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Bioprospecting for plant and fungus extracts with systemic effect to control the cucumber powdery mildew

Bioprospektion von pflanzlichen und pilzlichen Extrakten mit systemischem Effekt zur Bekämpfung des Echten Mehltaupilzes an Gurken

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Summary

This work was aimed to select plant and fungus extracts showing systemic effect to control the cucumber powdery mildew. For that, ethanolic extracts were obtained from five basidiomycetes and 71 plant species and tested under greenhouse conditions. Cucumber plants cv. 'Safira' were sprayed with extracts at the first true leaf growth stage and inoculated with *Sphaerotheca fuliginea* at the second leaf growth stage. The number, diameter and sporulation rate of powdery mildew colonies were evaluated 6 days after inoculation. In an initial screening phase, the extracts of the following plants and fungi revealed some systemic effect against the powdery mildew: *Aloe vera* (aloe), *Bidens pilosa*, *Ganoderma* sp. (basidiomycete), *Hyptis crenata, Mascagania bethamiana, Ocotea suaveolens, Oudemansiella canarii* (basidiomycete), *Pinus taeda* (pine), *Richardia grandiflora* and *Vernonia polyanthes.* They were compared in three other experiments and those from *Oudemansiella*, *Ganoderma*, pine needles and aloe leaves were the most efficient reducing the number of colonies on the secondary leaf by 79, 65, 30 and 21 %, respectively. Extracts from fruiting bodies of *Oudemansiella* and *Ganoderma* also decreased the diameter of colonies by 20 % as well as the sporulation rate by 45 and 70 %, respectively. While *Ganoderma* did not show any direct effect *in vitro*, the extract of *Oudemansiella* (60 mg fresh weight ml^{-1}) reduced the germination of powdery mildew conidia at 48 h after inoculation by 71 %. Furthermore, *Oudemansiella*-extract strongly inhibited the mycelium growth of *Cladosporium oxysporum* in liquid medium. The possible modes of action of these extracts are discussed.

Key words: *Cucumis sativus;* cucumber; powdery mildew; *Sphaerotheca fuliginea;* induced resistance; extracts; *Ganoderma; Oudemansiella canarii*

Zusammenfassung

Ziel dieser Arbeit war, pflanzliche und pilzliche Extrakte, die den Echten Mehltau an Gurken systemisch bekämpfen können, zu selektieren. Dazu wurden ethanolische Extrakte aus fünf Basidiomyceten und 71 Pflanzenarten im Gewächshaus getestet. Gurkenpflanzen der Sorte 'Safira' wurden im Einblattstadium mit den Extrakten gespritzt und im Zweiblattstadium mit *Sphaerotheca fuliginea* inokuliert. Sechs Tage nach der Inokulation wurden die Anzahl, der Durchmesser und die Sporulationsrate der Mehltaukolonien ausgewertet. In der Anfangsphase des Screenings wiesen die folgenden pflanzlichen und pilzlichen Extrakte eine systemische Wirkung gegen den Mehltau auf: *Aloe vera* (Aloe), *Bidens*

pilosa, Ganoderma sp. (Basidiomycet), *Hyptis crenata, Mascagania bethamiana, Ocotea suaveolens, Oudemansiella canarii* (Basidiomycet), *Pinus taeda* (Kiefer), *Richardia grandiflora* und *Vernonia polyanthes.* Diese Extrakte wurden in weiteren Versuchen miteinander verglichen. Am wirksamsten waren die Extrakte aus *Oudemansiella*, *Ganoderma*, Kiefernadeln und Aloeblättern. Sie reduzierten die Anzahl der Mehltaukolonien am zweiten Blatt um 79, 65, 30 bzw. 21 %. Die Extrakte aus dem Fruchtkörper von *Oudemansiella* und *Ganoderma* haben sowohl den Durchmesser um 20 % als auch die Sporulationsrate der Mehltaukolonien um 45 bzw. 70 % erniedrigt. Während *Ganoderma* keine direkte Wirkung *in vitro* zeigte, hat der Extrakt von *Oudemansiella* (60 mg frisches Gewicht ml^{-1}) 48 h nach Inokulation die Konidienkeimung des Mehltaupilzes um 71 % reduziert. Außerdem hemmte der *Oudemansiella*-Extrakt das Myzelwachstum von *Cladosporium oxysporum* im flüssigen Medium stark. Die möglichen Wirkungsmechanismen dieser Extrakte werden diskutiert.

Stichwörter: *Cucumis sativus;* Gurken; Mehltau; *Sphaerotheca fuliginea;* induzierte Resistenz; Extrakte; *Ganoderma; Oudemansiella canarii*

1 Introduction

The powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend.: Fr.) Polacci is one of the most destructive foliar diseases of cucumber (*Cucumis sativus* L.), being mainly controlled by means of fungicides. However, the increasing interest in public health and environment has motivated the development of alternative methods for disease controlling, such as induction of resistance (Stadnik et al. 2001).

