THE BIOLOGICAL ACTIVITY OF PHENOLIC ACIDS OF NATURAL ORIGIN AND PERSPECTIVES FOR THEIR USE IN PLANT PROTECTION PRACTICE

Olga N. Shemshura, Svetlana A. Aytkeldieva, Nadia E. Bekmakhanova, Ajnur I. Seitbattalova, Elvira T. Ismailova

Institute of Microbiology and Virology, Ministry of Education and Science of the Republic of Kazakhstan, Almaty, Bogenbai Batyr Street 103, 050510, Kazakhstan

Abstract

The investigations the presence of phenolic acids in the extracts of mycelium of fungi Penicillium, Aspergillus, Trichoderma, Beauveria. Strains with a high content of ferulic, gallic, vanillic and benzoic acid were identified. It was established, that all discharged phenolic acids show antibiotic activity. The greatest range of antibiotic activity showed analogues of ferulic acid and benzoic. It is shown that phenolic acids extracted from fungi, in most cases, are not toxic to of wheat plants.

Keywords: *fungi, penicillium, aspergillus, trichoderma, beauveria, extracts, phenolic acids, antibiotic activity*

INTRODUCTION

Issues of protection of plants and increasing their resistance to various of diseases caused by microorganisms has not yet lost the urgency and remain the focus of of science and production (Korsak, 2010; Pavlyushin, 2010; Harman, 2011; Ashmarina et al., 2011).

It is known that microorganisms that suppress the development of plant pathogens and bacteria that stimulate the growth of plants, are producers of antibiotics, amino acids, vitamins, auxins and other physiologically active substances have been widely applied in crop production (Farniev et al., 2010; Voronkova, 2011; Senatorova, 2012; Shternshis, 2013; Hristova et al., 2013; Davidyants, 2013). Among the producers of biologically active compounds microscopic fungi take the leading place, many of them may be the basis for the creation of effective plant protection products.

In the process of cultivation on artificial nutrient media, in the biomass and fungal culture broth accumulates a number of natural substances of different classes, but have one common property - the biological activity (Darhanova, 2010; Pattaeva, 2011; Kurakov, 2011; Sadykova, 2011). The most common agents having antimicrobial activity include antibiotics, alkaloids, peptides, phenolics, lipids, terpenoids, polysaccharides and others.

Among the low-molecular substances phenolic compounds constitute the largest group. High reactivity, a wide spectrum of pharmacological effects, active participation in the processes of growth, high levels in tissues and relatively simple isolation from plant material - all this has led them to the attention of researchers from different disciplines: biology, chemistry, and medicine. The study of the functional role of this large group of natural compounds essentially just beginning (Maslennikov et al., 2013; Zagoskina et al.,2013). Therefore, the identification of phenolic compounds in microscopic fungi is of undoubted interest.

Feature of strains antagonists is their ability to synthesize and accumulate a considerable amount of biologically active substances as a separate class, and groups that in some cases, increase the effect of each other.

Therefore to investigate the possibility of using microbial agents for combating plant pathogens, it is necessary to conduct the separation of the biologically active compounds into classes and individual metabolites to assess the antibiotic activity of the individual components against pathogenic microorganisms.

MATERIALS AND METHODS

Objects of study were 10 strains of microscopic fungi that have a high antagonistic activity pertaining to the genus Penicillium (340, 7N, 947), Aspergillus (127, 140, 5M), Trichoderma (F-1, TX, ANT), Beauveria (BB).

Fungal strains were cultured during 5 days at a temperature of 280C deep way on the soy medium. Analysis of the biomass of microscopic fungi was performed by conventional chromatographic methods (Heise I.M., Macek K., 1962; Akhrem, Kuznetsov, 1965; Mikesh, 1982; Becker, 1988) .

Obtained biomass was triturated with sand and extracted with 70% ethanol. Isolation of phenolic fractions was performed by the following sequence. To the concentrated 70% ethanol extract of mycelium was added an equal volume of sodium bicarbonate. Phenolic acids extracted with ethyl acetate. The obtained extract was washed in a separatory funnel several times to remove the mineral acid. Separation was carried out on a chromatographic paper in the benzene / methanol / acetic acid (45: 8: 4). A visualizing reagent - 0.2% solution of ferric alum. As a matter of standards used company «Sigma»: propionic, gentisic, ferulic, gallic, vanillic, resorcylic, veratric, benzoic, purple acid.

Fractions analogous standard compounds eluted in ethanol, evaporated and converted into a 5% ethanol solution for testing and phytopathogenic plant. For comparison, in all experiments using standard compounds phenolic.

Testing was conducted on phytopathogenic using the method of "holes" (Egorov, 2004). As test microorganisms were used: *Penicillium casei, Phitophtora infestance, Bacillus subtilis, Bacillus mycoides, Bacterium carotovorum, Pseudomonas alcoligenes*.

