Plant Diseases Containment and Growth Stimulators of Fungal Origin

N Pavlova¹, D Malygin¹, S Sokornova¹*

¹All-Russian Institute of Plant Protection, Laboratory of Toxicology and Biotechnology,
196608, Saint Petersburg, Russia

E-mail: svsokornova@vizr.spb.ru

Abstract. It is well known that mycopesticides have significant potential for a plant protection. For example, pathogenic fungi can induce nonspecific defence reactions and stimulate growth of non-host plants. The aim of this work is to evaluate the off-target activities of potential mycopesticides which affect germination, growth and development of crops. The strains of Stagonospora cirsii C-163, Calophoma complanata 32.121, Alternaria sonchi S-102, Beauveria caledonica BB16, BSc13Vg18, BSc25Vg18, Beauveria pseudobassiana BCu22, Beauveria bassiana T7, BHy1-06 were obtained from the pure cultures collection of the All-Russian Institute of Plant Protection. The most significant leaf spot containment (near 70%) was shown by mycelium extract S. cirsii C-163. In the case of leaf rust, the disease containment effect was lower and was observed for mycelium-based suspension of S. cirsii (near 35%). The maximum growth-promoting effect was observed for mycelium-based suspension of C. complanata. Multivariate analysis data showed that the species of B. caledonica and C. complanata are able to inhibit the development of wheat diseases and promote plant growth. Thus, in our opinion, the screening of producer strains should include the assessment of some nonspecific effects on the development and cultivation of plants.

1. Introduction

Mycopesticides have been proven to have considerable potential for successfully applied in the integrated plant protection strategies [1-4]. In addition to the main activity, pathogenic fungi can induce nonspecific defense reactions and stimulate germination, growth and development of crops, show antifungal activity etc. [5-9].

For example, the mycelium extract of Penicillium chrysogenum induces early defense-related responses such as an extracellular alkalisation in cell cultures and ethylene production in leaf plants, including Arabidopsis thaliana, tomato, tobacco and rice [10]. This strategy is being applied against Fusarium wilt of melon etc. [11-13]. The mycelium Penicillium frequentans PI909 protects the fruit against brown rot in stone fruit caused by Monilinia spp. [14]. Other isolates of P. frequentans from Picea glehnii seedling roots produce antibiotic penicillic acid [15].

Some pathogenic fungi can adopt an endophytic lifestyle. It is interesting, that fungal endophytes can limit pathogen damage in a tropical forest. In that respect, it is believed that the plant diseases containment is not caused by inducing systemic defense, because the protection of plant was primarily localized to endophyte-infected tissues [16]. The strains of Metarhizium brunneum and Beauveria bassiana demonstrates in vitro the effect against Verticillium dahlia and Cadophora helianthi, causal
agents of sunflower wilts. The competition and/or antibiosis types of antagonism were observed being dependent on the strain [17]. In contrast, for some *Beauveria* species, it has been shown that endophytic colonization of plant tissues can stimulate their growth and enhances the resistance of host-plant to drought and heat [18]. On other hand, the crude extracts of fungi can enhance plant secondary metabolites production [19, 20]. Thus, the mechanisms of the pathogenic fungi action on non-specific host plants are quite diverse.

The aim of this work is to evaluate the off-target activities of potential mycopesticides which affect germination, growth and development of crops.

2. Materials and methods

2.1. Fungal strain

The strains of *Stagonospora cirsii* С-163, *Calophoma complanata* 32.121, *Alternaria sonchi* S-102, *Beauveria caledonica* BB16, BSc13Vg18, BSc25Vg18, *Beauveria pseudobassiana* BCu22, *Beauveria bassiana* T7, BHy1-06 were obtained from the pure cultures collection of the All-Russian Institute of Plant Protection (table 1). The strains were stored at 5°C on potato-glucose agar (PGA).

