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Article

Efficacy of *Origanum* essential oils for inhibition of potentially pathogenic fungi

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This study aimed to assess the efficacy of *O. vulgare L. and O. majorana L.* essential oil in inhibiting the growth and survival of potentially pathogenic fungal strains and also sought to evaluate the possible mechanisms involved in the establishment of the antifungal property of the tested essential oils through assays of osmotic stability and morphogenesis. Test strains included in this study were *Candida albicans* ATCC 7645, *C. tropicalis* LM-14, *C. krusei* LM-09, *Cryptococcus neoformans* FGF-5, *Aspergillus flavus* LM-02, *A. fumigatus* IPP-21, *T. rubrum* ATCC 28184, *T. mentagrophytes* LM-64, *Microsporum gypseum* ATCC 184, *M. canis* LM-36 and *Cladosporium herbarium* ATCC 26362. *O. vulgare* essential oil presented a MIC value of 80 µL/mL, while for *O. majorana* this was 160 µL/mL. *C. krusei* LM-09 was the only strain resistant to all assayed concentrations of both essential oils. *O. vulgare* and *O. majorana* essential oil at their MIC values provided a cidal effect against *C. albicans* ATCC 7645 after 4 h of exposure. *O. vulgare* essential oil at 80 µL/mL exhibited 100 % inhibition of the radial mycelia growth of *T. rubrum* ATCC 28184 and *M. canis* LM-36 for 14 days. Assayed fungus strain protected by sorbitol (osmo-protectant agent) grew in media containing higher concentrations of *O. vulgare* and *O. majorana* essential oil in comparison to media without sorbitol, suggesting some specificity of these essential oils for targeting cell wall in the fungi cell. Main morphological changes observed under light microscopy provided by the essential oil of *O. vulgare* in *A. flavus* LM-02 were decreased conidiation, leakage of cytoplasm, loss of pigmentation and disrupted cell structure indicating fungal wall degeneration. These results suggest that essential oils from *Origanum* could be regarded as a potential antifungal compound for controlling the growth of pathogen fungi and the occurrence of mycoses.

Uniterms: Essential oil. Pathogen fungi. *Origanum* sp./antifungal property.

O objetivo deste estudo foi observar a eficácia do óleo essencial de *O. vulgare L. e O. majorana L.* na inibição do crescimento e sobrevivência de cepas de fungos potencialmente patogênicas, bem como avaliar os possíveis mecanismos envolvidos no estabelecimento da propriedade antifúngica dos óleos essenciais testados através do ensaio de estabilidade osmótica e morfogênese. As cepas fúngicas utilizadas neste estudo foram *Candida albicans* ATCC 7645, *C. tropicalis* LM-14, *C. krusei* LM-09, *Cryptococcus neoformans* FGF-5, *Aspergillus flavus* LM-02, *A. fumigatus* IPP-21, *T. rubrum* ATCC 28184, *T. mentagrophytes* LM-64, *Microsporum gypseum* ATCC 184, *M. canis* LM-36 e *Cladosporium herbarium* ATCC 26362. O óleo essencial de *O. vulgare* apresentou valor de CIM de 80 µL/mL, enquanto o óleo essencial de *O. majorana* apresentou valor de CIM de 160 µL/mL. *C. krusei* LM-09 apresentou-se como a única cepa resistente a todas as concentrações ensaiadas de ambos os óleos essenciais. Os óleos essenciais testados quando ensaiadas em seu valor de CIM causaram um efeito fungicida contra *C. albicans* ATCC 7645 após 4 h de exposição. O óleo essencial de *O. vulgare* na concentração de 80 µL/mL exibiu uma total inibição do crescimento micelial radial de *T. rubrum* ATCC 28184 e *M. canis* LM-36

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ao longo de 14 dias. As cepas fúngicas ensaiadas quando tratadas com sorbitol (agente osmo-protetor) foram capazes de crescer em meio adicionado de mais altas concentrações dos óleos essenciais quando comparados ao meio não adicionado de sorbitol, sugerindo especificidade destes produtos a parede celular como alvo na célula fúngica. As principais alterações causadas pelo óleo essencial de *O. vulgare* sobre a morfologia de *A. flavus* LM-02 foram diminuída conidiação, perda de citoplasma, perda de pigmentação e ruptura da estrutura celular indicando degeneração da parede celular fúngica. Estes resultados sugerem que óleos essenciais de espécies de *Origanum* poderiam ser considerados como potenciais antifúngicos para o controle de fungos patógenos e ocorrência de micoses.

