Europ.J.Hort.Sci., **75** (4). S. 147–152, 2010, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Control Effects of some Plant Extracts against Cucumber Powdery Mildew (*Sphaerotheca fuliginea*) and their Stability Study

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Summary

Powdery mildew is a serious cucumber disease. The plant kingdom represents an enormous reservoir of various biologically active molecules; twenty-two Chinese plants were screened using a detached leaf-disc assay for preventive effect against powdery mildew (*Sphaerotheca fuliginea*). Four plant extracts, *Euphorbia humifusa* Willd, *Robinia pseudoacacia* L., *Photinia serrulata* Lindl and *Cucurbita moschata* (Duch.) Poiret, showed high preventive effects. Further *in vivo* potted seedlings assays of these four plant extracts showed *E. humifusa* and *R. pseudoacacia* extracts had not only preventive effect but also curative effect. The control effect of *E. humifusa* extracts was confirmed in the greenhouse test. The stability assays of *E. humifusa* and *R. pseudoacacia* extracts showed these two extracts are relatively stable under 40 °C, alkali condition, and treatment of light, ultraviolet treatment in short period. These results may be a basis for further developing a natural fungicide against cucumber powdery mildew.

Key words. antifungal activity – control effect – *Euphorbia humifusa* – plant extracts – *Robinia pseudoacacia* – *Sphaerotheca fuliginea*

Introduction

Powdery mildew, caused by *Sphaerotheca fuliginea*, is one of the most serious diseases of cucumber, resulting in significant yield losses on fruit (Kiss 2003). This disease is mainly controlled in the field by applying a large number of chemical synthetic fungicide sprays. Although these fungicides are efficacious, their use is discouraged because of potentially negative effects, such as environmental pollution, phytotoxicity, and the residue in the soil and in the food which affect the human health. Moreover, long-time usage of these fungicides results in the development of resistance in the pathogen populations. Recently, alternative measures have been developed to control fungal diseases, including the use of mineral salts, essential oils, plant extracts, and biological agents (Simmonds et al. 1992; Copping and Menn 2000; Belanger and Labbe 2002; Istvan 2002; Kiss 2003). Plant extracts have been receiving increasing interest because of their useful characteristics: No-residue, easy degradation, non-environmental pollution and less resistant strains produced by pathogen.

Plant-derived compounds have been reported to protect cucumber plants from powdery mildew. The extracts of *Rumex crispus* L. roots were reported to inhibit the development of cucumber powdery mildew as effectively as the fungicide Feranimol (Kim et al. 2004). Ts-86 extracts (Tang et al. 2004) and *Rheum palmatum* extracts (Tang et al. 2003) were found to have efficacy against *S. fuliginea*. In another study, 1 % Osthol was also shown to display antifungal activity against *S. fuliginea* (Wang et al. 2005).

The plant kingdom represents an enormous reservoir of various biologically active molecules. Plant extracts have been demonstrated to contribute to the control of pathogenic fungi in crude or purified form. Milsana®, a plant extract of *Reynoutria sachalinensis* (Schmidt) Nakai that induces powdery mildew resistance in plants, is reported to effectively protect cucumber plants against powdery mildew (Herger and Klingauf 1990; Dik and Van Der Staay 1995). However, the application of Milsana® is restricted to some extent because of the cost of production (Daayf and Belanger 1995; Menzies and Belanger 1996).

After a document research of Poisonous Plants of China (Chen and Zhen 1987), twenty-two plants were collected in Shanghai Area. In this study, we screened twenty-two plants for their antifungal activity against *S. fuliginea*, among which the extracts of *E. humifusa* and *R. pseudoacacia* showed efficacy against *S. fuliginea* in controlled conditions. The activity of *E. humifusa* extracts was also confirmed in further greenhouse conditions test. The stability assay of these two extracts was also done, which will benefit the application of the two extracts in the agriculture for the further exploitation of a phytochemical fungicide.

Materials and Methods

Materials

Twenty-two plants (Table 1) were collected at Shanghai Area, China, on July 2006. The isolate of the cucumber

Table 1. Screened plants, collected part and their preventive effects against the cucumber powdery mildew in the detached leaf disc bioassays.

