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# Inhibitory effect of 10 natural phenolic compounds on *Fusarium verticillioides*. A structure–property–activity relationship study

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ABSTRACT

Fusarium is a ubiquitous hyalohyphomycete fungus usually isolated from food, which is widespread in different environments (plant, grain, soil) and present at all latitudes. This genus has been widely studied due to its ability to infect, cause tissue destruction and produce mycotoxins on important crops such as corn, wheat and other small grains. Over recent years, much effort has been directed at the search for new antifungal materials from natural sources, and many antimicrobial compounds coming from plants have been identified. Although the essential oils and their components have been recommended as fumigants for preservation of food commodities, little is known about the molecular properties related to the antifungal activity. In the present study, we performed a QSAR study for the inhibition of *Fusarium verticillioides* growth by ten natural phenolic compounds, which could serve as a guide for the rational design of further inhibitors. The results of the experimental determinations demonstrated that in terms of the antifungal activity of natural phenolic compounds on *F. verticillioides*, the following order was found: carvacrol > thymol > isoeugenol > eugenol > vanillin > creosol > m-cresol > o-cresol, p-cresol, and guaiacol. Mathematical models based on molecular properties, Lipophilicity, Molar refractivity and Saturated area were found to be the descriptors that best explained the antifungal activity of these compounds. These models could be used in future to predict the activity of new compounds and to guide the search for the synthesis of phenolic compounds with the capacity to alter *F. verticillioides* growth.


## 1. Introduction

Fusarium is a ubiquitous hyalohyphomycete fungus usually isolated from food, which is widespread in different environments (plant, grain, soil) and present at all latitudes. This genus has been widely studied due to its ability to infect and cause tissue destruction on important crops such as corn, wheat and other small grains on the field. In addition, several species of Fusarium may produce mycotoxins on the crops both in the field and in storage grains. In general, synthetic fungicides are used for the control of pathogenic and toxigenic fungi. However, potential toxicological problems (Chen, Moore, & Nesnow, 2008) and considerable deterioration of the environmental quality and human health (Cutler & Cutler, 1999, p. 299) has generated considerable interest in the preservation of grains by the use of naturally occurring compounds (Sofos, Beuchat, Davidson, & Johnson, 1998). Over recent years, much effort has been directed at the search for new antifungal materials from natural sources, and many antimicrobial compounds coming from plants have been identified, with their essential oils and components being recommended as fumigants the for preservation of food commodities (Singh et al., 2010). Several publications have reported the antifungal activity of some phenolic components of essential oils such as thymol, carvacrol and eugenol on *Fusarium verticillioides* (Dambolena et al., 2008; Menniti, Gregori, & Neri, 2010). However, little is known about the molecular properties related to the antifungal activity.

The quantitative structure–activity relationship (QSAR) is a method by which the chemical structure is quantitatively correlated with a well-defined process, such as biological activity or chemical reactivity. The QSAR approach employs extra-

*Abbreviations:* FW, formule weight; MR, molar refractivity; MV, molar volumen; ST, surface tension; *P*, polarizability; log *P*, logarithm of the octanol/water partition coefficient; PSA, polar surface area; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; pKa, logarithm of dissociation constant; PA, polar area; TA, total area; SA, saturated area; UA, unsaturated area; VdWSA, Van der Walls surface force; DPPH, free radical-scavenging activity; *K'*, phospholipid interaction capacity; Solv. acc., solvent accessible surface area.

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thermodynamically derived and computational-based descriptors to correlate biological activity in isolated receptors, cellular systems, and in vivo (Verma & Hansch, 2006). QSAR studies are widely used to predict the activity of new chemical compounds and to establish a mechanism for a better understanding of chemical–biological interactions in drug-design process. Although QSAR studies on the biological action of phenols have been reported for different biological systems (Dambolena, Zygadlo, & Rubinstein, 2011; Greenberg, Dodds, & Tian, 2008; Verma & Hansch, 2006; Voda, Boh, & Vrtacnik, 2004), no QSAR studies on *F. verticillioides* growth have yet been performed.

The aim of this investigation was to determine the effect of phenolic compounds on *F. verticillioides* growth and to evaluate which of the molecular properties of the natural phenolic compounds are important in antifungal activity. In the present work, we performed a QSAR study on the inhibition of *F. verticillioides* growth by natural phenolic compounds, which could serve as a guide for the rational design of further inhibitors.

