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Table: 2, Figs : 8

Allelopathic inhibitory effects of *Penicillium griseofulvum* produced patulin on the seed germination of *Orobanche cumana* Wallr. and *Phelipanche aegyptiaca* Pers.

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ABSTRACT

We studied the effects of identified metabolites and the dry residue from *Penicillium griseofulvum* (DRPG) on the seed germinations of *O. cumana* and *Phelipanche aegyptiaca* Pers. (*Orobanche aegyptiaca* Pers.). Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) assays were used to isolate and quantify the chemicals in the culture filtrate of *P. griseofulvum* (CFPG). Results showed that both patulin (a metabolite of *P. griseofulvum*) and the DRPG solutions at 1.0 mg mL^{-1} completely inhibited the seed germination of *O. cumana* and *P. aegyptiaca*. The colour and R_f value of patulin were also similar to the fourth band of dry residue of *P. griseofulvum* in the TLC assay. Moreover, the shape and retention time of patulin peak in the HPLC assay was similar to the main peak of the DRPG. In conclusion, the patulin is the main allelochemical produced from *P. griseofulvum*, which inhibited the seed germination of *O. cumana* and *P. aegyptiaca*.

Key words: Allelopathy, inhibitory effect, *Orobanche cumana* Wallr., patulin, *Penicillium griseofulvum*, *Phelipanche aegyptiaca*, seed germination.

INTRODUCTION

Root parasitic weeds *Orobanche* spp. causes substantial losses in crop yields of many crops (15,30). Two such parasitic weeds (*O. cumana* and *P. aegyptiaca*) severely threatens the growth of sunflower (*Helianthus annuus* L.) and Solanaceae plants, respectively (5,25). In North China [Shaanxi Province, Heilongjiang Province and Inner Mongolia Autonomous Region], *O. cumana* adversely affects the yield and quality of sunflower (3,9,14,36). The *P. aegyptiaca* adversely affects the production of tomato in Xinjiang Uygur Autonomous Region (40). The *Orobanche* spp. is difficult to control due to special life cycle and production of large number of tiny seeds (10). Since most damage to hosts has already been done before the *Orobanche* spp. emergence from the soil, hence, to control them at the seed

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germination stage is the promising control strategy (13,17,25,31).

Seed germination of *Orobanche* spp. is inhibited by the microorganisms [*Fusarium oxysporum* (26), *Myrothecium verrucaria* (11), *Rhizobium leguminosarum* (21), *Pseudomonas fluorescens* (38), arbuscular mycorrhizal fungi (12,18)] and certain soil-borne antagonistic microorganisms (*Streptomyces enissocaesilis* and *Penicillium griseofulvum*) (8). Many mechanisms illustrate the inhibitory effects of microorganisms on parasitic weeds through the production of compounds to inhibit the seed germination of parasitic weeds. Mabrouk *et al.* (20) found that *R. leguminosarum* strains controlled the *Orobanche crenata* through the accumulation of gallic acid and naringenin, which could inhibit the seed germination of *O. crenata* that were induced by GR24 (a synthetic strigol analogue). Results from Miché *et al.* (24) showed that the inhibitory effect of *Azospirillum brasilense* on the seed germination of *Striga hermonthica* induced by GR24 may due to the production of small lipophilic compounds. In addition, verrucarin A, epiepoformin, sphaeropsidin A and cytochalasans, which are metabolites of certain microorganisms also inhibits the seed germination of *Orobanche* spp. (10).

In our previous study, one soilborne fungus (*P. griseofulvum*) severely inhibited the seed germination of *O. cumana*, which was induced either by synthetic germination stimulant (GR24) or sunflower released stimulants (8). However, the allelochemicals produced by *P. griseofulvum* and inhibitory to *O. cumana* and *P. aegyptiaca* are still unknown. This study aimed to extract, isolate and identify the chemical constituents from the culture filtrate of *P. griseofulvum* (CFPG) and to find the inhibitory effects of identified allelochemicals on the seed germination of *O. cumana* and *P. aegyptiaca*.

MATERIALS AND METHODS

This study was done at the Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province, 712100, China (Longitude : 34° 27' N, Latitude 108° 07' E, Mean height above sea level : 497 m, Maximum and minimum temp : 37°C and -13°C, respectively, Annual rainfall : 635 mm).

Source of fungal and plant materials

The fungus *P. griseofulvum* used in this study was provided by Prof. Quanhong Xue, Microbial Resources Laboratory, College of Natural Resources and Environment of our University. *P. griseofulvum* was isolated from the healthy strawberry (*Fragaria ananassa* Duchesne) soil in Shaanxi Province, China and it inhibits the growth of soil-borne phytopathogens (34). Seeds of *Orobanche cumana* were collected from an infected sunflower field, Dingbian County, Shaanxi Province, China in 2010. Seeds of *Phelipemche aegyptiaca* were collected from an infected tomato (*Lycopersicon esculentum* Mill.) field, Yanji County, Uyghur Autonomous Region in 2011. GR24, a synthetic parasitic weed germination stimulant, was obtained from Dr. Christopher McErlean, School of Chemistry, University of Sydney, New South Wales, Australia.

Surface sterilization and preconditioning of *O. cumana* and *P. aegyptiaca*.

Seeds of *O. cumana* and *P. aegyptiaca* were surface sterilized by dipping in 1.0 % (w/v) NaClO solution for 3 min and then in 75% ethanol for 3 min (19). Seeds of *O. cumana* and *P. aegyptiaca* do not germinate unless conditioned under warm and moist conditions for few days. After thorough rinsing and air drying, 30 to 60 seeds were uniformly sown on each glass fibre filter disk (Whatman GF/A, 8 mm dia.), laid on two layers of moistened filter papers placed in Petri dishes (9 cm dia.). These Petri dishes were then sealed and incubated at 25°C in dark for conditioning for 4-days (34).

Germination assay of *O. cumana* and *P. aegyptiaca*

Twenty µL sample solution (culture filtrate of *P. griseofulvum*, solutions of *P. griseofulvum*, metabolites or methanol extracts from TLC prepared below), a pre-conditioned *O. cumana* or *P. aegyptiaca* seed disk and 20 µL GR24 (0.1 mg L^{-1}) were sequentially added to glass fibre disk (8 mm), placed in Petri dish (9 cm dia.). Autoclaved distilled water (20 µL) or GR24 (20 µL, 0.1 mg L^{-1}) were treated separately as the negative or positive controls to check the seeds viability. These Petri dishes were then sealed and incubated at 25°C in dark for 10 days (37). Thereafter, the germination number and total number of *O. cumana* and *P. aegyptiaca* seeds were counted by binocular dissecting microscope. The seed was considered germinated, when the germ tube protruded from the seed coat. Each treatment was replicated thrice in complete random block design. The germination rate and the germination inhibition rate were calculated as under:

Germination rate (GR) = (GN / TN) × 100%

GR: Germination rate of *Orobanche* spp. seeds; GN: Number of germinated *Orobanche* spp. seeds; TN: Total number of *Orobanche* spp. seeds

