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# Effects of menthol stereoisomers on the growth, sporulation and fumonisin $B_1$ production of *Fusarium verticillioides*

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ABSTRACT

Menthol is a naturally occurring cyclic terpene alcohol of plant origin from the *Lamiaceae* family. It has three chiral centres, implying eight possible different stereoisomers, which in turn define four pairs of enantiomers. This is the first work that reports on the stereoselective antifungal and antitoxigenic activities of the menthol stereoisomers on *Fusarium verticillioides*, with the (−)-menthol and (+)-menthol enantiomers found to be the most active inhibitors of fungal growth and sporulation. The results obtained suggest the importance of the presence of these substituents in the equatorial positions of menthol stereoisomers in the antifungal activity. The stereoisomer (−)-menthol, followed by (+)-menthol, were the most active compounds in the inhibition of fumonisin $B_1$ ($FB_1$) biosynthesis. The different antitoxigenic activities of (−)-menthol and (+)-menthol revealed that the molecular requirements to affect the $FB_1$ production were dependent not only on the presence of the substituents in the equatorial positions, but also on their spatial arrangements.

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## 1. Introduction

Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide (Marasas, Kriek, Fincham, & van Rensburg, 1984), with several species from this genus being economically damaging. This arises from their ability to infect and cause tissue destruction in important crops such as corn, wheat and other grains. However, their scope is not limited to the crops in the field, as they can also produce mycotoxins in storage silos. Fumonisins and trichothecenes are two important *Fusarium* mycotoxins that have received considerable attention related to food safety concerns from regulatory agencies, with the former being mainly produced by the fungi *Fusarium verticillioides* (Sacc.) Niremberg (e.g. *Fusarium moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Niremberg. Fumonisin $B_1$ ($FB_1$) is generally the most abundant fumonisin analogue (Leslie, Plattner, Desjardins, & Klittich, 1992). $FB_1$ have immunotoxic, neurotoxic, hepatotoxic, nephrotoxic and carcinogenic properties in animals (Stockmann-Juvala & Savolainen, 2008). Considerable interest has developed in recent years concerning the preservation of grains by the use of essential oils, in order to reduce fungal growth and mycotoxin production. Several publications have reported that some essential oils cause reduction in fumonisin production by *F. verticillioides* (López, Theumer, Zygadlo, & Rubinstein, 2004; Velluti, Sanchis, & Ramos, 2004), and some of the antitoxigenic properties of these essential oils might be principally attributed to monoterpenoids (Lambert, Skandamis, Coote, & Nychas, 2001). Menthol (2-isopropyl-5-methyl-cyclohexanol) exhibits numerous pharmacological properties (Ruiz del Castillo, Blanch, & Herraiz, 2004), including antifungal activity against yeast and toxigenic fungi (Soković et al., 2009). It is a naturally occurring compound of plant origin, which produces a typical minty smell and flavour in plants of the *Mentha* species. In fact, it is present in the volatile oil of several species of mint plants, such as peppermint (*Mentha piperita* and cornmint oil *Mentha arvensis*) (Galeotti, Di Cesare Mannelli, Mazzanti, Bartolini, & Ghelardini, 2002), and is classified as a cyclic terpene alcohol that exhibits three stereogenic centres (Fig. 1). Concerning its isomers, (−)-menthol is the most widespread natural one. Therefore, it is sometimes referred to as the "natural" menthol, despite all the other isomers also being found in peppermint oils (Oertling, Reckziegel, Surburg, & Bertram, 2007). It is endowed with the peculiar property of being both a fragrance and flavour compound. For this reason, it is widely used in flavouring for toothpaste, oral hygiene products and chewing gum (Galeotti et al., 2002). The chirality of the odorants, in addition, exerts an influence on their mode of action, indicating that the antipodes may behave differently (Lahlou, 2004). Moreover, differences in the sensory properties and biological activities between pairs of menthol enantiomers have also been previously reported (Corvalán, Zygadlo, &

