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# Fumigant activity of volatiles of *Streptomyces globisporus* JK-1 against *Penicillium italicum* on *Citrus microcarpa*

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ABSTRACT

Antifungal activity against *Penicillium italicum* of volatile substances from *Streptomyces globisporus* JK-1 grown on autoclaved wheat seed was studied *in vitro* and in planta. Fungal spore germination and mycelial growth of *P. italicum* cultures as well as sporulation and disease incidence on fungal-inoculated fruit were suppressed in the presence of the volatiles. For naturally infected fruit, disease incidence was reduced from 25% to 7.5%. Suppression of the infection process of *P. italicum* on Shatang Mandarin fruit (*Citrus microcarpa*) was observed via scanning electronic microscopy, showing inhibited spore germination on the Shatang Mandarin, and abnormal morphology for conidiophores and hyphae exposed to the volatiles. Based on gas chromatography/mass spectrophotometric analyses, 41 volatile organic compounds were identified from the volatiles of *S. globisporus* JK-1, and the most abundant compound was trans-1,10-dimethyl-trans-9-decalol (geosmin), an earthy smelling substance. Among these, technical grade formulations of eight were chosen for further study: phenylethyl alcohol, caryophyllene, dimethyl disulfide, dimethyl trisulfide, acetophenone, D-limonene, isoledene, and aromadendrene. D-Limonene, isoledene and aromadendrene showed no observable antifungal activity *in vitro* and in planta at tested concentrations. Both phenylethyl alcohol and caryophyllene showed weak inhibitory activity *in vitro* but no significant efficacy against *P. italicum* on Shatang Mandarin. Dimethyl disulfide or dimethyl trisulfide showed antifungal activity *in vitro* and efficacious control on Shatang Mandarin at a concentration of 100 $\mu L L^{-1}$ of airspace in treatment containers. Acetophenone showed antifungal activity *in vitro* at a concentration of 100 $\mu L L^{-1}$ and efficacious control on Shatang Mandarin at the highest concentration of 1000 $\mu L L^{-1}$. Volatiles from *S. globisporus* JK-1 have potential for control of blue mold of citrus species through fumigant action.



## 1. Introduction

Citrus (*Citrus* spp.) is the world's most common crop grown in over 100 countries world wide (Smilanick et al., 2005). Citrus fruits are enjoyed for their taste, nutritional value, and relatively low price. The control of postharvest disease of citrus is vital for maintaining quality and shelf life since several weeks or even longer are needed to transport the fruit from producers to consumers. Blue mold, caused by *Penicillium italicum*, is one of the most economically important postharvest diseases of citrus. It is estimated that 30–50% of citrus fruit is lost due to blue mold infection in China (Long et al., 2005).

Traditionally, fungicides such as thiabendazole, sodium O-phenylphenate, and imazalil are applied to minimize postharvest decay. However, the use of chemicals is becoming increasingly restricted in consideration of environmental and health concerns, as well as the cost of developing new pesticides to overcome resistance developed by pathogens (Bus et al., 1991; Holmes and Eckert, 1999). Several fungicides are no longer used for postharvest treatment or have been removed from the market altogether because of their possible toxicological risks (Meziane et al., 2006). Some alternatives investigated include post-inoculation treatment with hot water or with sodium carbonate, sodium bicarbonate or potassium sorbate (Montesinos-Herrero et al., 2009; Palou et al., 2001, 2002; Smilanick et al., 1999), or through biological control using microorganisms (Long et al., 2005; Meziane et al., 2006), as well as with combinations of these (Montesinos-Herrero et al., 2009; Obagwu and Korsten, 2003; Smilanick et al., 1999, 2005, 2008).

For control of blue mold, compounds such as sodium carbonate and sodium bicarbonate can be used with no regulatory restrictions in the European Union or the USA (Montesinos-Herrero et al., 2009). However, disposal of spent solutions is an issue because of the sodium content and the high pH and conductivity of the

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solutions of sodium carbonates (Smilanick et al., 2008). Among non-conventional treatments, several naturally occurring volatiles in the aromatic component of fruit and vegetable products (Kulakiotu et al., 2004; Tripathi and Dubey, 2004), or in essential oils of spices and herbs (Katharina and Rainer, 1998; Yahyazadeh et al., 2008) or in fungi (Grimme et al., 2007; Strobel et al., 2007) or bacteria (Bruce et al., 2003; Chen et al., 2008; Kai et al., 2007) have shown antifungal activity against fungal diseases. Previous studies have reported that volatiles from Actinomycetes could cause alterations in morphology of conidiophores and hyphae in several fungi (Moore-Landecker and Stotzky, 1973) and inhibit germination of *Cladosporium cladosporioides* (Herrington et al., 1985). Recently, studies on antifungal activity of *Streptomyces* volatiles have focused on control of some plant diseases such as seedling blight of rice, leaf blight of oilseed rape and fruit rot of strawberry (Wan et al., 2008). However, little information is available on the activity and efficacy of volatile compounds from *Streptomyces* against *P. italicum*. The objectives of this study were as follows: (i) to study the antifungal activity of volatiles produced by *Streptomyces globisporus* JK-1 against *P. italicum*; (ii) to determine the disease suppression efficacy of volatiles from *S. globisporus* JK-1 on Shatang Mandarin; (iii) to microscopically observe the effect of volatiles on the infection process of *P. italicum* on Shatang Mandarin; (iv) to identify volatile compounds produced by *S. globisporus* JK-1 which may be involved in disease suppression; (v) to investigate the effects of individual compounds from the mixture of volatiles of *S. globisporus* JK-1 against *P. italicum* and against blue mold on Shatang Mandarin.

## 2. Materials and methods

### 2.1. Microorganisms

*S. globisporus* JK-1 was obtained from the indoor air spora in the Plant Protection Building of Huazhong Agricultural University, Wuhan, China. The isolate JK-1 was identified as *S. globisporus* by morphological and physiological methods as well as by its 16S rDNA sequence. Preliminary inhibition tests against fungi were conducted using dual cultures (both *S. globisporus* and a test culture on the same plate). For short-term storage, *S. globisporus* JK-1 was streaked into test tube slants of potato dextrose agar, incubated for 5–7 d under continuous fluorescent light and stored at 4 °C. For long-term storage, *S. globisporus* JK-1 was maintained at −70 °C in potato dextrose broth supplemented with 25% glycerol. To produce the volatile substances, autoclaved wheat seeds in conical flasks (250 mL) were inoculated with a spore suspension of *S. globisporus* JK-1 at 1 mL per 100 g of wheat seeds, and the flasks were incubated at 28 °C for 14 d (Wan et al., 2008).

