THE INHIBITORY EFFECT OF TROPOLONE AND HINOKITIOL ON THE GROWTH AND DEVELOPMENT OF FUSARIUM OXYSPORUM F. SP. TULIPAE

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Abstract

The effect of tropolone and hinokitiol (β-thujaplicin) on in vitro growth of Fusarium oxysporum f. sp. tulipae was determined. Tropolone and hinokitiol greatly inhibited the growth of F. oxysporum f. sp. tulipae mycelium on potato dextrose agar (PDA). Total inhibition of the mycelium growth took place at tropolone concentration of 100 μg/cm³ and at hinokitiol concentration of 50.0 μg/cm³. Fungicidal doses of tropolone and hinokitiol for the F. oxysporum f. sp. tulipae mycelium growth were also documented. The results are discussed with data available in literature on the antifungal action of tropolone and hinokitiol on other species of pathogenic fungi.

Key words: Fusarium oxysporum f. sp. tulipae, mycelium growth, tropolone, hinokitiol (β-thujaplicin)

Introduction

Hinokitiol (β-thujaplicin) is a tropolone(2-hydroxy-2,4,6-cycloheptatrien-1-one)-related compound that is present in the heartwood of several Cupressaceae trees, such as Chamaecyparis obtusa, Cupressus lusitanica, Juniperus chinensis, Thuja occidentalis, Thujopsis dolabrata var. hondai (Haluk and Rousell 2000, Chedgy et al. 2007). Some other hinokitiol-related compounds were identified in above mentioned species, and other species of Cupressaceae trees, particularly α-thujaplicin, γ-thujaplicin, β-thujaplicinol, β-dolabrin, nootkatin (Haluk and Rousell 2000, Chedgy et al. 2007). The occurrence of hinokitiol and its related compounds in several Cupressaceae trees was reviewed in detail by Haluk and Rousell (2000).
Antifungal activity of hinokitiol, tropolone and their related compounds is well documented in literature. The fungicidal activity of tropolone was investigated towards in vitro growth of different white and brown rot fungi involved in wood biodegradation: *Coriolus versicolor*, *Phanerochaete chrysosporium*, *Poria placenta* and *Gloeephyllum trabeum*. It was showed that tropolone strongly inhibited the growth of these fungi on malt agar and antifungal activity of tropolone was similar to that of commercially available fungicides (Baya et al. 2001).

Tropolone and hinokitiol also inhibited in vitro mycelium growth of *Pythium aphanidermatum*, *Thanatephorus cucumeris*, *Fusarium solani*, *Botryotinia fuckeliana*, *Phomopsis obscurans*, *Colletotrichum lagenarium* and *C. orbiculare*, and their minimum inhibitory concentration (MIC) values ranged from 6.0 to 50.0 µg/l (Morita et al. 2003).

Saniewska and Saniewski (2007) documented the inhibitory effect of tropolone and hinokitiol on in vitro mycelium growth of *Phoma narcissi*, a pathogen of the Amaryllidaceae plants. The compounds, applied preventively, substantially inhibited the growth of the pathogen on excised scales and leaves of *Hippeastrum* (Saniewski et al. 2007).

Not only tropolone and hinokitiol have antifungal properties: related compounds with antifungal activity were isolated from heartwood of several Cupressaceae species. α-Thujaplicin, β-dolabrin, γ-thujaplicin and 4-acetyl-tropolone – components of *T. dolabrata* var. *hondai* showed strong antifungal activity against seven species of plant pathogenic fungi (Morita et al. 2004 a, b) earlier tested by Morita et al. (2003).

In the work a strong inhibitory effect of tropolone and hinokitiol on the mycelium growth of *Fusarium oxysporum* f. sp. *tulipae*, a severe pathogen of tulip bulbs, was demonstrated.

**Materials and methods**

The stock culture of *F. oxysporum* f. sp. *tulipae* (isolates *F.o.x.t.* 17 and *F.o.x.t.* 218) was maintained on potato dextrose agar (PDA, Merck) slants at 25°C in the dark.

