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Original Article

Morphological study of *Gelasinospora* from dung and antagonistic effect against plant pathogenic fungi *in vitro*

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ABSTRACT

Animal dung samples were collected from Surin and Suphan Buri provinces, Thailand. The alcohol treatment technique was used for fungal isolation. Identification of the genus *Gelasinospora* was based on morphological characteristics of ascomata, asci and ascospore ornamentation when grown on potato dextrose agar. Microscopic features were examined under stereo and compound microscopes and ascospores were observed using a scanning electron microscope. Four species of *Gelasinospora* were recorded: *G. calospora*, *G. hippopotama*, *G. indica* and *G. stellata*. The generic diagnostic description of each species was recorded. The species *G. hippopotama* and *G. stellata*, which were isolated from cow and buffalo dung are new records for Thailand. The *in vitro* antagonistic activity tests were conducted using isolates of each of the four species of *Gelasinospora* against seven genera of plant pathogenic fungi. All isolates of *Gelasinospora* inhibited 100% on the mycelial growth of *Phytophthora palmivora* and also inhibited more than 75% of the mycelial growth of *Alternaria alternata*, *Colletotrichum capsici* and *Curvularia lunata*. All isolates failed to inhibit the mycelial growth of *Rhizoctonia oryzae* and *Sclerotium rolfsii*, except for *G. hippopotama* KUFC6898, which inhibited 75.5% of the mycelial growth of *R. oryzae*.

Introduction

The genus *Gelasinospora* belongs to the family Sordariaceae of the Ascomycota. The important and well-known genera in this family are the *Gelasinospora*, *Neurospora* and *Sordaria* and these genera differ in the ornamentation of the ascospore wall (von Arx, 1982; Krug et al., 1994; Guarro et al., 2012). The Sordariaceous species have been used extensively as model organisms in various biological, biochemical, ecological, genetic and evolutionary studies (Cai et al., 2006; Guarro et al., 2012; Kruys et al., 2015). Most species of the *Gelasinospora* can be isolated most frequently from soil and dung in tropical and subtropical regions (Horie and Udagawa, 1974; Khan and Krug, 1989; Domsch et al., 1993; Manoch et al., 1999; Jeamjitt et al., 2007; Basumatary and McDonald, 2017). Several species of *Gelasinospora* produce a number of secondary metabolites, which are of potential use as anticancer pharmaceuticals (Fujimoto et al., 1998). This genus can produce immunosuppressive components including kobiin, kobifuranones A, B and C, and multiforisins A, B, C, D and E; which was demonstrated in a mouse spleen lymphocytes system (Fujimoto et al., 1998; 1999; 2006). Thus, the genus *Gelasinospora* has many interesting properties. The aim of the present study was to investigate the morphology of *Gelasinospora* species isolated from animal dung samples and to test the antagonistic activity against seven genera of plant pathogenic fungi *in vitro*.

Materials and methods

Sampling and fungal isolation

Animal dung samples were collected from Surin province, Northeast Thailand and for Suphan Buri province, Central Thailand. The alcohol treatment technique was used to isolate fungi from the dung samples. This technique was undertaken to stimulate ascospore germination on agar media (Warcup and Baker, 1963). Each 1 g sample was soaked in 9 mL of 65% ethyl alcohol in a test tube and incubated at 25–30°C for 15 min. Samples were mixed thoroughly and then spread onto Gochenaur's glucose ammonium nitrate agar (1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.03 g rose Bengal, 1 g yeast extract, 5 g glucose, 15 g agar, 4 mL streptomycin solution, 1 L distilled water) in a Petri dish (Gochenaur, 1964). The plates were incubated at 25–30°C for 3 d in the dark. Hyphal tips were then selected and inoculated onto slant potato dextrose agar

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(Merck; Darmstadt, Germany) and kept as pure cultures for identification. Pure cultures of *Gelasinospora* and dry specimens of dung samples have been deposited at the Kasetsart Fungal Culture Collection (KUFC) in the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok.