*P. italicum* was obtained from a naturally infected citrus fruit from the citrus orchard of Huazhong Agricultural University, Wuhan, China. The fungus was maintained on potato dextrose agar (PDA) at 4 °C. For use in experiments, the fungus was subcultured onto PDA in 9-cm-diameter Petri dishes, and incubated at 25 °C under continuous fluorescent light for 7–10 d. Conidia of *P. italicum* were harvested by adding 20 mL of water containing 0.05% Tween 80 into a Petri dish, gently rubbing the surface with a sterile rod, and passing the suspension through three layers of cheese cloth. The suspension was diluted with water to approximately $10^6$ conidia $mL^{-1}$ as assessed with a hematocytometer. This density is recommended for evaluation of postharvest treatments to control blue and green molds (Eckert and Brown, 1986).

### 2.2. Fruit

Freshly harvested, mature and healthy seedless Shatang Mandarin fruit (*Citrus microcarpa*) from Guangdong province with similar size and color (~30 g per fruit, ~4 cm across) were used in these experiments. The fruit were free from visible wounds and rot. Before each trial, fruit were washed with water and surface sterilized in 70% ethanol for 60 s, rinsed in sterile water and air-dried.

### 2.3. Antifungal activity of volatiles from S. globisporus JK-1

An antifungal bioassay was established to study the effects of volatiles on radial growth and sporulation, using small Petri dish bottoms, 60 mm diameter and 15 mm tall, placed inside larger Petri dishes, 150 mm diameter and 30 mm tall with 0.5 L total airspace. The smaller dishes contained 5 mL of PDA inoculated with a 5-mm-diameter plug from the periphery of an actively growing culture of *P. italicum*. Into each single larger dish, three smaller dishes with *P. italicum* cultures were placed, along with one smaller dish containing wheat seed inoculum of *S. globisporus* JK-1 prepared as described above. The larger dish was lidded and then sealed with parafilm, which allowed for free gas exchange between colonies while preventing direct contact. Different amounts of wheat seed culture of *S. globisporus* JK-1 were used, varying from 3.75 g to 60 g of culture per liter of airspace in treatment containers. Non-inoculated autoclaved wheat seeds were used as a control. After 5 d incubation at 25 °C, the diameters of the colonies in the smaller plates were recorded, and the total number of the conidia per plate was assessed. For each treatment, there were three replicates. The experiment was repeated three times.

To study the activity of volatiles on conidial germination, 50 μL aliquots of conidial suspension ($10^4$ conidia $mL^{-1}$) were spread onto PDA in smaller plates. Wheat seed cultures of *S. globisporus* JK-1 were place alongside the conidial suspensions inside larger plates and sealed as above. Twelve hours later, conidial germination was examined. To test whether the volatiles could kill conidia or just delay the germination, conidia exposed to the volatiles were examined daily for 3 d. Furthermore, the spores exposed to the volatiles that did not germinate after 3 d of exposure were washed by gentle repeated pipetting in 2 mL sterile water, centrifuged, and re-suspended in sterile water, spread onto fresh PDA plates, and observed for 6 d. For each treatment, there were three replicates, and the experiments were repeated three times.

### 2.4. Control of blue mold of Shatang Mandarin by the volatiles

Fourteen Shatang Mandarin fruit of similar size and color were placed on a metal sieve (20 cm diameter × 5 cm height). These sieves with test oranges were put on solid metal sieve bottoms of the same size, which contained 15 g, 30 g, 60 g, 120 g or 240 g of autoclaved wheat seeds or wheat seed culture of *S. globisporus* JK-1 per liter of airspace in the sieve apparatus. Six wounds, approximately 0.5 mm wide and 5 mm deep, were made in the region opposite the calyx (button) of each fruit with a steel needle. These six wounds formed a round circle with a diameter of 10 mm. A 20 μL droplet of conidial suspension ($10^6$ conidia $mL^{-1}$) of *P. italicum* was placed in the center of the wounded area. Sieves were covered with metal lids and sealed with parafilm followed by incubation at 25 °C. The relative humidity inside sieves was kept high with free water inside the containers. As controls, different amounts (15 g $L^{-1}$, 30 g $L^{-1}$, 60 g $L^{-1}$, 120 g $L^{-1}$ or 240 g $L^{-1}$) of autoclaved wheat seeds without *S. globisporus* JK-1 were used. The percent infected fruit, as well as lesion diameters, were determined at 5, 10, 15, 20 d post-inoculation. For each treatment, there were two replicates (14 fruit each), and the experiments were conducted three times.

To determine the effect of the volatile substances against naturally occurring blue mold of citrus, freshly harvested non-inoculated fruit were exposed to 120 g $L^{-1}$ autoclaved wheat seeds

or wheat seed culture of *S. globisporus* JK-1 in sealed metal sieve systems, and incubated at 25 °C for 10 d. After that, the wheat seed treatments were removed, and the fruit were further incubated for 5 d at 25 °C in enclosed but non-sealed environments to observe whether they were infected with *P. italicum*. The percentage of decayed fruit was evaluated after treatments, and for each treatment, there were nine replicates (total 126 fruit per treatment). The experiments were conducted twice.

### 2.5. Electron microscopy of treatment with volatiles

Shatang Mandarin oranges were placed in the metal sieve system and inoculated as described above. The sieves with test fruit were put on metal sieve bottoms of the same size, which contained 120 g $L^{-1}$ autoclaved wheat seeds or wheat seed culture of *S. globisporus* JK-1. Tissue samples (0.5 cm × 0.2 cm × 0.3 cm) were collected at various time intervals (0 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, 24 h, 36 h, or 48 h after treatment) from separate containers. For each treatment, three different Mandarin fruit were sampled, with two subsamples from each fruit. The samples were fixed in phosphate-buffered 2.5% glutaric dialdehyde in graded aqueous series of ethanol (30%, 50%, 70%, 85%, 95%, 100%) and critical point dried with $CO_2$ (13200-AB, SPI supplies, Japan) using isoamyl acetate as the intermediate fluid. Pieces of sample were sputter-coated with gold palladium (JEE-420, NTC, Japan) and the spores or mycelia of *P. italicum* were examined with a JSM-6090/LV scanning electronic microscope (NTC, Japan). The experiment was conducted twice.