The effect of tropolone and hinokitiol (β-thujaplicin) (purchased from Sigma-Aldrich Chemicals) on the growth of *F. oxysporum* f. sp. *tulipae* mycelium on PDA medium was investigated. The compounds were used at following final concentrations: 5.0, 10.0, 25.0, 50.0 and 100.0 µg/cm³ in PDA medium. Hinokitiol was dissolved in small amounts of 50% ethanol and tropolone was dissolved in distilled and sterilized water, and then they were added to PDA medium (sterilized at about 50°C). 5 mm diameter plugs were taken from seven-day-old culture of *F. oxysporum* f. sp. *tulipae*, and placed in the middle of 90 mm Petri dishes containing PDA medium supplemented with the tested compounds. Control plates contained the culture growing on PDA, without any additions and supplemented with ethanol at appropriate concentration. Five Petri dishes were used as an experimental unit and the trial was repeated twice. After two, four and six days of incubation (in
darkness at 25°C) the diameter of fungal colonies was measured in two perpendicular directions.

Additionally, mycelium plugs from which the colonies did not develop, were transferred into plates containing clean PDA and observed for six days.

The data were subjected to analysis of variance and Duncan’s multiple range test at 5% of significance was used for means’ separation.

Results and discussion

Tropolone and hinokitiol, applied directly to the PDA medium, exerted great inhibitory effect on the growth of *F. oxysporum* f. sp. *tulipae* mycelium. After eight days of incubation tropolone at concentration of 25.0 \( \mu g/cm^3 \) and 50.0 \( \mu g/cm^3 \) limited the mycelium growth of *F. oxysporum* f. sp. *tulipae* (isolate *F.oxt*. 17) in 42.6 and 65.3%, respectively and of isolate *F.oxt*. 218 in 28.2 and 100.0%, respectively. Tropolone at a concentration of 100 \( \mu g/cm^3 \) totally inhibited the mycelium growth of isolate *F.oxt*. 17 (Fig. 1, Phot. 1). It should be mentioned that 100 \( \mu g/cm^3 \) of tropolone were fungicidal. When the disks of *F. oxysporum* f. sp. *tulipae* mycelium (both tested isolates) were incubated for eight days on PDA with tropolone (100 \( \mu g/cm^3 \)) and transferred to clean PDA, the mycelium did not grow at all (Phot. 2).

Hinokitiol in the PDA medium at a concentration of 5.0, 10.0 and 25.0 \( \mu g/cm^3 \) limited the mycelium growth of *F. oxysporum* f. sp. *tulipae* (F.ox.t. 17) in 15.5, 33.2 and 82.1%, respectively, after eight days of incubation, while a concentration of 50.0 \( \mu g/cm^3 \) and higher totally inhibited the mycelium growth (Fig. 2, Phot. 3). It was also documented that hinokitiol at a concentration of 50.0 \( \mu g/cm^3 \) had a fungicidal action (unpublished 2008).

Thus, in the case of *F. oxysporum* f. sp. *tulipae*, hinokitiol had a much stronger inhibitory effect on the mycelium growth than tropolone (Figs. 1, 2, Photbs. 1, 2, 3).

It should be also mentioned that isolate *F.oxt*. 218 was inhibited by hinokitiol and tropolone to higher degree than isolate *F.oxt*. 17 (Figs. 1, 2, Photbs. 1, 2).

