Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*

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**ABSTRACT**

**Background:** Biocontrol agents are regarded as promising and environmental friendly approaches as agrochemicals for phytodiseases that cause serious environmental and health problems. *Trichoderma* species have been widely used in suppression of soil-borne pathogens. In this study, an endophytic fungus, *Trichoderma gamsii* YIM PH30019, from healthy *Panax notoginseng* root was investigated for its biocontrol potential.

**Methods:** *In vitro* detached healthy roots, and pot and field experiments were used to investigate the pathogenicity and biocontrol efficacy of *T. gamsii* YIM PH30019 to the host plant. The antagonistic mechanisms against test phytopathogens were analyzed using dual culture, scanning electron microscopy, and volatile organic compounds (VOCs). Tolerance to chemical fertilizers was also tested in a series of concentrations.

**Results:** The results indicated that *T. gamsii* YIM PH30019 was nonpathogenic to the host, presented appreciable biocontrol efficacy, and could tolerate chemical fertilizer concentrations of up to 20%. *T. gamsii* YIM PH30019 displayed antagonistic activities against the pathogenic fungi of *P. notoginseng* via production of VOCs. On the basis of gas chromatography-mass spectrometry, VOCs were identified as dimethyl disulfide, dibenzofuran, methanethiol, ketones, etc., which are effective ingredients for antagonistic activity. *T. gamsii* YIM PH30019 was able to improve the seedlings’ emergence and protect *P. notoginseng* plants from soil-borne disease in the continuous cropping field tests.

**Conclusion:** The results suggest that the endophytic fungus *T. gamsii* YIM PH30019 may have a good potential as a biological control agent against notoginseng phytodiseases and can provide a clue to further illuminate the interactions between *Trichoderma* and phytopathogens.

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1. Introduction

*Panax notoginseng* (Burk.) F.H. Chen (Araliaceae), an important member of the genus *Panax* [1], is a valued traditional Chinese medicinal herb. Because of its low adaptive capacity, which strictly depends on climate and environment, *P. notoginseng* is found in middle and high elevation areas around the subtropical zone [2], mainly in Wenshan, Yunnan, China. With increasing demand in pharmaceutical industries, *P. notoginseng* has been domestically cultivated on a large scale [3]. However, severe soil-borne diseases...
have significantly limited the yield and quality of *P. notoginseng*. The root-rot disease caused by a plethora of phytopathogens [4,5], such as *Fusarium solani*, *Fusarium oxysporum*, *Phoma herbarum*, and *Rhizoctonia solani* [6,7], is the most destructive among the phyto-
diseases of notoginseng, and is thought to be a main cause for the continuous cropping obstacles for *P. notoginseng*. To combat the disease and increase output, chemical fertilizers and pesticides have been extensively applied. These agro approaches resulted in soil salinization [8,9], changes in soil microbial biodiversity, and environmental pollution. Hence, there is an urgent need for sustain-
table and ecologically safe ways to control *P. notoginseng* diseases.

Biological control, which includes the use of specific organisms to control phytopathogens, is a nature-friendly ecological approach. *Trichoderma* spp. distributed widely in soil have been developed as a source of biocontrol agents for years. They are remarkable for their rapid growth, utilization of diverse substrates, resistance to biotic and abiotic stresses, and promotion of plant growth [10–12]. Some *Trichoderma* species show effective antagonistic activities toward plant pathogens, such as *Fusarium* spp., *Phoma* (*Pleospora*) betae, and *Rhizoctonia solani* [13,14], with the mechanisms of mycoparasitism, competition for nutrition and space, and indirect inhibition through volatile organic compounds (VOCs) [15].