Plant	Family	Collected part	Preventive effect (%)[a]
Robinia pseudoacacia L.	*Leguminosae*	leaf	88.46 ± 3.02 a
Euphorbia humifusa Willd	*Euphorbiaceae*	whole plant	84.62 ± 6.32 ab
Cucurbita moschata (Duch.) Poiret	*Cucurbitaceae*	leaf	80.77 ± 7.54 ab
Triadimefon emulsifiable (1:1000)[b] (CK)			80.77 ± 2.11 ab
Photinia serrulata Lindl.	*Rosaceae*	leaf	73.07 ± 6.72 b
Taraxacum mongolicum Hand.-Mazz	*Conpositae*	whole plant	50.00 ± 2.42 c
Gleditsia sinensis Lam.	*Leguninosae*	whole plant	50.00 ± 5.98 c
Pinus massoniana Larmb	*Pinaceae*	pine needle	38.46 ± 4.49 cd
Robinia pseudoacacia L.	*Leguninosae*	branch	34.62 ± 2.24 d
Platycladus orientalis (L.) Franco	*Cupressaceae*	leaf	26.93 ± 3.54 de
Humulus scandens (lour.) Merr.	*Cannabinaceae*	whole plant	26.93 ± 3.78 de
Setaria viridis (Linn.) Beauv.	*Gramineae*	whole plant	23.08 ± 2.89 de
Cerastium arvense Linn.	*Caryophyllaceae*	whole plant	23.08 ± 2.54 de
Eichnorniacrassipes (Mart) Solms Laub	*Pontederiaceae*	whole plant	23.08 ± 2.72 de
Magnolia denudata Desr.	*Magnoliaceae*	leaf	19.23 ± 1.34 e
Polygonum cuspidatum L.	*Polygonaceae*	whole plant	19.23 ± 2.83 e
Ranunculus japonicus Thunb.	*Ranunculaceae*	whole plant	11.54 ± 2.16 ef
Apium graveolens L.	*Unbelliferae*	whole plant	11.54 ± 2.94 ef
Morus alba L.	*Moraceae*	leaf	7.69 ± 0.93 ef
Lindera communis Hemsl.	*Lauraceae*	leaf	7.69 ± 2.21 ef
Solidago decurrens Lour.	*Conpositae*	leaf	7.69 ± 1.24 ef
Podocarpus macrophyllus (Thumb.) D.Don	*Podocarpaceae*	leaf	3.85 ± 0.22 f
Diospyros kaki Thunb.	*Ebenaceae*	leaf	3.85 ± 0.47 f

[a] The values represent means of three different experiments.
[b] Triadimefon emulsifiable is a commercial chemical pesticide, which was served as the positive control in this study.

powdery mildew pathogen used in these experiments was collected at Qibao Farm. The pathogen was identified as *S. fuliginea* by Dr Kang Suzhen, using a diagnostic guide (Li and Xu 2001). The cucumber cv. Baoyang, 5th, (a Chinese cultivar supplied by Shanghai Baoshan Vegetables Science Institute) was used in all experiments. The commercial fungicide, Triadimefon (20 %), as a chemical fungicide control was purchased from Yancheng Limin Chemical Plant, China.

Preparation of plant extracts

Fresh plant leaves were oven-dried at 40 °C and comminuted to 40 mesh in a comminuter (Shanghai Dianjiu herb Machinery manufacture Co., Ltd. Shanghai, China). Two hundred g of the plant powders were extracted with 2 L ethanol at room temperature for three periods of 24 h each. The macerated plant suspension was filtered and the marc was rinsed twice with 1 L ethanol, followed by filtration. The filtrate was concentrated *in vacuo* at 40 °C by a rotary evaporator (Shanghai Shengsheng Science-technology Co. Ltd. Shanghai, China) to obtain a dry residue. The concentrated extracts were dissolved in 200 mL distilled water and then filtered. To avoid phytotoxicity to cucumber seedlings, the crude extracts were diluted with water 2 times of extract volumn.

Cucumber seedling cultivation

Cucumber seedlings were cultivated in 8×8 cm plastic pots. Two seeds were sown in each pot. The pots were watered carefully and kept in a growth room at 25 °C (day) and 18 °C (night) and 16 h light : 8 h dark photoperiod, these culture conditions were used throughout the laboratory experiments. The seedlings were used for the experiment when the first true leaf of cucumber seedlings fully expanded.