Fallik and Grinberg (1992) documented that hinokitiol inhibited *in vitro* spore germination and mycelial growth of *Botrytis cinerea* and *Alternaria alternata*. In general, *Botrytis* was more sensitive than *Alternaria*, and spore germination of both fungi was more affected than their mycelium growth. The mycelium growth of *Botrytis* was completely inhibited at a concentration of 250 \( \mu l \), whereas inhibition of mycelium growth of *Alternaria* required 750 \( \mu l \) of hinokitiol per 1 l. A considerable reduction in decay development was observed when commercially harvested eggplants and red peppers were dipped in hinokitiol solution at concentration of 750 \( \mu l/l \). *Alternaria alternata* and *B. cinerea* were the main pathogens that developed during storage and shelf-life on eggplant and red peppers fruits. Hinokitiol was used to control postharvest disease in peach (Sholberg and Shimizu 1991). The effective dose of hinokitiol that reduced fungal spore germination by 50% (ED\(_{50}\)) was 30.0, 14.7 and 18.1 \( \mu l/l \), respectively for *B. cinerea*, *Monilinia fructicola* and *Rhizopus oryzae*. The mycelium growth of these fungi was greatly inhibited at low concentrations of hinokitiol (Sholberg and Shimizu 1991).
Fig. 1. Inhibitory effect of tropolone on in vitro growth of *Fusarium oxysporum* f. sp. *tulipae*; numbers upon the bars show the percent of mycelium growth inhibition in comparison to control after eight days of incubation.
Phot. 1. Influence of tropolone on in vitro growth of *Fusarium oxysporum* f. sp. *tulipae* (*F. ox t. 17*) cultured on potato dextrose agar (PDA, Merck); upper row: control, 25.0 µg/cm³, lower row: 50.0 µg/cm³, 100.0 µg/cm³ (photo by A. Saniewska)

Phot. 2. Lack of growth of *Fusarium oxysporum* f. sp. *tulipae* (*F. ox t. 17*) on PDA after previous incubation on PDA with tropolone at concentration of 100.0 µg/cm³ (left) and development of the pathogen previously cultured only on clean PDA (right) (photo by A. Saniewska)
Fig. 2. Inhibitory effect of hinokitiol on in vitro growth of Fusarium oxysporum f. sp. tulipae; numbers upon the bars show the percent of mycelium growth inhibition in comparison to control after eight days of incubation.
Preliminary results showed that \textit{in vitro} hinokitiol at a concentration of 200 µl/l completely inhibited spore germination and mycelial growth of \textit{R. stolonifer} (Fallik and Grinberg 1992).

Aharoni et al. (1993) showed that hinokitiol at a concentration of 750 µg/cm³ in wax controlled the decay-causing fungi on “Galia” melons (\textit{Cucumis melo} cv. ‘Reticulatus’) and had no phytotoxic effect on the fruit. Hinokitiol at concentrations of 200 and 300 µg/cm³ completely inhibited the mycelium growth of \textit{A. alternata} and \textit{Fusarium} spp., respectively.

Recently, Manter et al. (2007) documented that tropolone-related compounds, hinokitiol and nootkatin, isolated from heartwood of \textit{T. plicata} and \textit{C. nootkatensis} (syn. \textit{Callitropsis nootkatensis}), respectively, greatly inhibited the growth and development of \textit{Phytophthora ramorum}.

Thus, hinokitiol, a natural biologically active substance, is a very promising agent for control of a range of pathogens in fruits, vegetables and ornamental plants.
Conclusion

Tropolone and hinokitiol exerted great inhibitory effect on the mycelium growth of *Fusarium oxysporum* f. sp. *tulipae* when applied to PDA medium and further studies in vivo are necessary for their use against the pathogen development on tulip bulbs.

Streszczenie

HAMUJĄCE DZIAŁANIE TROPOLONE I HINOKITIOLU NA WZROST GRZYBNI *FUSARIUM OXYSPORUM* F. SP. *TULIPAE*

Celem badań było określenie wpływu tropolone i hinokitiolu (β-tujaplicyn) na wzrost i rozwój *Fusarium oxysporum* f. sp. *tulipae*. Tropolone i hinokitiol silnie hamowały wzrost grzybni *F. oxysporum* f. sp. *tulipae* na pożywce agarowo-ziemniaczano-glukoüzowej (PDA). Całkowite zahamowanie wzrostu grzybni *F. oxysporum* f. sp. *tulipae* nastąpiło przy stężeniu tropolone 100,0 µg/cm³ i przy stężeniu hinokitiolu 50,0 µg/cm³. Fungicydalne stężenia tropolone i hinokitiolu w stosunku do grzybni *F. oxysporum* f. sp. *tulipae* zostały również określone. Wyniki badań własnych zostały przedyskutowane z dostępnymi w literaturze danymi na temat antygrzybowego działania tropolone i hinokitiolu na inne gatunki grzybów patogenicznych.

Literature


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