VOCs can permeate and travel in soil pores for long distances [16]. VOCs emitted by *Trichoderma* spp. are crucial in controlling plant pathogens [17,18], activating plant immunity, and enhancing plant growth [19]. These potential biological values and features have attracted the attention of researchers in recent years [20–23]. However, *Trichoderma* species, substrates, and nutrient conditions can influence their biocontrol viability [24,25]. In screening antagonistic microbes against *notoginseng* diseases from the bio-
niches of *P. notoginseng*, an endophytic fungus with mycoparasitic activity was obtained from a healthy *notoginseng* root. In this study, experiments were carried out to estimate: (1) the pathogenicity of *Trichoderma gamsii* YIM PH30019 to its host plant; (2) biocontrol efficacy to phytopathoses in the continuous field experiments; (3) tolerance capacity to chemical fertilizers; (4) mycoparasitism and induced VOCs antagonistic activities against phytopathogens; (5) VOCs identified with gas chromatography-mass spectrometry (GC-MS).

2. Materials and methods

2.1. Microorganism culture

*T. gamsii* YIM PH30019 was isolated from the root of a healthy 2-
y-old healthy *P. notoginseng* plant collected in July 2012 from Wenshan, China. The root samples were thoroughly washed with running water to remove soil particles, treated with Tween 20 for 1 h, sterilized with 70% ethanol for 1 min, and finally washed with sterilized distilled water for three times. Roots were crushed in an autoclaved mortar and pestle. The paste was serially diluted to 10⁻⁴ with sterilized distilled water, and a 1-ml dilution was coated on the plate containing 20 ml potato dextrose agar (PDA) medium. The discrete colonies were transferred and purified on fresh PDA plates. Identification was based on morphological and ITS mole-
cular phylogenetic analysis. The ITS sequence of *T. gamsii* was sub-
mitted to GenBank with the accession no. KP715352. The pathogenic fungi—*Phoma herbarum* (YIM PH30340), *Fusarium floccigerum* (YIM PH30355), *Scytalidium lignicola* (YIM PH30004), and *Epichloe nigrum* (YIM PH30306)—used in this study were isolated from the rotten root of *P. notoginseng*. Their pathogenicity was confirmed using the method described by Miao et al [6]. All strains were maintained on PDA medium, and the voucher specimens were preserved at Yunnan Institute of Microbiology, Kunming, China.

The deactivated cell walls of *E. nigrum*, *S. lignicola*, *P. herbarum*, and *F. floccigerum* were prepared according to Yang et al [26] with modifications. Briefly, a 6-mm mycelial disk was cut from the edges of actively growing colonies of test pyttopathogens and transferred into a 500-ml conical flask containing 200 ml potato dextrose broth (PDB) medium. The flasks were incubated on a shaker at 28°C for 2 h, and mycelia were collected by filtering the culture broth. Cell walls were lyophilized and powdered. Antago-
nistic activities caused by VOCs were tested on the deactivated cell wall agar medium (DCWA) (15 g deactivated cell walls, 6.9 g NaH₂PO₄, 2.0 g KH₂PO₄, 1.4 g (NH₄)₂SO₄, 1.0 g peptone, 0.3 g MgSO₄·7H₂O, 0.3 g urea, and 15.0 g agar in 1 L distilled water, pH not adjusted), and VOCs were collected by culturing *T. gamsii* YIM PH30019 in the deactivated cell wall broth medium (DCWB). Controls were inoculated in the above media without the deactivated cell walls.

2.2. Pathogenic test of *T. gamsii* YIM PH30019

The pathogenic abilities of *T. gamsii* YIM PH30019 was estimated with 1-y-old healthy *P. notoginseng* in greenhouse tests. Three *P. notoginseng* seedlings were planted in pots with the size of 10 L (0.6 × 0.5 × 0.125 m, L/W/H) containing 6 L sterilized soil. Spores of *T. gamsii* YIM PH30019 were collected from the PDA plates. A 0.2-
ml spore solution (1 × 10¹²) of *T. gamsii* YIM PH30019 was applied to the soil around roots. The treatment without inoculation with YIM PH30019 was set as control. Six replicates were used for treatment and control. The pots were incubated at 25°C for 2 mo. Six replicates were used for each treatment. The pots were kept in a shed similar to the field-planting condition. Spores of *T. gamsii* and test pathogenic fungi were collected from PDA plates. Ten milliliter spores (1.0 × 10¹⁰) of *T. gamsii* YIM PH30019, pathogenic fungus, and the mixture of *T. gamsii* YIM PH30019 and pathogenic fungus were added into the autoclaved soil and mixed together thoroughly. The surface-sterilized healthy *notoginseng* roots were laid into the soil at a depth of 5 cm. Roots treated with pathogenic fungus or *T. gamsii* YIM PH30019 were set as controls. The biocontrol efficacy of PH30019 and virulence of test phytopathogens were checked every week for 1 mo (Fig. 1 in Support Information).

