11 Phytoanticipins: The Constitutive Defense Compounds as Potential Botanical Fungicides

Gyula Oros and Zoltán Kállai

Abstract

Present control technologies of plant pathogenic fungi decouple the pathogen's life cycle mainly in two points of ontogeny, either by destroying spores prevent the infection or inhibit the biotrophic thallus, thus anticipating the formation of new infective propagules. Although, nowadays, the only tool for credible control of cultivated plants is the use of synthetic chemicals, the calculability of yield sureness has been worldwide threatened by the emergence of acquired tolerance to this group of pesticides as well as anxious feelings for their undesirable side effects. This situation urges the development of efficient alternative control agents, as threatening the net return even 10% disease incidence can cause economic loss. One approach to discover newer antimicrobial compounds is to search for their presence in natural sources exploiting the defense strategies of plants against their pathogens. Contrary to phytoalexins that are synthesized *de novo* after the plant is exposed to microbial attack, i.e., being produced in response of elicitors or stressors, the phytoanticipins are not formed in the tissue or released from preexisting plant constituents. These substances are plant antibiotics presented in tissue prior to infection, serving as the basis of pest tolerance. Several thousands of such molecules of different structure have been identified; however, few of them met practical application. In this chapter, we focus on constitutive mechanisms that might be used for controlling phytopathogenic fungi with special regard to organic substances, which might serve either as botanical fungicides or as lead compounds for molecular design.

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Consequently, the introduction of alien phytoanticipins and precursors of phytoalexins into the proper host/parasite system can represent a prospective tool for disease management. We summarized the results and experiences of past three decades searching for candidates for biofungicides useful in pest management practices. The efficacy of over 100 plant species used as either spices or preparations in traditional medicine or culinary was demonstrated in vitro against 25 phytopathogenic fungi, and possible use of promising candidates was discussed.

Keywords
Phytoanticipin · Fungicide · Phytopathogen · Defense · Yeast · Herb · Spice

11.1 Introduction

Plants have evolved finely regulated complex of metabolic processes to sustain homeostatic balance as well as constitutive and inducible defense mechanisms to help in both wound healing and defense against attack by microbes and herbivores. The constitutive defenses include static structures, such as lignified cell walls, mineral or organic crystals that create physical barriers as well as wide variety of organic compounds. The inducible defenses are also multitudinous, involving gene activation-linked *de novo* enzyme syntheses and various metabolites called phytoalexins. The role of phytoalexins in defense mechanisms was intensively studied (Van Etten et al. 2001), while to constitutive compounds has been paid less attention. In this chapter, we focus on constitutive mechanisms that might be used for controlling phytopathogenic fungi with special regard to organic substances, which might serve either as botanical fungicides or as lead compounds for molecular design.

Contrary to phytoalexins that are synthesized *de novo* after the plant is exposed to microbial attack, i.e., produced in response of elicitors or stressors, the phytoanticipins are not performed in the tissue or released from preexisting plant constituents, but are plant antibiotics presented in tissue prior to infection, serving as the basis of pest tolerance. Several thousands of such molecules of different structure have been identified; however, few of them met practical application. These compounds represent heterogeneous chemical structures, and significant part of them is synthesized via polyketide, isoprenoid, shikimate, and phenylpropanoid pathways (Pedras and Yaya 2015). The progress in separation and analytical techniques has allowed the rapid identification of plant secondary metabolites. The screening of their biological activities combined with molecular genetic techniques elucidated various roles in defense mechanisms (Mazim et al. 2011; Carere et al. 2016).

Present control technologies of plant pathogenic fungi decouple the pathogen's life cycle mainly in two points of ontogeny. The applied chemicals either destroy spores, preventing the infection or inhibit the biotrophic thallus, anticipating the formation of new infective propagules. Although the tolerance of cultivated plants can be enhanced by diverse methods, the possibilities of biocontrol, as well as the enhancement of plant resistance with chemical treatment, are limited; none of these approaches resulted in the economically acceptable level of control for long term of application in recent plant cultivation technologies, contrary to modern synthetic pesticides. Nowadays, the only tool for creditable control of cultivated plants is the use of synthetic chemicals. However, the calculability of yield sureness has been worldwide threatened by the emergence of acquired tolerance to this group of chemicals as well as by anxious feelings for undesirable side effects. All these are major causes of concerns as even 10% disease incidence can cause economic loss threatening the net return. This situation urges the development of efficient alternative control agents. One approach to discover newer antimicrobial compounds is to search for their presence in natural sources exploiting the defense strategies of plants against their pathogens. Microbial species or strains that do not invade the plant are usually more sensitive to the components of performed barriers than a viable pathogen of this plant. Consequently, the introduction of alien phytoanticipins and precursors of phytoalexins into the proper host/parasite system can represent a prospective tool for disease management (Piasecka et al. 2015).

The possible use of botanicals in pest control technologies intrigued big expectations hitched up by social movements. Indeed, in some special cases, these preparations performed well.

However, in comparative studies, the new generation of synthetics surpassed the botanicals at some orders of magnitude (Table 11.1). The use of natural compounds as lead molecules is seemingly more prospective, and the new techniques of

Table 11.1 Antisporulant activity of commercial fungicides and reference substances

Treatment		Concentration (%) of substances				
Substances	Form[a]	0.0005	0.005	0.05	0.5	5
Dimethomorph	A	−	+	+	++	++
Metalaxyl	A	−	+	+	++	++
Mikal	B	−	−	+	+	++
Digitonin	A	−	−	+	+	++
Podophyllotoxin	A	−	−	−	+	++
Veratrin	A	−	−	−	+	+
Nutri-Neem	B	−	−	−	+	+
Milsana	B	−	−	−	+	++

Test organism: *Sclerospora graminicola* (Sacc.) J. Schröt

The antisporulant activity was evaluated by the following scale; full inhibition (++), partial inhibition (+), and no inhibition (−)

[a]A = 25% methanolic stock solution of active ingredients containing 1% of Tween 20 was used for preparing dilution series. The methanol and Tween 20 did not exhibit any inhibitory effect alone when applied at maximum doses (5 and 0.2%, respectively). B = Commercial preparations were used (Deepak et al. 2005)

molecular design help to map the parts of the active molecule that respond for the desired biological effect. In past decades, the losses caused by peronosporaceous pathogens are increasing, and only a few synthetics are available to control them at an economically acceptable level. Unfortunately, the populations of pathogens rapidly adopt to these highly active monosite inhibitors. Some natural compounds in model experiments exhibited notable antiperonospora effect, especially in their abiotrophic stages of ontogeny (Deepak et al. 2007).

Some natural compounds in model experiments exhibited notable antiperonospora effect, especially in their abiotrophic stages of ontogeny, among them the known Na^+ ion channel activator ceveratrum alkaloids effectively inhibited the systemic invasion of the parasitizing thallus as well (Oros 2010). These amphiphilic steroid alkaloids are thought to act by direct incorporation into the microbial membrane disrupting its structural and functional integrity. Examination of the effect of veratridine on the alkali metal salt tolerance of *Plasmopara halstedii* showed that this steroid alkaloid dramatically impaired the tolerance of microbes to Li^+, Na^+, Cs^+, and, especially, to K^+. Modifying its structure synthetically, the sporicidal activity was successfully increased about thousand times (Oros and Ujváry 1999). The non-steroidal analogues of ceveratrum alkaloids designed by molecular modeling have an anti-oomycetes activity that depends significantly on the chemical structure and is confined to certain biotrophic and abiotrophic developmental forms of *P. halstedii* (Table 11.2).

Interestingly, the main structural features of these non-steroidal compounds presented here bear a certain resemblance to known commercial fungicides such as fenpropimorph and fenpropidin as well as to the experimental diaryltetrahydropyridines (Takayama et al. 1995). Thus, the new compounds, on the one hand, refine the structure–fungicidal activity relationship for substituted piperidines and, on the other hand, define an extended structural scaffold for new fungicide development (Ujváry and Oros 2002). The ecological role of the botanical steroid alkaloids is not fully known. Nevertheless, it can be assumed that these substances have multiple functions in the wild plants among them to protective against herbivores and diseases (Wink 1993). In this context, it is interesting that digitonin, α-solanine, and their aglycones showed activity against the asexual spores of *P. halstedii* and *S. sclerospora* even though it is generally believed that cleavage of the glycoside bond of plant glycoalkaloids represents a deactivation process utilized by glycoalkaloid-resistant fungi. It should be emphasized, however, that *P. halstedii* and *S. sclerospora* are specific and obligate pathogens, and their host plants have not been shown to contain glycoalkaloids; thus, these pathogens are unlikely to have evolved such deactivation mechanism.

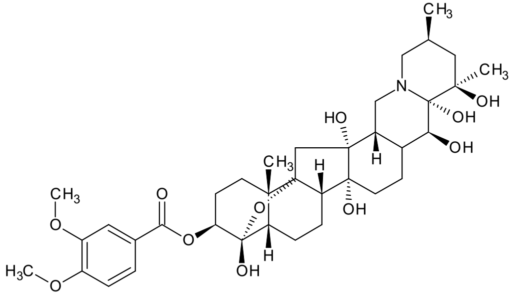
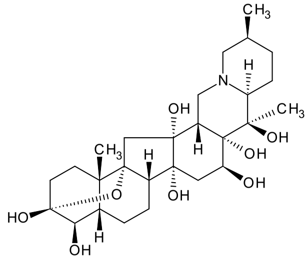
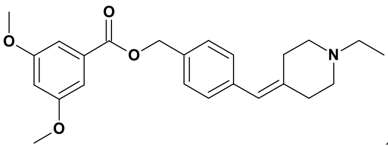
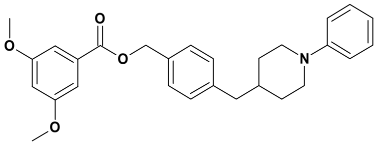
From now on, we summarize results of the past two decades searching for promising candidate botanicals useful in pest management practices. The selected for screening plants are attractive for humans, because of their characteristic organoleptic properties (smell and taste). Most of them are cultivated plants being part of the human diet. Their features are well known, and the marketed samples refer to traditionally accepted standards, that is important, as these plants exceptionally rich in secondary compounds of divergent structure (Table 11.3).

