

Control of Plant Diseases by Extracts of *Inula viscosa*

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ABSTRACT

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Leaves of *Inula viscosa* were collected from the field, dried, and extracted with a mixture of acetone and n-hexane. The oily, water-insoluble pastes obtained after evaporation of the solvents were used for the control of foliar diseases in growth chambers. The pastes, either dissolved in acetone or emulsified in water, effectively controlled downy mildew of cucumber, late blight of potato or tomato, powdery mildew of wheat, and rust of sunflower. Mean effective dose (concentration) required for 90% inhibition of disease values for acetone solutions and water emulsions

ranged from 0.68 to 1.02% and 0.65 to 1.00% (wt/vol), respectively. Dry matter content in fresh leaves, paste-extract yield in dry leaves, and disease control efficacy of paste extracts were similar in leaves of *I. viscosa* collected during May to October, suggesting that, for practical use, harvests can be conducted during most of the growing season. The results show that *I. viscosa* may be used as an herbal source for fungicidal preparations against foliar diseases caused by pathogens belonging to the families Oomycetes, Ascomycetes, and Basidiomycetes.

Additional keywords: *Blumeria graminis* f. sp. *tritici*, herbal extracts, *Phytophthora infestans*, *Pseudoperonospora cubensis*, *Puccinia helianthi*.

Inula viscosa (L.) Aiton (syn. *Cupularia viscosa* G. et G., *Dittrichia viscosa* Greuter) (*Compositae*) (common name "sticky fleabane") is a perennial weed, native to the Mediterranean Basin. It grows on hillslopes, damp habitats, and roadsides. In folklore medicine, this plant is used for therapeutic purposes, such as a diuretic, topical anti-inflammatory, and haemostatic (10). Aqueous extracts of *I. viscosa* were shown to exhibit antifungal activity in vitro (12,18), and organic solvent extracts were shown to be antibacterial (7). Cohen et al. (4) provided evidence for the antifungal activity in planta of extracts made with organic solvents, including methanol, ethanol, ethylacetate, acetone, chloroform, and n-hexane. Using thin-layer chromatography overlay assays, seven inhibitory zones against *Cladosporium cucumerinum* were observed in the extracts (4). In a recent study (21), it was found that leaf extracts of *I. viscosa* were highly effective in controlling downy mildew of grapevine, caused by *Plasmopara viticola*. In the present study, we were interested in following the efficacy of extracts obtained from *I. viscosa* during the active growing period in the field in controlling downy mildew of cucumber, late blight of potato or tomato, powdery mildew of wheat, and rust of sunflower. Another goal of this study was to compare the efficacy of acetone solutions made with the dry pastes and pastes emulsified in water.

MATERIALS AND METHODS

Growing of *I. viscosa* and collection of leaves. *I. viscosa* seed (collected in situ at Tirat-Yehuda, 20 km east of Tel-Aviv) were sown in Speedling trays (Hishsttil, Petach-Tiqwa, Israel) containing a mixture of peat and perlite (1:1, vol/vol), and grown in the greenhouse. When they reached the four- to six-leaf stage (≈ 2 months after sowing), plants were transplanted in the field at

Keramim (2 acres, Loess soil) in March 2001 and at Bar-Ilan Farm (four rows, each 50 m long, sandy-loam soil) in March 2002. Planting was done with a distance of 0.2 m between plants and 1 m between rows. During the dry season, plants were irrigated by drip irrigation. Water was supplemented with of N-P-K fertilizer (20:20:20) at 5 kg/ha once every 2 weeks.

To save labor and solvents, *I. viscosa* leaves should first be dried before extraction. For this purpose, we determined the content of dry matter in fresh leaves. At Keramim, 10 kg of leaves were collected in April, June, and September 2002, air dried for 1 week, and stored in paper bags at room temperature until used. At Bar-Ilan, leaves were collected at biweekly intervals from 10 to 15 plants in each row, with each sample composed of ≈ 0.25 kg of leaves, for a total of 1 kg/harvest. Consecutive harvests each were done from another set of plants. Harvests were done during 1 April to 15 October 2003. Leaves from each harvest were pooled, weighed, dried in an oven at 60°C for 24 h, weighed again to determine dry matter content, and kept in plastic bags until used for extraction.

Extraction and formulation. For each harvest, a 100-g sample of dry leaves was extracted at 35°C for 3 h by shaking in a mixture of acetone and n-hexane (9:1, vol/vol) at a ratio of 1:10 (wt/vol, dry leaves/solvent). The extract was filtered through Whatman No. 1 filter paper and vacuum dried at 45 to 50°C to obtain an oily, dry, green paste. The paste was weighed to determine the percentage of paste in the dry leaves.

Results obtained from the two harvests made at Bar-Ilan during each month of 2003 were used to obtain the mean (and standard deviation) of dry matter content in fresh leaves and paste yield in dry leaves, by using Super Anova (Abacus Concepts Inc., Berkeley, CA).

