THE USE OF ACTIDIONE ON THE CONTROL OF RICE BLAST[1]

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Actidione, discovered in a culture filtrate of *Streptomyces grieseus*, is remarkably effective on a wide range of fungi and yeast. Since 1952 it was in commercial use against turf disease caused by various fungi (Ford, 1958, Smith & Guyer 1961). Many studies concerned with the control of powdery mildew disease on beans, graps, dewberries, raspberries, wheat (Ford *et al.*, 1958), roses and tuberous begonia (palmer, 1959) were reported. In most of those cases phytotoxicity was a paramount problem. Its use against plant disease was possible only in cases where extremely low concentrations were already effective or where the treatted plant were selectively resistant to this antibiotic. A preliminary investigation was found that the mycelial growth and spore germination of *P. oryzae* were completely inhibited at the low concentration of 1 ppm of actidione, and a green house test also showed that it was quite effective against *P. oryzae*. Unfortunately phytotoxicity was still a problem to the rice seedlings. However, recently many actidione derivatives were developed and they were effectively used to avoid phytotoxicity. Anderson & Bowell (1962) used actidione semicarbozone derivative and comfirmed the persistance of protective activity against wheat stem rust for relatively long periods. Ark and Thompson (1958) Wilson and Ark (1958) proposed that foliage injury by this antibiotic was drasticall reduced by the addition to the antibiotic spary of 0.1 per cent Na-K chlorophyllin. It is possible that actidione may be used for the control of rice blast. The following experiment was undertaken with the objective of providing some information for the control of rice blast with actidione.

Materials and Methods

For the tests of the effect of actidione on spore germination, spores from 7 day old culture grown on Misato medium were used. Spore suspensions were

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mixed with water and various concentrations of the actidione on the hole slides. They were kept in petri dish saturated with water and incubated in 28°C. After 10 hr the spore germination was examined. For the tests of the effect of actidione on mycelial growth, Tanaka's synthetic medium (Tanaka 1963) was used. The various concentrations of actidione in water were added to sterile molten medium and poured into petri dishes. Each dish was seeded with a 1 mm disc of mycelium in the center of the dish. The growth was measured after incubation at 28°C for 7 days. Test organisms used were physiologic races of *P. oryzae* No. 1, 2, 5, 13. The actidione used was purchased from Nutritional Biochemicals Corporation.

For the test on therapeutic effect against rice blast, the spore suspensions of rice blast were sprayed directly to rice plants (Taichung 65) of 2 week-old seedlings and the rice plants were kept in the green house for 2 days. Then the various concentrations of actidione were sprayed. Usually the disease symptom started to appear at the fourth day after inoculation. The extension of lesions was examined after one week. For the test of the protective effect against rice blast, actidione was applied before inoculation. The stage of rice plant and the applied chemicals were the same as the above. After 2 days of application of actidione, the treated plants were sprayed with spore suspension. The lesions were examined after one week.

For the measurement of antibiotic activity a bioassay method of Whiffen (1948) was adapted. *Saccharomyces cerevisiae* was used as a test organism. In general *S. cerevisae* was sensitive to approximately 0.02 μg actidione applied to the disc (i.e., 1 μg/ml of test solution). Two plants were excised at a point on the stem just above the soil level and sterilized by exposure to propylene oxide fumes for 24 hr. They were then transferred to the surface of agar seeded with the test organism. A second procedure consisted of grinding excised plants in chloroform in a mortar. Filter paper disc were immersed in the chloroform extract and transferred to the surface of agar freshly seeded with the test organism. After an incubation period of 24 hr at 30°C the plates were observed for inhibition in growth of the test organism.

Paper chromatography was employed to characterized the active material present in plant grown in quartz sand and supplied with Hoagland's solution (Hoagland and Arnon 1938) containing 50 ppm actidione. Following the extraction of the active substance with chloroform, the plant extract was compared with a solution of actidione in chloroform by means of decending paper chromatography. The solution were spotted on strips of Watman No 1 filter paper. One *M* phosphate buffer at pH 6.8 was used as the developing solvent. After development the strips were air dried and transferred to the surface of agar seeded with the test organism. Following an incubation period

of 24 hr, inhibition zones were located and the Rf values of both the active substance in plant extracts and the pure antibiotic were calculated.

Results and Discussion

Inhibitory levels of actidione on the *P. oryzae:* The effect of actidione on the spore germination and mycelial growth of four physiologic races of *P. oryzae* was examined at concentration levels ranging from 0.001–10 ppm. As indicated in Table 1. the inhibition of spore germination was only slightly different physiologic races of *P. oryzae.* However spore germination was completely inhibited at the concentration of 1 ppm. At the concentration 0.1 ppm about 50% of spore germination was inhibited. As indicated in Table 2, race No 1 and 13, the mycelial growth was completely inhibited at the concentration of 1 ppm of actidione. With race 2 and 5, they have a little growth at the concentration of 1 ppm.