Propagation of cucumber powdery mildew

The cucumber powdery mildew pathogen was collected at Qibao Farm. The pathogen was cultured on leaves of potted cucumber. *S. fuliginea* was recultured every three weeks by tapping the infected leaves over leaves of 4–5 week old potted cucumber. The cucumber powdery mildew pathogen was maintained on leaves of potted cucumbers at 25 °C (day) and 18 °C (night) and 16 h light : 8 h dark photoperiod.

The conidial suspension was prepared according to Jeong et al. (2007). Spores of *S. fuliginea* were harvested from highly infected cucumber leaves by washing leaves with sterilized distilled water and filtering with sterilized cheese cloth. Spore suspensions were adjusted to a concentration 10^5 conidia ml^{-1} for inoculating cucumber

seedlings. This spore suspension can be used only during 1 h, because of the collapse of the spores in water during more than 1 h.

Bioassays

Detached leaf disc bioassay. The detached leaf-disc assay was performed according to the procedure of COHEN (1993). The leaves from 2nd to 5th leaves of plants were detached and the leaf-discs (1 cm in diameter) were obtained by a punch. The leaf disc was randomly used, 15 discs were emerged into each of the 22 plant extracts for 5 s and placed adaxial side up on the filter paper soaked with a nutrient solution in culture dishes. Full-strength nutrient solution contains the following amount of salts per 1000 mL of distilled water: 950 mg $Ca(NO_3) \cdot 4H_2O$; 810 mg KNO_3; 500 mg$SO_4 \cdot 7H_2O$; 155 mg $NH_4H_2PO_4$; 3 mg H_3BO_3; 2 mg $ZnSO_4 \cdot 7H_2O$; 0.05 mg $CuSO_4 \cdot 5H_2O$; 0.02 mg $NaMoO_4$ and 25 μg NaFeEDTA (PRAMANIK et al. 2000). Each dish contains 5 discs and 3 dished for a treatment. Leaf-discs soaked with distilled water and 20 % Triadimefon emulsifiable (chemical fungicide) at 1000 times dilution were used as negative and positive controls, respectively. Twenty four hours later, 10 μL of *S. fuliginea* conidial suspension was placed onto the centre of the leaf-discs, which were then incubated at 25 °C (day) and 18 °C (night) and 16 h light : 8 h dark photoperiod for 7 days. Disease severity was assessed as percentage of infected area, using a 0–3 index, where 0: no symptoms; 1: <30 % (leaf-disc area infected); 2: >30 % (leaf-disc area infected); 3: powdery mildew colonies cover the whole leaf-disc. The preventive effect of plant extracts was measured with the following equation (FANG 1998):

Disease index (%) = [∑(The number of disease leaves in this range × the disease range)/(Total leaves investigated × the highest range)] ×100
Preventive effect (%) = $[(D_C - D_T)/D_C] \times 100$
where, D_T = disease index of treatment, D_C = disease index of negative control

Preventive and curative assays at potted seedlings. The preventive assay was performed using the Chinese National Standard Operation Procedure of the National Research & Development Center for Pesticide, China (HU et al. 2007). The first expanded true leaves of cucumber seedlings were sprayed with the plant extracts of *E. humifusa*, *R. pseudoacacia*, *P. serrulata* and *C. moschata* until run-off. Control seedlings were sprayed with distilled water for negative control and 20 % Triadimefon emulsifiable (chemical fungicide) at 1000 times dilution for positive control. The treated leaves were inoculated with *S. fuliginea* fresh spore suspensions (concentration 10^5 conidia ml^{-1}) 24 h after treatment. The planted pots were arranged as a randomized complete block with four replicates per treatment (8 seedlings per treatment), maintained at 25 °C (day) and 18 °C (night), 16 h light : 8 h dark photoperiod and a relative humidity of 70±15 %. The experiments were repeated for three times.

The curative assay was similar to the preventative assay except cucumber seedlings at the same stage were inoculated with *S. fuliginea* spore suspensions, and 24 h later, sprayed with one of the four extracts, distilled water and 20 % Triadimefon emulsifiable (chemical fungicide) at 1000 times dilution.