Since the 1970s, the phenomenom of induced resistance in cucumber plants has been studied (Hammerschmidt and Yang-Cashman 1995). The classic example of induction of systemic resistance to several diseases in cucumber is that obtained by necrotizing agents, such as *Colletotrichum lagenarium* and the *Tobacco necrosis virus* (TNV) (Bashan and Cohen 1983; Hammerschmidt and Yang-Cashman 1995). For instance, Bashan and Cohen (1983) verified that the pre-inoculation of cucumber with TNV induces systemic resistance to the powdery mildew. Without causing necrosis, the following synthetic compounds activate plant resistance genes to powdery mildews: α-amino-isobutyric acid (Vogt and Buchenauer 1997); 2,6-dichloroisonicotinic acid (Hijewegen and Verhaar 1995), benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (Stadnik and Buchenauer 2000), phosphate salts and several micronutrients (Reuveni et al. 1997). The broad chemical diversity of inducers indicates that there is no common structural characteristic determining their activity.

Some plant extracts can also induce resistance in plants to pathogens and in the case of *Azadirachta indica* and *Reynoutria sachalinensis*, the commercial products Neemazal® (Singh and Prithiviraj 1997) and Milsana® (Daayf et al. 1997) were successfully developed to control the powdery mildew in pea and cucumber, respectively. However, the bioprospecting of plants to obtain new bioactive compounds, either acting directly or inducing resistance, has been relatively little explored for use in plant protection.

Fungi are another important source for supplying new bioactive compounds. In nature, they compete with different micro-organisms for the same substrates or habitats. To survive under these conditions, they developed several strategies, such as the production of toxic metabolites towards their competitors (Anke 1997). Although biologically active metabolites are produced by a great number of organisms, most bioprospecting programs have been limited to certain groups of soil fungi and, therefore, the real potential of other fungus groups remain little studied. Furthermore, the bioprospecting for new antibiotics have been mainly conducted with organisms from temperate regions. For these reasons, the potential of tropical plants and fungi for producing new bioactive metabolites is still unknown.

Although Brazil has a high diversity of plants and micro-organisms, little is known about their potentiality for controlling plant diseases. However, in the last years, financial sponsors and scientific communities have concluded that Brazilian biodiversity is a big patrimony of new bioactive molecules. In this context, some extracts of plants from Pantanal region have been successfully used to combat several human diseases and, therefore, aroused interest of both chemical and pharmacological industry (Pott and Pott 1994).

The main objective of this work was to do the bioprospecting for plant and fungus extracts from Pantanal with systemic effect, i. e., possibly increasing plant resistance, to control the cucumber powdery mildew.

2 Material and methods

The bioprospecting for fungus and plant extracts was carried out under greenhouse conditions in a continuous system of 50 tests following the method described by Stadnik and Buchenauer (1997).

2.1 Plant material and growing conditions

Cucumber plant hybrids (cv. 'Safira') were grown in a powdery mildew-free greenhouse under natural light conditions at 30/20 ± 5 °C (day/night). For that, five seeds were sown in 9-cm diameter plastic pots filled with loamy organic soil. Before use, soil was solarized and its pH adjusted with lime. The seeds were equidistantly put on the soil surface and immediately covered with 30 ml of the same substrate. At the cotyledon growth stage (c. 6 days after sawn), seedlings were thinned out and two of them were allowed to grow in each pot. Thereafter, urea was applied obtaining a fertilization level of 30 mg N kg^{-1} soil.

2.2 Origin and preparation of extracts

Most extracts were prepared using common weeds, medicinal and spice plants, all growing locally. The plants collected from far-away places, i. e., from the Pantanal region, were identified after Pott and Pott (1994) and their exsiccatae are deposited in the herbarium of the Pantanal Agricultural Research Center, Corumbá-MS. The following plant parts were used in the preparation of extracts: aerial part (ap), bulb (bu), flower (fl), fruit (fr), leaf (le), root (ro), rhizome (rh) or stem (st). Fruiting bodies of some macroscopic basidiomycete fungi (bf) were also used. The extracts were obtained by different methods and grouped as follows:

2.2.1 Latex and essential oils

To obtain the latex from 1: *Euphorbia tirucallii*, 2: *Poinsettia pulcherrima* and 3: *Plumeria* sp., leaves were detached and their latex was collected in a glass tube till reaching the necessary volume for essays. 4: *Eucalyptus citridora* essential oil and 5: pyroligneous extract were also tested. Eucalyptus leaf oil was supplied by Três Barras distillery (Torrinha, SP), while the pyroligneous extract, a complex acid liquid obtained in the dry distillation of eucalyptus wood, was supplied by Ecopirol Ltd. (São Paulo, SP). Prior use, all latex and oils were diluted with ethanol (10 %) to 1 : 10 and 1 : 20.

