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Antifungal activity of thymol against the main fungi causing fruit rot in *in vitro* conditions

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Abstract

Pomegranate fruit rot by the fungi *Penicillium* spp., *Aspergillus* spp., *Botrytis cinerea*, *Rhizopus* spp., *Nematospora* spp. and *Coniella* spp. In present study, the antifungal effects of thymol on the growth of *Aspergillus niger* and *Penicillium commune* isolated from pomegranate fruits investigated in *in vitro* conditions. The experiment was performed as a factorial based on completely randomized design (CRD) with three replicates. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of thymol for both fungi were 250 and 500 µg mL^{-1}, respectively. The lowest diameter of *Penicillium commune* colony (6.66 mm) was found at concentration of 250 µg mL^{-1} after 168 h, however it was not significantly ($P \leq 0.01$) different with the diameter of *Aspergillus niger* colony at the same time. Thymol at the concentration of 500 µg mL^{-1} had a similar effect as a fungicidal agent compared with thiabendazole (1500 µg mL^{-1}).

Keywords: *Aspergillus niger*, Minimum inhibitory concentration, Minimum fungicidal concentration, *Penicillium commune*

Introduction

One of the important issues in agriculture is the loss of quantity and quality of crops due to fungi and pests, which endangers the health of people. Pathogenic fungi and bacteria are the main factors affecting the nutritional quality and mycotoxin contamination of products that determine their shelf life and commercial value (Cortes-Higareda *et al*., 2019). Mycotoxins are toxic secondary metabolites of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium* (Venkataramana *et al*., 2014). Control of pathogens with fungicides causes consumer concern, as well as biological imbalance and environmental pollution (Palou, 2018). Therefore, the need for new strategies to control the diseases has led to more research on essential oils.

Materials and methods

Determination of MIC and MFC

Minimum inhibitory concentration (MIC) of thymol was determined by micro-broth dilution method; different concentrations of thymol were diluted serially from 2000 to 7.81 µg mL^{-1} and mixed with 200 µL of potato dextrose broth (PDB) medium. Minimum fungicidal concentration (MFC) of thymol was determined by the plate assay in PDA medium (Plodpai *et al*., 2013). Considering that MIC and MFC of thymol for both fungi were 250 and 500 µg mL^{-1}, respectively.

Measurement of the diameter of the fungus colony

Thymol at different concentrations was added to PDA culture medium and the mixture was poured into petri plates. The control group was PDA medium including 1500 µg mL^{-1} thiabendazole, PDA medium with 10% ethanol and PDA medium without thymol. The petri plates were incubated with 20 µL fungus spore at concentration 1×10^6 microconidia per mL. Afterward, petri plates were incubated at 28 ˚C for 168 h and the diameter of the fungus colony assessed every 24 h.

Statistical analysis

The experimental data were analyzed according to factorial design based on completely randomized design (CRD) with three replicates. Data were subjected to the analysis of variance (ANOVA) using SAS software ver. 9.4 (Statistical Analysis System, SAS Institute Inc., 1985). The means were evaluated using Duncan's multiple range tests to analyze the difference between treatments and intervals at a 95% confidence level of each variable.

Results and Discussion

The MIC and MFC of thymol for *Aspergillus niger* and *Penicillium commune* was 250 µg mL^{-1} and 500 µg mL^{-1}, respectively. Results of variance analysis showed that the main effect, interaction effects of two-and three-fold treatments on diameter of colony were significant ($P \leq 0.01$). After 168 h, the largest diameter of colony (54.66 mm and 53.66 mm) observed in positive control and ethanol 10% v/v, respectively, inoculated with *Aspergillus niger* with significant ($P \leq 0.01$) difference with the other treatments at the same time. After 168 h, smallest diameter of colony (6.66 mm) observed in media containing 250 µg mL^{-1} thymol inoculated with *Penicillium commune*, however it was not significantly ($P \leq 0.01$) different with the diameter of *Aspergillus niger* colony at the same time (Table. 1).