Pastes freely dissolved in acetone, producing greenish, transparent solutions. Such solutions were applied as a spray to the foliage of potted plants to assess their efficacy in controlling disease. Acetone solutions were prepared by dissolving 2 g of each paste in 100 ml of acetone and diluting with acetone into a series of concentrations (2, 1, 0.5, and 0.25%, wt/vol). A water emulsion was made by melting 34.4 g of a paste (derived similarly from

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leaves of *I. viscosa* collected at Keramim in June 2002) in a water bath (50°C), adding 65.6 g of emulsifier into the paste, and vortexing the mixture for 2 min to form a 34.4% emulsified concentrate (EC) (for details, see patent application IL149,716, May 2002) This product formed a stable emulsion when mixed with water.

Plants and pathogens. Cucumber (*Cucumis sativum* L.) 'Nadyojni', susceptible to *Pseudoperonospora cubensis* (Berk. & Curt. et de Toni) Rost.; potato (*Solanum tuberosum* L.) 'Mondial' and tomato (*Lycopersicon esculentum* Mill.) 'ZH', both susceptible to *Phytophthora infestans* Mont. (de Bary); wheat (*Triticum sativum* L.) 'Sharon', susceptible to *Blumeria graminis* f. sp. *tritici* (DC.) Speer; and sunflower (*Helianthus annuus* L.) 'H-567', susceptible to *Puccinia helianthi* Schwein. were grown from seed (tubers for potato) in 1-liter pots containing peat + perlite (1:1, vol/vol) in the greenhouse. Plants were used for disease control assays at 3 to 5 weeks after seeding.

Cucumber leaves infected by pathotype 3 of *Pseudoperonospora cubensis* (6) were collected from field-grown cucumbers at Bar-Ilan Farm in spring 2001. Leaves were kept on wet filter papers in 20-by-20-by-3-cm plastic trays at 20°C for 2 days (12 h of light/day) to stimulate sporulation of the pathogen. Isolate 367 (A₂ mating type, resistant to metalaxyl) (3) of *Phytophthora infestans* was kept and transferred on detached leaves of tomato. Sporangia of *Pseudoperonospora cubensis* and *Phytophthora infestans* were collected with a fine brush into cold distilled water, the concentration was adjusted to 1×10^3 sporangia/ml, and the suspensions were used for inoculation.

Sunflower leaves carrying urediniospores of *Puccinia helianthi* were obtained from Hazera Genetics, Brorim, Israel. Urediniospores were suspended in water containing 0.01% Tween 20, their concentration was adjusted to 1×10^4 spores/ml, and the suspension was used for inoculation of sunflower plants.

B. graminis f. sp. *tritici* was obtained from the Institute of Cereal Research, Tel-Aviv University, and maintained on wheat seedlings ('Sharon'). Fresh conidia produced on wheat seedlings were used for inoculation by gentle dusting over the test plants.

Activity of extracts dissolved in acetone. Pastes were dissolved in acetone and diluted into four concentrations (2, 1, 0.5, and 0.25%, wt/vol) as described above. Three replicate plants of cucumber, potato, tomato, and sunflower and three pots having 10 wheat plants/pot were used per treatment. The solutions were sprayed onto the upper leaf surface (both surfaces in wheat) of the plants. Plants treated with acetone and untreated plants served as controls. At about 30 min after treatment, when the acetone had evaporated, treated plants and control plants were inoculated with their respective pathogens. Inoculated plants, except wheat, were kept in a dew chamber for 12 to 16 h and then incubated in growth chambers at 20°C (12 h of light/day, $100 \mu\text{E m}^{-2} \text{s}^{-1}$) for 6 to 10 days, depending on the pathosystem. Wheat plants were placed in growth chambers immediately after inoculation. The percentage of infected leaf area in each plant was estimated visually. In sunflower, the number of pustules on each plant was counted. Control efficacy was calculated as $100(1 - X/Y)$, where X = percent infected leaf area in treated plants and Y = percent infected leaf area in acetone-control plants. The same calculation was applied for sunflower, except pustule numbers per plant were used for the calculation. Linear regression analysis was conducted between the log concentration of the extract (%) and probit of control efficacy (%) using SPSS 11.5 for Windows. Effective dose (concentration) required for 90% inhibition of disease (ED₉₀) for each extract was calculated based on the linear regression equations. For each pathosystem, the mean percentage of infected leaf area (pustule number for sunflower rust) and the standard deviation of the means of the seven monthly harvests were calculated with Super Anova. The mean ED₉₀ values, the standard deviation of the mean of the two harvests made each month for each pathosystem, and those for all harvests for each pathosystem were

calculated similarly. Analysis of variance was performed followed by Fisher's protected least significant difference (LSD) test to establish significant differences ($P = 0.05$) in ED₉₀ values among pathosystems.

