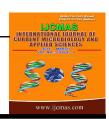
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Original Research Article

Antifungal Activities of *Cuminum cyminum* and *Pimpinella anisum*Essential Oils

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ABSTRACT

Keywords

Essential oils, antifungal activity, anise oil, cumin oil. Investigations were conducted to evaluate the antifungal activity of anise and cumin essential oils on mycelia growth of 90 isolates of fungi. The agar-well diffusion method was used to evaluate fungal growth inhibition at a concentration of 100%. Cumin oil was highly effective against all the isolates of tested fungi. It was completely inhibited mycelial growth of all fungi when added to solid medium. Anise oil had no inhibition, partial inhibition and completely inhibition against fungal isolates. Fifty-five isolates (61.1% of total isolates) were shown no inhibition, twenty-six isolates (28.9% of total isolates) were inhibited with different degrees and nine isolates (10% of total isolates) were completely inhibited by anise oil.

Introduction

Spices are aromatic or pungent vegetable substances used in minute quantities to enrich, alter or mask the flavour of food (Al-Mofleh, 2010). Spices have been traditionally used since ancient time for the preservation of food product as they have been reported to have antiseptic and disinfectant properties (Dawar *et al.*, 2008; Hashem and Alamri, 2010). They prolong the storage life of foods by preventing rancidity and oxidation of lipids (kelen and Tepe, 2008) or through bacteriostatic or bactericidal activity (Nazef *et al.*, 2008) and they perform the antifungal activity (Kotzekidou *et al.*, 2008). Spices and their

extracts were had various medicinal properties (Ayodele et al., 2009), they are affect digestion processes differently. Most of them stimulate the secretion of saliva. Curcuma, cayenne pepper, ginger, anis, mint, onions, fenugreek, and cumin enhance the synthesis of bile acids in the liver and their excretion in bile, what beneficially effects the digestion and absorption of lipids. Most of the prelisted spices stimulate the function of pancreatic enzymes (lipases, amylases and proteases), some also increase the activity of digestive enzymes of gastric mucosa (Srinivasan, 2005). Besides the effect on bile synthesis and enzyme activity, extracts from herbs

and spices accelerate the digestion and shorten the time of feed/food passage through the digestive tract (Suresh and Srinivasan, 2007).

Each spice has a unique aroma and flavor, which derive from compounds known as phytochemicals or secondary compounds (because they are secondary to the plant's basic metabolism). These chemicals evolved in plants to protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites (Walker, 1994). Anise oil (Anisi aetheroleum) is the essential oil obtained by steam distillation from dry ripe fruits of anise (P. anisum L.) or star anise (Illicum verum Hook. fil.) Pharmacopoeia, (European Essential oil of anise fruits contains from 80 to 95%, or more, trans-anethole as the main compound, followed by chavicol methyl ether (estragole), anisaldehyde and cis-anethole (Hansel et al., 1999; Tyler et al., 1988). Oil is used as an expectorant and carminative, especially in pediatrics, in cough mixtures and as a flavouring and spice. The important phenylpropanes, such as trans-anethole and chavicol methyl ether (estragole), have a stabilizing influence on the autonomous nervous system. Anise oil has been used in Iranian folk medicine for the treatment of some diseases, including seizures and epilepsy (Pourgholami et al., 1999). Anise has mild estrogenic effects, which explains the use of this plant in folk medicine for increasing milk secretion (Czygan, 1992) and for amenorrhea. Products that contain anise fruits extracts or anise essential oil may cause contact dermatitis, probably due to their anethole content (Martindale, 2002).

Composition of the cumin seed oil was extensively investigated many years ago. In 1981, Takahashi *et al.* (1981) reported

that cumin oil contained mint sulfide as a trace constituent. Twelve years later, Anon (1993) and Shaath and Azzo (1993) reported that the main constituents of Egyptian cumin seed oil were cumin aldehyde, b-pinene, g-terpinene, r-mentha-1,3- dien-7-al, r-mentha-1,4-dien-7-al and p-cymene. Composition of the cumin seed oil of Turkish origin was investigated by (Baser *et al.* 1992; Borges and Pino 1993) who found that Turkish cumin seed oil was characterized by high amount of cumin aldehyde, p-mentha-1,3-dien-7-al, p-mentha-1,4- dien-7-al, g-terpinene, p-cymene, b-pinene and perilla aldehyde.