2.3. Biocontrol estimation of root-rot disease

The biocontrol efficacy of *T. gamsii* YIM PH30019 was estimated in vitro with detached healthy *notoginseng* roots. YIM PH30019, pathogenic fungi, and the mixture of YIM PH30019 and test path-
ogenic fungus were inoculated with a healthy root in a pot containing 400 g soil autoclaved at 121°C for 60 min. Four replicates were set for each treatment. The pots were kept in a shed similar to the field-planting condition. Spores of *T. gamsii* and test pathogenic fungi were collected from PDA plates. Ten milliliter spores (1.0 × 10¹⁰) of *T. gamsii* YIM PH30019, pathogenic fungus, and the mixture of *T. gamsii* YIM PH30019 and pathogenic fungus were added into the autoclaved soil and mixed together thoroughly. The surface-sterilized healthy *notoginseng* roots were laid into the soil at a depth of 5 cm. Roots treated with pathogenic fungus or *T. gamsii* YIM PH30019 were set as controls. The biocontrol efficacy of PH30019 and virulence of test phytopathogens were checked every week for 1 mo (Fig. 1 in Support Information).

The disease coverage of type 0, 1, 2, 3, and 4 lesions for each root was evaluated following indicators [27] with modification, where 0 = no lesions, 1 = one to several lesions (roots blacking < 25%), 2 = extensive lesions or several entire roots necrotic (25–50% roots blacking), 3 = lesions on roots and dark-
ening of crown (50–75% root blacking), 4 = extensive darkening of crown (75–100% roots blacking).

2.4. Tolerance capacity to chemical fertilizers

To determine the effect of chemical fertilizers on the growth of *T. gamsii* YIM PH30019, three chemical fertilizers were selected—anmonium chloride, potassium nitrate, and ammonium dihydrogen phosphate—and mixed at a ratio of 1:1:1 (w/w/w). A 6-
mm plug of actively growing *T. gamsii* YIM PH30019 was placed on the center of a petri dish containing 25 mL PDA medium amended with chemical fertilizers at the following concentrations (w/v): 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, and 30%. Each concentration was repeated thrice. The petri dishes were incubated at 28°C for 1 wk. The morphology and the growth diameter were recorded daily.

### 2.5. Dual cultures and observations of mycoparasitism

The antagonism of *T. gamsii* YIM PH30019 against pathogens was investigated with dual culture tests [28]. The mycoparasitism of *T. gamsii* YIM PH30019 was observed by collecting the hyphae from the interaction zone between *T. gamsii* and test phytopathogens. The hyphal sample was transferred to glass coverslips, fixed with 2.5% glutaraldehyde, dehydrated in a series of ascending ethanol concentrations (50–100%) (v/v), dried in desiccator, and coated with gold in a sputter-coater (SCD 005; BAL-TEC, Switzerland); then, it was examined with a scanning electron microscope (Quanta 200FEG; FEI Company, Hillsboro, Oregon, USA).