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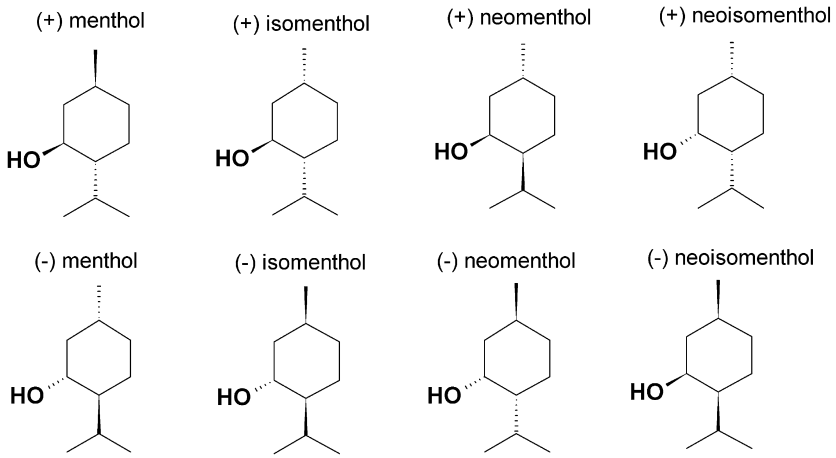


**Fig. 1.** Chemical structures of menthol stereoisomers.

García, 2009; Perillo & Zygadlo, 2005; Turina, Nolan, Zygadlo, & Perillo, 2006). However, the stereoselective activities of the menthol stereoisomers on the fungal growth and secondary metabolite biosynthesis have not been extensively explored. The fact that phospholipids are inherently chiral due to their *sn*-2-carbon atoms (Inagaki, Shibakami, & Regen, 1997) raises the intriguing possibility that stereospecific interactions between phospholipids and monoterpenes may produce a differential antifungal activity. Therefore, the objective of this work was to study the lipophilicity/hydrophobicity of the menthol stereoisomers and their selective activity on the *F. verticillioides* growth, sporulation and production of fumonisin $B_1$.

## 2. Materials and methods

### 2.1. Material

(1R,2S,5R)(−)-5-methyl-2-(1-methylethyl)-cyclohexanol ((−)-menthol), (1S,2R,5S)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol ((+)-menthol), (1S,2R,5R)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol ((+)-isomenthol), (1S,2S,5R)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol ((+)-neomenthol), and (1R,2R,5S)(−)-5-methyl-2-(1-methylethyl)-cyclohexanol ((−)-neomenthol) were purchased from Fluka-Kahlbaum-Germany (Fig. 1).

(−)-Isomenthol, (+)-neoisomenthol and (−)-neoisomenthol were not included in this work because they are not commercially available.

### 2.2. Fungal strain

A isolate of *F. verticillioides* MRC 4316 PROMEC, from the Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg; Republic of South Africa, grown on carnation leave agar by monosporic isolation, was used in all the experiments. This isolate had previously been shown to be a highly fumonisin producer in liquid culture (Vismer, Snijman, Marasas, & van Schalkwyk, 2004).

### 2.3. Determination of the octanol–water partition coefficient ($K_{ow}$)

The quantification of log $K_{ow}$ was performed following a modified Shake Flask Method (Griffin, Wyllie, & Markham, 1999). Briefly, solutions of a known terpenoid concentration were prepared using ultrapure water (type 1 water) which was pre-saturated with 1-octanol for 24 h prior to use. Equivalent volumes of water and octanol (pre-saturated with type I water for 24 h before use) were added together. The resulting two-phase mixture was repeatedly inverted for 1 h. After mixing, samples were centrifuged for 30 min (6000 rpm) to avoid emulsion formation. Both fractions of the sample were analysed using a Clarus 500 Perkin Elmer GC system, fitted with a DB-5 capillary column (30 m 250 mm) and a flame ionisation detection (FID) system. The GC operating parameters for the analysis were as follows: inlet temperature: 240 °C, carrier gas: nitrogen at 50 mL/min, detector temperature: 280 °C, initial oven temperature: 100 °C for 1 min (increased by 10 °C/min to 240 °C). The sample (1 μm) was injected with 1:50 split ratio. Quantitation was achieved by the use of external standards for each of the terpenoids.

