GROWTH-INHIBITORY EFFECTS OF FARNESOL AGAINST *SCEDOSPORIUM BOYDII* AND *LOMENTOSPORA PROLIFICANS*

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Abstract. *Scedosporium boydii* and *Lomentospora prolificans* are filamentous fungi reported to cause infection in immunocompromized individuals. We studied the effect of farnesol to inhibit growth of *S. boydii* and *L. prolificans* by measuring colony diameter and determining minimal effective concentration (MEC). *S. boydii* and *L. prolificans* were grown on Sabouraud dextrose agar (SDA) at 37°C for 5 days. Conidia were collected and adjusted to a concentration of 10^4 conidia/ml. Twenty microliters of conidia suspension was placed in each well of a six-well plate containing serial dilutions of farnesol (10 μM, 100 μM, 1,000 μM, and 10,000 μM) in SDA. Colony morphology and diameter were observed on days 1, 2, 3, and 4. Farnesol at concentrations of 1,000 μM or higher caused the colony diameter of both *S. boydii* and *L. prolificans* to be smaller than untreated controls in a dose-dependent manner. The MEC of farnesol to inhibit growth of both *S. boydii* and *L. prolificans* was 3.2 mM. This study reveals the antifungal property of farnesol against *S. boydii* and *L. prolificans*, which can be used for further study as an alternative antifungal agent against these fungal infections.

Keywords: *Scedosporium boydii, Lomentospora prolificans*, farnesol, minimal effective concentration

INTRODUCTION

Scedosporium boydii and *Lomentospora prolificans* are saprophytic fungi isolated from areas where human have had an influence, including playgrounds, in sewage and in industrial and agricultural areas (Harun *et al*, 2010; Rougeron *et al*, 2015). These fungi can cause life-threatening infections among immunocompromized individuals. *S. boydii* has been isolated from patients with cystic fibrosis (Cooley *et al*, 2007). *S. boydii* can cause mycetomas, arthritis, meningitis, brain abscesses, pneumonia and disseminated disease (Cortez

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et al, 2008). A fatal case of central nervous system infection caused by *S. boydii* was reported in an immunocompetent person who nearly drowned in polluted water (Kowacs *et al*, 2004). Infection of the bone has been reported in a post-traumatic immunocompetent patient **(**Steinbach *et al*, 2003**).** However, disseminated infection is more common in immunocompromized patients (Rabodonirina *et al*, 1994; Husain *et al*, 2005; Holmes *et al*, 2013).

S. boydii and *L. prolificans* have been found to be resistant to many antifungal drugs; voriconazole, miconazole, ketoconazole, itraconazole, fluconazole, flucytosine and amphotericin B have been shown to exhibit low growth-inhibitory antifungal activities (high MIC) against *L. prolificans* (Cuenca-Estrella *et al*, 1999; Meletiadis *et al*, 2002). Eventhough, *S. boydii* is susceptible to several antifungal drugs such as voriconazole, posaconazole, miconazole itraconazole, ketoconazole, and amphotericin B (Cuenca-Estrella *et al*, 2008), resistance to fluconazole and amphotericin B has been reported (Muñoz *et al*, 2000).

Quorum sensing (QS) is a process of cell-cell communication involving in the production, secretion, and detection of chemically signal molecules in a population dependent manner (Albuquerque and Casadevall, 2012; Rutherford and Bassler, 2012). Some microorganisms communicate with each other by releasing small substances called quorum sensing molecules (QSM). Farnesol ($C_{15}H_{26}O$; molecular weight, 222.37) was originally isolated from *Candida albicans* (Hornby *et al*, 2001). It plays an important role in several biological activities of yeasts and molds. Several studies have shown that farnesol can block germ tube formation and biofilm development in *C. albicans* (Ramage *et al*, 2002; Mosel *et al*, 2005), inhibit macroconidia germination (Semighini *et al*, 2008), induce apoptosis (Shirtliff *et al*, 2009; Wang *et al*, 2014) and inhibit fungal growth (Derengowski *et al*, 2009; Cordeiro *et al*, 2012; Yu *et al*, 2012). Moreover, farnesol has been shown to be involved in preventing overpopulation and nutrient competition, particularly in biofilm community (Ramage *et al*, 2002).