Germination inhibition rate % (GIR) = [(GC - GT) / GC]× 100%

GIR: Germination inhibition rate over the control; GC: Germination rate of *Orobanche* spp. seeds in the control; GT: Germination rate of *Orobanche* spp. seeds in the treatments

Preparation of culture filtrates and extracts of *P. griseofulvum*

For seed germination assay, the culture filtrate of *P. griseofulvum* (CFPG) was prepared as per Chen *et al.* (8) and four slants were prepared. *P. griseofulvum* was cultured in potato dextrose agar (PDA) medium slants for four days. Then, 3.0 mL autoclaved distilled water was added to each of four test tubes and the spores in the media slants were scraped off. The suspension of spores in each test tube was then transferred to separate 200 mL conical flasks containing 50 mL autoclaved potato dextrose liquid medium. The conical flasks were incubated at 28°C for 7-days at 110 RPM and the suspensions in each conical flask were then vacuum filtered through 0.45 µm millipore filters. The filtered culture filtrate in each conical flask was adjusted with autoclaved distilled water to get an equivalent concentration based on the mycelia dry weight (3.5 mg mL^{-1}). Then the adjusted culture filtrates were diluted to 0.35 mg mL^{-1}, 0.035 mg mL^{-1}, 0.0035 mg mL^{-1} and 0.00035 mg mL^{-1}. Each strain was replicated four times.

For isolation and identification of *P. griseofulvum* metabolites, the CFPG was prepared as per Chen *et al.* (8) with modification. Five mL autoclaved distilled water was added to each test tube (18 cm × 18 mm) and the spores were scraped off from the media slants. The spores suspension was then transferred to separate 500 mL conical flasks containing 250 mL autoclaved potato dextrose liquid medium. The conical flasks were incubated at 28°C for 10-days at 110 RPM and the suspensions (7.5 L) were then vacuum filtered through 0.45 µm millipore filters.

To determine the inhibitory activities of dry residue from *P. griseofulvum* (DRPG) culture filtrate on *Orobanche* and *Phelipanche* seed germination, CFPG were prepared by liquid culture as per Chen e*t al.* (8) with modification. Ten slants were used and the final vacuum-filtrated CFPG was extracted thrice with ethyl acetate (the volume ratios of culture filtrate to ethyl acetate were 1:1, 2:1 and 2:1). Then, all the ethyl acetate phases were mixed together and the anhydrous sodium sulphate was used to remove the excess moisture. After that, the filtered solution was vacuum evaporated to dryness at 35°C. Finally the DRPG was weighed (0.3339 g), dissolved into 4.0 mL methanol and then stored at 4°C until the use.

Inhibitory effects of CFPG on seed germination

In germination assay, the CFPG prepared above were used to test their inhibitory effects on the seed germination of *O. cumana* and *P. aegyptiaca*. The whole experiment was repeated twice.

Extraction, isolation and identification of *P. griseofulvum* metabolites

After fermentation was finished, the filtered CFPG was extracted by petroleum ether (PE) and ethyl acetate to yield 3 g and 13 g extractions, respectively. The PE extraction was chromatographed on three silica gel columns successively. The first column was prepared with a bed volume of Φ 52 × 260 mm and eluted by 3.3 L $CHCl_3$:MeOH 50:1, v/v). The main fraction was subject to the second column (Φ 42 × 160 mm), which was eluted by 1.2 L PE-acetone solvent (5:1, v/v). The main product was further chromatographed on the third silica gel column (Φ 21 × 120 mm) eluted by 0.6 L $CHCl_3$:MeOH (100:1, v/v). Finally, the extract was purified by Sephadex LH-20 (LH-20, 40−70 µm, Amersham Pharmacia Biotech AB, Uppsala, Sweden, (Φ 21 × 1500 mm) eluted with 0.6 L methanol gave the final product (92 mg) which was numbered as chemical compound 1.

The ethyl acetate extract (13 g) was separated into two fractions by silica gel column (Φ 52 × 260 mm) and eluted by 4.0 L $CHCl_3$:MeOH (50:1, v/v). The first fraction was further purified by silica gel column (Φ 21 × 120 mm) with 0.6 L chloroform:methanol (1:1, v/v) and then subjected to Sephadex LH-20 (LH-20, methanol) to give a compound (831 mg) which was numbered as chemical compound 2. The second fraction was recrystallized from methanol (21 mg) and numbered as chemical compound 3. The chemical structures of the compounds 1, 2 and 3 were elucidated using nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR, 500 and 125 MHz NMR instruments, Bruker Daltonics Inc., Bremen, Germany) and mass spectroscopy.

Inhibitory activities of DRPG, patulin and griseofulvin on seed germination

During the preparation of DRPG, the aqueous phase and ethyl acetate phase after each extraction by ethyl acetate and together with the undiluted CFPG were sampled. The inhibitory effects of these sampled solutions on the seed germination of *O. cumana* and *P. aegyptiaca* were tested based on the methods described above. The inhibitory effects of DRPG, the two main secondary metabolites identified in this study (patulin and griseofulvin) from *P. griseofulvum* were tested on the seed germination of *O. cumana* and *P. aegyptiaca*. Patulin used in this study was purchased from Toronto Research Chemicals, Toronto, Canada. It was dissolved in chromatograph grade methanol to make the final concentration of 1.0 mg mL^{-1}. Griseofulvin used in this study was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. It was dissolved in N, N- dimethyl formamide to make the final concentration of 10.0 mg mL^{-1}. Solutions (DRPG, patulin and griseofulvin) were diluted into 1.0 mg mL^{-1}, 0.1 mg mL^{-1} and 0.01 mg mL^{-1} concentrations. The inhibitory effects of these concentrations were determined on the seed germination of *O. cumana* and *P. aegyptiaca*. This experiment was repeated twice.

Thin Layer Chromatography (TLC) assay

Solutions of DRPG (dissolved with methanol to 50.0 mg mL^{-1}), patulin (1.0 mg mL^{-1}) and griseofulvin (10.0 mg mL^{-1}) were used in the TLC assay. Twenty µL DRPG solution (1.0 mg), 20 µL patulin solution (0.02 mg) and 10 µL griseofulvin solution (0.1 mg) were applied to respective TLC silica gel plates (5.0 cm wide, 18.0 cm long, Qingdao Shenghai Chemical Company Ltd of China). Each solution had two TLC silica gel plates. These plates were developed with mixture of ethyl acetate: hexane (7:3, v/v, 50 mL) and then two independent experiments were done.

Experiment 1: One plate of each solution was marked into 1.0 cm horizontal strips without exposure to Ultra Violet (UV) light. The coating on each strip (30 mg silica gel) was scraped and packed into a 1.5 mL centrifuge tube.

Experiment 2: The other plate of each solution was exposed to UV light (254 nm) and each fluorescent band was marked. Then the R_f values of these fluorescent bands were measured and the coating of these fluorescent bands were scraped and packed into a 1.5 mL centrifuge tube, respectively. After adding 1 mL methanol, these tubes from experiment 1 and 2 were sonicated for 20 min and then centrifuged for 1 min at 6000 RPM. The supernatants from experiment 1 were diluted 10-folds with methanol and the supernatants from experiment 2 were diluted two-folds with methanol, respectively. The inhibitory effects of these diluted and undiluted solutions were tested on the seed germination of *O. cumana* and *P. aegyptiaca* as described above. The TLC assay was repeated twice.