Unitermos: Óleo essencial. Patógenos fúngicos. *Origanum* sp./propriedade antifúngica.

INTRODUCTION

Over recent years, the incidence of important infections caused by fungi has increased. Opportunistic mycoses attack mainly immunologically suppressed individuals where this suppression is mainly secondary to degenerative diseases (e.g. cancer, diabetes), use of immunological suppressors or antibiotic therapy (Moellering Jr. *et al*., 2007). Most current antifungal drugs are either toxic (e.g. amphotericin B) or fungistatic (e.g. azoles) and new compounds have been actively sought (Frost *et al*., 1995).

Candida and Criptococcus genus are known as the yeasts most frequently involved in the etiology of mycotic infections (Lima *et al*., 2006). *Trichophyton*, *Microsporum* and *Epidermophyton* have the ability to invade keratinized tissues, such as hair, skin or nails, of humans causing dermatophytosis including tinea corporis, tinea pedis and onychomycosis (Weitzman, Summerbell, 1995; Koroishi *et al*., 2008). *Aspergillus flavus* and *A. parasiticus* causes different clinical manifestations of human aspergillosis such as cutaneous aspergillosis, aspergillar otomycosis, aspergillar onychomycosis, invasive lung aspergillosis and aspergillar sinusitis (Dubey *et al*., 2006).

The advent and widespread use of synthetic antimicrobials in the last century led to a lack of interest in plants as natural sources for antimicrobial drugs (Cowan, 1999). In recent years however, the situation has changed and the systematic screening of the biological interaction between microorganisms and plant products has been recognized as a valuable source of several compounds able to control the survival of pathogen microorganisms (Al-Fatimi *et al*., 2007). An extensive body of research has demonstrated that essential oils and their main components possess a wide spectrum of biological activity, which may be of great importance in several fields, from food chemistry to pharmaceutics (Knowles *et al*., 2000; Cristiani *et al.*, 2007).

Several plant families, especially the Lamiaceae, present prominent amounts of essential oil (yield > 2 %) (Baser, 1993; Baser, 1994). Given their broad variety of chemical characteristics and aroma, different species and biotypes of *Origanum* are widely used by the pharmaceutical and cosmetic industries, as a food flavor, for fragrance in perfumes and in alcoholic beverages (Sivropoulou *et al*., 1996; Novak *et al*., 2003; Aligianis *et al*., 2001). The *Origanum* species grow abundantly on stony slopes and rocky mountain areas at a wide range of altitudes (0-400 m) (Sahin *et al*., 2004).

O. vulgare L. (oregano) and *O. majorana* L. (marjoram) besides over fifty other types of the *Oregano* genus have been used in folk medicine to treat many illnesses as a spasmodic, antimicrobial, digestive, expectorant and aromatic for whooping and convulsive coughs (Dorman, Deans, 2000; Novak *et al.*, 2003). Some studies have found interesting antimicrobial activity in *Origanum* species, while *O. vulgare* and *O. majorana* have shown positive results in inhibiting the growth of pathogen microorganisms and in the synthesis of microbial metabolites (Marino *et al*., 2001; Baydar *et al.*, 2004).

The fungal cell wall acts as a protective barrier, prevents osmotic bursting from protoplast turgor and confers shape. The cell wall consists of many macromolecules such as β-glucans, chitin mannoproteins and other proteins. Many of these macromolecules are essential to the fungi and enzymes that synthesize these constituents could be important antifungal targets (Yamagushi *et al.*, 1982; Varona *et al.*, 1983). Damage to essential cell wall components by antifungal agents will lyse cells in the absence of an osmo-protectant, but cells will continue to growth if a suitable stabilizer is present in the medium. Reiss *et al.* (1992) and Frost *et al.* (1995) found that antifungal-treated fungi were viable if broth cultures were protected with sorbitol, but lysed if plated out on agar without osmotic support. According to these authors, assays of osmotic stability have been applied in fungi for assessing the mode of action of classical or emerging antibiotics. The sorbitol assay is claimed to be compatible with a range of natural products and pure chemicals (Kirsch *et al.*, 1986).

