ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 5, pp. 523–530. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © A.O. Berestetskiy, E.L. Gasich, E.V. Poluektova, E.V. Nikolaeva, S.V. Sokornova, L.B. Khlopunova, 2014, published in Mikrobiologiya, 2014, Vol. 83, No. 5, pp. 534–542.

EXPERIMENTAL ARTICLES

Biological Activity of Fungi from the Phyllosphere of Weeds and Wild Herbaceous Plants

A. O. Berestetskiy[1], E. L. Gasich, E. V. Poluektova, E. V. Nikolaeva, S. V. Sokornova, and L. B. Khlopunova

All-Russian Institute of Plant Protection, St. Petersburg, 196608 Russia

Received January 9, 2014

Abstract—Antimicrobial, phytotoxic, and insecticidal activity of 30 fungal isolates obtained from leaves of weeds and wild herbaceous plants was assessed. Antibacterial, antifungal, phytotoxic, and insecticidal activity was found in over 50%, 40, 47, and 40% of the isolates, respectively. These findings may be important for toxicological assessment of potential mycoherbicides, and may also provide a basis for investigation of the patterns of development of phyllosphere communities affected by fungal metabolites.

Keywords: antimicrobial activity, phytotoxicity, aphicidal activity, fungi, phyllosphere

DOI: 10.1134/S0026261714050051

Phyllosphere fungi, in particular, endophytic micromycetes, which are thought to include mildly pathogenic and saprotrophic species of the genera *Altermaria, Colletotrichum, Phoma,* and *Phomopsis*, have lately been attracting researchers' attention as potential producers of biologically active compounds. Several recent reviews describe the structure and properties of bioactive metabolites produced by endophytes whose hosts are mostly either medicinal plants or trees of tropical forests [1–4]. However, fungi from the phyllosphere of weeds and wild herbaceous plants have been insufficiently studied.

In the present work, 30 isolates of anamorphic ascomycetes (both phytopathogenic and endophytic) obtained from above-ground organs of weeds and wild plants were screened to identify potential producers of bioactive compounds. By choosing this group of fungi, we aimed to address several tasks: (1) to search for producers of herbicidal, antimicrobial, and insecticidal metabolites; (2) to evaluate the environmental safety of potential mycoherbicides; (3) to investigate the role of fungal secondary metabolites in the biocenosis of the herbaceous plant phyllosphere.

MATERIALS AND METHODS

The study was performed with 30 fungal isolates from the pure culture collection of the Mycology and Phytopathology Laboratory of the Institute of Plant Protection, including nine endophytes and 21 phytopathogens. Cultures were stored at 5°C on a standard potato glucose agar (PGA) medium. To obtain the inocula, fungal strains were grown on PGA for 2 weeks at 24°C. The fungal species studied are listed in the table.

Antimicrobial properties of fungi were studied using the following microorganisms: gram-negative *Pseudomonas syringae* pv. *lachrymans* strain 11 (Institute of Phytopathology), gram-positive *Bacillus subtilis* strain VNIISKM 78, and *Candida tropicalis*. They were cultured on PGA at 30°C.

Phytotoxic activity of fungal extracts was tested on cut-off leaves of *Arabidopsis thaliana* and 4-cm-long leaf fragments of couch grass (*Elytrigia repens*). Plants were grown in pots containing a standard soil mixture at 24°C (day) and 20°C (night) with artificial illumination (16 : 8 h). Cut leaves were incubated in a humid chamber, i.e., a transparent plastic container lined with moist filter paper.

To perform a primary screening for producers of antibiotic compounds using the agar block method, fungi were cultured on three media containing 1.5% agar–agar: PGA, Czapek's medium supplemented with vitamins (CZA), and yeast extract–peptone–glucose (YEPG) medium. Fungi were incubated at 24°C for 3 weeks.

For liquid-surface cultures, fungi were grown in liquid YEPG. The medium (100 mL in 500-mL conical vessels) was sterilized at 109°C for 30 min and inoculated with a 5-mm bullet excised at the border of the plated culture. Fungal cultures were incubated at the constant temperature of 24°C for 14 days without shaking.

Fungal extracts were obtained by filtering the cultural liquid (100 mL) to remove the mycelium and thrice extracting the liquid with 50, 100, and 150 mL of methylene chloride. The extracts were combined and dehydrated by filtering through water-free sodium

