Citral inhibits mycelial growth of *Penicillium italicum* by a membrane damage mechanism

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**A R T I C L E  H I S T O R Y**

Received 29 October 2013
Revised in revised form 8 January 2014
Accepted 9 January 2014

**R e s e a r c h  A r t i c l e**

1. **Introduction**

Citrus fruits are produced in subtropical areas that are distant from consumer markets and often must be stored if the market economy so dictates (Zhu et al., 2013). Months-long delay between harvest and consumption may result in postharvest losses due to pathological and physiological diseases (Scora & Scora, 1998). Blue mold, caused by *Penicillium italicum*, is one of the most damaging postharvest diseases of citrus fruit (Droby et al., 2008). Conventional synthetic fungicides are highly effective against this pathogen and remain a main means of control. However, intense application of synthetic fungicides creates concerns over the environment, human health, and proliferation of fungicide-resistant strains, and safer approaches are currently being sought (Tyagi, Gottardi, Malik, & Guerzoni, 2013; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008).

The use of terpenoids in postharvest decay control as an alternative to synthetic fungicides has attracted keen interest in the past decades, and several studies have demonstrated the potential of certain terpenoids (Kouassi, Bajji, & Jijakli, 2012; du Plooy, Regnier, & Combrinck, 2009; Yahyazadeh, Zare, Omidbaigi, Faghih-Nasiri, & Abbasi, 2009; Zhou, Tao, & Jia, 2014). Citral, a naturally occurring isoprenoid with two isomers (geranial and neral), reportedly exerts anti-fungal activity against some citrus postharvest pathogens such as *P. italicum* and *Penicillium digitatum* (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998; Linde, Combrinck, Regnier, & Virjievic, 2010; Scora & Scora, 1998; Wolken, Tramper, & Werf, 2002). Klieber and Wuryatmo (2002) reported that 15,000 µL/L citral solutions can completely inhibit the growth of *P. italicum*, *P. digitatum*, and *Geotrichum candidum*. The vapor of citral and its two isomers generated from 15 µL/L aqueous solutions in Petri dishes can inhibit the development of *P. digitatum*, *P. italicum*, and *G. candidum*, and concentrations ranging from 2 µL/L to 6 µL/L are also effective against *P. italicum*. Vapors of citral and geranial from 15 µL/L solutions exhibit fungicidal effects on *P. digitatum* and *G. candidum*, whereas neral exerts fungicidal effects on *G. candidum* (Wuryatmo, Klieber, & Scott, 2003). A recent study revealed that the spore germination and germ tube growth of *P. italicum* can be inhibited by citral at concentrations higher than 0.1 µL/plate, and complete inhibition occurs at 1.0 µL/plate (Droby et al., 2008).

As mentioned above, extensive and developmental studies have been carried out to investigate the anti-fungal activities of citral, but there is little study related to the investigation of its exact mechanism of the anti-fungal action. Park et al. (2009) found that the cell membrane and organelles of *Trichophyton mentagrophytes* were irreversibly damaged at 0.2 mg/mL citral. The lemon grass oil (rich in citral) at a concentration of 32.7 µL/L could cause serious morphological changes such as roughness, complete rupture, and complete degradation of *Candida albicans* cells (Tyagi & Malik, 2010a, 2010b). In our recent paper, citral was illustrated to possess the ability to destroy the membrane integrity of *Geotrichum citri-aurantii* (Zhou et al., 2014). To the best of our knowledge, however,
the anti-fungal mechanism of citral on Penicillium was rather limited. This study aimed to determine the anti-fungal efficacy of citral against the mycelial growth of *P. italicum*. The anti-fungal mechanism was also investigated by determining the following: (i) the morphology of cell membranes using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), (ii) the release of 260 nm absorbing cellular components, (iii) the extracellular electrical conductivity and pH, and (iv) the total lipid and ergosterol contents.

2. Materials and methods

2.1. Fungal species

The fungal pathogen *P. italicum* was isolated from infected citrus fruit and maintained on potato dextrose agar (PDA) at 28 ± 2 °C. The spores' concentration was adjusted to 5 × 10⁵ cfu/mL using a haemocytometer.

2.2. Chemicals

Citral (95%) and ergosterol (95%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Cholesterol (95%) and phosphovanillin (98%) were purchased from TCI Shanghai (Shanghai, China). All the chemicals were analytic grade.