Investigation of growth stimuliryuschey activity of selected fractions was performed on seedlings of wheat cultivar "tall-2." Seeds were soaked in the test solution for 4 hours. As a control, 2.5% ethanol and distilled water. At the end of holding time in solution, seeds were removed, washed in distilled water and 10 pieces were placed in Petri dishes on agar medium Kovrovtseva. Repeated experience for each variant 3-fold. The dish was placed in an oven at 28^0C for 2 days, after time hatched seed cups transferred under continuous artificial lighting. In 8-day-old seedlings, measurements of the linear length of the root and stem.

The level of significance was evaluated by the values of the deviations of the normalized value (Student's coefficient), equal to the quotient between the average value (M) and its error (m) for the significance level of 0.05 and 0.01 (P = 0.95 and 0.99).

RESULTS AND DISCUSSION

Investigation of phenolic content in extracts of mycelium of fungi studied strains revealed a high content of ferulic (strains 140, TX 340), gallic, vanillic (F-1 strain) benzoic (strains 127, IV) acid.

Phenolic acids such as propionic acid, contained in strains F-1, 127, ANT, TX; veratric revealed in strains 127, ANT, TX, BB, and purple - in strains 6M, 140, TX 947 were found in trace amounts. Gentisic and resorcylic acid in the studied extracts of fungi are absent (Table 1).

Table 1. The presence of phenolic in the extracts of mycelium microfungi

strain	propionic	gentisic	ferulic	gallic	vanillic	resorcylic	veratric	benzoic	purple
F-1	+	-	+	++	++	-	-	+	-
127	+	-	+	-	+	-	+	++	-
BB	-	-	+	-	+	-	+	++	-
ANT	+	-	-	-	-	-	+	-	-
6M	-	-	+	+	+	-	-	+	+
140	-	-	++	+	-	-	-	-	+
TX	+	-	++	+	-	-	+	-	+
7N	-	-	+	-	-	-	-	-	-
340	-	-	++	-	-	-	-	-	-
947	-	-	-	+	+	-	-	-	+

Note: (++) - high content of (+) - trace amount of (-) - absent

It should be noted that the microscopic fungi such as ferulic phenolics, benzoic acid, propionic acid, was first identified.

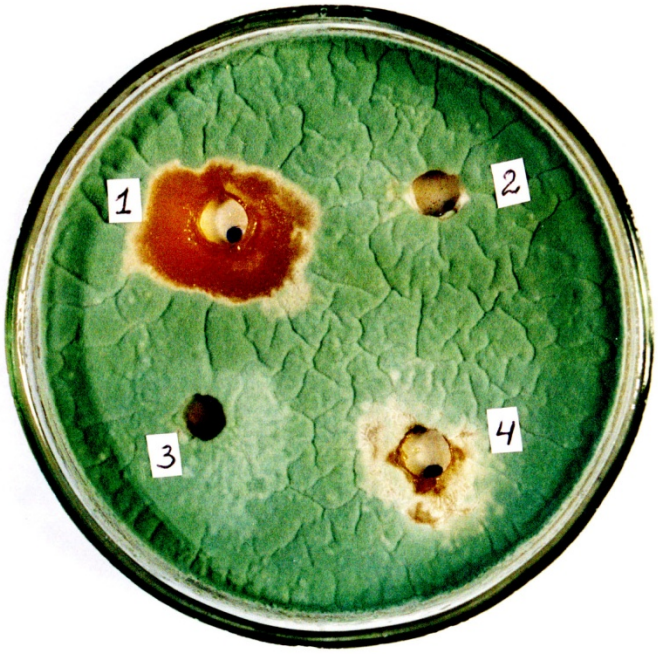
To study the biological activity of the fractions were identified with a high content of phenolic strains F-1, 127, 7N, TX, 340, BB.

It was established that all selected fractions in varying degrees, have antibiotic activity. The highest range of action relative to the test microorganisms turned in the fractions X-ph; S-ph and B-ph, is an analogue of benzoic and ferulic acid (Table 2, Figure 1-4).

Thus, the data show that the antibiotic activity of compounds of the standard in most cases does not exceed that of their counterparts derived from extracts of fungi.