Disease severity was investigated 6 and 12 days after inoculation. The incidence of powdery mildew was evaluated visually on individual leaf as percentage of diseased area, using an index of 0, 1, 3, 5, 7 or 9, where 0: no symptoms; 1: <1 %; 3: 2–5 %; 5: 6–20 %; 7: 21–40 %; and 9: >40 % (LIANG et al. 2005). Preventive effect or curative effect was measured as described in Section detached leaf-disc assay of 22 plant extracts. The data were analyzed by the post hoc Newman-Keuls test.

Greenhouse trial of *E. humifusa* extracts. The effect of *E. humifusa* extracts was tested in a greenhouse trial. When the first symptoms of powdery mildew occurred naturally, 10 mg mL^{-1} of extract was sprayed on cucumber plants in a greenhouse. Control plants were sprayed with distilled water and 20 % Triadimefon emulsifiable at 1000 times dilution. A second spray was applied 7 d later. Each treatment consisted of three replicates with 6 plants each and was arranged in a randomized complete block design. A total of 18 plants were treated in each variant. The disease severity was rated on 10 leaves (3–12 leaves) per plant. The incidence of powdery mildew on each leaf was visually evaluated and ranked as percentage of diseased area. The control effect was measured as described in Section preventive and curative assays at potted seedlings. The data were analyzed by the post hoc Newman-Keuls test.

Stability testing

In the stability testing, 15 mL of *E. humifusa* and *R. pseudoacacia* extracts were treated with different environmental factors and then used for the preventive assay as described in preventive assays at potted seedlings to know their stability. In a thermal stability assay, the extracts were incubated in 0, 20, 40, 60, and 80 °C water bath for 1 h; in acidbase stability assay, the extracts were adjusted into pH 3, 5, 7, 9 and 11 by NaOH or HCl and allowed to stand for 24 h, control seedlings were sprayed with HCl and NaOH (0.001 mol L^{-1}); in light stability assay, the extracts were maintained in 5000 lux light at 25 °C for 0, 24, 48, 72 or 96 h; in the assay of stability under UV illumination, the extracts were treated with 365 nm Ultraviolet for 0, 1, 2, 3 or 4 h.

Results

Screening for plant extracts against cucumber powdery mildew

Through detached leaf-disc assay, four extracts, *E. humifusa* whole plant, *R. pseudoacacia* leaf, *C. moschata* leaf and *P. serrulata* leaf, showed higher preventive activity as compared to other extracts with preventive effect of 84.62, 88.46, 80.77 and 73.07 %, respectively (Table 1), which were almost similar to the activity of Triadimefon emulsifiable (positive control).

Effect of E. humifusa, R. pseudoacacia, C. moschata *and* P. serrulata *extracts against cucumber powdery mildew in potted seedlings assays*

C. moschata and *P. serrulata* extracts, as well as the fungicide Triadimefon, showed significantly less efficacy as

Table 2. The preventive and curative effects of the four plant extracts against the cucumber powder mildew.

Treatment	Preventive effect (%)[a]		Curative effect (%)	
	6 days	12 days	6 days	12 days
Euphorbia humifusa	76.81 ± 7.08 a[b]	46.29 ± 7.72 a	50.92 ± 9.20 b	3.71 ± 2.71 bc
Robinia pseudoacacia	73.22 ± 2.44 a	27.78 ± 4.35 b	76.86 ± 8.52 a	53.73 ± 8.50 a
Cucurbita moschata	28.60 ± 8.07 b	5.56 ± 3.71 c	75.92 ± 8.43 a	31.48 ± 7.09 ab
Photinia serrulata	26.78 ± 7.55 b	3.71 ± 1.86 c	−7.42 ± 6.42 c	0.00 ± 0.00 c
Triadimefon emulsifiable	26.78 ± 5.43 b	1.85 ± 1.85 c	45.36 ± 8.85 b	0.00 ± 0.00 c

[a] The values represent means ± standard errors.
[b] The same letters indicate no significant difference (P≤0.05) by analysis of variance with the post hoc Newman-Keuls test.

Table 3. Control effect of the extract of *Euphorbia humifusa* in greenhouse condition.