### 2.4. Testing for antifungal activity. Minimum inhibitory concentration (MIC)

For the evaluation of antifungal activities, experiments were performed using a modified semisolid agar antifungal susceptibility method (SAAS) (Provine & Hadley, 2000). Briefly, five-milliliter aliquots of semisolid brain–heart infusion broth (Difco Laboratories, Detroit, Mich) containing 0.5% agar (w/v) (Bacto Agar, Difco Laboratories) at pH 7.4 (without dextrose, buffer or indicator) were prepared in sterility in 16 by 125 mm glass tubes, with or without the addition of menthol stereoisomers. These terpenes were dissolved with dimethyl sulfoxide (DMSO), and then added to the different tubes in order to obtain concentrations of 0.1; 0.2; 0.5; 1.0; 1.5 and 2 mM in the culture medium. The final concentration of DMSO was adjusted to 5 μl/ml in all the tubes. As control, a terpene-free medium with a 5 μl/ml final concentration of DMSO was used. Menthol solutions were mixed with the medium at 45 °C, and then the media were stored at 4 °C until solidification. In addition, one tube with uninoculated monoterpene-free medium was included as a sterility control. A conidia suspension ($1 \times 10^6$ ml), prepared with a *F. verticillioides* culture grown for 2 weeks in V-8 juice agar and Tween 20 at 2.5% (v/v) in sterile water, was used as inoculums. A standard loopful (0.001 ml) of this conidia suspension was inserted deeply into each tube of medium containing a known concentration of monoterpenes, as well as into the monoterpene-free medium, by a centred up-down motion to form a two-dimensional inoculum. Sterile mineral oil (0.5 ml) was layered onto the inoculated medium to inhibit sporulation, and then the tubes were tightly capped. In order to check the suspension purity and the conidia viability, a loopful of the inoculum suspension was streaked onto Sabouraud dextrose agar. All cultures were incubated for 48 h at 28 °C, or until good growth was apparent in the monoterpene-free control. Within 48 h, when by visual inspection a good growth of the *F. verticillioides* in the

monoterpene-free medium was already detected, this growth in all tubes was visually compared with that of the monoterpen-free control in order to determine inhibition. Growth was scored in the following manner: 4+, growth comparable to that of the monoterpene-free control; 3+, growth approximately 75% of that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less than that of the control; and 0, no visible growth. Each treatment had 5 replications, with the average giving the degree of mycelial development.

### 2.5. Effect of menthol stereoisomers on mycelial growth and sporulation

The antifungal activity of the menthol stereoisomers was tested using a radial growth of the fungal colony and conidial production inhibition technique following a methodology proposed by Meriles, Vargas Gil, Haro, March, and Guzman (2006). Briefly, Menthol stereoisomers were dissolved with dimethyl sulfoxide (DMSO), and then added to Czapek-Dox Agar to obtain a concentration of 1.00 mM. Menthol solutions were mixed with the medium at 45 °C, and then poured into the Petri dishes (9.0 cm in diameter). The final concentration of DMSO was adjusted to 5 μl/ml in all the Petri dishes. As control, a terpene-free medium with a 5 μl/ml final concentration of DMSO was used. Conidia suspensions ($1 \times 10^6$ ml), prepared as described in the testing for antifungal activity, were used as inoculums. 10 μl of these conidial suspensions were added aseptically to the centre of the Petri dishes and incubated in the dark at 25 °C. The colony diameter of *F. verticillioides* was measured after 3, 8 and 12 days of incubation, respectively, and each colony area (radial growth) was calculated using the formula for the area of a circle ($\pi \times r^2$). After incubation, the conidia were harvested twice by adding 15 ml of sterile distilled water per plate, and then gently scraping the medium surface with a soft paintbrush. The number of conidia was determined with a haemocytometer.