High Performance Liquid Chromatography (HPLC) assay

Solutions of DRPG, patulin and griseofulvin were analysed by HPLC (Shimadzu LC-6AD HPLC; system controller: CBM-20A; pump: LC − 6AD / 7A; and detector: SPD-20AV). Sample solutions were filtered through a membrane filter (0.45 µm) prior to injection into the sample loop. Twenty microliter of the DRPG (50.0 mg mL^{-1}, 1 mg), patulin solution (1.0 mg mL^{-1}, 0.02 mg) or griseofulvin (10.0 mg mL^{-1}, 0.2 mg) were loaded on a C

18 reverse phase HPLC column (25 cm × 4.6 mm, 5 μm), respectively. The column was washed with 80% methanol in Mille-Q water at a 0.5 mL min^{-1} flow rate of the mobile phase. UV absorption of the column effluents was monitored at 245 nm. The weight percentage content of patulin in the DRPG solution was calculated by the formula 3.

Formula 3: CD (%) = CS × (AD / AP)

Where, CD (%): Weight percent of patulin in the DRPG solution; CS (%): Weight percent of patulin in patulin solution; AD: Corresponding peak area of patulin in peak spectrum DRPG; AP: The peak area of patulin solution in peak spectrum patulin.

Statistical analysis

A one-way ANOVA was assessed to examine the effects of various treatments on different variables by using the DPS v 9.50 software (DPS Soft Inc., Tang, Hangzhou, China). Treatment means were compared using the least significant difference (LSD) test with a Bonferroni correction at the $P < 0.05$ level. Line figures were drawn by the use of SigmaPlot 10.0 software.

RESULTS AND DISSCUSSION

In all seed germination assays, neither *O. cumana* nor *P. aegyptiaca* germinated when treated with autoclaved distilled water alone.

Inhibitory effect of CFPG on the seed germination of *O. cumana* and *P. aegyptiaca*

The germination rates of *O. cumana* and *P. aegyptiaca* treated by GR24 were 88.7% and 88.8% in this experiment, respectively. The CFPG at 3.5 mg mL^{-1} and 0.35 mg mL^{-1} inhibited the seed germination of *O. cumana* by 100.0% and 98.9% than control, respectively. The inhibitory effects decreased when CFPG was diluted to 0.035 mg mL^{-1}, 0.0035 mg mL^{-1} or 0.00035 mg mL^{-1}. In *P. aegyptiaca*, the CFPG at 3.5 mg mL^{-1} completely inhibited the germination (100.0% inhibition) than control (Figure 1). The microorganisms may be used to control the *Orobanche* spp. (1,4,6,7). Results showed that *P. griseofulvum* (CF3) inhibited the seed germination of *O. cumana* and *P. Aegyptiaca* (Figure 1).

Chemical identification of metabolites from CFPG

Three chemical compounds were isolated from the CFPG and two of them were identified as griseofulvin (compound **1**) and patulin (compound **2**).

Chemical compound 1: White powder; $C_{17}H_{19}ClO_6$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.13 (1 H, s, H-5), 5.52 (1 H, s, H-3′), 4.02 (3 H, s, H-4 OMe), 3.97 (3 H, s, H-6 OMe), 3.60 (3 H, s, H-2′ OMe), 3.01 (1H, dd, J = 16.7 Hz, 13.4 Hz, H-6′), 2.83 (1 H, m, H-5′), 2.41 (1 H, dd, J = 4.7 Hz, 16.7 Hz, H-5′), 0.95 (3 H, d, J = 6.7 Hz, H-6′ Me); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.1 (C-4′), 192.5 (C-3), 170.8 (C-2′), 169.6 (C-6), 164.7 (C-7a), 157.8 (C-4), 105.2 (C-3a), 104.9 (C-3′), 97.3 (C-7), 90.8 (C-1′), 89.6 (C-5), 57.1 (C-4 OMe), 56.7 (C-2′ OMe), 56.5 (C-6 OMe), 40.1 (C-5′), 36.5 (C-6′), 14.3 (C-6 CH_3); MS (ESI^+) m/z 355.18 $[M+H]^+$. These data match the reported data for griseofulvin (16) (Figure 2).

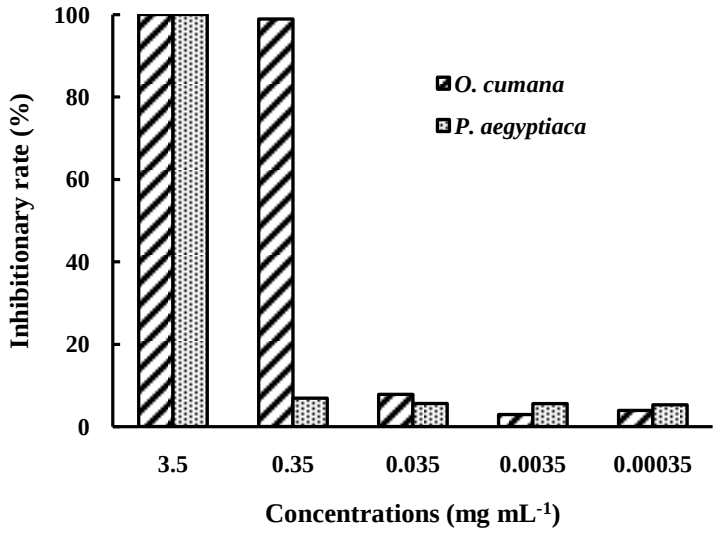
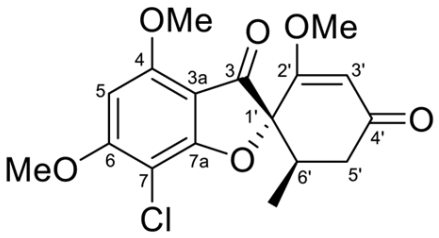


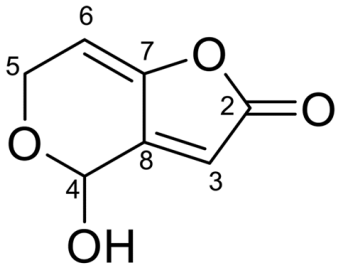
Figure 1. Inhibitory effects of *O. cumana* and *P. aegyptiaca* treated by CFPG. CFPG: culture filtrate of *P. griseofulvum*. Error bar is SD for six replicates (combined results of the two repeated experiments). For *O. cumana* or *P. aegyptiaca*, different lowercase letters indicate significant differences ($P < 0.05$) among treatments based on the LSD test with a Bonferroni correction.



7-chloro-2',4,6-trimethoxy-6'-methyl-3H,4'H-spiro[1-benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione

Figure 2. Chemical structure of compound 1 (griseofulvin) extracted from CFPG.

