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Alain Castellan

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In vitro inhibitory effect of tetrahydrocurcuminoids on *Fusarium proliferatum* growth and Fumonisin B1 biosynthesis

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3 ***In vitro* inhibitory effect of tetrahydrocurcuminoids on *Fusarium***
4 ***proliferatum* growth and Fumonisin B₁ biosynthesis**
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Abstract

Many plant-pathogens often produce toxic metabolites when growing on food and feed. Some antioxidative components seem to prevent fungal growth and mycotoxin formation. Recently, we synthesised a new class of powerful antioxidative compounds, *i.e.* tetrahydrocurcuminoids, and structure/antioxidant activity relationships have been established. The South West of France is known to produce large amounts of corn, which can be infected by *Fusarium* species, and particularly *F. proliferatum*. In this context, the efficiency of tetrahydrocurcuminoids, which can be obtained from natural curcuminoids, was investigated to control *in vitro* the growth of *F. proliferatum* and the production of its associated mycotoxin fumonisin B₁. The relation between structure and antifungal activity was studied. Tetrahydrocurcumin **THC1**, with two guaiacyl phenolic subunits, showed the highest inhibitory activity (measured as the radial growth on agar medium) against the *F. proliferatum* development (67% inhibition at a concentration of 13.6 $\mu\text{mol.mL}^{-1}$). The efficiencies of **THC2** (36% at a concentration of 11.5 $\mu\text{mol.mL}^{-1}$), which contains syringyl phenolic units and **THC3** (30% at a concentration of 13.6 $\mu\text{mol.mL}^{-1}$), which does not have any substituent on the aromatic rings were relatively close. These results indicate that the simultaneous presence of guaiacyl phenols and the enolic function of the β -diketone moiety play an important role in the inhibition mechanisms. The importance of this combination was confirmed by using n-propylguaiacol and acetylacetone as molecular models. Under the same conditions, ferulic acid and eugenol, other natural phenolic antioxidants, were less efficient in inhibiting fungal growth. **THC1** also reduced fumonisin B₁ production in liquid medium by approximately 35%, 50%, and 75% at concentrations of 0.8, 1.3, and 1.9 $\mu\text{mol.mL}^{-1}$, respectively. These very low inhibitory concentrations show that tetrahydrocurcuminoids could be one of the most promising biobased molecules for the control of mycotoxinogen fungal strains.

Keywords: *Fusarium proliferatum*; Fumonisin B₁; Tetrahydrocurcuminoids; Tetrahydrocurcumin; Phenolic compounds; Natural antioxidants; Antioxidants

Introduction

Fusarium species are among the most studied plant-pathogenic fungi with several species causing diseases on maize, wheat, barley, and other food and feed grain (Placinta et al 1999; Leslie et al 2006; Desjardins 2006). Climatic conditions at harvest and post-harvest treatment of grain are key determinants of fungal infection and spread. Poor post-harvest management can lead to a rapid deterioration in nutritional quality of seeds. Microbial activity can cause undesirable effects in grain, can contribute to heating and losses in dry matter through the utilization of carbohydrates, as energy source, degradation of lipids and proteins, production of volatile metabolites, and reduction of the quality of animal feed and seed (Magan and Aldred 2007). Decreased yield, as well as diminished quality and value of grains, results in significant worldwide economic losses and some methods of preservation have been proposed (Magan and Aldred 2007; Jouany 2007).

In addition, some fungi such as some *Fusarium* species, can produce mycotoxins on the crops, in the field and during the storage. These compounds are toxic, e.g. they can be lethal or carcinogens, mutagens, immunosuppressants, teratogens or oestrogens (Theumer et al 2002). *Fusarium proliferatum* is one of the most prevalent and toxigenic *Fusarium* species in freshly harvested corn in the South West of France. Fumonisin B₁ (FB₁) is generally the most abundant member of the family of mycotoxins generated by *F. proliferatum* (Leslie et al 1992).

Different strategies have been considered to reduce the contamination of human and animal food chains by fumonisins. Efficient drying of maize after harvesting is one of the most effective control strategies (Jouany 2007). However, maize is usually harvested wet (Singh et al 2001) and a delayed drying step results in a significant increase in fumonisin

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3 contamination. At present, post-harvest strategies evaluated to date are mainly based on the
4 use of chemical antifungal agents or modified atmospheres (Jouany 2007). Antioxidants also
5 are known to inhibit fungal growth and associated mycotoxin production for several toxigenic
6 *Fusarium* species (Siranidou et al 2002, Boutigny et al 2008). Among synthetic antioxidants,
7 esters of *para*-hydroxybenzoic acid (paraben) and butylated hydroxy-anisole (BHA) reduced
8 fungal growth and mycotoxin production by these strains (Farnochi et al 2005) whereas
9 butylated hydroxy-toluene (BHT) was less effective on *Aspergillus* ones (Passone et al 2005,
10 2008, 2009). Consumer perception that the use of industrially synthesized food preservatives
11 may be associated with potential toxicological problems has led to interest in the use of
12 natural antioxidants such as cyclic terpenes (Dambolena et al 2008), vanillic and caffeic acids
13 (Samapundo et al 2007), resveratrol (Aldred et al 2008) and various natural extracts (Sisti et
14 al 2008).