Identification of fungal isolates

Identification of the fungal isolates was based on morphological characteristics. The isolates were cultured on potato dextrose agar (PDA). Five replicate plates of each isolate were incubated at 25–30°C for 14 d. Macroscopic features were recorded such as colony diameter, colony color, presence of exudates, soluble pigment production and presence of ascomata. Microscopic features were examined such as the morphological characteristics of the ascomata, asci, and ascospores. A fine mounted needle was used to pick ascomata, which were placed on slides using sterile distilled water as the mounting medium and then examined under a stereo microscope (Olympus; Tokyo, Japan) and compound microscope (Carl Zeiss; Jena, Germany) and compared with species descriptions in identification keys. The ascospores were observed also using a scanning electron microscope (JSM–5600LV; JEOL Ltd; Tokyo, Japan), with air-dried ascospores being mounted on aluminum stubs using carbon tape, coated with gold and then examined at a 10 kV operating voltage.

Test for in vitro antagonistic activity

Isolates of *Gelasinospora* were selected to test for antagonistic activity against seven genera of plant pathogenic fungi: *Alternaria alternata* causing fruit rot of pear (*Pyrus pyrifolia*), *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annuum*), *Curvularia lunata* causing leaf spot of corn (*Zea mays*), *Phytophthora palmivora* causing root and stem rot of durian (*Durio zibethinus*), *Pythium aphanidermatum* causing damping-off of cucumber (*Cucumis sativus*), *Rhizoctonia oryzae* causing sheath rot of rice (*Oryza sativa*) and *Sclerotium rolfsii* causing southern blight of potato (*Solanum tuberosum*). Fungi were cultivated as dual cultures on PDA at 25–30°C for 14 d. The young mycelia from the colony margin of each *Gelasinospora* isolate and the specific plant pathogenic fungus were cut using a sterile cork borer (0.5 cm diameter) and transferred onto PDA, 6 cm apart. Plates were incubated at 25–30°C for 14 d. Each treatment was replicated five times. The inhibition levels as a percentage were calculated using the formula $[(A_o\text{-}A_i)/A_o] \times 100$, where A_o and A_i are the colony radius of the plant pathogenic fungus in the absence of the *Gelasinospora* isolate and of the dual culture test, respectively. Statistical analysis of the data was undertaken and the treatment means were compared using Duncan's multiple range test.

Results

Morphological characteristics and taxonomy of Gelasinospora species

Eight isolates of *Gelasinospora* were isolated from buffalo and cow dung samples from Surin and Suphan Buri province with six from cow dung and two from buffalo dung. All isolates were cultivated on PDA and kept as pure cultures using the following specimen codes: KUFC6852, KUFC6855, KUFC6856, KUFC6873, KUFC6876, KUFC6877, KUFC6895 and KUFC6898. The most representative characteristic features of colony morphology, ascomata, asci and ascospores were similar in all species. The ornamentation of the ascospore wall is considered to be a key character to identify fungal species. Four species of *Gelasinospora* were recorded including *G. calospora*, *G. hippopotama, G. indica,* and *G. stellata*, whose generic diagnosis description is amended as follows:

Gelasinospora calospora (Mouton) C. Moreau & M. Moreau, 1949 (Fig. 1)
(Synonyms: *Neurospora calospora*)

Colonies on PDA growing rapidly, reaching 9 cm diameter at 25–30°C in 7 d. *Mycelium* brown to dark brown, forming ascomata within 7–10 d. *Ascomata* ostiolate, scattered, superficial or immersed, pear shape, pale brown to dark brown at maturity, 500–750 × 350–600 μm. *Asci* 8-spored, cylindrical, colorless, 125–155 × 15–25 μm. *Ascospores* one–celled, ellipsoidal, hyaline when young, becoming olivaceous brown to dark brown, 21.5–29 × 12.5–15.5 μm, wall with circular and inwardly pits 1–1.5 μm diameter, one germ pore at each end.

Strain examined: KUFC6895.
Habitats: cow dung, Surin province, Thailand.

Gelasinospora hippopotama J.C. Krug, R. S. Khan and Jeng, 1994 (Fig. 2)
(Synonyms: *Neurospora hippopotama*)

Colonies on PDA growing rapidly, reaching 9 cm diameter at 25–30°C in 7 d. *Mycelium* hyaline to pale brown hyphae, forming ascomata within 5–7 d. *Ascomata* ostiolate, immersed, pale brown to dark brown at maturity, 450–750 × 350–500 μm. *Asci* 8-spored, cylindrical, colorless, 200–230 × 25–50 μm. *Ascospores* one-celled, ellipsoidal or subglobose to almost globose, hyaline when young, becoming olivaceous brown to dark brown, 40.5–46.3 × 29.5–33 μm, wall with circular, pits 1–2.5 μm diameter, 4–8 germ pores which may be scattered or concentrated near the end.