Table 11.2 Effect of steroid alkaloids and non-steroid analogues on sunflower downy mildew and on the asexual spores of *Plasmopara halstedii*

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
	Alkaloids with cevane skeleton					
Veratridine	60 de	73 c	>1000	100	100	10
Veracevine	93 ab	73 c	>1000	100	10	100
	Alkaloids with pregnane skeleton					
α-Solanine	95 a	30 g	>1000	1000	10	1
Solanidine	85 b	48 e	1000	100	10	1
	Non-steroidal analogues					
A	−28 i	84 b	100	10	1	0.1
B	100 a	0 h	>1000	>1000	10	100
C	60 de	80 b	100	10	1	0.01
D	0 h	100 a	>1000	>1000	10	10
	Reference fungicides[b]					
Tridemorph	70 cd	30 g	>1000	1000	10	10
Metalaxyl	100 a	73 c	>1000	>1000	>1000	1000

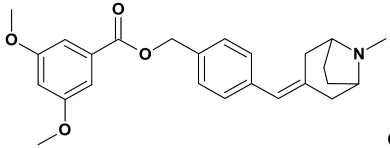
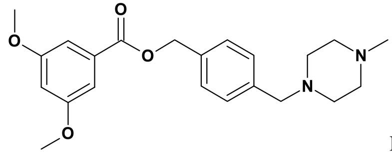
(continued)

Table 11.2 (continued)

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
 Veratridine			 Veracevine			
Veratridine			Veracevine			
 A			 B			

(continued)

Table 11.2 (continued)

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
 C			 D			

[a]One μM test compound per plant germling was applied. The activities marked with the same letter did not differ at P = 5% level (Fisher's test)
[b]Data from Ujváry and Oros (2002)

The composition can largely vary within samples. Chemotypes—chemically distinct entities within plant species on genetic variation—are exceptionally frequent for secondary compounds and can influence the quality of plant materials, which property has been largely used in chemotaxonomy.

From the agroindustrial point of view, the herbs have special advantages as their effects on mammalians are well known. Thus, the risk of elaboration of botanical preparation for pest control is significantly lower and less risky than the introduction of the plant with unknown biological effects in details. The use of pure compounds is more favorable; however, their production in industrial scale frequently meets difficulties and unprofitable. Thus, the herbal preparations may have a place in pest control technologies. Moreover, the protective effect can be resulted by synergic joint action of several secondary metabolites that phenomenon needs further studies.

11.2 Standard Operating Protocols

The growth response of 25 filamentous fungi pathogenic to 100 herbal preparations and seven culinary mushrooms was compared in model experiment applying poisoned agar technique following, in general, the route of Walker et al. (1937).

The herbal preparations of plant species listed in Table 11.3 were either home prepared of the plants collected in Protected Landscape Area of Buda Mountains (N 47°33′00″, E 18°52′60″) following traditional manners or purchased in drug store (Herbaria Co., Budapest). The desiccated plants were stored protected of light at ambient conditions over silica gel. The dry material was micronized before use.

The test fungi listed in Fig. 11.1 were maintained on potato dextrose agar slants at 22–25 °C (CM0139B, OXOID, Basingstoke) amended with two gL^{-1} casein digest (Difco, Detroit, USA), vitamins, and mineral salts (Oros and Naár 2018). All strains were isolated from various sources in Hungary and deposited in the Mycology Collection (WDCM824) of PPI.

Toxicity test: The conidia of fungi for inoculation of agar plates were washed up with sterile distilled water containing 0.05% Tween 20 of 8-day-old colonies grown up on PDA slants.

The herbal preparation was mixed with the agarized medium (2500 mg in 100 mL) and poured into Petri dishes (20 mL into a 90-mm-diameter dish). Then these plates were overlayered with 5 mL sterile agar (1.5 gL^{-1} in distilled water) and after solidification were inoculated with conidial suspensions (10^5 cell per mL) using a multipoint inoculator, and subsequently incubated at 20–22 °C. The intensity of colony growth was evaluated after 24 and 48 h by the following four-grade scale: 0 = no growth, 1 = growth on the limit of visual apperception, 2 = apparent but retarded growth as related to the untreated control, and 3 = the colony is not visually distinguishable from the untreated control and −1 = stimulation.

Table 11.3 Inhibitory effect of plant preparations on the growth of phytopathogenic fungi

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
Fungi Ascomycota						
	Agaricales					
1	*Agaricus bisporus* L.	Button mushroom	77	74	24	E8, E12, Y1, S36, E27, S26, B5, B1, S43, O1, S17
2	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Oyster mushroom	73	79	20	E4, E12, E29, Y1, E27, Y8, S37, S32, D3
3	*Lentinula edodes* (Berk.) Pegler	Shiitake	100	*n*	0	E29, Y1, O10
4	*Marasmius quercophylus* Pouzar	White-rot fungus	86	132	5	No
Basidiomycota						
	Auriculariales					
5	*Auricularia auricula-judae* (Fr.) Quel.	Wood ear	100	160	21	E4, E29, Y1, E27
	Boletales					
6	*Boletus edulis* Bull.	Penny bun	68	71	9	S25, S26
	Cantharellales					
7	*Cantharellus cibarius* Fr.	Cantharelle	72	75	24	E4, Y1, L4, Y8, S26, B2
Moss Lecanorales						
8	*Cetraria islandica* (L.)	Iceland moss	79	76	27	Y1
Plants Equisetales						
9	*Equisetum arvense* L.	Field horsetail	61	54	19	Y1, O10, L2, L4
	Cycadales					
10	*Zamia floridana* A.D.C.	Arrowroot	76	73	15	No
	Taxales					
11	*Taxus baccata* L.	English yew	88	83	39	D15, S22, Y1

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Pinales					
12	*Araucaria heterophylla*	Norfolk pine	83	87	27	No
13	*Juniperus communis* L.	Common juniper	75	72	20	E8, E30, Y1, E26, D17, E29, E25, E8
14	*Cupressocyparis leylandii*	Leylandii	85	90	16	D15, S22, S35
15	*Thuja occidentalis*	Arbor vitae	88	90	26	D15, D17, D19, S26, B8, O12
16	*Chamaecyparachilis obtusa*	Hinoki	89	117	12	Y1, D15, O6, B10, S7, L10, D29, S36, S35
17	*Sequoiadendron giganteum* J. Buchh.	Giant redwood	90	113	14	E8, W1, E29, Y7
18	*Picea pungens* Engelm.	Colorado spruce	64	53	23	No
19	*Picea abies* (L.) H. Karst.	Norway spruce	83	95	13	E29, L4, O4, O11, B22, O5, S4, D12, E21, S31
20	*Pinus nigra* J.F. Arnold	Black pine	88	111	18	E8, Y1, S22, E18, Y6, D5, S40, B9
	Austrobayleales					
21	*Illicium verum* Hook	Chinese Star anis	86	143	2	Y1, E26, S39, E25, E18, D16, S23, D18, O2, E9, B12, S31
	Acorales					
22	*Acorus calamus* L.	Sweet flag	80	95	6	E8, E4, M3, E30, Y1, L4, D15, S30, E26, D17, B10, E27, D16, E12, B3, B4, Y8, E19, D9, S19, D14, S25, D7, E1

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (R %)			Reported activity[b]
			PA	SA	PD	
Monocotyledones Zingiberales						
23	*Elettaria cardamomum* Maton	Cardamom green	83	93	22	E8, E4, E26, D17, S39, Y6, Y8, D9, S19, Y3, Y4, E25, B14, M2
24	*Elettaria cardamomum* Maton	Cardamom black	80	79	14	
25	*Zingiber officinale* L.	Ginger	80	83	10	E8, E29, E30, Y1, D15, S39, E27, B4, Y8, E25, Y9, E5, M2, S26, S1, S5, O12
26	*Curcuma longa* L.	Turmeric	89	98	19	E8, E4, E29, E30, D17, D19, S7, E25, E27, S23, M2, S26, S11, S29, S25, S32, D23, S13, D27, D2, S5, S8, D8, E10
27	*Kaempferia galanga* L.	Galangal	100	*n*	0	E8, E4, E29, B10, E5
	Asparagales					
28	*Allium schoenoprasum* L.	Chives	68	58	12	E8, L4, L10, S26, E17, L6
Dicotyledones Ranunculales						
29	*Chelidonium majus* L.	Greater celandine	70	59	20	E29, Y1, L3, L4, D15, S22, S30, E26, Y6, S23, E25, E5, D4, L7, D30, S38
30	*Clematis vitalba* L.	Old men's beard	77	96	7	E8, E4, Y1, Y8, M2, S26, E20

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (R %)			Reported activity[b]
			PA	SA	PD	
	Magnoliales					
31	*Myristica fragrans* Houtt.	Nutmeg	89	140	7	E4, Y1, O10, D15, S39, B10, S7, L1, L10, M2, S26, E9, S11, S29, B27, B26
32	*Flos myristicae*	Nutmeg	85	80	23	
	Fagales					
33	*Alnus glutinosa*	Black alder	77	67	10	Y4
34	*Juglans regia* L.	Walnut	95	187	3	E8, E4, E29, E30, E26, Y7, E27, E25, E11
	Laurales					
35	*Laurus nobilis* L.	Bay laurel	87	112	15	E8, E13, Y1, D35, D15, Y7, O6, B10, L10, D16, S23, B4, Y8, D9, S26, E9, E11, E15, S25, B8, D7, L8, B17, E14, B16, B13, B21, D26, D10, B27
36	*Cinnamomum verum* J. Presl.	Cinnamon	100	*n*	0	E4, Y1, L2, L4, D15, D17, D19, E22, O6, L10, Y8, D9, S19, S16, Y4, M2, S26, E16, D23, S1, D30, E14, D26, D10, D13, D25, B20, B15

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Piperales					
37	*Piper nigrum* L.	Black pepper	86	100	25	E8, E4, Y1, D15, S22, D19, E22, S36, Y8, S19, S26, E9, D24, S13, E10, B27, O12
	Caryophillales					
38	*Rumex patientia* L.	Patience dock	76	116	−6	E29, E28
	Cucurbitales					
39	*Momordica charantia* L.	Balsam pear	83	80	32	Y1, D15, S30, E26, S26, S11, D7, S6
	Brassicales					
40	*Sinapis alba* L.	Yellow mustard	100	*n*	0	E8, S26
41	*Wasabia japonica* (Miq.) Matsum	Wasabi root	100	707	1	D27
	Ericales					
42	*Arctostaphylos uvaursi* (L.) Spreng.	Bearberry	77	70	16	Y1, E29
43	*Camellia sinensis* L.	Green tea	69	52	12	Y1, E27, Y6, B4, Y4, O01, B17, B9
44	*Camellia sinensis* L.	Black tea	77	67	27	E29, Y1, E27, S25, S42, O01, B17, B9, B18, S44
45	*Primula veris* L.	Cowslip	90	125	19	No
46	*Vaccinium myrtillus* L.	Bilberry	80	73	33	Y1, D15, L10, S16, Y8, M2, S26
	Malvales					
47	*Hibiscus sabdariffa* L.	Red sorrel	89	145	9	D17, D19, E7
48	*Tilia cordata* P. Mill.	Lime	79	78	25	No

