Anti-mildew Spectrum of Mildiomycin*

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(Received January 11, 1979)

Mildiomycin (MIL) was effective by foliar application against eight genera of powdery mildew fungi on fifteen kinds of plants. Its effect at 80 ppm was equal to that of quinomethionate on cucumber, and surpassed quinomethionate and the other fungicides on almost every other plants tested. MIL was also effective against benomyl-resistant strain of *Sphaerotheca fuliginea*, causative fungus of cucumber powdery mildew. Phytotoxicity was not observed on any plants tested.

The systemic property of MIL was shown under the soil-free condition by root-soaking. Translaminar activity of MIL, which was proved on tobacco leaves seemed to explain its good control effect against *Leveillula taurica*, an endoparasitic fungus causing powdery mildew of green pepper.

INTRODUCTION

Mildiomycin (MIL) is a new antibiotic^{1,2)} of low mammalian toxicity,³⁾ produced by *Streptovercillium rimofaciens* B-98891.⁴⁾ Its activity against powdery mildew fungi was found through the screening by root-soaking of barley seedlings.⁵⁾ The range of anti-mildew activities, systemic and translaminar property of this antibiotic are reported in this paper.

MATERIALS AND METHODS

1. Chemicals

Mildiomycin® (MIL) (purity 97%, Takeda Chem. Ind. Ltd.) (Fig. 1)¹⁾ and TF-138 WP (MIL 8%) were used. Reference chemicals were Karathane® 37% EC (dinocap, mixture of dinitro octyl-phenyl crotonates), Mores-tan® 25% WP (quinomethionate, S,S-6 methylquinoxaline-2,3-diyl dithiocarbonate), Acricid® 50% WP (binapacryl, 2-*sec*-butyl 4,6-dinitrophenyl 3-methylcrotonate), Polyoxin AL 10% WP (polyoxin, polyoxin B complex), Topsin M® 70% WP (thiophanate-methyl, dimethyl 4,4'-*o*-phenylene-3,3'-dithiodial-

lophanate), Benlate® 50% WP (benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate), Milcurb® 12.5% LQ (dimethirimol, 5-*n*-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine), Udonkol® 30% WP (CE-CA, *N*-(2-cyanoethyl)monochloroacetamide) and Corona® 75% WP (wetable sulfur, sulfur).

2. Plants

Cucumber, melon, rose, tobacco, tomato, barley, garden pea, oxalis, apple (seedling) and green pepper were tested in the glass house or the plastic house in our laboratory. They were grown in 1/10,000a plastic pots excepting for roses which were grown in larger pots (diameter: 30 cm). Strawberry was tested in a farmer's plastic house in Tokushima prefecture. Red clover, mulberry, grape and oak were tested in the field.

3. Inoculation and Application

Inoculation was conducted by dusting conidia off the diseased leaves or by setting diseased plants adjacent to the test plants on the dates shown in Table 1–4. Chemical solutions were applied one to four times with weekly intervals on the dates shown in Table 1–4 by

* Studies on Mildiomycin (Part 2)

Fig. 1 Chemical structure of mildiomicin.

spray nozzle (pressure: 1 kg/cm²) or hand sprayer in enough volume to cover the surfaces of the plants.

4. Evaluation

The effects of the chemicals were determined by the percent area of mildew on the leaves. In the test of anti-mildew spectrum, percent disease control was calculated using following formula:

$$\text{percent disease control} = \left(1 - \frac{\text{percent area of mildew on treated plants}}{\text{percent area of mildew on untreated plants}} \right) \times 100$$

The dates of evaluations are shown in Table 1.