Efficacy of extracts emulsified in water. Paste derived from leaves of *I. viscosa* collected in June 2002 was formulated in June 2003 into a 34.4% EC product. Formulated paste and blank controls (containing the same rate of emulsifier only) were diluted with water into emulsions containing 1, 0.5, 0.25, or 0.125% paste (wt/vol). Emulsions were sprayed onto the foliage of plants, with three replicate plants (or pots for wheat) per concentration. Plants treated with water only were used as an untreated control. Treated plants, blank-control plants, and water-treated controls then were inoculated with their respective pathogens and incubated as mentioned above. The percentage of infected leaf area on each plant, control efficacy, and ED₉₀ values were calculated as described above. For each pathosystem, the mean percentage of infected leaf area (pustule number for sunflower rust) and standard deviation of the mean were calculated. Analysis of variance was performed followed by Fisher's protected LSD test to establish significant differences ($P = 0.05$) in disease severities among plants treated with different concentrations of each paste and between treated and untreated control plants for each pathosystem.

Thin-layer chromatography separation of paste. Paste (500 mg, Keramim, June 2002) was dissolved in 0.5 ml of acetone. The solution was streaked on three thin-layer chromatography (TLC) plates (Silica 60, Merck 5725) and run in chloroform:methanol, 9:1 (vol/vol), to a distance of 17 cm. Based on iodine vapor detection, six major colored regions (regions 1 to 6) were identified, corresponding to the following Rf values: 1 = 0 to 0.15, 2 = 0.15 to 0.43, 3 = 0.43 to 0.58, 4 = 0.58 to 0.78, 5 = 0.78 to 0.84, and 6 = 0.84 to 0.94. Silica of each region was scraped and eluted in acetone. The acetone was evaporated and the weight of each component was determined. Components were emulsified in water (see above), diluted into 250, 125, and 62.5 µg/ml, and applied to 10 tomato leaf disks (12 mm in diameter) in a petri dish, five 10-µl droplets per disk. Droplets were allowed to dry for 1 h and each leaf disk was inoculated with a 10-µl droplet of sporangial suspension (2,000 sporangia/ml) of *Phytophthora infestans*. Plates were incubated at 18°C in the dark for 16 h and then transferred to a 20°C growth cabinet as above. Percentage of leaf disk area occupied with mycelia and sporangia of the pathogen was recorded 7 days after inoculation. Untreated inoculated leaf disks and emulsifier-treated inoculated leaf disks served as controls. Percentage of inhibition of late blight development was calculated relative to the untreated control inoculated disks.

RESULTS

Dry matter and paste contents. Dry matter content in fresh leaves collected at Keramim in 2002 was 16, 22, and 25% in April, June, and September, respectively. Dry matter content in fresh leaves collected at Bar-Ilan during 2003 ranged from 16.7 to 27.8%, depending on the time of harvest (Fig. 1A). It was lower in leaves collected in April than in leaves collected from May to October. In October, when plants started to bloom, dry matter content was higher than in July but similar to the other harvests, except April.

After extracting the dry leaves, the solvent mixture was evaporated leaving an oily, water-insoluble, green paste. Weighing the paste derived from 100 g of dry leaves enabled us to obtain the percentage of extractable paste in the dry leaves (paste yield). At Keramim, paste yield in April, June, and September was 9.0, 13.0, and 15.1, respectively. Paste yield obtained from dry leaves collected at Bar-Ilan during 2003 ranged from 6.8 to 15.4% (Fig. 1B). The paste yield in leaves collected in April was lower than in leaves collected in the following 6 months.

Efficacy of pastes dissolved in acetone. Paste extracts derived from leaves collected at Keramim in April, June, and September 2002 were dissolved in acetone (1%, wt/vol) and tested for disease control. Mean control efficacy against late blight in tomato,

late blight in potato, and powdery mildew in wheat ranged between 70 to 80% for paste extracts derived in April, compared with 90 to 95% for extracts derived in June or September (data not shown).

Figure 2 presents data for one extract derived from leaves collected at Bar-Ilan on 15 June 2003. It reveals that the severity of downy mildew of cucumber, late blight of potato or tomato, powdery mildew of wheat, and rust of sunflower gradually decreased when treated with increasing doses (0.25 to 2%, wt/vol) of paste in acetone solution. At 0.25%, there were significant reductions in disease severity relative to acetone-treated plants, and at a concentration of 1% the efficacy reached 90% or more. No differences in disease severity were observed between untreated inoculated plants and acetone-treated inoculated plants (data not shown).