### 2.6. Collection and analysis of volatiles produced by S. globisporus JK-1

Volatiles from *S. globisporus* JK-1 after 7 or 14 d of incubation were collected and analyzed as described by Wan et al. (2008) with minor modifications. Briefly, volatiles from the head space in 250 mL conical flasks containing 40 g autoclaved wheat seeds or wheat seed culture of *S. globisporus* JK-1 were sampled by means of solid-phase micro-extraction (SPME) and analyzed with a gas chromatograph mass spectrometer (GC/MS, Agilent 7890A, American) equipped with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent, Santa Clara, CA, USA). Mass spectra were obtained using the scan modus (total ion count, 45–650 $m/z$). Confirmation of compound identity was done by comparison of mass spectra and retention times with those of available standards in the Library of the National Institute of Standards and Technology (NIST). In addition, the baseline volatile compounds from autoclaved wheat seeds were measured and subtracted. The experiments were conducted three times.

### 2.7. Effect of volatile compounds on the mycelial growth, sporulation and conidial germination in vitro

To study the effects of select individual volatile compounds (Table 1) on radial growth and sporulation, the antifungal bioassay described above with four small dishes within a larger dish was used. Three dishes contained a fungal culture, while the fourth dish contained a piece of autoclaved filter paper (square, 1.5 cm × 1.5 cm), to which one of the select compounds was added. Different amounts of individual volatile compounds (technical grade) were used, varying from 1 μL $L^{-1}$ to 1000 μL $L^{-1}$ of airspace in treatment containers. Equivalent amounts of sterile distilled water were used as controls. After 5 d of incubation at 25 °C, colony diameter and spore counts were assessed. The effect of select compounds on conidial germination was also assessed using the antifungal bioassay described above.

**Table 1**
Technical grade compounds detected in the volatiles from wheat seed culture of *Streptomyces globisporus* JK-1, and used *in vitro* and fruit studies for control of *Penicillium italicum*.

| Compound | Source | Purity |
|---|---|---|
| Dimethyl disulfide | Sigma | ≥98% (GC) |
| Dimethyl trisulfide | Tokyo chemical industry | ≥98.0% (GC) |
| Caryophyllene | Sigma–Aldrich | ≥80%, FCC, Kosher, FG |
| Aromadendrene | Sigma–Aldrich | Purum, ≥97.0% (sum of enantiomers, GC) |
| D-Limonene | Fluka | ≥99.0% (sum of enantiomers, GC) |
| Acetophenone | Fluka | puriss. p.a., standard for GC, ≥99.5% (GC) |
| Isoledene | Sigma–Aldrich | Purum, ≥95.0% (sum of enantiomers, GC) |
| Phenylethyl alcohol | Sigma–Aldrich | ≥99%, FCC, Kosher, FG |

### 2.8. Effect of volatile compounds on the control of P. italicum on Shatang Mandarin

Inoculated Shatang Mandarin fruit were placed in the metal sieve systems as described above. Individual select compounds were added to sterile filter paper discs (11 cm in diameter) and placed on the metal sieve bottoms to obtain a final concentration of 0 μL $L^{-1}$, 10 μL $L^{-1}$, 100 μL $L^{-1}$ or 1000 μL $L^{-1}$ of airspace in metal sieve containers. The sieves with test fruit were placed over the sieve bottoms containing the filter paper, covered with metal lids, and sealed with parafilm. For all the treatments, the fruit were incubated at 25 °C for 5 d, followed by the removal of the filter paper discs and sieve bottoms without resealing. Then after incubation at 25 °C for another 5 d, the percent infected fruit was determined. For each treatment, there were six replicates, involving a total of 84 fruit for each treatment.

### 2.9. Statistical analyses

The data were subjected to analyses of variance (ANOVA) using SPSS 13.0 software for windows (SPSS Inc., Chicago, USA). An arcsine transformation was applied to data of conidial germination and disease incidence prior to analyses of variance. Mean comparisons were performed by Fisher's Protected Least Significant Difference (LSD) test ($P = 0.05$).

## 3. Results

### 3.1. Isolation of S. globisporus

In August 2007, a small contaminant colony appeared in a PDA plate of *Magnaporthe grisea* in a plant pathology laboratory at Huazhong Agricultural University, and it showed strong inhibition of the fungus. The colony was transferred onto a fresh plate, grown for identification using morphological and molecular methods, and named *S. globisporus* JK-1. Preliminary screening showed that the actinomycete was antagonistic to several phytopathogenic fungi in dual culture plates (data not shown). Colony morphology of the fungi was also affected since fungi such as *M. grisea* and *Phoma citricarpa* which normally darken with age, stayed white in the presence of *S. globisporus* JK-1.

### 3.2. Antifungal activity of volatiles from S. globisporus JK-1 on P. italicum

Radial mycelial growth and sporulation of *P. italicum* were greatly suppressed by the volatiles from wheat seed cultures, whereas autoclaved wheat seed had no observable effects on

**Table 2**
Effect of volatiles produced by wheat seed culture of *Streptomyces globisporus* JK-1 on conidial germination, mycelial growth and sporulation of *Penicillium italicum*.

| *Streptomyces globisporus*[a] ($g\,L^{-1}$) | Mycelial growth[b] (cm) | Sporulation[b] ($\times 10^5$ spores per plate) | Germination[c] (%) |
|---|---|---|---|
| 0 | 5.2a | 3200a | 95.3a |
| 3.75 | 5.0b | 683b | 89.7b |
| 7.5 | 4.9b | 215c | 83.6c |
| 15 | 2.0c | 14.8d | 68.4d |
| 30 | 0.7d | 0.7e | 31.3e |
| 60 | 0.0e | 0.0f | 3.4f |

[a] Grams of culture per liter of airspace.
[b] Mycelial growth and sporulation were measured after incubation for 5 d at 25 °C. Means based on 27 replicates followed by the same letters within the column were not significantly different ($P < 0.05$) according to the Least Significant Difference test.
[c] Conidial germination was determined after 12 h at 25 °C.

