ (min)}$	Name of compound	Relative amount (%)	M.Wt
1	7.39	Artemisia ketone	0.37	152
2	13.05	Estragole	1.18	148
3	18.12	Cedrene	0.28	2.71
4	18.37	α-Curcumene	0.58	202
5	18.74	2,5- Piperazinedione,3-(phenylmethyl)-	0.72	204
6	19.04	α-Farnesne	0.51	204
7	20.47	Dendrolasin	2.49	218
8	22.12	Longipinocarveol, trans-	0.89	220
9	22.20	(-)-Isosativene	0.69	204
10	22.67	α-bisabolol oxide b	3.46	238
11	22.96	2-Isoamylpyrazine	3.55	150
12	23.09	B-Chamigrene	1.87	204
13	23.66	Chamzulene	2.33	184
14	25.22	α-bisabolol oxide a	49.09	238
15	25.77	Propanamide,N-cyclopropyl-3-(3-indolyl)-	0.36	228
16	26.75	En-yn-dicycloether	8.12	200
17	27.76	2-Cyano-3-methyl-3-(4-nitrophenyl)-propenoic acid, ethyl ester	0.69	260
18	30.03	Cyclobutane,1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-	2.25	512
19	32.29	bis(4 ethylphenyl)	1.35	280
20	33.01	Linoleic acid	2.10	252
21	34.61	13-Heptadecyn-1-ol	0.71	296
22	35.38	Heneicosane	0.27	338
23	36.11	Tetracosane	2.34	369
24	37.22	2,7-Dipropoxy-fluoren-9-onethiosemicarbazone Heptacosane	0.36	380

- P.N: Peak number, t_R: Retention time, M.Wt: Molecular weight
- The sum is not 100% because a lot of small unidentified peaks, making the whole sum.

TABLE 2. Quantitative chemical composition of *Zingiber officinale* Roscoe essential oil.

P.N	$t_{R\ (min)}$	Name of compound	Rrlative amount (%)	M.Wt
1	7.36	Artimisia ketone	0.5	152
2	13.04	Benzo[b]thiophene,2-methyl-	1.42	148
3	18.10	β-Sesquiphallendrene	10.26	204
4	18.37	Zingiberene	3.5	204
5	20.41	(-)-Spathulenol	1.75	220
6	22.18	Dilaurylthiodipropionate	3.01	514
7	22.62	12-Oxa[tetracyclo[5.2.1.1(2,6).1(8,11)]dodecan-10-ol,3-	1.57	238
8	25.19	acetoxy-	53.92	238
9	25.50	Isopulegol acetate	0.36	220
10	25.66	Aromadendrene oxide-(1)	0.36	238
11	25.73	α-bisabolol oxide a	0.32	228
12	26.71	Benzaldehyde,3-(4-methoxyphenoxy)-	9.19	174
13	27.72	Gramine	0.76	214
14	29.97	6-Methoxyharmalan	2.24	256
15	32.27	Palmitic acid	1.35	280
16	33.04	Linoleic acid	4.86	316
17	34.60	5-Heptadecene,1-bromo-	0.82	324
18	35.38	Tricosane	0.30	338
19	36.11	Tetracosane	2.67	101
20	37.22	1,3-Butanediamine	0.71	380
		Heptacosane		

- P.N: Peak number, t_R: Retention time, M.Wt: Molecular weight.
- The sum is not 100% because a lot of small unidentified peaks, making the whole sum**.**