| Collection number | Species               | Location                                |
|-------------------|-----------------------|-----------------------------------------|
| BB16              | *B. caledonica*       | Soil, Moskow Oblast                    |
| BSc13Vg18         | *B. caledonica*       | Vologodskaya Oblast, 2018               |
| BSc25Vg18         | *B. caledonica*       | Vologodskaya Oblast, 2018               |
| T7                | *B. bassiana*         | Soil, Kazakhstan                       |
| BHy1-06           | *B. bassiana*         | Hymenoptera adult, foothills of Zailiyskiy Alatau, Kazakhstan |
| BCu22             | *B. pseudobassiana*   | Weevil beetle adult, Kazakhstan        |
| C-163             | *S. cirsii*           | North Ossetia, Arkhonka, 2002           |
| S-102             | *A. sonchi*           | Krasnodar Krai, 2004                    |
| 32.121            | *C. complanata*       | Razmetelevo, Vsevolozhsky district, Leningrad Oblast |

2.2. Culture fluid

The 10-day inoculum was obtained in Petri plates on PGA media. Mycelium was obtained via cultivation in 250-mL flasks with 50 mL of liquid nutrient medium (sucrose - 30 g/l, soybean meal - 14 g/l, KH₂PO₄ - 1 g/l, MgSO₄×7H₂O – 0.5 g/l). The culture fluids were incubated on the orbital shaker Innova 42 (Edison, NJ, USA) at 180 rpm (revolution per minute) a temperature of 24°C for seven days. After 7 days of the cultivation at 24°C the mycelium in the stationary phase was harvested. The culture fluid was separated from the mycelium by passing through 5 layers of gauze. The filtrates were diluted 100 times with 0.001% Tween 80. The mycelium was pressed to 85% moisture by filtering through cheesecloth using a vacuum pump.
2.3. Mycelium-based compositions

2.3.1. Mycelium-based suspension. 25 mg mycelium (85% humidity) was crushed and homogenized with 0.001% Tween 80. Both the original and the autoclaved mycelium-based suspensions were processed this way.

2.3.2. Alcoholic extract. The 200 mg of mycelium was extracted with 10 ml 80% ethanol at 80°C. The precipitate was separated by centrifugation at 7000 g for 10 min. The solutions were filtrated and the solvent was evaporated. The crude extracts were tested at a concentration 0.02%.

2.4. Treatment of plants

The plants were grown at a 16-hour photoperiod, illumination 1000-1500 lux, temperature 22-24°C, during 7 day.

The plants susceptible to diseases of Saratovskaya 29 and Sudarynya varieties were inoculated with a suspension of Cochliobolus sativus spores (4×10³ spores/ml) and Puccinia tritici pustules (2000 pustules/ml). To assess the diseases containment effects the plants were sprayed with mycelium-based compositions 24 h prior to pathogen inoculation (30 ml per 100 plants) [21].

2.5. Evaluation of disease development

The development of the disease was assessed on 7th and 14th days after inoculation by the area of necrosis and its prevalence in relation to the control (%). 0.001% Tween 80 was used as a control.

2.6. Evaluation of growth stimulating effect

Evaluation of growth stimulating activity was carried out according to the growth rate of roots and seedlings. The 100 grains were placed between two sheets of filter paper in rolls. These rolls were placed into 500 ml glasses, each containing 50 ml of composition [22]. The compositions were used: 1 – mycelium alcoholic extract (100-fold); 2 – culture fluid (100-fold); 3 – mycelium-based suspension (humidity 85%, 25 mg / ml); 4 – autoclaved mycelium-based suspension (25 mg / ml).

2.7. Data analysis

The experiments were carried out in two biological and three analytical repeats. The data were processed using analysis of variance, statistical and multivariate analysis in Excel with the XLSTAT.

3. Results and discussion

The disease containment effect of the different compositions varied depending on the disease type. The most significant leaf spot containment (near 70%) was shown by mycelium extract S. cirsii C-163. In the case of leaf rust, the disease containment effect was lower. It was observed for mycelium-based suspension of S. cirsii at the rate of approx. 35 (figures 1-2).

The maximum growth-stimulating effect was observed for mycelium-based suspension of C. complanata. Alcoholic extracts of B. bassiana, B. pseudobassiana, S. cirsii и A. sonchi had a negative effect on the growth of wheat roots (figure 3). We believe that this is due to the fact that alcoholic extracts may contain compounds with phytotoxic activity. For example, it was previously shown that some extracts of B. bassiana BBL and B. pseudobassiana demonstrated phytotoxic activity against Elytrigia repens и Sonchus arvensis [23]. In turn, compounds with phytotoxic activity against Sonchus arvensis were obtained for the strains of S. cirsii S-47 and A. sonchi S-102 [24, 25]. At the same time, alcoholic extracts of B. caledonica and C. complanata had no effect on wheat development.