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17 Recently, we have studied a new class of natural phenolic antioxidants,
18 tetrahydrocurcuminoids (THCs) (Castellan et al 2007; Portes et al 2007; Portes et al 2009).
19 They are derived from curcuminoids extracted from the roots of *Curcuma longa* L.,
20 commonly called turmeric. THCs are colorless and more potent than yellow curcuminoids as
21 antioxidants (Portes et al 2007); they could, therefore, be used in colorless foods and
22 cosmetics, where synthetic antioxidants are commonly used (Majeed et al 1990, 2008; see
23 also www.tetrahydrocurcuminoids.com). Curcuminoids and tetrahydrocurcuminoids involve
24 similar mechanisms for their antioxidative action (Portes et al 2007), but
25 tetrahydrocurcuminoids are much more photostable than curcuminoids (Castellan et al 2007).
26 The potential antimicrobial activity of tetrahydrocurcuminoids against several Gram-positive
27 bacteria (Venkateswarlu et al 2000) has also attracted interest because these compounds may
28 offer protection against *Listeria monocytogenes*, a human pathogenic bacterium. According to
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3 this promising perspective, we (Portes et al 2009) prepared films with both antibacterial and
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6 antioxidative properties from renewable sources that incorporate two efficient
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8 tetrahydrocurcuminoids derivatives, **THC1** and **THC2** (Figure 1), into a protonated chitosan
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10 film matrix.

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15 The study described in this paper was carried out to determine the time dependence effects of
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17 three tetrahydrocurcuminoids at different doses and in two different conditions (agar solid
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19 media or liquid medium) on the growth of a particular strain of *F. proliferatum* and on the
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21 biosynthesis of the FB₁ mycotoxin. Antifungal properties will be tentatively correlated to
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THCs structures.

Material and Methods

Chemicals and Reagents

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Compounds involved in the study are shown in Figure 1: THCs were synthesized as
previously reported (Portes et al 2007); acetylacetone (**AA**), eugenol (**E**) and ferulic acid (**FA**)
were commercial products obtained from Sigma-Aldrich France (Saint Quentin Fallavier); 4-
n-propylguaicol (**PG**) was prepared by hydrogenation of eugenol in methanol with Pd/C
(10%) under hydrogen pressure (80 bars) for 24 hours at 40° C according to Ruffin et al
(2002). The solvents were obtained from Sigma-Aldrich France (Saint Quentin Fallavier) and
were used as received. Fumonisin B₁ was purchased from Sigma-Aldrich (St Louis MO,
USA).

Insert Figure 1

Microorganisms and Culture Conditions

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The *F. proliferatum* strain, INRA 212, was from the INRA-MycSa laboratory collection. In
liquid cultures, the INRA 212 strain produced predominantly FB₁. Stock cultures were

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3 maintained on Potato dextrose agar (PDA) (Sigma-Aldrich) slants at 4°C. When inoculum
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5 was required, the *F. proliferatum* strains were grown on PDA slants at 25°C, in the dark, for 8
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7 days and spore suspension was prepared by adding sterile distilled water to slants followed by
8
9 gentle shaking. Spores concentrations were evaluated using a Thoma cell.
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12 13 14 15 *Antifungal Activity of Compounds*

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17 The antifungal activity of the phenolic compounds was determined as follows:

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19 (i) A THC solution (1 mL) at a known concentration was placed on the PDA surface and the
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21 medium dried in a laminar flow hood at 25 °C for 30 min. A growth control was performed
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23 with water in place of the THCs. The bioactivity of tetrahydrofuran (THF), the solvent used to
24
25 dissolve the biocides, was determined from experiments with THF in place of THCs. Five
26
27 microliters of spore suspension at 10^4 spores mL⁻¹ was deposited in the middle of each Petri
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29 dish (90 mm diameter) and incubated at 25°C and 75% relative humidity (RH) for fourteen
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31 days, that corresponds to 80 % of the colonized area in THF control Petri dishes.
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35 (ii) Fungal growth was determined by measuring the colony radial growth (RG), which was
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37 given as the average of two perpendicular diameters length measurements (in cm).
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41 (iii) Radial Growth (RG) was used to calculate inhibition percentages as:

$$42 \text{ Inhibition \%} = 100 (\text{RG}_{\text{THF control}} - \text{RG}_{\text{sample}}) / \text{RG}_{\text{THF control}}$$