### 2.6. Antagonistic assay of VOCs from *T. gamsii* YIM PH30019 against pathogens

The antagonistic effect of VOCs to pathogens was evaluated using the method described by Dennis and Webster [17] with modifications. A 6-mm plug of *T. gamsii* YIM PH30019 was cut from the actively growing cultures, then placed on the center of a 90-mm petri dish containing 25 mL DCWA. The lid of plates with YIM PH30019 was replaced by the bottom containing PDA inoculated with pathogenic fungus. The dishes were taped together with the center of a petri dish containing 5 mL DCWB medium. The antagonism of VOCs to pathogens was evaluated with chemical fertilizers at the following concentrations (w/v): 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, and 30%. The hyphal sample was transferred to glass coverslips, fixed with 2.5% glutaraldehyde, dehydrated in a series of ascending ethanol concentrations (50–100%) (v/v), dried in desiccator, and coated with gold in a sputter-coater (SCD 005; BAL-TEC, Switzerland); then, it was examined with a scanning electron microscope (Quanta 200FEG; FEI Company, Hillsboro, Oregon, USA).

### 2.7. VOC analysis by GC-MS

For the identification of VOCs from *T. gamsii* YIM PH30019, spore suspension was prepared to a final concentration of 5 × 10^7 spores/mL in sterile water. A 200-µL spore suspension was transferred into a 15-mL conical flask containing 5 mL DCWB medium. The flasks were incubated on a shaker at 28°C, 180 rpm for 4 d. Extraction was carried out at 50°C for 30 min with preconditioned PA fiber (85µM, polyacrylate) in the headspace. The VOCs were desorbed by placing the fiber into the GC injection port for 1 min at 250°C. Compounds were resolved in the following conditions: helium flow, 1.0 mL/min; oven temperature, 50°C (2 min), 6°C/min to 180°C (1 min), then 6°C/min to 260°C (5 min); and mass spectrometer monitoring in full scan mode (m/z 35–550) operated in the electron ionization mode at 70 eV with a source temperature of 220°C. Compounds were tentatively identified by the mass spectra using the National Institute of Standards and Technology database.

### 2.8. Biocontrol efficacy in continuous cropping field

Field experiments were conducted in a continuous cropping artificial shed in which crops of *P. notoginseng* harvested in the previous year were used. The experimental field is located at Xiangshuilong Village, a traditional notoginseng cultivation center in Wenshan, Yunnan. Rice bran was mixed with equal mass water, and autoclaved at 121°C, for 60 min. *T. gamsii* YIM PH30019 was inoculated in the autoclaved rice bran until the final *T. gamsii* YIM PH30019 spores concentration was up to 1.0 × 10^10 spore/g. The field soil was treated with chemical fumigants as described by Gao et al [29] prior to planting notoginseng. Fermented rice bran with *T. gamsii* YIM PH30019 was applied to each plot (treated with Trichoderma, Tt) (1.3 × 2.0 m) as the basal fertilizer with the use of 0.15 kg/m², and 112 1-y-old healthy notoginseng seedlings were planted according to the description of Sun et al [30] in January 2015. Experimental plots applied only with autoclaved rice bran were set as controls (CK). Treatment (Tt) and control (CK) (Fig. 2 in Support Information) were conducted in triplicate. After the emergence of notoginseng, 0.5 mL spore (1 × 10^5 spores/mL) of *T. gamsii* YIM PH30019 was applied to the soil by root irrigation for further protection. Seedling emergence and dead seedling rate were recorded from the emergence of notoginseng.

### 3. Results

#### 3.1. Pathogenicity to *P. notoginseng*

*T. gamsii* YIM PH30019 showed no pathogenic activity (Fig. 1). After coinoculation for 2 mo, either treated with *T. gamsii* YIM PH30019 or the control, *P. notoginseng* plants maintained their healthy growing status. The roots showed no symptoms on the
Table 1

