BIOASSAY OF CERTAIN FUNGICIDES FOR THE RICE BLAST FUNGUS, *PIRICULARIA ORYZAE*(1)

by

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Blast disease due to *P. oryzae* is widely distributed in rice growing countries, particularly in Taiwan, and is considered as the most important rice disease, accordingly the studies on this disease have been made from various angles in major rice-growing countries. In connexion with its control, application of fungicides is now generally considered as one of the important cultural practices and also largely put into practice in many countries. In Taiwan, fungicides now recommended for controlling the disease are mercury compounds. Granosan used for seed treatment; Ceresan-lime or Fumiron tablet used for controlling the leaf and neck blast in the field. Mercury compounds recently used may cause leaf injury on *Indica* type of rice, although at present it is almost satisfactorily controlled by applying with organic mercury compounds mentioned above.

In the present paper, certain resultant data of the laboratory experiments on evaluation of the fungicidal action of the certain commercial fungicides recently recommended for controlling the rice diseases in Japan against rice blast fungus are reported.

Materials and Methods

Fungicides studied and method of assay: The following fungicides were tested.

Commercial name	Active ingredients	Manufacturer
Fumiron Tablet	Phenyl mercuric p-toluene sulfonanilide Ethyl mercuric phosphate Ethyl mercuric urea } 5%	Japan
Takeda Mer Tablet	Phenyl mercuric dinaphthyl methane disulfonate 4.08%	Japan
Simmer Tablet	Phenyl mercuric acetate 5.6% Phenyl mercuric chloride 1.6% Phenyl mercuric dinaphthyl methane disulfonate 0.8%	Japan
Mergon Powder	Ethyl mercuric p-toluene sulfonanilide 7.92%	Japan

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Blaes M Powder	Blasticidin 1% Phenyl mercuric acetate 1.66%	Japan
Blaes S_3 Powder	Blasticidin 2%	Japan
Dithane 31 Powder	Maneb (manganese ethylene bisdithiocarbamate) 53.0%	U.S.A
Mei-ko-nung Emul.	Phenyl mercuric acetate 5%	Taiwan

These fungicides were diluted with dist. water at the different concentrations of the active ingredient. Bioassay was conducted by means of the method in vitro (dipping and fumigating of spore and hyphae), in semivivo (infection on cut sheath in a petri-dish with Takahashi's sheath cell inoculation test) and in vivo (inoculation with potted seedling plants). In the Petri-dish experiments, the sheath (8 cm in length) were dipped in the fungicidal solutions for a moment and placed in a moistened large petri-dish (15 cm in diameter) after disiccation of the coated fungicide. In the pot experiments, however, the test plant was sprayed with the fungicidal solutions or suspensions of the definite quantity.

The fungus employed and method of inoculation: the fungus which was supplied by the laboratory of physiological specilization of rice blast fungus in our institute and designated as 0S-45S isolate (P group, 13 race), was employed throughout the present experiment. It was cultured sucessively on the yeast starch agar plate at 28°C. Spores were harvested from the 10-day culture by agitation with distilled water and glass beads and used for germination test and inoculation. A round piece of hyphal thread was cut from the 14-day-culture by use of a cork borer and used for drag tolerance test. In the petri-dish experiment, inoculation was conducted by means of Takahashi's sheath cell inoculation test. In the pot experiments, the seedlings at 3–5 leaves stage were inoculated by spraying with spore suspension which contain 10^6 spores per ml under a specially designed greenhouse with controlled moisture and temperature.

Experimental results

A. Drag tolerance of spore germination

1. Inhibition to spore germination by direct dipping:

The fungus was cultured in a yeast starch agar slant for 10 days. Then 10 ml of the fungicidal solution at 100 p. p. m. was poured into the slant so as to make the spore suspension at the concentration of 1×10^6/ml. The spore dipping in the fungicidal solutions or suspensions was tested on slide glass in a moistened Petri-dish at 28°C. After 24 hours, the germinability of thus spore was inspected.

Table 1. Tolerance of spore of the rice blast fungus to the different fungicidal solutions or suspensions at 100 p. p. m.