Similar experiments were conducted with all other pastes derived from the field at Bar-Ilan during 2003. Disease severity data were collected, percent efficacy was calculated, and ED₉₀ values were derived after log-probit transformation. Goodness of fit of the linear regressions used to calculate the ED₉₀ values ranged, for the different harvests, between 0.889 and 0.997 for cucumber downy mildew, 0.906 and 0.998 for potato late blight, 0.916 and 0.993 for tomato late blight, 0.871 and 0.999 for wheat powdery mildew, and 0.838 and 0.997 for sunflower rust. The results (Fig. 3A to E) indicate differences between pastes and among pathosystems. In most pathosystems, the efficacy of the paste obtained in April was lower compared with the pastes obtained later in the season. Mean ED₉₀ values calculated from the data of Figure 3 are given in Figure 4. Mean ED₉₀ values ranged from 0.68 to 1.02%, greater than the value of <0.18% for grape downy mildew in a previous study (21). The order of sensitivity to the pastes, in terms of mean ED₉₀, was wheat powdery mildew < sunflower rust < potato and tomato late blight < cucumber downy mildew (Fig. 4).

Efficacy of paste emulsified in water. A paste, derived from leaves harvested in June 2002, was formulated with the aid of an emulsifier and sprayed, at various concentrations, onto the foliage of the test plants. The percentage of infected leaf area (or, for sunflower rust, pustule numbers) for the various pathosystems is

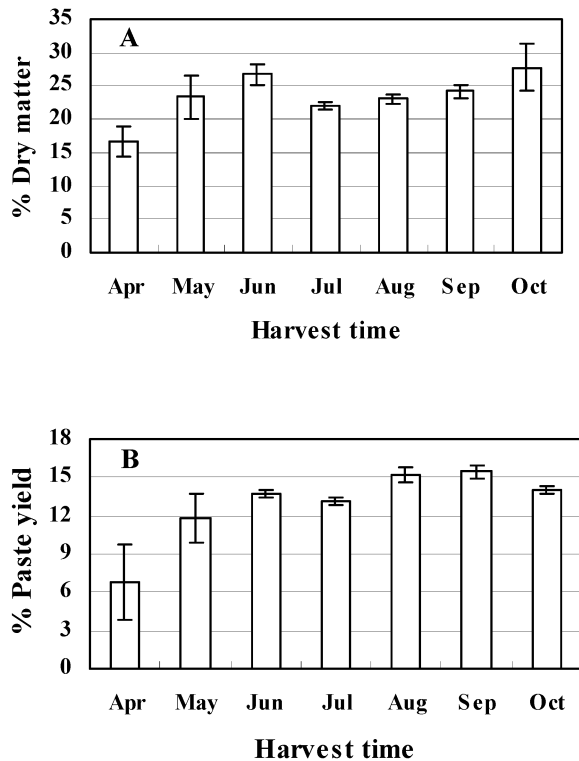


Fig. 1. A, Dry matter content in fresh leaves of *Inula viscosa* harvested at bi-weekly intervals from April to October 2003. Values are means and standard deviations of two harvests per month. B, Yield of paste extract in dry leaves of *I. viscosa* harvested at bi-weekly intervals from the field during 2003 and dried at 60°C. Values are means and standard deviations of two harvests per month.