In our study, the antifungal activity of farnesol against *S. boydii* and *L. prolificans* was determined by minimal effective concentration (MEC). MEC is a method to access the lowest concentration, which the fungus reveals microscopic morphological changes, especially for echinocandins group (Imhof *et al*, 2003).

No published paper described the effect of farnesol against *S. boydii* and *L. prolificans*. Therefore, we aimed to study the growth inhibitory effects of farnesol against *S. boydii* and *L. prolificans* in order to determine a potentially antifungal agent against these fungi.

MATERIALS AND METHODS

Fungal strains

The fungal specimens used for our study were *S. boydii* CBS 120157 and *L. prolificans* CM 324 which were kindly provided by Dr Ana Alastruey-Izquierdo (Servicio de Micología, Instituto de Salud Carlos III, Madrid, Spain).

Growth conditions

S. boydii CBS 120157 and *L. prolificans* CM 324 were grown on Sabouraud Dextrose Agar (SDA) (Oxoid, Hampshire, UK) slant for 5 days at 37°C. Conidia were collected by washing with sterile phosphate-buffered saline (PBS, pH 7.2) and adjusted to a concentration of 10^4 conidia/ml.

Effect of farnesol on *S. boydii* and *L. prolificans* growth

Twenty microliters of conidia suspension was placed in each well of a six-well plate containing serial dilutions (10 μM, 100 μM, 1,000 μM, and 10,000 μM) of farnesol (Sigma-Aldrich, St Louis, MO) in SDA. A stock solution of 1 M farnesol was prepared by dissolving farnesol in ethanol. The stock solution was then diluted to the desired concentration using SDA. The plate was incubated at 25°C and the colony morphology was observed and the colony diameter was measured on days 1, 2, 3, and 4. The plates were monitored for 10 days. Wells containing 1% (v/v) ethanol (farnesol diluent) and farnesol-free SDA were also included as controls. The study was performed in triplicate.

Farnesol *in vitro* susceptibility testing

Antifungal susceptibility testing was performed using broth microdilution and observing for filamentous fungi, according to Clinical and Laboratory Standard Institute (CLSI) guideline M38-A2 (CLSI, 2008). We tested the antifungal efficacy of farnesol at concentrations ranging from 0.1 mM to 51.2 mM. Results were assessed after 72 hours of incubation at 37°C. We determined the minimum effective concentration (MEC) of farnesol by determining the lowest drug concentration that resulted in reduced growth of round and hyphal fungal forms compared to the hyphal growth seen in the control wells using a microtiter plate reading mirror (Cooke Engineering, Alexandria, VA). We conducted this study in duplicate.

Table 1
Colony diameters of farnesol-treated *Scedosporium boydii* and *Lomentospora prolificans* after incubation at 25°C.

Fungal strain/Culture condition	Colony diameter in mm (mean ± SD)		
	Day 2	Day 3	Day 4
S. boydii			
SDA	9.33 ± 0.58	15.0 ± 0	21.0 ± 0
SDA + ethanol	9.33 ± 0.58	15 ± 0	21 ± 0
SDA + 10 μM farnesol	8.67 ± 1.15	15 ± 0	21 ± 0
SDA + 100 μM farnesol	8.0 ± 1.0	14.67 ± 0.58	20.67 ± 0.58
SDA + 1,000 μM farnesol	NG[a,b,c]	8 ± 0[a,b,c]	13.67 ± 0.58[a,b,c]
SDA + 10,000 μM farnesol	NG[a,b,c]	NG[a,b,c]	13.0 ± 0[a,b,c]
L. prolificans			
SDA	7.67 ± 0.58	10.0 ± 0	15.0 ± 0
SDA + ethanol	7.67 ± 0.58	10.0 ± 0	15.0 ± 0
SDA + 10 μM farnesol	8.0 ± 0	10.0 ± 0	15.0 ± 0
SDA + 100 μM farnesol	6.67 ± 0.58[b]	10.0 ± 0	13.0 ± 0[a,b]
SDA + 1,000 μM farnesol	NG[a,b,c]	5.0 ± 0[a,b,c]	8.0 ± 0[a,b,c]
SDA + 10,000 μM farnesol	NG[a,b,c]	5.0 ± 0[a,b,c]	8.0 ± 0[a,b,c]

