Effects of Chalcones and Chromenes on Powdery Mildew and Anthracnose of Cucumber Plants

Keido Kō, Katsuhiko ISEKI,* Yumiko ADACHI, Kazuhiko KONNO,* Katsura YOKOYAMA* and Tomomasa MISATO

The Institute of Physical and Chemical Research, Wako, Saitama 351, Japan
* Mitsubishi Yuka Co., Ami-cho, Inashikigun, Ibaragi 300–03, Japan

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A group of chalcones and a group of chromenes were found to be effective to prevent the powdery mildew and anthracnose of cucumber plants. Several derivatives of them were tested by in vitro assays and pot test. Among these test compounds, 2-[cis-3-(2-hydroxyphenyl)]propenoylbenzoic acid and 1,2-benzopyran-2-spiro-3'-phthalide, both compounds showed remarkably effective on the plant diseases. However, the former was rather phytotoxic to some kinds of plants.

INTRODUCTION

On a series of the author’s screening test program with respect to naturally occurring substances for preventing plant diseases, some antibiotics, e.g. blasticidin S,1) bihoromycin,2) aabomycin3) etc. and extracts of plant cell components, e.g. antiviral substances from tissue-cultured cells of Phytolacca americana4) and Agrostemma githago5) and an antifungal substance, lecithin extracted from soy bean,6) were found that they were potentially effective to prevent development of several kinds of plant diseases. A group of chalcones was also one of the effective substances found by the authors. It inhibited a growth of several kinds of phytopathogenic fungi, e.g. Colletotrichum lagenarium, Glomerella cingulata, and Botrytis cinerea. However, the chalcones were somewhat phytotoxic on some kinds of plants. Therefore, a group of chromenes which was closely related to the chalcones in the chemical structure, was also tested for its effects on the development of plant diseases.

Chalcones are a series of flavonoid pigments occurred in plants as a yellow pigment, and also a precursor of the more common flavonoid pigments. The preventive effects of the chalcones and the chromenes on the development of plant diseases were detected by pot tests of powdery mildew and anthracnose of cucumber plants. This paper will present some results in respect of the efficacy of some derivatives of chalcones and chromenes on powdery mildew and anthracnose of cucumber plants.

MATERIALS AND METHODS

1. Test Chemicals

Many derivatives of chalcones, e.g. 2-[cis-3-(2’-hydroxyphenyl)]propenoylbenzoic acid etc. and of chromenes, e.g. 1,2-benzopyran-2-spiro-3’-phthalide etc. were prepared as listed in the tables presented later. According to the objects, 1% ethanol aqueous solutions of the test chemicals were prepared for spore germination test, 10% ethanol aqueous solutions for pot test and 99% ethanol solution for paper disk assay.

2. Test organisms

The causal fungus of cucumber anthracnose disease, Colletotrichum lagenarium was furnished from National Institute of Agricultural Sciences, and cultured on sweet corn agar medium which contained 200 g of canned
sweet corn and 15 g of agar in 1 liter distilled water. The sporulation of C. lagenarium was performed by culturing the fungus on agar plate of the sweet corn medium in 90 mm (in diameter) of petri-dishes and incubating for 10 days. Spores of the causal fungus of cucumber powdery mildew, Sphaerotheca fuliginea, were collected from diseased plants for inoculation successively kept their conidia on fresh plants by renewal inoculation at intervals of 2 weeks.

3. Test Plants
Cucumber plants (Sagamihanjiro) were cultivated in air-conditioned greenhouse for 2 weeks under 28°C in day-time and 23°C in night-time, 60% R.H.

4. Biological Assays
4.1 In vitro test
Two ways of in vitro test were used in this experiment. a) Ordinary method of the slide germination test was used for assaying fungicidal activity against C. lagenarium. b) Paper disk method: Disks of filter paper (8 mm in diameter and 1.5 mm in thickness) were used in this experiment. Potato dextrose agar medium was poured in petri-dish (90 mm in diameter). Each petri-dish was prepared with 10 ml of basal layer and 5 ml of upper layer which was seeded a certain number of spores of the test organism, C. lagenarium. After the assay-plate solidified, the drug-preimmersed paper disks were put on the plate. Then, after incubating for 48–72 hr, the diameter (mm) of inhibition zone developed on the plate was measured by vernier callipers.

4.2 In vivo test
Pot test was conducted as an in vivo test. Two week-grown seedlings of cucumber were used for the test. Inoculations of C. lagenarium and S. fuliginea on cucumber plants were performed respectively by spraying their conidia, after the test plants were treated by spraying the test chemicals. The concentrations of conidia for the inoculation were about 300 spores per ml of distilled water for C. lagenarium, and 5–10 spores per ml of distilled water for S. fuliginea.