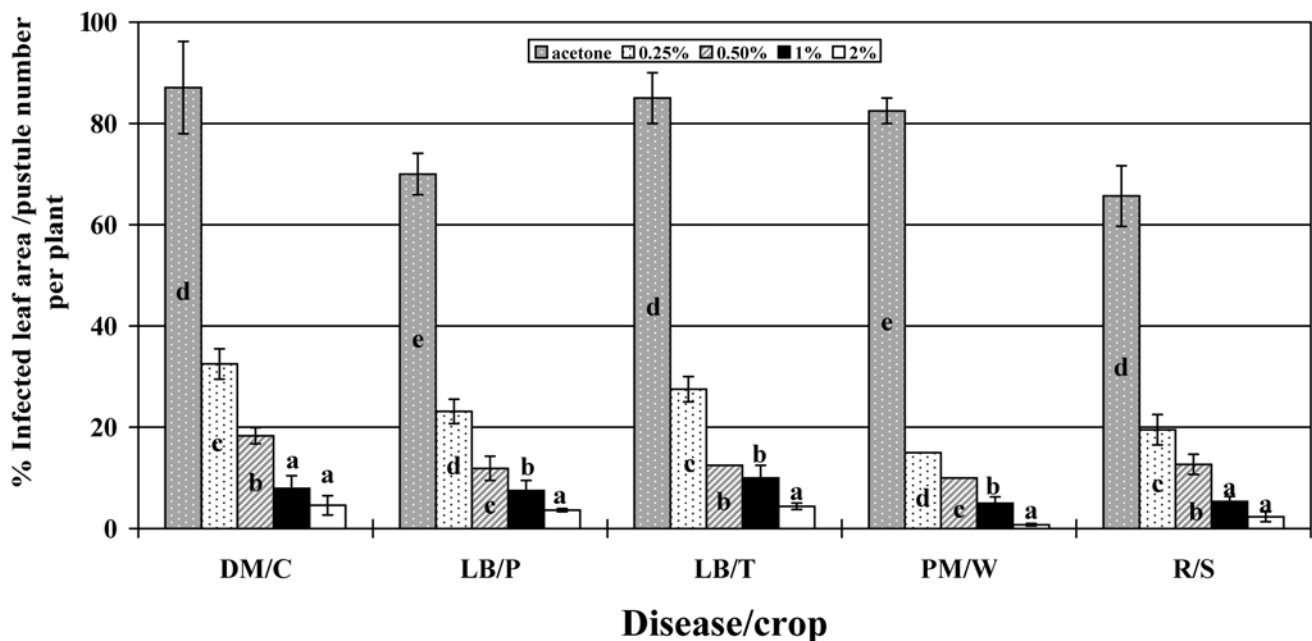


Fig. 2. Percentage of leaf area infected by *Pseudoperonospora cubensis*, *Phytophthora infestans*, and *Blumeria graminis* f. sp. *tritici* in cucumber, potato and tomato, and pustules of *Puccinia helianthi* on each sunflower plant after treatment with either acetone alone or acetone solution containing 0.25, 0.5, 1, or 2% paste of *Inula viscosa* leaves. DM/C, LB/P, LB/T, PM/W, and R/S stand for downy mildew/cucumber, late blight/potato, late blight/tomato, powdery mildew/wheat, and rust/sunflower, respectively. The test extract of *I. viscosa* was prepared from leaves collected on 15 June 2003. Values are means and standard deviations of three replicate plants (three pots of plants for wheat). For each pathosystem, means with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference test.

presented in Figure 5. The data indicate $\approx 50\%$ disease control with 0.125% of the emulsified paste. With 1% of the product, $\approx 90\%$ control was measured. The ED_{90} values for the different pathosystems were: downy mildew/cucumber, 1.01; late blight/potato, 0.97; late blight/tomato, 0.95; powdery mildew/wheat, 0.82; and rust/sunflower, 0.62%. The differences among the various pathosystems were similar to those obtained with the acetone solutions (Fig. 4). The percentage of infected leaf area (pustule number for sunflower) in emulsifier-treated control plants was similar to that in untreated control plants. The order of sensitivity of the pathosystems to the emulsified paste extract was similar to that of the acetone solution.

Activity of paste components. Data on the control efficacy of late blight in tomato leaf disks of the components derived from the paste (after TLC separation) are shown in Figure 6. Some components were more active than others, while the emulsifier itself enhanced disease development. Thus, at a concentration of 62.5 $\mu\text{g/ml}$, components derived from regions 1, 2, 3, 4, 5, and 6 of the TLC plates exhibited 0, 13, 41, 72, 82, and 78% inhibition of the disease, respectively. At 250 $\mu\text{g/ml}$, only components 3, 4, and 6 exhibited $>85\%$ inhibition, indicating that activity resides in nonpolar compounds. It should be noted that components derived from a region may contain more than one compound.

DISCUSSION

The present study shows that extracts made from leaves of *I. viscosa* possess broad-spectrum activity against foliar diseases

of crop plants. They were effective not only against grape downy mildew caused by *Plasmopara viticola* as shown previously (21), but also against cucumber downy mildew caused by *Pseudoperonospora cubensis*, late blight in potato and tomato caused by

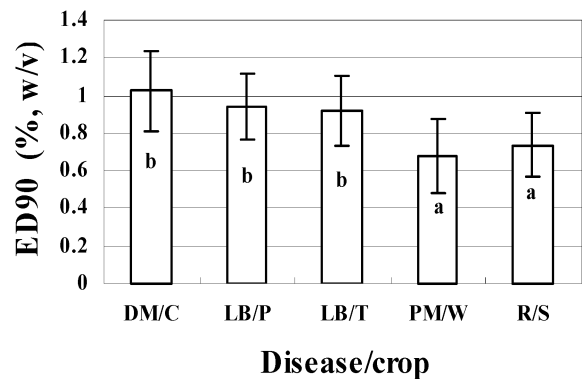


Fig. 4. Effective concentration (percent, wt/vol) of *Inula viscosa* paste required for 90% inhibition (ED_{90}) of *Pseudoperonospora cubensis* in cucumber (DM/C), *Phytophthora infestans* in potato (LB/P), *P. infestans* in tomato (LB/T), *Blumeria graminis* f. sp. *tritici* in wheat (PM/W), and *Puccinia helianthi* in sunflower (R/S). Potted plants were treated with acetone alone or acetone solution containing 0.25 to 2% pastes of *I. viscosa* leaves harvested in the field at biweekly intervals during April to October 2003. Values are means and standard deviations of ED_{90} values for all harvests during the growth season. Means with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference test.