2.2.2 Extracts by maceration

Local plants and fungi were collected and freshly used to extract bioactive compounds, as follows: Ten grams of fresh tissue were homogenized in a mixer containing 100 ml ethanol (95 %). After remaining 4 h in the ethanol solution, the extracts were filtered through a two-layer paper filter and the filtrates were dried in a exhaust chamber overnight. The residues were then diluted with 20 ml of a 10 % ethanol solution (v/v) to obtain a concentration of 500 mg fresh tissue per ml (1 : 2) and, finally, lower concentrated dilutions (1 : 4 and 1 : 8) were successively prepared. The following plant and fungus species were tested: 6: *Allamanda cathartica* (le), 7: *Allium sativum* (bu), 8: *Aloe vera* (le), 9: *Althernantera brasiliana* (le), 10: *Arnica montana* (le, fl), 11: *Atropa belladonna* (le), 12: *Azadirachta indica* (le), 13: *Baccharis trimera* (le), 14: *Bidens pilosa* (le, ro), 15: *Bouganvillea spectabilis* (le), 16: *Cajanus cajan* (le), 17: *Calendula officinalis* (le), 18: *Clavaria* sp. (bf), 19: *Cocos nucifera* (fr), 20: *Coriandrum sativum* (le), 21: *Cupressus* sp. (le), 22: *Cymbopogum citratus* (le), 23: *Cynara scolymus* (le), 24: *Desmodium tortuosum* (le), 25: *Euphorbia heterophylla* (le), 26: *Euphorbia spendlens* (le), 27: *Feijoa sellowiana* (le), 28: *Ganoderma* sp. (ba), 29: *Ilex paraguariensis* (le), 30: *Ipomea* sp. (le), 31: *Lentinula edodes* (bf), 32: *Matricaria*

chamomilla (le), 33: *Mentha piperita* (le), 34: *Mikania cordifolia* (le), 35: *Murraya paniculata* (le), 36: *Oudemansiella canarii* (bf), 37: *Paullinia cupana* (fr), 38: *Peumus boldus* (le), 39: *Phyllanthus tenellus* (le), 40: *Pimpinella anisum* (le), 41: *Pinus taeda* (le), 42: *Pisolithus tinctorius* (bf), 43: *Psidium guajava* (le), 44: *Pteridium aquilinum* (le), 45: *Ricinus communis* (le), 46: *Sansevieria zeylanica* (le), 47: *Sonchus oleraceus* (le), 48: *Symphitium officinalis* (le), 49: *Vernonia polyanthes* (le), 50: *Wedelia padulosa* (le), 51: *Zingiber officinale* (rh) and 52: *Lagenaria* sp. (le).

2.2.3 Extracts by percolation

Plant parts collected in Pantanal region were first dried with forced air circulation at 50 °C, finely powdered and then stored until use. For extraction, the dry powder (*c.* 250 g) was then put into a glass column and the extraction was done using ethanol or ethyl acetate, till the solvent did not present any visible solute in the solution. The extracts were concentrated with a rotary evaporator at 40 °C. To be able to apply similar concentrations of those extracts produced by maceration, the dilutions 1 : 2 and 1 : 4 were done after mathematical conversion based on the fresh weight of plant tissue. The tested plant species belonging to this group are: 53: *Agonandra brasiliensis* (le, ro), 54: *Alchornea disicolor* (le), 55: *Annona cornifolia* (st), 56: *Bauhinia bauhinioides* (le), 57: *Bunchosia paraguaiensis* (le), 58: *Couepia uiti* (le), 59: *Copaifera martii* (le), 60: *Curatella americana* (le), 61: *Davilla elliptica* (st), 62: *Diopyros hispida* (le, fr), 63: *Erytroxylum anguifugum* (le, st), 64: *Fagara hassleriana* (le, st), 65: *Gomphrena elegans* (ap), 66: *Hyptis crenata* (ap), 67: *Licania parvifolia* (le, st), 68: *Machaerium hirtum* (st), 69: *Mascagania bethamiana* (le), 70: *Melia azedarach* (fr), 71: *Mimosa chaetosphera* (ap), 72: *Ocotea suaveolens* (le, st), 73: *Rhamnidium elaeocarpum* (le), 74: *Richardia grandiflora* (ap), 75: *Tocoyena formosa* (st) and 76: *Unonopsis lindimanii* (st). Except for *M. azedarach*, all plant species in this group are native to Pantanal.

2.3 Extract application

Cucumber plants were treated at the first true leaf growth stage (i. e., 12–14 days after sowing). The extracts were applied on the adaxial surface of both cotyledons and first leaves using an air-pressurized sprayer and delivering a volume sufficient to cause run off (*c.* 1 ml/ plant). After treatment, plants were maintained in the powdery mildew-free greenhouse until inoculation.