NG, No growth. [a]$p < 0.05$ compared with the colony diameter of fungi grown on SDA alone. [b]$p < 0.05$ compared with the colony diameter of fungi grown on SDA containing 10 μM farnesol. [c]$p < 0.05$ compared with the colony diameter of fungi grown on SDA containing 100 μM farnesol.

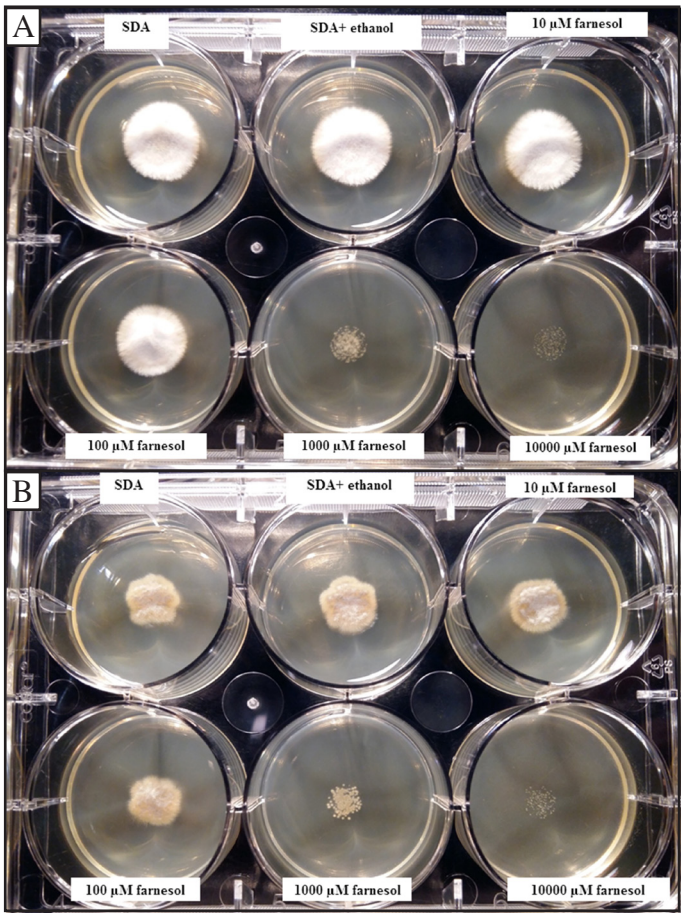


Fig 1A–Colony morphology of *Scedosporium boydii* (A) and *Lomentospora prolificans* (B) on SDA, SDA+ethanol and SDA+serial dilutions of farnesol after incubation at 25°C for 4 days.

Statistical analysis

The differences in the mean colony diameters of *S. boydii* and *L. prolificans* compared to controls at each concentration of farnesol were assessed with the Mann-Whitney test. A *p*-value of <0.05 was considered statistically significant.