| Chemical fertilizers' effect on the growth of Trichoderma gamsii YIM PH30019<sup>1)</sup> |
|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Concentration (% w/v)      | 1                | 2                | 3                | 4                | 5                | 6                |
| 0                           | 24.9 ± 0.3       | 60.2 ± 0.3       | 90.0 ± 0.0       | 90.0 ± 0.0       | 90.0 ± 0.0       | 90.0 ± 0.0       |
| 2                           | 19.9 ± 0.4       | 51.6 ± 0.1       | 90.0 ± 0.0       | 90.0 ± 0.0       | 90.0 ± 0.0       | 90.0 ± 0.0       |
| 4                           | 18.6 ± 0.1       | 40.1 ± 0.2       | 68.0 ± 0.3       | 90.0 ± 0.0       | 90.0 ± 0.0       | 90.0 ± 0.0       |
| 6                           | 14.0 ± 0.1       | 32.4 ± 0.2       | 54.1 ± 0.2       | 77.1 ± 0.1       | 90.0 ± 0.0       | 90.0 ± 0.0       |
| 8                           | 12.5 ± 0.8       | 19.4 ± 0.4       | 42.2 ± 0.1       | 63.1 ± 0.1       | 81.1 ± 0.1       | 90.0 ± 0.0       |
| 10                          | 11.5 ± 0.1       | 16.2 ± 0.1       | 37.0 ± 0.2       | 55.0 ± 0.2       | 69.0 ± 0.1       | 83.1 ± 0.2       |
| 12                          | 9.0 ± 0.2        | 12.0 ± 0.2       | 31.0 ± 0.2       | 45.0 ± 0.2       | 55.2 ± 0.2       | 67.3 ± 0.2       |
| 14                          | —<sup>3</sup>    | 9.1 ± 0.2        | 22.9 ± 0.2       | 34.1 ± 0.2       | 42.2 ± 0.1       | 50.1 ± 0.2       |
| 16                          | —                | 14.1 ± 0.2       | 23.4 ± 0.2       | 32.1 ± 0.2       | 39.1 ± 0.2       | 45.1 ± 0.2       |
| 18                          | —                | 10.0 ± 0.2       | 17.1 ± 0.1       | 22.1 ± 0.2       | 24.2 ± 0.2       | 29.6 ± 0.2       |
| 20                          | —                | —                | 10.1 ± 0.3       | 13.1 ± 0.2       | 15.2 ± 0.1       | —                |
| 22                          | —                | —                | —                | —                | —                | —                |
| 24                          | —                | —                | —                | —                | —                | —                |
| 26                          | —                | —                | —                | —                | —                | —                |
| 28                          | —                | —                | —                | —                | —                | —                |
| 30                          | —                | —                | —                | —                | —                | —                |

<sup>1</sup> Data were recorded for a 7-d inoculation.

<sup>2</sup> Colony diameter was an average value of three replicates.

<sup>3</sup> No growth was observed in our tests.
dibenzofuran, methanethiol, and ketones, may serve as chemical inhibitors to the growth of pathogenic fungi.

3.7. Biocontrol efficacy in continuous cropping field

Emergence of seedling in the continuous cropping field is very important in notoginseng planting. In our experiments, the average emergence rate in the treatments reached up to 57.8%, much more than that in the control (average 14.2%) at the end of March 2015 (Fig. 8). In the observation, dead seedlings occurred during the emergence of notoginseng, which was mainly caused by root-rot disease, and were removed from the field after they were recorded. Until the end of May 2015, the average percent mortality in the treatment plots was 23.9% and 61.3% in the controls (Fig. 9), indicating that T. gamsii YIM PH30019 may have protective effects on notoginseng seedlings against root-rot disease in the field experiments (Fig. 2 in Support Information). In early May, a small-scale disease breakout caused the mortalities to increase up to 20% in both the treatment plots and the controls. After root irrigation with T. gamsii YIM PH30019, the seedling death rate in treatment became significantly lower than that in CK.