2.4 Pathogen and inoculation

The isolate cnpma-1999-1 of *S. fuliginea* was maintained on young cucumber plants cv. 'Safira'. One day before conidia were required as inoculum, heavily sporulating leaves were shaken to displace old conidia and ensure high conidial viability.

In the initial screening phase, plants were transferred to another greenhouse at the second leaf growth stage (*c.* 3 days after treatment) and the pathogen conidia were homogeneously spread on cucumber plants by tapping infected leaves about 30 cm above them (Stadnik et al. 2001).

In the final screening phase, plants were artificially inoculated with a suspension of 8×10^4 conidia ml^{-1} at the second leaf growth stage and incubated under greenhouse conditions until evaluation.

2.5 Evaluation and selection criteria

The number of powdery mildew colonies (NC) on both primary (treated) and secondary (untreated) leaves was counted 7 days after inoculation. When the disease severity was too high, the percent diseased leaf area was estimated. The disease severity was expressed in percent of the severity values of the control plants. The selection criteria were the degree of systemic effect, i. e. the disease reduction on the secondary leaf, and the necessary dosage to obtain such effect. Only extracts statistically differing from controls were selected in the initial screening.

The extracts selected in the initial screening were compared in a set of three additional independent experiments (final screening phase). For this purpose, cucumber plants were exactly grown as described above and treated at the first leaf growth stage with the diluted extract 1 : 4 (v/v). Plants were inoculated with a conidial suspension at the second leaf growth stage and incubated in a disease-free greenhouse.

After 6 days, the number (NC), the diameter (DC) and the daily sporulation rate (SR) of powdery mildew colonies were evaluated. The diameter was measured using a binocular stereoscopic microscope equipped with an ocular micrometer. To estimate the sporulation rate, 110-mm diameter leaf discs were removed from the interveinal areas of secondary leaves and the number of colonies on them counted. Only areas with no more than three colonies were chosen for the essay. Immediately after counting, leaf discs were laid with the adaxial surface uppermost on 500 µl sterile distilled water in 10 ml glass vials. After an incubation time of 48 h under natural light at 25 °C, the discs were turned over and the vials were then quickly shaken for 2 min, to release conidia in water. Thereafter, the conidial concentration in each vial was determined by counting the spores in a Neubauer's haemocytometer. Finally, the sporulation was calculated and expressed in number of conidia/colony/day.

2.6 Conidial germination of *Sphaerotheca fuliginea*

Cucumber plants were exactly grown as described in 2.1. At the first leaf growth stage, five plants were treated with the extracts of *Ganoderma* (100 mg fw/ml) and *Oudemansiella* (60 mg fw/ml), respectively. Control plants were treated with a 10 % ethanol solution. Plants were artificially inoculated with a suspension of 8×10^4 conidia ml^{-1} 1 day after treatment and incubated in a disease-free greenhouse under natural light conditions at 30/20 ± 5 °C (day/night). From the same leaf, two discs (110 mm diameter) were sampled at 12, 24 and 48 h after inoculation and processed as described by Stadnik and Buchenauer (2000): The leaf discs were incubated in three subsequent steps: (1) with the adaxial surface up on the paper moistened with ethanol: glacial acetic acid (3 : 1, v/v) solution for about 24 h to fix and bleach the leaf tissues; (2) on paper moistened with water for 4 h; and (3) on paper moistened with lactoglycerol (equal parts lactic acid, glycerol and water) for clearing and storage. All steps were carefully carried out to avoid displacement of conidia and loosely attached germlings. Fungal structures were stained by transferring a drop of 0.1 % trypan blue in lactophenol onto the leaf surfaces, and after covering the stained discs with a coverslip they were examined with a light microscope (Axioskop, Zeiss, Germany) for determination of the germination rates of *S. fuliginea*-conidia. Germination was defined as the formation of either one germ tube three times longer than the conidium length or of multiple germ tubes. At each evaluation time, five replicates were used for each treatment and about 100 conidia were examined for each replication composed of two leaf discs.

2.7 Mycelial growth of *Cladosporium oxysporum*

The effect of *Ganoderma* and *Oudemansiella* extracts on the mycelial growth of *Cladosporium oxysporum* was tested in potato-dextrose liquid medium (PD) (100 ml potato extract and 20 g of dextrose per liter; pH 6.0). For this purpose, the concentrated extracts were sterilized by filtration (Millipore, 0.22 µm) and added to the liquid medium, obtaining a final concentration of 100 mg and 60 mg of fresh weight l^{-1} for *Ganoderma* and *Oudemansiella,* respectively. Thereafter, 100 µl of a suspension of 3×10^4 conidia of *C. oxysporum* ml^{-1} was pippeted in each Erlenmeyer flask containing 100 ml liquid medium. The fungus was cultured for 8 days at 20 °C on a shaker with a setting of 150 rpm. The mycelium was collected on filter paper, dried at 105 °C for 24 h and, finally, its weight was determined.