[1] Corresponding author; e-mail: aberestetski@yahoo.com

List of fungal isolates studied

Species	Isolate	Ecological group	Host plant	Geographical origin
Acremonium sp.	16.5	Endophyte	*Convolvulus arvensis*	St. Petersburg
Epicoccum nigrum	5.14	Endophyte	*Cirsium arvense*	Leningrad oblast
Vermicularia sp.	13.20	Endophyte	*Heracleum sosnowskyi*	Leningrad oblast
Botrytis cinerea	47.1	Phytopathogen	*Galinsoga parviflora*	Sumy oblast, Ukraine
Sclerotinia sclerotiorum	Scl	Phytopathogen	*Cannabis sativa*	Penza oblast
Ascochyta chenopodiicola	17.28	Phytopathogen	*Chenopodium album*	Penza oblast
Bipolaris sorokiniana	39.3	Phytopathogen	*Atriplex* sp.	Penza oblast
Chaetopyrena sp.	52.2	Endophyte	*Chenopodium album*	Harbin, China
	52.5	Endophyte	*Convolvulus arvensis*	Krasnodar krai
Colletotrichum gloeosporioides	13.3	Phytopathogen	*Galinsoga parviflora*	Sumy oblast, Ukraine
Coniothyrium sp.	53.6	Endophyte	*Chenopodium album*	Harbin, China
Passalora dubia	38.23	Phytopathogen	*Ch. album*	Samara oblast
Phoma sp.	9.247	Phytopathogen	*Artemisia vulgaris*	Vladivostok
	17.19	Phytopathogen	*Convolvulus arvensis*	Chui oblast, Kyrgyzstan
	32.49	Phytopathogen	*Chenopodium* sp.	Penza oblast
	32.97	Phytopathogen	*Arctium tomentosum*	Khabarovsk krai
	32.136	Phytopathogen	*Arctium* sp.	Irkutsk
	22.2	Phytopathogen	*Chenopodium urbicum*	Oryol oblast
	22.5	Phytopathogen	*Ch. urbicum*	Krasnodar
	32.137	Phytopathogen	*Chenopodium* sp.	Irkutsk
	17.18	Phytopathogen	*Ch. album*	Leningrad oblast
	17.52	Phytopathogen	*Ch. album*	Lipetsk oblast
Septoria atriplicis	9.105	Phytopathogen	*Ch. album*	Harbin, China
S. calystegiae	9.301	Phytopathogen	*Calystegia sepium*	St. Petersburg
	9.302	Phytopathogen	*Convolvulus arvensis*	Derbent, Dagestan
Stagonospora convolvuli	9.296	Phytopathogen	*C. arvensis*	St. Petersburg
Phomopsis asteriscus	61.4	Phytopathogen	*Heracleum sibiricum*	Leningrad oblast
Ph. albicans	32.22	Endophyte	*Lepidotheca suaveolens*	Valaam, Karelia
Ph. malvacearum	61.2	Endophyte	*Abutilon theophrastii*	Primorskii krai
Ph. morphaea	3.1	Endophyte	*Papaver rhoeas*	Stavropol krai

sulfate. After the solvent was evaporated, the solid residue was weighted and its biological activity was analyzed.

Antibiotic activity of extracts was assessed using the standard agar block and paper disk techniques (100 μg extract per disk) [5]. Phytoxicity of 5 mg/mL extract solutions was determined using the leaf disk method [6]. Contact insecticidal activity of 0.4% extract solutions was assessed in larvae of the vetch aphid [7].

Experiments were performed in at least three replicates. Mean values were compared using the least significant difference test (LSD) at the probability level of 95%. Calculations were performed with Statistica 6.1.

RESULTS AND DISCUSSION

Evaluation of antimicrobial activity using the agar block method. We found that antimicrobial activity against the three test microorganisms—as assessed using the agar block method—varied significantly among the 30 fungal isolates. Antibacterial activity was