4. Discussion

Biocontrol is the most ecofriendly approach to the management of plant disease. Further understanding of the biocontrol
mechanisms from different aspects is the critical role for agricultural applications in the future [31]. The most important genus used as a biocontrol agent is the *Trichoderma* [32], which has an outstanding interaction with plant and plant pathogens [33]. The interactions include antagonism toward fungal pathogens, plant growth promotion, plant defense responses, and protection of plants from environmental stresses, such as salinity and drought [34,35]. Continuous cropping obstacles significantly affect *P. notoginseng* seedling emergence and cause severe mortality to seedlings [36]. In our field experiments with continuous cropping soil, *T. gamsii* YIM PH30019 showed desirable biocontrol potential compared to plots without inoculation of *T. gamsii* YIM PH30019 (Fig. 2 in Support Information).

Frequent use of chemical fertilizers results in soil salinization and can also cause loss of protective efficacy for some biocontrol agents [37,38]. To achieve higher yield, multiple chemical fertilizers are widely used in *notoginseng* planting. Potential microbial agents should be hyperosmotic in controlling the diseases of *P. notoginseng*. The hyperosmotic property was evaluated with a series of composite chemical fertilizers. The results showed that *T. gamsii* YIM PH30019 can grow well at 0–12% (w/v) and bear up to 20% (w/v) of chemical fertilizers that are usually used in agriculture. This characteristic may ensure that *T. gamsii* YIM PH30019 can exert its biocontrol efficacy in hyperosmotic soil.

Dual culture and induced VOCs assay presented effective antagonism of *T. gamsii* YIM PH30019 on test pathogenic fungi associated with root-rot diseases of *P. notoginseng* (Figs. 3–6). Other *T. gamsii* isolates also showed antagonistic activity to phytopathogens [39,40]. The recognition process was thought as the precondition necessary to inhibit the pathogens during the mycoparasitism that happened between *Trichoderma* and pathogens [41,42]. The mycoparasitism process of coiling, which depends on recognition, was also detected in both dual culture and SEM observations in our study.

*Trichoderma* spp. can produce VOCs that inhibit the growth of plant pathogenic fungi via soil air diffusion [43] or induce a defense response in plants [44,45]. The VOC profiles of *Trichoderma* species include alcohols, ketones, alkanes, furanes, pyrones, and terpenes [24], which have varying degrees of antagonistic activity against pathogenic fungi. Cocultivation with pathogenic fungus could significantly enrich the metabolites of *T. harzianum* in comparison to the pure culture [46]. The deactivated pathogenic cell walls could induce *T. harzianum* to produce greater levels of some proteins [47]. *T. gamsii* YIM PH30019 produced VOCs induced in the deactivated cell wall medium. These VOCs also presented antagonistic activity to the phytopathogens. However, the *T. gamsii* antagonism of VOCs induced by pathogenic fungi in the process of biocontrol has been poorly studied. In this study, deactivated cell walls of pathogenic fungi could induce *T. gamsii* YIM PH30019 to produce different VOC profiles, including dimethyl disulfide, dibenzofuran, methanethiol, and ketones. It is noteworthy that dimethyl disulfide is reported to
Fig. 7. The induced volatile organic compounds (VOC) profiles produced by *Trichoderma gamsii* YIM PH30019. VOCs were collected from *T. gamsii* inoculated in four deactivated cell wall broth (DCWB) media and control without the deactivated cell walls, respectively. Peaks of compounds were recorded and are identified in Table 2.
have favorable antagonistic activity against insects and pathogens associated with many important plants and crops [48,49]. Many chemical fumigants are used to control plant disease at present [50], but they could change the biological equilibrium [51], such as eradicating beneficial organisms [52] and increasing pathogen populations [53]. Nonchemical methods that effectively control plant diseases are highly desirable. The results give us insight that the deactivated cell walls of pathogenic fungi could be an effective activator for *T. gamsii* YIM PH30019 to produce more VOCs that inhibit the growth and metabolism of pathogenic fungi. This interesting phenomenon warrants further investigation in ongoing studies.