2.8 Experimental design and statistical analysis

In each screening test, three to seven extracts arranged in a completely randomized design were evaluated. Three replications composed by two plants each were used per treatment. Standard analysis of variance (ANOVA) was carried out using the SAS statistical package, version 8. For the initial screening, significance of mean differences between extract treatment and control within each test was determined using Student's t-test. ANOVA was followed by Tukey's test ($P < 0.05$) for other experiments. When necessary, appropriate transformations of the values were performed to normalize data and stabilize the variance throughout the data range prior to analysis of variance.

3 Results

While most tested extracts did not significantly affect the powdery mildew, some of them increased and others decreased the disease severity. Thus, for example, the spray of the following diluted extracts: 7: *A. sativum* (1 : 4), 2: *P. pulcherrima* (1 : 20) and 45: *Ricinus communis* (1 : 4) significantly enhanced the powdery mildew infection on the secondary leaf by 51, 391 and 76 %, respectively (Table 1). This disease-increasing effect was also observed to a lesser extent at both lower and higher extract dilutions.

Although no decrease of the powdery mildew severity was found on the secondary leaf, highest latex concentrations (1 : 10) of *P. pulcherrima* (Euphorbiaceae) and *Plumeria* sp. (Apocynaceae) strongly reduced the disease severity on the primary leaf (treated) by 73 % and 32 %, respectively. Also the pyroligneous extract (1 : 10; 1 : 20) showed a local effect reducing the disease by 34 % on the primary leaf (data not shown).

All extracts showing systemic effect also cause a significant reduction of the powdery mildew on the primary leaf. Extracts from *B. pilosa* L. (hairy beggar-ticks), *H. crenata* Pohl (wild peppermint), *P. taeda* (pine), *R. grandiflora* (C. et S. Steud.) (bernarda) and *V. polyanthes*, and the fungus extract of *Ganoderma* sp reduced the number of powdery mildew colonies on the untreated leaf (systemic effect). Therefore, these extracts were used at the same dilution (1 : 4) and compared again in another experiment (Table 2). Among the extracts presented in the Table 2, those of *H. crenata*, *R. grandiflora* and *V. polyanthes* did not significantly affect the powdery mildew, as it had been observed in the initial screening. However, the extracts of *Ganoderma* sp., *P. taeda* and *B. pilosa* showed the best systemic effect reducing the number of powdery mildew colonies on the secondary leaf (untreated) by 56, 36 and 31 %, respectively. Furthermore, the extracts of *Ganoderma* sp. and *P. taeda* reduced the sporulation rate of colonies by 60 and 40 %, respectively. Although not significant in relation to the control, these fungal extracts also trended to reduce the colony diameter (DC).

Table 1. Effect of plant extracts on the number of colonies (NC) of powdery mildew *(Sphaerotheca fuliginea)* on primary and secondary leaves of cucumber (*Cucumis sativus* L.) 7 days after inoculation

Selected extract	NC (% of control)	
	Primary leaf (treated)	Secondary leaf (untreated)
Allium sativum	240*	151*
Poinsettia pulcherrima	17*	491*
Plumeria sp.	68*	94
Ricinus communis	314*	176*

* indicates that mean significantly differs to its control according to the Student's t-test ($P < 0.05$).

Table 2. Effect of selected extracts on the number (NC), diameter (DC) and sporulation rate (SR) of colonies of powdery mildew *(Sphaerotheca fuliginea)* on the secondary leaf (untreated) of cucumber plants (*Cucumis sativus* L.)

Selected extract	NC	DC (mm)	SR (conidia/colony/day)
Control	97 ab*	1,8 ab	622 abc
Bidens pilosa	67 cd	1,7 ab	399 cde
Ganoderma sp.	43 e	1,2 b	250 e
Hyptis crenata	75 bcd	1,7 ab	531 bcd
Pinus taeda	64 de	1,1 b	354 de
Richardia grandiflora	89 bc	2,1 a	839 a
Vernonia polyanthes	117 a	1,9 a	725 ab

* Means within a column followed by the same letter are not significantly different according to the Tukey test ($P < 0.05$).

Table 3. Effect of selected extracts on the number (NC), diameter (DC) and sporulation rate (SR) of powdery mildew colonies *(Sphaerotheca fuliginea)* on the secondary leaf (untreated) of cucumber (*Cucumis sativus* L.)

Selected extract	NC	DC (mm)	SR (conidia/colony/day)
Control	120 a*	2,1 a	732 a
Aloe vera	57 bc	1,9 ab	469 ab
Mascagnia bethamiana	73 b	2,2 a	360 b
Ocotea suaveolens	95 ab	1,9 ab	641 a
Oudemansiella canarii [a]	22 c	1,4 b	234 c

* Means within a column followed by the same letter are not significantly different according to the Tukey test ($P < 0.05$).
[a] Plants were treated with 60 mg fresh weight mycelium ml^{-1}.