This study aimed to evaluate the efficacy of *O. vulgare* and *O. majorana* essential oils in inhibiting the

growth/survival of fungi, including moulds and yeasts, recognized as potential etiological agents of human mycosis. The investigation also sought to suggest possible mechanisms involved in the establishment of the antifungal property of the tested essential oils through the assay of osmotic stability and morphogenesis.

MATERIALS AND METHODS

Essential oils

The essential oils extracted from the leaves of *O. vulgare* and *O. majorana* were obtained from Ferquima Ind. e Com. Ltda. (Vargem Grande Paulista, São Paulo, Brazil) and their quality parameters (appearance, color, purity, odor, density - 20°C, refraction index - 20°C) were described in an accompanying technical report. The essential oils were tested in the range of 320 – 0.03 μL/mL. Solutions of essential oils were prepared in Sabouraud Broth (SB) using bacteriological agar (0.15 g/100mL) as the stabilizing agent according to Bennis *et al.* (2004).

Fungi

Candida albicans ATCC-7645, *C. tropicalis* LM-14, *C. krusei* LM-09, *Cryptococcus neoformans* FGF-5, *Aspergillus flavus* LM-02, *A. fumigatus* IPP-21, *T. rubrum* ATCC-28184, *T. mentagrophytes* LM-64, *Microsporum gypseum* ATCC 184, *M. canis* LM-36 and *Cladosporium herbarium* ATCC 26362 were used as test microorganisms. These strains were supplied by the Laboratory of Mycology, Department of Pharmaceutical Sciences, Federal University of Paraíba, João Pessoa, Brazil. Stock cultures were kept on Sabouraud Agar (SA) slants under refrigeration.

For antifungal assays, the fungi were cultivated on SA slants for 24-48 h at 37 ºC and for 10-14 days at 28 ºC, for yeasts and moulds, respectively. Suspensions of the microorganisms were prepared in sterile saline solution (0.9 g/100mL) with added Tween 80 (1 g/100 mL). Suspensions were adjusted to contain approximately 10^6 count forming unit per mL (cfu/mL) and 10^6 spores per mL (spores/mL) for yeasts and moulds, respectively, according to Cleeland and Squires (1991) and Carmo *et al.* (2008).

Synthetic antifungals

The sensitivity of the fungal strains to the standard antifungals amphotericin B, nistatin, fluconazole, 5-fluorocytosine and ketoconazole (Table I) was assessed by the solid medium diffusion procedure using filter paper discs (CECON Ltda, São Paulo, Brazil).

Minimal Inhibitory Concentration (MIC)

A solid medium diffusion technique employing wells in dishes was used to determine the MIC of the essential oils. A 1 mL quantity of the fungal suspension was uniformly spread in sterile SA Petri dishes. After the inoculum

TABLE I - Synthetic compounds applied in the antifungal assays

Antifungal	Assayed Concentration	Diameter of inhibition zones	Interpretation *
5-Fluorocytosine	1 mcg	> 20	Sensitive
(5- FC1)		20-10	Intermediary
		<10	Resistant
Amphotericin B	100 mcg	>10	Sensitive
(AB)		≤10	Intermediary or Resistant
Nistatin	100 U.I.	>10	Sensitive
(NI)		≤10	Resistant
Fluconazole	25 mcg	>19	Sensitive
(FLU)		19-14	Intermediary
		≤14	Resistant
Ketoconazole	50 mcg	>20	Sensitive
(KET)		20-10	Intermediary
		≤10	Resistant
5-Fluorocytosine *	1 mcg	>10	Sensitive
(5-FC1)		≤10	Intermediary or Resistant

*According to NCCLS (1997); ** only for *A. fumigatus*

absorption by SA, wells were made using sterile glass stems (diameter 6 mm) into which 50 µL volumes of the essential oils solutions were added. Subsequently, the system was incubated for 48 h at 37 ºC and for 7 days at 28 ºC for yeasts and moulds, respectively. At the end of the incubation period, the inhibition zones were measured. The lowest concentration of the essential oils able to develop inhibition zones with a diameter greater than or equal to 10 mm was considered the MIC (Hadacek, Greger, 2000).