43 44 45 46 47 48 *Mycotoxin and FB₁ Analyses in Liquid Medium*

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50 Liquid-culture experiments were performed in GYEP (Glucose, Yeast Extract, Peptone)
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52 medium (Miller et al 1983). Sterile Petri dishes (55 mm diameter) containing 7 mL of GYEP
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54 medium were inoculated with the spore suspension to achieve a final concentration of 10^4
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56 spores/mL. One milliliter of **THC1** solution, using THF/water (1v/1v) as the solvent, was
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58 added to the medium at an appropriate concentration. Cultures were incubated in the dark at
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3 25°C and 75% RH for 25 days. Mycelium was pelleted by centrifugation (10 000 rpm for 10
4
5 min at 4°C), stored at -80°C for two days and then lyophilized and weighted. The culture
6
7 medium was decanted and stored at -20°C until analyzed for FB₁.
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11 Fumonisin were extracted from culture filtrates by using an adaptation of a described
12
13 protocol (Shephard et al 1990). Briefly, 2 mL of culture filtrate was adjusted to pH 6.5 with
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15 NaOH (10 mol.L⁻¹) and fumonisins were purified by using Bond Elut Strong Anion Exchange
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17 (SAX) cartridges (Varian, Palo Alto, CA, USA). Fumonisin were eluted using 4 mL of
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19 methanol/acetic acid (99/1, v/v) and evaporated to dryness under a nitrogen stream. Dried
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21 samples were dissolved in 200 µL of methanol before high performance liquid
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23 chromatography analysis with fluorescence detection (HPLC-FLD). Ten µL of sample were
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25 derivatized with 90 µL of *o*-phthalaldehyde reagent. Twenty µL of this solution were
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27 injected into the HPLC system within one minute of derivatization. HPLC-FLD analysis was
28
29 performed as previously described (Shephard et al 1990). The HPLC system consisted of an
30
31 Agilent LC 1100 series apparatus equipped with a quaternary pump (G1311A), an
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33 autosampler (G1329A), a fluorimeter (G1314A) and the ChemStation chromatography
34
35 manager software (Agilent, Massy, France) with an Equisorb ODS2 5 µm column (length 150
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37 mm; i.d. 4.6 mm). Quantification of FB₁ was based on calibration with solutions prepared
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39 from commercially available standards. Results were expressed as µg of FB₁ per mg of
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41 mycelium.
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49 *Analysis of Results*

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51 Antifungal activity of THCs was evaluated using three repetitions conducted in parallel, in
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53 triplicate. The fungal radial growth was measured after 2, 3, 6, 7, 8, 9, 10 days of incubation.
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55 For mycelium and fumonisin B₁ production, data were obtained from five experiments. For
56
57 each experiment, five Petri dishes were prepared according to the previously described
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59 procedure with *F. proliferatum*, using respectively water, water/THF (1v/1v), and solutions of
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3 **THC1** in water/THF at different concentrations (0.8, 1.3, 1.9 and 2.7 $\mu\text{mol.mL}^{-1}$). Another
4 series of Petri dishes was prepared some days later followed by a third one. The experiments
5 were repeated with three replicates per treatment being destructively sampled at days five, ten,
6 fifteen, twenty and twenty five for analysis.
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12 A statistical one- or two-way (treatment, incubation time) ANOVA test was made on all the
13 data.
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16 17 18 19 20 **Results**

21 *Antifungal properties of THCs.*

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23 The effects of **THC1** at three different concentrations ranging from 2.7 to 13.4 $\mu\text{mol.mL}^{-1}$
24 against the *F. proliferatum* growth are reported in Figure 2. A lag-phase occurred whatever
25 the concentration. This latent period did not exceed 2 days in all cases. After the lag-phase,
26 the growth rates were affected by **THC1**.
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34 Insert Figure 2

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36 The comparison with control cultures using water, indicated that the solvent (THF) used to
37 dissolve tetrahydrocurcuminoid plays a role in decreasing the fungal growth during the first 7
38 days ($p < 0.05$). Analysis of all the results displayed in Figure 2 show that the growth of *F.*
39 *proliferatum* was statistically different when **THC1** was present ($p < 0.01$) and, as the THC
40 concentrations increase, the fungal growth decreases significantly ($p < 0.05$). The inhibition
41 factors were respectively close to 2, 4 and 6 for 2.7, 8.0 and 13.4 $\mu\text{mol.mL}^{-1}$ **THC1**
42 concentrations, respectively. Except for the lowest **THC1** concentration (2.7 $\mu\text{mol.mL}^{-1}$), the
43 inhibitory effect of the biocide was still detectable 10 days after the inoculation for both
44 higher concentrations.
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3 The efficiency of **THC1** to inhibit the *F. proliferatum* development was compared to that of,
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5 **THC2** and **THC3**, two other tetrahydrocurcuminoid derivatives (Figure 3)
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8 Insert Figure 3
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10 All THC derivatives inhibited the fungal growth after 10 days of incubation, and higher the
11 concentration, higher the inhibitory properties were. The best results were obtained by **THC1**
12 since the inhibition percentage reached 70% in cultures supplemented with a $13.4 \mu\text{mol.mL}^{-1}$
13 concentration. The lowest inhibition percentage, close to 30%, was obtained by **THC3**. A
14 comparison of the three THC profile curves showed that **THC2** showed a particular behavior:
15 the inhibition percentage was the lowest at low concentrations (8% at $2.2 \mu\text{mol.mL}^{-1}$) and was
16 greater than that of **THC3** at higher concentrations (40% compared to 30% at $13.4 \mu\text{mol.mL}^{-1}$).
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29 *Action of **THC1** against fumonisin B₁ production.*

