Bjerkandera adusta M1 inhibits Fusarium oxysporum and prevents the wilt incidence in Brassica napus

xiao feng

Southwest University <https://orcid.org/0000-0002-9341-8996>

Yong Li (✉ liyongwf@swu.edu.cn)

<https://orcid.org/0000-0001-6435-4906>

Suping Li

Southwest University

Yifan Lu

Southwest University

Jingjie Zhang

Southwest University

Yunyun Zhu

Southwest University

Hongjun Yang

Southwest University

Xinhua He

Southwest University

Research article

Keywords: Biocontrol, carbendazim, disease-resistant enzymes, fermentation broth, fungicide

Posted Date: December 5th, 2019

DOI: <https://doi.org/10.21203/rs.2.18233/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

8 Highlights

- 9 • *B. adusta* M1 inhibits the growth of *Fusarium oxysporum* f. sp. *conglutinans*
- 10 • *B. adusta* M1 & its FB decreases the incidence and disease index of fusarium wilt
- 11 • Control effects on fusarium wilt are equal to or greater than fungicide carbendazim
- 12 • *B. adusta* M1 improves the activity of relevant leaf enzymes against fusarium wilt
- 13 • Mycoparasitism & possible antifungal compounds from *B. adusta* M1 prevent
- 14 fusarium wilt

Abstract

Background: *Bjerkandera adusta* degrades polycyclic aromatic compounds, such as cellulose and lignin, with the production of laccase and peroxidase. However, its effect on plant disease is unknown.

Results: In this study, both the confrontation culture and greenhouse pot experiments were carried out to address the biocontrol mechanisms of *B. adusta* M1, which was isolated from a unique purple soil (Eutric Regosol), on the growth of pathogenic *Fusarium oxysporum* f. sp. *conglutinans* (FOC) and incidence of fusarium wilt in *Brassica napus*. Results showed that the hyphal growth rate of *B. adusta* M1 was significantly greater than that of FOC, indicating a strong competitiveness by *B. adusta* M1. In addition, the *B. adusta* M1 fermentation broth significantly inhibited the growth of FOC hyphae by $62.79 \pm 1.80\%$, which was greater than an inhibition rate of $40.63 \pm 1.68\%$ by the chemical fungicide carbendazim. The image from a scanning electron microscope showed the hyphae of FOC directly was penetrated by the *B. adusta* M1 hyphae, indicating a strong mycoparasitism by *B. adusta* M1. Besides, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth reduced the incidence and disease index of the fusarium wilt in *Brassica napus* leaves, and the control effects of different treatments against fusarium wilt were 57.09% and 47.67%, respectively, which were comparable better to 46.11% of the chemical fungicide carbendazim. Furthermore, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth increased the activity of superoxide dismutase, catalase, peroxidase and phenylalanine ammonia-lyase, which related to an enhancement of disease resistance. Similarly, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth decreased the cell membrane permeability and malondialdehyde contents, thereby reducing the cell membrane damage from the pathogenic fungus.

Conclusion: In summary, results from this present study demonstrated that both the *Bjerkandera adusta* M1 and *B. adusta* M1 fermentation broth inhibited the growth of

FOC and decreased the incidence and disease index of the fusarium wilt disease in *Brassica napus*. Therefore, *B. adusta* M1 could be applied as a potential biocontrol fungus to against the fusarium wilt disease.

Keywords: Biocontrol; carbendazim; disease-resistant enzymes; fermentation broth; fungicide

1. Background

Fusarium oxysporum, a globally saprophytic fungus, can survive in soil for years or decades[1,2]. The fungus invades the host roots and colonizes in the xylem tissues to cause yellowing or wilting disease termed fusarium wilt[1].

Brassica napus is an important economic oil crops grown in China, Canada, Britain and Germany etc[3]. Brassica crops are severely infested by various fungal pathogens, particularly *Fusarium oxysporum* f. sp. *conglutinans* (FOC), which causes the fusarium wilt. That lead to serious loss of quality and quantity of the oilseed brassica plants[4,5]. The symptoms of the fusarium wilt disease include yellowing, wilting, stunting, and necrosis of leaf tissues and suppressed root development appeared in irregular-shaped patches, and finally led to the death of whole plant[2,6, 7]. Since fusarium wilt was observed in Beijing in 2001[8], the disease has presented increasingly severe damage to the production and quality of *Brassica napus* as well as economic losses in northern China[1].

At present, the control methods for fusarium wilt are mainly based on breeding disease-resistant varieties[7,9] and crop rotation[10], whereas soil fumigation with broad-spectrum biocides such as methyl bromide could pose great threat to the ecological environment[11]. In addition, the transgenic strains has showed enhanced resistances to *F. oxysporum* with delayed disease development by the Host-Delivered RNA interference (HD-RNAi) technology[9], but it is still not applied in the field.