Measure of fungal viability

The influence of *O. vulgare* essential oil (80 µL/mL), *O. majorana* essential oil (160 µL/mL) and ketoconazole (50 µL/mL) on the viability of *C. albicans* ATCC-7645 was determined using the viable cell count procedure. For this, 5 mL volumes of sterile double strength SB were inoculated with 1 mL of the yeast inocula. Subsequently, 4 mL of the antifungals solutions were added to the system and followed by shaking using a Vortex for 30 s. The system was incubated at 28 ºC. At different time intervals (0, 1, 2, 4, 6, 8, 10, 12 and 24 h) post-incubation, 1 mL of the suspension was serially diluted in sterile peptone buffer and spread on sterile SA Petri dishes, followed by incubation for 48 h at 28 ºC. Control flasks without essential oil or ketoconazole were tested in the same manner. After the incubation period, the mean number of cfu/mL was counted (Souza *et al*., 2007). The detection limit for viable cell count was 2 log cfu/mL.

Measurement of radial mycelial growth

Inhibition by *O. vulgare* essential oil (80 µL/mL) and ketoconakole (50µg/mL) of the radial mycelial growth of *T. rubrum* and *M. canis* was assessed using the poisoned substrate technique (dilution in solid medium). For this, a 2 mm plug taken from a 10 day-old mould culture grown on SA slants at 28 °C was placed in the center of a sterile SA Petri dish along with antifungals added at a suitable concentration and incubated at 28 °C. At different intervals (0, 2, 4, 6, 8, 10, 12 and 14 days) after incubation, the radial mycelial growth was measured (mm) using calipers. Control flasks without essential oil or ketoconazole were tested in the same manner (Daferera *et al*., 2003).

Sorbitol assay

The sorbitol assay was carried out in an attempt to unveil a possible mechanism involved in the establishment of the antifungal property of the tested essential oils. *A. flavus* LM-02 was included in this assay as a test strain. For this, 1 mL of the fungal suspension was added to 4 mL of the essential oil solutions at the suitable concentrations. Subsequently, 5 mL of double strength SB with the addition of the osmotic protectant sorbitol (final concentration 0.8 M) was introduced to the system followed by shaking for 30 s using a Vortex. The system was statically incubated for 7 days at 28 °C. MIC was defined as the lowest concentration of the essential oil required to completely prevent visible fungal growth. Control flasks of SB without sorbitol (standard media) were tested in the same manner. Based on the ability of sorbitol to act as an osmotic protector of fungi cell wall, the higher MIC values observed in the media with added sorbitol compared to the standard media implicated the cell wall as one of the possible cell targets of the essential oils (Frost *et al*., 1995).

Fungal morphogenesis study

In order to evaluate morphological alterations caused by the essential oil from *O. vulgare* in *A. flavus* LM-02, a sample of mycelium was taken from the periphery of a 10-day-old fungal colony grown on SA at 28 ºC containing the essential oil (80 µL/mL). The samples were fixed in lacto-phenol–cotton blue stain and observed under the microscope at 400 x to examine morphological abnormalities. The control assay without essential oil was tested in the same manner (Sharma, Tripathi, 2008).

All antifungal assays were carried out in triplicate and the results were expressed as an average of the two parallel assays.