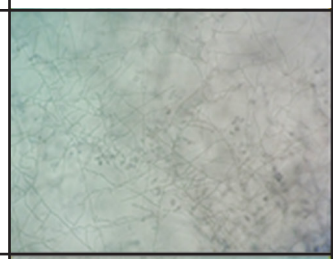
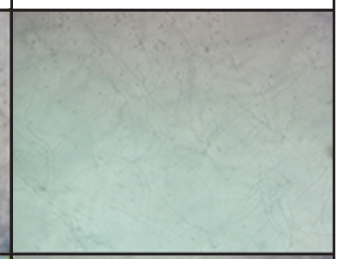
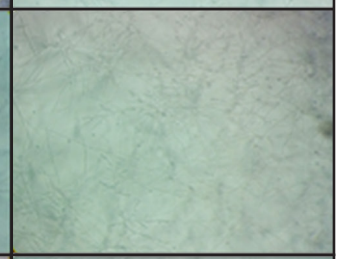
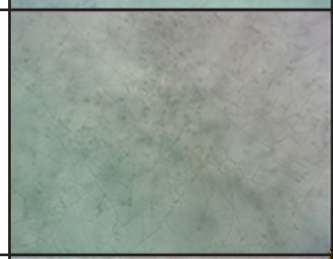
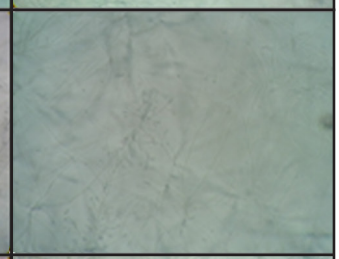
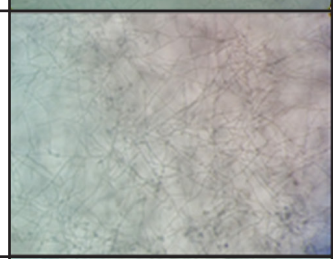
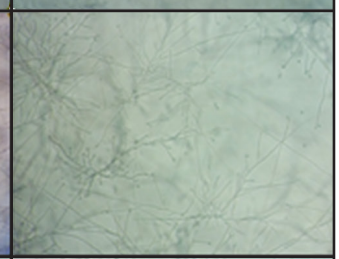
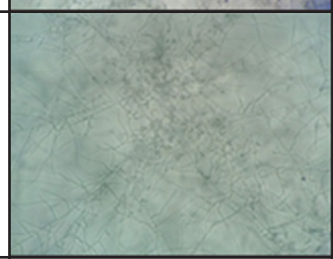
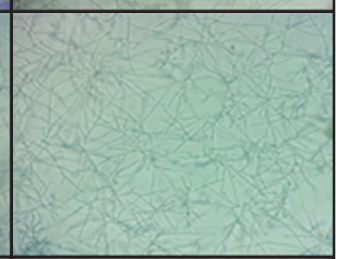
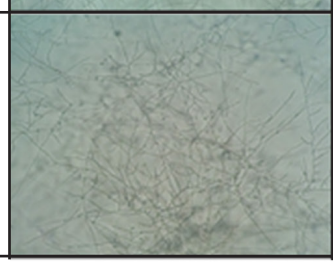
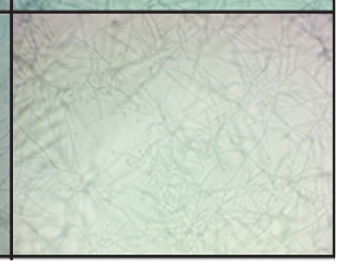
RESULTS

Effect of farnesol on the growth of *S. boydii* and *L. prolificans*

Fungal colonies were seen after 2 days incubation. When *S. boydii* was grown on SDA containing 1% (v/v) ethanol (SDA + ethanol) (control), the colony diameter increased from day 2 to day 4 in the same rate as the colonies grown on SDA alone (Table 1). When *S. boydii* was grown on the lowest tested concentration of farnesol (10 µM), the colony diameters increased at the same rate as the colonies grown on SDA and SDA + ethanol. However, at higher concentrations (1,000 and 10,000 µM) of farnesol, the colony of *S. boydii* grew at significantly slower rates than the colonies grown on SDA ($p<0.05$). At lower concentrations (10 and 100 µM), farnesol did not inhibit growth of *S. boydii* but at higer concentrations (1,000 and 10,000 µM), farnesol did slow growth of *S. boydii*.

When *L. prolificans* was grown on SDA alone, the colonies grew at the same rate as colonies grown on SDA + ethanol (Table 1). At a farnesol concentration of 10 µM, *L. prolificans* grew at the same rate as colonies grown on SAD and SAD + ethanol. At farnesol concentrations of 100 µM or higher, *L. prolificans* grew at significantly slow rates than colonies grown on SDA and SDA + ethanol ($p<0.05$) (Table 1).