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31 Stress factors, e.g. efficient biocides, can increase the production of secondary metabolites in
32 fungal cultures (Edreva et al; 2008). Thus, it was essential to measure the impact of the most
33 active THC on FB₁ production. This study was performed in GYEP liquid cultures
34 supplemented or not with **THC1**. Preliminary results were conducted in order to clarify the
35 antifungal efficiency using four tetrahydrocurcuminoid concentrations (0.8, 1.3, 1.9, 2.7
36 $\mu\text{mol.mL}^{-1}$) in water/THF (1v/1v). The mycelium growth in water/THF solutions, with or
37 without **THC1**, vs incubation time is shown in Figure 4a. A comparison with control cultures
38 in water indicate that THF plays a significant role ($p < 0.05$) in the decrease of mycelium
39 production during the first 20 days. Statistical analysis of results showed that mycelium
40 weights were statistically different ($p < 0.05$) in the presence and in the absence of **THC1**
41 (water/THF), with the noticeable exception of day 15. They depended both on **THC1**
42 treatment and incubation time ($p < 0.05$). Nevertheless, the general tendency was an increase of
43 the mycelium production between day 5 and day 15, followed by a slow decrease after day
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3 15. As the amount of **THC1** increased, the mycelium weight decreased ($p < 0.05$). In addition,
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5 a complete inhibition was observed with the highest **THC1** concentration ($2.7 \mu\text{mol.mL}^{-1}$).
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9 Insert Figure 4

10 To investigate the specific effect of **THC1** on FB_1 biosynthesis, **THC1** concentrations ≤ 1.9
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12 $\mu\text{mol.mL}^{-1}$ were used. Fumonisin accumulation was detected as early as day 5 in water
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14 control cultures and day 10 in water/THF control cultures. This could be due to a too low
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16 fungal growth in cultures, except in water controls. Taking into account this observation, the
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18 determination of **THC1** impact on fumonisin FB_1 production will be only discussed from day
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20 10 (Figure 4b). In the water/THF control and in the **THC1** supplemented-cultures at 0.8, 1.3,
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22 $1.9 \mu\text{mol.mL}^{-1}$, the total fumonisin production increased almost linearly, from day 10 to day
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24 20, with calculated R^2 of 0.985, 0.999, 0.999 and 0.678, respectively. After day 20, a small
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26 decrease of the fumonisin production was observed. This could be due to a stationary
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28 production of FB_1 associated to a possible degradation or complexing of the mycotoxin with
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30 chemicals of the medium (Meca et al 2010). Statistical analysis of data indicated that all the
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32 results obtained after **THC1** treatments were significantly different from those of the control
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34 solvent ($p < 0.01$). All the **THC1** treatments had significant effects after day 10 ($p < 0.05$). For
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36 day 10, the impact of **THC1** concentrations was not significant ($p \geq 0.06$). The general
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38 tendency was a decrease in fumonisin production when **THC1** was present; as **THC1**
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40 concentrations increased, the fumonisin amounts decreased, which was statistically evidenced
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42 after 10 days of incubation.
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52 FB_1 production measured in 15, 20 and 25 days-old cultures supplemented with **THC1** was
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54 respectively reduced by 31, 37 and 32 % (**THC1** concentration: $0.8 \mu\text{mol.mL}^{-1}$); 41, 47 and
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56 58 % (**THC1** concentration: $1.3 \mu\text{mol.mL}^{-1}$) and close to 75% (**THC1** concentration: 1.9
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58 $\mu\text{mol.mL}^{-1}$).
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Discussion

Antifungal activities of tetrahydrocurcuminoids

A comparison of the antifungal activities of **THC1**, **THC2** and **THC3** suggested that their behavior was strongly related to their structures. The presence of the phenol and methoxy groups in the *ortho* position of the benzene ring was essential in the antifungal activity of THCs. Nevertheless, a second methoxy group in the *ortho* position of the phenol group (**THC2**) led to reduce antifungal activity. Similar behavior on photochemical reactivity and antioxidant properties were observed when **THC2** was incorporated into carboxymethylcellulose (Castellan et al, 2007) or chitosan (Portes et al, 2009) solid-state matrices. The high antifungal activity of the non-phenolic **THC3** (Table 1) was surprising and suggested that the enolic part of the molecule can have an important role in the bioactivity of THCs (Sugiyama et al, 2006). We also conducted similar studies with *n*-propylguaiacol (**PG**) to approximate the action of the guaiacyl nucleus; acetylacetone (**AA**) to approximate the action of the enol group; eugenol (**E**) and ferulic acid (**FA**) to assess the role of different substituents in the *para* position of the phenol (Figure 1). The selected concentration of **AA** was equal to that of **THC1** and **THC3** ($13.4 \mu\text{mol}\cdot\text{mL}^{-1}$). **PG**, **E** and **FA** were tested at twice the concentration ($26.8 \mu\text{mol}\cdot\text{mL}^{-1}$), because the THCs have two benzene rings and one enol group. Results are summarized in Table 1.