RESULT AND DISCUSSION

Results of the MIC values of *O. vulgare* and *O. majorana* essential oil against several potentially pathogenic fungi are shown in Table II. The findings reveal that both of the essential oils provided inhibition of most of the fungi strains tested. *O. vulgare* essential oil showed an MIC value of 80 µL/mL, while *O. majorana* had an MIC value of 160 µL/mL. Three tested strains were resistant to all assayed concentrations of *O. vulgare* essential oil, versus five for *O. majorana* essential oil. *C. krusei* was the only strain resistant to both essential oils. These results suggested a wider spectrum of antifungal activity for *O. vulgare* essential oil. Likewise, Oliveira *et al.* (2009) found lower MIC values for *O. majorana* essential oil against *Staphylococcus aureus* and *Enterobacter* spp. in comparison to *O. vulgare* essential oil.

Carmo *et al*. (2008) found MIC values of O. vulgare essential oil ranging from 20 to 80 µL/mL against the Aspergilli species. For *A. flavus* and *A. fumigatus* these

TABLE II - MIC of the essential oil from *O. vulgare* L. and *O. majorana* L. against some potentially pathogenic fungi*

Microorganisms	Essential oils (µL/mL)	
	O. vulgare L.	*O. majorana* L.
C. albicans ATCC 7645	80	160
C. tropicalis LM-14	80	160
C. krusei LM-09	R	R
C. neoformans FGF-5	80	160
T. rubrum ATCC 28184	80	R
T. mentagrophytes LM-64	R	160
M. gypseum ATCC 184	R	160
M. canis LM-36	80	R
C. herbarium ATCC 26362	80	R
A. flavus LM-02	80	160
A. fumigatus IPP-21	80	R

* results expressed in diameter (mm) of inhibition zones; R: resistant to all assayed concentrations

authors found MIC of 20 and 40 µL/mL, respectively. Souza et al. (2007) detected MIC values for O. vulgare oil of between 10 and 20 µL/mL against Candida species. Bussata *et al*. (2008) found average MIC values for O. majorana essential oil of 1.1 and 1.6 mg/mL against positive and negative Gram bacteria, respectively.

Earlier studies of *in vitro* antimicrobial activity reported in the literature have found terpinen-4-ol as the main component of *O. majorana* essential oil (Ezzeddine *et al*., 2001; Vági *et al.*, 2005; Busatta *et al.*, 2008). Main compounds of *O. vulgare* essential oil have been identified as carvacrol and thymol (Souza *et al.*, 2008; Barros *et al.*, 2009). Other compounds such as α-pinene, *p*-cymene, *γ*-terpinene, caryophyllene oxide, germacrene, linalool and *trans*-sabinene have been found in both oils at low percentages.

The effect of the MIC of *O. vulgare* and *O. majorana* essential oil on the viability of *C. albicans* ATCC-7645 is shown in Figure 1. Both essential oils produced a sharp drop in fungal count throughout the evaluated intervals establishing a cidal effect after 4 h of exposure. From 4 h onwards, no recovery in cell count was noted. A compound was considered as having a strong fungicidal effect when able to cause a decrease of 99.9% (3 log cycles) of the initial inoculum (Espinell Ingroff, 1992). No significant difference ($p>0.05$) was found among the yeast counts for the broth with added essential oils and the standard antifungal ketoconazole. Ketoconazole was included in the growth kinetic assay because no tested fungi presented resistance to it in solid medium diffusion assay. The other antifungal showed a weak capacity in inhibiting at least one tested strain (Table III).

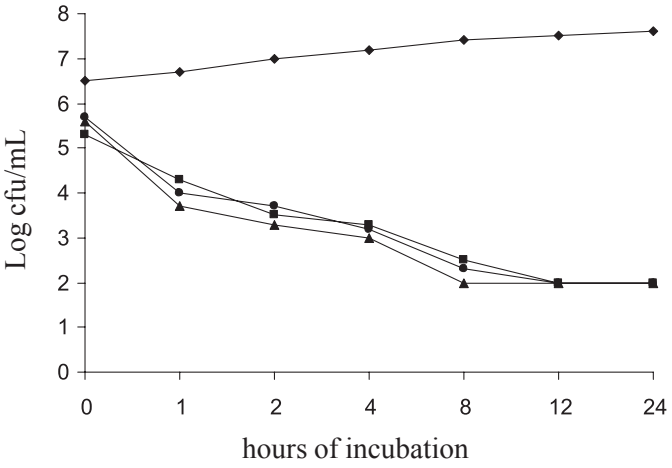

FIGURE 1 - Survivor curves for *C. albicans* ATCC 7645 in Sabouraud broth as a function of the antifungal compound: (◆): control (0 µL/mL); (▲): *O. vulgare* essential oil (80 µL/mL); (●): *O. majorana* essential oil (160 µL/mL; (■): ketoconazole (50 µg/mL).