RESULTS

Anti-mildew spectrum of MIL was examined using powdery mildews on 15 kinds of plants caused by the fungi of 13 species of 8 genera (Table 1). The percent disease control by 80 ppm of MIL was more than 90% against powdery mildews on cucumber, melon, strawberry, tomato, barley, tobacco, grape vine, apple, rose, red clover, oxalis and oak, and 80 to 90% on garden pea, mulberry and green pepper. In respect to parasites, MIL showed excellent control effects against *Sphaerotheca*, *Erysiphe*, *Podosphaera*, *Microsphaera*, *Uncinula* and *Cystotheca*. It was also effective against *Leveillula*, an endoparasitic fungus, and *Phyllactinia*. In comparison with the control effect of dinocap (148 ppm) against powdery mildews, 80 ppm of MIL was superior on 13 kinds of plants and inferior on mulberry. In comparison with quinomethionate (62.5–83 ppm), 80 ppm of MIL was equal or superior on strawberry, cucumber, barley and oak. In comparison

with the other fungicides on cucumber, the effect of MIL at 40 or 80 ppm was better than that of polyoxin (100 ppm), thiophanate methyl (700 ppm), benomyl (250 ppm), CECA (200 ppm) and binapacryl (250 ppm), and inferior or equal to wettable sulfur (2,500 ppm) according to the concentration. But severe leaf injury was observed with wettable sulfur.

As the effect of benomyl or thiophanate-methyl was rather insufficient against the powdery mildew of cucumber, a test in comparison with MIL was conducted using inocula from different sources, one from benomyl-treated plot and the other from untreated plot. As shown in Table 2, MIL was effective enough against both inocula, although benomyl and thiophanate methyl was a little effective only against the inoculum from untreated plot.

No phytotoxicity of MIL was observed with a spray of 160 ppm on cabbage, garden balsam, small red bean as well as the crops shown in Table 1. Besides, apple, strawberry and cucumber was not injured by the several times of sprays of MIL at 320, 320 and 1,280 ppm, respectively.

A systemic property of MIL was expected from the screening process⁹⁾ through which MIL was found out. But, MIL was not effective against powdery mildew of barley by five hours of seed soaking in 10,000 ppm solution, nevertheless, good control equal to that of 0.01 ppm solution of the systemic fungicide, dimethirimol, was produced by root soaking of cucumber seedlings in 10 ppm solution of MIL (Table 3). Drench application was tried in vain at the rate of 0.025% (w/v) of MIL to the soil of potted cucumber.

In relation to the systemic property, trans-laminar property was examined by spraying the upper surfaces of tobacco leaves with 80 ppm of MIL two days after inoculation on the lower surfaces. Although the upper surfaces were exposed to daily infections of conidia, MIL controlled powdery mildew on that surface remarkably, being a little superior to 83 ppm of quinomethionate. And on the lower surfaces, effective control, if not a remarkable one, was obtained by MIL, being superior in comparison with the quinomethionate whose control was unsatisfactory (Table 4). Similar results were also produced on cucumber.

Table 1 Effect of mildiomycin on control of powdery mildews.