Several studies have been shown that essential oils of spices and herbs demonstrate antifungal effects as reported by several researchers (Gulcin *et al.*, 2003; Angioni *et al.*, 2004; Lopez *et al.*, 2005; Rasooli *et al.*, 2006; Cruz *et al.*, 2007; Chen *et al.*, 2008; Mekawey *et al.*, 2009; Skrinjar *et al.*, 2009). This work was conducted to investigate the antifungal activity of anise oil and cumin oil on the growth of fungi.

Materials and Methods

The fungal strains used in this study that isolated from spices (anise and cumin) samples.

Inculation

The strains (eighty-seven species and 3 species varieties belonging to 32 genera) were cultivated on Czapek's agar medium for about ten days at 28°C until they were well sporulated, then by a sterile cork borer with 10 mm in diameter cut one disk from the pure culture and inoculated into 100 ml Erlenmeyer flask contained 50 ml sterilized distillated water to make spore suspension.

Antifungal screening:

The modified Collins *et al.* (1995) agarwell diffusion method was employed to determine the antifungal activity of oils; these essential oils were obtained from Ouss factory.

Agar-well diffusion method

Using modified Collins *et al.* (1995), one ml of spore suspension poured into sterile plate, 20 ml of sterile Czapek's medium poured into a sterile culture plate and allowed to set at room temperature for about 30 minutes, with a sterile cork borer, three bores about 10 mm in diameter were punched on the plates, 0.5 ml of essential oil was poured into each bore and the plates were incubated at 28° C for ten days.

Results and Discussion

The study reported here deals with the inhibitory effects of two spices essential oils on the growth of various fungi that

isolated from anise and cumin seeds (90 isolates).

The data presented in (Table 1), clearly show that cumin oil was highly effective against all the isolates of fungi tested. It is completely inhibited mycelial growth of all fungi when added to solid medium. Anise essential oil showed variable effects on fungal growth ranging from complete inhibition in case of sensitive isolates to limit or no inhibition on resistant isolates.

Nine isolates (10% of total isolates) were completely inhibited with anise oil and these were: Chaetomium oblatum, Drechslera erythrospila, D. euphorbiae, Epicoccum purpurascens, Fusarium sulphureum, Gibberella tricincta, Scopulariopsis brevicaulis, Setosphaeria rostrata and Stemphylium solani.

Twenty-six isolates (28.9% of total isolates) were inhibited by anise oil but with different effects and these were: Aspergillus clavatus, A. sydowii, A. ustus, A. versicolor, Botryotrichum piluliferum, Circinella *C*. funicola, muscae, Cladosporium Curvularia musae, brachyspora, Emericella nidulans var. Eurotium chevalieri, Fusarium oxysporum, Memnoniella subsimplex, Myrothecium verrucaria, Nectria Paecilomyces haematococca, carneus, aurantiogriseum, Р. Penicillium brevicompactum, P. duclauxii, funiculosum, P. spinulosum, Stachybotrys state of Melanopsamma pomiformis, Syncephalastrum racemosum, Trichoderma hamatum. T. viride and Ulocladium chartarum. The remaining fungal isolates (55 isolates) were not inhibited by anise oil.

Several studies have shown that essential oils of anise and cumin seeds and other

Table.1 Inhibition effect of essential oils (anise and cumin oils) on the growth of various fungal isolates, (diameter of inhibition zone in mm).

Fungal isolates	Inhibation zone in mm	Inhibation zone in mm
	Anise oil	Cumin oil
Acremonium furcatum	N.I	C.I
A. kiliense	N.I	C.I
A. rutilum	N.I	C.I
A. strictum	N.I	C.I
Alternaria alternata	N.I	C.I
A. brassicicola	N.I	C.I
A. chlamydospora	N.I	C.I
A. raphani	N.I	C.I
Aspergillus candidus	N.I	C.I
A. clavatus	14	C.I
A. flavus	N.I	C.I
A. fumigatus	N.I	C.I
A. niger	N.I	C.I
A. ochraceus	N.I	C.I
A. sulphureus	N.I	C.I
A. sydowii	30	C.I
A. tamarii	N.I	C.I
A. terreus var. aureus	N.I	C.I
A. ustus	17	C.I
A. versicolor	17	C.I
Botryotrichum piluliferum	20	C.I
Chaetomium anguipilium	N.I	C.I
C. atrobrunneum	N.I	C.I
C. citrinum	N.I	C.I
C. crispatum	N.I	C.I
C. dreyfussii	N.I	C.I
C. funicola	39	C.I
C. globosporum	N.I	C.I
C. globosum	N.I	C.I
C. jabalpurense	N.I	C.I
C. spiralotrichum	N.I	C.I
C. oblatum	C.I	C.I
C. subspirilliferum	N.I	C.I
C. uniporum	N.I	C.I
Circinella muscae	15	C.I
Cladosporium cladosporioides	N.I	C.I
C. musae	23	C.I
C. sphaerospermum	N.I	C.I
C. variabile	N.I	C.I
Cochliobolus lunatus	N.I	C.I
C. spicifer	N.I	C.I
C. tuberculatus	N.I	C.I
Cunninghamella echinulata	N.I	C.I
Curvularia brachyspora	26	C.I
C. lunata var. aeria	N.I	C.I