Antifungal properties of essential oils

Agar disc diffusion method

The results revealed that the two selected EOs were potentially effective in suppressing fungal growth of tested pathogenic fungal isolates with variable potency. This was evidenced by the clear zone of inhibition produced by EO extracts against the tested fungi. Chamomile EO was found to be most efficacious and showed a strong antimycotic activity against all the selected strains when compared to ginger EO (Figs. 1, 2). The maximum inhibition zone (5.15±0.07cm) was exhibited by chamomile EO against *Penicillium chrysogenum*. This was followed in order by *Aspergillus niger* and *Aspergillus flavus* with values of 4.9±0.14 and 2.05 ±0.21cm, respectively. *P. chrysogenom* was found to have the highest susceptibility with the clear zone (4.15±0.07cm) followed by *A. flavus* and *A. niger,* respectively concerning to ginger EO. Similar to our results, the antifungal activity of the essential oil of *Matricaria chamomilla* is related to its terpenes type components (Pauli-Magnus & Meier, 2006). Ayran et al. (2018) reported that α-bisabolol from *Matricaria chamomilla* might inhibit fungal growth via specific inhibition of ergosterol biosynthesis. The results of this study agree with those of other researchers who explained that chamomile EO exhibited antimicrobial activity against a wide variety of bacteria and fungi (Tolouee et al., 2010; Roby et al., 2013; Kazemi, 2014; Al-Snafi, 2016; Stanojevic et al., 2016; Göger et al., 2018; Alireza, 2012). Also, these results are in accordance with recent works of (Attiya et al., 2018; Baratta et al., 1998; Ali et al., 2005; Senhaji et al., 2007; Ionica et al., 2016; Mir & Qureshi, 2017; Hussein & Joo, 2018), who showed that ginger essential oil exhibited an inhibitory effect against a wide range of pathogenic bacteria and fungi. It appears that there is a relationship between the chemical constituents of oil and its antimicrobial potential. Chamomile essential oil was rich in sesquiterpene hydrocarbon while ginger oil was rich in monoterpene hydrocarbon. Because both oils had different chemical profiles, differences in antimicrobial activity could be expected. The antimicrobial activity of chamomile and ginger essential oils are due to some of the major components present in the oil can penetrate the microbial membrane and react with the membrane proteins and enzymes as well as phospholipids bilayer, which cause disturbance of microbial enzyme system and disruption of the function of genetic material (Farag et al., 1989; El-Baroty et al., 2010; Abd El-Baky & El-Baroty, 2008; İşcan et al., 2016).

Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)

The effective concentrations, MIC and MFC values of chamomile and ginger essential oils against *Penicillium chrysogenum, Aspergillus flavus,* and *Aspegillus niger* are summarized in Table 3. The EO of chamomile showed the highest activity against fungal strains in variable degrees with MIC values ranging from (1.25 to 2.5µg/ml) comparing with ginger volatile oil (2.5 to 5µg/ml). The values of MIC and MFC for chamomile oil were 1.25 and 2.50µg/ml against *Penicillium chrysogenum*, respectively, which were significantly lower than those of ginger oil. In general, MFC value was higher than their respective MIC values by 2 fold approximately in the case of chamomile oil. The lower value of MIC compared to MFC value indicated that chamomile oil is fungistatic at lower concentrations and fungicidal at higher concentrations. Our results of MICs and MFCs were similar to those of Kazemi (2014), El-Baroty et al. (2010), Nerilo et al. (2016) and Sharma et al. (2016) who also observed antimicrobial activities of chamomile and ginger EOs against bacteria and fungi but with different values. Variation of the antimicrobial potentiality of different EOs may originate from differences in their chemical composition and volatile nature (Perczak et al., 2019). Also, it maybe due to the variety of test microorganism implicated and the type of medium used to evaluate antimicrobial potency. However, the present study suggested that chamomile and ginger EOs proved to be potentially effective against all test pathogenic fungi with variable degrees and can be used as natural preservatives to control food borne-pathogens.

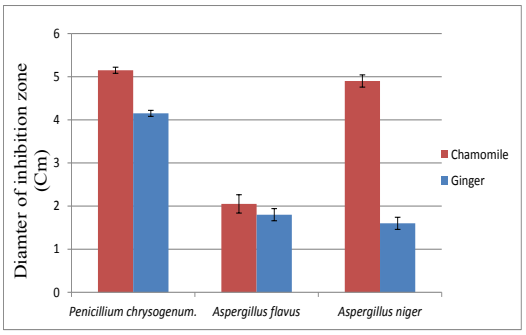

Fig. 1. Antifungal potential of chamomile and ginger essential oils by disc diffusion method against pathogenic fungal isolates.