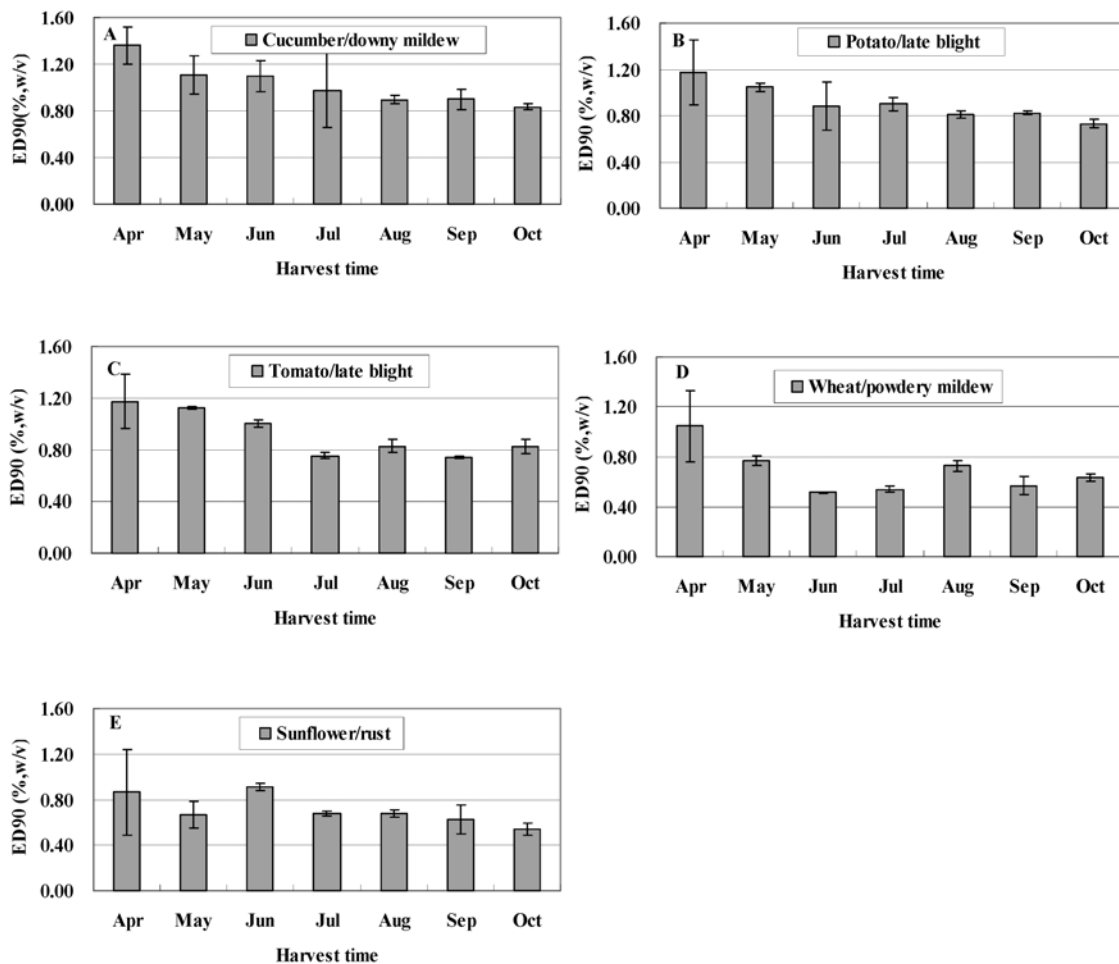


Fig. 3. Effective concentration (percent, wt/vol) of *Inula viscosa* paste required for 90% inhibition (ED_{90}) of **A**, *Pseudoperonospora cubensis* in cucumber, **B**, *Phytophthora infestans* in potato, **C**, *P. infestans* in tomato, **D**, *Blumeria graminis* f. sp. *tritici* in wheat, **E**, and *Puccinia helianthi* in sunflower. Plants were treated with acetone alone or acetone solution containing 0.25 to 2% pastes of *I. viscosa* leaves harvested in the field at biweekly intervals from April to October 2003. Values are means and standard deviations of ED_{90} values for two harvests per month.

Phytophthora infestans, wheat powdery mildew caused by *B. graminis*, and sunflower rust caused by *Puccinia helianthi*. The results corroborate earlier observations (4) showing that extracts of *I. viscose* made with organic solvents are useful in disease control. These findings may be significant to the agricultural industry when fungal strains resistant to site-specific fungicides prevail (5,8), as well as to organic farming where synthetic pesticides are prohibited.

The data showed that paste extracts dissolved in acetone are effective in controlling diseases. Such paste extracts kept their high antifungal activity after storage of 3 years at room temperature (B. H. Ben-Daniel, unpublished data). However, because acetone cannot be used in the field, we developed a technique to emulsify the paste obtained after extraction. The developed formulated product contains 34.4% paste. The emulsified product was effective in controlling all five diseases in potted plants, whereas the emulsifier itself was ineffective at corresponding doses. This EC product also was effective against downy mildew on detached grapevine leaves and in grapevines grown in the field (21). The 34.4% EC was effective after 1.5 years of storage at room temperature and resisted heating or freezing for 2 weeks (B. H. Ben-Daniel, unpublished data). Dried leaves of *I. viscosa* stored at room temperature for 1 year produced an extract as effective as newly harvested dry leaves or as dry leaves kept on the bench for 4 years (unpublished data). These findings show that the active ingredients are stable, which is valuable information for industrial production of the extracts.