The extensive use of chemical pesticides such as copper sulfate solution, carbendazim and thiophanate-methyl suspension to control the fusarium wilt has

posed threat to human health and environment, and resulted in pathogen resistance [12,13]. In this context, biological control, the potential of applying beneficial microorganisms to control the fusarium wilt could provide an alternative to the use of chemical pesticides[14,15]. However, limited biological control methods have been developed to prevent *Brassica napus* from fusarium wilt disease. Nevertheless, studies have shown that non-pathogenic *Fusarium oxysporum* inhibited spore germination and induced resistances by competing for nutrient and space, which might prevent the infection of FOC[3].

Bjerkandera adusta, a white rot fungus belonging to the family *Polyporaceae*, generally parasitizes on plant branch, fallen or dead trees[16,17]. At present, studies on *Bjerkandera adusta* primarily focus on the production of laccase, lignocellulose decomposition[18], and degradation of organic pollutants such as daunomycin and humic acids[19]. At present, limited information is available for the role of *B. adusta* in the biological control of plant diseases, particularly the fusarium wilt disease. The *B. adusta* M1 has been isolated from a purple soil (Eutric Regosol, FAO Soil Classification System) locating in the Southwest University campus in Chongqing, China, and the biocontrol effects of *B. adusta* M1 and *B. adusta* fermentation broth on *Didymella bryoniae* or watermelon vine blight was greater than those of carbendazim[20]. However, no studies have addressed the biocontrol effects of *Bjerkandera adusta* on the fusarium wilt disease, particularly for the vegetable oil crops including *Brassica napus*. The present study explored the effects and mechanisms of *B. adusta* M1 on controlling the fusarium wilt in the *Brassica napus* under both the laboratory culture and greenhouse pot experiments. The generated results would provide some theoretical and practical bases on the biocontrol of *B. adusta* M1 to the fusarium wilt disease in the field.

2. Results

2.1. Competition of *B. adusta* M1 on FOC

In the confrontation culture test, the growth rate of *B. adusta* M1 was greater than that of FOC. The hyphae of two fungi begin to contact each other after inoculation for 4 days, until 8th day the growth of FOC was obviously inhibited by *B. adusta* M1 (Fig. 1).

2.2. Inhibitory effects of the *B. adusta* M1 fermentation broth on FOC

Inoculation of *B. adusta* M1 fermentation broth or carbendazim significantly inhibited the growth on FOC, being indicated by a smaller colony radius on the plate (Table 1). Besides, the inhibitory effect on FOC was greater under the *B. adusta* M1 fermentation broth than under the chemical fungicide carbendazim.

2.3. Mycoparasitism effects of *B. adusta* M1 on FOC

The image from the scanning electron microscopy showed that the hyphae of *B. adusta* M1 were directly interspersed and pierced (Fig. 2) that of FOC, indicating that the latter might be expanded and even deformed.

2.4. Control effects of *B. adusta* M1 on FOC

The incidences of the fusarium wilt treated with the *B. adusta* M1 fermentation broth was significantly decreased (Table 2). Additionally, the disease index of the fusarium wilt were significantly decreased under the *B. adusta* M1, *B. adusta* M1 fermentation broth and carbendazim, though the *B. adusta* M1 showed a higher decrease effect than others treatments.

2.5. The activities of enzymes related to disease resistances

Compared to the basic broth media, the activity of SOD or CAT under the *B. adusta* M1 and *B. adusta* M1 fermentation broth was significantly increased by 25.92% and 22.38% or 78.80% and 82.28%. While the activity of POD or PAL was significantly increased by 46.64% or 44.23% under *B. adusta* M1 than under basic

broth media (Fig.3).

2.6. The cell membrane permeability and malondialdehyde contents

By contrast with the control, electrical conductivity was significantly decreased by 48.68% and 33.71% under the *B. adusta* M1 and the *B. adusta* M1 fermentation broth (Fig. 4a). Besides, a considerable decrease of 71.04% in the malondialdehyde contents was observed under the *B. adusta* M1 than under the control (Fig. 4b).

3. Discussion

Bjerkandera adusta, was a white rotting fungi growing on branch and fallen wood. Recent studies have focused on its enzymatic properties and application, such as laccase synthesis[21], lignocellulose decomposition[17], biodegradation of synthetic dyes[16,22] and the wastewater treatment[23]. In contrast, few reports have been done if *B. adusta* could control the fusarium wilt disease. The strain of *Bjerkandera adusta* M1 could effectively inhibit *Didymella bryoniae*[20]. Both the *B. adusta* M1 and *B. adusta* M1 fermentation broth showed a significant inhibition on the growth of FOC, and a better inhibition effect than the chemical fungicide, carbendazim (Fig. 1 vs Table 2). Besides, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth decreased the incidence and disease index of fusarium wilt, and their control effects were superior or equivalent to that of carbendazim (Table 2). The above results showed that *B. adusta* M1 could be a biological fungus possessing the ability to control fusarium wilt disease, though this remains to be tested in field trials.