Strains examined: KUFC6898, KUFC6935.
Habitats: cow and buffalo dung, Surin province, Thailand.

Gelasinospora indica (J. N. Rai, Wadhwani & J. P. Tewari) Arx, 1973 (Fig. 3)
(Synonyms: *Neurospora indica*)

Colonies on PDA growing rapidly, reaching 9 cm diameter at 25–30°C in 7 d. *Mycelium* brown to dark brown, forming ascomata within 10–14 d. *Ascomata* scattered, superficial or immersed, globose to subglobose, brown to dark brown, ascomata non-ostiolate, 170–230 μm. *Asci* 8-spored, cylindrical, colorless. *Ascospores* one-celled, broadly ellipsoidal to subglobose, hyaline when young, becoming olivaceous brown to dark brown, 20.5–22.5 × 21.5–27.5 μm, wall reticulate with angular pits 3–3.5 μm diameter, one germ pore at each end.

Strains examined: KUFC6852.
Habitats: cow dung, Surin province, Thailand.

Gelasinospora stellata Cailleux, 1971 (Fig. 4)
(Synonyms: *Neurospora stellata*)

Colonies on PDA growing rapidly, reaching 9 cm diameter at 25–30 °C in 7 d. *Mycelium* hyaline to pale brown hyphae, forming ascomata in 4–7 d. *Ascomata* non-ostiolate, scattered, immersed, globose to subglobose, pale brown to dark brown at maturity, 170–230 μm diameter. *Asci* 8-spored, cylindrical, colorless. *Ascospores* one-celled, broadly ellipsoidal to sub-globose, hyaline when young, becoming olivaceous brown to dark brown, 27.5–30 × 25.5–27.5 μm, wall reticulate with angular to sub-angular pits 4 μm diameter, one germ pore at each end.

Strains examined: KUFC6873, KUFC 6934, KUFC 6939, KUFC6950.

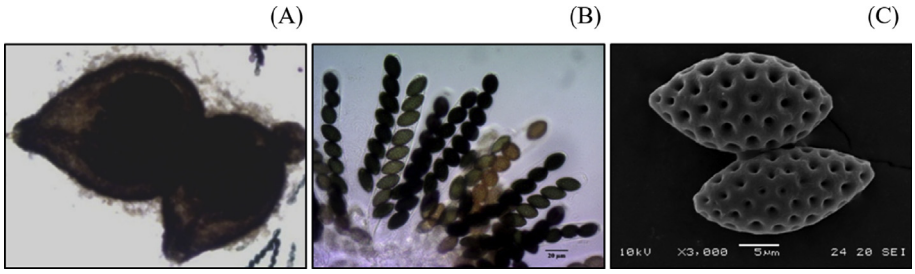

Fig. 1. *Gelasinospora calospora*: (A) ostiolate ascomata; (B) asci containing eight spores each inside; (C) scanning electron micrograph of ascospores with circular and inwardly pitted walls.

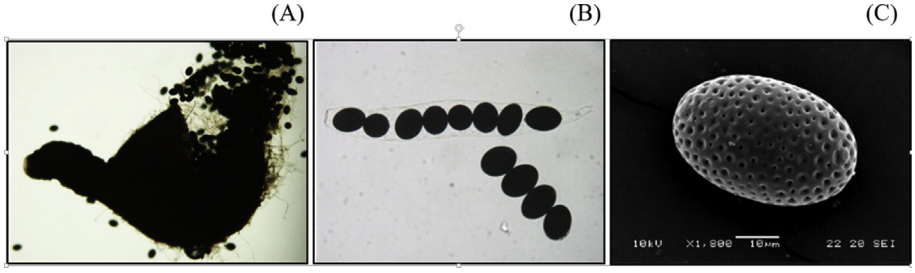

Fig. 2. *Gelasinospora hippopotama*: (A) ostiolate ascoma; (B) ascus containing eight spores each inside; (C) scanning electron micrograph of an ascospore with circular pitted walls and scattered germ pores.