The test chemicals dissolved in 10% ethanol aqueous solutions were sprayed on the test plants by foliar treatment with spray-gun under 2 kg/cm². The treated plants inoculated with C. lagenarium were incubated in high-moist chamber for 24 hrs, in the dark under 25°C. And further incubation was continued under 25°C, 60% R.H. and 5,000 lux of artificial illumination for 72 hrs. After 4 day-incubation, the disease-development was investigated by counting their diseased spots.

As to the disease-development of cucumber powdery mildew, incubation was carried under 25°C, natural illumination for 10 days. The preventive value was calculated by the following equation. All test chemicals listed in Tables 1–5, were applied at the concentration of 1,000 ppm for the pot tests.

\[
\text{Preventive value (\%) = } \left(1 - \frac{\text{Total number of diseased spots on the treated plants}}{\text{Total number of diseased spots on the untreated plants}}\right) \times 100
\]

RESULTS
1. Effects of some Derivatives of 2-(Cis-3-phenylpropenoyl)benzoic Acid on Powdery Mildew and Anthracnose of Cucumber Plants
As shown in Table 1, all of these compounds, more or less, showed anti-fungal activities in vitro and in vivo. Among these derivatives of 2-(cis-3-phenylpropenoyl)benzoic acid, the 2'-hydroxy and 4'-methoxy derivatives were the most effective against both powdery mildew and anthracnose of cucumber plants. This comparison of fungicidal activities was made by the pot test under the concentration of 1,000 ppm and by the spore germination test and paper disk assay under the concentration of 100 ppm. The 2'-hydroxy derivative was found rather preventive against powdery mildew than to anthracnose, while the 4'-methoxy derivative showed vice versa. However, these two compounds were almost completely preventive against the test diseases. For the in vitro test, the 2'-hydroxy derivative was much more effective to inhibit the spore germination of C. lagenarium.
2. Effects of Some Derivatives of Cis-3-phenylpropenoylbenzene on Powdery Mildew and Anthracnose of Cucumber Plants

As shown in Table 2, among these derivatives of cis-3-phenylpropenoylbenzene, 2-[cis-3-(2-hydroxyphenyl)]propenoylbenzoic acid and allyl-2-(cis-3-phenylpropenoyl)benzoate were the most effective to prevent powdery mildew of cucumber plant and to inhibit the spore germination of Colletotrichum lagenarium. However, the former showed an inhibitory zone on the agar plate of C. lagenarium, when tested with the paper disk method, while the latter did not. Ethyl 2-[cis-3-(3-methylphenyl)]propenoylbenzoate was found specifically effective to prevent the disease development of anthracnose of cucumber plant.

3. Effects of Some Derivatives of Cis-3-(4-chlorophenyl)propenoylbenzene on Powdery Mildew and Anthracnose of Cucumber Plants

So far as the tested compounds, as shown in Table 3, the chlorination of cis-3-phenylpropenoylbenzene at the 4'-position seems to be little use for enhancing the inhibitory effect of the compounds on the test organisms. The 2-carboxyl derivative showed a remarkable
inhibition on the spore germination of *C. lagenarium*. However, its preventive effects on the disease developments of powdery mildew and anthracnose of cucumber plants were considerably low.

4. Effects of Some Derivatives of Cis-3-phenylpropenoyl-4-methoxybenzene on Powdery Mildew and Anthracnose of Cucumber Plants

As shown in Table 4, paper disk soaked by cis-3-phenylpropenoyl-4-methoxybenzene made an inhibition zone on the agar plate of *C. lagenarium* in diameter of 15 mm, whereas, there was no inhibitory activity on the tested disease. As for the 4'-methoxy-, 3'-nitro-, 4'-hydroxy- and 2-bromo-derivatives, they were specifically effective on anthracnose of cucumber plants. However, there was no inhibitory activity in vitro.

![Chemical structure](image)

Table 4 Effects of some derivatives of cis-3-phenylpropenoyl-4-methoxybenzene on powdery mildew and anthracnose of cucumber plants.