Photomicrographs of *S. boydii* and *L. prolificans* are shown in Fig 1A and 1B. When *S. boydii* was grown on SDA, SDA + ethanol, and low concentrations of farnesol (10 or 100 µM), the colonies on day 4

Farnesol concentration (mM)	*S. boydii*	*L. prolificans*
Control		
0.1 mM		
0.2 mM		
0.4 mM		
0.8 mM		
1.6 mM		

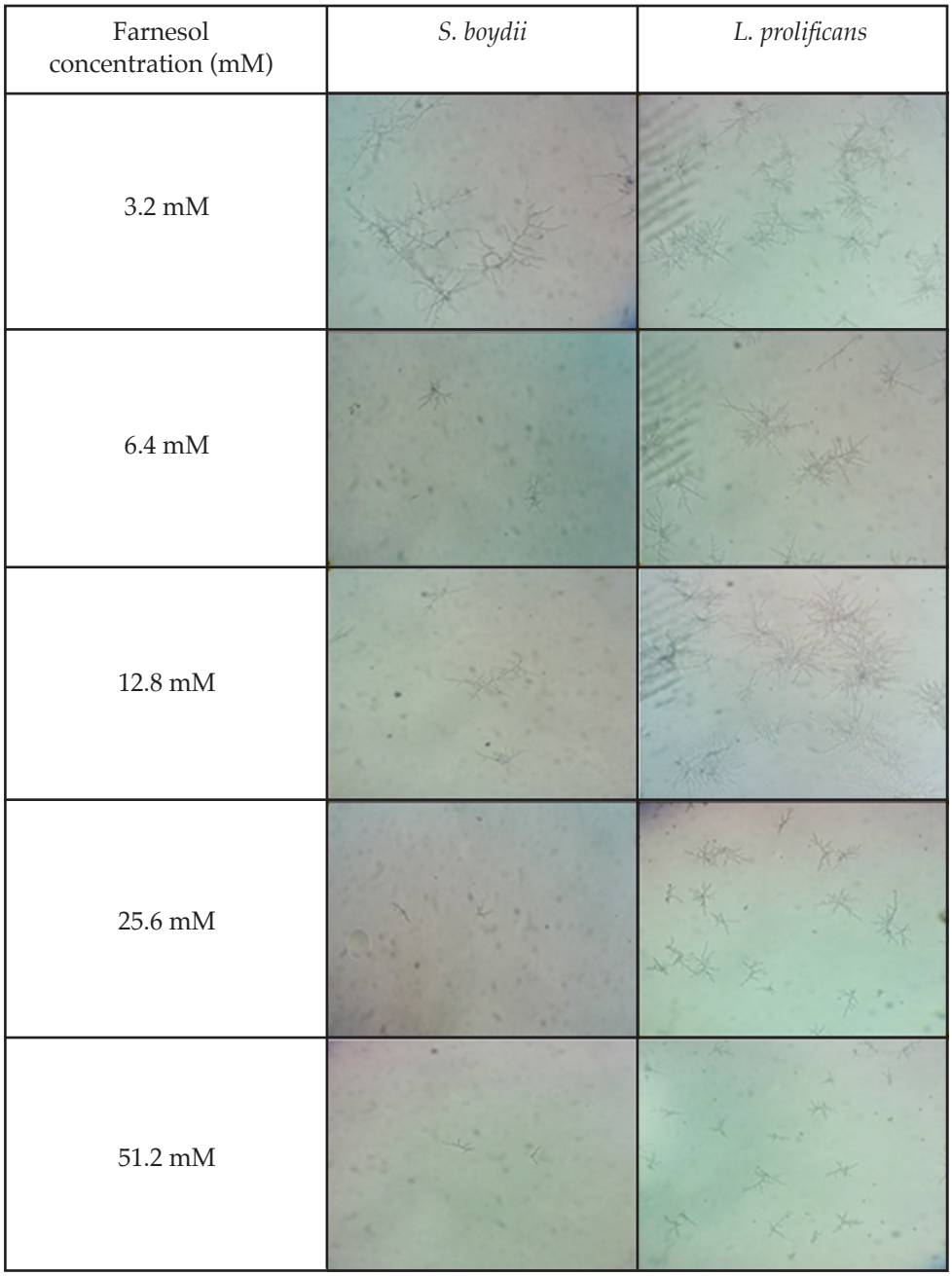


Fig 2–Farnesol *in vitro* susceptibility testing. Serial dilutions of farnesol from 0.1 mM to 51.2 mM were carried out. After incubation at 37 °C for 72 hours, we investigated the minimum effective concentration (MEC), resulting in inhibited growth of round and hyphal fungal forms.

were white and cottony in texture. When *S. boydii* was grown on higher concentrations of farnesol (1,000 and 10,000 μM) colony growth was inhibited (Fig 1A).

When *L. prolificans* was grown on SDA, SDA + ethanol and a low concentration of farnesol (10 μM), brownish colonies were formed. When *L. prolificans* was grown on higher concentrations of farnesol (100-10,000 μM) colony growth was inhibited (Fig 1B).

Farnesol *in vitro* susceptibility testing

The MEC of farnesol that caused morphological alteration in both *S. boydii* and *L. prolificans* hyphae was 3.2 mM for both fungi. At concentrations of 0.1-1.6 mM farnesol, the morphology of the tested fungi was not affected (Fig 2).

DISCUSSION

Other studies have investigated the effect of farnesol on the growth and development of yeast and filamentous fungi (Mosel *et al*, 2005; Semighini *et al*, 2008; Derengowski *et al*, 2009), but ours is the first study to evaluate the effect of farnesol on *S. boydii* and *L. prolificans*. In our study, farnesol inhibited the growth of these fungi in a concentration-dependent manner. A previous study reported farnesol inhibited the switch from yeast to hyphae in *C. albicans* but did not affect preexisting hyphae (Nickerson *et al*, 2006). Farnesol has also been shown to affect the growth of *C. albicans* growing as yeast (Uppuluri *et al*, 2007). Farnesol has also been shown to impair colony development of *Penicillium expansum*: farnesol-treated colonies were smaller than control colonies (Liu *et al*, 2009).