Fungicde \ Item	No. of spore examined	No. of germinated spore	rate of germination	index number (CK 100)
Fumiron	1,085	16	1.47	1.49
Takeda Mer	645	16	2.48	2.52
Simmer	1,367	18	1.32	1.34
Blaes S_3	344	31	9.01	9.16
Blaes M	339	12	3.54	3.60
Mergon	695	4	0.58	0.58
Dithane 31	905	614	67.85	68.99
Mei-ko-nung	908	7	0.77	0.78
CK	545	536	98.35	100.00

As shown by table 1, under the adequate condition of temperature and moisture, 98 per cent spores of the fungus germinated within 24 hours after incubation. All of the fungicides exerted the inhibitory effect on spore germination. Among the fungicides tested, Mergon and Mei-ko-nung showed to be the most effective and the Dithane 31 did not show so much effect.

2. Inhibition to spore germination by gas:

The spore obtained from a yeast starch agar slant which was cultured for 10 days was tested on slide glass in a Petri-dish which was moistened with filter paper containing fungicidal solution or suspension at 100 p. p. m. 24 hours after incubation, the effectiveness of gas inhibition to spore germination was read with counting the number of germinated spores.

Table 2. Tolerance of spore of the rice blast fungus to the gases of different fungicidal solutions or suspensions at 100 p. p. m.

Fungicide \ Item	No. of spores examined	No. of germinated spore	rate of germination	index No. (CK 100)
Fumiron	433	229	52.80	58.00
Takeda Mer	549	413	75.23	83.64
Simmer	550	366	66.55	73.99
Blaes S_3	629	596	94.75	105.33
Blaes M	751	441	58.72	65.23
Mergon	859	12	1.40	1.56
Dithane 31	739	648	87.68	97.48
Mei-ko-nung	640	31	4.84	5.38
CK	368	331	89.95	100.00

According to Table 2, among the fungicides tested, Mergon and Mei-ko-nung showed to be the most effective as same as in the previous experiment. However Fumiron, Takeda Mer, Simmer and Blaes M, showed so less effective and Blacs S_3, Dithane 31 were ineffective.

So far as the results were concerned, Mergon and Mei-ko-nung were much more efficacious in both direct dipping and gas fumigating on the inhibitory effect of spore germination.

B. Drag tolerance of hyphae

1. Inhibition to hyphal growth by direct dipping:

According to Hashioka (1961), the fungus was cultured in a Petri-dish for 2 weeks. Then 50 ml of the fungicidal solution or suspension at 100 p. p. m. was poured into the dish so as to make the hyphal thread soak in the solution or suspension for 10, 20, 30, and 60 min., respectively. After appropriate time of immersion, the hyphal thread was cut with cork borer at the size of 7 mm in diameter, and wash twice with dist. water. 8 days after incubation, the viability of thus-treated hyphal piece was read by means of transferring it onto the agar plate.

Table 3. Tolerance of hyphae of the rice blast fungus to the different fungicidal solutions at 100 p. p. m.

Fungicide	Time of dipping (min)			
	10	20	30	60
Fumiron	++*	+	−	−
Takeda Mer	+	+	−	−
Simmer	+	+	−	−
Blaes S_3	+++	+++	+++	+++
Blaes M	++	+	−	−
Mergon	−	−	−	−
Dithane 31	+++	+++	+++	+++
Mei-ko-nung	++	+	−	−
CK	+++	+++	+++	+++

*Number of − + marks indicates the grade of mycelial development.

As shown by table 3, at 100 p. p. m. the hyphae are killed by brief dipping in Mergon whereas Fumiron, Takeda Mer, Simmer, Blaes M and Mei-ko-nung require 30 min to kill hyphae. While the suspensions of Blaes S_3 and of Dithane 31 are not lethal to hyphae within 60 min.

2. Inhibition to hyphal growth by gas:

The round piece of hyphal thread from 2-week-culture was cut by use of a cork-borer and transferred on the potato sucrose agar plate. Then the inoculated Petri-dish was reversed and the filter paper containing fungicides was put on the inner of the cover so to fumigate the agar plate. It was inspected two days interval after incubation.

As shown by table 4, the fungicides tested except Blaes S_3 and Dithane 31 could comparatively prevent the hyphal growth in the early stage of incubation. Afterward the hyphal growth started because the gas of fungicide gradually de-

Table 4. Toleance of hyphae of the rice blast fungus to the gases of different fungicidal solutions or suspensions at 100 p. p. m.