Table 1. *Effect of actidione on the spore germination of P. oryzae.*

Strains	Concentration of actidione (ppm)	Percentage of germination	Percentage of inhibitions
1	0	96	—
	0.001	82	6.8
	0.01	77	12.5
	0.1	49	44.3
	1	0	100.0
5	0	92	—
	0.001	85	9.5
	0.01	78	17.0
	0.1	60	36.2
	1	0	100.0
13	0	98	—
	0.001	80	16.6
	0.01	70	27.1
	0.1	54	43.7
	1	0	100.0

In general the inhibition occurred at the concentration of 0.001 ppm for the spore germination and at concentration of 0.01 ppm for the mycelial growth.

Therapeutic and protective effect against rice blast by the application of actidione: The therapeutic and protective effects of actidione were ascertained in the greenhouse. The results were shown in the Table 3 and 4.

Table 2. *Effect of actidione on the mycelial growth of P. oryzae.*

Strains	Concentration of actidione (ppm)	Mycelium growth (diameter cm)	Percentage of inhibition (%)
1	ck	2.3	0.0
	0.001	1.9	17.0
	0.01	1.7	26.0
	0.1	1.6	30.5
	1	0	100.0
	10	0	100.0
2	ck	2.4	0.0
	0.001	2.4	0.0
	0.01	1.5	36.4
	0.1	1.3	45.7
	1	0.2	81.6
	10	0	100.0
5	ck	2.5	0.0
	0.001	2.5	0.0
	0.01	2.4	4.0
	0.1	2.0	32.0
	1	0.2	90.2
	10	0	100.0
13	ck	2.4	0.0
	0.001	2.4	0.0
	0.01	2.4	0.0
	0.1	1.7	24.2
	1	0.2	99.0
	10	0	100.0

Measured after 7 days.

Table 3. *Therapeutic effect of actidione on rice blast.*

Spray concentration (ppm)	Number of lesions measured	*Number of lesions extend	Percentage of lesions extend	**Therapeutic value
ck	145	104	71.8	
1	106	27	25.5	64.4
10	79	4	8.0	90.0
25	62	0	0	100.0
50	0	0	0	0
100	0	0	0	0

* The small dot lesions started to appear at the fourth day, then some lesions continued to extend to larger spindle shape and some lesions stayed in original small dot lesions.

** Theropeutic value $= \dfrac{\left(\begin{smallmatrix}\%\text{ of lesion growth}\\ \text{in control plant}\end{smallmatrix}\right) - \left(\begin{smallmatrix}\%\text{ of lesion growth}\\ \text{in treated plant}\end{smallmatrix}\right)}{\left(\begin{smallmatrix}\%\text{ of lesion growth}\\ \text{in control plant}\end{smallmatrix}\right)} \times 100$

Table 4. *Protective effect of actidione on rice blast.*

Spray concentration (ppm)	Number of total lesions	Number of lesions per leaf	*Protective value
ck	164	5.4	—
1	86	2.8	48
10	36	1.2	77
25	3	0.1	98
50	0	0	100
100	0	0	100

* The protective value $= \frac{\left(\begin{smallmatrix}\text{number of lesion per a}\\ \text{leaf in control plant}\end{smallmatrix}\right) - \left(\begin{smallmatrix}\text{number of lesion per a}\\ \text{leaf in treated plant}\end{smallmatrix}\right)}{\left(\begin{smallmatrix}\text{number of lesion per a}\\ \text{leaf in control plant}\end{smallmatrix}\right)} \times 100$

Systemic transport of actidione in rice seedling: Actidione residues behaved quite differently on the several plant species (Wallen and Millar 1957, Starzyk and Mitchell 1963). The systemitic transport was demonstrated in bean and wheat, but not in corn, cowpea and cucumber. The behavior in rice plant is unknown. Rice seedlings grown in quartz sand in the greenhouse were supplied with Hoagland's solution until one week after they had emerged. Following this period, Hoagland's solution containing actidione at concentrations of 10, 25, 50 and 100 ppm was supplied to the plants. Four days after application of actidione the seedlings were excised and sterlized with propylene oxide fumes. The plants then were assayed for antibiotic activity by transferring them to petri plates containing seeded assay agar. The degree of antibiotic activity exhibited by plants treated with actidione depended on the concentration of antibiotic supplied to the plants. A gradual increase in zone size occurred with each increase in concentration of actidione. The systemic nature of the antibiotic material is clearly indicated in Fig. 1.

