Fungicidal preparations from *Inula viscose*

Extrakte aus *Inula viscose* zur Bekämpfung von Pilzkrankheiten

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Abstract

Inula viscosa is a perennial plant native to the Mediterranean Basin. Extracts made from the shoots of this plant exhibited a strong fungicidal activity *in vitro* and *in vivo*. TLC analyses revealed at least 7 fungicidal compounds. Most are lipophilic. When such extracts were sprayed on the leaf surface of crop plants they effectively controlled downy mildew in grape, cucumber and tobacco; late blight in potato and tomato; gray mold in cucumber and tomato; and, powdery mildew in cucurbits and cereals. Field experiments conducted with grape vine resulted in effective control of *Plasmopara viticola*. The data suggest that *Inula viscosa* is a useful source of herbal fungicidal preparations for agricultural use.

Keywords:

Organic farming; natural fungicides; disease control

Introduction

Inula viscosa (Compositeae) is a perennial weed native of the Mediterranean Basin. It has sticky (viscous) leaves with a typical odor. Normally, no pests harbor on this plant nor animals feed on it. In folklore medicine its extracts are used to hill inflammations and its dry shoots to repel insects. Extensive chemical analyses of *I. viscosa* revealed a series of terpenoid compounds, with some exhibiting antifungal activity. The purpose of this study was to examine the efficacy of various extracts of *I. viscosa* in controlling fungal diseases of crop plants.

Methods and Materials

Shoots of *I. viscosa* were collected from various locations in Israel during the summer season and used either fresh or dry, for extraction. Extraction was made by shaking the plant material with a solvent (at a ratio of 1:10, w/v) for various periods of time (see Results). The extract was filtered through 3 cheesecloth to remove plant debris and then through Whatman N°. 1 filter paper. Extracts were used as such, or evaporated to dryness (at 35 °C in vaccuo) to obtain a paste. The paste was dissolved (0.01–1%) in an appropriate solvent and then applied to crop plants for testing.

Plant extracts were spotted on TLC plates, ran with chloroform:methanol (90:10, v/v) and developed with I_2 vapor. Other TLC plates were developed by spraying with spore suspension of *Cladosporium cucumerinum* in 2% PDB. White spots on a black background of the plate indicated antifungal zone. Extracts were also subjected to HPLC and GC-MS to identify some of the compounds present.

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To examine fungicidal activity *in planta* the treated plants were inoculated with an appropriate fungal pathogen and symptom production was evaluated 1 week later.

Results

Table 1 presents the paste yields of extracts obtained from *Inula* shoots using various solvents and procedures. When fresh shoots were used, yields ranged between 0.55-6.64% whereas when dry shoots were used yields ranged between 3.52-29.76%. The lowest yield was obtained with n-hexane and the highest with methanol.

TLC analysis of a chloroform extract showed 10 bands with iodine vapor, of which 7 were inhibitory to *C. cucumerinum* (Table 2).

The chloroform extract was applied to depressions of microscope glass slides and after the solvent has evaporated sporangia of *Phytophthora infestans* or conidispores of *Botrytis cinerea* were applied to the depressions. Table 3 indicates a strong inhibitory effect of the extract on germination of both fungi.

Chloroform extract of various concentrations was sprayed onto potato and cucumber plants which were thereafter inoculated with the late blight and powdery mildew pathogens, respectively. Table 4 shows 50% control of the disease with 0.05-0.1% and 95-96% control with 0.4% solutions of the extract (dissolved in acetone).

The efficacy of 0.4% solutions, of extracts prepared with the aid of 7 different solvents, in controlling 5 different fungal diseases is presented in Table 5. The data show poor efficacy of the water extract as against high efficacy of organic solvent extracts. Powdery mildew in cucumber was better controlled with extracts made with ethylacetate or n-hexane than with those made with acetone or chloroform.

Efficacy of 4 extracts prepared with dual mixtures of organic solvents is shown in Table 6.

Emulsions of acetone extract were further examined against *Plasmopara viticola* in grape leaves in both the greenhouse and the field. Results from the greenhouse show >90% control of the disease with 0.1% of such emulsions. Three sprays in the field resulted with about 90% control of downy mildew in grapevines treated with 0.25% emulsion relative to control untreated vines.