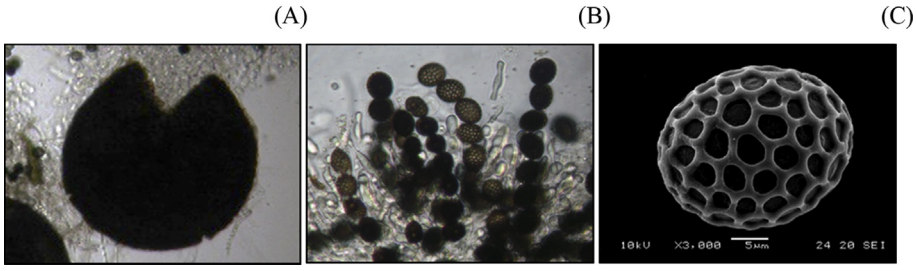

Fig. 3. *Gelasinospora indica*: (A) non-ostiolate ascoma; (B) asci containing eight spores each inside; (C) scanning electron micrograph of an ascospore with reticulate angular pitted walls.

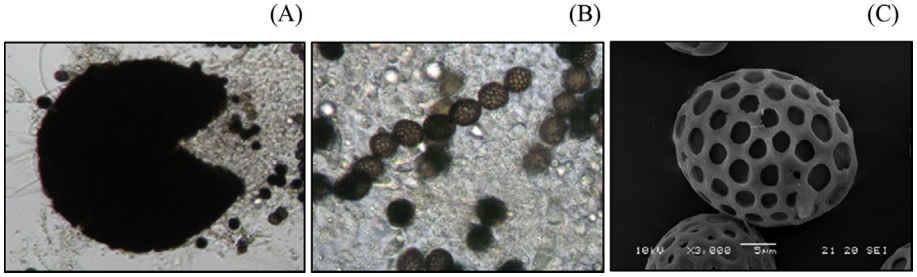

Fig. 4. *Gelasinospora stellata*: (A) non-ostiolate ascoma; (B) asci containing eight spores each inside; (C) scanning electron micrograph of an ascospore with reticulate angular to subangular pitted walls.

Habitats: cow and buffalo dung, Surin province, Thailand; cow dung, Suphan Buri province, Thailand.

Antagonistic effect of Gelasinospora against plant pathogenic fungi

Based on the colony growth rate as well as the potential for ascospore formation, four isolates of *Gelasinospora* species (*G. calospora* KUFC6895, *G. indica* KUFC6852, *G. hippopotama* KUFC6898 and *G. stellata* KUFC6873) were selected to test for antagonistic activity against seven genera of plant pathogenic fungi (Fig. 5). All isolates of *Gelasinospora* completely inhibited mycelial growth of *Phytophthora palmivora* and also inhibited mycelial growth of *Colletotrichum capcisi* ranging from 90.5% to 100%. Moreover, all isolates could inhibit mycelial growth of *Alternaria alternata* and *Curvularia lunata* ranging from 75.5% to 100%. *G. hippopotama* KUFC6898 could inhibit mycelial growth of *Rhizoctonia oryza*e by