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Apiales					
49	*Foeniculum vulgare Mill.*	Fennel	84	87	23	Y1, O10, L4, D15, B10, M2, M2, E15, D7, B6, B27
50	*Apium graveolens* L.	Celery	75	64	16	B22, B27, B20, B15
51	*Anethum graveolens* L.	Dill	73	62	29	E8, E4, Y1, E7, S19, S12, M4
52	*Petroselinum crispum*	Parsley	86	87	31	E4, S37, E16, E21, B27
53	*Levisticum officinalis* L.	Lovage	79	68	27	L4, S22, B10, B22
54	*Carum carvi* L.	Caraway	84	92	15	E4, E13, Y1, D15, Y7, S19, D2, D7
55	*Coriandrum sativum* L.	Coriander	69	66	23	E8, E13, E29, Y1, L4, Y6, S29, E15, S41, O7, D1
56	*Pimpinella anisum* L.	Anis	88	121	16	E8, E13, E29, E30, Y1, E26, E22, Y7, O6, B10, L10, E27, Y4, E15, S25
57	*Panax ginseng* C. Meyer	Ginseng	84	95	24	E29, E30, S24, S30, S39, E25, S23, S26, S18, B7, D11, D22, O9
	Malpigiales					
58	*Hypericum perforatum* L.	St. Johnswort	73	59	21	E29, E30, S24, E26, E27, E25, S41
	Fabales					
59	*Galega officinalis* L.	Goat's rue	65	60	24	E8, Y1, D30
60	*Ononis spinosa* L.	Cammock	83	87	23	Y1
	Myrtales					

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
61	*Epilobium parviflorum* Schreb.	Willow herb	64	54	19	Y1
62	*Pimenta officinalis* L.	Allspice	83	93	2	No
63	*Punica granatum* L.	Pomegranate	99	196	18	E8, E4, E29, E30, Y1, L4, D15, E11, E26, D17, S26, E15, O1, M4, L8, E14, Y2, B2, E2, S28, E28
64	*Punica flos*	Pomegranate	85	87	22	
65	*Syzygium aromaticum* L.	Clove	100	*n*	0	E8, E4, Y1, D15, S22, D19, S23, B4, Y8, S26, D23, E14, O9, E10, D13, B20, B15
	Rosales					
66	*Alchemilla alpina* L.	Lady's mantle	80	80	19	No
67	*Frangula alnus* P. Mill.	Buckthorn	80	115	−12	No
68	*Humulus lupulus* L.	Hop	82	111	2	E8, E4, E13, E12, M3, E29, E30, M1, D15, S22, E22, D16
69	*Kerria japonica* (L.) DC.	Japanese rose	87	98	27	No
70	*Rosa canina* L.	Dog briar	74	69	30	E4, Y1, D17, D19, E22, E7, B4
71	*Urtica dioica* L.	Great nettle	73	65	23	E26, E25, B16, B21
	Sapindales					
72	*Citrus lemon* L.	Lemon	89	97	28	E8, S30, D17, D19, E18, S26, D30, E14, O12
73	*Schinus terebinthifolius* Raddi	Pink peppercorn	90	107	20	E4, Y1, L4, S22, S4, B4, M2, S26, B25, E28

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Vitales					
74	*Vitis vinifera* L.	Wine grape	86	84	25	S24, O11, B22, L2
	Asterales					
75	*Achillea millefolium* L.	Common yarrow	63	56	19	D15, S22, D9, D20
76	*Arctium lappa* L.	Great burdock	76	71	20	E8, Y1, E22
77	*Artemisia dracunculus* L.	Tarragon	98	140	25	E8, E4, E30, Y1, E26, S39, L10, E27, S4, E5, M2, S26, E3
78	*Calendula officinalis*	Marigold	67	58	21	E8, E4, E29, E30, D15, S30, E26, D17, E7, Y7, B10, L10, E27, E25, S26, S41, Y5, D7, B6
79	*Carthamus tinctorius* L.	Safflower	80	80	30	E8, Y7, E11
80	*Cnicus benedictus* L.	Holy thistle	68	64	15	No
81	*Echinacea purpurea* (L.) Moench	Echinacea	69	62	24	Y1, L4, Y6, B4, Y8
82	*Matricaria chamomilla* L.	Chamomile	91	109	30	E8, E4, E13, S24, D15, E22, M2, E15, S41, Y5, B16, D13
83	*Taraxacum officinale* L.	Dandelion	67	54	25	No
	Dipsacales					
84	*Sambucus nigra* L.	European elder	68	55	18	Y8, S38
	Gentianales					
85	*Asperula odorata* L.	Sweet woodruff	66	61	18	No
86	*Centaurium erythreae* Rafn.	Centaury	85	121	2	E8, E4, O10, D15, D9, E9, E11, S37
87	*Coffea arabica* L.	Coffee	68	56	24	E8, Y1, E22, E9, D24, B23

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Boraginales					
88	*Myosotis sylvatica* Hoffm.	Forget-me-not	67	59	27	No
	Lamiales					
89	*Hyssopus officinalis* L.	Hyssop	64	53	14	S26, S34
90	*Lavandula officinalis* L.	Lavender	75	70	24	Y1, O10, S30, E22, D16, E9, S29, S37, B8, L5, M4, L9, B17, B16, B21, B11, O7, D1, D13, D21
91	*Majorana hortensis* Moench.	Marjoram	70	60	33	E4, E13, Y1, L4, D15, O3
92	*Marrubium vulgare* L.	White horehound	66	55	28	E8, Y1, L4
93	*Melissa officinalis* L.	Common balm	100	*n*	0	E8, S22, Y8, S26, M4, B16, E23
94	*Mentha piperita* L.	Peppermint	75	69	33	E29, Y1, S1, E7, O6, B10, L10, S36, S23, S19, S26, E9, S29, S25, S17, S32, D30, Y5, B16, B13, B21, O7, D1, O3
95	*Ocimum basilicum* L.	Basil	81	79	31	E8, E4, E29, Y1, E30, L4, S30, E26, E7, Y7, B10, E27, E25, M2, E9, B24, S10, M4, S2, B27, O12, L3
96	*Origanum vulgare* L.	Oregano	83	94	23	E8, E13, E29, Y1, O10, L4, D15, S30, E22, B10, S36, E18, O4, S16, M2, S26, E9, S29, E15, S17, S37, S32, M4, L8, O8, E14, E10, S2, O7, D1, D25, D21, O3, S38, B20, B15

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
97	*Plantago major* L.	Common plantain	82	83	29	Y1
98	*Rosmarinus officinalis* L.	Rosemary	97	130	24	E8, Y1, O10, S24, D15, D17, E22, D16, Y4, M2, S26, E9, S29, E15, S41, S37, S32, Y5, E10, S2, O7, D1, B27, D21, O3
99	*Salvia officinalis* L.	Garden sage	81	88	6	Y1, O10, L4, D15, D17, O11, D16, S23, Y8, Y4, Y9, M2, S26, E15, S17, E23, S2
100	*Satureia hortensis* L.	Summer savory	89	147	2	E8, E4, L4, E5, M2, S26, S33, O9
101	*Syringa vulgaris*	Common lilac	81	78	27	No
102	*Thymus vulgaris* L.	Thyme	88	110	25	E8, E4, E13, M3, E29, Y1, O10, L4, D15, S22, S30, E22, B10, S36, E18, S23, Y8, S16, M2, M2, S26, E9, S29, E15, S25, S34, S17, L8, D30, O8, E14, E1, O7, D1, D13, B20, B15
103	*Verbascum phlomoides* L.	Mullein	84	86	31	Y1, D17, E22, Y4

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (R %)			Reported activity[b]
			PA	SA	PD	
104	*Verbena officinalis* L.	Common vervain	84	174	13	E13, L4, E18, S4, S16, O8
105	*Veronica officinalis* L.	Speedwell	83	87	20	No
	Solanales					
106	*Capsicum annuum* L.	Pepper	82	69	24	E8, E4, Y1, O10, D15, O6, S26
107	*Ipomoea tricolor* Cav.	Morning glory	98	112	14	No

The cases where the only mammalian pathogens have been mentioned are not included into the references, but those where a phytopathogenic or food rotting species were tested and pathogenic species included into the tests the latter are mentioned

[a]Potential activity values (PA) have been calculated by potency mapping technique according to Lewi (1976); the largeness of activity spectrum (SA) refers to the set of fungi tested, which is negatively proportional to the given value, i.e., the n means complete inhibition of all strains tested; PD = the intensity of degradation (%) of the effect during 24 h of incubation, where zero means no deterioration of the efficacy

[b]The fungal species with reported sensitivity to extracts of the given plants are as follows:

Zygomycota: M1—*Mucor rouxii* (Shigeyuki and Yuko 1985), M2—*Mucor sp.* (bin Jantan et al. 2003; Szakiel et al. 2011; Abdolahi et al. 2010), M3—*Rhizopus oryzae* (Ujváry and Oros 2002; Niknejad et al. 2015; Khan et al. 2017; Nabigol and Farzaneh 2010), M4—*Rhizopus sp.* (Ali et al. 2017; Camele et al. 2010; Tehranifar et al. 2011; Lopez et al. 2007)