Insert Table 1

The percentage inhibition of **THC3** was more than three fold higher than **AA**, which shows that the benzene rings have an important role in the antifungal mechanism. A significant antifungal activity was ascribed to **PG** (49% of growth inhibition after ten incubation days), suggesting that the guaiacyl unit played a considerable role in the bioactive properties. The biocide efficiency of **THC1** was different from the cumulative antifungal activities of **PG** and

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2
3 **AA**, which indicated that the constituting units, **PG** and **AA**, do not well mimic the behavior
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5 of **THC1**. A similar observation was made on the electronic absorption of **THC1** (Castellan
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7 et al 2007).
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12 Ferulic acid (**FA**) and eugenol (**E**) were introduced in this comparative study, owing to their
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14 similarities in structures (Fig. 1) with parts of the THC molecule: **FA** contains both cinnamic
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16 acid and guaiacyl structures; **E** has a guaiacyl unit and a non-conjugated double bond. Recent
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18 papers showed some interesting data concerning these compounds: **FA** inhibits type B
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20 trichothecene biosynthesis (Boutigny et al, 2009); cinnamic acid inhibits the fungal growth of
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22 *Neurospora crassa* (Said et al, 2004), *Rhodotorula minuta* (Fujii et al, 2002), *F. oxysporum*
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24 (Wu et al, 2008) and **E** has known antifungal activity against *Candida albicans* (Braga et al,
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26 2007). In this study, **E** and **FA** did reduce the fungal growth of *F. proliferatum* by practically
27
28 the same inhibition percentage (*ca* 30 %) but largely lower than the inhibition induced by
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30 THC derivatives. So, it seemed that the presence of either the carbon-carbon double bond or
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32 the carboxylic acid function in the lateral chain of the guaiacyl unit reduced the antifungal
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34 activity.
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44 Some differences appear when comparing antioxidant and antifungal activities of **THC1**,
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46 **THC2** and **THC3**. Antioxidant properties need the presence of a phenol group in the meta- or
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48 para-position of the linking chain and a phenol or a methoxy group as neighbour; moreover,
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50 the involvement of the β -diketone enolic part should be weak (Portes et al 2007). Antifungal
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52 properties of the THCs seem to be related to the presence of benzene rings, phenol and
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54 methoxy groups in the *ortho* position of the benzene rings and also to β -diketone enol. The
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56 presence of a syringyl unit (two methoxy groups near the phenol function) in the THC
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3 structure appears unfavorable. Thus, it is likely that antioxidant and antifungal activities of
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structure appears unfavorable. Thus, it is likely that antioxidant and antifungal activities of
THCs do not involve the same molecular mechanisms.

*Action of **THC1** against fumonisin B₁ Production.*

THC1, which performed the best antifungal activity, affected the fungal growth in liquid
medium and led to a complete inhibition at a relatively low concentration (2.7 $\mu\text{mol.mL}^{-1}$).
When the fungal strain was not inhibited, **THC1** still influenced FB₁ production, mainly after
ten days of incubation. These results suggested that both fungal growth and fumonisin
biosynthesis were likely modified by **THC1**. Similar results were observed with (i) artificial
antioxidants, such as BHA and propylparaben, which limited the fungal growth of *Aspergillus*
flavus, *Aspergillus parasitus* and *Fusarium* and the production of associated mycotoxins
(Thompson 1992, 1996) and (ii) on acid-type natural antioxidants (caffeic and vanillic acids)
on *Fusarium verticillioides*, *F. proliferatum* and *Aspergillus* and FB₁ metabolite (Samapundo
et al 2007; Beekrum et al 2003). It is likely that the fungal membrane was affected by **THC1**,
as described by Torres et al (2003) and Braga et al (2007).

Tetrahydrocurcuminoids, particularly **THC1**, appear as a new class of compounds that can
inhibit *F. proliferatum* growth and toxic FB₁ biosynthesis at a relative low *in vitro*-
concentration. The mechanisms involved in this inhibition require to be elucidated and the
potentiality of *in vivo* applications has to be investigated. **THC1** is a metabolite (Pan et al,
1999) of the natural well-known non-toxic curcumin used in food, and its preparation by
hydrogenation of the latter can be performed in a large scale for many industrial applications
(Majeed M, Badmaev V, 2002; Seyler et al, 2003).

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Figure Captions

Figure 1: Chemical structures of compounds involved in the study (THCs: tetrahydrocurcuminoids; **PG**: n-propylguaiacol; **E**: eugenol; **AA**: acetylacetone; **FA**: ferulic acid)

Figure 2: *F. proliferatum* radial growth versus time of incubation in presence of **THC1** at different concentrations: (-◇-) THF; (-●-) **THC1** 2.7 $\mu\text{mol.mL}^{-1}$; (-×-) **THC1** 8.1 $\mu\text{mol.mL}^{-1}$; (-■-) **THC1** 13.4 $\mu\text{mol.mL}^{-1}$ (THF is the control-solvent, in absence of **THC1**).

Figure 3: Inhibition percentage of *F. proliferatum* growth in presence of biocides at different concentrations after 10 days incubation at 25 °C and 75 % RH: (-◆-) **THC1**; (-×-) **THC2**; (-▲-) **THC3**.