Discussion

Extracts derived from *Inula viscosa* with organic solvents are shown here to contain antifungal compounds. Water extracts showed a poor yield of such compound suggesting that those fungicidal materials are mostly lipophilic. *In vitro* and *in planta* studies revealed broad-spectrum efficacy of these extracts in controlling various diseases including Oomycetes, Ascomycetes, Basidiomycetes (bean and sunflower rusts, data not shown) and Fungi Imperfecti.

Field studies also revealed efficacy of *Inula* extracts in controlling downy mildew in grape leaves. The data suggest that *Inula viscosa* may serve as an efficient source for herbal fungicides.

Table 1: Yield of different methods of preparing *Inula* extracts

Solvent	Dry weight of extract (grams per 100 g starting material) Extraction Procedure		
	Freshly cut shoots, dipping for 10 sec*	Freshly cut shoots, shaking for 30 min**	Dried shoots, shaking for 30 min***
Water		0.96	12.80
Methanol		4.48	29.76
Ethanol		4.96	20.80
Acetone	5.04	4.80	19.84
Ethylacetate	4.87	6.24	22.08
Diethylether	4.50	4.00	14.40
Chloroform	6.64	4.64	16.00
n-Hexane	0.55	1.60	3.52

* 100 g freshly cut shoots extracted in 0.25 liter of solvent
** 100 g freshly cut shoots extracted in 1 liter of solvent
*** 10 g dried and ground shoots extracted in 0.1 liter of solvent

Table 2: Results of thin-layer chromatography separation and bioassay of the fungicidal properties of *Inula* extract

Compound Number	R_f of Compound*	I_2 Staining Intensity	Width of Zone of Inhibited Growth of *C. cucumerinum* (mm)
1	0.00	–	0
2	0.31	++	7
3	0.35	++	0
4	0.37	++	0
5	0.42	++	5
6	0.49	+++	11
7	0.53	++++	15
8	0.64	++++	10
9	0.72	++++	15
10	0.81	+	3

*Note: R_f values may vary 10% between experiments

Table 3: The effect of *Inula* extract on zoospore discharge and cytospore germination of ***Phytophthora infestans***, and spore germination of *Botrytis cinerea*

	Concentration of *Inula* extract (%)		
	P. infestans		***B. cinerea***
Inhibition of Fungal Activity (%)	**Zoospore Discharge**	**Cytospore Germination**	**Conidial Germination**
50% Inhibition	0.075	0.05	0.1
100% Inhibition	0.2	0.1	0.3

Table 4: The protective effect of *Inula* extract against *Phytophthora infestans* in potato and *Pseudoperonospora cubensis* in cucumber

Concentration of *Inula* Extract (%)	**Protection Against Blight (%)**	**Protection Against Downy Mildew (%)**
0 (acetone control)	0	0
0.00125	14	26
0.0250	20	42
0.05	40	51
0.1	50	68
0.2	65	83
0.4	95	96

Table 5: Protection against plant diseases by 0.4% extracts derived from dried, ground *Inula* shoots

	% Control of the Disease				
Solvent Used for Extraction	**Late Blight in Potato**	**Downy Mildew in Cucumber**	**Powdery Mildew in Wheat**	**Powdery Mildew in Cucumber**	**Gray Mold in Cucumber**
Water	26	43	45	37	
Methanol	89	99	90	75	
Ethanol	94	99	93	95	
Ethylacetate	91	91	83	100	
Acetone	99	95	93	75	100
Chloroform	83	100	86	70	
n-Hexane	97	91	93	97	85

Table 6: Efficacy of 0.4% *Inula* dual extracts against fungal plant pathogens

	% control of the disease			
Solvent mixture used for extraction 1+1 v/v	**Late blight in potato**	**Powdery mildew in wheat**	**Gray mold in cucumber**	**Downy mildew in grapes**
Chloroform + n-hexane	76	94	100	*nt*
Ethylacetate + n-hexane	82	94	100	*nt*
Ethanol + n-hexane	72	89	100	*nt*
Acetone + n-hexane	95	92	*nt*	87

nt = not tested