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Effects of thymol on the morphology of the main fungi causing pomegranate fruit rot

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Abstract

Pomegranate fruit rot leads to lose of significant quantity of fruit worldwide. In present study, the antifungal effects of thymol on the morphology of *Aspergillus niger* and *Penicillium commune* as the main fungi causing pomegranate fruit rot investigated in *in vitro* conditions. Examination of cell morphology using scanning electron microscope (SEM) in *Aspergillus niger* colony showed that cell deformation was observed due to destruction of the cell membrane and loss of cell wall strength at concentration of 250 µg mL^{-1} (FC50) after 168 h. Produced hyphae had irregular branching and no spore production was observed. Evaluation of *Penicillium commune* colony cell morphology using SEM showed that thymol at concentration of 250 µg mL^{-1} (FC50) caused superficial wrinkles, bifurcation of hyphal apex and no spore production was observed.

Keywords: Antifungal effects, *Aspergillus niger*, *Penicillium commune*, Scanning electron microscopy

Introduction

Postharvest losses of fresh fruits and vegetables are estimated at about 55% (Eguillor, 2019; FAO, 2019). Fungi are the most common postharvest fruit and vegetable pathogens worldwide (Xiao and Rogers, 2004). Control of pathogens with fungicides causes consumer concern, as well as biological imbalance and environmental pollution (Arias and Toledo, 2018). The active ingredients of medicinal plants are proposed to control postharvest rot due to lack of pathogen resistance, low production costs, degradability and non-pollution of the environment. In addition, pH, low temperature and oxygen levels in storage conditions of horticultural products improve their antifungal activity (Burt, 2004). Therefore, the aim of this research was to investigate the antifungal potential of thymol and study of morphological changes in fungi.

Materials and methods

Morphological analysis of fungus growth

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. For morphological analysis, 20 µL of fungi spores inoculated in PDA medium treated with thymol at IC_{50} and FC_{50} 125 µg mL^{-1} and 250 µg mL^{-1}, respectively against control (*Aspergillus niger* and *Penicillium commune* inoculated in PDA medium without thymol) at 28 °C for 168 h. Subsequently, washing with 0.1 mM phosphate buffer saline (PBS) (pH 7) was performed. Then, 2.5% glutaraldehyde solution (diluted in PBS) was added to each sample and again washed twice with PBS. The samples were dehydrated in ethanol solution with increasing concentration (50-100%), and finally subjected to drying under CO_2 (Zhang *et al*., 2019). Prior to imaging, the samples were coated with a thin layer of gold and morphological characteristics of the microconidia were determined with a scanning electron microscope (SEM) (TESCAN vega3, Czech).

Results and Discussion

Examination of cell morphology in *Aspergillus niger* using SEM showed that cell morphology was normal in the control samples (Fig 1a, 1b, 1c). Also, hyphae with normal structure, long and smooth surface with constant diameter and abundant spores were observed (Fig 1a, 1b). Cell morphology was normal in samples treated with 125 µg mL^{-1} of thymol (IC50) (Fig 1d, 1e, 1f), a lower density and dispersion of the spore-producing structure was observed (Fig 1d, 1e). Cell deformation was observed in samples treated with 250 µg mL^{-1} of thymol due to destruction of the cell membrane and loss of cell wall strength (Fig 1g, 1h, 1i). Furthermore, hyphae production with irregular branching was observed (Fig 1g, 1h), and spore production was prevented (Fig 1i). Examination of cell morphology in *Penicillium commune* using SEM showed that cell morphology was normal in the control samples (Fig 2a, 2b, 2c), spores were round and chain-like were placed on the mycelia (Fig 2b). At the concentration of 125 µg mL^{-1} of thymol (IC50), the number of spores decreased and the fungal mycelia were visible (Fig 2d, 2e). Furthermore, superficial wrinkles in the fungal hyphae and less spores were observed (Fig 2f). At the concentration of 250 µg mL^{-1} of thymol (FC50), superficial wrinkles and tip bulge out in the fungal hyphae, bifurcation of hyphal apex (Fig 2g, 2h) and no spore production was observed (Fig 2i).

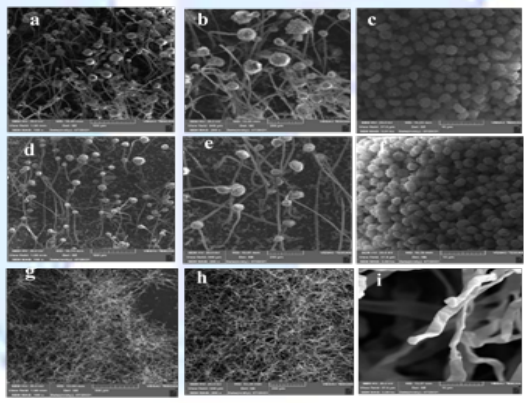

Figure 1. Scanning electron microscope images of *A. niger* mycelia and conidia after 168 h (a, b, c), *A. niger* mycelia and conidia in the presence of 125 µg mL^{-1} thymol (IC50) after 168 h (d, e, f), and *A. niger* mycelia and conidia in the presence of 250 µg mL^{-1} thymol (FC50) after 168 h (g, h, i).

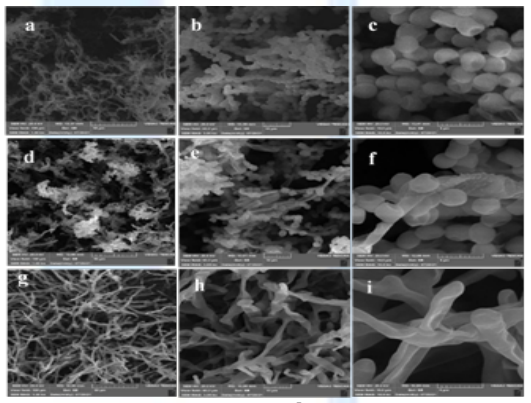

Figure 2. Scanning electron microscope images of *P. commune* mycelia and conidia after 168 h (a, b, c), *P. commune* mycelia and conidia in the presence of 125 µg mL^{-1} thymol (IC50) after 168 h (d, e, f), and *P. commune* mycelia and conidia in the presence of 250 µg mL^{-1} thymol (FC50) after 168 h (g, h, i).

Thyme essential oil completely prevents the growth of green mold due to the presence of secondary compounds of thymol and carvacrol (Gholchinno *et al*., 2018). According to our results, changes in morphology and surface structure such as superficial wrinkles, flaking and destruction in the fungus hyphae, loss of cytoplasm and plasma membrane has also been observed (Oliveira *et al*., 2019).

References

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