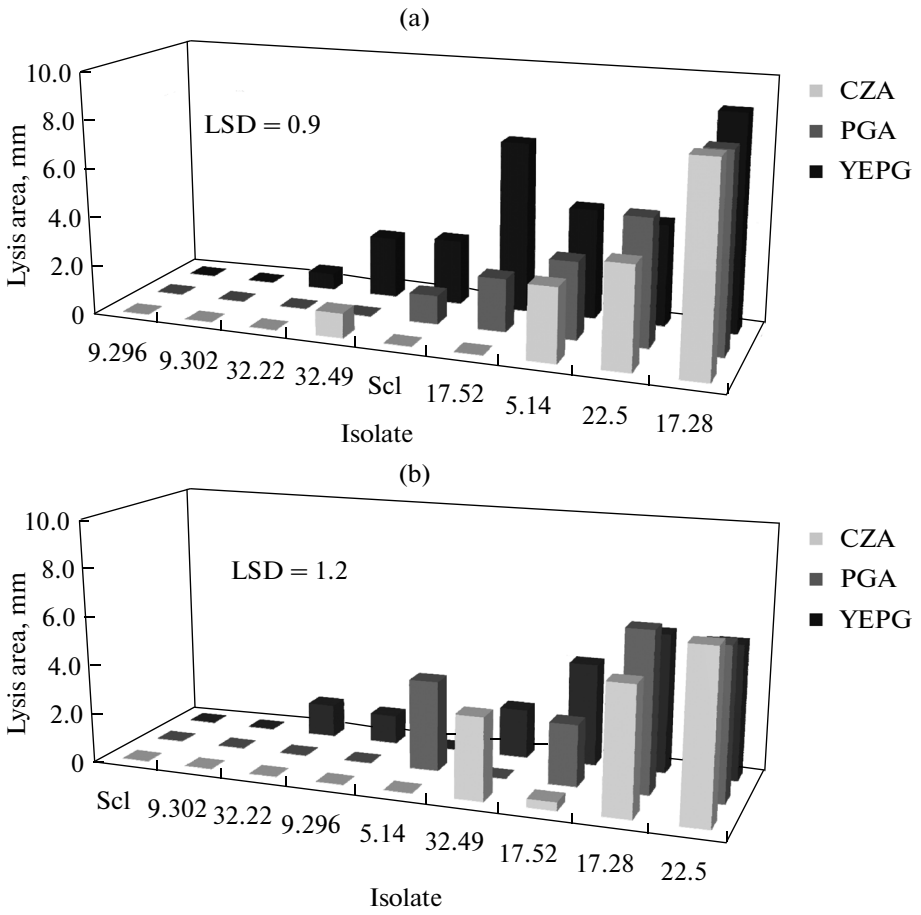

Fig. 1. Antibacterial activity of phylloshere fungi against *Bacillus subtilis* (a) and *Pseudomonas syringae* (b). Here and in Figs. 2–5, the results shown differ from control at the significance level of $P = 0.05$.

observed in nine isolates (30% of all micromycetes studied). However, only seven fungal isolates (23%) were active against both bacterial species tested. On the whole, the highest level of antibacterial activity was observed for *Ascochyta chenopodiicola* 17.28 and *Phoma* sp. 22.5 (Fig. 1). The same isolates, along with *Epicoccum nigrum* 5.14, strongly inhibited the growth of *Candida tropicalis* (Fig. 2).

Antibiotic activity of fungi depended considerably on the composition of the agar-containing culture medium. Producers of antibacterial metabolites were detected best when grown on YEPG, whereas PGA cultures were best suited to identify antifungal activity. There was a significant interaction between the medium composition and the fungal isolate. For instance, the growth of both species of test bacteria was inhibited most by *A. chenopodiicola* 17.28 and *Phoma* sp. 17.52 cultures grown in YEPG. Antibacterial properties of *Phoma* sp. 22.5 were most pronounced in CZA and PGA cultures (Fig. 1a). *Epicoccum nigrum* 5.14 cultures grown in all three media were active against *Bacillus subtilis* but only PGA cultures were active against *Pseudomonas syringae* (Fig. 1). *Sclerotinia sclerotiorum* inhibited the growth of *B. subtilis* when cultured on PGA and YEPG, whereas *P. syringae* was not at all sensitive to its exometabolites. *Phoma* sp. 32.49 inhibited both bacterial species when grown on CZA and YEPG (Fig. 1a). Cultures of *Stagonospora convolvuli* 9.296 and *Phomopsis albicans* 32.22 grown on YEPG showed weak activity against *P. syringae* (Fig. 1b).

The highest antifungal activity was observed for *E. nigrum* 5.14 and *A. Chenopodiicola* 17.28 grown on PGA and CZA; *Phoma* sp. 22.5 was active when grown on PGA and YEPG. *Phoma* sp. 32.49 showed moderate activity on all culture media used. The growth of *C. tropicalis* was also inhibited by *S. convolvuli* 9.296 and *Septoria calystegiae* 9.302 (Fig. 2).

These results suggest that screening for potential producers of antimicrobial metabolites should be performed using different types of growth substrates.

This study has been the first to detect antimicrobial activity of *A. chenopodiicola*. Antimicrobial properties of *Ascochyta* spp. are usually due to ascochitine, an azaphilonoid compound [8]. Fungi of the genus *Phoma* produce a range of antimicrobial compounds of various structures [9]. Antimicrobial (in particular,

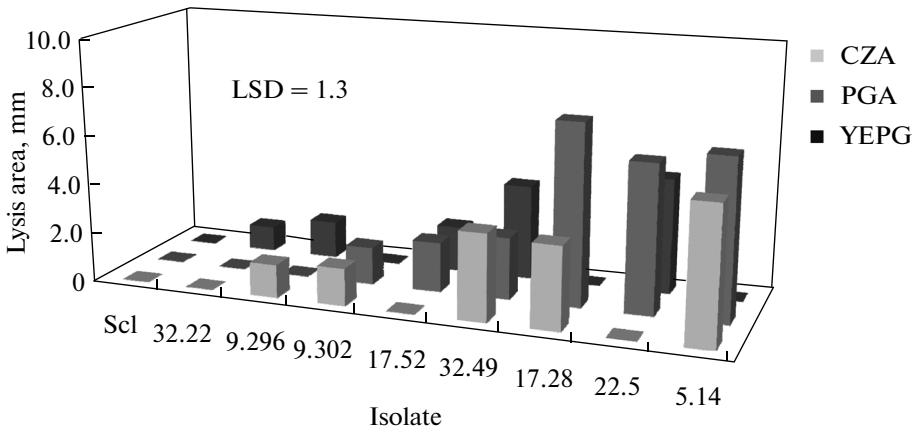

Fig. 2. Antifungal activity of phyllosphere fungi against *Candida tropicalis*.

antifungal) properties of the genus *Epicoccum* have been reported previously [10] and are currently being extensively investigated [11, 12]; they are also used for biofungicide development [13, 14].