Table 1. Interaction effects of fungi, thymol and time on diameter of colony. Data are the mean ± SE (n=99). Duncan's multiple range test ($P \leq 0.01$).

Time	Concentrations of thymol (µg mL^{-1})	fungi		Time	Concentrations of thymol (µg mL^{-1})	fungi		Time	Concentrations of thymol (µg mL^{-1})	fungi		Time	Concentrations of thymol (µg mL^{-1})	fungi	
		A. niger	*P. commune*			*A. niger*	*P. commune*			*A. niger*	*P. commune*			*A. niger*	*P. commune*
24 h	0	9.66loknm	8.33 qopnm	72 h	0	33.66 kl	14.66 gef	120 h	0	44.33 fg	19.66ywxuvz	168 h	0	53.66 a	23 ts
	7.81	7.66 qopnr	8.33 qopnm		7.81	30.66 nm	13.33 gh		7.81	42.66 hg	17.33 cabdz		7.81	51.33 b	20ywxuv
	15.62	7.33 qopr	7.66 qopnr		15.62	30 po	12.33 jih		15.62	40.66 hi	17.33 cabdz		15.62	50.33 cb	18.66 ywxabvz
	31.25	7.33 qopr	6.66 qpsr		31.25	28.33 rq	11.66 jkih		31.25	39 ji	16.66 ceabd		31.25	49 cd	18 ycxabz
	62.5	6 qsr	3.66 tu		62.5	27.66 r	6.33 qsr		62.5	36.66 kl	11.33 ljkih		62.5	47.33 ed	15.66 cefd
	125	4.66 ts	0		125	17 ceabd	4.66 ts		125	23 ts	9.33 loknm		125	28.33 rq	12.33 jih
	250	0	0		250	0	0		250	0	0		250	8 qopn	6.66 qpsr
	500	0	0		500	0	0		500	0	0		500	0	0
	1000	0	0		1000	0	0		1000	0	0		1000	0	0
	2000	0	0		2000	0	0		2000	0	0		2000	0	0
	thiabendazole	0	0		Thiabendazole	0	0		Thiabendazole	0	0		Thiabendazole	0	0
	10% ethanol	9 lopnm	9.66 loknm		10% ethanol	32.66 no	13.66 gfh		10% ethanol	42.66 hg	19.66ywxuyz		10% ethanol	54.66a	21.66tu
48 h	0	21 tuv	11.66 jkih	96 h	0	39 ji	17.66 ycabz	144 h	0	49.33 cbd	20.33 wxuv				
	7.81	19 ywxavz	11.33 ljkih		7.81	37.33 jkl	15.66 cefd		7.81	48.66 cd	18.33 wxuv				
	15.62	18 ycxabz	10.66 ljkim		15.62	36.66 kl	14.66 gef		15.62	48.33 ced	17.66 ycabz				
	31.25	16.33 cebd	8.33 qopnm		31.25	35.66 ml	13.66 gfh		31.25	48.33 ced	17.33 cabdz				
	62.5	15 gefd	4.33 tsu		62.5	33.33n	7.66 qopnr		62.5	46.33 fe	13.33 gh				
	125	9.66 loknm	2.33 u		125	18.33 ywxabz	6 qsr		125	24.66 s	10 ljknm				
	250	0	0		250	0	0		250	5.33 tsr	2.33 u				
	500	0	0		500	0	0		500	0	0				
	1000	0	0		1000	0	0		1000	0	0				
	2000	0	0		2000	0	0		2000	0	0				
	Thiabendazole	0	0		Thiabendazole	0	0		Thiabendazole	0	0				
	10% ethanol	20.33 wxuv	10.66 ljkim		10% ethanol	38.33 jk	17 ceabd		10% ethanol	46.33 fe	20.66 wuv				

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In this research, thymol had a good capacity to prevent the growth of pomegranate decay fungi, which was consistent with other reports on the antifungal activity of rosemary and thyme essential oils on gray mold due to the high concentration of thymol (Sokovic and Van Griensven, 2006).

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