Fungicide	Days after inoculation			
	4	6	7	8
Fumiron	1 mm*	2 mm	4 mm	6 mm
Takeda Mer	—	—	2	3
Simmer	—	—	—	—
Blaes S_3	11	15	20	25
Blaes M	2	3	5	7
Mergon	—	—	—	—
Dithane 31	10	12	18	22
Mei-ko-nung	—	—	0.5	0.5–1
CK	11	15	20	25

*Length of mycelia.

creased. On 8th day, when the filter paper impregnated with fungicides became dry the fungicides were entirely ineffective. It is clear from the resultant data the hyphae are completely killed by the gases of Simmer and Mergon. Such a tendency is roughly killed by the gases of Simmer and Mergon. Such a tendency is roughly coincide with that in the experiment of spore germination test stated above.

C. Experiments with the cut sheath

The sheaths of a lowland rice variety, Taichung 65, at heading stage were cut and dipped in different fungicidal solutions or suspensions at 50 and 100 p. p. m. for a moment. After desiccation of the coated solutions or suspensions, those sheaths were inoculated and incubated by means of Takahashi's (1958) sheath cell inoculation test. Spore tested was obtained from a yeast starch agar tube which was cultured for 10 days.

Table 5. Preventive action of the different fungicidal solutions or suspensions at 50 and 100 p. p. m., to the rice infection.

Concentration / Fungicide	50 ppm			100 ppm		
	Germination	Appresorium	Infection	Germination	Appresorium	Infection
Fumiron	—	—	—	—	—	—
Takeda Mer	—	—	—	—	—	—
Simmer	+	+	+	—	—	—
Blaes S_3	+	+	+	+	+	+
Blaes M	—	—	—	—	—	—
Mergon	—	—	—	—	—	—
Dithane 31	+	+	+	+	+	+
Mei-ko-nung	—	—	—	—	—	—
CK	+	+	+	+	+	+

No germination, appresorium formation and infection were observed under microscopic test on the inner surface of cut sheath treated by Fumiron, Takeda Mer, Blaes M, Mergon and Mei-ko-nung at 50 p. p. m.. However Simmer at 50 p. p. m., Blaes S_3 and Dithane 31 at 100 p. p. m. the spore germination, formation of appresorium and infection were as well as that of control. From the result mentioned above, it is clearly recognized that the preventive action, in semi-vivo was roughly coincide with that in the experiment of spore germination. If the fungicide can exert the inhibitory effect to spore germination, it may be concerned possessing efficacy for preventive action to blast infection on rice.

D. Experiments with the potted plant

The susceptible variety of Taichung 65 to the blast fungus of isolate 0S-45S was grown in fungus free soil in iron flat (30cm×30cm in size) and top-dressed twice each with 50 ml of 2% ammonium sulfate solution. The seedlings at 3-5 leaves stage were inoculated by spraying spore suspension which contain 10^6 spores per ml. under a specially designed green house with controlled moisture and temperature. In the experiments concerning the preventive effect, the seedlings were atomized by the solution or suspension of the respective fungicide at 100 p. p. m. 5 hours prior to inoculation, whereas in those of therapeutic effect they were sprayed 24 hours after inoculated plants were kept in an inoculation chamber in which the moisture (90-05%) and temperature (25-28°C) were controlled. About 24 hours after inoculation the flats were returned to the green house. Four or five days later, the inoculated seedling was examined and the type of infection read according to Abumiya's scale proposed in 1955.

Table 6. Effects of preventive and therapeutic spraying of the different fungicides on the blast infection on the seedling rice at 100 p. p. m.

Fungicide	Preventive spraying		Therapeutic spraying	
	Infection [a]	Phytotoxicity [b]	Infection [a]	Phytotoxicity [b]
Fumiron	−	−	+	−
Takeda Mer	−	+	+	+
Simmer	+	+	+	++
Blaes S_3	+	+	++	+
Blaes M	−	++	−	++
Mergon	−	−	−	−
Dithane 31	++	−	++	−
Mei-ko-nung	−	−	−	−
CK	++	−	++	−

a −mark shows no infection. +mark shows resistant type. ++mark shows susceptible type.
b +marks shows the grade of phytotoxicity.