Multivariate analysis data using agglomerative hierarchical clustering showed that the species of B. caledonica and C. complanata are able to inhibit the development of wheat diseases and promote plant growth.
Thus, in our opinion, the screening of producer strains should include the assessment of some nonspecific effects on the development and cultivation of plants.

**Figure 1.** Evaluation of the development of spot blotch, caused by *Cochliobolus sativus*, on the 7th day after inoculation. 1 – mycelium alcoholic extract (100-fold); 2 – culture fluid (100-fold); 3 – mycelium-based suspension (humidity 85%, 25 mg / ml); 4 – autoclaved mycelium-based suspension (25 mg / ml). Least significant difference (LSD) 0.05 = 6.4, bars indicate standard deviation at p = 0.05.

**Figure 2.** Evaluation of the development of leaf rust caused by *Puccinia triticina*, on the 14th day after inoculation. 1 – mycelium alcoholic extract (100-fold); 2 – culture fluid (100-fold); 3 – mycelium-based suspension (humidity 85%, 25 mg / ml); 4 – autoclaved mycelium-based suspension (25 mg / ml). Least significant difference (LSD) 0.05 = 6.2, bars indicate standard deviation at p = 0.05.
Figure 3. Evaluation of the growth stimulating activity of the compositions, on the 4th day after treatment. 1 – mycelium alcoholic extract (100-fold); 2 – culture fluid (100-fold); 3 – mycelium-based suspension (humidity 85%, 25 mg / ml); 4 – autoclaved mycelium-based suspension (25 mg / ml). Least significant difference (LSD) 0.05 = 5.7, bars indicate standard deviation at p = 0.05.

4. References

[1] Bailey K L, Boyetchko S M and Langle T 2010 Social and economic drivers shaping the future of biological control: a Canadian perspective on the factors affecting the development and use of microbial biopesticides Biol. Contr. 52 3 221–9

[2] Lengai G and Muthomi J 2018 Biopesticides and their role in sustainable agricultural production J. Biosci. Med. 6 7–41

[3] Berestetskiy A and Sokornova S 2018 Production and stabilization of mycoherbicides Biological approaches for controlling weeds Publisher: IntechOpen pp 63-88

[4] Levchenko M V, Kononchuk A G, Gerus A V and Lednev G R Differential susceptibility of locusta migratoria and schistocerca gregaria (orthoptera: acrididae) to infection with entomopathogenic fungi 2020 Plant Protection News 103 2 150-52

[5] Chen M, Zeng H, Qiu D, Guo L, Yang X and Sh H 2012 Purification and characterization of a novel hypersensitive response-inducing elicitor from Magnaporthe oryzae that triggers defense response in rice PLoS One 5 1–12

[6] Wiesel L, Newton A C, Elliott I, Booty D, Gilroy EM, Birch PRJ and Hein I 2014 Molecular effects of resistance elicitors from biological origin and their potential for crop protection FPLS 5 1–13

[7] Zhang Y, Yang X, Zeng H, Guo L, Yuan J and Qiu D 2014 Fungal elicitor protein PebC1 from Botrytis cinerea improves disease resistance in Arabidopsis thaliana Biotechnol. Lett. 36 1069–78

[8] Chen Z Z, Wang J G, Li Y, Zhong Y, Liao J G, Lu S G, Wang L, Wang X W and Chen S Y
2018 Dry mycelium of *Penicillium chrysogenum* activates defense via gene regulation of salicylic acid and jasmonic acid signaling in arabidopsis *Physiol. Mol. Plant Pathol.* **103** 54–61

[9] Ramos Y, Taibo AD, Jiménez JA and Porta O 2020 Endophytic establishment of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* larvae. *Egypt. J. Biol. Pest Co* **30** 1–6

[10] Thuerig B et al 2006 An aqueous extract of the dry mycelium of *Penicillium chrysogenum* induces resistance in several crops under controlled and field conditions *Eur. J. Plant Pathol.* **114** (2) 185–97

[11] Dong H and Cohen Y 2001 Extracts of killed *Penicillium chrysogenum* induce resistance against *Fusarium* wilt of melon *Phytoparasitica* **29** 421–30