A biocontrol fungi could activate various mechanisms to cope with the threats from pathogens, including competition[24], secretion of antimicrobial substance [25], induced systemic resistance[26], and mycoparasitism[27].

In general, after the two fungi came into contacts, the biological *Trichoderma* spp. expressed inhibitory effect on the growth of the pathogenic fungus, *Serpula lacrymans* through space and nutrient competition[24,28]. In our results, the growth rate of *B. adusta* M1 was higher than that of FOC by competing for nutrient and space,

and the growth of FOC was obviously inhibited by *B. adusta* M1 (Fig. 1).

The mycoparasitism possesses an important role in the suppression of pathogenic fungi by biocontrol fungi[27,29]. The mechanisms of mycoparasitism of *Trichoderma* mainly refer to the process of contact, infection, recognition, entanglement, penetration and degradation against plant pathogenic fungi[30]. *Trichoderma* would establish a parasitic relationship with a pathogenic fungus after successful recognition, and show substantial parasitic ability in the penetration of pathogenic fungal mycelia, and the production of cell wall degrading enzymes including beta-1,6-glucanase and protease, could finally lead to death of pathogenic fungus[31, 32]. In addition, microbial antagonists, such as *Trichoderma harzianum* and *T. asperellum*, are widely applied because of their ability to control plant fungal diseases that were induced by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*[14,29,33]. In the present study, the *B. adusta* M1 hyphae had directly penetrated the FOC hyphae, which might cause the latter to swell, deform and even rupture, showing a strong mycoparasitism (Fig. 2).

The induced systemic resistance (ISR), typically results in an adapted and efficient defense response to pathogens under both biotic and abiotic stress [13,34]. A biocontrol fungus could induce plant response to pathogenic fungus by secreting resistant secondary metabolites, which involves the activity of SOD, CAT, PAL and POD enzymes, thus enhances plant disease resistance[13,35,36]. For instance, after the inoculation with *T. asperelloides* T203, the defense system was activated with the increase of PAL in cucumber[37]. The activity of SOD, POD, CAT and PAL was significantly increased under 1.5 mL methyl jasmonate, and the disease resistance was then improved[38]. In the present study, the *B. adusta* M1 significantly improved the activity of leaf SOD, CAT, POD and PAL, and the *B. adusta* M1 fermentation broth significantly increased the activity of leaf SOD and CAT (Fig. 3), indicating that both the *B. adusta* M1 and *B. adusta* M1 fermented broth could increase the plant's resistance on fusarium wilt with the

increasing activity of defense enzymes.

Plant cell membrane is regarded as an important channel for material exchange between cells and the external environment, and it exerts vital role during plant growth and development[39]. The electrolyte of plant cell is leaked under stress including low temperature and water deficit, leading to a decrease of osmotic potential but an increase of the conductivity of plant cell membrane[40,41]. Malondialdehyde (MDA), an excessive product of membrane lipid peroxidation on the plant cell, and its content is directly related to damage degree of plant cell membrane[39]. In general, the cell membrane permeability or malondialdehyde content was associated with plant resistances[42]. In the current experiment, a significant decrease of both the leaf malondialdehyde content and the conductivity was observed under both the *B. adusta* M1 and *B. adusta* M1 fermentation broth (Fig. 4). As a consequence, the decrease of structural damage of plant cell membrane was related to an increase of disease resistance by *Brassica napus*.

The extracellular proteins of 110 and 17 kDa produced by *Trichoderma viride* TvMN7 could inactivate RS toxin, a carbohydrate compound containing mainly alpha-glucose and mannose, which produced by the rice sheath blight pathogen, *Rhizoctonia solani*[43]. Observation from the confocal laser scanning microscope indicated that, the metabolite of *Pseudomonas chlororaphis* PCL1391, phenazine-1-carboxamide, inhibited the infection of *Fusarium oxysporum* on tomato[44]. In the present study, the *B. adusta* M1 fermentation broth significantly inhibited the growth of FOC (Table 1), thus the brassica plants become more resistant on fusarium wilt infected by FOC (Table 2). As a result, the *B. adusta* M1 exerted great role in controlling the fusarium wilt disease.

4. Conclusion

Our results indicated that both the *B. adusta* M1 and *B. adusta* M1 fermentation broth inhibited the growth of FOC, and decreased the incidence and disease index of

the fusarium wilt disease, indicating that *B. adusta* M1 might be a potential biocontrol fungus against the fusarium wilt disease.