In another experiment (Table 3), other extracts selected in the initial screening were compared, i. e., those from the plants *A. vera* L. (aloe), *M. bethamiana* (Gris.) Anderson (white liana), *O. suaveolens* Hassl. (black cinnamon) and the fungal fruiting bodies of *O. canarii*. Extracts obtained from *A. vera* L., *M. bethamiana* and *O. canarii* showed systemic effect, strongly impairing the development of powdery mildew on the secondary cucumber leaves. They reduced the number of powdery mildew colonies by 53, 39 and 82 %, respectively. However, only the *Oudemansiella* extract significantly reduced the colony diameter from 2.1 mm in the control to 1.4 mm in the treated plants. The sporulation rate of powdery mildew colonies was significantly reduced only at *M. bethamiana*- and *Oudemansiella* treatments, by 51 and 68 %, respectively (Table 3). *Oudemansiella* extract was applied at a lower concentration than other treatments because it was phytotoxic at concentrations higher than 100 mg fresh weight mycelium ml^{-1}. Plants treated with higher concentrations showed total leaf necrosis already 6 h after treatment.

Based on results from Table 2 and 3, the four best selected extracts, i. e., those from the fungi *Ganoderma* sp. and *O. canarii* and of the plants *A. vera* and *P. taeda*, were compared in a final screening experiment. Among them, *Ganoderma* sp. and especially *Oudemansiella*, showed the best effect in reducing systemically the number, diameter and sporulation rate of powdery mildew colonies (Fig. 1). The number of colonies (NC) was the most affected variable by all extracts, while both diameter and sporulation rate of colonies were not significantly influenced by the extracts of aloe and pine. Only the effects of *Ganoderma* and *Oudemansiella* extracts could be confirmed again, and therefore, they were further investigated.

The conidial germination evaluated on leaf discs became higher with increasing time periods after inoculation with *S. fuliginea*. Thus, the germination on control leaves was at 48 h after inoculation two-fold higher than at 12 h. The germination test revealed a strong inhibitory effect of *Oudemansiella* extract and, when compared to the control, the germination of *S. fuliginea* conidia was reduced on the treated leaves by 64, 74 and 71 % at 12, 24 and 48 h after inoculation, respectively. On the other hand, the *Ganoderma* extract did not significantly affect the conidial germination (Fig. 2).

In liquid medium, the mycelium growth of *C. oxysporum* was strongly inhibited by the extract from *Oudemansiella* fruit bodies, but not by the extract from *Ganoderma* (Fig. 3). The addition of extract from fruiting bodies of *Oudemansiella* (60 mg fresh weight ml^{-1}) reduced the *Cladosporium* mycelium weight by 84 %.

4 Discussion

Since powdery mildews are biotrophic fungi sensibly affected by host conditional nutrition, the spraying of some extracts, such as *A. sativum* and *R. communis* may have caused a higher availability of nutrients to the parasitic fungus. This could explain the disease increase on the secondary leaf when treated with such extracts. Another explanation for systemic disease enhancement is the inhibition of active plant defense mechanisms. Different modifications in host metabolism as well as the suppression

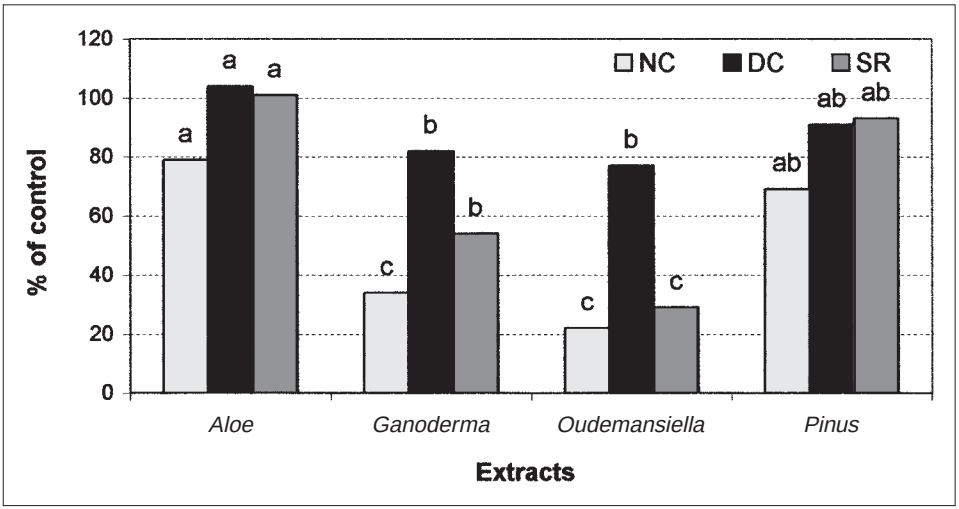

Fig. 1. Effect of extracts from *Aloe vera* leaves, *Ganoderma*- and *Oudemansiella*-fruiting bodies, and *Pinus taeda* needles on the number (NC), diameter (DC) and sporulation rate (SR) of powdery mildew colonies *(Sphaerotheca fuliginea)* an the secondary leaf of cucumber plants (*Cucumis sativus* L:). Columns followed by the same letter did not differ by the Tukey test ($P < 0.05$) within a same variable.