In a similar experiment, the cocentration of antibiotic in plants supplied with various concentrations of actidione was estimated by the following method. The active substance from plants (fresh weight 1 gm) was extracted in 5 ml of chloroform. The chloroform fractions were evaported to dryness in a desiccator in vacuum at room temperature. The residues were taken up in 0.1 ml cholroform. 0.01 ml of this fraction were used for assaying as described before. Known concentrations of actidione were also prepared, and these were assayed at the same time. The inhibition zone was proportional to the concentration of actidione used in the range 1 ppm to 100 ppm. The curve obtained was used as the standard curve.

The inhibition zone produced by the chloroform extracts from plants supplied with actidione at 50 ppm was slightly larger than the inhibition zone produced by a 1 ppm solution of actidione in chloroform (Table 5). It was

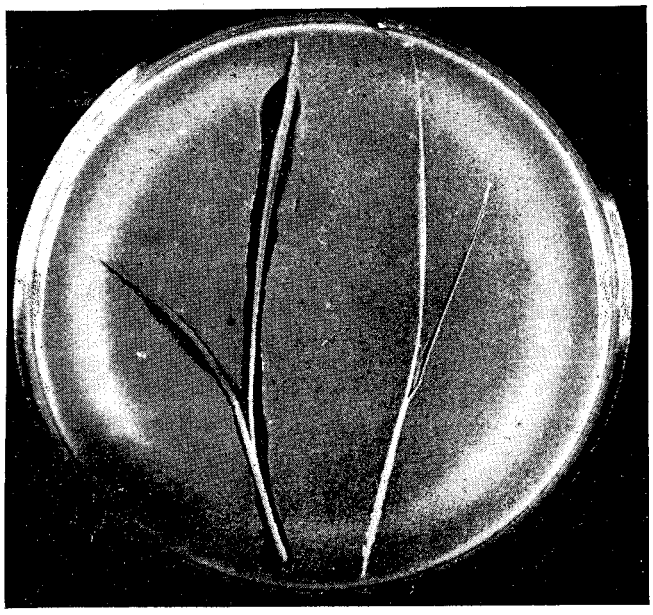

Fig. 1. Inhibition zones produced by rice seedlings grown in quartz sand and supplied with actidione, left: treated-seedling, right: check.

assumed therefore, that there was at least 10 ppm of active material in plants supplied with 50 ppm of actidione.

Table 5. *Antibiotic activity of control concentrations of actidione and of chloform extracts from plants supplied with the same concentrations of actidione.*

Concentration (ppm) of actidione in controls and in solutions supplied to plant.	mm of inhibition zone on agar seeded with *S. cerevisiae*	
	Controls	Plant extracts
0	0	0
1	16	0
10	27	0
25	33	8
50	37	18
100	40	21

Paper chromatography was employed to characterized the active material present in the plant. Only one active spot was obtained from the sample extracted from actidione-treated seedlings. The Rf. value obtained for the active substance in plant extracts and the pure antibiotic were 0.50 and 0.71 respectively. The above data support the tentative conclusion that the active substance present in plant extracts may not be the actidione itself but it is a closely related compound.

Summary

Actidione at concentration of 1 ppm inhibited both spore germination and mycelial growth of *P. oryzae*. The therapeutic and protective effects of actidione were ascertained in the green house. Both effects were very effective at the concentration 50 ppm of actidione applied, but phytotoxicity was still a problem. The application of antibiotic solution above 50 ppm concentration induced the production of phyotoxicity. The systemic activity of actidione in rice seedlings was also demonstrated by a bioassay method. When actidione was supplied to the roots of seedlings growing in quartz sand, the antibiotic was absorbed by the roots and translocated throught the stem and leaf tissue. The amount of antibiotic absorbed by the seedlings depended on the concentration of actidione supplied to the root. Paper chromatography of the active material contained in the chloroform extracts obtained from actidione treated plant yielded a Rf value which had a little difference from the value obtained for pure solution of the antibiotic.

利用 Actidione 防治水稻稻熱病

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抗生素 Actidione 是一種很有效之殺菌劑，其在 1 ppm 之濃度下能完全抑制稻熱病孢子之發芽及菌系之生長，在溫室利用水稻幼苗測定其治療及保護效果，發現兩者之效果都很好。但可惜藥害很强，在 50 ppm 之濃度下就有藥害發生。Actidione 在水稻體內是系統性的，能自根部吸收後傳佈整株水稻，水稻體內之抗生素抽出後利用濾紙色析法初步定性結果發現其 Rf 值與原來之 Actidione 不相同。可能以另一種狀態存在於水稻體中。

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