5. Materials and Methods

5.1. Materials.

Brassica napus was (*Brassica napus* L. cv. Xinde Miscellaneous Oil No. 9) purchased from the Mianyang Special Research Seed Industry Co., Ltd, Sichuan, China. The growth media is a purple soil (Eutric Regosol, FAO Soil Classification System) from the National Key Field Station for Purple Soil Fertility and Fertilizer Monitoring Base, locating in the Southwest University campus (E 106°24'37"; N 29°48'32") Beibei, Chongqing, China. The collected purple soil at 5-10 cm depth were thoroughly mixed after the removal of debris, dried at 30°C and sieved over 2mm, and then autoclaved for 2 h. The basic soil chemical properties were shown in Table 3.

Two fungus strains: *Bjerkandera adusta* M1 was isolated from the above-mentioned rhizosphere soil (5-10 cm depth) in April 2016. *Fusarium oxysporum* f. sp. *conglutinans* (FOC) was donated by the Institute of Plant Ecology and Pathology of Southwest University.

Chemical fungicide: carbendazim [50% N-(benzimidazolyl-2) methyl carbamate] was purchased from the Guoguang Agrochemical Company, Jianyang, Sichuan, China.

The potato dextrose agar (PDA) medium was used for cultivating both the *B. adusta* M1 and FOC[45], and it contained 200 g shelled potato, 20 g dextrose, 15~20g agar, 1000 mL distilled water. The basics broth medium (20 g dextrose, 10 g NH₄Cl, 1 g MgSO₄, 1 g CaCl₂, 2 g KH₂PO₄, 1000 mL distilled water) was used for preparing the fermentation broth of *B. adusta* M1 and FOC.

5.2. Methods

5.2.1. Preparation the spore suspension of *B. adusta* M1 and FOC

B. adusta M1 and FOC were respectively inoculated on the PDA plate, cultured at 28 °C for 5 d, subsequently added 5 mL sterilized water and then gently scraped off the conidia with an inoculating spear. The spore suspension was transferred to a sterile 50 mL triangle bottle and thoroughly shaken, then filtered twice through eight layers of gauze. The concentration of filtrate was immediately determined by a Globulimeter (The Qiujiang Biochemical Reagent Instrument Co., Ltd, Shanghai, China) under a SMART Biological Microscope (Auto Optical Instrument Co., Ltd, Chongqing, China), and then diluted to 4.5×10^5 pcs/mL water.

5.2.2. Preparation of fermentation broth of *B. adusta* M1

Five mycelial discs (5mm diameter) of *B. adusta* M1 inoculated on 100 mL basic broth medium inside a 250 mL triangle bottle were shaken for 5 days (150r/min and 28 °C) and centrifuged (3,000 r/min for 1 min). The filtrate from the supernatant (0.22 mm sterile filter) was then used as the fermentation broth of *B. adusta* M1.

5.2.3. Determination of inhibitory effect of *B. adusta* M1 fermentation broth on FOC

The antifungal test was applied to determine the fungistatic effect of *B. adusta* M1 fermentation broth on the growth of FOC, and the following formula 1 was used to calculate the related inhibition rate[46]. The process was as follows: inoculated a mycelial disc of FOC on a PDA plate containing 3 mL *B. adusta* M1 fermentation broth, and 3 mL 0.4 mg/mL carbendazim as chemical fungicide treatment. After 5 days of culture at 32 °C, the radius of the FOC colony was determined.

The inhibition rate (%) = (colony radius of control group - colony radius of treatment group) / colony radius of control group $\times$ 100% (1)

5.2.4. Competition and mycoparasitism of *B. adusta* M1 against FOC

Two experiments were carried out to address competition and mycoparasitism of *B. adusta* M1 against FOC. Both *B. adusta* M1 and FOC mycelial discs with a diameter of 5 mm were inoculated on the same PDA plate, but separated by 3 cm each

other to form a two-point confrontation. One group of these PDA plates was applied to observe the competitive effect of *B. adusta* M1 on FOC, cultivated for 8 days in the dark at 32 °C, then observed and taken pictures. In another group of these PDA plates, a 2 cm × 2 cm aluminum foil was placed between the two strains on the surface of each PDA plate, incubated at 32 °C for about 48 h until the two strains were in contact with each other on the aluminum foil. The mycelial sample from the interactive regions was prepared as a microscope slide[47] then the mycoparasitism effect of *B. adusta* M1 on FOC was observed under a scanning electron microscope (Nikon Co., Ltd, Shanghai, China).