Treatment	Control effect (%)[a]
Euphorbia humifusa	59.44 ± 9.20 a[b]
Triadimefon emulsifiable concentrate	59.72 ± 8.85 a
Control (distilled water)	–

[a] The values represent means ± standard errors.
[b] The same letters indicate no significant difference (P≤0.05) by analysis of variance with the post hoc Newman-Keuls test.

compared to *E. humifusa* and *R. pseudoacacia* in preventive assays at potted seedlings, *E. humifusa* and *R. pseudoacacia* extracts showed preventive efficacy with preventive effects of 76.81 and 73.22 % respectively, at 6 d after inoculation (Table 2). Moreover, the preventive efficacy partly remained apparent after 6 more days in culture, while Triadimefon emulsifiable *C. moschata* and *P. serrulata* extracts lost their preventive effects.

E. humifusa, *R. pseudoacacia* and *C. moschata* extracts showed higher curative efficacy than Triadimefon emulsifiable with curative effects of 50.92, 76.80 and 75.92 % respectively, at 6 d after inoculation (Table 2). The curative efficacy of *E. humifusa* extracts was not evident after 6 more days of culture, while the efficacy of *R. pseudoacacia* and *C. moschata* was still observed (Table 2). *P. serrulata* extracts showed no curative effects (Table 2).

Greenhouse trial of E. humifusa *extracts*

E. humifusa extracts were bioassayed in the greenhouse and the results showed that the control effect of *E. humifusa* extracts, 59.44 %, showed the same effect as Triadimefon emulsifiable 59.72 % (Table 3).

Stability assay of R. pseudoacacia *and* E. humifusa *extracts*

The increasing of pretreatment temperature from 0 °C to 40 °C had little effects on the efficacy of *R. pseudoacacia* and *E. humifusa* extracts. The preventive effect of *E. humifusa* extracts decreased significantly when pretreated at the temperatures higher than 60 °C, while extracts of *R. pseudoacacia* were most effective at 60 °C (Fig. 1A), *E. humifusa* was more sensible to temperature than *R. pseudoacacia*. In acidbase stability assay, to avoid effects of NaOH or HCl to cucumber seedlings, control seedlings were sprayed with HCl and NaOH (0.001 mol L^{-1}), our tests show cucumber seedlings are not affected by HCl and NaOH with low concentration, the extract solution at safe concentrations were used for the test. Compared to temperature, pH dramatically influenced the efficacy of *R. pseudoacacia* and *E. humifusa* extracts. *R. pseudoacacia* and *E. humifusa* extracts had low efficacy (preventive effect <50 %) under acid condition, while gained high efficacy (preventive effect >70 %) under neutral and alkalic conditions (Fig. 1B). *R. pseudoacacia* extracts remained stable at different phases of light (Fig. 1C), while long-time exposure to light (>72 h) dramatically decreased the efficacy of *E. humifusa* extracts (Fig. 1C). *R. pseudoacacia* and *E. humifusa* remained stable at 1 h of UV (Fig. 1D), while long-time exposure to UV (>2 h) dramatically decreased the efficacy of extracts (Fig. 1D).

Discussion

In this study, twenty-two plants were screened for their preventive effect against powdery mildew fungus by leaf discs assay. The results showed that plant extracts of *E. humifusa*, *R. pseudoacacia*, *C. moschata* and *P. serrulata* had a preventive activity. However, in further assays with potted seedlings, *C. moschata* extracts exhibited poor preventive effect but good curative effect (Table 2). On the other hand, *P. serrulata* extracts presented both poor preventive effect and poor curative effect (Table 2). *In vivo* potted seedlings assays showed *E. humifusa* and *R. pseudoacacia* extracts had not only preventive effect but also curative effect (Table 2). This may be due to these two plant extracts contain not only the preventive and also curative active compound. It is normally considered that the plant extract is a mixture of the secondary metabolism compounds. The compounds responsible for these two effects will be isolated from the extract and further studied later.

