Original Articles

Comparison of the Antimicrobial Activity of Various Agricultural Chemicals against Phytopathogenic Bacteria and Fungi Recently Isolated from Diseased Plants

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The minimal inhibitory concentrations (MIC) values of various antibiotics and agricultural chemicals for isolates of *Erwinia carotovora*, *Pseudomonas lachrymans*, *Alternaria mali*, *Alternaria kikuchiana*, *Botrytis* sp. and *Sclerotinia* sp. collected in various localities of Japan were examined to determine the cross-resistance to various chemicals. To minimize the effects of various conditions, data on MIC values for isolates tested were pooled for 2 or 3 years, and the correlogram of the MIC values between an antimicrobial agent and the other agricultural chemicals were plotted in a single figure. The cross-resistance to an antimicrobial agent of isolates of the bacteria and fungi coincident with the resistance to the other agricultural chemicals was determined, and the relationships between MIC values for the isolates of the gray mold fungus (*Botrytis* sp.) and the control of the gray mold disease by benomyl and thiophanate methyl were found to be correlated each other.

The multiple resistance of organisms to various chemicals has been increasing over a long period of time, and it causes serious problems in the field of antibacterial drugs, veterinary drugs and insecticides. Recently, it was reported that some phytopathogenic bacteria and fungi resistant to antibiotics and synthetic fungicides were isolated from diseased plants in the fields and such resistant strains were rapidly distributed increasingly every year.^{1,2)}

The resistance to benomyl of some phytopathogenic fungi was reported to coincide with that to the thiophanate methyl derivatives and also to thiophanate methyl.³⁻⁶⁾ Uesugi *et al.*⁷⁾ reported that the resistance to S-benzyl *O,O*-diethyl phosphorothioate of *Pyricularia oryzae* coincided with the resistance to S-benzyl *O,O*-diisopropyl phosphorothioate and *O*-ethyl *S,S*-diphenylphosphorodithioate, except phosphoro-

amide acid ester, from studies on laboratory. We also reported that kasugamycin-resistant strains of *P. oryzae* had cross-resistance to blasticidin S and polyoxin D.^{8,9)}

Minimal inhibitory concentration (MIC) values of chemicals for the phytopathogenic bacteria and fungi obtained from diseased plants from 1971 to 1976 were studied. A method for establishing the appropriate MIC values was described in the previous paper,¹⁰⁾ and such values were used for grouping the isolates into sensitive and resistant strains. To minimize the effects of variable conditions, data on MIC values of chemicals for the isolates of the bacteria and fungi studied were pooled over 2 years, and the correlogram of the MIC values between an antimicrobial agent and other agricultural chemicals were plotted in a single figure, and the relationships between the MIC values for the isolates of the gray

mold fungus (*Botrytis* sp.) and the control of gray mold disease of the plants were investigated in the present study.

MATERIALS AND METHODS

1. Strains of Phytopathogenic Bacteria and Fungi

The phytopathogenic bacteria and fungi used for this study were obtained from diseased plants in various localities of Japan. They were 113 isolates of *Erwinia carotovora*, 93 of *Pseudomonas lachrymans*, 131 of *Alternaria mali*, 88 of *Alternaria kikuchiana*, 203 or 115 of *Botrytis* sp. and 82 of *Sclerotinia* sp. (Figs. 1 to 10).

2. Antibiotics and Fungicides

Antibiotics and fungicides used in this study were: Blsaticidin S (BcS), kasugamycin (KsM), polyoxin B (PoB), streptomycin (SM), tetrachloroisophthalonitrile (chlorothalonil), methyl 2-benzimidazolecarbamate (carbendazim), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl), dimethyl 4,4-*o*-phenylene bis(3-thioallophanate) (thiophanate methyl), *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropanedicarboximide (S-7131) and CuSO₄.

SENSITIVITY TEST (MIC)

MIC values of antibiotics and fungicides for the phytopathogenic bacteria and fungi were determined by the streak method for the bacteria, and the mycelial fragment method for the fungi using agar media with these chemicals in two-fold serial dilutions. For the bacteria, and fungi, King B medium,¹¹⁾ and the polyoxin B-potency-test medium¹²⁾ were used, respectively. Data were obtained after 24 hours incubation at 28°C for the bacteria, and 48 hours at 26°C for the fungi.