TABLE III - Sensitivity of potentially pathogenic fungi to standard antifungals

Fungi	Standard antifungals				
	AB	NI	5FC	FLU	KET
C. albicans ATCC 7645	S	S	R	S	S
C. tropicalis LM-14	R	S	I	I	S
C. krusei LM-09	R	S	I	R	S
C. neoformans FGF-5	S	S	R	R	S
T. rubrum ATCC 28184	R	S	R	R	S
T. mentagrophytes LM-64	R	S	R	R	S
M. gypseum ATCC 184	R	R	R	R	I
M. canis LM-02	R	S	R	R	S
C. herbarium ATCC 26362	R	S	R	R	S
A. flavus LM-02	R	S	R	R	I
A. fumigatus IPP-21	R	S	R	R	I

* results expressed in diameter (mm) of inhibition zones; R: resistant; S: Sensitive

The effect of *O. vulgare* essential oil on the radial mycelial growth of *T. rubrum* ATCC-28184 and *M. canis* LM-36 in solid medium is shown in Figure 2 and 3, respectively. The essential oil at 80 µL/mL exhibited a fumigant effect against both fungi. *O. vulgare* provided a 100% lethal effect against both fungi after 2 days. *T. rubrum* and *M. canis* presented a slight increase in radial growth when exposed to 50 µg/mL of ketoconazole. However, the mould growth was always lower than that noted in the control assay. The control assay showed

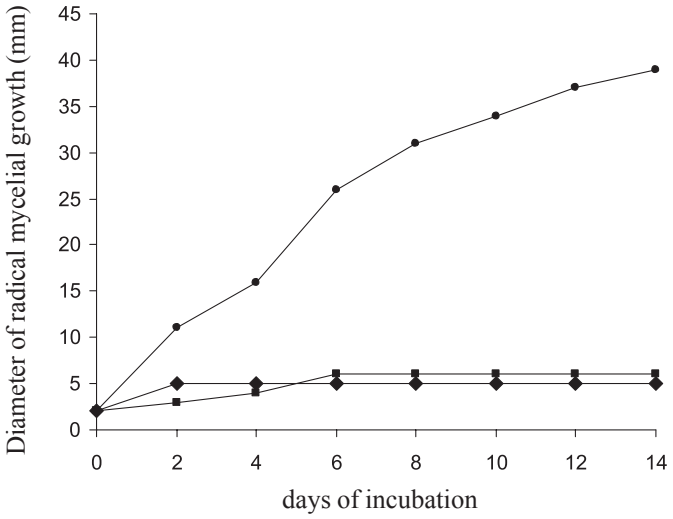

FIGURE 2 - Radial mycelia growth for *T. rubrum* ATCC 28184 as a function of the antifungal compound: (●): control (0 µL/mL); (♦): *O. vulgare* essential oil (80 µL/mL); (■): ketoconazole (50 µg/mL).