Leaf disc method was accomplished with leaf discs in the culture dishes, using less plant material, is suitable for a big scale screening. This can be considered as a primary step, to decide the plant extract effect, potted seedlings bioassay, even greenhouse trail are indispensable because this essay is more similar to the real situation. In

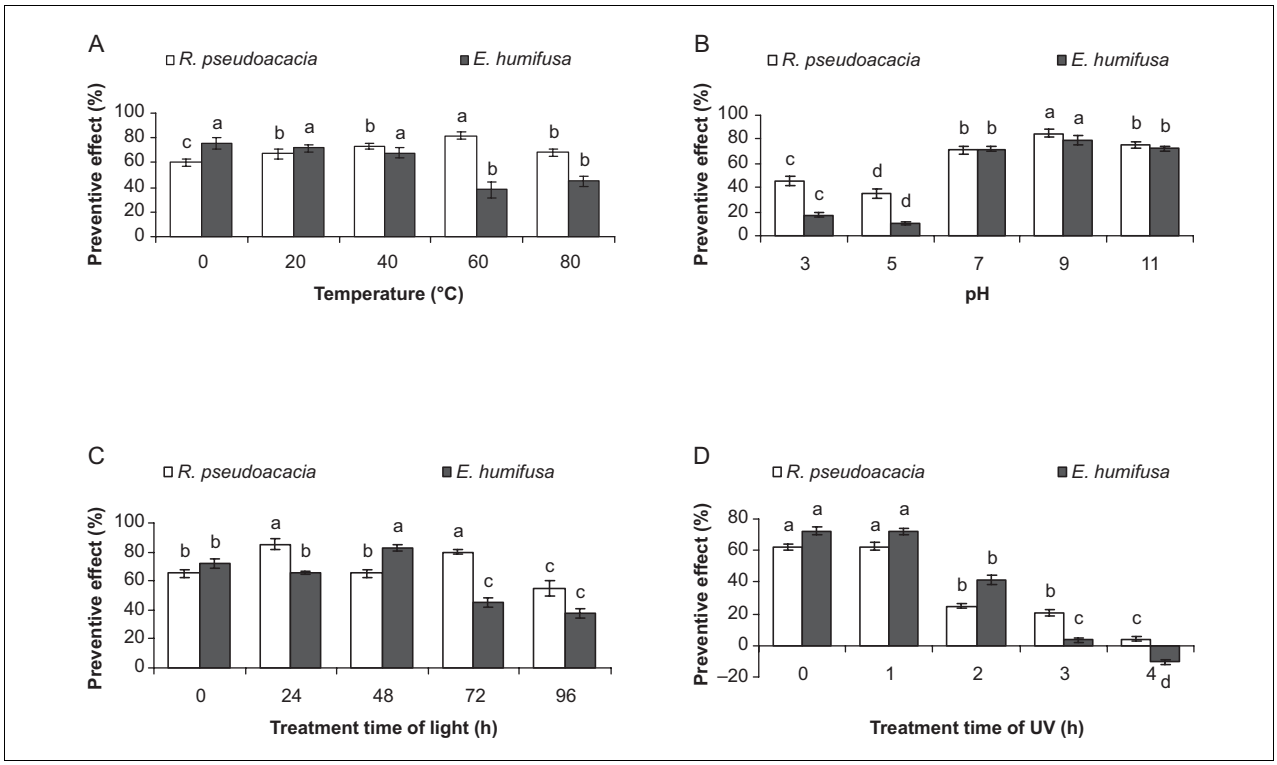


Fig. 1. The stability assay of *R. pseudoacacia* and *E. humifusa* extracts. *R. pseudoacacia* and *E. humifusa* extracts were treated with different factors and then used for the preventive assay. A. the thermal stability assay; B. the acidbase stability assay; C. the light stability assay; D. the assay of stability under UV illumination.
The values represent means ± standard errors. The same letters indicate no significant difference (P≤0.05) by analysis of variance with the post hoc Newman-Keuls test.

this experiment, some plant extracts showed the same (*E. humifusa* and *R. pseudoacacia* extract) and some different effect (*C. moschata* and *P. serrulata* extracts) between these two different assays. These may be because the assay with leaf discs was accomplished by soaking the leaf discs in the extract solution, while that with potted seedlings, the extract solution was sprayed on the leaves, the extract quantity absorbed by leaves may different between these two treatment ways. Moreover, assay with leaf discs was in cultural dishes which was a closed condition, while with potted seedlings one, it was a opened condition. Some extracts had the effect in the closed culture dished, may be due to the volatilization of the active compounds, may had no effect with the potted plants. These may explain some differences between these two assays.

Greenhouse trial of *R. pseudoacacia* extracts was accomplished by Zhang et al. (2008), the results showed that the water soluble extracts had antifungal efficacy in a dose-dependant manner. Greenhouse trial of *E. humifusa* extracts confirmed the results of potted seedling assay. It is most interesting that *E. humifusa* extracts showed almost the same efficacy as that of chemical control, Triadimefon in the greenhouse trial (Table 3), which provided a possibility of further developing this extract for the agricultural use.