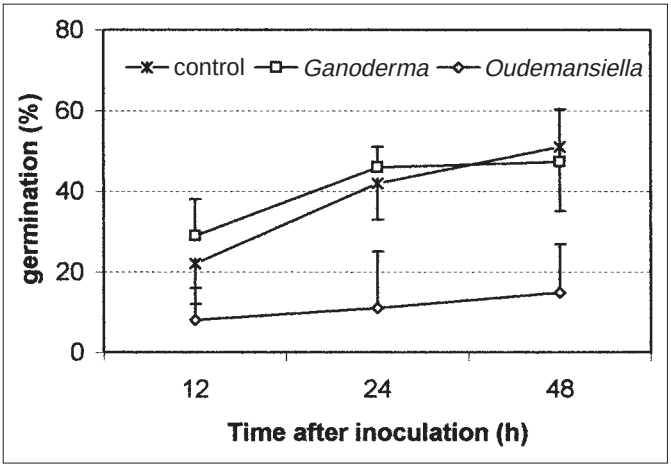

Fig. 2. Conidial germination of *Sphaerotheca fuliginea* at 12, 24 and 48 h after inoculation on cucumber leaf discs pre-treated with extracts from fuiting bodies of *Ganoderma* and *Oudemansiella.*

of biochemical resistance components have been related to a temporary increase of powdery mildew susceptibility in different hosts (Stadnik and Buchenauer 2000; Stadnik et al. 2001). In contrast to our results, Singh et al. (1995) reported that ajone, a chemical compound from garlic *(A. sativum)*, has shown significant control of powdery mildews under glasshouse conditions. Possibly, the concentration of ajone in the tested garlic extract did not reach levels enough for powdery mildew inhibition or this compound was eventually degraded during the time period between treatment and inoculation. Note that plants were inoculated 3 days after treatment.

The reduction of disease severity on primary leaves treated with latex from *P. pulcherrima* and *Plumeria* sp. was probably due to the presence of fungitoxic compounds, such as terpene(s) and alkaloid(s) in these extracts. Since no disease reduction was observed on the secondary leaf, such

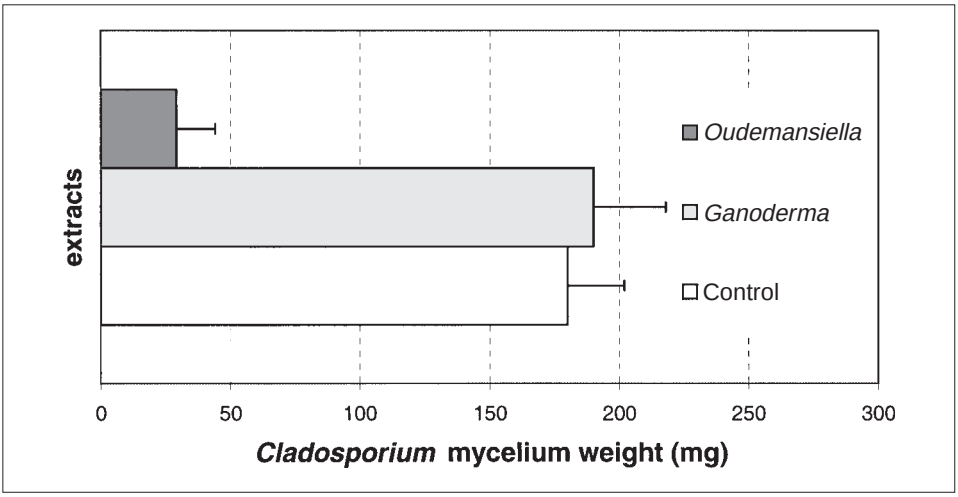

Fig. 3. Effect of the extracts from fruit bodies of *Ganoderma* sp. and *Oudemansiella canarii* on the mycelium weight of *Cladosporium oxysporum*, after eight days length incubation in 100 ml potato-dextrose liquid medium. Bars indicate the mean standard deviations.

fungitoxic compound(s) may be not translocatable. It is known that latex is a milky fluid composed by a rich mixture of agglomerative low-density materials and several terpenes, alkaloids, vitamins, lipids and amino acids (Lynn and Clevette-Radford 1987). Miyasaka et al. (2001) reported that a weekly spray with pyroligneous extract diluted to 1 : 100 controlled the downy mildew and apparently affected the physiology of lettuce plants, because their leaves became more vigorous and more brightly colored. However, its mode of action is still unknown.