Considering the effects of preventive and therapeutic action of various fungicides hitherto tested on the blast infection of the seedling rice, it is evident that the preventive action of organomercurials except Simmer is more distinct than that of nonorganomercurials. But in the therapeutic application, the Fumiron and Takeda Mer of organomercurials did not show so much effect. Although the gas or suspension of Simmer tested in vitro showed more or less effective to the hyphal growth and spore germination of blast fungus. As to phytotoxicity of each fungicides to the seedling rice tested, Simmer and Blaes M brought about more serious injury appearing brown spot on the leaves and sheaths of the seedling sprayed. Takeda Mer and Blaes S_3 also appeared a little injury.

Discussion and Conclusion

In general, the effectiveness of various fungicides against plant pathogens in field practice are varied with kinds of diseases. For example, Phenyl Mercuric Acetate against rice blast fungus in paddy field is more effective than that of rice sheath spot fungus, but the Urbazid is reverse (Inoue 1958; Hashioka 1961; Chen 1959; Chien 1962). However, a given fungicide possesses its special fungitoxicity against a certain pathogen in field. Sometimes in the field trials, we could detect the phenomenon that effectiveness of a certain fungicide in preventive action against a given plant disease differed with that in therapeutic application.

So far as the fungicides employed throughout the present experiments were concerned, the inhibitory effect on both preventive and therapeutic action was merely detected in those, Mergon, Mei-ko-nung and Blaes M. All of them were of organomercurials. Although Fumiron in vitro and semi-vivo gave distinct fungicidal action, nevertheless in vivo for therapeutic spraying, it did not show so much effect. Simmer in vitro both direct and gas inhibition showed considerable effect on spore germination and hyphal growth, while in semi-vivo and vivo, inoculation was made after desiccation of the coated suspension so that efficacy of Simmer under this condition having almost evaporated, its fungitoxicity diminished immediately before inoculation.

From the result stated above, it is evident that vapor action of certain organomercurials tested in laboratory against blast fungus repressed the spore germination and hyphal growtn rather distinctly. And then, we can draw the conclusion that the difference of effectiveness of those fungicides hitherto tested under the experimental conditions, is considered to be possible due to the differences of durability and vapor action on the foliage. Such phenomenon are coincide with the experiments made by Hashioka on bioassay of the organic fungicidal substances for the rice sheath spot fungus in 1961.

Summary

1. In the present paper, certain resultant data of the laboratory experiments on evaluation of the fungicidal action of the certain commercial fungicides recently

recommended for controlling the rice diseases in Japan against rice blast fungus are reported.

2. Fungitoxicity of the fungicides hitheto tested under the experimental condition for controlling the rice blast fungus, is considered possibly due to the differences of durability and vapor action of them on the foliage.

3. It was proved in vitro that the organomercurials were distinctly efficacious than nonorganomercurials.

4. So far as the experiments in vitro and in vivo were concerned, Mergon and Mei-ko-nung seemed could be determined as the practical fungicides. The efficacy of Mergon and Mei-ko-nung could cover that of the Fumiron recently recommended for controlling the rice blast in Taiwan.

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幾種殺菌劑對稻熱病菌之防治效力檢定

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摘　要

本試驗爲檢定 Fumiron 片劑，Takeda Mer 片劑，Simmer 片劑，Mergon 可濕性粉劑，Blaes M 可濕性粉劑，Blaes S_3 可濕性粉劑，Dithane 31 可濕性粉劑及 Mei-ko-nung (美谷濃) 乳劑等稻作病害常用之殺菌劑，對稻熱病菌之防治效力而擧行。於 vitro, semi-vivo 及 vivo 試驗中，由於實驗條件之不同，各藥劑之效果略有差異。但有機汞劑之效力均比非有機汞劑爲佳，其中 Mergon 可濕性粉劑及 Mei-ko-nung (美谷濃) 乳劑之防治與治療效果都比標準藥劑 Fumiron 片劑較顯著。其藥效之高低，似與藥劑之持續效果及蒸氣壓之差異，具有密切關係。