Basidiomycota: B1—*Malassezia furfur* (Waithaka et al. 2017), B2—*Rhodotorula mucilaginosa* (Visnjevec et al. 2017; Dulger et al. 2004), B3—*Cryptococcus gastricus* (Devi and Ganjewala 2009), B4—*Cryptococcus neoformans* (bin Jantan et al. 2003; Phongpaichit et al. 2005; Ewais et al. 2014; Shreaz et al. 2016; Pinheiro et al. 2017; Sigei 2013; Lewi 1976; Kovatcheva et al. 2011; Mir-Rashed et al. 2010; Thirach et al. 2003), B5—*Ustilago maydis* (Waithaka et al. 2017; Cardoso et al. 2017), B6—*Athelia rolfsii* (Fonseca et al. 2015a; Turkolmez and Soylu 2014), B7—*Coprinus comatus* (Ng and Wang 2001; Lam and Ng 2001), B8—*Pleurotus ostreatus* (Lelono et al. 2018), B9—*Schizophyllum commune* (Eberhardt and Young 1994), B10—*Rhizoctonia solani* (Fonseca et al. 2015a; Turkolmez and Soylu 2014; Kwon et al. 2017; Huang et al. 2010; Prasad et al. 2016; Yoon et al. 2013; Ojala et al. 2000; Osorio et al. 2010; Thobunluepop et al. 2009; Mullerriebau et al. 1995; Lee et al. 2007a), B11—*Gloeophyllum trabeum* (Sen and Yalcin 2010), B12—*Anthrodia sp.* (Hedenstrom et al. 2016), B13—*Ceriporiopsis subvermispora* (Sen and Yalcin 2010), B14—*Coriolus versicolor* (Shreaz et al. 2016), B15—*Laetiporus sulphureus* (Xie et al. 2017), B16—*Oligoporus placenta* (Sen and Yalcin 2010), B17—*Phanerochaete chrysosporium* (Arora and Ohlan 1997), B18—*Phlebia radiata* (Arora and Ohlan 1997), B19—*Sporotrichum pulverulentum* (Arora and Ohlan 1997), B20—*Trametes hirsuta* (Xie et al. 2017), B21—*Trametes versicolor* (Sen and Yalcin 2010), B22—*Heterobasidion parviporum* (Ojala et al. 2000; Kusumoto et al. 2014), B23—*Hemileia vastatrix* (de Colmenares et al. 1998), B24—*Peridiopsora mori* (Maji et al. 2005), B25—*Phakopsora pachyrhizi* (Bigaton et al. 2013), B26—*Puccinia triticina* (Cho et al. 2007), B27—*Uromyces appendiculatus* (Arslan et al. 2009)

Ascomycota: Saccharomycetales: Y1—*Candida albicans* (bin Jantan et al. 2003; Waithaka et al. 2017; Visnjevec et al. 2017; Devi and Ganjewala 2009; Ewais et al. 2014; Thirach et al. 2003; Millot et al. 2017; Uslu et al. 2013; Khan et al. 2013; Hong et al. 2004; Glisic et al. 2007; Sarac et al. 2014; Digrak et al. 1999; Yazdani et al. 2009; Houksey et al. 2010; Al Taweel 2007; Meng et al. 2009; Constantine 1966; Girardot et al. 2014; Iyer et al. 2017; Dulger et al. 2015; Nofouzi 2015; Roco Gauch et al. 2014; Ehssan and Saadabi 2012; Hearst et al. 2009; Castillo et al. 2018; Iwalokun et al. 2000; Verma et al. 2008; Dellamura and Edmar 2013; Ika et al. 2018; Alves et al. 2013; Al-Zubairi et al. 2017; Sasidhran and Menon 2010; Hammer et al. 1999; Jagessar et al. 2008; Namdar et al. 2014; Hirasawa and Takada 2004; Kosalec et al. 2005; Seidler-Łożykowska et al. 2013; Silva et al. 2011; Ertürk 2010; Altuner et al. 2010; Urziya et al. 2016; Bouterfas et al. 2016; Tonea et al. 2016; Johann et al. 2008; Endo et al. 2010; Hayouni et al. 2011; Kochthressia et al. 2012; Binns et al. 2000; Talib and Mahasneh 2010; Lopes-Lutz et al. 2008; Gundidza et al. 2009; Schmourlo et al. 2005; Giamperi et al. 2002; Bajer et al. 2017; Mejd et al. 2015; Bouterfas et al. 2016; Chen et al. 2013), Y2—*Candida glabrata* (Kochthressia et al. 2012), Y3—*Candida orthosilosis* (Badiee et al. 2012), Y4—*Candida parapsilosis* (Visnjevec et al. 2017; Sigei 2013; Nofouzi 2015; Roco Gauch et al. 2014; Seidler-Łożykowska et al. 2013; Hayouni et al. 2011; Badiee et al. 2012; Hofling et al. 2010), Y5—*Candida sp.* (Kasiri and Heidari-Soureshjani 2018; Ratha Bai and Kanimozhi 2012), Y6—*Candida tropicalis* (Sigei 2013; Digrak et al. 1999; Meng et al. 2009; Talib and Mahasneh 2010; Badiee et al. 2012; Taha and Shakour 2016), Y7—*Geotrichum candidum* (Seidler-Łożykowska et al. 2013; Silva et al. 2011; Lovecka et al. 2017; Bouzouita et al. 2003; Bibi et al. 2016), Y8—*Saccharomyces cerevisiae* (bin Jantan et al. 2003; Dulger et al. 2004; Phongpaichit et al. 2005; Mir-Rashed et al. 2010; Iwalokun et al. 2000; Sasidhran and Menon 2010; Namdar et al. 2014; Talib and Mahasneh 2010; Badiee et al. 2012; Wen 2009; Cioch et al. 2017; Farcasanu et al. 2006; Smith et al. 2008; Araujo et al. 2003; Barla et al. 2007; Baerlocher and Oertli 1978), Y9—*Torulopsis glabrata* (bin Jantan et al. 2003; Hofling et al. 2010). Hyphomycetales: W1—Aquatic hyphomycetes (Chantawannakul et al. 2005). Eurotiomycetes: E1—*Ascosphaera apis* (Boudegga et al. 2010; Kumar et al. 2010), E2—*Exophiala dermatitidis* (Visnjevec et al. 2017), E3—*Fonsecaea pedrosoi* (Gundidza et al. 2009), E4—*Aspergillus flavus* (Niknejad et al. 2015; Devi and Ganjewala 2009; Ewais et al. 2014; Castillo et al. 2018; Verma et al. 2008; Ika et al. 2018; Al-Zubairi et al. 2017; Binns et al. 2000; Gundidza et al. 2009; Schmourlo et al. 2005; Wen 2009; Kapoor et al. 2008; Al-Sohaibani et al. 2011; Uddin et al. 2003; Vânia et al. 2014; Simonić et al. 2014; Shiva Rani et al. 2013; Tian et al. 2012; Vokk et al. 2011; Dorman 1999; Skrinjar et al. 2009; Krishnamurthy et al. 2008; Mileva et al. 2014; Sagar et al. 2011; Tajehmiri et al. 2018; Preeti and Sudhir 2014; Dhingra et al. 2007; Cai et al. 2012; Rizwana et al. 2016), E5—*Aspergillus fumigatus* (bin Jantan et al. 2003; Binns et al. 2000; Tajehmiri et al. 2018; Babu et al. 2007), E6—*Aspergillus glaucus* (Simonić et al. 2014), E7—*Aspergillus nidulans* (Glisic et al. 2007; Preeti and Sudhir 2014), E8—*Aspergillus niger* (bin Jantan et al. 2003; Niknejad et al. 2015; Nabigol and Farzaneh 2010; Ali et al. 2017; Waithaka et al. 2017; Devi and Ganjewala 2009; Ewais et al. 2014; Glisic et al. 2007; Sarac et al. 2014; Verma et al. 2008; Sasidhran and Menon 2010; Altuner et al. 2010; Rakatama et al. 2018; Kochthressia et al. 2012; Binns et al. 2000; Gundidza et al. 2009; Taha and Shakour 2016; Mehrabian et al. 2000; Lovecka et al. 2017; Wen 2009; Kapoor et al. 2008; Uddin et al. 2003; Vânia et al. 2014; Simonić et al. 2014; Shiva Rani et al. 2013; Dorman 1999; Tajehmiri et al. 2018; Preeti and Sudhir 2014; Dhingra et al. 2007; Cai et al. 2012; Rizwana et al. 2016; Rodrigez 2017; Matthews and Haas 1993; Fierascu et al. 2018; Kawachi 2010; Singh et al. 2002; Saglam et al. 2009; Kloucek et al. 2012; Atta-Ur-Rahman Choudhary et al. 2000), E9—*Aspergillus ochraceus* (Verma et al. 2008; Cioch et al. 2017; Simonić et al. 2014; Houicher et al. 2016; Santos et al. 2014; Salem et al. 2016; Basilico and Basilico 1999; Stupar et al. 2014), E10—*Aspergillus parasiticus* (Saglam et al. 2009; Suganthi et al. 2013), E11—*Aspergillus versicolor* (Verma et al. 2008; Caputo et al. 2017), E11—*Penicillium chrysogenum* (Niknejad et al. 2015; Devi and Ganjewala 2009; Kumar and Yadav 2014), E12—*Penicillium citrinum* (Niknejad et al. 2015; Caputo et al. 2017; Boyraz and Ozcan 2005; De Martino et al. 2009; Felsociova et al. 2015), E13—*Penicillium digitatum* (Nabigol and Farzaneh 2010; Kloucek et al. 2012; Kharchoufi et al. 2018; Nicosia et al. 2016; Yahyazadeh et al. 2008; Vitoratos et al. 2013), E14—*Penicillium expansum*