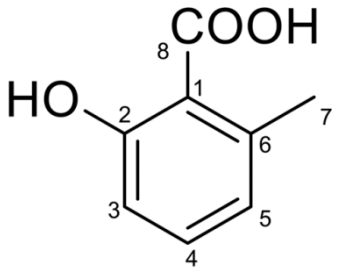
Chemical compound 2: Yellow oily paste; $C_7H_6O_4$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.04 (1 H, s, H-3), 6.01 (1 H, d, J = 0.9 Hz, H-4), 5.95 (1 H, m, H-6), 4.70 (1 H, ddd, J = 17.3, 3.0, 0.7 Hz, H-5a), 4.41 (1 H, ddd, J = 17.3, 3.0, 0.7 Hz, H-5b); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.2 (C-2), 150.3 (C-7), 146.3 (C-8), 111.1 (C-3), 108.1 (C-6), 88.9 (C-4), 59.6 (C-5); MS (ESI^+) m/z 155 $[M+H]^+$. These data match the reported data for patulin (33) (Figure 3).



4 - Hydroxy-4H-furo[3,2-c]pyran-2(6H)-one
Expansine Clavatin

Figure 3. Chemical structure of compound 2 (patulin) extracted from CFPG

Chemical compound 3: Colorless crystal; $C_8H_8O_3$; 1H-NMR (500 MHz, Acetone-d6) δ: 7.31 (1 H, t, J = 7.9 Hz, H-4), 6.77 (2 H, m, H-3, H-5), 2.56 (3 H, s, H-7); 13C-NMR (125 MHz, Acetone-d6) δ: 174.0 (C-8), 163.9 (C-2), 142.4 (C-6), 134.9 (C-4), 123.4 (C-5), 116.0 (C-3), 113.3 (C-1), 23.8 (C-7); MS (ESI+) m/z 153 [M+H]+. These data match the reported data for 2-hydroxy-6-methylbenzoic acid (23) (Figure 4).



2-hydroxy-6-methylbenzoic acid

Figure 4. Chemical structure of compound 3 extracted from CFPG

SEED GERMINATION OF *O. CUMANA* AND *P. AEGYPTIACA*

(i). Inhibitory effects of aqueous phases and ethyl acetate phases of *P. griseofulvum*

The germination rate of *O. cumana* induced by GR24 was 90.7% in this experiment. After three times extraction, most active materials that could inhibit the seed germination of *Orobanche* spp. were transferred to ethyl acetate phase. The inhibition rate of *O. cumana* germination treated by the undiluted aqueous phase solutions were dropped from 100% (in first extraction) to 33% (in third extraction). The undiluted ethyl acetate phase solutions (after the first and second extraction) completely inhibited (100% inhibition) the seed germination of *O. cumana*. But after the third extraction, the inhibition dropped to 6.3%. In 10-folds dilution, the aqueous phases after the first extraction reduced the seed germination

of *O. cumana* by 17.2% than control and there were no inhibitory effects after the second and third extraction. However, the 10-folds ethyl acetate phase solutions significantly reduced the seed germination of *O. cumana* after the first (94.9% inhibition) and second extraction (62.1% inhibition). For 100-folds dilutions, the aqueous phase solution reduced the seed germination of *O. cumana* only after the first extraction (by 9.1%) and the ethyl acetate phase solutions inhibited the seed germination of *O. cumana* both after the first (by 10.6%) and second (by 9.9%) extraction. Besides, the undiluted and 10-folds diluted mixture solutions of ethyl acetate phase reduced the seed germination of *O. cumana* by 100% ($P < 0.05$) and 65.4% ($P < 0.05$), respectively than control (Figure 5).

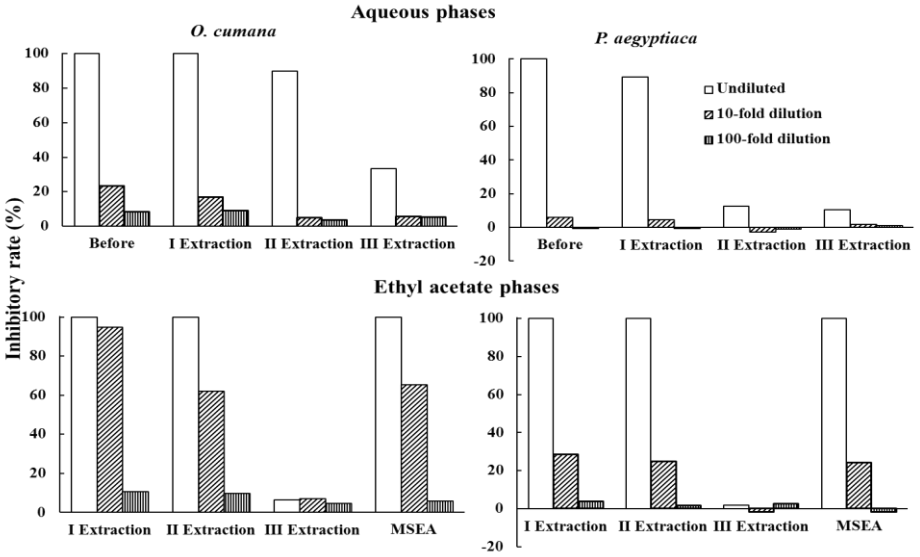


Figure 5. Inhibitory effects of aqueous phases and ethyl acetate phases of *P. griseofulvum* on the seed germination of *O. cumana* and *P. aegyptiaca*. MSEA: Mixture of the sampled ethyl acetate phases

The germination rate of *P. aegyptiaca* induced by GR24 was 90.5% in this experiment. Before extraction, the undiluted CFPG completely inhibited the seed germination of *P. aegyptiaca*. The undiluted aqueous phase solutions after the first, second and third extraction reduced the seed germination of *P. aegyptiaca* by 89.3% ($P < 0.05$), 12.5% ($P < 0.05$) and 10.4% ($P < 0.05$), respectively, relative to control. However, there were no inhibitory effects of 10-folds or 100-folds dilutions. For ethyl acetate phases, the undiluted solutions after the first and second extraction completely inhibited the seed germination of *P. aegyptiaca*, yet the inhibitory effect disappeared after the third extraction. The 10-folds ethyl acetate solutions after the first and second extraction inhibited the seed germination of *P. aegyptiaca* by 28.6% and 24.7%, respectively. In addition, the undiluted mixture of ethyl acetate phases also completely inhibited the seed germination of *P. aegyptiaca*. After

diluting 100-folds, none of the ethyl acetate phase solutions affected the seed germination of *P. aegyptiaca* significantly (Figure 5).

(ii). **DRPG, patulin and griseofulvin solutions**

The germination rate of *O. cumana* treated by GR24 was 89.6%. The DRPG reduced the germination of *O. cumana* by 100.0% and 29.9% at 1.0 mg mL^{-1} and 0.1 mg mL^{-1} concentrations, respectively. Patulin completely inhibited (100.0% inhibition) the seed germination of *O. cumana* at 1.0 mg mL^{-1} and 0.1 mg mL^{-1} concentrations and 11.6% inhibition at 0.01 mg mL^{-1} concentration. However, griseofulvin at 1.0 mg mL^{-1} concentration caused 12.7% inhibition on the seed germination of *O. cumana*.