I. viscosa is well known for its strong odor and the stickiness of its foliar parts. Extensive studies, therefore, have been conducted by researchers to elucidate the nature or biological activity of its water extracts, essential oils, and whole extracts in organic solvents. Extractions were done using different techniques: in water (12,18), in boiling water (22), by autoclaving in water and partitioning with organic solvents (11,13), by distillation in water to recover the essential oils (14), and by various organic solvents. These studies disclosed the presence of phenolics, flavonoids, terpenoids, sesquiterpene acids, sesquiterpene lactones, and other compounds (1,2,4,7,9,15,19,20,22,23).

Yegen et al. (23) reported that aqueous extracts and essential oils of *I. viscosa* were antifungal in vitro. Muller-Riebau et al. (15), on the other hand, found only small amounts of antifungal essential oils or phenolics, concluding that the plant has no economical value for producing antifungal preparations. These authors also reported (14) that the main compounds in the essential oil are p-cymene and carvarol. Perez-Alonso et al. (17) found that the yield of essential oils of aerial parts was 0.2%. The major constituents were borneol, bornyl acetate, and isobornyl acetate. Wollenweber et al. (22) identified 22 flavonoids in acetone extracts of *I. viscosa* leaves. Sanz et al. (19) analyzed methanol extracts of the foliage and identified flavonoids, sesquiterpene lactones, sesquiterpene acids, esters of 9-hydroxynerolidol, and eudesmane acids. Grande et al. (9) isolated 10 triterpenoids, free or esterified, from boiling acetone extracts of the aerial parts.

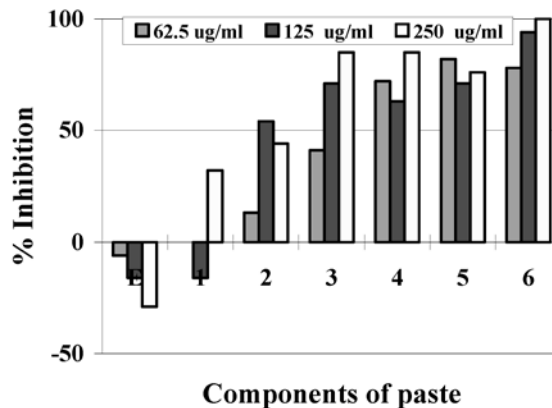


Fig. 6. Control of late blight on tomato leaf disks by components derived from *Inula viscosa*. Dry paste was dissolved in acetone, applied to thin-layer chromatography plates, and separated into six regions. Silica scraped from each region was extracted with acetone and applied at various doses to the leaf disks. Percent inhibition of disease development was calculated relative to untreated, inoculated disks. Standard deviations (data not shown) did not exceed 12% of the corresponding mean value.