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(Ali et al. 2017; Nofouzi 2015; Sasidhran and Menon 2010; Preeti and Sudhir 2014; Matthews and Haas 1993; Fierascu et al. 2018; Boyraz and Ozcan 2005; Felsociova et al. 2015; Mizhir et al. 2016; Nionelli et al. 2018), E22—*Penicillium verrucosum* (Ozcakmak et al. 2012), E23—*Penicliium pallidum* (Chen et al. 2013), E24—*Epidermophyton floccosum* (bin Jantan et al. 2003; Ewais et al. 2014; Cavaleiro et al. 2006; Yazdani et al. 2009; Wuthi-Udomlert et al. 2000; Massiha and Zolfaghar 2015; Xue et al. 2017), E25—*Microsporum canis* (Abdolahi et al. 2010; Devi and Ganjewala 2009; Ewais et al. 2014; Cavaleiro et al. 2006; Yazdani et al. 2009; Seidler-Łożykowska et al. 2013; Gundidza et al. 2009; Hemamalini et al. 2015), E26—*Microsporum gypseum* (Waithaka et al. 2017; Phongpaichit et al. 2005; Cavaleiro et al. 2006; Ika et al. 2018; Seidler-Łożykowska et al. 2013; Gundidza et al. 2009; Shiva Rani et al. 2013; Wuthi-Udomlert et al. 2000; Massiha and Zolfaghar 2015; Xue et al. 2017; Sharma and Sharma 2013; Cespedesa et al. 2006), E27—*Paracoccidioides brasiliensis* (Xu et al. 2014), E28—*Trichophyton mentagrophytes* (Shigeyuki and Yuko 1985; bin Jantan et al. 2003; Cavaleiro et al. 2006; Yazdani et al. 2009; Houksey et al. 2010; Mehrabian et al. 2000; Wuthi-Udomlert et al. 2000; Sharma and Sharma 2013; Wegiera et al. 2011; Rautio e al. 2012), E29—*Trichophyton rubrum* (Okubo et al. 1991; bin Jantan et al. 2003; Seidler-Łożykowska et al. 2013; Kochthressia et al. 2012; Gundidza et al. 2009; Massiha and Zolfaghar 2015; Xue et al. 2017; Lis-Balchin et al. 1998; Hemamalini et al. 2015; Cespedesa et al. 2006), E30—*Trichophyton verrucosum* (Ghosh 2006; Hemamalini et al. 2015), E31—*Trichosporium vesiculosum* (Cobos et al. 2015), E32—*Phaeomoniella chlamydospora* (Saha et al. 2005). Dothideomycetes: D1—*Pyrenochaeta lycopersici* (Bajer et al. 2017), D2—*Botryodiplodia theobromae* (Begum et al. 2013; Chu et al. 2005), D3—*Botryosphaeria berengeriana* (Pan et al. 2017), D4—*Botryosphaeria dothidea* (Sherwood and Bonello 2013), D5—*Diplodia pinea* (Burger et al. 2010), D6—*Diplodia seriata* (Saha et al. 2005), D7—*Macrophomina phaseolina* (Fonseca et al. 2015a; Turkolmez and Soylu 2014; Cobos et al. 2015; Ghosh 2006; Chu et al. 2005; Ghosh 2006; Lee et al. 2007b), D8—*Phyllosticta caricae* (Mungkornasawakul et al. 2002), D9—*Cladosporium cladosporioides* (Verma et al. 2008; Simic et al. 2004; Endah 2005; Bekhechi et al. 2011; Minova et al. 2015), D10—*Fulvia fulva* (Simic et al. 2004), D11—*Mycosphaerella arachidicola* (Lam and Ng 2001), D12—*Mycosphaerella fragariae* (Hoyos et al. 2012), D13—*Pseudocercospora griseola* (Krauze-Baranowskaa and Wiwa 2003), D14—*Septoria chrysanthemi* (Endah 2005), D15—*Alternaria alternata* (Kwon et al. 2017; Cho et al. 2007; Verma et al. 2008; Shiva Rani et al. 2013; Sagar et al. 2011; Rizwana et al. 2016; Saglam et al. 2009; Kloucek et al. 2012; Simic et al. 2004; Chen et al. 2013; Chu et al. 2005; Minova et al. 2015; Johann et al. 2010; Xu et al. 2014; Gupta et al. 2017; Thakur et al. 2013; Glazer et al. 2012; Badawy and Abdelgaleil 2014; Cabral et al. 2016; Fawzi et al. 2009; Bayar et al. 2018; Pane et al. 2016), D16—*Alternaria solani* (Huang et al. 2010; Baka 2010; Thobunluepop et al. 2009; Itako et al. 2008; Dellavalle et al. 2011), D17—*Alternaria sp.* (Glisic et al. 2007; Nofouzi 2015; Preeti and Sudhir 2014; Mizhir et al. 2016; Endah 2005; Babu et al. 2007; Fiori et al. 2000; Tonucci-Zanardo et al. 2015), D18—*Bipolaris maydis* (Huang et al. 2010), D19—*Curvularia sp.* (Preeti and Sudhir 2014; Singh et al. 2002; Bekhechi et al. 2011; Fiori et al. 2000), D20—*Didymella bryoniae* (Wang et al. 2018), D21—*Epicoccum nigrum* (Stupar et al. 2014), D22—*Exserohdurn turcicum* (Rizvi et al. 1980), D23—*Exserohilum rostratum* (Bekhechi et al. 2011), D24—*Helminthosporium sp.* (Bekhechi et al. 2011; Smid et al. 2013), D25—*Phoma foveata* (Pedras and Sorensen 1998), D26—*Phoma helianthi* (Simic et al. 2004), D27—*Phoma sp*. (Bekhechi et al. 2011; Nagy et al. 2014), D28—*Stemphylium botryosum* (Badawy and Abdelgaleil 2014), D29—*Stemphylium solani* (Kwon et al. 2017), D30—*Venturia inaequalis* (Cho et al. 2007), Letiomycetes: L1—*Blumeria graminis* (Cho et al. 2007), L2 —*Erysiphe necator* (Pazmiño-Miranda et al. 2017), L3—*Phyllactinia corylea* (Maji et al. 2005), L4—*Botrytis cinerea* (Abdolahi et al. 2010; Nabigol and Farzaneh 2010; Camele et al. 2010; Tehranifar et al. 2011; Dulger et al. 2004; Ojala et al.

2000; Lee et al. 2007a; Lopez-Reyes et al. 2013; Cai et al. 2012; Nicosia et al. 2016; Vitoratos et al. 2013; Yilmaz et al. 2016; Matos et al. 2011; Chen et al. 2013; Endah 2005; Hoyos et al. 2012; Pazmiño-Miranda et al. 2017; Ikeura and Fumiyuki Kobayashi 2015; Pârvu et al. 2008; Dafarera et al. 2003; Bouchra et al. 2003; Corato et al. 2010; Elshafie et al. 2016; Zarai et al. 2011; dos Santos et al. 2010; Li et al. 2011; Parvu et al. 2013), L5—*Botrytis fabae* (Itako et al. 2008; Baka 2010), L6—*Botrytis peaoniae* (Cai et al. 2012), L7—*Botrytis tulipae* (Hussein and Joo 2017), L8—*Monilia laxa* (Nicosia et al. 2016; Elshafie et al. 2016; Li et al. 2011; Lopez-Reyes et al. 2013), L9—*Sclerotinia nivalis* (Thomidis and Filotheou 2016), L10—*Sclerotinia sclerotiorum* (Fonseca et al. 2015a; Kwon et al. 2017; Yoon et al. 2013; Mullerriebau et al. 1995; Cai et al. 2012; Chen et al. 2013; Pane et al. 2016; Thomidis and Filotheou 2016). Orbiliomycetes: O01—*Monacrosporium ambrosium* (Maji and Banerji 2015). Sordariomycetes: S1—*Pestalotiopsis theae* (Begum et al. 2013; Bekhechi et al. 2011), S2—*Pilidiella granati* (Meepagala et al. 2002), S3—*Valsa mali* (Zhang et al. 2006), S4—*Colletotrichum acutatum* (Hoyos et al. 2012; Zarai et al. 2011; Johnny et al. 2011), S5—*Colletotrichum cameliae* (Begum et al. 2013; Yanar et al. 2011a), S6—*Colletotrichum capsici* (Karimi et al. 2016), S7—*Colletotrichum coccodes* (Kwon et al. 2017; Cho et al. 2007; Xu et al. 2014; Schnee et al. 2013), S8—*Colletotrichum falcatum* (Singh et al. 2002), S9—*Colletotrichum gloeosporioides* (Lee et al. 2007a; Cho et al. 2007; Cho et al. 2007; Simonić et al. 2014; Dhingra et al. 2007; Rizwana et al. 2016; Santos et al. 2014; Yilmaz et al. 2016; Bekhechi et al. 2011; Xu et al. 2014; Pârvu et al. 2008; Johnny et al. 2011), S10—*Colletotrichum lindemutianum* (Caputo et al. 2017), S11—*Colletotrichum musae* (Simonić et al. 2014; Dhingra et al. 2007; Rodrigez 2017), S12—*Colletotrichum nymphaeae* (Arslan and Dervis 2010), S13—*Colletotrichum orbiculare* (Bekhechi et al. 2011), S14—*Colletotrichum sublinenola* (Owaid et al. 2017), S15—*Colletrotrichum truncatum* (Osorio et al. 2010), S16—*Verticillium dahliae* (Owaid et al. 2017), S17—*Verticillium fungicola* (Sokovic and VanGriensven 2006; Atmaca et al. 2017), S18—*Cylindrocarpon destructans* (Rizvi et al. 1980), S19—*Fusarium sp*. (Ali et al. 2017; Chu et al. 2005; Endah 2005; Badawy and Abdelgaleil 2014; Bouchra et al. 2003), S20—*Fusarium avenaceum* (Johann et al. 2010), S21—*Fusarium clamidosporum* (Chen et al. 2013), S22—*Fusarium culmorum* (Ojala et al. 2000; Dorman 1999; Golah et al. 2013; Johann et al. 2010; Zhang et al. 2006; Kumar et al. 2016), S23—*Fusarium graminearum* (Huang et al. 2010; Houicher et al. 2016; Chen et al. 2013; Rizvi et al. 1980; Zhang et al. 2006; Tomescu et al. 2015; Santamarina et al. 2016; Sales et al. 2015), S24—*Fusarium guttiforme* (Pinto et al. 2007), S25—*Fusarium moniliforme* (Sigei 2013; Thobunluepop et al. 2009; Mullerriebau et al. 1995; Singh et al. 2002; Ghosh 2006; Houicher et al. 2016; Cobos et al. 2015; Imtiaz 2016), S26—*Fusarium oxysporum* (Bowers and Locke 2000; Szakiel et al. 2011; Waithaka et al. 2017; Dulger et al. 2004; Fonseca et al. 2015a; Ng and Wang 2001; Lam and Ng 2001; Lee et al. 2007a, b; Verma et al. 2008; Al-Zubairi et al. 2017; Simonić et al. 2014; Singh et al. 1994; Dorman 1999; Rizwana et al. 2016; Fierascu et al. 2018; Cabral et al. 2016; Bayar et al. 2018; Pane et al. 2016; Fiori et al. 2000; Zhang et al. 2006; Imtiaz 2016; Rongai et al. 2017; Merali et al. 2003; Sesan et al. 2017; Matsubara et al. 2015; Elsherbiny et al. 2010), S27—*Fusarium poae* (Chen et al. 2013), S28—*Fusarium sambucinum* (Joseph et al. 2008), S29—*Fusarium semitectum* (Simonić et al. 2014; Dhingra et al. 2007), S30—*Fusarium solani* (Fonseca et al. 2015a; Sagar et al. 2011; Baka 2010; Preeti and Sudhir 2014; Itako et al. 2008; Bouchra et al. 2003; Zhang et al. 2006; Shuzhen et al. 2016; Liu et al. 2017; Singh and Rai 2000), S31—*Fusarium udum* (Milovanović et al. 2014), S32—*Fusarium verticillioides* (da Silva Bomfim et al. 2015; Avanço et al. 2017; Lopez et al. 2004; Mehrparvar et al. 2016), S33—*Lecanicillium fungicola* (Glamoclija et al. 2005), S34—*Mycogone perniciosa* (Potocnik et al. 2010), S35—*Trichoderma atroviridae* (Balkan et al. 2017), S36—*Trichoderma harzianum* (Sokovic and VanGriensven 2006; Yeo et al. 2009; Sasidhran and Menon 2010; Oros et al. 2010; Atmaca et al. 2017; Balkan et al. 2017), S37—*Trichoderma viride* (Verma et al. 2008; Vânia et al. 2014; Shiva Rani et al. 2013; Linde et al. 2016), S38—*Trichothecium roseum* (Endo et al. 1990), S39—*Pyricularia oryzae* (Cho et al. 2007; Xu et al. 2014; Rizvi et al. 1980; Zhang et al. 2006; Engelmeier et al. 2004; Fonseca et al. 2015b), S40—*Ceratocystis coerulescens* (Eberhardt and Young 1994), S41—*Chalara paradoxa* (Xue et al. 2017; Pinto et al. 2007), S42—*Chaetomium globosum* (Cabral et al. 2016), S43—*Humicola grisea* (Kumar and Yadav 2014), S44—*Daldinia concentrica* (Arora and Ohlan 1997)