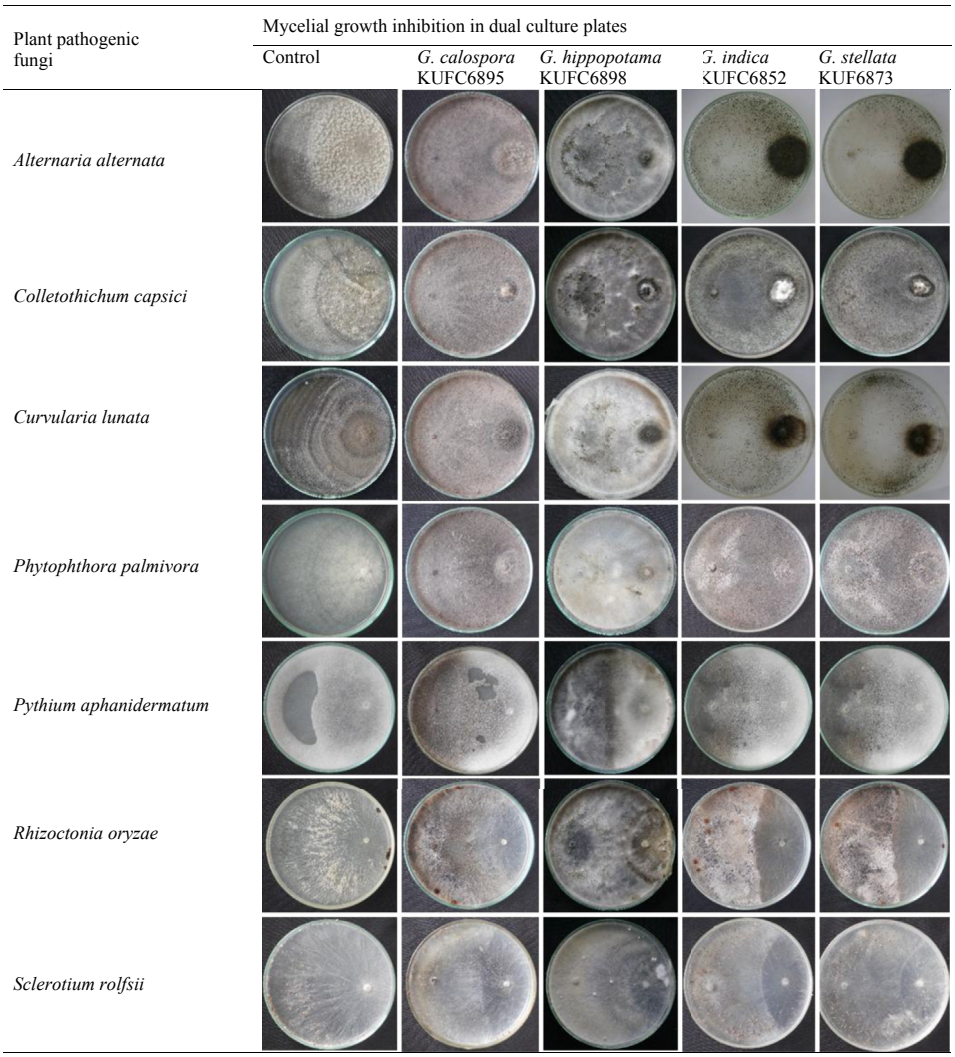

Fig. 5. Antagonistic activity test of four isolates of *Gelasinospora* (left) against seven genera of plant pathogenic fungi (right) cultivated as dual cultures on PDA.

75.5%. Four species of *Gelasinospora* could not inhibit mycelial growth of *Pythium aphanidermatum*, *R. oryza*e and *Sclerotium rolfsii* as they only had inhibition levels ranging from 0% to 54.5% (Table 1).

Discussion

The genus *Gelasinospora* was proposed by Dowding (1933) and is a genus belonging to the order Sordariales (Kirk et al., 2008). It is the largest genus of Sordariomycetes, which is coprophilous, soilborne and also found on plant debris (Cai et al., 2006; Kruys et al., 2015) and plays an important role in ecosystems, functioning as saprobic and endophytic fungi (Domsch et al., 1993; Basumatary and McDonald, 2017). This genus differs from other genera because it forms characteristic pitted ascospores, while *Neurospora* has longitudinally ribbed ascospores (Cain, 1950; Khan and Krug, 1989; Krug et al., 1994). The genera *Neurospora* and *Gelasinospora* are conventionally distinguished by differences in ascospore ornamentation, with

Table 1
Efficacy of *Gelasinospora* spp. to inhibit mycelial growth of seven plant pathogenic fungi in dual culture tests.

Plant pathogenic fungi	% Inhibition[†]			
	G. calospora KUFC6895	*G. hippopotama* KUFC6898	*G. indica* KUFC6852	*G. stellata* KUF6873
Alternaria alternata	80.5^{b}	100^{a}	75.5^{d}	75.5^{d}
Colletotrichum capsici	100^{a}	100^{a}	90.5^{b}	95.5^{b}
Curvularia lunata	80.7^{b}	100^{a}	79.8^{c}	79.5^{c}
Phytophthora palmivora	100^{a}	100^{a}	100^{a}	100^{a}
Pythium aphanidermatum	36.7^{c}	54.5^{c}	35^{e}	34.7^{e}
Rhizoctonia oryzae	0^{d*}	75.5^{b}	0^{f*}	0^{f*}
Sclerotium rolfsii	0^{d*}	0^{f*}	0^{f*}	0^{f*}
Coefficient of variation (%)	0.97	0.48	3.46	2.37