Table 2. Antibiotic activity of phenolic fractions of microscopic fungi (inhibition zone test phytopathogens, mm)

fractions	strain	concentration, %	phytopathogens					
			Penicillium casei	Phitopftora infestans	Bacillus subtilis	Bacillus mycoides	Bacterium carotovorum	Pseudomonas alcoligenes
S-ph	F-1	1.2	6.5±0.76	0	6±1.0	0	11±0.76	8±1.04
B-ph	127	1.1	11±1.9	6±0.58	20±1.0	21.5±1.16	13±1.76	0
R-ph	340	2.0	0	0	0	0	0	9±1.32
K-ph	BB	2.7	5.5±1.04	7±1.16	0	13±1.32	0	0
X-ph	TX	1.0	6.5±0.87	7.5±0.5	21.5±1.2	0	22±1.32	5±1.04
Z-ph	140	3.3	6±0.58	6.5±1.0	0	0	0	5.5±0.87
E-ph	7N	1.4	8±1.16	0	0	0	0	6±0.76
benzoic	standard	1.0	5±0.5	7±1.04	6±1.04	0	12.5±1.04	10±1.26
gallic	standard	1.0	7.5±1.26	0	0	0	11±1.04	7.5±1.0
vanillic	standard	1.0	0	5±0.76	0	0	5.5±1.0	5±0.5
ferulic	standard	1.0	6±1.0	4.5±0.5	0	0	0	5.5±1.04
ethanol	control	2.5	0	0	0	0	0	0

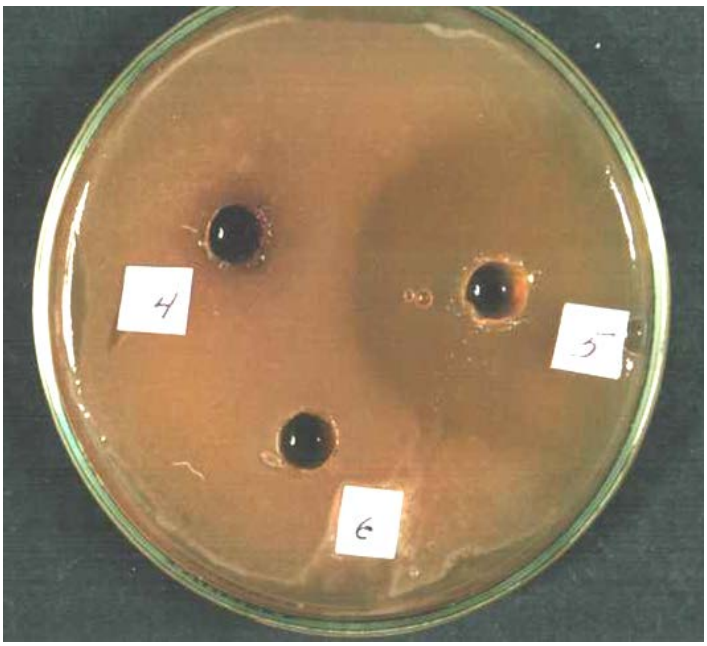


1- B-ph (analogue of benzoic acid);

2- S-ph(analogue of vanillic acid); 3- 2.5 ethanol (control);

4- benzoic (standard)

Figure 1. Antibiotic activity of phenolic fractions against *Penicillium casei*



4- ferulic acid (standard);

5- X-ph (analogue of ferulic acid); 3- 2.5 ethanol (control)

Figure 2. Antibiotic activity of phenolic fractions against *Bacterium carotovorum*

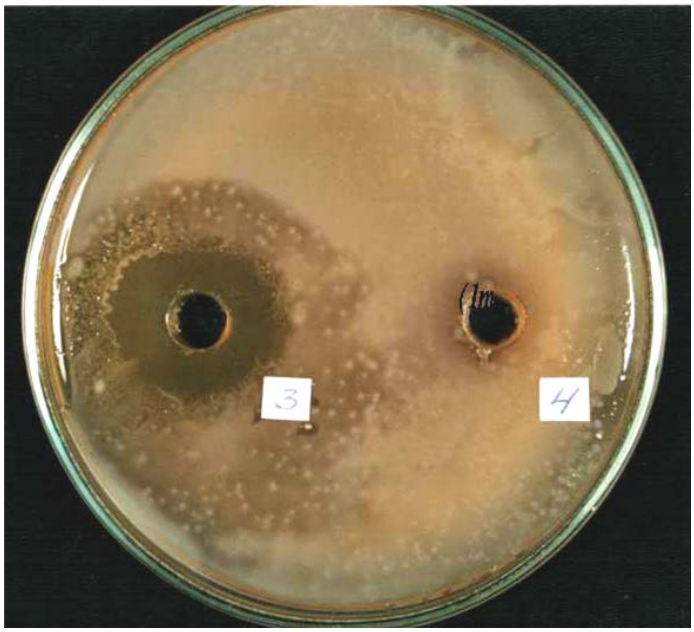
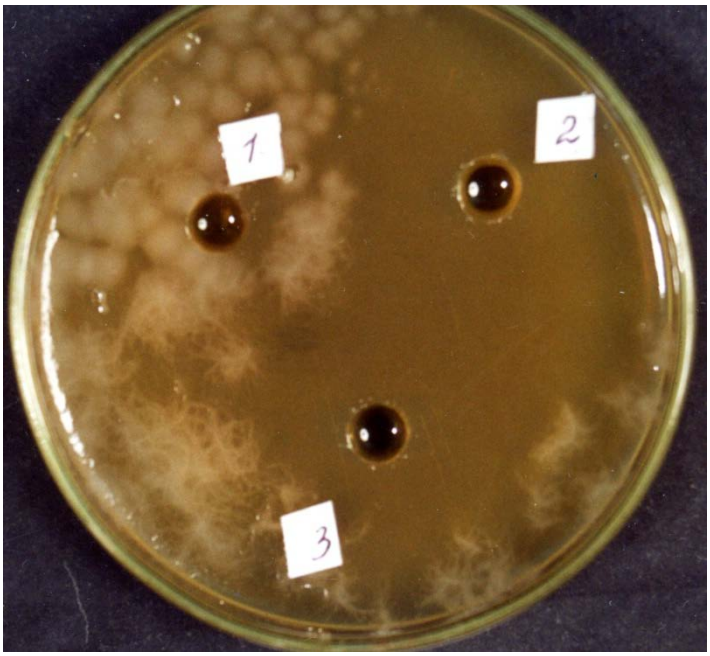