Oomycota: Peronosporales: O1—*Pythium sp*. (Osorio et al. 2010; Oros et al. 2010), O2—*Pythium aphanidermatum* (Huang et al. 2010), O3—*Pythium insidiosum* (Lee et al. 2007a; Kozlowski and Métraux 1999), O4—*Pythium ultimum* (Shenvi et al. 2011), O5—*Phytophthora cactorum* (Hoyos et al. 2012), O6—*Phytophthora capsici* (Shreaz et al. 2016; Mullerriebau et al. 1995; Zhao et al. 2004; Garcia et al. 2018; Bohinc et al. 2015), O7—*Phytophthora cinnamomi* (Bajer et al. 2017), O8—*Phytophthora citrophthora* (Camele et al. 2010), O9—*Phytophthora megasperma* (Rizvi et al. 1980), O10—*Phytophythora infestans* (Cho et al. 2007; Itako et al. 2008; Yanar et al. 2011b; Soylu et al. 2006a, b; Baka 2010; Godeanu-Matei et al. 2016), O11—*Plasmopara viticola* (Pazmiño-Miranda et al. 2017; Gabaston et al. 2017; Dagostin et al. 2011), O13—*Sclerospora graminicola* (Deepak et al. 2005)

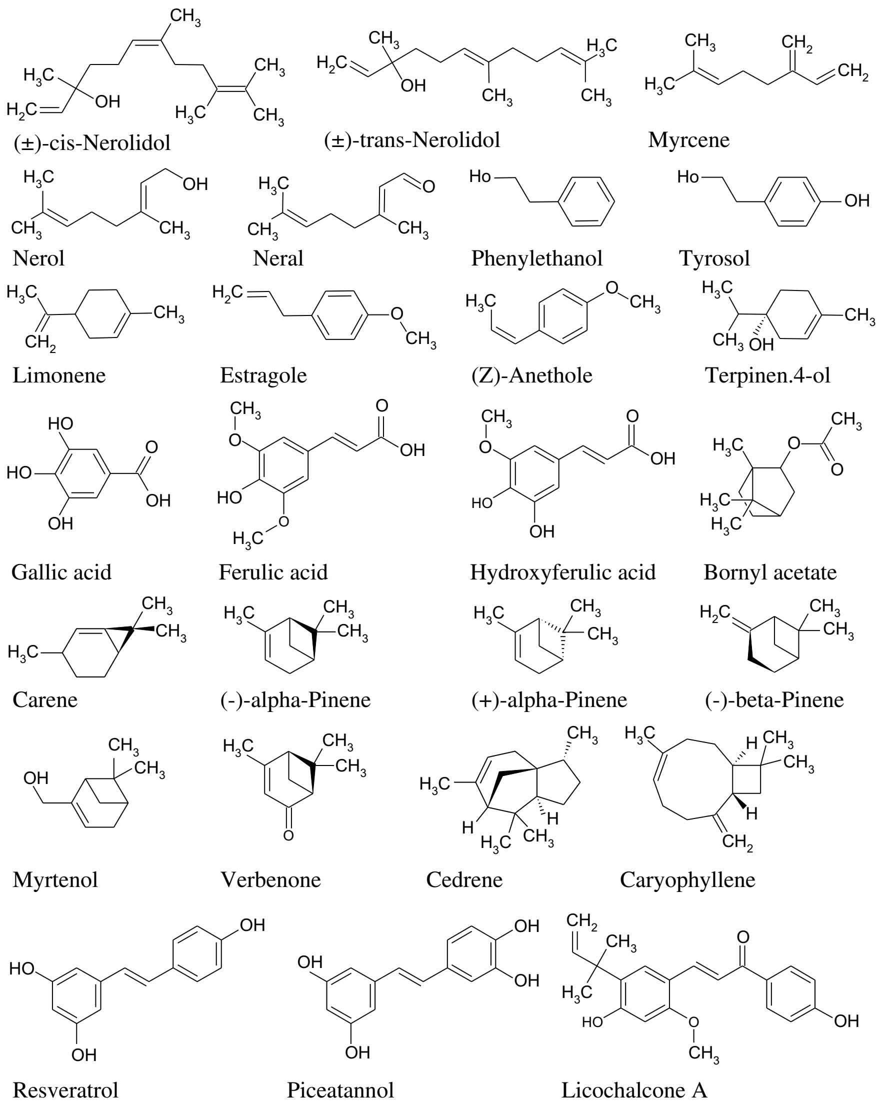


Fig. 11.1 Major secondary metabolites with approved antifungal effect of the most potent culinary and medical herbs tested. R^1—gluocose, R^2—galactose-glucose[xylose]-galactose-glucose. The presence of as minimum as one of listed compounds has been demonstrated in proper marketed spice or herb at more than 10% of active ingredients

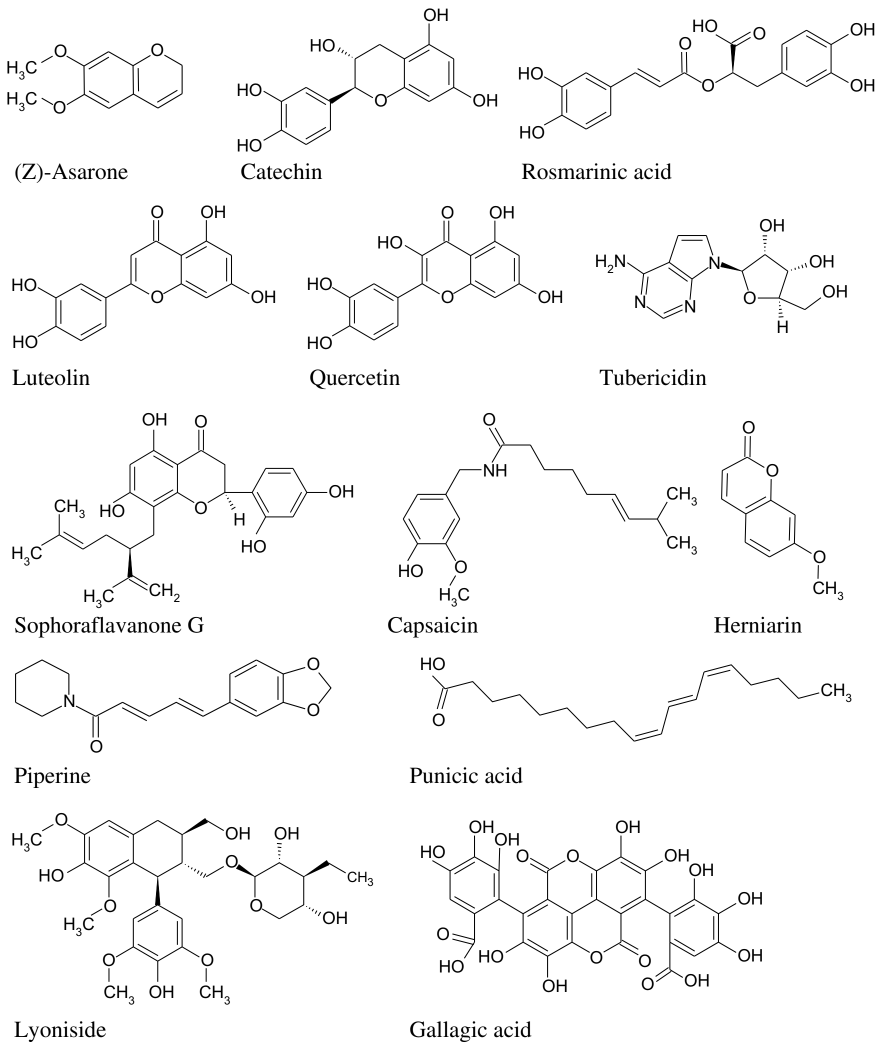


Fig. 11.1 (continued)

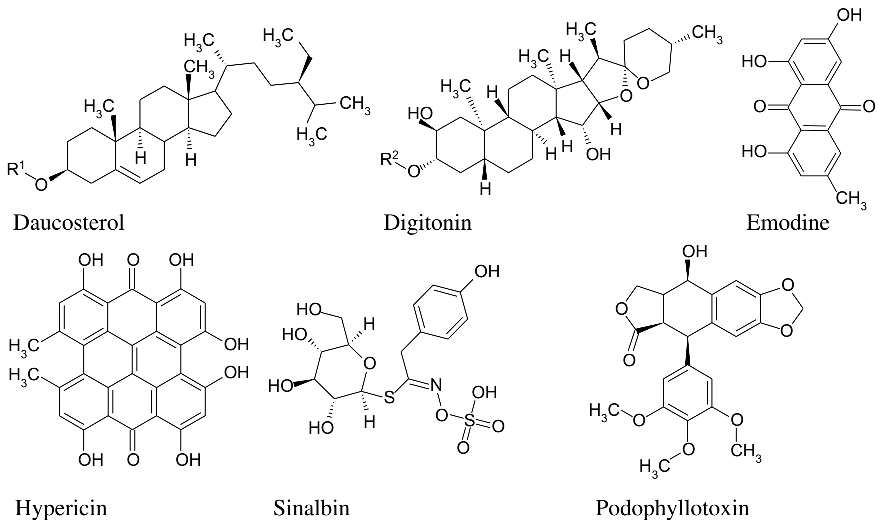


Fig. 11.1 (continued)

Data Analysis Fisher's test was applied to evaluate the significance of differences between variants at $p = 0.05$ level. The basic data matrix (107 preparations × 25 target strains × 2 evaluations) comprising response values by the scale of evaluation was subsequently analyzed with multivariate statistical methods following previously described scheme to elucidate the number of factors affecting the selective response of target fungi to toxic principles (Magyar and Oros 2012).