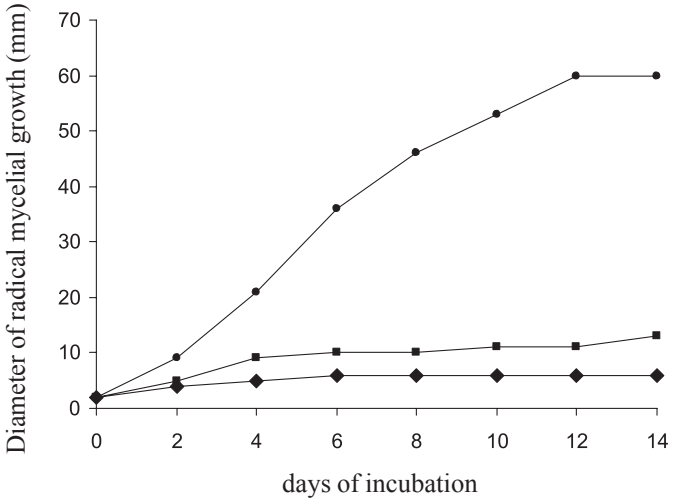

FIGURE 3 - Radial mycelia growth for *M. canis* LM-36 as a function of the antifungal compound: (-): control (0 µL/mL); (◆): *O. vulgare* essential oil (80 µL/mL); (■): ketoconazole (50 µg/mL).

a steady rate of mycelia growth for all the time points evaluated.

Few studies have focused on the effect of essential oils on the radial mycelial growth of pathogenic fungi. Carmo *et al.* (2008) found that the essential oil from *Cinnamomum zeylanicum* (20 – 80 µL/mL) caused inhibition of the mycelial growth of *A. niger* for 14 days.

The MIC of the essential oil from *O. vulgare* and *O. majorana* against *A. flavus* LM-02 by macrodilution assay in the presence and absence of sorbitol in the growth media are shown in Table IV. The MIC values found for + and – sorbitol treatments differed. The MIC without sorbitol was 0.125 and 4 µL/mL for *O. vulgare* and *O. majorana*, respectively, whereas for the assay with sorbitol values were 1.0 and 16. According to these findings, the assayed fungus strain protected by sorbitol grew in the presence of up to 0.06 and 8 µL/mL of *O. vulgare* and *O. majorana* essential oil (regarding the use of serially diluted solutions), respectively. Since sorbitol is known as an osmo-stabilizer protecting the cell wall from lysis caused by antifungal agents, these findings suggests some specificity of these essential oils for targeting cell wall in the mould cell.

TABLE IV - MIC of the essential oil from *O. vulgare* L. and *O. majorana* L. against *A. flavus* LM-02 in presence and absence of sorbitol

Essential oil	MIC (µL/mL)	
	– sorbitol	+ sorbitol
O. vulgare L.	0.125	4.0
O. majorana L.	0.06	8.0

(-): without sorbitol; (+): with sorbitol

According to Frost *et al.* (1995) the protection of fungal growth with sorbitol is not limited to β-(1,3) glucan synthesis inhibitors but can also be applied to inhibitors of synthesis of other cell wall polymers, and the mechanisms controlling cell wall synthesis. These authors also stated that the sorbitol protection assay is known as a broad spectrum screen for finding not only compounds that directly interfere in cell wall synthesis and assembly but also regulatory mechanisms involved in this process.

Macrodilution in broth assay was used for detecting the MIC in sorbitol assay. The MIC values for both assayed essential oils found in sorbitol assay were lower than those seen in solid medium diffusion assay. This disparity in results could largely be related to the variation of the neat essential oil on the disc or in the well, of the disc or well size, agar composition or the volatility of the essential oil in an open air system when using the solid medium diffusion technique (Pattnail *et al.*, 1996; Viljoen *et al.*, 2003). However, it is well established that the antifungal property of different essential oils ranges from a narrow to wide spectrum depending on the assayed essential oil, its concentration and fungal target (Burt, Reinders, 2003). Moreover, the oil volatility during the incubation period could possibly be responsible for higher MIC values when using the solid medium diffusion procedure in comparison to dilution assays (Sahin *et al.*, 2004; Duarte *et al.*, 2005).

Cells treated with compounds that interfere with cell wall biosynthesis often have distinct morphological characteristics. The changes in morphology could suggest the possible target or mode of action of the inhibitor. Observations of *A. flavus* LM-02 under the light microscope at 400 x magnification after exposure to 80 µL/mL of *O. vulgare* essential oil showed some morphological abnormalities (Figure 4).