**Table 3**
Blue mold incidence and disease severity on inoculated seedless Shatang Mandarin in the presence or absence of the volatiles produced by *Streptomyces globisporus* JK-1 in an enclosed 2 L sieve system.

| *Streptomyces globisporus* ($g\,L^{-1}$) | Disease incidence[b] (%) | | | | Lesion diameter[c] (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 5 | Day 10 | Day 15 | Day 20 | Day 5 | Day 10 | Day 15 | Day 20 |
| 0[a] | 100a | 100a | 100a | 100a | 4.0a | 4.0a | 4.0a | 4.1a |
| 15 | 100a | 100a | 100a | 100a | 1.8b | 2.3b | 3.5b | 4.1a |
| 30 | 70.6b | 100a | 100a | 100a | 0.8c | 1.6c | 2.8c | 3.7b |
| 60 | 19.0c | 59.5b | 100a | 100a | 0.1d | 0.5d | 1.7d | 2.3c |
| 120 | 0.0d | 0.0c | 26.2b | 66.7b | 0.0d | 0.0d | 0.1e | 0.5d |
| 240 | 0.0d | 0.0c | 21.5b | 59.5c | 0.0d | 0.0d | 0.1e | 0.4d |

[a] Control treatments consisted of 15 $g\,L^{-1}$, 30 $g\,L^{-1}$, 60 $g\,L^{-1}$, 120 $g\,L^{-1}$ or 240 $g\,L^{-1}$ autoclaved wheat seeds without *S. globisporus* JK-1.
[b,c] Means based on 6 replicates followed by the same letters within each column were not significantly ($P < 0.05$) different according to the Least Significant Difference test.

**Table 4**
Volatile organic compounds from *S. globisporus* JK-1 produced on 7-d-old wheat seed culture detected by GC–MS analysis.

| Possible compound[a] | RT[b] (min) | Area (%) |
|---|---|---|
| 5,5-Dimethyl-1,3-hexadiene | 9.76 | 0.61 |
| Oxime-, methoxy-phenyl- | 12.74 | 0.05 |
| 2-Pentyl-Furan | 15.61 | 0.36 |
| D-Limonene | 17.38 | 0.23 |
| Benzoic acid, methyl ester | 20.72 | 2.21 |
| Naphthalene | 24.88 | 0.02 |
| 2-Cyclopenten-1-one, 3,4-dimethyl- | 26.67 | 0.06 |
| 1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3aα,7aβ) | 27.87 | 1.11 |
| Cyclohexane, 1,1,4,4-tetramethyl-2,6-bis(methylene)- | 28.44 | 0.12 |
| 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)- | 31.67 | 0.03 |
| Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)- | 31.90 | 0.61 |
| 3a,7-Methano-3aH-cyclopentacyclooctene, 1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl-, [3aR-(3aα,7α,9aβ)]- | 32.17 | 0.01 |
| α-Cubebene | 32.43 | 0.31 |
| Copaene | 33.37 | 0.14 |
| Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]- | 34.38 | 2.28 |
| α-Cubebene | 34.54 | 0.22 |
| Trans-1,10-Dimethyl-trans-9-decalol | 34.88 | 15.22 |
| Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- | 35.43 | 3.04 |
| 1H-Cyclopropa[a]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1aR(1aα,7α,7aα,7bα)]- | 35.96 | 2.85 |
| 1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3aα,7aβ) | 36.22 | 1.13 |
| Seychellene | 36.43 | 0.99 |
| Caryophyllene | 36.83 | 0.39 |
| 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR(1aα,4aβ,7α,7aβ,7bα)]- | 37.00 | 3.45 |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aα,8aα)- | 37.24 | 0.22 |
| 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- | 38.12 | 18.02 |
| Cedrene | 38.52 | 1.31 |
| 1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,7α,7aβ,7bα)]- | 38.70 | 3.36 |
| Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- | 38.87 | 0.18 |
| 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- | 39.12 | 0.13 |
| 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1aα,4α,4aβ,7bα)]- | 39.29 | 1.36 |
| Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- | 39.67 | 3.18 |
| Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | 40.03 | 1.76 |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aα,8aα)- | 40.19 | 0.41 |
| Naphthalene, 1,2-dihydro-1,1,6-trimethyl- | 40.40 | 0.07 |
| (+)-Epi-bicyclosesquiphellandrene | 41.48 | 0.17 |
| α-Muurolene | 42.09 | 0.69 |
| Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- | 43.12 | 1.19 |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]- | 43.68 | 0.14 |
| Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]- | 43.87 | 0.24 |
| Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R(1α,3aβ,4α,7β)]- | 44.14 | 1.78 |

[a] Minor compounds were detected in autoclaved wheat seeds and are not included in this table.
[b] RT, retention time.