<table>
<thead>
<tr>
<th>Substituents</th>
<th>2</th>
<th>3'</th>
<th>4'</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
<th>D (mm)</th>
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<td></td>
<td></td>
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<td>0</td>
<td>0</td>
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<tr>
<td>2. CH₃</td>
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<td></td>
<td>CH₃</td>
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<td>33</td>
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<td>0</td>
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<td></td>
<td>Cl</td>
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<tr>
<td>8. Br</td>
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<td></td>
<td></td>
<td>0</td>
<td>92</td>
<td>0</td>
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A, Powdery mildew of cucumber plant; B, Anthracnose of cucumber plant; C, Inhibition ratio of spore germination of *Colletotrichum lagenarium* in 100 ppm solution of the test chemicals; D, Inhibition zone (mm) by paper disk method.

5. Effects of Some Derivatives of 1,2-Benzopyran-2-spiro-3'-phthalide on Powdery Mildew and Anthracnose of Cucumber Plants

As shown in Table 5, the inhibitory activities of the 2-phenyl-, the 5-methoxy-, and the 7-bromo-derivatives on the test organisms and the tested diseases were far less effective than that of the mother skeleton, 1,2-benzopyran-2-spiro-3'-phthalide.

![Chemical structure](image)

Table 5 Effects of some derivatives of 1,2-benzopyran-2-spiro-3'-phthalide on powdery mildew and anthracnose cucumber plants.

<table>
<thead>
<tr>
<th>Substituents</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
<th>D (mm)</th>
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<tr>
<td>1.phenyl</td>
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<td>20</td>
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<tr>
<td>3. Br</td>
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A, Powdery mildew of cucumber plant; B, Anthracnose of cucumber plant; C, Inhibition ratio of spore germination of *Colletotrichum lagenarium* in 100 ppm solution of the test chemicals; D, Inhibition zone (mm) by paper disk method.

6. Comparison of Some Biological Effects between 2-[Cis-3-(2'-hydroxyphenyl)]propenoylbenzoic Acid and 1,2-Benzopyran-2-spiro-3'-phthalide

A comparison was made with the fungicidal activities and the preventive activities of 2-[cis-3-(2'-hydroxyphenyl)]propenoylbenzoic acid and 1,2-benzopyran-2-spiro-3'-phthalide which were the most effective compounds among the chalcones and the chromenes respectively, on the disease development of the test plant. As shown in Table 6, in comparison of the inhibitory effects of the compounds on anthracnose and powdery mildew of cucumber plants, 1,2-benzopyran-2-spiro-3'-phthalide was much more effective than 2-[cis-3-(2'-hydroxyphenyl)]propenoylbenzoic acid. However, the effects of both compounds on the spore germination of *C. lagenarium* and the inhibition zone formation taken by paper disk method were alike each other.

Furthermore, some phytotoxicities were observed, when the test plants were sprayed
by 2-[(cis-3-(2’-hydroxyphenyl)]propenoylbenzoic acid at the concentration more than 500 ppm. Whereas, there was no visible phytotoxicity observed on the same treatment of 1,2-benzopyran-2-spiro-3’-phthalide.

DISCUSSION

It is hard to put the above results in order as an universal doctrine about the relationship between chemical structures and biological activities. However, as for the chalcones, 2’-hydroxy-derivative is the most effective compound in 2-(cis-3-phenylpropenoyl)benzoic acid. Furthermore, when OH radical was transited from 2’-position to 3’- or 4’-position, the biological activities on these compounds were much less effective than the 2’-hydroxy-derivative. Whereas, the transition of OCH₃ radical among 2’-, 3’- and 4’-positions seemed to be of no influence to the biological activities.

As shown in Table 2, the biological activities of some esterified derivatives were less effective than the acyl derivative. And it is very interesting that allyl 2-[(cis-phenylpropenoyl)benzoate was specifically effective on powdery mildew of cucumber plant.

Among these derivatives of chalcone, 2-[(cis-3-(2’-hydroxyphenyl)]propenoylbenzoic acid was the most effective compound to prevent the disease development of powdery mildew and anthracnose of cucumber plants. However, this compound was phytotoxic to cucumber plant. The treated levels became otiolated, and then some necrotic spots were observed, when the compound was applied at the concentration more than 500 ppm. Occasionally, the treated leaves were rolled up by the treatment of the compound. In order to reduce the phytotoxicity caused by 2-[(cis-3-(2’-hydroxyphenyl)]propenoylbenzoic acid, a chromene, 1,2-benzopyran-2-spiro-3’-phthalide, which is a dehydrated form of 2-[(cis-3-(2’-hydroxyphenyl)]propenoylbenzoic acid, was used for the test.

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benzoic acid の 10% アルコール 500 ppm 水溶液の
葉面散布で、幼葉に黄化、褐斑などの薬害が認められ
た。それに対して、1,2-benzopyran-2-spiro-3’-phthalide は同様の処理では薬害が認められない。

REFERENCES
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