Antimicrobial activity of fungal extracts. For most fungal isolates studied, the yield of extractives from the culture filtrate did not exceed 50 mg/L. However, for some isolates (*Chaetopyrena* sp. 52.2, *Phomopsis morphaea* 3.1, *Ph. asteriscus* 61.147, *Passalora dubia* 38.23, *Sclerotinia sclerotiorum*, and *Phoma* sp. 32.49), the yield of extractives was higher and ranged from 50 to 100 mg/mL. The highest yield was obtained for *Bipolaris sorokiniana* 39.3 (175 mg/mL) and *Coniothyrium* sp. 53.6 (402 mg/mL).

Extracts of 13 fungal isolates (43%) significantly inhibited the growth of *B. subtilis*; the strongest activity was observed for the extracts of *B. sorokiniana* 39.3, *Coniothyrium* sp. 53.6, and *P. dubia* 38.23 (Fig. 3a).

The number of fungal extracts active against *P. syringae* was considerably lower: there were eight such isolates (27%). The growth of *P. syringae* was most efficiently inhibited by extracts from culture filtrates of *Coniothyrium* sp. 53.6, *Phoma* sp. 32.136, and *Phoma* sp. 32.137 (Fig. 3b).

Only five isolates (17%) produced extracts that showed antifungal activity against *Candida tropicalis*; among them, extracts of *Bipolaris sorokiniana* 39.3 and *Coniothyrium* sp. 53.6 were the most efficient ones (Fig. 3c).

The results of these experiments did not correlate with the data on antimicrobial activity of fungi as assessed using agar blocks. Most probably, this fact reflects the different chemical nature of antimicrobial compounds produced by the fungi. Extraction with methylene chloride results in the isolation of mainly lipophilic compounds, which cannot easily diffuse through an agar-containing medium.

In the available sources, there is little information on antimicrobial properties of *B. sorokiniana*, a phytopathogenic soil fungus. Some data suggest that it produces sterigmatocystin, a mycotoxin also possessing antibacterial activity [15, 16]. Fungi of the genus *Coniothyrium* are known producers of bioactive compounds. In particular, a hyperparasitic fungus *C. minitans* produces a macrolide antibiotic macrosphelide A, which suppresses the growth of fungi and gram-negative bacteria [17]. However, the extract of *Coniothyrium* sp. 53.6 was also active against *B. subtilis*, a gram-positive bacterium. Antimicrobial properties of *Passalora dubia*, an agent causing leaf spots in different *Chenopodium* species, have not been previously described. A closely related peanut pathogen, *P. arachidicola*, produces dothistromin, an anthraquinone pigment exhibiting light-dependent antimicrobial activity [18].

Thus, two different tests of antimicrobial activity showed that many phyllosphere fungi can produce antibiotic compounds. Altogether, 57% of fungal isolates studied produced antibacterial metabolites, and 40% of isolates produced antifungal metabolites. On the whole, antimicrobial metabolites were produced by six of nine (67%) endophytic fungi and by 11 of 21 (52%) phytopathogenic isolates.

Phytotoxic activity of fungal extracts. Significant phytotoxic activity against *A. thaliana* was observed for 14 extracts of fungal isolates (47% of the total number), and 12 isolates (40%) were active against *E. repens* (Fig. 4). Among endophytic fungi, extracts of five isolates were active against both plant species tested (56% of all endophytes studied), and there were ten such isolates among phytopathogens (47%). Interestingly, nearly all phytotoxic extracts also exhibited antimicrobial properties. Extracts of *B. sorokiniana* 39.3, *Coniothyrium* sp. 53.6, and *P. dubia* 38.23 showed the highest level of nonselective toxicity in the plants tested (Fig. 4).

B. sorokiniana is known to produce terpenoid toxins [19]; phytotoxins of *P. dubia* have not been studied so far, and dothistromin, the above-mentioned mycotoxin of *P. arachidicola*, is also phytotoxic [18]. We