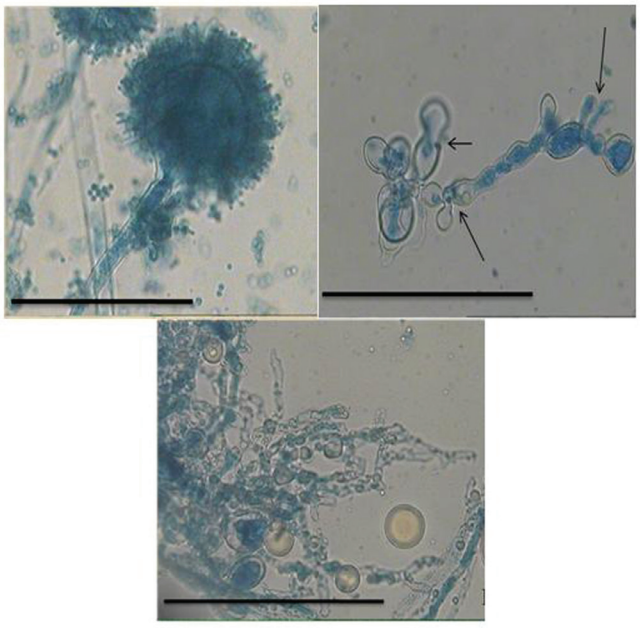

FIGURE 4 - Light microphotographs of *A. flavus* LM-02 mycelium growing on AS with or without *O. vulgare* (80 µL/mL) essential oil during 7 days of incubation at 25-28 °C. (a) Control conidial head of *A. flavus*, large and radiated, development of vesicle on conidiophore, conidia clearly visible, Bar 100 µm. (b) (b-c) Hyphae modification induced by *O. vulgare* essential oil showing anomalous structure with bud-like formation and loss of pigmentation; clear leakage of cytoplasm content and destruction of cell structure evidenced by disrupted hyphae integrity, Bar 100 µm.

Microscopic examination of the control mycelium (untreated cell) of *A. flavus* LM-02 revealed regular cell structure with homogenous cytoplasm, clearly visible conidia and profuse conidiation on a large and radiated conidial head. The mycelia cultivated in the medium with added essential oil appeared to present morphological changes with distortion of hyphaes. The morphological changes found were loss of cytoplasm content (empty hyphae), loss of pigmentation, distorted development of hyphae and empty hyphae. The hyphae become distorted with swelling in their structure and budded apical tips. In addition, the oil caused a clear absence of conidiation.

De Billeberk *et al.* (2001) and Rasooli and Abyaneh (2004) stated that these kinds of morphological changes in moulds caused by exposure to essential oils suggest that the mode of antifungal activity could include an attack on the cell wall and retraction of the cytoplasm in the hyphae ultimately resulting in death of mycelium. Moreover, Carmo *et al.* (2008) reported that these changes could also be related to the interference of the essential oil components in enzymatic reactions of cell wall synthesis affecting the fungal growth and morphogenesis.

These results are in agreement with the findings of the sorbitol assay, reinforcing the theory of the cell wall as a target for the antifungal activity of the assayed essential oil. Frost *et al.* (1995) stated that combining the analysis of growth with sorbitol assay and the study of morphology characteristics of cells (Sorbitol Protection and Analysis of Morphology – SPAM) could lead to a suitable procedure for detection of cell wall-acting antifungal agents.

Velluti *et al.* (2003) suggested that antimicrobial activity of essential oil depends on the chemical structure of its components. Phenolic compounds known to be constituents of *O. vulgar*e and *O. majorana* essential oil have antimicrobial properties attributable to the presence of an aromatic group known to be reactive and to form hydrogen bonds with active sites of target enzymes (Dimitriević *et al.*, 2007; Souza *et al.*, 2007). However, it has also been suggested that the effectiveness of complete essential oils is higher than the activity of each separate compound (Milos *et al.*, 2000).

In conclusion, our results indicate that essential oils from *Origanum* species could have a practical and promising use in the inhibition of fungal growth. More specifically, the broad inhibition of fungal growth by the essential oils from *O. vulgare* and *O. majorana* justifies their possible rational use as an alternative antifungal compound to control the growth of pathogen fungi and the occurrence of mycoses.

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