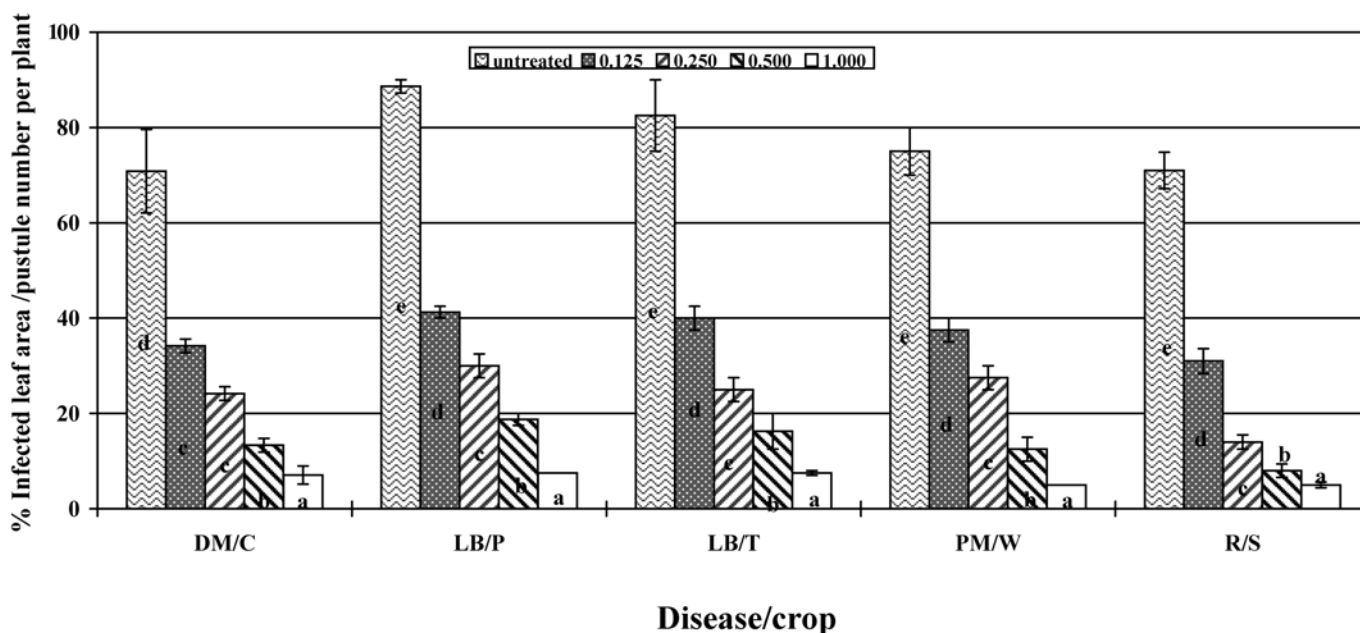


Fig. 5. Percentage of leaf area infected by *Pseudoperonospora cubensis*, *Phytophthora infestans*, and *Blumeria graminis* f. sp. *tritici* in cucumber, potato and tomato, and pustule numbers of *Puccinia helianthi* in each sunflower plant. Plants were treated with either water (untreated) or emulsion in water containing 0.125, 0.25, 0.5, or 1% paste of *Inula viscosa* leaves or water emulsion containing the same rate of emulsifier only (blank control). DM/C, LB/P, LB/T, PM/W, and R/S stand for downy mildew/cucumber, late blight/potato, late blight/tomato, powdery mildew/wheat, and rust/sunflower, respectively. Values are means and standard deviations of three replicate plants (three pots of plants for wheat). For each pathosystem, means with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference test.

Chemical analyses conducted on our paste samples showed the presence of tomentosin, inuviscolide (sesquiterpene lactones) (B. H. Ben-Daniel and Y. Cohen, *unpublished data*), costic acid, and iso-costic acid. TLC analysis revealed four regions with high activity against late blight. The nature of the compounds residing in these regions is currently being investigated. Costic acid and iso-costic acid were shown previously by us to control root-knot nematodes in tomato and cucumber (16). Iso-costic acid (12-carboxy-eudesma-3,11 (13)-diene) from *I. viscosa* was reported by Shtacher and Kashman to possess antifungal activity in vitro (20).

Various extracts were reported to possess differing levels of antimicrobial activities in vitro against phytopathogenic fungi, dermatophytic fungi, yeasts, and bacteria. However, only limited data are available on the antimicrobial activity of such extracts in planta (4,21,24). The present study confirms efficacy of extracts made with organic solvents against members of Oomycetes, Ascomycetes, and Basidiomycetes in planta. Our previous data showed poor activity of water extracts against plant disease (4). Ziv (24) showed that aqueous extracts obtained by boiling of old leaves were inhibitory to fungi in vitro and against *Botrytis cinerea* on fruits of grapes and tomato. Ali-Shtayeh et al. (2) used aqueous and ethanolic extracts and showed activity against several bacteria and *Candida albicans* in vitro. Maoz and Neeman (13) demonstrated that the organic phase of an aqueous extract possessed antifungal activity in vitro as well as inhibitory effect on chitin synthesis of dermatophytes and *C. albicans*. The same extract was reported by these authors (11) to contain the sesquiterpene tayunin. Their earlier study (12) showed that extracts made by autoclaving in water were inhibitory to bacteria and dermatophytic fungi with minimal inhibitory concentration values of 1.25 and 0.625 to 2.5% (dry weight of leaves in water, wt/vol), respectively. Similar results were obtained by Ali-Shtayeh and Abu-Ghadeib (1).

Our observations at Keramim in 2002 indicated that leaves harvested in April contain less dry matter or paste and exhibited lower control efficacy compared with leaves harvested in June or September. At Bar-Ilan we measured the (i) dry matter content in fresh leaves, (ii) paste (dry extract) yield, and (iii) efficacy of the extracts in disease control. We assumed that fluctuations in these variables, if they occur, should be considered to maximize the economical value of *I. viscosa* as a source for antifungal products. Therefore, extracts were prepared from leaves harvested at biweekly intervals from 1 April to 15 October 2003, the period of active growth of *I. viscosa* in Israel. The results corroborate our preliminary observations. They proved minor differences in dry matter content, paste yield, and efficacy of the different extracts, except for leaves collected in April, in which all the above values were lower compared with the other harvests. For practical use, therefore, harvesting can be done during almost the entire growth period.

Other data (data not shown) indicate that, after each harvest, the newly emerging shoots developing within \approx 1 month also were suitable for getting effective extracts. This means that harvesting can be done repeatedly until late October, when plants start to flower.

The availability of herbal extracts, such as from *I. viscosa*, in the market may fulfill the need for a suitable product for organic farming to combat destructive diseases in crop plants.

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