5.2.5. Effects of *B. adusta* M1 on fusarium wilt

After *Brassica napus* seeds were soaked in 75% ethyl alcohol for 5min, in 20% sodium hypochlorite for 3 min, rinsed with the sterile water several times, and then cultivated at 28 °C for 3 d in an incubator. The germinated seeds were sowed in a seedling tray (18 cm × 24 cm) containing sterilized soil media. Three seedlings with two cotyledons were transplanted to a flowerpot (18 cm × 24 cm) containing 2kg sterilized soil media. The *Brassica napus* seedlings were irrigated with 10 mL FOC spore suspension after 20 d slow growth without nutrient supplements.

After *Brassica napus* seedlings had been infected by *B. adusta* M1 (after inoculation for five days), to test the control effects of *B. adusta* M1 on fusarium wilt under four treatments (3 replicates each) : (1) 10 mL basic broth media (Control); (2) 10 mL *B. adust* M1 spore suspension; (3) 10 mL *B. adusta* M1 fermentation broth; (4) 10 mL chemical fungicide carbendazim (0.08 mg/mL). After 24 h treatment, the incidence and disease index of *Brassica napus* and the control effects of *B. adusta* M1 on fusarium wilt were investigated every five days according to the following respective formulas[48].

Incidence (%) = the number of plants infected / total number of plants × 100%

(2);

$$\text{Disease index} = \frac{\sum (\text{the number of leaves at all levels} \times \text{relative series})}{(\text{total number of leaves} \times \text{maximum series})} \times 100 \quad (3);$$

$$\text{Control effects} = \frac{(\text{disease index of control group} - \text{disease index of treatment group})}{\text{disease index of control group}} \times 100\% \quad (4).$$

5.2.6. Effects of *B. adusta* M1 on disease resistance of *Brassica napus*

Brassica napus seedlings were sowed and managed as described as 5.2.5. After growth for 20 days, three treatments (3 replicates each) were respectively done: (1) 10 mL basic broth media (Control); (2) 10 mL *B. adust* M1 spore suspension; (3) 10 mL *B. adust* M1 fermentation broth. After 24h treatments, the above-mentioned *Brassica napus* seedlings were irrigated with 10 mL of FOC spore suspension. After 22 days treatment, the activity of leaf superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and phenylalanine ammonia lyase (PAL) in *Brassica napus* seedlings was respectively measured by the nitroblue tetrazole method and guaiacol method, using a spectrophotometry or UV spectrophotometer[49]. In addition, the cell membrane permeability and malondialdehyd content in *Brassica napus* leaves were detected by an electrolyte infiltration[50] and a thiobarbital (TBA) colorimetry[51], respectively.

5.2.7. Statistical analysis

Data (means $\pm$ LSD, $n = 3$) were subjected to one-way analysis of variance (ANOVA) and significant differences between treatments were compared by the least significant differences at $P < 0.05$. All significance tests and correlation analyses were done using the SPSS 10.00 software, and the mean performed using a SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).

Abbreviations

B. adusta M1: Bjerkandera adusta M; FB: *B. adusta* M1 fermentation broth; *Fusarium oxysporum* f. sp. *conglutinans*: FOC

Acknowledgements

We would thank Dr. Yunxia Pan, the Greenhouse Manager, for supporting our pot experiments. We also would thank the suggestion of the experiment design from Professor Jianguo Huang at the Southwest University, Chongqing, China.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Authors ' contributions

Xiao Feng designed the study and wrote the manuscript. Xiao Feng, Suping Li, Yi-Fan Lu performed the experiments. Xin-Hua He and Yong Li modified this manuscript. All authors reviewed and approved the manuscript.

Funding

The study was supported by an Agricultural Science and Technology Achievements Transformation Fund, the Ministry of Science and Technology in China (2013GB2F100396) and the Program of Basic Research and Frontier Exploration, the Chongqing Municipal City in China (cstc2018jcyjAX0616).

Ethics approval and consent to participate

Ethical approval is not applicable in the case of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lv HH, Yang YH, Liu X, Ling J, Fang ZY, Yang LM, Zhang YY, Wang Y. Draft genome sequence of FGL03-6, a race 1 strain of *Fusarium oxysporum* f. sp. *conglutinans*, the causal agent of cabbage fusarium wilt. Microbiol Resour Ann. 2018;6 (16):e00191-18.
2. Liu X, Xing MM, Kong CC, Fang ZY, Yang LM, Zhang YY, Wang Y, Ling J, Yang YH, Lv HH. Genetic diversity, virulence, race profiling, and comparative

