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Research Article

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Antifungal Activities of B-Ionone, Carvone and 1,8-Cineole Essential Oil Components Against *Aspergillus niger* Spores

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ABSTRACT

Many substances have been reported as antibacterial agents and some as antifungal in the scientific literature. The aim of this study was to evaluate the antifungal potential of three main compounds of essential oils, β-Ionone; carvone and 1,8-cineole, against the growth of spores of Aspergillus niger strain. For that purpose, the spores of the studied strain were exposed to successive concentrations of these three compounds to determine their minimum inhibitory (MIC) and fungicidal (MFC) concentrations against this pathogenic strain by the microdilution method using the 96 well microtiter plates. The obtained results have shown the capacity of three compounds to inhibit the growth of A. niger spores with low values of CMIs determined for β-Ionone (2.5%), carvone (0.625%) and 1,8-cineole (1.25%). Furthermore, the determination of MFCs allowed to find that minimum concentrations, necessary to eliminate definitively the initial inoculum of A. niger spores, were 10% for β-Ionone when that of carvone was only 5%. The MFC of 1,8-cineole compound was not determined even at 10% of concentration. This study, which showed for the first time the antifungal potential of these three compounds against this pathogenic fungi, also allow to highlight the possibility of using these three compounds in formulation of commercial antifungal products.

Keywords: 1,8-cineole; β-Ionone; Antifungal activity; *Aspergillus niger*; Carvone; Spore

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INTRODUCTION

Many substances have been reported as antibacterial agents and some as antifungal in recent decades in the scientific literature. Some of these substances are natural chemical molecules, or are modified by chemical synthesis; several are antibiotics produced by certain microorganisms against others; or still substances extracted from plants (essential oils, plant extracts, etc.) and which have proved effective against pathogenic microorganisms. Among these, the antimicrobial potential of essential oils has been frequently reported in recent years [1-6]. With volatile properties, the essential oils are natural products with complex chemical compositions. They are used for centuries for their various antibacterial, antioxidant, antifungal, anti-inflammatory, insecticidal properties [7-11]. Their properties are also used, more industrially in recent decades, in various fields such as cosmetics, pharmaceuticals, or in food industries. Being composed by several tens of different molecules, some of these latter are in very high proportions in the essential oils and also confer them their bioactive properties against microorganisms [12]. The *Aspergillus* genus is one of the most known genera of fungi with *Penicillium* and *Paecilomyces* [13]. This fungal genus consists of over 300 species, the most known are *A. flavus*, *A. terreus*, *A. fumigatus*, *A. nudilans*, or *A. niger*. The species of this genus are, among other, responsible for invasive aspergillosis [14] and can produce ochratoxin [15], a secondary metabolite with possible carcinogenic effects for human health [16]. Considered as the main agent that may cause invasive aspergillosis, *A. niger* is involved in post-harvest fruit losses in the storage sites [15]. This strain is also a frequently reported pathogen in food contamination. The spores of *A. niger*, which are a asexual form of

reproduction of this strain, are easily dispersible and also represent their resistance form in extreme environmental conditions. Indeed, they are protected by rigid cell walls [17,18]. The inactivation of these spores is therefore a challenge to limit, or eliminate, the risk of pathogenicity that represents the strain of *A. niger* as well as in food industries and in medical field. The main objective of the present work is to contribute to evaluate the antifungal potential of three essential oils compounds against the growth of the spores of these pathogen fungi. Thus, the spores of *A. niger* were confronted with successive concentrations of β-Ionone, carvone and 1,8-cineole in order to determine the minimum inhibitory (MIC) and fungicidal (MFC) concentrations of these three essential oil compounds.

MATERIALS AND METHODS

Fungal Strain

The studied *Aspergillus niger* strain was isolated in our laboratory [19] from cedar wood decay (*Cedrus atlantica*) from an old house located in Derb Lamté in the old Medina of Fez (Morocco).

Aspergillus niger Strain, Growth Conditions and Harvesting Spores

The *Aspergillus niger* strain was cultivated on the malt extract agar medium and its growth was obtained at 25°C. The *A. niger* spores were then harvested by scraping a 7 days fungal culture surface in a sterile 0.1% of Tween-80. The spore suspension were concentrated by centrifugation at 10,000 g for 15 min at 4°C until a concentration of 10^6 spores/ml (counted with a hemacytometer) [20].