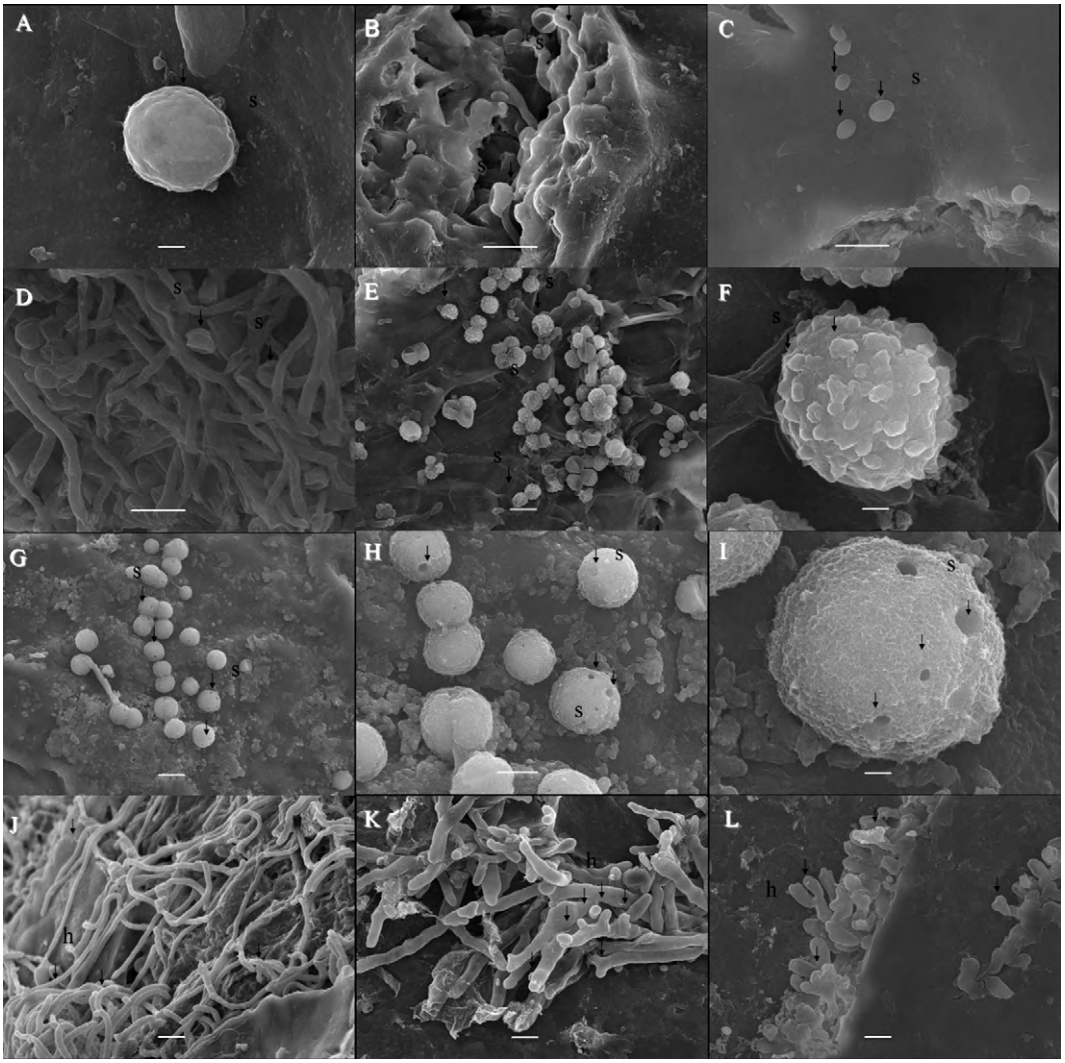


**Fig. 1.** Scanning electronic micrographs of *P. italicum* growing on the surface of Shatang Mandarin in the presence or absence of the volatiles incubation at 25 °C. (A) The spores of *P. italicum* without any treatment. (B and C) 12 HPI in the absence (B) or presence (C) of volatiles. (D–F), 18 HPI in the absence (D) or presence (E and F) of volatiles, and F is a higher magnification of E. (G–I) 48 HPI in the presence of volatiles, and I is a higher magnification of H. (J) Mycelial growth without being exposed to the volatiles. (K and L) Mycelial growth exposed to the volatiles for 10 d. Bars: B–E, G, J, K and L represent 10 μm; A, F and I represent 1 μm; H represents 5 μm.

growth. After 5 d in the presence of 15 g $L^{-1}$ or 30 g $L^{-1}$ wheat seed culture of *S. globisporus* JK-1, the mycelial growth (less the 5 mm diameter of the inoculum plug) was 2 cm and 0.7 cm, respectively, and the sporulation was $1.48 \times 10^6$ and $7.0 \times 10^4$ spores per plate, respectively. In comparison, the mycelial growth was 5.2 cm and sporulation was $3.2 \times 10^8$ spores per plate in the non-inoculated controls. No mycelial growth was observed in the presence of volatiles produced by 60 g $L^{-1}$ *S. globisporus* JK-1 cultures, nor were spores found; however, the dormant mycelial plugs which had been exposed to the volatiles for 5 d, produced new growth after being transferred to fresh PDA plates.

There was also more than 50% inhibition of conidial germination in the presence of volatiles produced by 30 g $L^{-1}$ *S. globisporus* JK-1 cultures, and the growth rate of germ tubes was also reduced. Spore germination was only 3.4% when the *S. globisporus* JK-1 culture was increased to 60 g $L^{-1}$. In contrast, germination of the conidia in the control treatment (autoclaved wheat seed) was as high as 95.3% (Table 2). In addition, the spores exposed to the volatiles, at either 30 g $L^{-1}$ or 60 g $L^{-1}$ for 3 d did not germinate after being transferred to fresh PDA plates even after washing.

### 3.3. Control of blue mold of Shatang Mandarin by the volatiles

For the Shatang Mandarin fruit obtained from Guangdong, naturally occurring rots on the non-inoculated test fruit were mainly caused by *P. italicum*, *P. digitatum*, *Colletotrichum gloeosporioides* and other minor pathogens affecting over 30% of the fruit (data not shown). A significant reduction of the percentage of decayed fruit was achieved by fumigating with 120 g $L^{-1}$ volatiles from wheat seed culture of *S. globisporus* JK-1. After incubation at 25 °C for 10 d, the disease incidence of decayed fruit naturally infected with *P. italicum* and subjected to wheat seed culture of *S. globisporus* JK-1 was 7.5% while the untreated control was 25.3%.

The incidence of blue mold on Shatang Mandarin after wound inoculation was greatly decreased in the presence of volatiles (Table 3). On non-treated inoculated fruit, there was 100% disease incidence by 5 d of incubation at 25 °C, whereas exposure 30 g $L^{-1}$ or 60 g $L^{-1}$ wheat seed culture *S. globisporus* JK-1 gave 70.6% and 19.0% disease incidence respectively, and no symptoms or spores were observed in the presence of 120 g $L^{-1}$ or 240 g $L^{-1}$. With increased incubation periods to 10 or 15 d, the disease incidence continued to increase. However, even after 20 d incubation at 25 °C, 33.3% and 40.5% of the fruit were not diseased in the presence of 120 g $L^{-1}$ or

240 g $L^{-1}$ wheat seed culture of *S. globisporus* JK-1. Fruit from all other treatments showed 100% disease incidence by that time, but the disease severity was strongly reduced in the presence of the volatiles (Table 3). In addition, no rind blemishes were observed in non-inoculated fruit exposed to volatiles.