-
- 336 genomic analysis of the *Fusarium oxysporum* f. sp. *conglutinans* strains infecting
337 cabbages in China. Front Microbiol. 2019;10:373.
- 338 3. Zhang QH, Zhang J, Yang L, Zhang L, Jiang DH, Chen WD, Li GQ. Diversity and
339 biocontrol potential of endophytic fungi in *Brassica napus*. Biol Control. 2014;72:
340 98-108.
- 341 4. Ramirezvillupadua J, Endo RM, Bosland P, Williams PH. A new race of *Fusarium*
342 *oxysporum* f.sp *conglutinans* that attacks cabbage with type-a resistance. Plant Dis.
343 1985;69 (7):612-613.
- 344 5. Abdel-Farid IB, Jahangir M, van den Hondel CAMJJ, Kim HK, Choi YH,
345 Verpoorte R. Fungal infection-induced metabolites in *Brassica rapa*. Plant Sci.
346 2009;176(5):608-615.
- 347 6. Gaetan SA. Occurrence of fusarium wilt on canola caused by *Fusarium oxysporum*
348 f. sp. *conglutinans* in Argentina. Plant Dis. 2005; 89 (4):432-432.
- 349 7. Pu ZJ, Shimizu M, Zhang YJ, Nagaoka T, Hayashi T, Hori H, Matsumoto S,
350 Fujimoto R, Okazaki K. Genetic mapping of a fusarium wilt resistance gene in
351 *Brassica oleracea*. Mol Breeding. 2012;30 (2):809-818.
- 352 8. Li MY, Zhang TT. Li XH, Yan H. Fusarium wilt on cruciferous vegetables and
353 identification of their pathogens. Plant Protect (in Chinese). 2003;29 (3):44-45.
- 354 9. Hu, ZL, Parekh U, Maruta N, Trusov Y, Botella JR. Down-regulation of *Fusarium*
355 *oxysporum* endogenous genes by Host-Delivered RNA interference enhances
356 disease resistance. Front Chem. 2015;3:UNSP 1.
- 357 10. Wang BB, Li R, Ruan YZ, Ou YN, Zhao Y, Shen QR. Pineapple-banana rotation
358 reduced the amount of *Fusarium oxysporum* more than maize-banana rotation
359 mainly through modulating fungal communities. Soil Biol Biochem. 2015;86:77-86.
- 360 11. Fravel D, Olivain C, Alabouvette C. *Fusarium oxysporum* and its biocontrol. New
361 Phytol. 2003;157:493-502.
- 362 12. Ren Z. Analysis on the occurrence and control methods of main pests and diseases
363 of rapeseed in Anhui Province. J Agric Dis Res (in Chinese). 2016;6 (07):1-4.

-
- 364 13. Vos CMF, De Cremer K, Cammue BPA, De Coninck B. The toolbox of
365 *Trichoderma* spp. in the biocontrol of *Botrytis cinerea* disease. Mol Plant Pathol.
366 2015;16 (4):400-412.
- 367 14. Qualhato TF, Lopes FAC, Steindorff AS, Brandao RS, Jesuino RSA, Ulhoa CJ.
368 Mycoparasitism studies of *Trichoderma* species against three phytopathogenic
369 fungi: evaluation of antagonism and hydrolytic enzyme production. Biotechnol Lett.
370 2013;35 (9):1461-1468.
- 371 15. Zhang FL, Ge HL, Zhang F, Guo N, Wang YC, Chen L, Ji X, Li CW. Biocontrol
372 potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum*
373 in soybean. Plant Physiol Bioch. 2016;100: 64-74.
- 374 16. Sugawara K, Igeta E, Amano Y, Hyuga M, Sugano Y. Degradation of antifungal
375 anthraquinone compounds is a probable physiological role of DyP secreted by
376 *Bjerkandera adusta*. Amb Express. 2019; 9:56.
- 377 17. Moody SC, Dudley E, Hiscox J, Boddy L, Eastwood DC. Interdependence of
378 primary metabolism and xenobiotic mitigation characterizes the proteome of
379 *Bjerkandera adusta* during wood decomposition. Appl Environ Microb. 2018;84
380 (2):UNSP e01401-17.
- 381 18. Bouacem K, Rekik H, Jaouadi NZ, Zenati B, Kourdali S, El Hattab M, Badis A,
382 Annane R, Bejar S, Hacene H, Bouanane-Darenfed A, Jaouadi B. Purification and
383 characterization of two novel peroxidases from the dye-decolorizing fungus
384 *Bjerkandera adusta* strain CX-9. Int J Biol Macromol. 2018;106:636-646.
- 385 19. Belcarz, A., Ginalska, G., Kornilowicz-Kowalska, T., 2005. Extracellular enzyme
386 activities of *Bjerkandera adusta* R59 soil strain, capable of daunomycin and humic
387 acids degradation. Appl Microbiol Biot. 68 (5), 686-694.
- 388 20. Zhang XH, Zhang HN, Li Y, Wang WQ. Screening and identification of
389 biocontrol fungi against *Didymella bryoniae* and optimization of fermentation
390 conditions. Chinese J Bioeng (in Chinese). 2017;37 (05):76-86.
- 391 21. Rojas-Verde G, Salazar-Alpuche RY, Pereyra-Alferez B, Galan-Wong L,