Figure 4: a) Dry weight mycelium obtained by incubation with *F. proliferatum* after 5, 10, 15, 20 and 25 days in presence of various quantities of **THC1** in liquid medium. (-□-): THF/water 1v/1v; (-△-): **THC1** 0.8 $\mu\text{mol.mL}^{-1}$; (-×-): **THC1** 1.3 $\mu\text{mol.mL}^{-1}$; (-+-): **THC1** 1.9 $\mu\text{mol.mL}^{-1}$; (-○-): **THC1** 2.7 $\mu\text{mol.mL}^{-1}$ (For clarity smoothed line were drawn between points). b) FB_1 metabolite produced by *F. proliferatum* after 11, 15, 20 and 25 days in presence of various quantities of **THC1** in liquid medium. (-□-): THF/water 1v/1v; (-△-): **THC1** 0.8 $\mu\text{mol.mL}^{-1}$; (-×-): **THC1** 1.3 $\mu\text{mol.mL}^{-1}$; (-+-): **THC1** 1.9 $\mu\text{mol.mL}^{-1}$.

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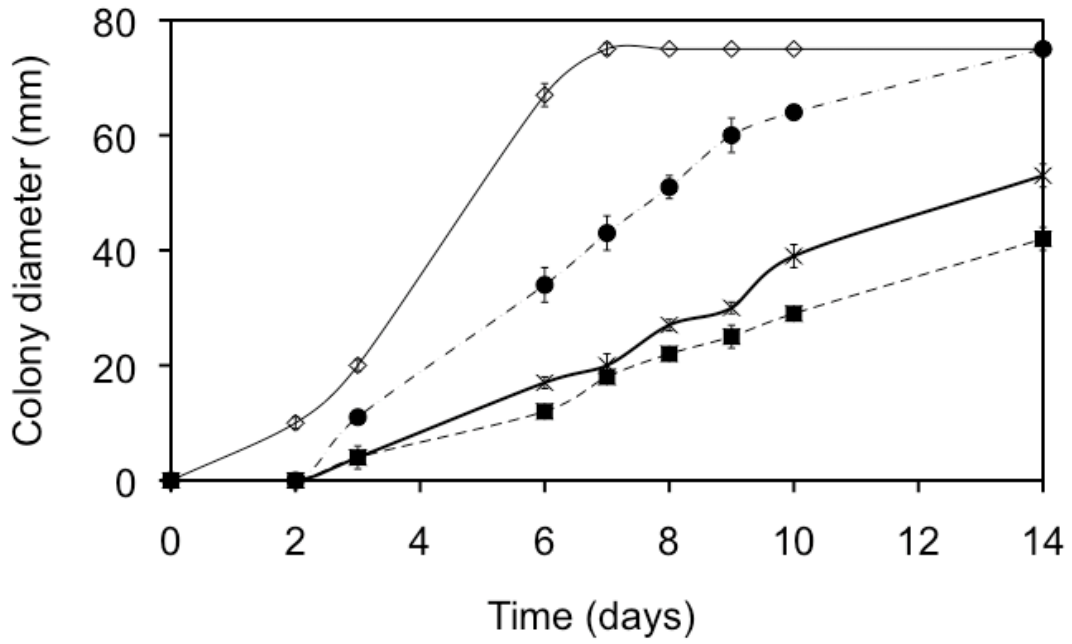


Figure 2

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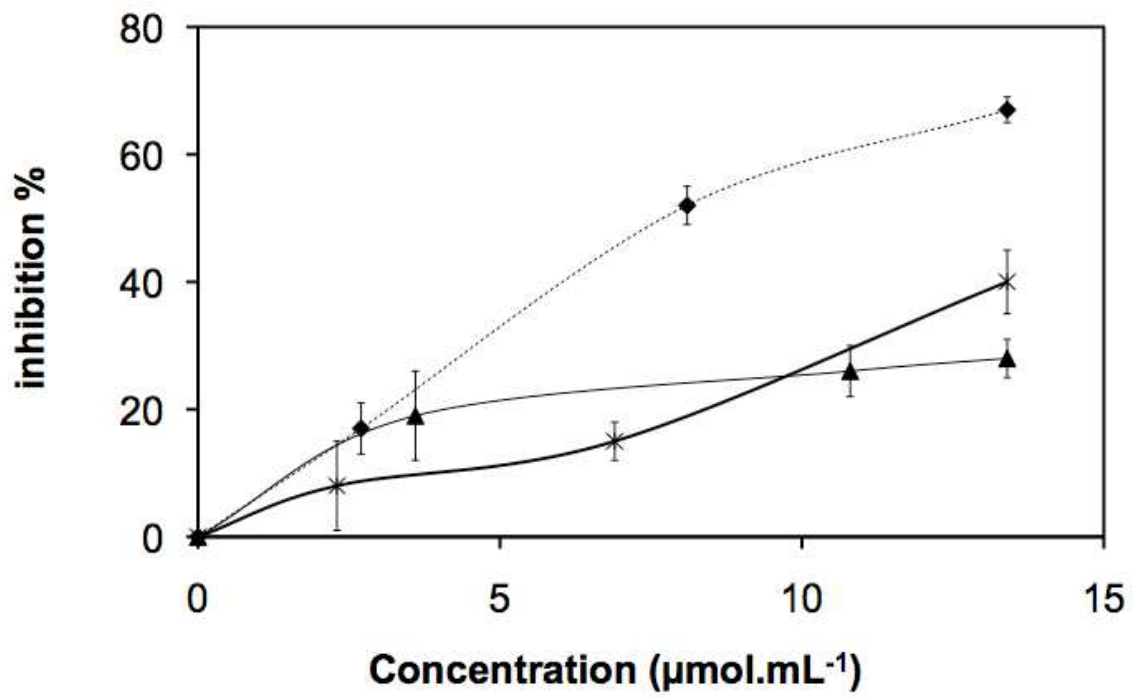