## 2. Material and methods

### 2.1. Materials

5-Isopropyl-2-methylphenol (carvacrol), 2-isopropyl-5-methylphenol (thymol), 2-methylphenol (ortho-cresol), 3-methylphenol (meta-cresol), 4-methylphenol (para-cresol), 2-methoxy-4-prop-2-enylphenol (eugenol), 2-methoxy-4-[(E)-prop-1-enyl]phenol (isoeugenol), 2-methoxy-4-methylphenol (creosol), 2-methoxyphenol (guaiacol) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) were purchased from Fluka-Kahlbaum-Germany (Fig. 1).

### 2.2. Fungal strain

One isolate of *F. verticillioides* MRC 4316 PROMEC (from the Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg; Republic of South Africa) grown on carnation leaf agar by monosporic isolation was used in all the experiments.

Other studies have demonstrated the ability of *F. verticillioides* MRC 4316 to produce fumonisins under different culture conditions (Vismer, Snijman, Marasas, & van Schalkwyk, 2004).

### 2.3. Testing for antifungal activity

#### 2.3.1. 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-millilitre aliquots of semisolid brain–heart infusion broth (Difco Laboratories, Detroit, Michigan, USA.) 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 phenolic compounds. These compounds were dissolved with dimethyl sulfoxide (DMSO), and then added the different tubes in order to obtain concentrations of 0.2; 0.32; 0.4; 0.65; 0.8; 1.0; 1.2; 1.6; 2.0; 2.4 and 3.0 mM in the culture medium. The final concentration of DMSO was adjusted to 5 μl/ml in all the tubes. As control, a compound-free medium with a 5 μl/ml final concentration of DMSO was used. Phenolic compound solutions were mixed with the medium at 45 °C, and then the media were stored at 4 °C until solidification. *F. verticillioides* was previously grown on Czapek-Dox agar in Petri plates for 7 days at 28 °C in the dark to allow profuse sporulation. Sterile distilled water was added to each plate and a conidia suspension ($1 \times 10^6$ conidia/ml) was obtained by scraping the colony surface with a sterile Drigalsky spatula and then filtering it through a cheese cloth. A standard loopful (0.001 ml) of this conidia suspension was inserted deeply into each tube of medium containing a known concentration of phenolic compounds, as well as into the compound-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. All cultures were incubated in the dark for 72 h at 28 °C. Then, the growth in all tubes was visually compared with that of the control in order to determine inhibition and scored in the following manner: 4+, growth comparable to that

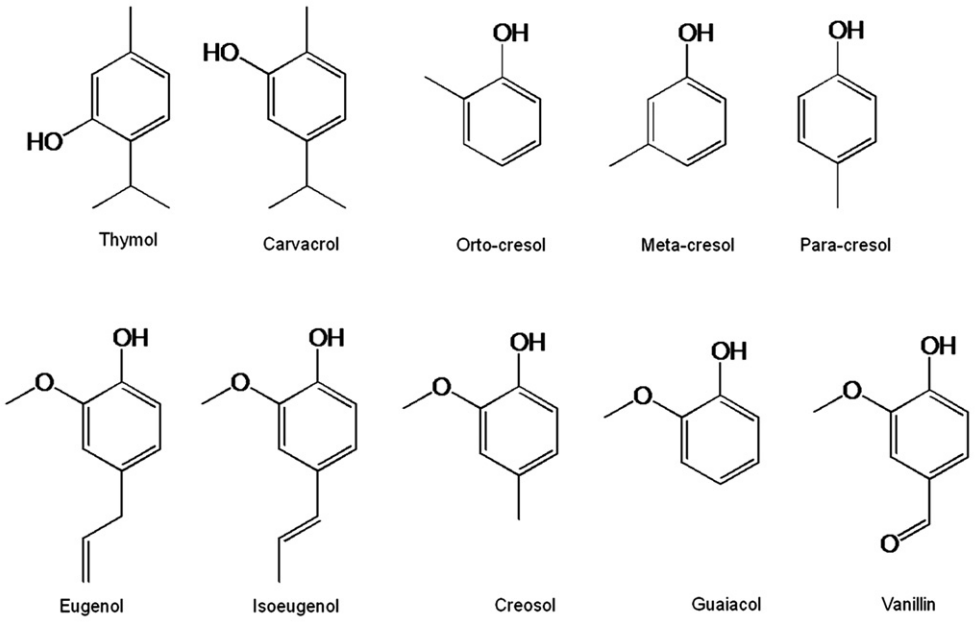


**Fig. 1.** Chemical structures of natural phenolic compounds studied in the present work.