The germination rate of *P. aegyptiaca* treated by GR24 was 91.7%. DRPG and patulin solutions completely inhibited the seed germination of *P. aegyptiaca* at 1.0 mg mL^{-1} concentrations. Patulin at 0.1 mg mL^{-1} concentration inhibited the seed germination of *P. aegyptiaca* by 10.8% than control. However, griseofulvin solutions did not inhibit the seed germination of *P. aegyptiaca* at three tested concentrations (Figure 6).

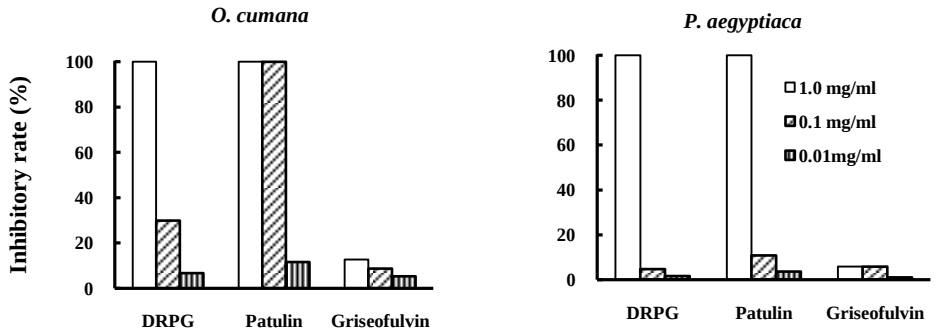


Figure 6. Inhibitory effects of the DRPG, patulin and griseofulvin solutions on the seed germination of *O. cumana* and *P. aegyptiaca*. DRPG: Dry residue of *P. griseofulvum*

It was reported that metabolites (*epi*-Epoformin, sphaeropsidin A, cytochalasans, gallic acid and naringenin) produced by certain biocontrol microorganisms could inhibit the seed germination of *Orobanche* spp. (10,20). In this study, three metabolites were isolated from CFPG. Two main metabolites were identified as patulin and griseofulvin, and the patulin proved very inhibitory to the seed germination of *O. cumana* and *P. aegyptiaca*.The DRPG and patulin solutions inhibited the seed germination of *O. cumana* and *P. aegyptiaca*, the inhibition magnitude of these solutions to *O. cumana* and *P. aegyptiaca* were different. Seeds of *O. cumana* treated by these solutions above were less likely to germinate than that of the *P. aegyptiaca*. Hence it was proposed that *P. griseofulvum* is more suitable to control the *O. cumana* than *P. aegyptiaca*.

TLC ASSAY

The DRPG solutions of number 9 and 10 samples at 30 mg mL^{-1} completely inhibited (100% inhibition) the seed germination of *O. cumana* and by 91.5% inhibition, respectively

than control (Table 1). These two solutions at 30 mg mL^{-1} also inhibited the seed germination of *O. cumana* by 35.5% and 33.3% than control. However, there was no

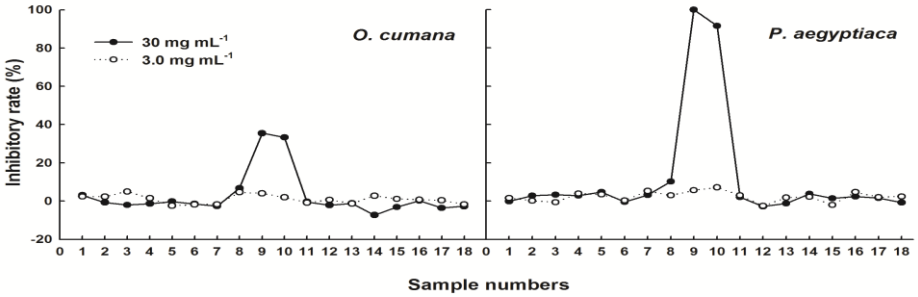


Figure 7. Inhibitory effects of DRPG at TLC plates on the seed germination of *O. cumana* and *P. aegyptiaca*. Error bar is SD for eight replicates (combined results of the two repeated experiments). DRPG: dry residue of *P. griseofulvum*

Table 1. Inhibitory effects of patulin and griseofulvin on the seed germination of *O. cumana* and *P. aegyptiaca* under TLC assay

Strip number	Patulin (1.0 mg mL^{-1})				Griseofulvin (10.0 mg mL^{-1})			
	O. cumana		*P. aegyptiaca*		*O. cumana*		*P. aegyptiaca*	
	GR [a] %	PR [b] %	GR%	PR%	GR%	PR%	GR%	PR%
Control (GR24)	87.6 ± 3.5 a	-	89.7 ± 4.4 a	-	87.6 ± 3.5 a	-	89.7 ± 4.4 ab	-
1	85.8 ± 6.0 a	4.3	91.0 ± 4.3 a	-1.5	84.3 ± 4.7 a	3.7	92.1 ± 2.8 a	-2.7
2	84.9 ± 5.8 a	5.4	91.3 ± 2.2 a	-1.8	85.0 ± 8.2 a	3.0	89.3 ± 4.9 ab	0.4
3	86.6 ± 3.7 a	3.4	94.3 ± 3.6 a	-5.1	86.4 ± 5.4 a	1.4	93.1 ± 2.6 a	-3.8
4	86.6 ± 5.8 a	3.4	91.6 ± 4.4 a	-2.1	83.9 ± 6.4 a	4.3	91.3 ± 4.9 ab	-1.8
5	85.1 ± 4.2 a	5.1	89.6 ± 3.9 a	0.1	86.2 ± 4.0 a	1.6	90.9 ± 6.4 ab	-1.3
6	86.9 ± 4.9 a	3.2	93.5 ± 5.4 a	-4.2	84.5 ± 5.4 a	3.6	89.7 ± 4.0 ab	0.0
7	88.3 ± 8.3 a	1.5	93.0 ± 2.8 a	-3.7	86.1 ± 5.7 a	1.7	84.2 ± 9.1 b	6.1
8	88.2 ± 7.0 a	1.7	87.1 ± 7.8 a	2.9	88.0 ± 4.6 a	-0.5	89.7 ± 4.6 ab	0.0
9	87.0 ± 8.5 a	3.0	89.6 ± 5.6 a	0.1	84.5 ± 4.4 a	3.5	91.7 ± 3.9 a	-2.2
10	85.3 ± 3.3 a	4.9	93.5 ± 3.6 a	-4.2	86.7 ± 6.9 a	1.0	92.5 ± 5.6 a	-3.2
11	87.7 ± 7.3 a	2.3	88.5 ± 5.8 a	1.3	86.1 ± 4.9 a	1.7	92.5 ± 3.5 a	-3.1
12	86.4 ± 3.8 a	3.7	91.3 ± 6.1 a	-1.8	87.3 ± 6.7 a	0.4	93.0 ± 2.9 a	-3.7
13	86.3 ± 2.7 a	3.7	90.3 ± 4.0 a	-0.6	90.9 ± 5.6 a	-3.8	88.9 ± 4.7 ab	0.9
14	83.8 ± 5.7 a	6.5	91.9 ± 4.5 a	-2.5	87.8 ± 2.5 a	-0.2	92.9 ± 4.2 a	-3.6
15	84.0 ± 4.9 a	6.4	92.0 ± 6.1 a	-2.6	84.9 ± 4.7 a	3.1	92.6 ± 3.7 a	-3.2
16	82.9 ± 5.0 a	7.6	89.5 ± 3.8 a	0.2	85.7 ± 5.5 a	2.2	91.1 ± 3.9 ab	-1.6
17	87.3 ± 3.0 a	2.7	89.8 ± 4.7 a	-0.1	88.8 ± 5.9 a	-1.4	90.5 ± 4.8 ab	-0.9
18	84.8 ± 4.0 a	5.5	93.0 ± 4.6 a	-3.7	85.0 ± 4.5 a	3.0	89.9 ± 4.9 ab	-0.2