Figure 3

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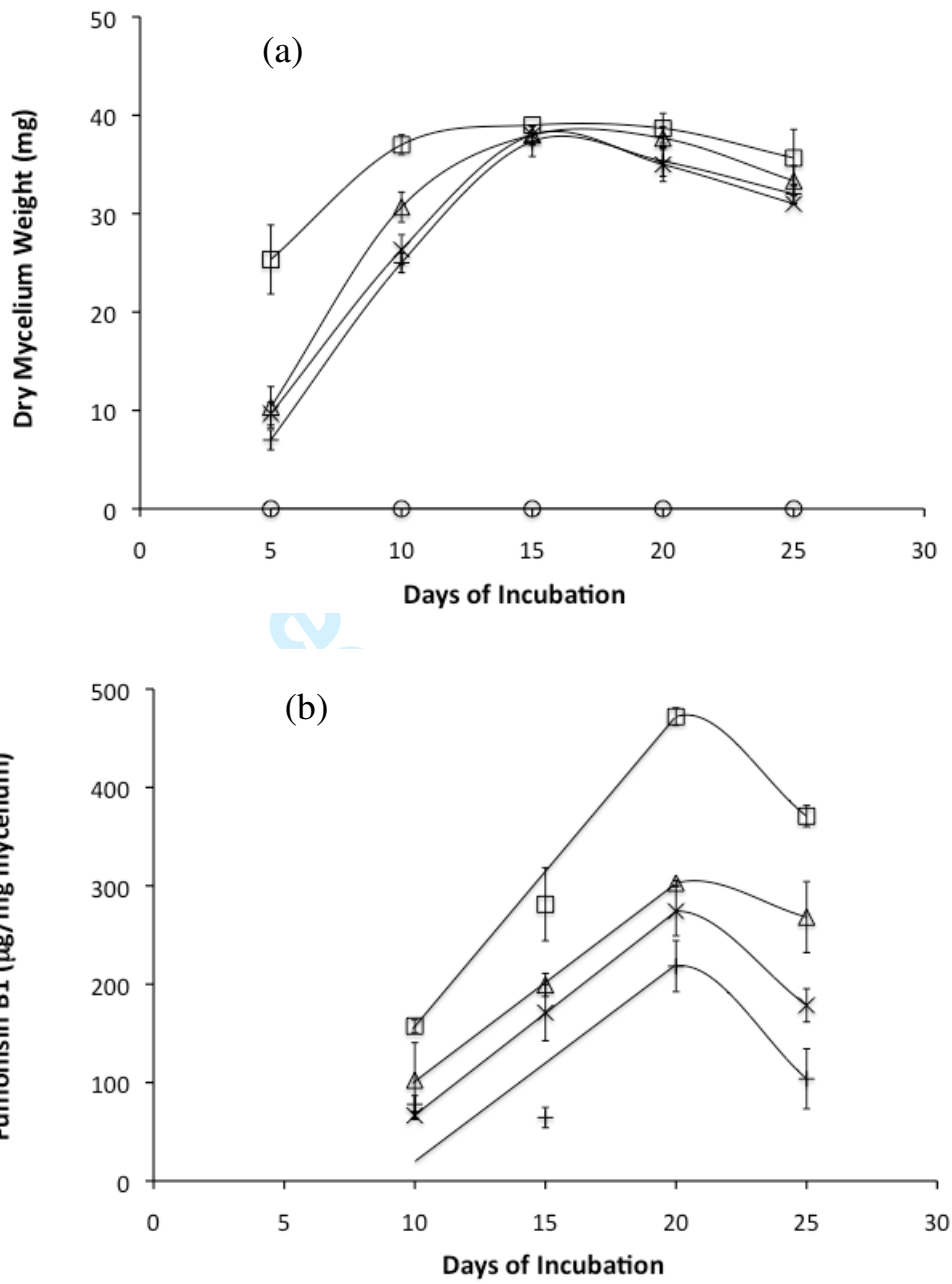