-
- 392 Arevalo-Nino K. Lignolytic enzyme production and RBBR decolorization by
393 native white rot fungi. J Biotechnol. 2007;131:S170-S170.
- 394 22. Eichlerova I, Homolka L, Nerud F. Decolorization of high concentrations of
395 synthetic dyes by the white rot fungus *Bjerkandera adusta* strain CCBAS 232.
396 Dyes Pigments. 2007;75 (1):38-44.
- 397 23. Anastasi A, Spina F, Prigione V, Tigini V, Varese GC. Textile wastewater
398 treatment: scale-up of a bioprocess using the fungus *Bjerkandera adusta*. J
399 Biotechnol. 2010;150:S52-S52.
- 400 24. Verma M, Brar SK, Tyagi RD, Surampalli RY, Valero JR. Antagonistic fungi,
401 *Trichoderma* spp.: panoply of biological control. Biochem Eng J. 2007;37 (1):1-20.
- 402 25. Saravanakumar K, Yu CJ, Dou K, Wang M, Li YQ, Chen J. Synergistic effect of
403 *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on
404 enhanced biocontrol of *Fusarium oxysporum* f. sp *cucumerinum*. Biol Control.
405 2016;94: 37-46.
- 406 26. Gillmeister M, Ballert S, Raschke A, Geistlinger J, Kabrodt K, Baltruschat H,
407 Deising HB, Schellenberg I. Polyphenols from rheum roots inhibit growth of
408 fungal and oomycete phytopathogens and induce plant disease resistance. Plant Dis.
409 2019;103 (7):1674-1684.
- 410 27. Adams PB. The Potential of Mycoparasites for Biological-Control of Plant-
411 Diseases. Annu Rev Phytopathol. 1990;28:59-72.
- 412 28. Srinivasan U, Staines HJ, Bruce A. Influence of media type on antagonistic modes
413 of *Trichoderma* spp. against wood decay basidiomycetes. Material und
414 Organismen. , 1992;27(4):301-321.
- 415 29. Gomes EV, Costa MD, de Paula RG, de Azevedo RR, da Silva FL, Noronha EF,
416 Ulhoa CJ, Monteiro VN, Cardoza RE, Gutierrez S, Silva RN. The Cerato-Platanin
417 protein Epl-1 from *Trichoderma harzianum* is involved in mycoparasitism, plant
418 resistance induction and self-cell wall protection. Sci Rep-Uk. 2015;5:17998.
- 419 30. Elad Y, Barak R, Chet I. Possible role of lectins in mycoparasitism. J Bacteriol.

1983;154 (3):431-1435.

31. Djonovic S, Pozo MJ, Kenerley CM. Tvbg3, a beta-1,6-glucanase from the biocontrol fungus *Trichoderma virens*, is involved in mycoparasitism and control of *Pythium ultimum*. *Appl Environ Microb*. 2006;72 (12):7661-7670.
32. De Marco JL, Felix, CR. Characterization of a protease produced by a *Trichoderma harzianum* isolate which controls cocoa plant witches' broom disease. *Bmc Biochem*. 2002;3(1):1-7.
33. Kurniawan E, Panphon S, Leelakriangsak M. Potential of marine chitinolytic *Bacillus* isolates as biocontrol agents of phytopathogenic fungi. *IOP Conf Ser.: Earth Environ Sci*. 2018;217:12044.
34. Tuzun S. The relationship between pathogen-induced systemic resistance (ISR) and multigenic (horizontal) resistance in plants. *Eur J Plant Pathol*. 2001;107 (1):85-93.
35. Liu X, Chi H, Yue M, Zhang XF, Li WJ, Jia EP. The regulation of exogenous jasmonic acid on UV-B stress tolerance in wheat. *J Plant Growth Regul*. 2012;31 (3):436-447.
36. Fauteux F, Remus-Borel W, Menzies JG, Belanger RR. Silicon and plant disease resistance against pathogenic fungi. *Fems Microbiol Lett*. 2005;249:1-6.
37. Yedidia I, Shoresh M, Kerem Z, Benhamou N, Kapulnik Y, Chet I. Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. *Appl Environ Microb*. 2003;69 (12):7343-7353.
38. Sun DQ, Lu XH, Hu YL, Li WM, Hong KQ, Mo YW, Cahill DM, Xie JH. Methyl jasmonate induced defense responses increase resistance to *Fusarium oxysporum* f. *sp cubense* race 4 in banana. *Sci Hortic-Amsterdam*. 2013;164: 484-491.
39. Wang B, Liu F, Li Q, Xu S, Zhao XZ, Xue PL, Feng X. Antifungal activity of zedoary turmeric oil against *Phytophthora capsici* through damaging cell membrane. *Pestic Biochem Phys*. 2019;159:59-67.