### 3.4. Electron microscopy of treatment with volatiles

Scanning electron micrographs of *P. italicum* spores in the untreated control showed a normal morphology with a smooth surface (Fig. 1A). Untreated spores germinated and invaded host tissue (Fig. 1B), but during 12 h incubation with volatiles from *S. globisporus,* spore germination was inhibited (Fig. 1C). At 18 h post-inoculation (HPI), abundant mycelial growth of untreated samples was apparent on the plant surface with ramification throughout the parenchymal tissue beneath the wound sites (Fig. 1D). In contrast, the spores exposed to volatiles for 18 h did not germinate and appeared to release protoplasm (Fig. 1E and F), and became pitted (Fig. 1G–I). In treated samples, some spores were able to germinate and invade the host. However, mycelial growth was suppressed and terminal hyphae showed stunted tips (Fig. 1K and L) compared to the untreated control (Fig. 1J).

### 3.5. GC/MS analysis of volatile produced by S. globisporus JK-1

GC/MS analysis showed that the most frequent 41 volatile organic compounds from 7-d-old and 14-d-old wheat seed culture of *S. globisporus* JK-1 all had similarity indices (SI) > 90% with ones from the NIST library (Tables 4 and 5). These compounds mainly fell into several classes: alkenes, aromatic hydrocarbons, alcohols, sulfides, and lipids. Most of the volatiles from the 7-d-old and 14-d-old cultures of *S. globisporus* JK-1 were the same but differed in quantity (Tables 4 and 5). Trans-1,10-dimethyl-trans-9-decalol (geosmin) was detected as the major component in volatiles from both 7-d-old and 14-d-old cultures of *S. globisporus* JK-1, at 15.2% and 13.9% of all compounds, respectively. The second most abundant compound in the volatiles from 7-d-old culture was 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-[s-(E,E)], at 13.0%, while in 14-d-old cultures, it was naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl) at 9.13%. However, when tested in mass, the volatiles from 7-d-old cultures showed much less antifungal activity than 14-d-old cultures (data not shown). Dimethyl disulfide, dimethyl trisulfide, phenylethyl alcohol, acetophenone, isoledene, and aromadendrene were found in the volatiles from 14-d-old cultures but not in the volatiles from 7-d-old cultures. In addition, we found that d-limonene and caryophyllene were produced in significantly larger quantities (up to 10-fold-greater) in 14-d-old than in 7-d-old cultures. Hence, these particular volatile compounds were selected for further individual testing of their antifungal activity.

### 3.6. Effect of volatile compounds on mycelial growth, sporulation and conidial germination in vitro

Among the eight volatile compounds tested, only dimethyl disulfide, dimethyl trisulfide and acetophenone completely inhibited mycelial growth, sporulation and conidial germination of *P. italicum in vitro* at both tested concentrations of 100 μL $L^{-1}$ and 1000 μL $L^{-1}$ (Tables 6–8). Phenylethyl alcohol showed weak inhibitory activity against mycelial growth and sporulation as well as conidial germination, while caryophyllene only showed weak inhibitory activity against sporulation and conidial germination at a concentration of 1000 μL $L^{-1}$ (Tables 6–8). D-Limonene, aro-

**Table 5**
Volatile organic compounds from *S. globisporus* JK-1 produced on 14-d-old wheat seed culture detected by GC–MS analysis.

| Possible compound[a] | RT[b] (min) | Area (%) |
|---|---|---|
| Dimethyl disulfide | 5.59 | 0.90 |
| 5,5-Dimethyl-1,3-hexadiene | 9.76 | 0.12 |
| Oxime-, methoxy-phenyl- | 12.88 | 0.14 |
| Dimethyl trisulfide | 14.44 | 0.81 |
| D-Limonene | 17.39 | 3.42 |
| Acetophenone | 19.41 | 0.20 |
| Phenylethyl alcohol | 21.81 | 3.04 |
| Naphthalene | 24.84 | 0.25 |
| 2-Cyclopenten-1-one, 3,4-dimethyl- | 26.24 | 0.36 |
| 1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3aα,7aβ) | 27.85 | 0.26 |
| 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)- | 31.88 | 0.46 |
| α-Cubebene | 32.42 | 0.08 |
| 3a,7-Methano-3aH-cyclopentacyclooctene, 1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl-, [3aR-(3aα,7α,9aβ)]- | 32.77 | 0.06 |
| Copaene | 33.56 | 2.83 |
| Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]- | 34.23 | 1.14 |
| Trans-1,10-Dimethyl-trans-9-decalol | 34.78 | 13.9 |
| 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1aα,4α,4aβ,7bα)]- | 34.99 | 0.44 |
| Caryophyllene | 35.42 | 4.79 |
| 1H-Cyclopropa[a]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1aR(1aα,7α,7aα,7bα)]- | 35.95 | 7.82 |
| Aromadendrene | 36.30 | 0.28 |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]- | 36.39 | 1.36 |
| Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- | 36.67 | 0.38 |
| 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- | 36.80 | 0.82 |
| 1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,7α,7aβ,7bα)]- | 36.95 | 3.33 |
| 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR(1aα,4aβ,7α,7aβ,7bα)]- | 37.09 | 0.29 |
| Isoledene | 37.63 | 0.79 |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aα,8aα)- | 37.74 | 0.69 |
| 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- | 37.92 | 1.38 |
| (+)-Epi-bicyclosesquiphellandrene | 38.37 | 0.25 |
| α-Muurolene | 38.72 | 4.49 |
| Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- | 39.25 | 0.19 |
| Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- | 39.64 | 6.05 |
| Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | 40.00 | 9.13 |
| Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R(1α,3aβ,4α,7β)]- | 44.10 | 0.41 |

[a] Minor compounds were detected in autoclaved wheat seeds and are not included in this table.
[b] RT, retention time.