Figure 4

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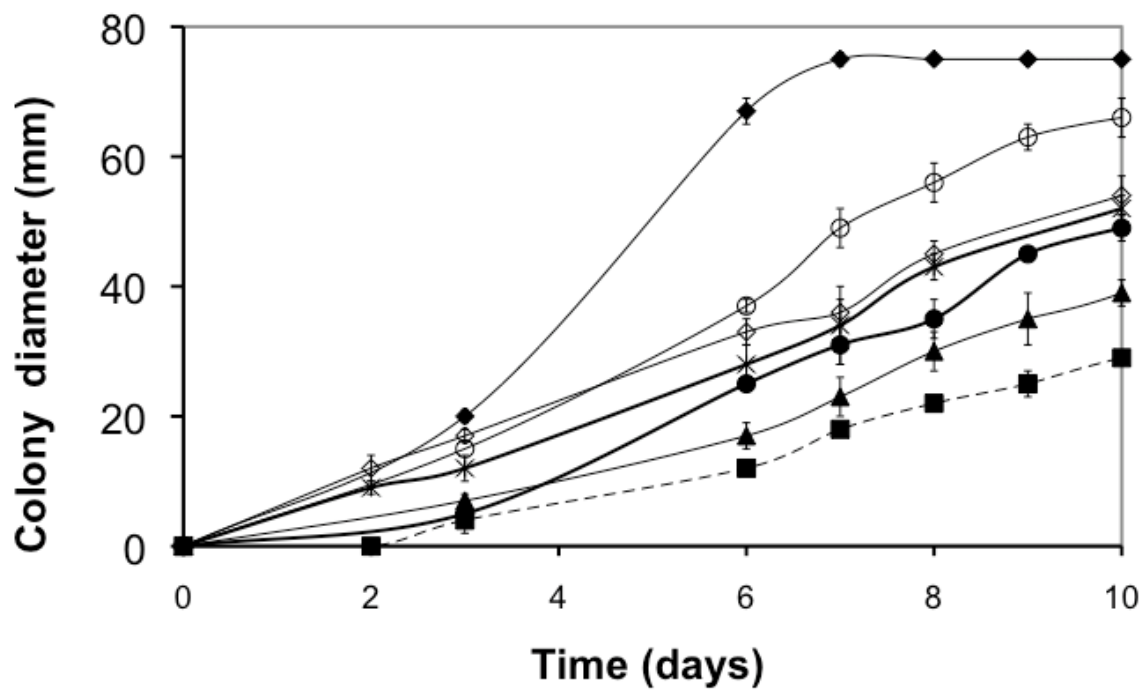


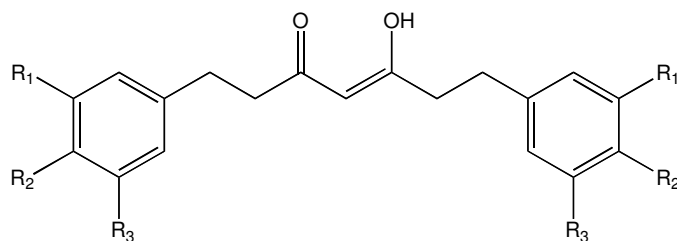
Figure 5

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Table 1: Inhibition percentage of *F. proliferatum* radial growth after 10 days of incubation in presence of biocides (water and THF are control experiments) at 25 °C and 75 % RH.^a

Compounds ^a	Inhibition % (\pm SD)
Water	0 ^b
THF	0 ^b
THC1 (13.4 $\mu\text{mol.mL}^{-1}$)	67 \pm 2
THC2 (13.4 $\mu\text{mol.mL}^{-1}$)	40 \pm 5
THC3 (13.4 $\mu\text{mol.mL}^{-1}$)	28 \pm 3
AA (13.4 $\mu\text{mol.mL}^{-1}$)	9 \pm 4
PG (26.8 $\mu\text{mol.mL}^{-1}$)	49 \pm 4
E (26.8 $\mu\text{mol.mL}^{-1}$)	31 \pm 4
FA (26.8 $\mu\text{mol.mL}^{-1}$)	29 \pm 5

^a: See scheme 1; ^b: Petri dishes entirely covered by *F. proliferatum*



THC1 R₁ = OCH₃ ; R₂ = OH ; R₃ = H

THC2 R₁ = OCH₃ ; R₂ = OH ; R₃ = OCH₃

THC3 R₁ = R₂ = R₃ = H

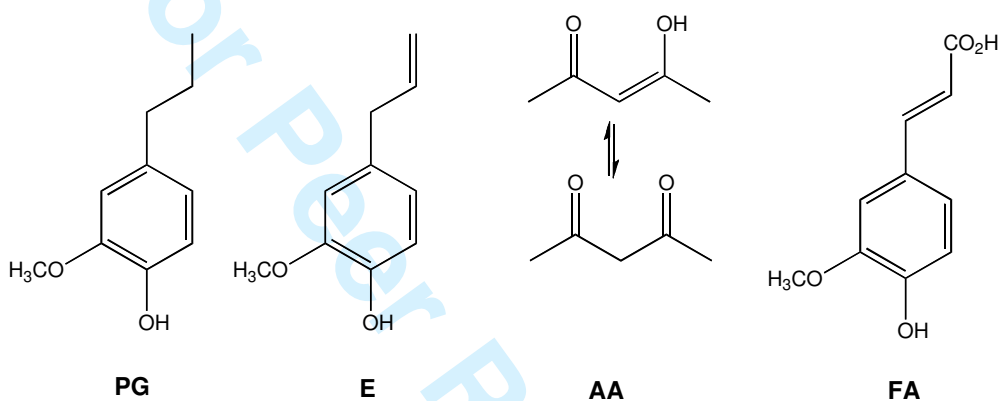


Figure 1