Potency mapping (PM) and spectral component analysis (SCA) were employed to disclose differences between both antifungal activity of preparations and sensitivity responses of test strains following Lewi (1976). The SCA separates the basic data matrix into two part; the first is a vector proportional to overall strength of responses (PM), while the second is a matrix of spectral components (Spectral Map, SPM) characterizing the spectrum of activity or sensitivity.

PCA was carried out on the correlation matrix calculated of basic data matrix, and only the components having an eigenvalue greater than one were included into the evaluation of data to demonstrate potential number of factors influencing on sensitivity responses of target fungi. Moreover, principal component regression analysis (PCRA) was employed to reveal changes in weight of influencing factors during the incubation, i.e., time dependence of the growth inhibitory effect.

Box plot analysis was applied to demonstrate time-dependent alterations in sensitivity responses. Cluster analysis (CA) combined with SCA was used to reveal relationships among the spectrum of sensitivity responses of phytopathogenic fungi to preparations.

Statistical functions of Microsoft Office Excel 2003 (Microsoft, Redmond, USA) and Statistica5 program (StatSoft 5.0., Tulsa, USA) were used for analysis of data. The graphical presentation of result of data analysis was edited uniformly in MS Office PowerPoint 2003.

11.3 Results

The conidia of all strains germinated and start to form well-distinguishable colonies within 24 h after inoculation, and the intensity of radial growth corresponded to character of species on untreated control plots. The differences between parallels did not surpass 1 mm, so their growth was near synchronous.

The germination of conidia of all strains was inhibited by various degree by herbs after 24 h of inoculation with the exception of *Alternaria* that start to form colony growing on *Clematis vitalba*: Therefore at given dose, all herbs exhibited outstanding antifungal effect being the *Hyssopus officinalis* the least active (Table 11.3). However, this situation changed dramatically after 24 h when the only ten herbs inhibited the growth of all strains (Table 11.4). The loss of activity varied within large limits, and no pattern could be recognized about the taxonomic position of plants (details of the analysis of SMP are not shown). The increase of inhibition as compared to untreated control was observed in 99 cases of 2675 pairs; the more than half of such cases were observed in Ranunculales, Caryophyllales, Myrtales, and Rosales (7, 8, 7, and 23, respectively), and no cases occurred in culinary fungi and moss. The relationship between the initial activity of herbal preparations and activation process needs further studies, although, seemingly the moderately active herb suffered the major deterioration of their antifungal effect.

The sensitivity response of strains varied in large limits; however, none of them was inhibited completely by all preparations. With exception of *Colletotrichum musae* and *Gliocladium catenulatum,* all strains activated as minimum as one of herbs, taking into the consideration the 99 of 2675 pairs, so this process seems to be highly specific and depends on target fungus. Clustering the fungi based on daily changes in their response to herbal preparation (A_{24}-A_{48}), two big clusters have been separated (Fig. 11.2). The strains of soil origin and the insect pathogen were separated of those isolated of foliage. The abilities to either deteriorate or activate the antifungal effect seemingly were not related to taxonomic position of target fungi, as, for example, *Geotrichum candidum* and *Trichotecium roseum* formed a close cluster, or two *Glomerella cingulata* anamorphs (Sour Cherry 1 and 2) have been linked into two different subclusters. The clusters A and B forming a super-cluster comprise more sensitive strains than C, D, and E; moreover, the latter are more heterogeneous in respect of the origin of strains. Thus, one can suppose that former environmental adaptation takes more influence on their sensitivity responses to herbs than traits formed during phylogeny.

Table 11.4 Similarity of hidden variables influencing the performance of growth response of target strains

First day		Principal components second day							
PCs	No.	1	2	5	8	10	11	12	13
No.	Weight	54.6	10.6	4.2	2.7	2.3	1.9	1.6	1.5
1	49.6	**−0.843**	−0.167	−0.011	0.147	0.278	0.075	0.099	0.126
2	9.6	0.094	**0.734**	−0.049	0.117	0.203	0.071	0.263	0.088
3	3.8	0.281	−0.135	−0.083	0.007	**0.527**	0.063	0.175	0.095
7	2.5	−0.091	−0.072	**−0.618**	0.053	0.012	−0.069	−0.070	0.033
8	2.1	0.114	−0.050	−0.180	**0.507**	0.177	−0.103	−0.239	−0.021
10	1.8	−0.066	0.130	−0.100	−0.226	0.058	**−0.531**	0.051	0.447
11	1.5	−0.107	0.205	0.292	−0.196	0.290	−0.019	**−0.564**	−0.244
12	1.4	0.096	0.079	0.162	0.037	0.198	0.145	0.003	**0.573**

The weight of principal components is given in percents of total variation they comprised. Significance of correlation coefficients: $r_{0.01} = 0.5256$; $r_{0.02} = 0.4815$

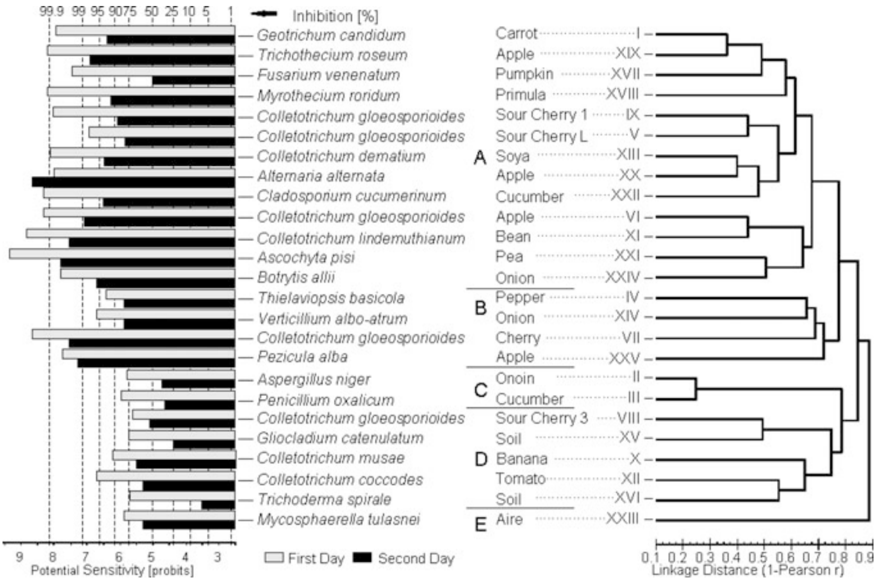
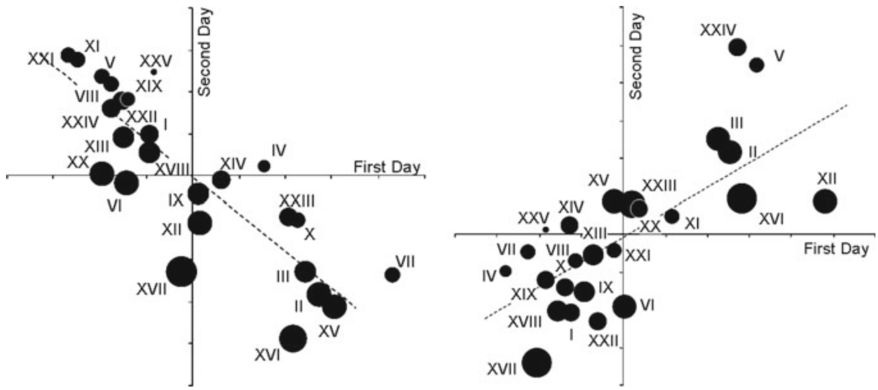


Fig. 11.2 Relationship between spectrum of deterioration of inhibitory substances and growth response of test fungi. Prior to SCA, the growth responses were converted to probit values. Potency mapping technique was used to calculate potential overall sensitivity of strains (growth response to strength of herbal action). The similarity of the sensitivity spectra of strains was analyzed applying unweighted pair group averages method based on correlation matrix of spectral variables. Subclasses were sorted at $p < 0.05$ level

The principal component analysis revealed high number of factors determining the action of herbal preparations. The response of fungi was influenced during conidial germination and germ tube elongation, i.e., start of colony formation (first day of evaluation) by sixteen principal components (PCs) having an eigenvalue greater than one, which comprised 95% of total variation, and among them four hidden factors were seemingly responsible at 70% of the inhibitory effect of herbs. After subsequent incubation (second-day evaluation), the growth response of the same set altered as it was delineated above (see Table 11.3); the PCA elucidated 13 relevant PCs comprising 93% of total variation, where three of them related to 73% of inhibition of colony formation.

This time-dependent reduction of the number of PCs (hidden variables) that influences significantly the performance of strains growing on poisoned agar plates indicates that some factors were eliminated of the medium. Indeed, comparing sets of data recorded at first and second evaluations by means of PCRA sorted out eight PCs in both sets (Table 11.4), which were correlated significantly and explaining majority of acting hidden factors (72 and 81%, respectively). In both sets were separated five PCs which did not show similarity (explaining 19 and 12% of total variation, respectively). The increase of the weight of similar hidden variables as well as decrease of their number (three PCs of 3.7% weight) as compared to the first



A - First PCs explaining 49,6 and 54,6 percents of total variation

B - Second PCs explaining 9,6 and 10,6 percents of total variation

Fig. 11.3 Similarities between hidden variables influencing the performance of sensitivity responses of target strains at various phases of their ontogeny. The Roman numerals indicate strains listed in Fig. 11.2. The size of pies is proportional to potential capacity of strain to deactivate the growth inhibitory effect of herb incorporated into the medium. The path coefficients of the fitness of regression lines in graphs A and B are 0.7099 and 0.7845, respectively

evaluation might indicate the changes in the level of active compounds in the medium resulted by metabolic activity of target fungi. As the activity of various herbs was affected by strain-dependent manner, only some general aspects of the character of major hidden variables could be postulated. Plotting strains as PC variables by intercorrelating the major PCs of two sets (Fig. 11.3) elucidated remarkable selectivity of interaction between herbs and strains. The first pair (Fig. 11.3a) negatively influenced the performance of herbs, so it can be most probably related to metabolic degradation of active principles, while the second pair (Fig. 11.3b) affected positively, which may indicate the increase of importance of permanent target sites in expression of antifungal effect (characterized by intensity of growth inhibition).