Host plants (cultivar)	Pathogenic fungi	Chemicals* & concn. (a.i. ppm)	Disease control (%)	Date		
				Inoculation	Spraying	Evaluation
Cucumber (Shinko A)	<i>Sphaerotheca fuliginea</i>	MIL 40	95.5	Nov. 14	Nov. 16 Nov. 22	Nov. 29
		MIL 80	99.1			
		DINO 148	82.7			
		QUINO 62.5	100.0			
		TH-M 700	86.6			
		BENO 250	67.8			
		CECA 200	77.1			
		POLY 100	95.4			
		W.S. 2500	100.0****			
Melon (Ginsen)	<i>S. fuliginea</i>	BINA 250	86.0	Feb. 12	Feb. 14	Feb. 29
		MIL 40	80.4			
		MIL 80	92.8			
Rose (Arlene Francis)	<i>S. pannosa</i>	DINO 148	94.8	—	Mar. 11 Mar. 18	Apr. 1
		MIL 40	96.0			
		MIL 80	97.8			
Strawberry (Hogyoku)	<i>S. humuli</i>	DINO 148	63.2	—	Nov. 21 Nov. 29	Dec. 6
		MIL 40	96.0			
		MIL 80	96.3			
		DINO 148	78.9			
		QUINO 83	50.0			
Tobacco (MC)	<i>Erysiphe cichoracearum</i>	POLY 100	80.9	—	Feb. 8	Feb. 19
		MIL 40	92.1			
		MIL 80	93.2			
		DINO 148	94.0			
Tomato (Fukujyu No. 2)	<i>E. cichoracearum</i>	MIL 40	87.0	Jun. 10	Jun. 12	Jul. 2
		MIL 80	94.8			
		DINO 148	83.3			
Barley (Shiga- hakkoku 5)	<i>E. graminis</i>	MIL 40	90.0	Mar. 12	Mar. 14	Mar. 26
		MIL 80	94.5			
		QUINO 83	90.0			
Garden pea (Usuimi endo)	<i>E. pisi</i>	MIL 40	87.1	Jun. 28	Jul. 1	Jul. 10
		MIL 80	88.4			
		DINO 148	87.4			
Red clover	<i>E. polygoni</i>	MIL 40	85.1	—	Jun. 20	Jun. 27
		MIL 80	90.3			
		DINO 148	46.1			
Oxalis	<i>Microsphaera ruselli</i>	MIL 40	97.0	Jun. 28	Jun. 29	Jul. 6
		MIL 80	98.7			
		DINO 148	83.5			
Apple (Rall's Janet)	<i>Podosphaera leucotricha</i>	MIL 40	87.9	May 22	May 24	May 31
		MIL 80	91.8			
		DINO 148	92.7			
		POLY 160	81.9			
Mulberry (Ichinose)	<i>Phyllactinia moricola</i>	MIL 40	81.6	—	Sep. 3 Sep. 10	Oct. 8
		MIL 80	82.5			
		DINO 148	88.9			
Grape (Muscut berry A)	<i>Uncinula necator</i>	MIL 40	100.0	—	Jun. 26 Jul. 3 Jul. 10 Jul. 17	Aug. 7
		MIL 80	100.0			
		TH-M 476	66.7			
Green pepper (Ise)	<i>Leveillula taurica</i>	MIL 80	86.5	Apr. 18	Apr. 19	Apr. 29
		DINO 148	41.5			
Oak	<i>Cystotheca wrightii</i>	MIL 40	50.3	—	Apr. 26	May. 3
		MIL 80	93.4			
		DINO 148	89.2			
		QUINO 83	55.1			

* MIL : Mildiomycin 8% WP,
 DINO : Dinocap 37% EC,
 QUINO : Quinomethionate 25% WP,
 TH-M : Thiophanate methyl 70% WP,
 BENO : Benomyl 50% WP,
 ** +++ : Chlorosis

CECA : CECA 30% WP,
 POLY : Polyoxin-AL 10% WP,
 W.S. : Wettable sulfur 75% WP,
 BINA : Binapacryl 50% WP,

Table 2 Effect of mildiomyacin on control of benomyl-resistant strain of *Sphaerotheca fuliginea* on cucumber.

Chemicals	Concn. (ppm)	Area mildewed (%)	
		Sources of inoculum	
		Benomyl plot*	Control plot
Mildiomyacin	80	0	0
Benomyl	250	89.2	59.2
Thiophanate methyl	250	95.0	48.3
Dinocap	148	0	0
Control	—	96.7	88.3

* Inocula plants were sprayed with benomyl (250 ppm) 15 times.
Inoculation: May 10, Spray: May 11,
Evaluation: May 17

Table 3 Effect of mildiomyacin on control of powdery mildew on cucumber seedlings by root soaking.

Chemicals	Concn. (ppm)	Area mildewed (%)	
		14 days after inoculation	
		Cotyledon	Leaf
Mildiomyacin	0.1	100.0	100.0
	1.0	72.5	55.0
	10.0	0	0
Dimethirimol	0.01	0	0
Control	—	100.0	100.0

Table 4 Effect of mildiomyacin on control of powdery mildew of tobacco by the spray to the upper surfaces of the leaves.