[a] GR: Germination rate. [b] PR: Percent reduction over the control. Data are represented as mean ± SD (n = 8, combined results of two repeated experiments). Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$) based on the least significant difference (LSD) test with a Bonferroni correction.

inhibition at 3 mg mL^{-1} concentration. The other samples of DRPG at 30 mg mL^{-1} or 3 mg mL^{-1} did not affect the seed germination of *O. cumana* or *P. aegyptiaca* (Figure 7). Likewise

the samples of patulin and griseofulvin also did not affect the seed germination of *O. cumana* or *P. aegyptiaca* (Table 1).

TLC assay indicated that the DRPG showed an active inhibition zone for the seed germination of *O. cumana* and *P. aegyptiaca* (Table 2). Among the four fluorescent bands of DRPG, only the fourth band inhibited the seed germination of *O. cumana* and *P. aegyptiaca* significantly. The undiluted solution of this band completely inhibited the seed germination of *O. cumana* and *P. aegyptiaca* . Its two-folds dilution also reduced the seed germination of *O. cumana* and *P. aegyptiaca* by 100.0% ($P < 0.05$) and 52.5% ($P < 0.05$), respectively, than control. Similarly, the undiluted fluorescent band solution of patulin inhibited the seed germination of *O. cumana* and *P. aegyptiaca* by 46.0% and 31.7% over the control and the two-folds diluted solution reduced the seed germination of *O. cumana* by 11.0% ($P < 0.05$). However, the fluorescent band solution of griseofulvin had negligible effects on the seed germination of *O. cumana* or *P. aegyptiaca* (Table 2).

Table 2**.** Inhibitory effect of the fluorescent bands of DRPG, patulin and griseofulvin on the seed germination of *Orobanche* spp. under TLC assay

Samples	Bands	R_f value	*O. cumana*				*P. aegyptiaca*			
			Un-diluted		Two-folds dilution		Un-diluted		Two-folds dilution	
			GR [a] (%)	IR [b] (%)	GR (%)	IR (%)	GR (%)	IR (%)	GR(%)	IR (%)
Control (GR24)			87.3 ± 3.6 a	-	87.3 ± 3.6 a	-	90.9 ± 5.2 a	-	90.9 ± 5.2 a	-
DRPG [c]	1	0.26	83.9 ± 5.3 a	3.9	86.0 ± 4.1 a	1.5	91.5 ± 2.2 a	-0.6	89.9 ± 3.0 a	1.2
	2	0.32	84.0 ± 2.3 a	3.8	84.6 ± 5.9 a	3.1	91.6 ± 3.7 a	-0.8	90.3 ± 3.0 a	0.6
	3	0.39	87.4 ± 7.5 a	-0.1	84.3 ± 6.1 ab	3.5	89.0 ± 9.7 a	2.1	92.6 ± 4.0 a	-1.9
	4	0.48	0.0 ± 0.0 c	100.0	0.0 ± 0.0 c	100.0	0.0 ± 0.0 c	100.0	43.2 ± 17.0 b	52.5
Patulin	1	0.47	47.2 ± 4.5 b	46.0	77.7 ± 5.2 b	11.0	62.1 ± 5.0 b	31.7	87.0 ± 4.5 a	4.3
Griseofulvin	1	0.34	82.0 ± 5.1 a	6.1	85.6 ± 6.2 a	2.0	88.6 ± 3.0 a	2.5	89.0 ± 6.6 a	2.0

[a] GR: Germination rate. [b] IR: Inhibition rate of germination relative to control. [c] DRPG: Dry residue of *P. griseofulvum*. Data are represented as mean ± SD (n = 8, combined results of the two repeated experiments). Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$) based on the LSD test with a Bonferroni correction

When the DRPG was exposed to UV light (254 nm), four fluorescent bands were shown. The fourth fluorescent band of DRPG contained the number 9 and 10 samples in Figure 7. The R_f value of this band was 0.48, the most closer to patulin fluorescent band (R_f = 0.47). The R_f value of griseofulvin fluorescent band was 0.34 which was closer to the second fluorescent band of DRPG (R_f = 0.32) (Table 2). In addition, the colour of patulin fluorescent band under 254 nm UV light was black which was similar to fourth fluorescent band of DRPG and the colour of griseofulvin fluorescent band under 365 nm UV light was blue which was same like the second fluorescent band of DRPG.

The TLC assay showed that only the samples 9 and 10 (fourth fluorescent band) of

the DRPG inhibited the seed germination of *O. cumana* and *P. aegyptiaca*. Furthermore, the colour and R_f values of the fluorescent band of patulin were similar to the fourth fluorescent band of the DRPG. Hence, it was concluded that patulin was the main metabolite contributing to the allelopathic inhibitory effect of *P. griseofulvum* on the seed germination of *O. cumana* and *P. aegyptiaca*. Although the inhibitory effects of the third metabolite (2-hydroxy-6-methylbenzoic acid) of *P. griseofulvum* were not tested on the seed germination of *O. cumana* or *P. aegyptiaca*we, but it was speculated that this third metabolite had no or negligible effect on the seed germination of these test species.

HPLC ASSAY

Four peaks were observed in the HPLC profile of DRPG. One main peak was observed in the HPLC profile of patulin and the retention time was 3.387 min, which was very close to the first peak of the DRPG (3.364 min). Two main peaks were exhibited in the HPLC profile of griseofulvin solution, the first peak was N, N- dimethyl formamide (the solvent used to dissolve griseofulvin) in preliminary experiment, thus the second peak was considered as griseofulvin. The retention time of the second peak of the griseofulvin solution was 4.593 min which was close to that of the third peak of the DRPG (4.252 min) (Figure 8). By weight the patulin (%) in DRPG was 33.46% based on the HPLC profiles of patulin and DRPG. The DRPG solutions reduced the seed germination of *O. cumana* by 100.0% and 29.9% at 1 mg mL^{-1} and 0.1 mg mL^{-1}(equal to patulin at 0.335 mg mL^{-1} and 0.034 mg mL^{-1}), respectively. In addition, the patulin solutions at 1.0 mg mL^{-1}, 0.1 mg $mL^{-1}$and 0.01 mg mL^{-1}inhibited the seed germination of *O. cumana* by 100.0%, 100.0% and 11.6%, respectively. It was concluded that the inhibitory effects of patulin on the seed germination of *O. cumana* depended on the change of patulin concentration, not only in patulin solutions but also in the DRPG solutions. Thus, patulin mainly contributed to the allelopathic inhibitory effects of *P. griseofulvum* on the seed germination of *O. cumana* and *P. aegyptiaca*.