### 2.6. Effect of terpenes on $FB_1$ production

For $FB_1$ production, $1 \times 10^6$ ml of conidia suspension of *F. verticillioides*, prepared as described in the testing for antifungal activity, were inoculated into 50 ml Myro liquid medium (Blackwell, Miller, & Savard, 1994) and incubated in the dark at 28 °C for 21 days. Menthol estereoisomers were dissolved with DMSO, in order to obtain concentrations of 1.00 mM in the culture medium. As control, a terpene-free medium with a 5 μl/ml final concentration of DMSO was used. The solutions were added to the different flasks on the fifth day post-inoculation, because it was of the evaluated application day of higher antitoxigenic activity (data not shown). Five replications of each treatment were performed, with this experimental procedure being repeated.

### 2.7. $FB_1$ quantification

Samples (1000 μl) from the liquid cultures were centrifuged for 15 min to 9000*g*. The supernatants obtained were diluted with acetonitrile (1:1), and the quantification of the samples was performed following a methodology proposed by Shephard, Sydenham, Thiel, and Gelderblom (1990). Briefly, an aliquot (50 μl) was derivatized with 200 μl of a solution prepared by adding 5 ml of 0.1 M sodium tetraborate and 50 μl of 2-mercaptoethanol to 1 ml of methanol containing 40 mg of *o*-phthaldialdehyde. The derivatized samples were then analysed by means of a Hewlett Packard HPLC equipped with a fluorescence detector. The wavelengths used were 335 nm and 440 nm for excitation and emission, respectively. An analytical reverse phase column C18 (150 mm × 4.6 mm internal diameter and 5 μm particle size) connected to a precolumn C18 (20 mm × 4.6 mm and 5 μm particle size) was also used. The mobile phase was methanol, $NaH_2PO_4$ 0.1 M (75:25), with the pH being set at 3.35 ± 0.2 with orthophosphoric acid, and a flow rate of 1.5 ml/min used. The quantification of fumonisin $B_1$ was carried out by comparing the peak areas obtained from samples with those corresponding to the analytical standards of 10.5, 5.25 and 21 2.625 μg/ml of $FB_1$ (PROMEC, Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg; Republic of South Africa).

### 2.8. Statistical evaluation

Statistical analyses were conducted using INFOSTAT/ Proffesional 2005p.1 (F.C.A.-Universidad Nacional de Córdoba, Argentina) at $P = 0.05$. Data from these studies were analysed by a one-way analysis of variance (ANOVA). Normality of data was tested using the Shapiro–Wilk test. Comparisons between treatments were performed by the DGC (Di Rienzo, Guzmán and Casanoves) test (Di Rienzo, Guzman, & Casanoves, 2002). Results giving *P* values < 0.05 were considered significant.

## 3. Results

### 3.1. Octanol–water partition coefficient ($K_{ow}$) of menthol

Menthol stereoisomers showed statistically significant differences in their lipophilicity ($P < 0.0001$), when measured by the log $K_{ow}$ values (Table 1). Concerning the menthol diasteromers, (+)-menthol and (−)-menthol enantiomers were the most lipophilic compounds found amongst the stereoisomers studied, followed by (+)-isomenthol. (+)-neomenthol and (−)-neomenthol were the most hydrophilic compounds. However, no significant differences were encountered between the log $K_{ow}$ values of the menthol enantiomers.

### 3.2. Testing for antifungal activity — minimum inhibitory concentration (MIC)

Menthol stereoisomers exhibited varying levels of antifungal activity against *F. verticillioides* (Table 2). At very low concentrations (0.25 mM), none of the steroisomers of menthol significantly altered the fungal growth. The most active inhibitors were (+)-menthol and (−)-menthol, with a MIC value of 1.50 mM, followed by (+)-neomenthol at 2.00 mM. Although, (−)-neomenthol and (+)-isomenthol had little effect on the fungus development, they did not completely inhibit its growth (Table 2).