Two effects, preventive and curative were investigated *in vivo* assay with potted seedlings. These results may help the trail in the greenhouse, for the plant extract possessing the preventive effect may be applied before or at the same time as the appearance of the symptom, while for one with curative effect, can be applied after the appearance of the symptoms. In this experiment, the extract of *E. humifusa* was demonstrated to have the two effects in the potted seedlings assay. In the greenhouse trail, the extract was applied immediately after the first appearance of the symptom, and prevented the development of the symptom.

Environmental factors often influence the efficacy of fungicide (Qasem and Abu-blan 1995). In this study, we tested whether temperature, light, pH and ultraviolet radiation influence the efficacy of *R. pseudoacacia* and/or *E. humifusa* extracts. As our data showed, high temperature (Fig. 1A), low pH (Fig. 1B) and long exposure to UV (Fig. 1D) decreased the efficacy of *E. humifusa* extracts; while the efficacy of *R. pseudoacacia* extracts was mainly influenced by pH (Fig. 1B) and UV (Fig. 1D). These results indicated that both *R. pseudoacacia* and *E. humifusa* extracts are best used under alkali condition in the afternoon or in the evening when the UV rays are weaker. At the same time, *E. humifusa* extracts could be better used as a preventive agent, and *R. pseudoacacia* extracts as curative agent.

Although the mechanisms of *R. pseudoacacia* and *E. humifusa* extracts function against powdery mildew fungus still remain elusive, our finding of the preventive effect of some extracts may probably be an induced resistance, extract may contain some compounds as elicitors, could induce some resistant reactions, which reduce the disease severity. The curative effect, may due to some

active compounds can absorbed by plant cells and can inhibit the development of the mycelium, so that can control the disease.

TANG et al. (2003) found that 100-fold *Rheum palmatum* extract had a preventive effect of 90.14 % against cucumber powdery mildew, superior to the chemical control Triadimefon in the potted plant assay. KIM et al. (2004) showed that the extracts of *Achyranthes japonica* and *Rumex crispus* were as efficient as the fungicide Fenarimol against the powdery mildew in the greenhouse trail. One year later, WANG et al. (2005) demonstrated that 3 sprays of 1 % Osthol aqueous emulsion could control cucumber powdery mildew by 98.51 % in the greenhouse trail. The efficacy of the plants extracts in this study was less than those of these three references. Wang used 1 % pure active compound Osthol's formulation, which may easily spread on the surface of the leaves and make it more efficient. For increase the activity of the extract in this experiment, the active compound's purification, isolation, and formulation study will be necessary to be taken.

The preventive efficacy of *E. humifusa* extracts in the greenhouse trial provides the possibility of developing these extracts for agricultural use, especially in the greenhouse condition. For the use in the field may be difficult due to its poor stability under UV light. Moreover, *E. humifusa* is well distributed in several provinces of China and is easy to regenerate. So the agricultural utility of *E. humifusa* would not interfere with the ecological environment.

Acknowledgements

We are very grateful to Kang Suzhen, Zhang Zhiyuan and Li Yanbo (Shanghai Jiao Tong University) for their assistance and advice. Thank Dr. M. Nicole of UMR RPB, Resistance des plantes, IRD-CIRAD-UM2, BP 64501, 34394 Montpellier Cedex 5, France for his revision of this manuscript. We also thank all the members from Natural Product and Healthy Plant Laboratory of Shanghai Jiao Tong University and the members from National R&D Center for Pesticide in Shanghai for their advice, assistances and technical help. The research was supported by the Transformation Project of Agricultural Science and Technology Achievements, Ministry of Science and Technology China, and by Shanghai Leading Academic Discipline Project (No. B209).

References

BELANGER, R.R. and C. LABBE 2002: Control of powdery mildews without chemicals: prophylactic and biological alternatives for horticultural crops. In: BELANGER, R.R., W.R. BUSHNELL, A.J. DIK and T.L.W. CARVER (eds.): The Powdery Mildews: A Comprehensive Treatise. American Phytopathological Society, St. Paul, USA, 256–267.