2.3. Anti-fungal activity

Effects of citral on mycelial growth of *P. italicum* were tested in vitro by agar dilution method (Yahyazadeh, Omidbaigi, Zare, & Taheri, 2008). PDA (20 mL) was poured into sterilized Petri dishes (90 mm diameter) and measured amounts of citral were added to PDA mediums (with 0.05% Tween-80) to give desired concentrations of 0.00, 0.06, 0.13, 0.25, 0.50, 1.00, and 2.00 µL/mL. A 6 mm diameter disc of inocula was cut from the periphery of actively growing culture on PDA plates with a punching bear, and then was placed at the center of each new Petri plate. Culture plates were then incubated at 28 ± 2 °C for 48 h. Each treatment was performed in triplicates. The percentage of inhibition of mycelial growth (MGI) was calculated according to the following formula:

\[ \text{MGI(\%)} = \frac{(dc - dt)}{dc} \times 100 \]

where dc (cm) is the mean colony diameter for the control sets and dt (cm) is the mean colony diameter for the treatment sets. The lowest concentration that completely inhibited the growth of the fungus was considered the minimum inhibitory concentration (MIC). The minimum fungicidal concentration (MFC) was regarded as the lowest concentration that prevented growth of the pathogen after a following 72 h incubation at 28 ± 2 °C in a fresh PDA plate, indicating more than 99.5% killing of the original inoculums (Talibi et al., 2012).

2.4. Scanning electron microscopy (SEM)

The 4-day-old fungal cultures on PDA treated with citral at various concentrations (0, MIC and MFC) were used for all SEM observations (Helal et al., 2007). Small pieces of *P. italicum* mycelia (2 mm diameter) treated by citral at various concentrations (0, MIC and MFC) were fixed in 3.0% (v/v) glutaraldehyde in 0.05 mol/L phosphate buffer containing 3.0% (v/v) glutaraldehyde for 3 h, washed thrice with 0.2 mol/L phosphate buffer of pH 7.4 for 45 min, then post fixed in 1.0% osmium tetroxide for 2 h. After that, the cells were washed again with the phosphate buffer for 30 min. All the steps of fixation were carried out at 4 °C. Samples were dehydrated in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95% and 100%). They were passed in three changes of acetone ethanol (1:2, 1:1 and 2:0) for 10 min each. The specimens were further treated with propylene oxide twice each for 30 min as a transitional fluid, and embedded in Spurr’s resin. Ultrathin sections (approximately 70 nm in thickness) were made by an ultramicrotome with a diamond knife. The sections were mounted on copper grids, and stained with 2% uranyl acetate and lead citrate each for 30 min. The stained sections were examined in a JEOL JSM-1230 SEM (JEOL, Ltd., Tokyo, Japan) operated at an accelerating voltage of 80 kV.

2.5. Transmission electron microscopy (TEM)

The 4-day-old fungal cultures on PDA treated with citral at various concentrations (0, MIC and MFC) were used for all TEM observations (Helal et al., 2007). Small pieces of *P. italicum* mycelia (2 mm diameter) treated by citral at various concentrations (0, MIC and MFC) were fixed in 3.0% (v/v) glutaraldehyde for 3 h, washed thrice with 0.2 mol/L phosphate buffer of pH 7.4 for 45 min, then post fixed in 1.0% osmium tetroxide for 2 h. After that, the cells were washed again with the phosphate buffer for 30 min. All the steps of fixation were carried out at 4 °C. Samples were dehydrated in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95% and 100%). They were passed in three changes of acetone ethanol (1:2, 1:1 and 2:0) for 10 min each. The specimens were further treated with propylene oxide twice each for 30 min as a transitional fluid, and embedded in Spurr’s resin. Ultrathin sections (approximately 70 nm in thickness) were made by an ultramicrotome with a diamond knife. The sections were mounted on copper grids, and stained with 2% uranyl acetate and lead citrate each for 30 min. The stained sections were examined with a transmission electron microscope (JEM-1230; JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 80 kV.