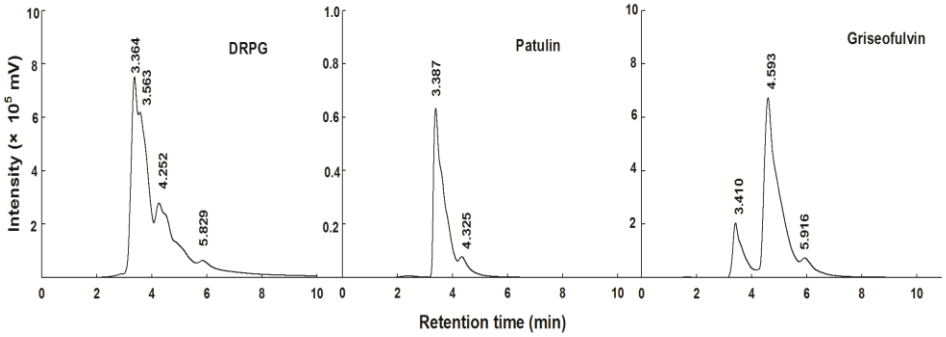


Figure 8. HPLC profile of DRPG, patulin and griseofulvin. DRPG: dry residue of *P. griseofulvum*

Patulin is usually found in fruit products or rotten fruits and known as mycotoxin (22,32). *In-vitro*, it also inhibits the growth of some plant pathogenic fungi [*Saccharomyces*

cerevisiae (35), *Rhizoctonia solani* (28), *Botrytis cinerea* and *Pythium ultimum* (39)]. The fungus *P. griseofulvum* was isolated from the rhizosphere soil of healthy strawberry plant, it inhibited the growth of soil-borne pathogens and also promotes the plant growth (34). *P. griseofulvum* may be applied to the soil to control the *Orobanche* spp. Until now, there is no evidence that patulin transfer from the soil, through the plants to humans or animals can be harmful to the human health. Besides the griseofulvin, another metabolite of *P. griseofulvum* is common antibiotic for plant pathogens (2,27,29), hence, the release of griseofulvin to the soil might be good for the crops health. All above indicate the feasibility of using *P. griseofulvum* to control the *Orobanche* and *Phelipanche* spp.

Bio-pesticide had drawn more attention recently as it is harmless to environment and human health. In addition to direct application of *P. griseofulvum* into the soil, the active metabolite of *P. griseofulvum*, patulin, could also be produced industrially and applied to the soil as chemical herbicide to control *Orobanche* spp. However, the patulin stability in soil, its harmfulness to crops and the application dose still need further researches.

CONCLUSIONS

Patulin and griseofulvin were isolated and identified as the main metabolites from CFPG (culture filtrate of *P. griseofulvum*). These two metabolites inhibited the seed germination of *O. cumana* and *P. aegyptiaca*. Both patulin and the DRPG solutions at 1.0 mg mL^{-1} completely inhibited the seed germination of *O. cumana* and *P. aegyptiaca*. In TLC assay, only the fourth band of DRPG significantly inhibited the seed germination of *O. cumana* and *P. aegyptiaca*. The colour and R_f value of patulin were similar to the fourth band of DRPG in the TLC assay. The shape and retention time of patulin peak in the HPLC assay was similar to the main peak of the DRPG. Therefore, patulin is the main allelochemical from *P. griseofulvum* which is responsible for the inhibitory effects on the seed germination of *O. cumana* and *P. aegyptiaca*.

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REFERENCES

1. Aybeke, M., Şen, B. and Ökten, S. (2014). *Aspergillus alliaceus*, a new potential biological control of the root parasitic weed *Orobanche*. *Journal of Basic Microbiology* **54**: S93-S101.
2. Tang, H.Y., Zhang, Q., Li, H. and Gao, J.M. (2015). Antimicrobial and allelopathic metabolites produced by *Penicillium brasilianum*. *Natural Products Research* **29**: 345-348.
3. Bai, Q.J., Yun, X.P., Gao, Z.M., Xu, L.M., Li, Y.M., Zhang, H.L., Du, L. and Li, X.S. (2013). The occurrence and control techniques of sunflower broomrapes in Inner Mongolia. *Inner Mongolia Agricultural Science and Technology* **1**: 75-76. (Chinese).