CONTROL OF PLANT DISEASE

To examine the control of *Botrytis* sp. by benomyl or thiophanate methyl, detached leaves of Nijisseiki pear and of kidney bean, and sliced fruits of cucumber were used. Inocula were prepared by cutting 4 days-old mycelial mats of *Botrytis* sp. together with agar. The leaves and fruits were dipped in benomyl or thiophanate methyl solution for one hour before inoculation. Inoculation experiments were

conducted by placing inocula on the detached leaves of Nijisseiki pear and kidney bean, or sliced fruits of cucumber, and incubating in the moist chamber at 25°C. Data on the size of local lesions were obtained by daily observation for 3 days after inoculation.

RESULTS AND DISCUSSION

The correlogram of MIC values between an antimicrobial agent and the other agricultural chemicals for isolates of *E. carotovora*, *P. lachrymans*, *A. mali*, *A. kikuchiana*, *Botrytis* sp. and *Sclerotinia* sp. isolated from diseased plants in various localities of Japan was examined to clarify the cross-resistance in these isolates.

Therefore, the correlogram of the MIC values between an antimicrobial agent and the other agricultural chemicals were shown by pooling the data on the MIC values for the isolates of phytopathogenic bacteria and fungi for 2 or 3 years.

(1) *Erwinia carotovora*

As the MIC value of SM for grouping of isolates of *E. carotovora* into sensitive and resistant groups, the grouping value showed 50 µg/ml in the previous paper.¹⁰⁾ As shown in Fig. 1, the results demonstrate that SM-resist-

Fig. 1 Correlogram of MIC values between streptomycin and kasugamycin of *Erwinia carotovora*. (113 isolates)

Fig. 2 Correlogram of MIC values between streptomycin and kasugamycin of *Pseudomonas lachrymans* (93 isolates).

Fig. 3 Correlogram of MIC values between polyoxin B and blasticidin S of *Alternaria mali* (131 isolates).

ant strains of *E. carotovora* were insensitive to KsM.

(2) *Pseudomonas lachrymans*

In *P. lachrymans*, the grouping value for SM showed 50 µg/ml in the previous paper.¹⁰⁾ As shown in Fig. 2, the results demonstrate that SM-resistant strains of *P. lachrymans* were sensitive to KsM except two isolates.

(3) *Alternaria mali*

In *A. mali*, the MIC value of antibiotics for grouping of isolates into sensitive and resistant groups, the grouping value for PoB showed 50 µg/ml. For MIC of BcS, the grouping value showed 100 µg/ml in the previous paper.¹⁰⁾ As shown in Fig. 3, the results demonstrate that PoB-resistant strains of *A. mali* were sensitive to BcS.

(4) *Alternaria kikuchiana*

The grouping value for PoB and BcS determined by pooling the value in isolates of *A. kikuchiana* showed 100 µg/ml and 100 µg/ml respectively, in the previous paper.¹⁰⁾ As shown in Fig. 4, the results demonstrate that PoB-resistant strains of *A. kikuchiana* were sensitive to BcS.

(5) *Botrytis* sp.

In *Botrytis* sp., the determination of the

Fig. 4 Correlogram of MIC values between polyoxin B and blasticidin S of *Alternaria kikuchiana* (88 isolates).

grouping value for carbendazim showed 0.78 µg/ml or 1.56 µg/ml and for chlorothalonil showed 25 µg/ml in the previous paper.¹⁰⁾ As shown in Figs. 5, 6, 7 and 8, carbendazim-resistant strains of *Botrytis* sp. were sensitive

Fig. 9 Correlogram of MIC values between polyoxin B and chlorothalonil of *Sclerotinia* sp. (82 isolates).

to carbendazim, chlorothalonil, PoB and CuSO_4 (Figs. 9 and 10).

The relations between MIC of carbendazim for the isolates of *Botrytis* sp. and the control of the gray mold disease by both benomyl and thiophanate methyl *in vivo* were correlated each other. Carbendazim-resistant strains of *Botrytis* sp. had cross-resistance to benomyl and thiophanate methyl on the control of gray mold disease (Tables 1 and 2). These results were similar to those of both *in vitro* and *in vivo*

Fig. 10 Correlogram of MIC values between carbendazim and CuSO_4 of *Sclerotinia* sp. (82 isolates).

activity of KsM and BcS against *Pyricularia oryzae*.⁸⁻¹⁰⁾

The *in vitro* activity of benomyl and some chemically related compounds against the fungi tested clearly demonstrates the resistance to the other benzimidazole derivatives and also to thiophanate methyl, and cross-resistance to benomyl and thiophanate methyl. These results may be explained by the fact that thiophanate methyl in tap water, plant tissues and soil gives rise to carbendazim, and the same compound is formed rapidly in aqueous solution of benomyl.¹²⁻¹⁸⁾

Table 1 The relation between MIC of carbendazim in isolates of *Botrytis* sp. and preventive effect of the gray mold disease by benomyl on the plant.