Chemicals	Concn. (ppm)	Area mildewed (%)			
		Dec. 29		Jan. 5	
		Upper*	Lower**	Upper*	Lower**
Mildiomyacin	80	1.3	2.6	0.7	5.0
Quinomethionate	83	1.5	5.2	2.3	16.3
Control	—	23.9	12.4	54.7	19.1

* & ** The asterisks show the upper surfaces and lower surfaces of the leaves, respectively.
Spraying: Dec. 23 and 29, Inoculation: Dec. 21

DISCUSSION

A group of plant disease known as powdery mildews are caused by the members of a family *Erysiphaceae* which consists of 18 genera.⁹⁾ Many of the economically important plants are listed as the hosts of this disease. Diversity of causative fungi and host plants makes it difficult to control the powdery mildews by a single fungicide. Unsatisfactory effects by several chemicals against the powdery mildew of tobacco, strawberry or oak were observed in spite of their potential effectiveness against cucumber powdery mildew. On the other hand, MIL was found to be effective against wide range of powdery mildews; the mildews of barley (*Erysiphe graminis*), grape vine (*Uncinula necator*), apple (*Podosphaera leucotricha*) rose (*Sphaerotheca pannosa*) and tobacco (*Erysiphe cichoracearum*) which are listed

among the most serious parasites.⁷⁾ MIL was also effective against the powdery mildew of green pepper which is caused by an endoparasitic fungus, *Leveillula taurica*, a good control of which was difficult with the commercial fungicides.

There are several other problems with the mildewcides; the occurrence of russet fruits of grape⁸⁾ and apple⁹⁾ or chlorosis of cucumber leaf or abnormal growth of strawberry leaves by triforine; seediness of strawberry by sulfur dust¹⁰⁾; necrosis on cucumber leaves by dimethirimol; phytotoxicity under high temperatures by quinomethionate,^{8,11)} or dinocap; development of resistant strain of powdery mildew fungi by benomyl,^{12,13)} thiophanate methyl¹⁴⁾ or dimethirimol;¹⁵⁾ and so on. As to MIL, it proved to be highly active against powdery mildews without accompanying any phytotoxicity. MIL also proved to be active

enough against benomyl-resistant strain of *Sphaerotheca fuliginea*. The problem whether resistant strain is induced by continuous use of MIL or not is under study. But no indication of decrease in efficacy has been observed so far.

The poor results of drench or seed dipping and the effectiveness of root soaking suggest that MIL has essentially a systemic property but sufficient amount was not taken by the plant with drench or seed dipping application. Effectiveness of MIL on the lower surfaces of tobacco leaves by the spray which was confined to the upper surfaces suggests that translaminar property is equipped with MIL, which seems to explain its effective control of the powdery mildew of green pepper. Bassino *et al.*¹⁶⁾ observed rather weak effects of several chemicals against the powdery mildew of melon particularly on the lower surfaces. We observed alike phenomena on cucumber plants when thorough spray application was difficult because of thick leaves. Translaminar property of MIL will be a favorable character in such a case.

The wide range of anti-mildew spectrum, no phytotoxicity, low mammalian toxicity³⁾ and translaminar property of MIL may provide the ease of its application as a fungicide for powdery mildew control.

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要 約

ミルディオマイシンの各種うどんこ病に対する効果

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Streptovercillium rimofaciens B-98891 により産生される抗生物質ミルディオマイシンのうどんこ病防除効果を検討した。ミルディオマイシンは、15種の供試植物上で、8属13種の病原菌によるうどんこ病に有効であり、植物への薬害も認められなかった。本剤の80 ppm 液の散布による防除効果はキノメチオネートにほぼ同等で、かつ他の供試薬剤にまさった。菌糸内生の *Leveillula tausica* によるピーマンうどんこ病にも有効であり、かつ、タバコ、キュウリ等において処理葉面の裏側の発病抑制も認められたことから、本剤は葉片通過作用をもつと考えられた。また、本剤はベノミル耐性の *Sphaerotheca fuliginea* によるキュウリうどんこ病にも有効であった。

これらの性質から、ミルディオマイシンは新しいうどんこ病防除剤の好素剤として期待されえるものと結論された。