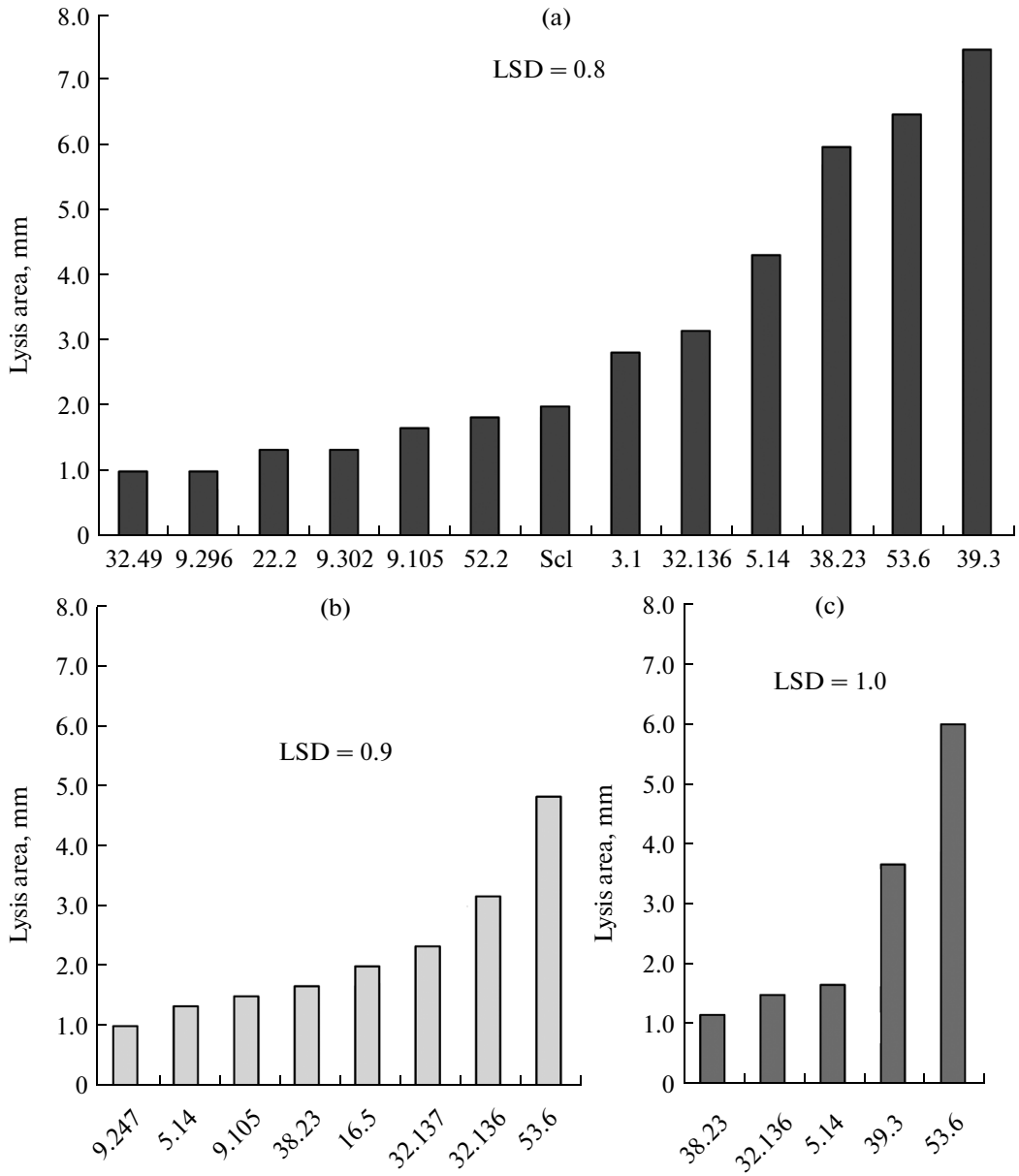

Fig. 3. Antibiotic activity of extracts fungal culture filtrates against *Bacillus mesentericus* (a), *Pseudomonas syringae* (b), and *Candida tropicalis* (c).

did not find any information on the phytotoxic properties of *Coniothyrium* spp. in the available sources.

Phytotoxin production is a typical trait of phytopathogenic ascomycetes [20, 21], but it has been little studied in endophytic fungi. However, our results show that fungi of this ecological group (e.g., *E. nigrum* 5.14, *Chaetopyrena* spp. 52.2 and 52.3, *Phomopsis* spp.) are also capable of producing phytotoxic metabolites.

Insecticidal activity of fungal extracts. Twelve fungal isolates (40% of the total number) showed a significant, although rather low, insecticidal activity against larvae of the vetch aphid. Two of them were endophytic fungi (22% of all endophytes studied) and ten were phytopathogens (50% of all plant-pathogenic fungi studied). Only three fungal isolates (*Phoma* sp. 22.2, *Acremonium* sp. 16.5, and *Colletotrichum gloeosporioides* 13.3) produced extracts that killed at least 20% of vetch larvae (Fig. 5). These three isolates also exhibited other types of biological activity (Figs. 1–4).

It was reported previously that endophytic fungi of the genus *Acremonium* can produce alkaloids with insecticidal properties [22, 23]. In phytopathogenic *Colletotrichum* and *Phoma* spp., insecticidal activity has been observed for the first time. Insecticidal activ-