### 3.3. Effect of menthol stereoisomers on mycelial growth and sporulation

The radial growth of the *F. verticillioides* colony (Fig. 2) was reduced by (+)-menthol, (−)-menthol and isomenthol. However, neomenthol enantiomers did not have any significant effects on radial growth. Furthermore, except for isomenthol, the effect of menthol stereoisomers on fungal sporulation was similar to that which

**Table 1**
Log $K_{ow}$ values of menthol stereoisomers determined using Shake Flask Method.

| Menthol stereoisomers | Measured log $K_{ow}$ [A] |
|---|---|
| (+)-Isomenthol | 3.17 ± 0.07 b |
| (+)-Menthol | 3.32 ± 0.04 a |
| (−)-Menthol | 3.36 ± 0.03 a |
| (+)-Neomenthol | 2.87 ± 0.05 c |
| (−)-Neomenthol | 2.78 ± 0.05 c |

[A] Values are presented as mean ± SE. Values having different letters are significantly different from each other according to DGC multiple range test at $P < 0.05$. Five replications were done for each treatment.

**Table 2**
Testing for antifungal activity. Minimum inhibitory concentration (MIC).

| Menthol stereoisomers | Concentrations of menthol stereoisomers evaluated | | | | |
|---|---|---|---|---|---|
| | 0.25 mM | 0.50 mM | 1.00 mM | 1.5 mM | 2.00 mM |
| (+)-Isomenthol | 4 | 4 | 4 | 3 | 2 |
| (+)-Menthol | 4 | 4 | 3 | 1[a] | 1 |
| (−)-Menthol | 4 | 4 | 2 | 1[a] | 1 |
| (+)-Neomenthol | 4 | 3 | 2 | 2 | 1[a] |
| (−)-Neomenthol | 4 | 4 | 3 | 2 | 2 |

Values are presented as 0 – no visible growth, 1 – growth 25% or less than control, 2 – growth approximately 50% of control, 3 – growth approximately 75% of control, 4 – growth comparable to that of control. The experiment was performed five times.
[a] Score of minimal inhibitory concentration.

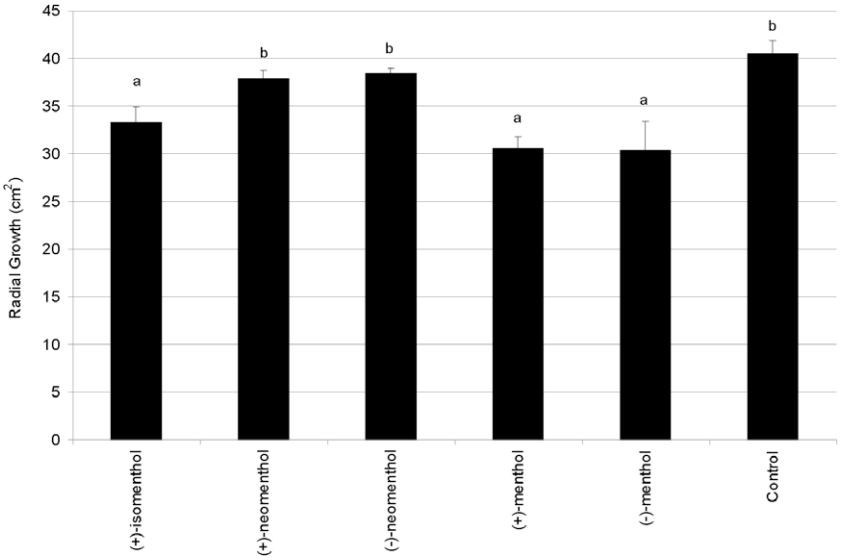


**Fig. 2.** Effects of menthol stereoisomers on radial growth of fungal colony (colony area). Aliquots of 10 μL suspension of *Fusarium verticillioides* were added aseptically to the centre of Petri and incubated in the dark at 28 °C. The menthol stereoisomers were evaluated at final concentrations of 100 mM. Radial grown of *F. verticillioides* colony was measured after 3, 8 and 12 days of incubation, respectively and each colony area was computed using the formula for the area of circle ($\pi \times r^2$). Values having different letters are significantly different from each other according to DGC multiple range test at $P < 0.05$ ($n = 5$).