C. pallescens	N.I	C.I
Drechslera australiensis	N.I	C.I
D. erythrospila	C.I	C.I
D. euphorbiae	C.I	C.I
D.state of		
Trichometasphaeria		
pedicellata	N.I	C.I
Emericella nidulans var. lata	33	C.I
Epicoccum purpurascens	C.I	C.I
Eurotium chevalieri	20	C.I
Fusarium dimerum	N.I	C.I
F. merismoides	N.I	C.I
F. oxysporum	22	C.I
F. semitectum	N.I	C.I
F. sulphureum	C.I	C.I
Gibberella fujikuroi	N.I	C.I
G. tricincta	C.I	C.I
Memnoniella subsimplex	33	C.I
Monographella nivalis	N.I	C.I
Mucor circinelloides	N.I	C.I
M. hiemalis	N.I	C.I
M. racemosus	N.I	C.I
Myrothecium verrucaria	14	C.I
Nectria haematococca	16	C.I
Paecilomyces carneus	35	C.I
Penicillium aurantiogriseum	23	C.I
P. brevicompactum	19	C.I
P. chrysogenum	N.I	C.I
P. citrinum	N.I	C.I
P. corylophilum	N.I	C.I
P. duclauxii	15	C.I
P. funiculosum	31	C.I
P. purpurogenum	N.I	C.I
P. spinulosum	17	C.I
Phoma eupyrena	N.I	C.I
Rhizopus stolonifer	N.I	C.I
Scopulariopsis brevicaulis	C.I	C.I
S. sphaerospora	N.I	C.I
Setosphaeria rostrata	C.I	C.I
Stachybotrys state of		
Melanopsamma pomiformis	26	C.I
Stemphylium solani	C.I	C.I
Syncephalastrum racemosum	50	C.I
Trichoderma hamatum	36	C.I
T. viride	16	C.I
Ulocladium alternariae	N.I	C.I
U. botrytis	N.I	C.I
U. chartarum	18	C.I

C.I= completely inhibition, N.I= No inhibition.

spices demonstrate antifungal effects. Matan and Matan (2008) studied the antifungal activities of anise oil, lime oil, and tangerine oil against molds identified from rubberwood surfaces (Aspergillus Penicillium chrysogenum, and niger, Penicillium sp.) and determined the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC). Anise oil was the strongest inhibitor with the MIC and MFC of 40 ulml⁻¹ against *Penicillium* sp. and *A. niger* and 60 µlml⁻¹ against P. chrysogenum. Lime oil and tangerine oil were also effective against those molds at higher concentrations of 100–180 µlml⁻¹.

Skrinjar and Nemet (2009) studied the antimicrobial activity of essential oils extracted from garlic, mustard, cinnamon, cumin, clove, bay, thyme, basil, oregano, pepper, ginger, sage, rosemary etc., against most common bacteria and fungi that contaminate food. Fungi tested includes *Aspergillus* and *Cladosporium* species. It was found that cinnamon, cloves and mustard have very strong antimicrobial potential. Oils from cumin, oregano, sage, thyme and rosemary show medium inhibitory effect, and spices such as pepper and ginger have weak inhibitory effect.

Naeini *et al.* (2009) studied the anti-Candida albicans activity by the essential oils of 16 Iranian medicinal plants. They found that essential oils of Zataria multiflora, Thymus kotschyanus, Cuminum cyminum and Plargonium graveolens showed significant activity against C. albicans (P < 0.05).

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