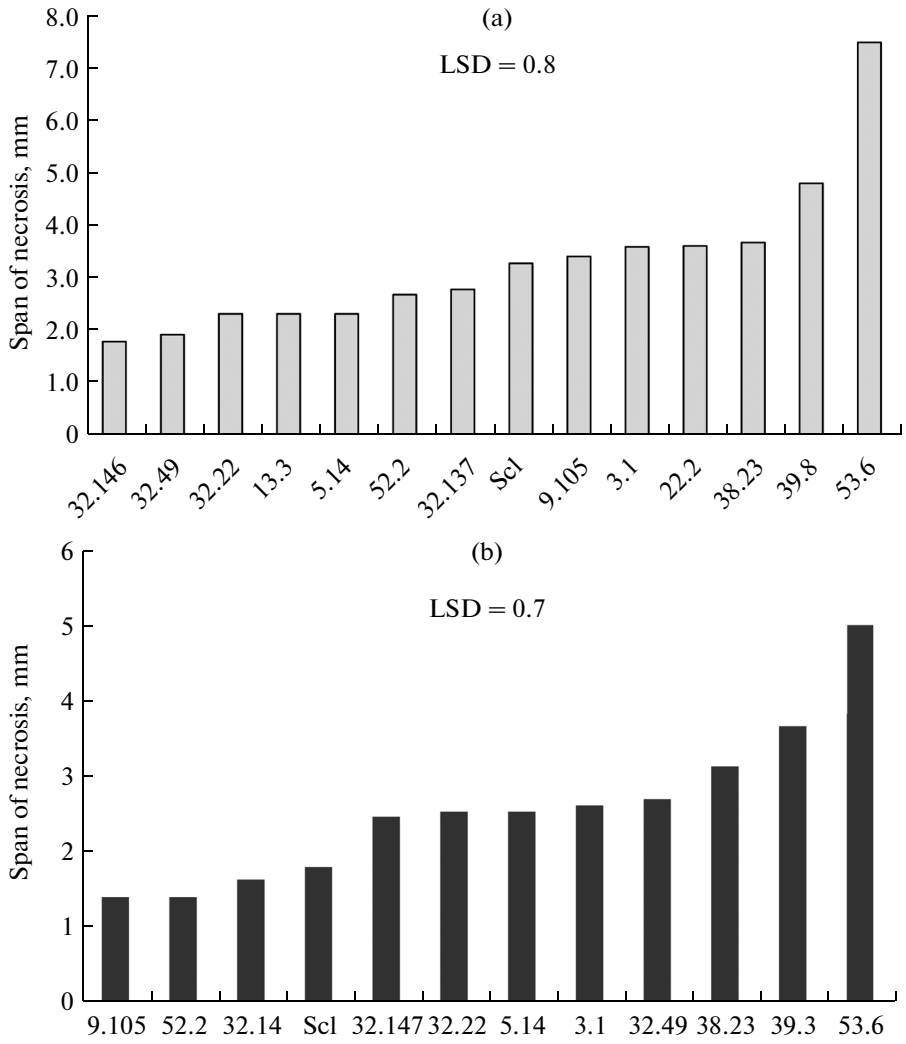

Fig. 4. Phytotoxic activity of extracts of fungal culture filtrates against *Arabidopsis thaliana* (a) and *Elytrigia repens* (b).

ity was also described in some other endophytic and phytopathogenic fungi [24, 25].

The technique used in this work to assess insecticidal activity against vetch aphid actually determined only the contact insecticidal activity of fungal extracts. However, it was recently reported that phytotoxins of some micromycetes can possess larvicidal [26] and deterrent properties [27]. Therefore, further investigation of insecticidal properties of phytopathogenic and endophytic fungi should involve a wider range of test objects and techniques appropriate for assessment of insecticidal activity of fungal metabolites [28].

Our study showed that phytopathogenic and endophytic fungi of the phyllosphere of weeds and wild herbaceous plants may exhibit a wide range of biological activity and produce bioactive compounds. Presumably, these fungi and their metabolites play an important role in phyllosphere communities by suppressing the growth of sensitive microorganisms (e.g., phytopathogenic bacteria) and by affecting the behavior of phytophagous insects. It seems rather interesting that the insecticidal potential was higher in phytopathogens than in endophytes, while phytotoxic properties of endophytes and phytopathogens fungi were similar. Our study also confirmed that plant-pathogenic fungi can act as producers of antimicrobial compounds [29]. However, the body of data collected is currently not large enough to clearly identify the ecological and taxonomic groups of fungi most promising for further screening studies.

Our results should be taken into account during the development of safety evaluation procedures for mycoherbicides, which are a priori considered less dangerous than chemical pesticides. Obviously, investigation of their potential effects on useful species should be just as thorough as toxicological evaluation of chemical pesticides. For instance, metabolites of *Ascochyta caulina*, a potential mycoherbicide against *Chenopodium album*, were classified as class II hazard-