4. Barghouthi, S. and Salman, M. (2010). Bacterial inhibition of *Orobanche aegyptiaca* and *Orobanche cernua* radical elongation, *Biocontrol Science and Technology* **20**: 423-435.
5. Barker, E., Press, M., Scholes, J. and Quick, W. (1996). Interactions between the parasitic angiosperm *Orobanche aegyptiaca* and its tomato host: growth and biomass allocation. *New Phytologist* **133**: 637-642.
6. Boari, A., Ciasca, B., Pineda-Martos, R., Lattanzio, V.M., Yoneyama, K. and Vurro, M. (2016). Parasitic weed management by using strigolactones-degrading fungi. *Pest Management Science* doi:10.1002/ps.4226.
7. Bouraoui, M., Abbes, Z., Rouissi, M., Abdi, N., Hemissi, I., Kouki, S. and Sifi, B. (2016). Effects of rhizobia inoculation, N and P supply on *Orobanche foetida* parasitising faba bean (*Vicia faba* minor) under field conditions. *Biocontrol Science and Technology* **26**: 776-791.
8. Chen, J., Xue, Q.H., McErlean, C.S.P., Zhi, J.H., Ma, Y.Q., Jia, X.T., Zhang, M. and Ye, X.X. (2016) Biocontrol potential of the antagonistic microorganism *Streptomyces enissocaesilis* against *Orobanche cumana*. *BioControl* **61**: 781-791.
9. Chen, X.F. (2010). A comprehensive treatment and the reason of sunflower broomrape occurring in Dingbian County. *Modern Agricultural Sciences* **15**: 215-217. (Chinese)
10. Cimmino, A., Fernández-Aparicio, M., Andolfi, A., Basso, S., Rubiales, D. and Evidente, A. (2014). Effects of fungal and plant metabolites on broomrapes (*Orobanche* and *Phelipanche* spp.) seed germination and radicle growth. *Journal of Agricultural and Food Chemistry* **62**: 10485-10492.
11. El-Kassas, R., El-Din, Z.K., Beale, M.H., Ward, J.L. and Strange, R.N. (2005) Bioassay-led isolation of *Myrothecium verrucaria* and verrucarin A as germination inhibitors of *Orobanche crenata*. *Weed Research* **45**: 212-219.
12. Fernandez-Aparicio, M., Garcia-Garrido, J.M., Ocampo, J.A. and Rubiales, D. (2010). Colonisation of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanche* and *Phelipanche* species seed germination. *Weed Resarch* **50**: 262-268.
13. Fernandez-Aparicio, M., Reboud, X. and Gibot-Leclerc, S. (2016). Broomrape weeds. Underground mechanisms of parasitism and associated strategies for their control: A Review. *Frontiers in Plant Science* **7**: 135.
14. Imerovski, I., Dimitrijevic, A., Miladinovic, D., Dedic, B., Jocic, S., Tubic, N.K. and Cvejic, S. (2016). Mapping of a new gene for resistance to broomrape races higher than F. *Euphytica* **209**: 281-289.
15. Joel, D.M. (2000). The long-term approach to parasitic weeds control: Manipulation of specific developmental mechanisms of the parasite. *Crop Protection* **19**: 753-758.
16. Levine, S.G., Hicks, R.E., Gottlieb, H.E. and Wenkert, E. (1975). Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. XXX. griseofulvin. *The Journal of Organic Chemistry* **40**: 2540-2542.
17. Lopez-Raez, J.A., Matusova, R., Cardoso, C., Jamil, M., Charnikhova, T., Kohlen, W., Ruyter-Spira, C., Verstappen, F. and Bouwmeester, H. (2009). Strigolactones: ecological significance and use as a target for parasitic plant control. *Pest Management. Science* **65**: 471-477.
18. Louarn, J., Carbonne, F., Delavault, P., Becard, G. and Rochange, S. (2007). Reduced germination of *Orobanche cumana* seeds in the presence of arbuscular mycorrhizal fungi or their exudates. *PLoS One* **7**: e49273.
19. Ma, Y.Q., Jia, J.N., An, Y., Wang, Z. and Mao, J.C. (2013). Potential of some hybrid maize lines to induce germination of sunflower broomrape. *Crop Science* **53**: 260-270.
20. Mabrouk, Y., Simier, P., Arfaoui, A., Sifi, B., Delavault, P., Zourgui, L. and Belhadj, O. (2007). Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with *Orobanche crenata*. *Journal of Phytopathology* **155**: 728-734.
21. Mabrouk, Y., Zourgui, L., Sifi, B., Delavault, P., Simier, P. and Belhadj, O. (2007). Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitised by *Orobanche crenata*. *Weed Research* **47**: 44-53.
22. Mahfoud, R., Maresca, M., Garmy, N. and Fantini, J. (2002). The mycotoxin patulin alters the barrier function of the intestinal epithelium: mechanism of action of the toxin and protective effects of glutathione. *Toxicology and Applied Pharmacology* **181**: 209-218.
23. Mandal, S.K. and Roy, S.C. (2008). Titanocene (III) chloride mediated radical-induced synthesis of 3, 4-dihydroisocoumarins: synthesis of (±)-hydrangenol, (±)-phyllodulcin,(±)-macrophyllol and (±) -thunberginol G. *Tetrahedron* **64**: 11050-11057.

24. Miché, L., Bouillant, M.L., Rohr, R., Sallé, G. and Bally, R. (2000). Physiological and cytological studies on the inhibition of *Striga* seed germination by the plant growth-promoting bacterium *Azospirillum brasilense*. *European Journal of Plant Pathology* **106**: 347-351.
25. Molinero-Ruiz, L., Delavault, P., Pérez-Vich, B., Pacureanu-Joita, M., Bulos, M., Altieri, E. and Domínguez, J. (2015). History of the race structure of *Orobanche cumana* and the breeding of sunflower for resistance to this parasitic weed: A review. *Spanish Journal of Agricultural Research* **13**: e10R01.
26. Muller-Stover, D., Kohlschmid, E. and Sauerborn, J. (2009). A novel strain of *Fusarium oxysporum* from Germany and its potential for biocontrol of *Orobanche ramosa*. *Weed Resarch* **49**: 175-182.
27. Napier, E.J., Turner, D.I. and Rhodes, A. (1956). The *in vitro* action of griseofulvin against pathogenic fungi of plants. *Annals of Botany* **20**: 461-466.
28. Nicoletti, R., De Stefano, M., S., De Stefano, S., Trincone, A. and Marziano, F. (2004). Antagonism against *Rhizoctonia solani* and fungitoxic metabolite production by some *Penicillium* isolates. *Mycopathologia* **158**: 465-474.
29. Park, J.H., Choi, G., Lee, H.B., Kim, K., Jung, H.S., Lee, S.W., Jang, K., Cho, K. and Kim, J.C. (2005). Griseofulvin from *Xylaria* sp. strain F0010, an endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. *Journal of Microbiology and Biotechnology* **15**: 112-117.
30. Parker, C. (2009). Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Management Science* **65**: 453-459.
31. Pérez-Bueno, M.L., Barón, M., García-Carneros, A.B. and Molinero-Ruiz, L. (2014). Diagnosis of the infection of sunflower by *Orobanche cumana* using multicolour fluorescence imaging. *Helia* **37**: 173-179.
32. Ritieni, A. (2003). Patulin in Italian commercial apple products. *Journal of Agricultural and Food Chemistry* **51**: 6086-6090.
33. Seijas, J., Pilar, V.T.M., Estévez, R., Castedo, L. and Riguera, R. (1989). New total synthesis of patulin. *Heterocycles* **29**:181-184.
34. Shen, G.H., Xue, Q.H., Zhang, J., Duan, J.L., Wang, D.S. and Yang, X.H. (2012). Screening, identification and biocontrol potential of antagonistic fungi against strawberry root rot and plant growth promotion. *Scientia Agricultura Sinica* **45**: 4612-4626. (Chinese)
35. Sumbu, Z.L., Thonart, P. and Bechet, J. (1983). Action of patulin on a yeast. *Applied and Environmental Microbiology* **45**:110-115.
36. Wang, W.J. (2010). Identification of resistance to *Orobanche* and high resistant germplasm selection of Heilongjiang sunflower resource. *Heilongjiang Agricultural Sciences* **9**: 39-41. (Chinese)
37. Ye, X.X., Zhang, M., Dong, S.Q. and Ma, Y.Q. (2016). Conditioning duration and agents involved in broomrape seeds responding to germination stimulants. *Plant Growth Regulation*. doi: 10.1007/s10725-016-0199-2
38. Zermane, N., Souissi, T., Kroschel, J. and Sikora, R. (2007). Biocontrol of broomrape (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.) by *Pseudomonas fluorescens* isolate Bf7-9 from the faba bean rhizosphere. *Biocontrol Science and Technology* **17**: 483-497.
39. Zhang, C.L., Zheng, B.Q., Lao, J.P., Mao, L.J., Chen, S.Y., Kubicek, C.P. and Lin, F.C. (2008). Clavatol and patulin formation as the antagonistic principle of *Aspergillus clavatonanicus*, an endophytic fungus of *Taxus mairei*. *Applied Microbiology and Biotechnology* **78**: 833-840.
40. Zhang, X.K., Yao, Z.Q., Zhao, S.F., Ding, L.L. and Du, J. (2012). Distribution, harmfulness and its assessment of *Orobanche aegyptiaca* in the Xinjiang Uygur Autonomous Region. *Plant Quarantine* **26**: 31-33. (Chinese)