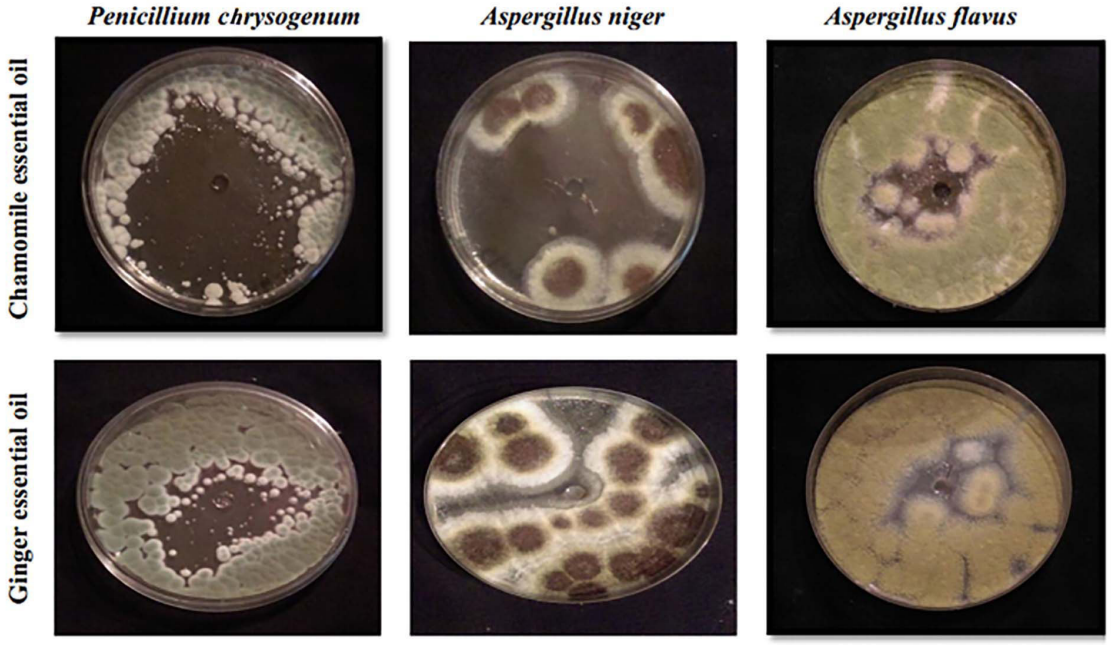

Fig. 2. Zones of growth inhibition (cm) showing the antifungal activity of chamomile and ginger essential oils against pathogenic fungal isolates.

TABLE 3. MICs and MFCs values of chamomile and ginger essential oils against pathogenic fungal strains.

Fungal strains	Essential oils (µg/ml)			
	Chamomile EO		Ginger EO	
	MIC (µg/ml)	MFC (µg/ml)	MIC (µg/ml)	MFC (µg/ml)
Penicillium chrysogenum	1.25	2.50	2.5	3.75
Aspergillus flavus	1.25	2.50	2.5	3.75
Aspergillus niger	2.50	5.0	5.0	5.50

Conclusion

The main goal of this study is to potentiate the antimicrobial efficiency of essential oil extracts against pathogenic seed-borne fungi. Results demonstrated promising use of both chamomile and ginger EOs as an environmental friendly botanical alternative against *Penicillium chrysogenum*, *Aspergillusniger,* and *Aspergillus flavus* as seed-borne isolates. EOs offer an alternative way to fight and control microbial contamination and indicate their natural sustainability to develop technologies eco-friendly, effective to protect against fungal and bacterial pathogens. However, the *in vitro* effects did not always provide anacceptable criterion. Further investigations should be conducted to detect the antimicrobial and antioxidant activities of EOs *in vivo*to verify their possible phytopathogenicity on plant/seed parts.