On the other hand, extracts of some plant species could systemically control the powdery mildew in cucumber plants. Thus, *H. crenata* showed in the initial screening phase some effect against the powdery mildew that could, however, not be confirmed in final experiments. Scramin et al. (2000) reported that essential oils from *Hyptys* are bioactive against insects and phytopathogenic fungi. They identify at least 15 volatile compounds in wild peppermint *(H. crenata)*. Essential plant oil, in which higher concentration of fungitoxic terpenoids are present, seems more appropriate for controlling fungi directly. This was described by Iwu et al. (1990) who verified the presence of 32 different terpenoids in essential oil from *Hyptis suaveolens* showing antimicrobial activity.

Discrepancies between the initial screening reported in the text and the follow-up tests presented in Table 2 and 3 were possibly due to influence of environmental factors and their interaction with cucumber plants. It is known that plants differently respond to resistance inducers when maintained in different environments (Stadnik and Buchenauer 1997). Note that cucumber plants were kept under greenhouse conditions, but some factors (e. g., light, water supply, etc.) could be not perfectly controlled. Even for extracts acting directly against the pathogen, these factors must also be taken into consideration to explain these discrepancies, because they distinctly affect the uptake, translocation and degradation of bioactive compounds.

Laboratory experiments have shown that extracts from different plant species inhibit the growth of bacteria, fungi and yeasts. For example, *Bidens* spp. has different bioactive compounds, such as poliacetilenes, triterpenes and essential oils, which show a strong antimicrobial activity, well-studied in the natural medicine. The main bioactive compound in leaves of *Bidens*, fenil-heptatriyne, is one of the poliacetilenes with strong antimicrobial and antiviral activity (Dharmananda 2002). However, always when plant extracts show systemic effect, the induction of resistance must be taken into consideration as a possible explanation for disease control as well.

Aloe is known to produce at least six different antiseptic compounds (Davis 1997). The presence of such antimicrobial compounds could be an explanation for a possible direct effect of the aloe extract on

S. fuliginea. On the other hand, Stangarlin et al. (1999), investigating the syntheses of phytoalexins in soybean cotyledons, found that aloe extract induces the accumulation of the deoxianthocianidine. This suggests that aloe extract can also induce resistance to plant pathogens.

The pine needle extract also showed activity against the cucumber powdery mildew and its control efficacy in the different experiments was, however, quite variable. Ouf et al. (1999) suggested that pine needle extract contain inhibitory substances that are either directly toxic to *Rhizoctonia solani* or indirectly inhibitory due to interference with pathogen metabolism.

The best results obtained in the bioprospecting for extracts with systemic effect were those with *Ganoderma* and Oude*mansiella.* The family Ganodermataceae is a quite numerous and diversified group of fungi. Some of them are plant pathogens causing wood rot in roots and stem of trees. The genus *Ganoderma* commonly occurs on urban trees which are exposed to different kinds of physiological stresses and wounding (Hirotani et al.1993; Smânia et al. 1999). Curiously, fruiting bodies of some *Ganoderma* species have been used for a long while in the oriental medicine as an anti-inflammatory agent and in the body defense against several diseases (Hirotani et al. 1993). Although it has been verified that some *Ganoderma*-isolates produce compounds with antimicrobial activity (Smânia et al. 1999), the extract from fruiting bodies of *Ganoderma* sp. used in this work neither reduced the germination of *S. fuliginea*-conidia nor inhibit the mycelial growth of *Cladosporium oxysporum*. Therefore, the induction of systemic resistance in cucumber plants may be involved in the reduction of the powdery mildew severity in *Ganoderma*-extract-treated plants.

O. canarii is a basidiomycete belonging to the Tricholomataceae family, common in tropical regions, growing on wooden trunk and branches of dead or living trees (Pegler 1983). *Oudemansiella* spp. produce large amounts of lipids and, in the case of *O. mucida* (a template specie), the total lipid content is negatively correlated with the production of antibiotic compounds. *O. mucida* also produces other active compounds known as strobilurins and oudemansins, which are strongly effective against a large spectrum of saprophytic and pathogenic fungi. Indeed, the strobilurins are one of the few cases in which a successful product, originating from a fungal metabolite was developed to protect plants against their pathogens (Anke 1997). Although extracts from fruiting bodies of *Oudemansiella* revealed *in vitro* activity against *S. fuliginea* (Fig. 2) and *C. cladosporium* (Fig. 3), more studies are necessary to identify the active metabolites and to elucidate their exact mode of action.

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