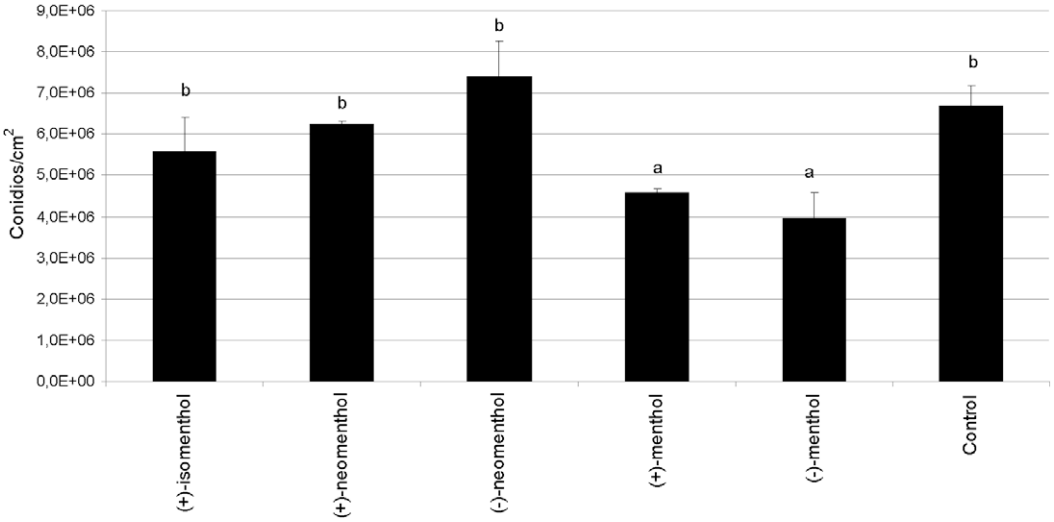


**Fig. 3.** Effects of menthol stereoisomers on fungal sporulation. Aliquots of 10 μL suspension of *Fusarium verticillioides* were added aseptically to the centre of Petri and incubated in the dark at 28 °C. The menthol stereoisomers were evaluated at final concentrations of 100 mM. After incubation, conidia were harvested twice by adding 15 ml of sterile distilled water per plate and gently scraping the medium surface with a soft paintbrush. The number of conidia was determined with a haemocytometer. Values having different letters are significantly different from each other according to DGC multiple range test at $P < 0.05$ ($n = 5$).

occurred on the radial growth of fungal colony, with (+)-menthol and (−)-menthol being the most active compounds in inhibiting the sporulation of *F. verticillioides* (Fig. 3). Both menthol compounds showed significant inhibitory effects compared with the