-
- 448 40. Plazek A, Dubert F, Marzec K. Cell membrane permeability and antioxidant
449 activities in the rootstocks of *Miscanthus X giganteus* as an effect of cold and frost
450 treatment. *J Appl Bot Food Qual.* 2009;82 (2):158-162.
- 451 41. Gunes A, Pilbeam DJ, Inal A, Coban S. Influence of silicon on sunflower cultivars
452 under drought stress, I: growth, antioxidant mechanisms, and lipid peroxidation.
453 *Commun Soil Sci Plan.* 2008;39 (13-14):1885-1903.
- 454 42. Kuk YI, Shin JS, Burgos NR, Hwang TE, Han O, Cho BH, Jung SY, Guh JO.
455 Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop*
456 *Sci.* 2003;43 (6):2109-2117.
- 457 43. Sriram S, Raguchander T, Babu S, Nandakumar R, Shanmugam V,
458 Vidhyasekaran P, Balasubramanian P, Samiyappan R. Inactivation of phytotoxin
459 produced by the rice sheath blight pathogen *Rhizoctonia solani*. *Can J Microbiol.*
460 2000;46 (6):520-524.
- 461 44. Bolwerk A, Lagopodi AL, Wijfjes AHM, Lamers GEM, Chin-A-Woeng TFC,
462 Lugtenberg BJJ, Bloemberg GV. Interactions in the tomato rhizosphere of two
463 *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxyspo*
464 *rum* f. sp. *radicis-lycopersici*. *Mol Plant Microbe In.* 2003;16 (11):983-993.
- 465 45. Cong ML, Zhang B, Zhang KY, Li GQ, Zhu FX. Stimulatory effects of sublethal
466 doses of carbendazim on the virulence and sclerotial production of *Botrytis cinerea*.
467 *Plant Dis.* 2019;103(9):2385-2391.
- 468 46. Campanile G, Ruscelli A, Luisi N. Antagonistic activity of endophytic fungi
469 towards *Diplodia corticola* assessed by in vitro and in planta tests. *Eur J Plant*
470 *Pathol.* 2007;117(3):237-246.
- 471 47. Kexiang G, Xiaoguang L, Yonghong L, Tianbo Z, Shuliang W. Potential of
472 *Trichoderma harzianum* and *T. atroviride* to control *Botryosphaeria berengeriana* f.
473 sp. *piricola*, the cause of apple ring rot. *J Phytopathol.* 2002;150 (4-5):271-276.
- 474 48. Smith VL, Wilcox WF, Harman GE. Potential for biological-control of
475 phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp.

-
- 476 Phytopathology. 1990;80 (9):880-885.
- 477 50. Yuan L, Kerim A, Zhang LQ. Effects of NaCl stress on active oxygen metabolism
478 and membrane stability in *Pistacia Vera* seedlings. Acta Phytoecologica Sonia.
479 2005;29: 985-991.
- 480 49. Qi SW, Guan CY, Liu CL. Relationship between activity of some enzymes and
481 antibacterial nuclear disease in *Brassica napus* L. Acta Agronomica Sinica (in
482 Chinese). 2004;3, 270-273.
- 483 51. Guo YanJun, Ni Yu, Han Long, Tang Hua. Changes in physiological and
484 biochemical indexes of alfalfa leaves under procymidone stress and *sclerotinia*
485 challenge. Acta Phytophylacica Sinica (in Chinese). 2010;37 (03):283-284.

Figures

Figure 1

The left is *Bjerkandera adusta* M1 and the right is *Fusarium oxysporum* f. sp. *conglutinans*.

Figure 2

The mycoparasitism effect of *Bjerkandera adusta* M1 on FOC. The hyphae of *B. adusta* M1 directly interspersed the hyphae of FOC. FOC: *Fusarium oxysporum* f. sp. conglutinans

Figure 3

Effects of *Bjerkandera adusta* M1 and *B. adusta* M1 fermentation broth on activities of enzyme related to disease resistance in *Brassica napus* leaves. M1: *Bjerkandera adusta* M1; FB: *B. adusta* M1 fermentation broth. Data are means $\pm$ SE, $n = 3$. Different letters in the same column indicate significant differences at $P < 0.05$ by the LSD test.

Figure 4

Effects of *Bjerkandera adusta* M1 and its fermentation broth on membrane permeability (a) and malondialdehyde (b) in *Brassica napus* leaves. M1: *Bjerkandera adusta* M1; FB: *B. adusta* M1 fermentation broth. Data are means $\pm$ SE, $n = 3$. Different letters indicate significant differences at $P < 0.05$ by the LSD test.