[12] Dong H and Cohen Y 2002 Dry mycelium of *Penicillium chrysogenum* induces resistance against *Verticillium* wilt and enhances growth of cotton plants *Phytoparasitica* **30** 2 147–57

[13] Tamm L, Thüürig B, Fliesbach A, Goltlieb A E, Karavani S and Cohen Y 2011 Elicitors and soil management to induce resistance against fungal plant diseases *Njas-Wagen.* *J. Life Sc.* **58** 131-37

[14] Guijarro B, Larena I, Casals C, Teixidó N, Melgarejo P and De Cala A 2019 Compatibility interactions between the biocontrol agent *Penicillium frequentans* Pf909 and other existing strategies to brown rot control *Biol. Contr.* **129** 45–54

[15] Yamaji K, Fukushima Y, Hashidoko Y and Tahara S. *Penicillium frequentans* isolated from *Picea glehnii* seedling roots as a possible biological control agent against damping-off 2005 *Ecol. Res.* **20** 1 103-07

[16] Arnold A E, Mejia L C, Kyllo D, Rojas E I, Maynard Z, Robbins N and Herre E A 2003 Fungal endophyte limit pathogen damage in a tropical tree *PNAS* **100** 15649-54

[17] McGuire A V and Northfield T D 2020 Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae* *FSUFS* **4** 6

[18] Kuzhuppillymyal-Prabhakarankutti L, Tamez-Guerra P, Gomez-Flores R, Rodriguez-Padilla MC and Ek-Ramos M J 2020 Endophytic *Beauveria bassiana* promotes drought tolerance and early flowering in corn *World J. Microb. Biot.* 36–47

[19] Liang C X, Chen C, Zhou P F, Xu L, Zhu J H, Liang J C, Zi J C and Yu R M 2018 Effect of *Aspergillus flavus* fungal elicitor on the production of terpenoid indole alkaloids in *Catharanthus roseus* cambial meristematic cells *Molecules* **23** 12 3276

[20] Salehi M, Moieni A, Safaie N and Farhad S 2019 Elicitors derived from endophytic fungi *Chaetomium globosum* and *Paraconiothyrium brasilienise* enhance paclitaxel production in *Corylus avellana* cell suspension culture *Plant Cell Tiss. Org.* **136** 1 161–71

[21] Mikhailova L A 2006 *Genetics of relationships of leaf rust pathogen and wheat* (St. Petersburg: VIZR Publ.) 80

[22] Likhachev B S 1975 Determination of the growth force of seeds of grain crops by morphophysiological assessment of seedlings (Leningrad: VASKhNIL) 15

[23] Berestetskiy A O, Ivanova A N, Petrova M O, Stepanycheva E A, Lednev G R, Prokof’eva D S and Usanov A M Comparative analysis of the biological activity and chromatographic profiles of the extracts of *Beauveria bassiana* and *B. pseudobassiana* cultures grown on different nutrient substrates 2018 *Microbiology* **87** 2 200-14

[24] Dalinova A, Dubovik V, Chisty L, Kochura D, Ivanov A, Smirnov S, Petrova M, Zolotarev A, Evidente A and Berestetskiy Á 2019 Stagonolides J and K and Stagochromene A, two new natural substituted nonenolides and a new disubstituted chromene-4.5-dione isolated from *Stagonospora cirsii* S-47 proposed for the biocontrol of *Sonchus arvensis* *J. Agr. Food Chem.* **67** 47 13040-50

[25] Dalinova A, Chisty L, D. Kochura D, Garnyuk V, Petrova M, Prokof’eva D, Yurchenko A, Dubovik V, Ivanov A, Smirnov S, Zolotarev A and Berestetskiy A 2020 Isolation and bioactivity of secondary metabolites from solid culture of the fungus *Alternaria sonchi* *Biomolecules* **10** 81
Acknowledgments
The research is supported by the Russian Science Foundation, project № 16-16-00085 (development of technologies for production and application of mycoherbicides for control of troublesome weeds) and 20-66-46009 (data analysis and interpretation), as well as The Federal Fundamental Scientific Research Program for 2013-2020, project № AAAA-A16-116080510099-8 (ecology phyllosphere fungal communities and biorational mycoherbicides). The research was performed using equipment of the Core Centrum “Innovative plant protection technologies” at the All-Russian Institute of Plant Protection (St. Petersburg, Russia).