**Table 6**
Effect of technical grade volatile compounds (droplets applied to filter paper in an enclosed Petri dish system with 0.5 L airspace) on the mycelial growth of *Penicillium italicum* after 5 d of incubation.

| Volatile compound | Mycelial growth (cm) at different concentrations of volatiles | | | | |
|---|---|---|---|---|---|
| | 0 μL L$^{-1}$ | 1 μL L$^{-1}$ | 10 μL L$^{-1}$ | 100 μL L$^{-1}$ | 1000 μL L$^{-1}$ |
| Dimethyl disulfide | 5.1a | 1.4b | 0.7c | 0.0d | 0.0d |
| Dimethyl trisulfide | 5.1a | 1.2b | 0.4c | 0.0d | 0.0d |
| D-Limonene | 5.1a | 5.1a | 5.1a | 4.8b | 4.7b |
| Acetophenone | 5.1a | 1.7b | 0.3c | 0.0d | 0.0d |
| Phenylethyl alcohol | 5.1a | 5.1a | 4.5b | 3.6c | 2.7d |
| Aromadendrene | 5.1a | 4.7b | 4.6b | 4.7b | 4.5c |
| Isoledene | 5.1a | 4.6b | 4.6b | 4.5c | 4.3d |
| Caryophyllene | 5.1a | 5.0a | 4.6b | 4.2c | 4.1c |

Means based on 27 replicates followed by the same letters within the column were not significantly different ($P < 0.05$) according to the Least Significant Difference test.

**Table 7**
Effect of technical grade volatile compounds (droplets applied to filter paper in an enclosed Petri dish system with 0.5 L airspace) on the sporulation of *Penicillium italicum* after 5 d of incubation.

| Volatile compound | Sporulation[a] ($\times 10^5$ spores per plate) at different concentrations of volatiles | | | | |
|---|---|---|---|---|---|
| | 0 μL L$^{-1}$ | 1 μL L$^{-1}$ | 10 μL L$^{-1}$ | 100 μL L$^{-1}$ | 1000 μL L$^{-1}$ |
| Dimethyl disulfide | 3207a | 1.5b | 0.9b | 0.0b | 0.0b |
| Dimethyl trisulfide | 3197a | 1.1b | 0.5b | 0.0b | 0.0b |
| D-Limonene | 3152a | 3145a | 2805b | 1034c | 1025d |
| Phenylethyl alcohol | 3140a | 2930a | 2258b | 508.3c | 475.0c |
| Acetophenone | 3055a | 772.9b | 0.5c | 0.0c | 0.0c |
| Aromadendrene | 3183a | 3023a | 2947a | 1279b | 760.5c |
| Isoledene | 3177a | 3117a | 2997ab | 1057b | 546.3c |
| Caryophyllene | 3163a | 3123a | 2083b | 833.3c | 43.0d |

[a] Means based on 27 replicates followed by the same letters within the column were not significantly different ($P < 0.05$) according to the Least Significant Difference test.

madendrene and isoledene had no significant affect on mycelial growth, sporulation or conidial germination of *P. italicum* at the concentrations tested.

### 3.7. Effect of volatile compounds on the control of P. italicum on Shatang Mandarin

The incidence of blue mold on Shatang Mandarin after wound inoculation was greatly decreased in the presence of dimethyl disulfide, dimethyl trisulfide and acetophenone, respectively (Table 9). On non-treated inoculated fruit, there was 100% disease incidence by 5 d of incubation at 25 °C. Dimethyl disulfide and dimethyl trisulfide inhibited disease incidence at both tested concentrations of 100 μL L$^{-1}$ and 1000 μL L$^{-1}$, while 1000 μL L$^{-1}$ acetophenone was needed to inhibit disease incidence. In contrast to the *in vitro* results, phenylethyl alcohol and caryophyllene did not significantly inhibit the *P. italicum* infections on Shatang Mandarin compared to untreated control. Treatments with D-limonene, aromadendrene and isoledene did not affect disease incidence at the concentrations tested.

## 4. Discussion

*Streptomyces* spp. are known to produce strong odors, and the accidental discovery of a contaminant isolate that was inhibitory to various phytopathogenic fungi in culture plates led us to investigate whether this isolate could be used in control of blue mold of citrus caused by *P. italicum*. In this study, 41 volatile compounds from *S. globisporus* JK-1 were identified by GC/MS analysis. An earthy smelling substance, trans-1, 10-dimethl-trans-9 decalol (geosmin) was found to be the major component. This volatile compound has also been found to be produced by *Penicillium* spp., *Aspergillus* spp., *Streptomyces* spp., *Noncyanobacteria* and *Cyanobacteria* (Wan et al., 2008). Six of the other volatile substances detected in this study have been reported to be the antimicrobial volatiles of the other organisms: D-limonene in the essential oil of *Calocedrus formosana* leaf and *Calocedrus macrolepis* var. *formosana* leaf (Chang et al., 2008; Cheng et al., 2004); caryophyllene in the essential oil of basil (*Ocimum basilicam*) and *C. macrolepis* var. *formosana* Florin leaf (Chang et al., 2008; Oxenham et al., 2005); phenylethyl alcohol in an endophytic fungus *Muscodor albus*

**Table 8**
Effect of technical grade volatile compounds (droplets applied to filter paper in an enclosed Petri dish system with 0.5 L airspace) on the conidial germination of *Penicillium italicum* after 12 h of incubation.

| Volatile compound | Conidial germination[a] (%) at different concentrations of volatiles | | | | |
|---|---|---|---|---|---|
| | 0 μL L$^{-1}$ | 1 μL L$^{-1}$ | 10 μL L$^{-1}$ | 100 μL L$^{-1}$ | 1000 μL L$^{-1}$ |
| Dimethyl disulfide | 95.1a | 29.7b | 1.7c | 0.0c | 0.0c |
| Dimethyl trisulfide | 95.1a | 20.1b | 1.3c | 0.0c | 0.0c |
| D-Limonene | 94.8a | 95.0a | 94.6a | 92.3a | 91.6a |
| Phenylethyl alcohol | 95.4a | 93.0a | 78.8b | 75.0b | 75.0b |
| Acetophenone | 95.0a | 70.3b | 2.1c | 0.0d | 0.0d |
| Aromadendrene | 95.3a | 95.3a | 93.7a | 93.7a | 93.3a |
| Isoledene | 95.0a | 94.5a | 94.0a | 92.7a | 91.7a |
| Caryophyllene | 95.3a | 91.4a | 22.7b | 9.4c | 9.0c |

[a] Means based on 27 replicates followed by the same letters within the column were not significantly different ($P < 0.05$) according to the Least Significant Difference test.