2.6. Release of cellular material

The release of cell constituents into the supernatants was measured according to the method described previously (Paul, Dubey, Maheswari, & Kang, 2011) with minor modifications. Briefly, mycelia from a 100 mL potato dextrose broth (PDB) culture of *P. italicum* were collected by centrifugation at 4000 g for 20 min, washed three times, and re-suspended in 100 mL phosphate buffered saline (pH 7.0). The suspensions were then treated by citral at various concentrations (0, MIC and MFC) and incubated at 28 ± 2 °C under agitation in an environmental incubator shaker for 0, 30, 60 and 120 min. Subsequently, 2 mL of samples was collected and centrifuged at 12,000 g for 2 min. To determine the concentration of the released constituents, 1 mL of supernatant was used to measure the absorbance at 260 nm with the UV–2450 UV/Vis Spectrophotometer (SHIMADZU international trade (Shanghai) Co. Ltd).

2.7. Measurement of extracellular pH

The measurements of extracellular pH of *P. italicum* cells were carried out using a Delta-320 pH-meter (Mettler-Toledo, Greifensee, Switzerland) following the instructions. Initially, 100 µL fungal suspensions (10⁵ cfu/mL) were added to 20 mL PDB and incubated in a moist chamber at 28 ± 2 °C for 2 d. The mixtures were centrifuged at 4000 g for 20 min and the resulting pellet was collected and washed for 2–3 times with sterilized double distilled water, and resuspended in 20 mL sterilized double distilled water. After the addition of citral at MIC or MFC, the extracellular pH of *P. italicum* cells was determined at 0, 30, 60 and 120 min of treatment. Control flasks without citral were also tested.
2.8. Potassium ions efflux

A previously described method was used to determine the amount of the potassium ions (Bajpai, Sharma, & Baek, 2013). The concentration of free potassium ions in suspensions of *P. italicum* was measured after the exposure of fungal cells to citral at MIC or MFC for 0, 30, 60 and 120 min. At each pre-established interval, the extracellular potassium concentration was measured by a photometric procedure using the flame atomic absorption spectroscopy (Shimadzu AA6300, Japan). Control flasks without citral were tested similarly. Results were expressed as amount of extracellular free potassium (µg/mL) in the growth media in each interval of incubation.

2.9. Determination of total lipid content

Total lipid content of *P. italicum* cells with citral at various concentrations (0, MIC and MFC) were determined using phosphovanillin method (Helal, Sarhan, Abu Shahla, & Abou Elkair, 2006). The 2-day-old mycelia from 50 mL PDB were collected and centrifuged at 4000 g for 10 min. Then the samples were dried with a vacuum freeze drier for 4 h. About 0.1 g of dry mycelia were homogenized with liquid nitrogen and extracted with 4.0 mL of methanol–chloroform–water mixture (2:1:0.8, v/v/v) in a clean dry test tube with vigorous shaking for 30 min. The tubes were centrifuged at 4000 g for 10 min. The lower phase containing lipids was thoroughly mixed with 0.2 mL saline solution and centrifuged at 4000 g for 10 min. Then, an aliquot of 0.2 mL chloroform and lipid mixture was transferred to a novel tube and 0.5 mL H2SO4 was added, heated for 10 min in a boiling water bath. After that, 3 mL phosphovanillin was added and shake vigorously, and then incubated at room temperature for 10 min. The absorbance at 520 nm was utilized to calculate total lipid contents from the standard calibration curve using cholesterol as a standard.

2.10. Determination of ergosterol content

Total ergosterol content of *P. italicum* cells with citral at various concentrations (0, MIC and MFC) were determined using HPLC method (Yuan, Wang, Liu, Kuang, & Zhao, 2007). The 2-day-old mycelia from 50 mL PDB were collected and centrifuged at 4000 g for 15 min. Then the samples were dried with a vacuum freeze drier for 4 h. About 0.1 g of dry mycelia were homogenized with liquid nitrogen and saponified by adding 4 mL of freshly prepared 30% (w/v) methanolic KOH and 8 mL of absolute ethanol at 90 °C for 2 h. The mixtures were extracted with 3 mL petroleum ether for three times and washed by saturated NaCl solution twice. The samples were then vacuum concentrated and dissolved in 10 mL absolute ethanol.