### 5. Conclusion

*T. gamsii* YIM PH30019, isolated as an endophytic fungus, showed no pathogenicity to the host plant of *P. notoginseng*, and presented favorable biocontrol efficacy *in vitro* and field evaluation with continuous cropping soil. It could grow well in high concentrations of chemical fertilizers, produce a plenty of VOCs to inhibit the growth of pathogenic fungi, and protect notoginseng against infection by phytopathogens. These results indicate that *T. gamsii* YIM PH30019 can be used as a promising biocontrol agent against the phytopathogenic fungi of *P. notoginseng*. In-depth studies should be carried out in different and complex field conditions to evaluate its biocontrol efficacy and influence on indigenous microbial communities, as well as the effect of agro approaches (chemical fertilizers, pesticides, fungicides, etc.) on *T. gamsii* YIM PH30019.

### Table 2

The induced VOCs produced by *Trichoderma gamsii* YIM PH30019

| Treatments                  | Retention time (min) | Peak Volatile compounds |
|-----------------------------|----------------------|-------------------------|
| Deactivated cell walls of *Epicoccum nigrum* | 0.514 | A Methanethiol |
| 2.811 | B Dimethyl disulfide |
| 5.114 | C 2-Hexanone, 4-methyl |
| 8.445 | D 3-Heptanone, 5-ethyl-4-methyl |
| 11.505 | E 2-Undecane |
| 16.725 | F 2-Undecane |
| 22.967 | G Fluorene |

| Deactivated cell walls of *Scytalidium lignicola* | 0.517 | A Methanethiol |
| 2.047 | B Methyl thiocetate |
| 2.799 | C Dimethyl disulfide |
| 5.146 | D Butanethioic acid, 5-methyl ester |
| 8.936 | E 3-Octanone |
| 14.241 | F Benzene, 2-methoxy-4-methyl-1-(1-methylethyl) |
| 16.220 | G 2-Undecanone |

| Deactivated cell walls of *Phoma herbarum* | 6.246 | A 2-Heptanone |
| 8.004 | B 2-Heptanone, 6-methyl-6-methyl- |
| 8.924 | C 3-Octanone |
| 10.107 | D β-Phellandrene |
| 11.783 | E 2-Nonanone |
| 11.996 | F 2-Nonanone |
| 13.452 | G 2-Decanone |
| 14.331 | H 2-Decanone |
| 15.884 | I 2-Undecanone |
| 16.731 | J 2-Undecanone |
| 18.154 | K 2-Dodecanone |
| 21.628 | L Dibenzofuran |
| 22.954 | M Fluorene |

| Deactivated cell walls of *Fusarium flocciferum* | 0.508 | A Methanethiol |
| 1.341 | B Methyl thiocetate |
| 2.797 | C Dimethyl disulfide |
| 8.914 | D 3-Octanone |
| 11.780 | E 2-Undecanone |
| 14.234 | F Benzene, 2-methoxy-4-methyl-1-(1-methylethyl) |
| 20.096 | G Bicyclo[5.2.0]nonane |
| 23.633 | H 5H-Inden-5-one |
| 15.873 | A 2-Undecane |
| 22.954 | B Fluorene |

1) *T. gamsii* YIM PH30019 was inoculated in the DCWB medium.
2) *T. gamsii* YIM PH30019 was inoculated in the above medium without deactivated cell wall.
DCWB, deactivated cell wall broth; VOCs, volatile organic compounds.

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Fig. 8. Average *Panax notoginseng* seedling emergence rates in continuous cropping field. Results are expressed as mean ± SD (n = 3). *Statistically significant differences (Duncan’s multiple range test, p < 0.05). CK, control; SD, standard deviation; Tt, treatment plot with *T. gamsii* YIM PH30019.
Fig. 9. Seedlings mortality until May 31, 2015. Trichoderma gamsii YIM PH30019 was applied to the treatment plots by root-irrigation in the 5th wk. Results are expressed as mean ± SD (n = 3). *Statistically significant differences (Duncan’s multiple range test, p < 0.05). CK, control; SD, standard deviation; Tt, treatment plot with T. gamsii YIM PH30019.

Conflicts of interest
The authors have no conflicts of interest with any parties or individuals.

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Appendix A Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2015.09.006.

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