**Table 9**
Effect of technical grade volatile compounds (droplets applied to filter paper in an enclosed metal sieve system with 2 L airspace) on control of *Penicillium italicum* on Shatang Mandarin after 5 d of incubation.

| Volatile compound | Disease incidence (%) at different concentrations of volatiles | | | |
|---|---|---|---|---|
| | 0 $\mu L L^{-1}$ | 10 $\mu L L^{-1}$ | 100 $\mu L L^{-1}$ | 1000 $\mu L L^{-1}$ |
| Dimethyl disulfide | 100.0a | 74.6b | 0.0c | 0.0c |
| Dimethyl trisulfide | 100.0a | 65.5b | 0.0c | 0.0c |
| D-Limonene | 100.0a | 100.0a | 100.0a | 100.0a |
| Phenylethyl alcohol | 100.0a | 100.0a | 100.0a | 100.0a |
| Acetophenone | 100.0a | 100.0a | 75.0b | 0.0c |
| Aromadendrene | 100.0a | 100.0a | 100.0a | 100.0a |
| Isoledene | 100.0a | 100.0a | 100.0a | 100.0a |
| Caryophyllene | 100.0a | 100.0a | 100.0a | 100.0a |

Means based on 6 replicates followed by the same letters within the column were not significantly different ($P < 0.05$) according to the Least Significant Difference test.

(Grimme et al., 2007; Strobel et al., 2007); dimethyl disulfide and dimethyl trisulfide in Garlic Oil (Ross et al., 2001); and aromadendrene in the essential oil of *Melaleuca alternifolia* (tea tree) leaf (Hammer et al., 2003; Porter and Wilkins, 1999). Five of the volatile compounds detected in this study, including isoledene, α-muurolene, (+)-epi-bicyclosesquiphellandrene, copaene and the second most abundant 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene, have been reported to be components of antimicrobial volatiles in essential oils of wood or volatile constituents of propolis, the resinous mixture that bees collect from botanical sources (Cavaleiro et al., 2006; Hocine et al., 2009; Porter and Wilkins, 1999; Pyun and Shin, 2006; Vukovic et al., 2007; Wan et al., 2008).

The results of present study showed that d-limonene, aromadendrene and isoledene had no significant affect on mycelial growth, sporulation or conidial germination of *P. italicum*, but these results differed from previous reports (Chang et al., 2008; Hammer et al., 2003; Porter and Wilkins, 1999). This may be due to the different treatment methods used. In this study, the compounds were used as fumigants rather than amended into culture medium as previously reported. Phenylethyl alcohol and caryophyllene showed weak antifungal activity *in vitro*, but no efficacy in tests against blue mold on Shatang Mandarin even at 1000 $\mu L L^{-1}$, the highest concentration tested. Only dimethyl disulfide, dimethyl trisulfide and acetophenone showed effective inhibitory activity both *in vitro* and in planta. This is the first report that the dimethyl disulfide, dimethyl trisulfide and acetophenone showed efficacy against a postharvest disease as fumigants. The possibility of synergistic effects of individual compounds in mixtures remains to be studied.

Previous studies showed that volatiles from *Bacillus amyloliquefaciens* IN937 and *B. subtilis* GB03 could promote growth of *Arabidopsis thaliana* (Ryu et al., 2003; Vespermann et al., 2007) and induce systemic resistance of *A. thaliana* against *Erwinia carotovora* subsp. *carotovora* (Ryu et al., 2004). Other studies showed that volatiles from bacteria and yeast inhibited pigment production by sapstain fungi (Bruce et al., 2003), and that *Aspergillus* volatiles can regulate aflatoxin synthesis in *Aspergillus parasiticus* (Roze et al., 2007). Significant differences between the volatiles compounds from *S. globisporus* JK-1 and some other Actinomycetes (Dickschat et al., 2005; Wilkins, 1996; Wan et al., 2008) were also found. Thus, different organisms and different species may release similar and different types of volatile substances, with a variety of effects.

The results of the present study indicated that volatile substances produced by *S. globisporus* JK-1 have a significant effect on the mycelial growth and sporulation as well as conidial germination of *P. italicum in vitro*. Previous studies had shown that volatiles substances produced by *S. griseoruber* inhibited spore germination (Herrington et al., 1985). Recently, suppression of mycelial growth of *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, as well as reduction in the incidence of seedling blight of rice, leaf blight of oilseed rape and fruit rot of strawberry has been reported for volatiles of *S. platensis* F-1 (Wan et al., 2008).

We found that the greater the mass of wheat seed culture of *S. globisporus* JK-1, the greater the inhibitory activity of *S. globisporus* JK-1 against *P. italicum* both in axenic culture (mycelial growth, spore germination and sporulation) and in planta (disease incidence, disease severity). Morphological changes in the fungal pathogen were also observed, such as hyphal distortion and shortening, and protoplasm leakage from spores on surface tissue of Shatang Mandarin.

In our study, germination of spores and mycelial growth from mycelial plugs were almost fully inhibited when exposed to 60 g $L^{-1}$ wheat seed culture during 3 d. These mycelial plugs were able to produce hyphae when transplanted onto fresh PDA plates demonstrating fungistatic effects on hyphal growth; but the spores, even after washing did not germinate when plated on fresh PDA, showing fungicidal effects on spores. This activity against spores of *P. italicum* is similar to the fungicidal activity of some plant volatile compounds against *P. expansum* (Neri et al., 2006, 2007).

The infection process of *P. italicum* on Shatang Mandarin was suppressed in the presence of volatiles. Scanning electronic microscopy showed that spores exposed to the volatiles did not germinate and that protoplasm was released from exposed spores. In addition, the apices of terminal hyphae were shortened and abnormally swollen when growing out of fruit in the presence of volatiles.

In conclusion, the present study showed that the volatiles from *S. globisporus* JK-1 and its components have efficacy against *P. italicum in vitro* and on citrus fruit, and could potentially be an effective alternative for the control of postharvest diseases by fumigant action. The effect of the volatiles on other organisms such as microbial of food safety concern or insects was not examined in this study, and their effect on fruit flavor is unknown; however, no rind blemishes were observed even after exposure to volatiles for 20 d. For practical use, further study on the effects on individual compounds and mixtures are needed, including the safety of these compounds to humans and the environment.

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