HPLC was conducted on a Shimadzu LC-20AT liquid chromatography system (Shimadzu Scientific Instrument, Japan) equipped with a model LC-20AT solvent delivery system, a model SPD-M20A photo diode array detection system, and an Empower Chromatography Manager. The sample extracts were separated and analyzed by using a C18 column (250 mm × 4.6 mm, 5 µm) at room temperature. The mobile phase consisted of solvent A (methanol/water, 80:20, v/v) and solvent B (methanol/dichloromethane, 75:25, v/v). A gradient procedure was used as follows: starting at sample injection, 0% of B for 5 min; a linear gradient from 0% to 100% of B for 19 min; 100% of B for 20 min. The flow rate was 1.0 mL/min. Chromatographic peaks were identified by comparing the retention times and spectra against the known standard. The detecting wavelength was set at 282 nm Aliquots of 20 µL were directly injected into the HPLC for the determination. All injections were repeated three times.

2.11. Statistical analysis

All data were expressed as the mean ± SD by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Duncan’s test was performed to test the significance of differences between means obtained among the treatments at the 5% level of significance using SPSS statistical software package release 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Anti-fungal activity

The effect of citral on the mycelial growth of *P. italicum* in vitro is shown in Table 1. The mycelial growth of *P. italicum* was strongly inhibited by citral in a dose-dependent manner. Citral inhibited one-third of the mycelial growth of *P. italicum* even at a relatively low concentration of 0.06 µL/mL. Citral at 0.25 µL/mL inhibited approximately two-thirds of the mycelial growth, with a radial growth inhibition of 64.70%. The mycelial growth of *P. italicum* was completely inhibited at 0.50 µL/mL citral, but fungicidal activity was induced at 1.00 µL/mL citral. Thus, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of citral were 0.50 and 1.00 µL/mL, respectively.

3.2. SEM

The effect of citral on the morphology of *P. italicum* was examined using SEM (Fig. 1). The control fungus grown on potato dextrose agar (PDA) had normal, tubular, regular, and homogenous hyphae (Fig. 1a). All mycelia of *P. italicum* treated with MIC or MFC of citral for 4 d showed considerable changes in hyphal morphology. *P. italicum* treated with MIC of citral showed a warty surface of mycelia (Fig. 1b). By contrast, the hyphae of *P. italicum* treated with MFC of citral appeared severely collapsed because of the lack of cytoplasm (Fig. 1c and d). Shrunken and distorted mycelia were also observed (Fig. 1c).

3.3. TEM

To elucidate further the nature of the killing mechanisms of citral, *P. italicum* treated with MIC and MFC of citral were analyzed by TEM. Compared with the control treatment, citral treatment resulted in clear morphological changes (Fig. 2b and c). The untreated cells displayed a smooth and compact surface (Fig. 2a). MIC of citral clearly induced the formation of depressed hyphae (Fig. 2b). By contrast, MFC of citral damaged the cell wall and cell membrane of *P. italicum*, which resulted in the loss of the cytoplasm and the extrusion of abundant materials from the outside of the cell wall (Fig. 2c).

<table>
<thead>
<tr>
<th>Concentration (µL/mL)</th>
<th>Mycelial growth inhibition (%)*</th>
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<tbody>
<tr>
<td>0.00</td>
<td>0.00 ± 5.10*</td>
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<tr>
<td>0.05</td>
<td>29.39 ± 0.00*</td>
</tr>
<tr>
<td>0.13</td>
<td>32.33 ± 5.10*</td>
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<tr>
<td>0.25</td>
<td>64.70 ± 0.00*</td>
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<tr>
<td>0.50</td>
<td>100.00 ± 0.00*</td>
</tr>
<tr>
<td>1.00</td>
<td>100.00 ± 0.00*</td>
</tr>
<tr>
<td>2.00</td>
<td>100.00 ± 0.00*</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SD. Data presented are the means of pooled data (n = 6). The column with different lowercase letters between different concentrations indicates significant differences according to Duncan’s test (P < 0.05).
3.4. Release of cell constituents

Results of the release of cell constituents when *P. italicum* was treated with MIC and MFC of citral for 0, 30, 60, and 120 min are shown in Fig. 3a. The release of cell constituents significantly increased \((P < 0.05)\) when *P. italicum* was treated with early exposure of MIC or MFC of citral. The OD\(_{260}\) value in *P. italicum* suspensions with MIC of citral for 30 min was 0.425, which was significantly higher \((P < 0.05)\) than that of the control \((0.058)\) but significantly lower \((P < 0.05)\) than that of MFC of citral \((0.612)\). The OD\(_{260}\) value of *P. italicum* suspensions treated with MIC and MFC of citral maintained a smooth ascending trend after 30 min of exposure.