11.4 Exploitation of Findings

The anthracnose caused by *Glomerella* anamorph has caused increasing losses in Hungarian sour cherry orchards since 2006. The pathogen rapidly acquired tolerance to most effective triazole fungicides. Because of the short tolerance period (maximum 6 days), the protection of sour cherry fruit is a special problem, and use of rapidly deteriorating fungicide is requested. The botanical preparations can stand this prerequisite. The possible use of ten herbs proved to be most active among tested ones (shiitake, galangal, cinnamon, yellow mustard, clove, oregano, summer flavory, wasabi root, wood ear, pomegranate) had been examined to control anthracnose of almond, bilberry, cherries, green pepper, grape, and tomatoes.

However, the promising results in model experiments could not be reproduced in large scale in the sour cherry orchard, where the situation was similar to those observed in the case of pathogenic *Glomerella* anamorphs (Oros et al. 2010). The only pomegranate preparation acted at acceptable way at 1 kg ha^{-1} rate in model experiments that means the preparation manufactured of aborted flowers can control the pathogen, while the others either should be applied at irrational for control doses

Table 11.5 Most important relationships to be evaluated for development and application of pest control agents

Exposed organisms			Therapeutic index	Persistence (days)
To be controlled		To be protected (P)		
Pest (C)	Traditional	*Homo sapiens*	No harm	Not
		Host plant	>5	1–30
		Vertebrates	>100	Not
		Bees	>100	Not
		Saccharomyces	>3	Not
	Future	Symbionts	>10	Not
		Antagonists	?	Not
		Predators	?	Not
		Ecosystems	?	?

Therapeutic index (T.I.) = MTD_P/MID_C, where MTD and MID are maximum tolerated and minimum inhibitory doses of control agent, respectively

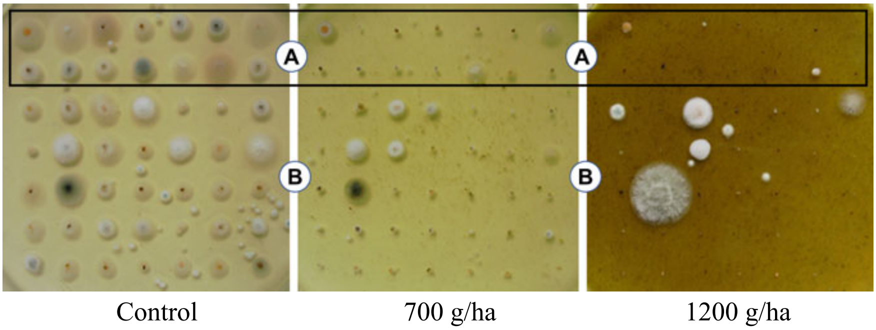


Fig. 11.4 Growth response of *Glomerella* anamorphs to various doses of pomegranate. The standard preparation made of micronized aborted pomegranate flowers was incorporated into PDA prior to pouring into Petri dishes (5-mm-thick layer) at rate mimicking the concentration of the spray to be used in field conditions. The strains in black bordered box (A) are various *Glomerella* anamorph (*Colletotrichum* species), while those of group B were isolated of sour cherry fruits collected in orchards of Fruitculture Research Institute (47°40′22.2″ N, 21°41′24.7″ E). The positions of the proper strains are identical on plates

or proved to be phytotoxic in effective dose, i.e., their therapeutic value lagged behind highly active synthetic monosite inhibitors (Table 11.5).

Unfortunately, 5 of 35 lines of anthracnose pathogen proved to be highly tolerant to prospected new biofungicide (Fig. 11.4). Most probably, in the case of other host/pathogen pairs, similar results can be expected that shows the limit of development of botanical fungicides based on crude preparations.

11.5 Discussion

The use of chemical fungicides is costly and potentially harmful to the environment. The trend toward the environmentally friendly pesticides has led to the search for new antifungal agents from various sources, including medicinal herbs, however, to plants of culinary use have paid less attention. Alternative control with herbal preparations showing the greatest antifungal potential could provide economical, safe, and non-hazardous tools for management of cultivated plants and increase food quality from sustainable production (Khaskheli et al. 2016). Most probably all plants have phytoanticipins of diverse molecular structure and size of simple myrcene or phenylethanol to steroid alkaloids, oligocarbohydrates, proteins, etc. There are increasing number of studies dealing with the isolation and chemical characterization of such molecules as well as their role in host–pathogen interactions. Several and successful efforts have been made to introduce compounds of plant origin (strychnine, rotenone, cevadine, pyrethrins) to use against pests; however, the botanicals of similar activity or formers active against phytopathogenic fungi have not been marketed yet. Here we investigated only the heat-tolerant compounds of low molecular weight.

There are increasing number of studies dealing with the isolation and chemical characterization of phytoanticipins as well as their role in host–pathogen interactions. Nevertheless, it seems to be clear that the defense molecules either predisposed or induced cannot be regarded as the agents of a single defense mechanisms. Very little detailed information is at our disposal about the multiple mechanisms for plant resistance against pests and pathogens, and these are still a matter of debate.

The Biopesticides and Pollution Prevention Division in the Office of Pesticide Programs of Environmental Protection Agency of USA encourages the development of biopesticides as well as the use of safer pesticides, including biopesticides. Since generally accepted that biopesticides tend to pose fever risks than conventional ones, EPA generally requires much less data to register them than latter. In fact, new biopesticide is often registered less than a year, compared with an average of more than three years for those based on synthetic chemicals. However, using any chemical in pest control management the same requirements have to be taken into the consideration, when these preparations aimed to be applied at large scale! Moreover, the selectivity of action also has to be evaluated by the same manner, independently of the character of active ingredient, and this requirement is more strict than those used in the case of pharmaceuticals (Table 11.5). For example, the bees meet regularly the essential oil flavonoids that are mighty attractants for pollinating animals. However, the dose in concerted activities is very low. It is well known; the essential oils might be detrimental for humans in elevated doses when inhaling for long-term exposure. Numerous reports support that content of preformed antifungal compounds correlates with disease resistance, for example, the fall of preformed antifungal compounds in strawberry fruits was correlated with a decline in natural disease resistance against *Botrytis cinerea* (Terry et al. 2004). Analogies of medicine frequently used as some botanical preparations are traditionally applied against dermatomycoses. However, the decision on therapeutic value is different: In medicine, some iatrogenic effect might be accepted, for example, the drug applied more harmful to cancer cells than regular cells, or the use of arsenic derivatives to eliminate parasitic protozoans. In these cases usually, the ration of ED50 or LD50 values is used. Contrarily, the adverse effects in the case of host/parasite pairs are rarely accepted, and the ration of maximum tolerated dose by host plant and minimum inhibitory dose for pathogen should be taken (Table 11.5); moreover, the decisionmaker should take account of suspected knowledge of users when recommends dose for practical applications, i.e., the three- to fivefold overdose cannot harm the exposed cultivated plant.

The separation and identification of active principles of herbs important as well as the use of well-identified molecules have advantages. However, the crude extracts and herbal material per se often differ in the activity being the latter more effective (Al-Sohaibani et al. 2011). The preparation may destroy the active principle, or separate synergically interacting substances (Kapoor et al. 2008). The content of single molecules and their ratios often differ batch to batch, which shows similar affectivity due to synergic interaction of component. This fact indicates that the use of homemade crude preparations may have advantages in special regards in microscale applications.

Dramatic advancement in biology can be seen within the last fifty years. The contemporary plant biology, which led through meristem culture to the clonal propagation as well as these procedures led to tissue culture techniques, which were utilized to grow cells in suspension cultures with subsequent ability to regenerate whole plants that created a whole new era in plant biology. Some efforts have been made, and there is an increasing interest to introduce alien genes coding performed defense molecules into cultivated plants. These new properties also should be approved by selectivity criteria that are identical to those requested in the case of synthetic pesticides (Table 11.2). The experiences are contradictory: Unexpected adverse effect has manifested both in biocoenoses and in pests themselves, mainly due to acquired tolerance in populations of target organisms, like Lepidopteras to thuringiensis toxins or innumerable weed species to herbicides. No doubt about that microbes of agricultural interests will also rapidly adapt to new properties. The introduction of toxic substances alien to edible plants can also induce serious damages as it was demonstrated in the case of galanthus toxin—all this underlines that the soft practice of EPA cannot be kept when the registration of biopreparations for large-scale use takes place. Nevertheless, the intentions to improve the plant resistance by rationally designed genetic manipulations using biotech methods are

Table 11.6 Expected application of gene engineering to approve efficiency of pest control by biorational way

Type of compound	Field of application		
	Food chain	Industrial plants	Wild areas
Phytoalexin	+	+	+
Phytoanticipin	?	+	+
Signal molecules	?	+	+

promising together with to develop botanical preparations to combat losses in agriculture (Table 11.6).

Some questions need answers, first of all problems of unwanted exposures. In spite of intensive studies on defense molecules our knowledge regarding their mode of action and the flow of signal transmitters from the pathogen to the plant cells is still poor. The protective functions are highly diversified, and the variegation of defense mechanism shows multifunctional character. The exposed population, being not uniform genetically, is a mixture of strains as well as the ratio of different isomers can vary in botanical preparation depending on source and mode of manufacturing, the strain-specific action and stereometric-dependent response may limit the usefulness of herbal preparations. Nevertheless, studies on the biological activities of herbs are increasingly important in the search for natural and safe alternative pesticides in recent years. There is a lot of to be done before their use in large scale. More in-depth knowledge of potentially useful plants can provide results of economic importance for food and even pharmacological industry.

The abundant use of antimicrobial agents resulted in the emergence of drug-resistant bacteria, fungi, and viruses both in medicine and agriculture. To overcome this threat, there is necessary to find new, effective antimicrobial agents with novel modes of actions. The plant defense molecules are promising candidates for lead compounds. Some compromise among yield sureness, quality, and number of products is requested for making the biorationally designed and carefully selected new varieties.

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