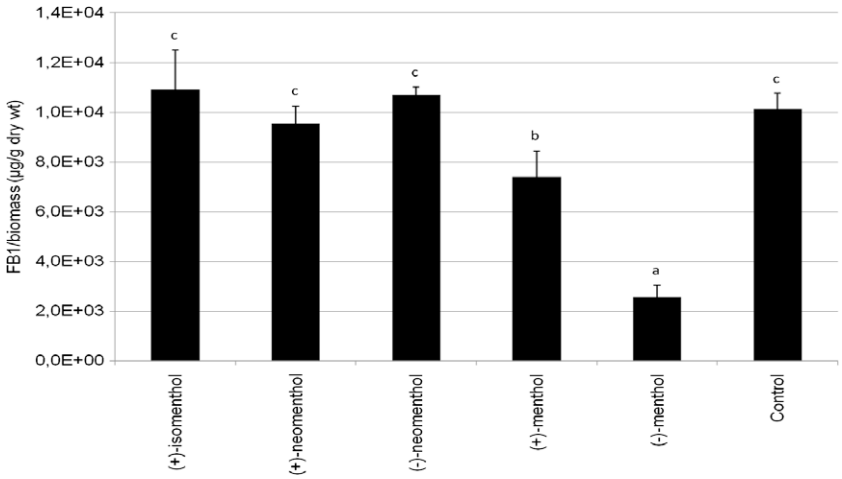


**Fig. 4.** Effects of menthol stereoisomers on FB1 production (µg/g dry wt). Aliquots of 500 µL suspension of *Fusarium verticillioides* were added and cultured in the dark at 28 °C for 21 days. The menthol stereoisomers were evaluated at final concentrations of 100 mM and were applied on the fifth day post-inoculation. The quantification was performed following the methodology proposed by Shephard et al. (1990). Five replications were done for each treatment. Values having different letters are significantly different from each other according to DGC multiple range test at $P < 0.05$ ($n = 5$).

control. However, neomenthol enantiomers and isomenthol did not have any significant effects on the fungal sporulation.

### 3.4. Effect of terpenes on $FB_1$ biosynthesis in liquid medium

To determine whether the menthol stereoisomers were active on $FB_1$ biosynthesis by *F. verticillioides,* we measured the $FB_1$ concentration and fungi biomass in the presence of each stereoisomer in liquid medium culture.

Examination of the cultures reveled the inhibitory effects of these terpenes on mycotoxin production (Fig. 4), with (−)-menthol and (+)-menthol enantiomers exhibiting significant inhibitory effects when compared with the control. (−)-Menthol was the most active stereoisomer in toxin biosynthesis inhibition, followed by (+)-menthol. However, the remaining menthol stereoisomers did not have any significant effects on the $FB_1$ biosynthesis.

## 4. Discussion

The compound menthol is a non-planar cyclohexane ring which has methyl, hydroxyl and isopropyl substitutes. There are two common conformations of cyclohexane, the chair and the boat form, with the chair type being thermodynamically stable. The substituting groups can occur in the axial or equatorial position. Menthol has three chiral centres, thus eight different stereoisomers are possible, which define the following four pairs of enantiomers; these are (+)-menthol and (−)-menthol; (+)-neomenthol and (−)-neomenthol; (+)-isomenthol and (−)-isomenthol; and (+)-neoisomenthol and (−)-neoisomenthol (Fig. 1) (Etzold, Jess, & Nobis, 2009). If two compounds are enantiomers of each other, they have the same physical properties, except for the sign of optical rotation. However, diasteromers seldom have identical physical properties. The (+) and (−)-menthols are the most stable pair of enantiomers, because all their substitutes are in the equatorial position.

The results obtained in the present work show that the menthol stereoisomers have different antifungal activities. Although the antifungal activity of the menthol monoterpene has been previously studied (Dambolena, Theumer, Zygadlo, & Rubinstein, 2008; Soković et al., 2009), no data have been reported regarding the stereoselective antifungal and antitoxigenic activities of menthol stereoisomers, with the toxic effects on the membrane structure and function generally being used to explain the antimicrobial action of monoterpenoid components. The specific mechanisms involved in the antimicrobial action of monoterpenos, however, remain poorly characterised (Trombetta et al., 2005). The permeability of cell membranes is dependent on the lipophilic character of the solutes that cross the membrane and the composition of the membrane (Turina et al., 2006). Related to this, the effect that a lipophilic compound has on the integrity of a membrane depends on the position in the membrane where it has accumulated. This is dependent on the hydrophobicity of the solute, which affects the depth of penetration into the bilayer, and the induced changes in the physico-chemical properties (Turina et al., 2006), resulting in a lower membrane integrity and an increase in the proton passive flux across the membrane. This effect has been particularly noted for compounds having a log $K_{ow}$ greater than 3 (Ben Arfa, Combes, Preziosi-Belloy, Gontard, & Chalier, 2006).