3.5. Extracellular pH

The extracellular pH of *P. italicum* cells exposed to citral is presented in Fig. 3b. The extracellular pH sharply decreased in the control. Conversely, the extracellular pH of *P. italicum* suspensions with MIC or MFC of citral rapidly increased in the initial 30 min of treatment, followed by a moderate ascending trend. The extracellular pH values in *P. italicum* suspensions after incubation with MIC and MFC of citral for 30 min were 5.84 and 5.79, respectively, which were significantly higher than that of the control \((4.70)\) \((P < 0.05)\).

3.6. Potassium ion efflux

Potassium ions \((K^+\) leaked from *P. italicum* cells incubated with citral (Fig. 3c). MFC of citral significantly induced the release of \(K^+\) in the initial 30 min of treatment, and the \(K^+\) concentration after 30 min was 2.02 \(\mu g/mL\). When the incubation time increased to 120 min, MFC of citral had no further effect. By contrast, incubation with MIC of citral did not result in more \(K^+\) release than from the control cells in the initial 30 min of treatment. After 30 min of incubation, \(K^+\) released by MIC of citral continuously increased and reached 1.80 \(\mu g/mL\) after 120 min of incubation.

3.7. Total lipid content

The effect of citral on the total lipid content of *P. italicum* cells is shown in Fig. 4a. The total lipid contents of *P. italicum* cells significantly decreased \((P < 0.05)\) with increasing citral concentration in the initial 30 min of treatment. The total lipid contents of *P. italicum* cells after 30 min of incubation with MIC and MFC of citral were 237.3 ± 6.3 and 215.1 ± 8.0 mg/g dry weight, respectively, which were significantly lower than that of the control \((265.6 ± 4.6\) mg/g dry weight) \((P < 0.05)\). Moreover, the total lipid contents of *P. italicum* cells treated with citral remained stable after 30 and 60 min of exposure but continuously decreased thereafter.

3.8. Ergosterol content

The ergosterol contents in *P. italicum* cells with citral continuously decreased during the entire period, whereas those in the untreated cells remained stable (Fig. 4b). The ergosterol contents of *P. italicum* cells incubated with MIC and MFC of citral for 30 min were 3.28 ± 0.01 and 2.56 ± 0.16 mg/g dry weight, respectively, which were significantly lower than that of the control \((4.93 ± 0.94\) mg/g dry weight). This change became more evident with increasing exposure time \((P < 0.05)\). At 120 min of exposure, the ergosterol contents in *P. italicum* cells treated with MIC and MFC of citral were 2.03 ± 0.02 and 1.35 ± 0.01 mg/g dry weight, respectively, which were significantly higher than that of the control \((4.38 ± 0.36\) mg/g dry weight).

4. Discussions

This study proved the effectiveness of citral in inhibiting the mycelial growth of *P. italicum*. The inhibitory effect was positively correlated with the citral concentration. The MIC and MFC of citral in this study were 0.5 and 1.0 \(\mu L/mL\), respectively (Table 1). These results were consistent with those of previous studies describing the anti-fungal activity of citral (Jia, He, Tao, & Zhou, 2013; Scora & Scora, 1998). However, concentrations lower than these values can
also inhibit spore germination or germ tube elongation in \textit{P. italicum}. For example, Wuryatmo et al. (2003) reported that the vapor of citral generated from 15 μL/L aqueous solutions in Petri dishes can completely inhibit the spore germination of \textit{P. italicum}. They also found that concentrations ranging from 2 μL/L to 6 μL/L are effective against \textit{P. italicum}. A recent study revealed that the spore germination and germ tube growth of \textit{P. italicum} can be inhibited by 6 μL/L citral, and complete inhibition occurs at 15 μL/L (Droby et al., 2008). This discrepancy could be due to the differences in the experimental approaches used in the two studies. We used PDA with citral to create an emulsion in which the mycelia were in direct contact with citral. By contrast, Wuryatmo et al. (2003) and Droby et al. (2008) applied variable quantities of citral on a paper disc attached to the top of a Petri dish to create different concentrations of volatiles in the Petri dish headspace. Our present result was similar to that reported by Klieber, Scott, and Wuryatmo (2002), who compared the anti-fungal efficiency of citral against \textit{P. italicum} by three different methods. They found that the incorporation of 3000 μL/L citral into a neutral Dox-yeast agar (NDYA) completely inhibits the spore germination of \textit{P. italicum}, whereas spores exposed to 15,000 μL/L citral solution for 1 h before inoculation can still germinate on NDYA. Moreover, they showed that 16,143 μL/L is the headspace concentration that completely inhibits spore germination in volatile citral. These findings strongly support the outcomes of the present study, and confirm that citral can act as a promising biological fungicide.