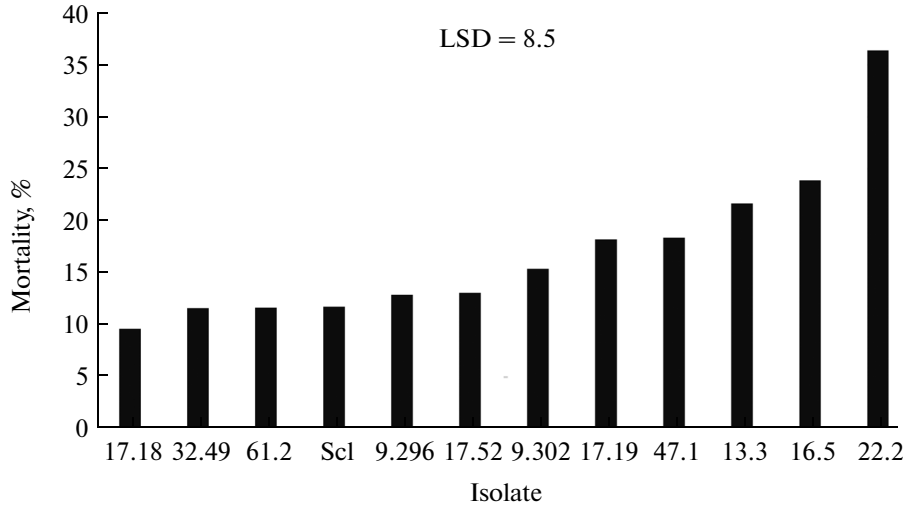

Fig. 5. Contact insecticidal activity of extracts of fungal culture filtrates against larvae of the vetch aphid.

ous materials based on toxicological tests in model species of soil and freshwater animals [30].

On the other hand, biological efficiency of mycoherbicides may be to a certain degree affected by the presence of endophytic fungi on particular target weeds. For instance, *E. nigrum* 5.14, isolated from leaves of the creeping thistle, showed a strong antifungal activity. Thus, mycobiota of target weeds requires detailed investigation, considering both phytopathogens (potential microherbicides) and endophytes (which protect their hosts from external enemies). For example, the activity of endophytic fungi inhabiting grapevines inhibits both saprotrophic and endogenous microflora [31].

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 12-04-00853).

REFERENCES

1. Firáková, S., Šturdíková, M., and Múcková, M., Bioactive secondary metabolites produced by microorganisms associated with plants, *Biologia*, 2007, vol. 62, no. 3, pp. 251–257.
2. Aly, A.H., Edrada-Ebel, R., Indriani, I.D., Wray, V., Müller, W.E.G., Totzke, F., Zirrgiebel, U., Schächtele, C., Kubbutat, M.H.G., Lin, W.H., Proksch, P., and Ebel, R., Cytotoxic metabolites from the fungal endophyte *Alternaria* sp. and their subsequent detection in its host plant *olygonum senegalense, J. Nat. Prod.*, 2008, vol. 71, pp. 972–980.
3. Hussain, H., Ahmed, I., Schulz, B., Draeger, S., and Krohn, K., Pyrenocines J-M: four new pyrenocines from the endophytic fungus, *Phomopsis* sp., *Fitoterapia*, 2012, vol. 83, pp. 523–526.
4. Wang, L.-W., Xu, B.-G., Wang, J.-Y., Su, Z.-Z., Lin, F.-C., Zhang, C.-L., and Kubicek, C.P., Bioactive metabolites from *Phoma* species, an endophytic fungus from the Chinese medicinal plant *Arisaema erubescens, Appl. Microbiol. Biotechnol.*, 2012, vol. 93, pp. 1231–1239.
5. Egorov, N.S., *Osnovy ucheniya ob antibiotikakh* (Basics of Antibiotics Science), Moscow: Nauka, 2004.
6. Berestetskii, A.O., Yuzikhin, O.S., Katkova, A.S., Dobrodumov, A.V., Sivogrivov, D.E., and Kolombet, L.V., Isolation, identification, and characteristics of the phytotoxin produced by the fungus *Alternaria cirsinoxia, App. Biochem. Microbiol.*, 2010, vol. 46, no. 1, pp. 75–79.
7. Mitina, G.V., Yuzikhin, O.S., Isangalin, F.Sh., and Yakimov, A.P., Isolation and chemical structural investigation of the insecticidal toxin from the fungus *Lecanicillium muscarium, Nauchn. Priborostr.*, 2012, vol. 22, no. 2, pp. 3–10.
8. Oku, H. and Nakanishi, T., A toxic metabolite from *Ascochyta fabae* having antibiotic activity, *Phytopathology*, 1963, vol. 53, pp. 1321–1325.
9. Poluektova, E.V. and Berestetskiy, A.O., Secondary metabolites of the *Phoma* spp. fungi: structure, activity, and practical importance, *Mikol. Fitopatol.*, 2013, vol. 47, no. 5, pp. 281–289.
10. Mallea, M., Pesando, D., Bernard, P., and Khoulalene, B., Comparison between antifungal and antibacterial activities of several strains of *Epicoccum purpurascens* from the Mediterranean area, *Mycopathologia*, 1991, vol. 115, pp. 83–88.
11. Kaliňak, M., Baratova, V., Gallova, E., Ondrušova, Z., and Hudecova, D., Secondary metabolite production of *Epicoccum* sp. isolated from lignite, *Acta Chimica Slovaca*, 2013, vol. 6, no. 1, pp. 42–48.
12. Talontsi, F.M., Dittrich, B., Schüffler, A., Sun, H., and Laatsch, H., Epicoccolides: antimicrobial and antifungal polyketides from an endophytic fungus *Epicoccum* sp. associated with *Theobroma cacao, Eur. J. Org. Chem.*, 2013, vol. 2013, no. 15, pp. 3174–3180.