In our study, intermediate concentrations (100 μM) of farnesol inhibited *L. prolificans* growth but not *S. boydii*. However, higher concentrations did affect growth of both *S. boydii* and *L. prolificans* in a concentration-dependent manner. The yeast-to-hyphae transition of *C. alibicans* was inhibited by farnesol in a concentration-dependent manner (Mosel *et al*, 2005).

In our study, a relatively high concentration of farnesol (MEC = 3.2 mM) was needed to inhibit studied fungi and cause morphological changes. Farnesol has been found to have antifungal activity against *Histoplasma capsulatum* (MIC range: 0.0078-0.00312 μM) (Brilhante *et al*, 2015) and *Coccidioides posadasii* (MIC range: 0.00171-0.01369 mg/l) (Brilhante *et al*, 2013). Farnesol has also been found to have antifungal activity against *Sporothrix* spp (MIC range: 0.003-0.222 mg/l). Derengowski *et al* (2009) also found farnesol had *in vitro* activity gainst *Paracoccidioides brasiliensis* with a MIC of 25 μM. Farnesol has also been shown to have inhibitory activity against *Cryptococcus neoformans* and *Cryptococcus gattii* (MIC range: 0.29-75.0 μM) (Cordeiro *et al*, 2012). Farnesol has been shown to have growth inhibition against *Candida* spp (MIC range: 4.68-150 μM for *C. parapsilosis*; 18.75-75 μM for *C. tropicalis*; 18.75-150 μM against *C. albicans* (Cordeiro *et al*, 2013).

Our study showed farnesol has an antifungal effects on *S. boydii* and *L. prolificans* at higher concentrations. Further studies are needed to confirm this activity *in vivo*.

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