Chemicals

The volatile terpene derivatives, used in this study for the evaluation of their antifungal potential, were β-ionone (99% pure), carvone (99% pure) and 1,8-cineole (99% pure). These three volatile derivatives are part of the chemical composition of several essential oils. The antifungal potential of these three molecules was compared with that of Fluconazole (a standard antifungal substance). The used components were purchased from Sigma-Aldrich.

Antifungal Activity Evaluation

The antifungal activities, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), of the tested essential oil components (β-ionone, carvone and 1,8-cineole) against *A. niger* spores were studied according to the microdilution method by using 96 well microtiter plates [21-24]. Briefly, the used essential oils components were diluted in a sterile solution at 1% Tween-80. The malt extract medium was distributed in the wells of the microplate. Then, the tested components were diluted successively from well to well and the concentration range was from 10 to 0.01%. In the control wells, an equivalent volume to that of the tested components was replaced by the sterile solution at 1% Tween-80. Then, a volume of 10 µl of the fungal spore suspension which was concentrated at 10^6 spores/ml was added to all of microplate wells.

Thus, the MIC that is defined as the lowest concentration without visible growth of the studied strain was determined after incubation for 48 h at 25°C. The determination of the MFC was conducted by depositing a 3 µL volume from microtiter plates containing 100 µL of broth per well for further incubation at 25°C for 72 h on malt extract agar. The MFC was defined as the lowest concentration with no visible growth, indicating 99.5% killing of the initial inoculum.

RESULTS

The antifungal evaluation results of the activities of the three tested essential oil components against the growth of *Aspergillus niger* spores are shown in Table 1. Thus, as can be seen in Table 1, the compounds used in this study had a significant antifungal effect against the growth of *A. niger* spores. Indeed, for the β-Ionone molecule, the first concentration at which no visible growth of the spores was observed after the incubation time was 2.5%. At the same time, the concentration corresponding to the MIC of the 1,8-cineole molecule against *A. niger* was 1.25% when that of the carvone was only 0.625%. Thus, among all the tested volatile terpene compounds with antifungal effect in the present work, the one that has shown the best efficacy against the growth of *A. niger* spores was the carvone molecule with the most lowest MIC found. It is obviously followed by 1,8-cineole and β-Ionone compounds, respectively. This reflects a greater sensitivity of the *A. niger* spores in the previously established order of the molecules used in the present work (carvone > 1,8-cineole > β-Ionone).

Table 1: Results of MIC antifungal activities for the tested essential oil components against *A. niger* spores

Wells	Concentrations	*Aspergillus niger* spores			
		β-Ionone % (v/v)	Carvone % (v/v)	1,8-cineole % (v/v)	Fluconazole mg/ml (w/v)
1	10	-	-	-	-
2	5	-	-	-	-
3	2.5	-	-	-	-
4	1.25	+	-	-	-
5	0.625	+	-	+	+
6	0.3125	+	+	+	+
7	0.15625	+	+	+	+
8	0.078125	+	+	+	+
9	0.0390625	+	+	+	+
10	0.01953125	+	+	+	+
11	0.009765625	+	+	+	+
12	Control (0)	+	+	+	+

+: presence of growth; -: absence of growth; positive control: fungal spores suspensions in Extract Malt Broth supplemented with agar (0.15% w/v)

On the other hand, the obtained results for the determination of minimum fungicidal concentrations with these compounds almost follow the same line as that of the MICs and are reported in Table 2. Indeed, we found that the corresponding concentration to MFC for the β-Ionone molecule, for which the initial inoculum of the *A. niger* spores was killed, was 10% (Table 2). However, until a concentration of 10%, the MFC of the 1,8-cineole compound was not able to be determined for the studied strain (Table 2).

Table 2: Results of the determined MFCs for the tested molecules against *A. niger* spores

	Aspergillus niger spores			
	β-Ionone % (v/v)	Carvone % (v/v)	1,8-cineole % (v/v)	Fluconazole mg/ml (w/v)
MFC	10	5	nd	nd

nd: not determined

However, as with its MIC *vis-à-vis* of the spores of the studied strain, the molecule of carvone showed the lowest MFC obtained compared with the two other molecules. The volume of 3 µl, which showed no visible growth on malt extract agar during the determination of the MFC, was from to the wells of the microplate whose concentration was 5% of the carvone compound (Table 2). These obtained results confirmed the very high sensitivity of the spores of this *A. niger* strain to the molecule of carvone. Compared to the antifungal potential of the tested essential oils compounds, a test was performed with fluconazole which is a recognized antifungal substance and used in the medical field. The antifungal evaluation results of the activities of fluconazole against the growth of *Aspergillus niger* spores are also shown in Table 1. Thus, the results obtained (Table 1) with the fluconazole showed that the MIC of this molecule against the growth of this fungal strain corresponded to 1.25 mg/ml. As regards to the MFC, it has not been determined even at a concentration of 10 mg/ml of fluconazole (Table 2).