13. Zhou, T. and Reeleder, R.D., Application of *Epicoccum purpurascens* spores to control white mold of snap bean, *Plant Dis.*, 1989, vol. 73, pp. 639–642.
14. Mari, M., Torres, R., Casalini, L., Lamarca, N., Mandrin, J.F., Lichou, J., Larena, I., De Cal, M.A., Melgarejo, P., and Usall, J., Control of post-harvest brown rot on nectarine by *Epicoccum nigrum* and physicochemical treatments, *J. Sci. Food Agric.*, 2007, vol. 87, pp. 1271–1277.
15. Perry, M.J., Adlard, M.W., and Holt, G., The isolation of a fungal metabolite which exhibits antimicrobial synergy with sterigmatocystin, *J. App. Bacteriol.*, 1982, vol. 52, no. 1, pp. 83–89.
16. Maes, C.M. and Steyn, P.S., Polyketide-derived fungal metabolites from *Bipolaris sorokiniana* and their significance in the biosynthesis of sterigmatocystin and aflatoxin B1, *J. Chem. Soc., Perkin Trans.*, 1984, vol. 1, pp. 1137–140.
17. Tomprefa, N., McQuilken, M.P., Hill, R.A., and Whipps, J.M., Antimicrobial activity of *Coniothyrium minitans* and its macrolide antibiotic macrosphelide A, *J. App. Microbiol.*, 2009, vol. 106, no. 6, pp. 2048–2056.
18. Stoessl, A., Abramowski, Z., Lester, H.H., Rock, G.L., and Towers, G.H.N., Further toxic properties of the fungal metabolite dothisrromin, *Mycopathologia*, 1990, vol. 112, pp. 179–186.
19. Kachlicki, P., *Helminthosporia Metabolites, Biology, Plant Diseases*, Cheł kowski, J., Ed., Poznan: Inst. Plant. Genet., 1995, pp. 1–26.
20. Berestetskiy, A.O., A review of fungal phytotoxins: from basic studies to practical use, *Appl. Biochem. Microbiol.*, 2008, vol. 44, no. 5, pp. 453–465.
21. Stergiopoulos, I., Collemare, J., Mehrabi, R., and De Wit, P.J., Phytotoxic secondary metabolites and peptides produced by plant pathogenic dothideomycete fungi, *FEMS Microbiol. Rev.*, 2013, vol. 37, no. 1, pp. 67–93.
22. Breen, J.P., Acremonium endophyte interactions with enhanced plant resistance to insects, *Annu. Rev. Entomol.*, 1994, vol. 39, pp. 401–423.
23. Popay, A.J., Tapper, B.A., and Podmore, C., Endophyte-infected meadow fescue and loline alkaloids affect Argentine stem weevil larvae, *New Zealand Plant Protection*, 2009, vol. 62, pp. 19–27.
24. US Patent no. 5491122, 1996.
25. Bills, G.F., González-Menéndez, V., Martín, J., Platas, G., Fournier, J., Peršoh, D., and Stadler, M., *Hypoxylon pulicicidum* sp. nov. (*Ascomycota, Xylariales*), a pantropical insecticide-producing endophyte, *PLoS ONE*, 2012, vol. 7, no. 10. e46687
26. Han, L.-R., Wang, Z.-H., Zhang, H.-J., Xue, L.-S., Feng, J.-T., and Zhang, X., Isolation of endophytic fungi from *Tripterygium wilfordii* and their insecticidal activities, *Afr. J. Microbiol. Res.*, 2013, vol. 7, no. 9, pp. 771–776.
27. Cimmino, A., Andolfi, A., Avolio, F., Ali, A., Tabanca, N., Khan, I.A., and Evidente, A., Cyclopaldic acid, seiridin, and sphaeropsidin A as fungal phytotoxins, and larvicidal and biting deterrents against *Aedes aegypti* (*Diptera: Culicidae*): structure-activity relationships, *Chem. Biodivers.*, 2013, vol. 10, no. 7, pp. 1239–1251.
28. Li, B., Yuan, H., Fang, J., Tao, L., Huang, Q., Qian, X., and Fan, Z., Recent progress of highly efficient *in vivo* biological screening for novel agrochemicals in China, *Pest Manag. Sci.*, 2010, vol. 66, no. 3, pp. 238–247.
29. Berestetskiy, A.O. and Kurlenya, A.S., Antimicrobial properties of phytopathogenic ascomycetes, *Mikol. Fitopatol.*, 2014, vol. 48, no. 2, pp. 121–132.
30. Fumagalli, P., Andolfi, A., Avolio, F., Boari, A., Cimmino, A., and Finizio, A., Ecotoxicological characterisation of a mycoherbicide mixture isolated from the fungus *Ascochyta caulina, Pest Manag. Sci.*, 2013, vol. 69, pp. 850–856.
31. Cueva, C. and Moreno-Arribas, M.V., Bartolomé, B., Salazar, Ó., Vicente, M.F., and Bills, G.F., Antibiosis of vineyard ecosystem fungi against food-borne microorganisms, *Res. Microbiol.*, 2011, vol. 162, pp. 1043–1051.

Translated by D. Timchenko