Figure 3. Antibiotic activity of phenolic fractions against *Bacillus mycoides*

3- B-ph (analogue of benzoic acid);

4- benzoic (standard)



1- 2.5 ethanol (control); 3- B-ph (analogue of benzoic acid);

4- X-ph (analogue of ferulic acid);

Figure 4. Antibiotic activity of phenolic fractions against *Bacillus subtilis*

In some cases the inhibition zone were less phytopathogens variants standards as compared to their natural analogues, or even lack of observed microbial suppression test in variants with standard substances.

For example, if ferulic acid no exhibits bactericidal effects on *Bacillus subtilis* and *Bacterium carotovorum*, then an analog fraction X-ph, isolated from the strain TX inhibits the growth of these pathogens. Also observed the opposite effect. Thus, standard of the gallic acid affects on *Penicillium casei* and *Bacterium carotovorum*, and an analog of R-ph strain 340 - is not. All this indicates selectivity of action of natural compounds. Overall, our results confirm the presence of the antibiotic activity and phenolic contrary to the literature data on the biological activity of phenolic compounds from plants (Kunaeva et al., 1995).

The results of studies of the effect on the growth of phenolic fractions of wheat seedlings have shown that phenolic acids derived from fungi at the concentrations tested, in most cases proved to be non-toxic (Table 3). The apparent inhibition of seedling growth was observed in the variant with a metabolite R-ph (gallic acid analogue) isolated from strain 340. Stimulating activity of phenolic fractions revealed in variants with metabolites S-ph (analogue vanillic acid), K - ph and B-ph (benzoic acid analogs) isolated from strains F-1, BB and 127, stem and root growth in these embodiments, averaged 2% -16% compared with the control (water).

In other variants the investigated metabolites stimulated or inhibited growth of stem and root growth (X-ph), or vice versa (Z-ph). As for standard compounds, they all were toxic to wheat seedlings, although studies have been taken to a concentration much lower than in the case of using their natural analogues.

Statistical analysis of the data in tables 2 and 3 show that the difference between the experimental and control options are significant at level of 0.95

Table 3. The growth of wheat seedlings after seed treatment phenolic fractions

sample	concentration, %	stalk		root	
		cm	%	cm	%
control 1 (water)	-	11.6±0.28	100	9.6±0.2	100
control 2 (ethanol)	2.5	10.6±0.31	91.4	8.7±0.22	90.6
S-ph (F-1)	1.47	13.4±0.18	115.5	10.4±0.2	108.3
B-ph (127)	1.35	13.5±0.26	116.4	11.2±0.23	116.7
R-ph (340)	2.15	9.9±0.23	85.3	8.2±0.21	85.4
K-ph (BB)	3.22	11.9±0.27	102.6	9.8±0.28	102
Z-ph (140)	1.2	11.5±0.27	99.1	9.9±0.31	103.1
E-ph (7N)	1.26	10.9±0.3	94	8.8±0.32	91.7
X-ph (TX)	2.4	11.7±0.28	100.8	9.5±0.25	98.9
benzoic (standard)	1.0	5.3±0.25	45.7	3.3±0.19	34.3
gallic (standard)	1.0	6.5±0.22	56	2.8±0.25	29.2
vanillic (standard)	1.0	10.5±0.24	90.5	6.5±0.25	67.7
ferulic (standard)	1.0	8.9±0.31	76.7	5.3±0.23	55.2

Note: for each embodiment used 30 seeds.

Thus, studies have shown the promise of further study of phenolic compounds of microbial origin as possible biological products for plant protection.

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