[†] Values followed by the same letters are not significantly different at the 95% confidence limit based on Duncan's multiple range test.
[*] Plant pathogenic fungi overgrew the colonies of *Gelasinospora* species.
The letters a-f are the statistical values. The values followed by the same letters did not significantly different at the 95% confidence limit based on Duncan's multiple range test.

elevated longitudinal ridges (ribs) separated by depressed grooves (veins) in *Neurospora* and spherical or oval indentations (pits) in *Gelasinospora*. Dettman et al. (2001) concluded that ascospore morphology was not an accurate predictor of phylogenetic relationships among genera.

Garcia et al. (2004) proposed that the names *Neurospora* and *Gelasinospora* are synonymized and the circumscription of the genus *Neurospora* was amended. Partial sequences of the 28S rDNA gene from 27 species of both genera were analyzed to infer their phylogenetic relationships. A synopsis and key to the 49 species of *Neurospora* based on ultrastructural and 28S rDNA sequence data are recognized. Two new species of *Neurospora* (*N. nigeriensis* and *N. uniporata* spp. nov.) were recently isolated from soils of Nigeria and Spain. In addition, the new genus *Pseudogelasinospora* was described to accommodate *P. amorphoporcata* from soil of Thailand (syn. *Gelasinospora amorphoporcata* comb. nov.). However, Cai et al. (2006) stated that *Anixiella* species are nested among *Gelasinospora* species. They also stated that although *Gelasinospora* and *Neurospora* are closely related and not resolved as a monophyletic group, there is insufficient evidence to place them into the same genus. Their study was based on the analysis of partial nuclear 28S ribosomal DNA, nuclear ITS ribosomal DNA, and partial nuclear β-tuberlin.

In Thailand, several species of *Gelasinospora* have been reported from soil, animal dung and plants. Manoch et al. (1999) and Manoch (2004) reported *Gelasinospora* from agricultural soil and forest soil such as *G. dictyophora*, which was isolated from pineapple plantation soil, Rayong province, while *G. hapsidophora* was isolated from forest soil in Phu Luang Wild Life Sanctuary, Loei province as well as *G. udagawae*, which was isolated from mangosteen plantation soil, Rayong province (Manoch et al., 2009). Some species of *Gelasinospora* have been reported from healthy plants as endophytes such as *G. calospora*, which was isolated from *Oroxylum indicum* in Phu Luang Wild Life Sanctuary (Kokaew, 2011). In addition, *G. brevispora* and *G. indica* have been recorded as coprophilous ascomycetes isolated from cow dung, Krabi province (Jeamjitt et al., 2007) and isolated from barking deer dung collected from Phu Luang Wild Life Sanctuary (Piasai and Manoch, 2009), respectively. In the present study, two noteworthy species (*G. hippopotama* and *G. stellata*) from cow and buffalo dung are new records for Thailand. However, the ornamentation of the ascospore walls of *G. stellata* is similar to *G. indica*, but *G. stellata* has bigger ascomata and slightly broader ascospores (Garcia et al., 2004; Guarro et al., 2012).

The potential of *Gelasinospora* species as biological agents against plant diseases has not been reported previously. In this preliminary investigation, the results showed that all isolates of *Gelasinospora* could completely inhibit mycelial growth of *Phytophthora palmivora*. They inhibited more than 70% on mycelial growth of *Alternaria alternata*, *Colletotrichum capsici* and *Curvularia lunata*, but only 30% on mycelial growth of *Pythium aphanidermatum*. All isolates failed to inhibit mycelial growth of *Rhizoctonia solani* and *Sclerotium rolfsii*, except for *G. hippopotami* that inhibited mycelial growth of *Rhizoctonia oryza*e by 75.5%. It may be assumed that the genus *Gelasinospora* could be used as natural inhibitors of the growth of plant pathogenic fungi. Therefore, further study is warranted of the mechanisms and potential of *Gelasinospora* for use in the biocontrol of plant pathogens.

Conflict of interest

The authors declare that there are no conflicts of interest.

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