The SEM image (Fig. 1) clearly shows the difference between the treated and untreated \textit{P. italicum} hyphae. The hyphae treated with MIC of citral shrank and formed a rough surface, whereas those exposed to a relatively higher concentration (100 μL/mL) were distorted and collapsed. These findings were verified by the TEM images of \textit{P. italicum} hyphae in which plasmalemma disruption and structural disorganization of the cytoplasm were observed in the citral-treated cells (Fig. 2). Our results were in agreement with those previously reported (Aiemsaard, Aiumlamai, Aromdee, Taweechaisupapong, & Khunkitti, 2011; Park et al., 2009; Tyagi & Malik, 2010a, 2010b, 2011). These changes in hyphae may be attributed to the increase in cell permeabilization, and they usually mean the leakage of small molecular substances and ions, lesions, and discrepancies in cell metabolism (Bajpai et al., 2013; Liu, Zong, Qin, Li, & Tian, 2010). Thus, the mechanism underlying the fungitoxic action of citral against \textit{P. italicum} could be through membrane disruption and cell growth hindrance.

Membrane permeability parameters, including loss of 260 nm absorbing materials, change in extracellular pH, and leakage of potassium ions, were used to verify this hypothesis. These parameters are commonly used to indicate gross and irreversible damage to the cytoplasmic and plasma membranes (Bajpai et al., 2013; Paul et al., 2011; Shao, Cheng, Wang, Yu, & Mungai, 2013; Turgis, Han, Caillet, & Lacroix, 2009). The release of cell constituents (Fig. 3a) and extracellular pH (Fig. 3b) in the fungal suspensions visibly increased with increasing citral concentration. The maximum release of cell constituents was observed in \textit{P. italicum} cell suspensions treated with MFC of citral after 30 min of exposure, showing an absorbance of 0.612. The extracellular pH of \textit{P. italicum} suspensions with MIC or MFC of citral visibly increased in the initial 30 min of exposure, whereas that of the control sharply decreased. Meanwhile, after 30 min of exposure, citral induced the leakage of...
intracellular potassium ions (Fig. 3c). These findings suggest that the cytoplasmic membranes of *P. italicum* incurred irreversible damage. The ions inside the cells leaked, and an imbalance in the osmotic pressure of the intra- and extracellular membranes occurred.

Lipids are one of the main components of biological membranes (Heaton & Randall, 2011). The decrease in lipid content usually suggests a reduction in membrane stability and an increase in permeability to water-soluble materials (Helal et al., 2006, 2007; Prashar, Hili, Veness, & Evans, 2003). In the present study, the addition of citral significantly decreased the lipid contents of *P. italicum* (Fig. 4a). This result suggests that citral can affect the cell membrane structure and inhibit *P. italicum* growth. The effect of citral on the amount of ergosterol was assessed to ensure the target of citral in the plasma membrane. Ergosterol, which is specific to fungi, is the major sterol component of the fungal cell membrane. Ergosterol is also responsible for maintaining cell function and integrity (Khan et al., 2010; Tian et al., 2012). Commercial fungicides against *P. italicum*, such as imazalil, thiabendazole, pyrimethanil, and fludioxonil, can disrupt the normal sterol biosynthetic pathways or significantly reduce the quantity of ergosterol in *P. italicum* (Ghossotha, Schmidt, Margosanb, & Smilanic, 2007; Sánchez-Torres & Tuset, 2011; Sun, Wang, Feng, Ma, & Li, 2011). In the present study, citral considerably impaired ergosterol biosynthesis in *P. italicum* cells (Fig. 4b). These findings suggest that the plasma membrane can be an important anti-fungal target of citral.

In this study, citral dramatically inhibited the mycelial growth of *P. italicum*. The anti-fungal activity of citral can be attributed to the disruption of cell membrane integrity and membrane permeability.

**Acknowledgments**

This study was supported by the National Natural Science Foundation of China (No. 31271964) and Research Foundation of Education Bureau of Hunan Province (No. 12B126).

**References**


