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BRIEF REPORT

Growth modeling to control (*in vitro*) *Fusarium verticillioides* and *Rhizopus stolonifer* with thymol and carvacrol

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KEYWORDS
Thymol;
Carvacrol;
Synergism;
Fungal growth

Abstract The aim of this study was to evaluate the antifungal activity (*in vitro*) of thymol and carvacrol alone or in mixtures against *Fusarium verticillioides* and *Rhizopus stolonifer*, and to obtain primary growth models. Minimal inhibitory concentration (MIC) was evaluated with fungal radial growth with thymol or carvacrol concentrations (0–1600 mg/l). Mixtures were evaluated using concentrations below MIC values. Radial growth curves were described by the modified Gompertz equation. MIC values of carvacrol were 200 mg/l for both fungi. Meanwhile, MIC values of thymol were between 500 and 400 mg/l for *F. verticillioides* and *R. stolonifer*, respectively. A synergistic effect below MIC concentrations for carvacrol (100 mg/l) and thymol (100–375 mg/l) was observed. Significant differences ($p < 0.05$) between the Gompertz parameters for the antimicrobial concentrations and their tested mixtures established an inverse relationship between antimicrobial concentration and mycelial development of both fungi. Modified Gompertz parameters can be useful to determine fungistatic concentrations.

PALABRAS CLAVE
Timol;
Carvacrol;
Sinergismo;
Crecimiento fúngico

Modelización del crecimiento *in vitro* para controlar *Fusarium verticillioides* y *Rhizopus stolonifer* con timol y carvacrol

Resumen El objetivo de este trabajo fue evaluar la actividad antifúngica *in vitro* del timol y del carvacrol, solos o en mezclas, contra *Fusarium verticillioides* y *Rhizopus stolonifer*, y

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obtener modelos primarios de crecimiento. Se evaluó la concentración inhibitoria mínima (CIM) con el crecimiento radial, se ensayaron concentraciones de timol y carvacrol de 0 a 1.600 mg/l. Las mezclas se evaluaron utilizando concentraciones por debajo de los valores de CIM. Las curvas de crecimiento radial fueron descritas por la ecuación de Gompertz modificada. Se obtuvieron los siguientes valores de CIM: carvacrol, 200 mg/l para las 2 especies; timol, 500 mg/l y 400 mg/l para *F. verticillioides* y *R. stolonifer*, respectivamente. Se observó un efecto sinérgico a concentraciones inferiores a las CIM para el carvacrol (100 mg/l) y el timol (100-375 mg/l). Hubo diferencias significativas ($p < 0,05$) entre los parámetros de crecimiento de Gompertz; se estableció que existe una relación inversa entre la concentración de los antimicrobianos y el desarrollo del micelio de ambos hongos.

Fusarium spp. and *Rhizopus* spp. are widespread and ubiquitous in the environment. They can contaminate food before harvest or under post-harvest conditions and are considered food spoilers. Some *Fusarium* species can produce mycotoxins, decreasing the commercial value of the affected products[1].

Fungal contamination, colonization and infection of plants are initiated by contact of the host with conidia (spores) and subsequent conidial germination. Germination and initiation of infections involve biochemical activities with an increase in metabolism and induction of morphological changes. Fungal survival and growth in food may lead to spoilage and toxin formation. These metabolites are structurally diverse compounds and represent an important category of natural toxins that can affect humans and result in economic losses worldwide[12].

For years, synthetic chemical additives were efficient to control food fungal growth; however, these products represent a potential hazard to human health. Currently, a lot of studies on food preservation by natural compounds are being carried out[8]. Many antimicrobial compounds from plants have been identified; several publications reported the antifungal activity of some phenolic components of essential oils such as thymol and carvacrol. Natural isopropyl cresols, carvacrol (5-isopropyl-2-methylphenol), and thymol (2-isopropyl-5-methylphenol) are the major components of oregano (*Origanum* spp.) and thyme (*Thymus* spp.) essential oils. Some researchers have pointed out the antimicrobial activity against bacteria, molds, and yeast[11] of these natural extracts. Carvacrol and thymol are Generally Recognized as Safe (GRAS) food additives, and are used as flavoring agents in baked goods, sweets, beverages, and chewing gum[3].

Food preservation trends indicate the use of new chemical preservatives simultaneously in mixtures to keep food safety and quality at lower antimicrobial doses[1]. These mixtures provide a wider range of increasing activity against different pathogenic microorganisms, or act on several points inside cells, which can enable a better control compared to individual agents[13]. Moreover, in order to evaluate antimicrobial action, predictive microbiology models are valuable. Different primary models describe either germination or mycelium growth kinetics of various fungal species on food products[15]. The modified Gompertz equation is a suitable predictive tool applied in nonlinear growth curves that describe quantitative parameters such as growth, lag phase and fungal growth rate[12].

Therefore, the aim of this study was to evaluate the antifungal activity (*in vitro*) of thymol and carvacrol alone or in mixtures against *Fusarium verticillioides* and *Rhizopus stolonifer* species, and to obtain predictive models of growth.

Microorganisms and preparation of cultures: *F. verticillioides* and *R. stolonifer* were obtained from the Facultad de Ciencias Químicas (Benemérita Universidad Autónoma de Puebla) collection. The microorganisms were maintained on Petri dishes containing sterilized potato-dextrose agar (PDA Merk, Mexico City, Mexico) and incubated in a dark environment at 28 °C for 5–6 days until fungal growth was observed. Fungal structures (conidia and mycelia) were observed using a Zeiss Primo Star microscope (Carl Zeiss AG, Göttingen, Germany) and identified according to taxonomic keys[4].

Minimum Inhibitory Concentration (MIC): thymol or carvacrol (Sigma–Adrich, Milwaukee, WI, USA) were mixed (using a vortex shaker) with sterilized PDA medium to achieve final concentrations of 100, 150, 200, 400, 800 and 1600 mg/l (concentrations were selected according to reference[1]). Agar solutions were poured into sterile petri dishes. Fungal spores were obtained by pouring 9 ml of sterile physiological water (0.90% w/v of NaCl) on the agar plate surface previously inoculated with each mold, followed by gentle scraping using a sterile rake to remove the maximum quantity of spores. Spore suspensions were transferred into sterile tubes. The number of spores present in the suspension was determined using a hemocytometer and an optical microscope (Zeiss Primo Star, Göttingen, Germany), and expressed as number of spores per milliliter (spores/ml). Suspensions were serially diluted to approximately 1000 spores/ml. Finally, plates were inoculated with 10 μl of spore suspension in the center of the plate and were incubated at 28 °C; radial growth was measured every 12 h during 84–96 h. A growth control was prepared in parallel to ensure that viable organisms were present. Every test was performed in triplicate. MIC values were determined as the lowest concentration at which no growth occurred[3].

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