Disclosure statement: No potential conflicts of interest were reported by the authors.

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تقييم النشاط المضاد للفطريات معمليا من مستخلصات الزيوت الطبيعية العطرية الصديقه للبيئة ضد فطريات الحبوب الغذائية الممرضة

غادة أمين يوسف[1]، أسماء صلاح محمد [2]

[1] قسم النبات والميكروبيولوجى – كلية العلوم – جامعة الإسكندرية – الإسكندرية – مصر، [2] معهد الدراسات العليا والبحوث (الدراسات البيئية) – جامعة الإسكندرية – الإسكندرية – مصر.

في الآونة الأخيرة ، كان هناك اهتمام متزايد باستخدام المواد الطبيعية للموارد النباتية كبدائل لمبيدات الفطريات الكيميائية التقليدية. تمتلك الزيوت الطبيعية (EOs) المستخلصة من النباتات نشاطًا كبيرًا كمضادً للميكروبات ولها تطبيق واسع في المجال الطبى. تركز هذه الدراسة على تقييم الخواص المضادة للفطريات لنوعين من الزيوت النباتية الطبيعية الاكثر شيوعا وهما الزنجبيل (*Zingiber officinale Roscoe*) والكاموميل (*Chamomilla recutita* L.). حيث تم تمييز الزيوت الطبيعية وتحليلها باستخدام التحليل الطيفي الكتلي للغاز (GC-MS). ووجد أن المركبات الأكثر شيوعًا التي تم تحديدها من مستخلص زيت البابونج وهي أكسيد ألفا-بيسابولول (49.09%) كمركب مهيمن، إين-ين-ديسيكلوثير (8.12%)، وأكسيد ألفا-بيسابولول (3.46%)، الدندرولاسين (2.49%) وشامزولين (2.33 %). وتم التعرف على المركب الرئيسي لزيت الزنجبيل الأساسي وهو أسيتات إيزوليجول (53.92%) يليه s-sesquiphallendrene(10.26%)، الجين (9.19%) والزنجبرين (3.50%) على التوالي. ولقد اجريت عدة تجارب معملية لتعين النشاط المضاد للميكروبات لهذه الإنواع من الزيوت النباتية الطبيعية باستخدام طريقة إنتشار قرص الأجار(agar disc diffusion method) ومقايسة التخفيف الجزئي للحد الأدنى من التركيزات المثبطة (MICs) والحد الأدنى لتركيز مبيدات الفطريات (MFCs). وتم تعين النشاط المضاد للفطريات ضد ثلاث سلالات فطرية ممرضة، *Penicillum chrysogenum*، *Aspergillus niger* و *Aspergillus flavus*. حيث تم عزل هذه السلالات المسببة للأمراض من أنواع مختلفة من البذور المخزنة مثل الفول ونرة الفشار والأرز. أشارت النتائج إلى أن زيت البابونج EO هو المستخلص الأكثر فعالية وكفأة وأظهر نشاطا عاليا مضاد للفطريات ضد جميع السلالات المعزولة الممرضة عند مقارنته بزيت الزنجبيل EO. حيث كان أعلى تثبيط (5.15 ± 0.07 سم) ضد *Penicillium chrysogenum* وأقل قيمة MIC تتراوح من (1.25 إلى 2.5) ميكروغرام / مل) مقارنة مع زيت الزنجبيل (2.5 إلى 5 ميكروغرام / مل). كان الحد الأدنى للتركيز المثبط والتركيز الأدنى لمبيد الفطريات لزيت البابونج EO في حدود 1.25-2.5 ميكروغرام / مل و 2.5-5.0 ميكروغرام / مل ، على التوالي. وعلى ذلك سوف يكون زيت الكاموميل مستخلصا مناسبًا لإجراء مزيد من الأبحاث للتحقق من دوره في التطبيقات الصيدلانية والأغراض الزراعية كعلاج أمن للبذور وصديق للبيئة.