Class of MIC of carbendazim	Cucumber (fruit)			Nijisseiki pear (leaf)			Kidney bean (leaf)		
	No. of isolates	Diameters of lesions tested*		No. of isolates	Diameters of lesions tested*		No. of isolates	Diameters of lesions tested*	
		Sensitive	Resistant		Sensitive	Resistant		Sensitive	Resistant
0.1	5	5	0	7	7	0	5	5	0
0.2	4	4	0	7	7	0	5	5	0
0.39	5	5	0	7	7	0	5	5	0
0.78	2	2	0	2	2	0	2	2	0
1.56	3	3	0	3	3	0	3	3	0
3.12	5	5	0	6	5	1	5	4	1
6.25	5	4	1	6	4	2	4	2	2
12.5	5	2	3	6	4	2	4	2	2
25	5	2	3	7	3	4	5	2	3
50	1	0	1	1	0	1	1	0	1
100	2	0	2	2	0	2	2	0	2
200	4	0	4	6	0	6	5	0	5
>200	4	0	4	5	0	5	4	0	4

* Percent decrease in diameter of local lesion per inoculation by application of a 250 µg/ml solution of benomyl.

Table 2 The relation between MIC of carbedazim in isolates of *Botrytis* sp. and preventive effect of the gray mold disease by thiophanate methyl on the leaf of kidney bean.

Class of MIC of carben-dazim	Kidney bean (leaf)		
	No. of isolates	Diameters of lesions tested*	
		Sensitive	Resistant
0.2	3	3	0
0.39	3	3	0
0.78	2	2	0
1.56	3	3	0
3.12	3	3	0
6.25	3	2	1
12.5	3	1	2
25	3	1	2
50	1	0	1
100	2	0	2
200	3	0	3
>200	3	0	3

* Percent decrease in diameter of local lesion per inoculation by application of a 460 µg/ml solution of thiophanate methyl.

A detailed comparison of the sensitivity and accuracy of both the MIC test and the control effect of the agricultural chemicals on the plant disease produced with many isolates of phytopathogenic bacteria and fungi has been made. Furthermore the correlogram of MIC values shown in this study might not be applicable at the concentrations of agricultural chemicals used in the fields. However, we reported that the relationships between MIC value in isolates and control of disease on the plant in which *E. carotovora* to SM, *A. kikuchiana* to PoB and *P. oryzae* to KsM and BcS were correlated to each other in the previous paper.¹⁰⁾ When used together with the knowledge of a method of application and of the pharmacokinetics of agricultural chemicals, the correlogram of MIC values between an antimicrobial agent and the other agricultural chemicals contribute information that would be useful for application of agricultural chemicals in the field.

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要 約

最近全国各地の罹病植物から分離された数種植物病原菌株の薬剤感受性値について、寒天平板希釈法によるMICで測定し、各菌株の示す数種薬剤間の感受性値を比較した。その結果、野菜類の軟腐病菌およびキュウリ斑点細菌病菌の示すストレプトマイシンおよびカスガイシン感受性値の間に相関はなかったが、ストレプトマイシン耐性キュウリ斑点細菌病菌のなかで、2菌株がカスガイシンに耐性を示した。リンゴ斑点落葉病菌およびナシ黒斑病菌の示すポリオキシシン B およびブラストサイジン S 感受性値の間に相関は認められなかった。灰色かび病菌において、carbendazim, chlorothalonil, ポオリキシシン B, S-7131 および硫酸銅に対する感受性値は各薬剤間に相関はなかった。しかし carbendazim および chlorothalonil に耐性を示す二剤耐性菌が分布していることが認められた。菌株病菌では供試薬剤に耐性を示す菌株は認められなかった。各菌株の示す MIC 値と植物体上における防除効果を調べた結果、灰色かび病菌において carbendazim の MIC 値とベノミルおよびチオファネートメチルの灰色かび病防除効果は相関することが認められたが、*in vitro* で中等度耐性菌と推定される菌株群のなかで、植物体上における防除効果が菌株によって感受性または耐性を示すという現象も観察された。

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