DISCUSSION

The involvement, frequently reported in the scientific literature, of the *Aspergillus niger* strain in invasive aspergillosis cases, in post-harvest decay of stored fruits or in food contamination, have made the inactivation of these spores a challenge.

The evaluation of the antifungal activity of three essential oil components, in the present study, showed the potential of each one of them against the growth of *Aspergillus niger* spores. Thus, it was observed that compared to MICs found, that of carvone is two times lower than that of the 1,8-cineole compound and four times lower than that of β-Ionone compound.

Similar results were reported by Stupar et al. [5] in their work on the antifungal activity of several essential oils and biocide benzalkonium chloride (BAC) against the fungi isolated from cultural heritage objects. Indeed, they showed that the essential oil of *Rosmarinus officinalis*, whose main compound is 1,8-cineole (44.28%), had a strong fungistatic effect (MIC=30 µl/ml) against the *A. ochraceus* strain but with a rather low fungicidal effect (MFC=100 µl/ml). The same test on the *A. niger* strain showed much less interesting values (MIC=MFC=100 µl/ml) than those found in the present study. The use of the biocide BAC had a very important antifungal effect on all tested fungi among which both *Aspergillus* strains with very low values (MIC=0.1 µl/ml and MFC=0.25 µl/ml for *A. niger*) and (MIC=0.1 µl/ml and MFC=0.5 µl/ml for *A. ochraceus*).

More recently, Tian et al. [15] indicated, in its work on the efficacy of the perillaldehyde in control of *A. niger* causing grape decay, that this substance had a significant antifungal effect against this strain with MIC at 0.25 µl/ml and MFC 1 µl/ml. On the other hand, the use of nano-metal to inhibit *A. niger* was reported by Yu et al. [25]. Indeed, the authors indicated that the critical Ag concentration needed to inhibit the germination and growth of the *A. niger* spores of 5 wt% nano Ag catalyst was 65 mg/ml. In addition, the MIC obtained from the use of fluconazole was 128 µg/ml [26]. However, about this standard antifungal, Shanmugaraj et al. [27] reported that it was ineffective against *Aspergillus*. On the other hand, if the antimicrobial potential of essential oils is well established in the scientific literature, it remains a major problem concerning the concentration and chemical composition of the molecules which constitute the essential oils of various plants. Indeed the latter are influenced by several factors including climate, geographical location, environmental, etc. thus, their antimicrobial potential may also be affected according to their extraction periods.

Therefore, it might be more interesting to evaluate the antibacterial and antifungal potential of these terpene compounds. Some of these molecules are present in very high proportions and constitute the major components of essential oils. It is also in this objective that the present work was realized. Unlike essential oils, the advantage, of the use of these compounds, would be to have molecules whose the biological properties as well as the chemical composition would not be influenced by the different factors mentioned above.

However, as with essential oils, the mechanism of action by which these molecules attack microorganisms is still very poorly explained in the scientific literature. Some authors have even suggested that these multitude of varied chemical molecules would not appear to have specific targets [28,29]. In fact, their lipophilic properties allow them to destabilize the structures of the macromolecules (polysaccharides, fatty acids and phospholipids, etc.) through the cell wall and the cytoplasmic membrane of microorganisms [30-32]. Thus, the destabilized membranes of the attacked microorganisms become permeable. What causes a loss of ions and thus induces a difference of potential at the membranaire level [33-36]. Always in the same vein, the lipids and proteins would be also affected [34,37]. However, the mechanism of action of some major essential oil components is better known in the literature. Indeed, this is the case for example for thymol and carvacrol which are two major compounds of the Thyme and Oregon essential oils. Thus, Xu et al. [38] reported in their work on the mechanism of action of these two molecules against *Escherichia coli* that they have the ability to permeabilize and depolarize the cytoplasmic membrane of this bacterial strain.

CONCLUSION

The obtained results in the present study demonstrated that these three compounds (β-Ionone; carvone and 1,8-cineole) have a real antifungal potential and they could be used as antifungal agents as well as to significantly reduce (or eliminate completely) the growth of *Aspergillus niger*. They could also be used as antifungal agents against the growth of other important pathogenic fungal which present major risks both in the food industries and in the medical field. However, additional research is needed to better understand their mechanisms of action against the fungi in order to lead to the formulation of a commercial antifungal product.

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