CHEN, J.S. and S. ZHEN 1987: Poisonous Plants of China, Science Press, Beijing.

COHEN, R. 1993: A leaf disk assay for detection of melons to *Sphaerotheca fuliginea* racel. Plant Dis. **77**, 513–517.

COPPING, L.G. and J.J. MENN 2000: Biopesticides: a review of their action, applications and efficacy. Pest Manage. Sci. **56**, 651–676.

DAAYF, F. and R.R. BELANGER 1995: The effects of plant extracts of *Reynoutria sachaliensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Dis. **79**, 577–580.

DIK, A.J. and M. VAN DER STAAY 1995: The effect of Milsana on cucumber powdery mildew under Dutch conditions. Meded. Fac. Landbouwwet. Univ. Gent **59**, 1027–1034.

FANG, Z.D. 1998: Research Method of Plant Pathology. 3th ed., China agricultural publishing house, Beijing.

HERGER, G. and F. KLINGAUF 1990: Control of powdery mildew fungi with extracts of the giant knotweed, *Reynoutria sachalinensis* (Polygonaceae). Meded. Fac. Landbouwwet. Univ. Gent **55**, 1007–1014.

HU, W.Q., W.G. ZHU, D.G. CHEN, J. CHEN and Y.H. ZHEN 2007: Biological Activity of Novel Strobilurin Compound ZJ 1621. Chin. J. Pesticide Sci. **9**, 240–244.

ISTVAN, U. 2002: Transforming natural products into natural pesticides-experience and expectations. Phytoparasitica **30**, 439–442.

KIM, J.J., M.S. GOETTEL and D.R. GILLESPIE 2007: Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Biological Control. **40**, 327–332.

KIM, J.C., G.J. CHOI, S.W. LEE, J.S. KIM, K.Y. CHUNG and K.Y. CHO 2004: Screening for antifungal extracts against various plant pathogenic fungi and control of powdery mildew with extracts of *Achyranthes japonica* and *Rumex crispus*. Pest Manage. Sci. **60**, 803–808.

KISS, L. 2003: A review of fungal antagonists of powdery mildews and their potential as biocontrol agents, Pest Manage. Sci. **59**, 475–483.

LIANG, Y.C., W.C. SUN, J. SI and V. RÖMHELD 2005: Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. Plant Pathol. **54**, 678–68.

LI, H.L. and J.Y. XU 2001: Agricultural plant pathology experiment guidance, China agricultural publishing house, Beijing. Pp. 128–133.

MENZIES, J.G. and R.R. BELANGER 1996: Recent advances in cultural management of diseases of greenhouse crops. Canadian J. Plant Pathol. **18**, 186–193.

PRAMANIK, M.H.R., N. MASAYUKI and A. TOSHIKI 2000: Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativus*) in hydroponic culture. J. Chem. Ecol. **26**, 1953–1967.

QASEM, J.R. and H.A. ABU-BLAN 1995: Antifungal activity of aqueous extracts from some common weed species. Ann. Appl. Biol. **127**, 215–219.

SIMMONDS, M.S.J., H.C. EVANS and W.M. BLANEY 1992: Pesticides for the year 2000: mycochemicals and botanicals. In: KADIR, A.S.A. and H.S. BARLOW (eds.). Pest Management and the Environment in 2000. CAB International, Wallingford, UK, 127–164.

TANG, R., X.H. ZHANG, T.L. HU and K.Q. CAO 2003: Control Effect of the Extracts from *Rheum palmatum* on Powdery Mildew of Cucumber. J. Anhui Agricult. Univ. **4**, 363–366.

TANG, R., X.H. ZHANG and K.Q. CAO 2004: The effect of extracts of some Chinese medicine plants against Powdery Mildew of Cucumber. Northern Hort. **4**, 84–85.

WANG, C.M., G.P. WU, Y.Z. WANG and Z.Q. SHI 2005: Study on Osthol against Powdery Mildew of Cucumber. Jiangsu Agricult. Sci. **4**, 57–58.

ZHANG, Z.Y., G.H. DAI, Y.Y. ZHUGE and Y.B. LI 2008: Protective effect of *Robinia pseudoacacia* Linn1 extracts againstcucumber powdery mildew fungus, Crop Protection **27**, 920–925.

Received January 11, 2010 / Accepted May 27, 2010

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