The menthol stereoisomers showed little effect on the fungal growth. However, the (−)-menthol and (+)-menthol enantiomers were the most active inhibitors. The chirality of the biomembrane components has a fundamental role in the organisation and biological functions of the cellular membrane (Bombelli et al., 2008). Thus, the chiral organisation of the biomembrane could be influenced by the interactions with chiral compounds (i.e. monoterpenes) (Bernardini et al., 2009). For this reason, the substituents in the equatorial position may be important in the antifungal activity of menthol stereoisomers.

Slight structural differences can be sufficient to affect the physical or chemical properties, such as log $K_{ow}$, and hence alter the antifungal activity. Considering that the (+)-menthol and (−)-menthol enantiomers are the most lipophilic compounds amongst the stereoisomers studied, their antifungal activity may be due to their capacity to accumulate inside the membrane and thereby induce changes in the physico-chemical properties. This is in agreement with Maffei, Camusso, and Sacco (2001), who suggested that decreasing the water solubility of monoterpenes makes it easier for terpenoids to interact with the root membrane and disrupt the integrity, thus causing a rapid and reversible membrane depolarisation. These authors also reported that (−)-menthol was more active than (+)-neomenthol in causing membrane depolarisation. Trombetta et al. (2005) reported an antimicrobial effect of (+)-menthol, suggesting that (+)-menthol may interact with the lipid fraction of the microorganism plasma membrane, resulting in alterations in its permeability, thereby allowing leakage of intracellular materials. Moreover, Turina et al. (2006) demonstrated

that menthol can penetrate artificial model membranes, usually by interacting with the polar head of the membrane lipids, thus increasing its differential polarity.

The results obtained in the present work show that (−)-menthol was the most active menthol stereoisomer in inhibiting fumonisin biosynthesis, followed by (+)-menthol. On the other hand, (+)-isomenthol, (−)-neomenthol and (+)-neomenthol did not have any significant effects on $FB_1$ production. The antitoxigenic activity of menthol monoterpene has been poorly explored. Dambolena et al. (2008) reported a low activity of menthol on $FB_1$ biosynthesis, perhaps due to the fact that corn grain *(Zea mays)* was used as the substrate or because a commercial mixture of menthol was utilised with unknown proportions of each stereoisomer. In the present study, the different antitoxigenic activities of (−)-menthol and (+)-menthol with respect to (+)-isomenthol, (+) and (−)-neomenthol, may be explained by the lipophilic character of the compounds, as described above. However, the differential activity of (−)-menthol and (+)-menthol cannot be due to their lipophilic character, because the enantiomers had the same physical properties.

Summing up, the results obtained allow us to speculate that the stereoselective antitoxigenic activities of (+)-menthol and (−)-menthol enantiomers resulted from a specific interaction with a target in the fungal membranes, favored by the spatial arrangement of their substituents. The fact that the regulation of aflatoxin biosynthesis is connected with calcium-dependent signalling (Juvvadi & Chivukula, 2006) and that (−)-menthol could play an important role in the entry of Ca2+ from the external medium (Maffei et al., 2001), raises the intriguing possibility that stereospecific interactions of (-)-menthol in the fungal membranes may contribute to a Ca2+ imbalance and thereby affect fumonisin production.

## 5. Conclusions

The present study constitutes the first contribution to the description of the stereoselective antifungal and antitoxigenic activities of the menthol stereoisomers on *F. verticillioides.* The finding on the stereospecific antifungal activity and on the enantiospecific antifumonisin activity of the menthol stereosisomers in *F. verticillioides,* raise new perspectives in the study of the mechanism of the antifungal and antimicotoxicogenic activity of the monoterpenes. Accordingly, the present findings highlight the importance of taking the chirality of the compound into account when studying the antifungal and antimicotoxicogenic mechanisms of the monoterpenes, due to the fact that the chiral organisation of the biomembrane could be differentially altered by interaction with chiral compounds.

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