

The Disinfection of Rice Seeds Affected by
Helminthosporium Oryzae, with Special Reference
to the Hot-Water Treatments.

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I. Introduction.

Sesame-leaf-spot or 'Gomahagare' disease of rice caused by *Helminthosporium Oryzae* is one of the most widely distributed of rice diseases, and its destructive nature may be classed as being comparable to that of blast or 'Imoti' disease. One of the causes of the primary outbreak of this disease is attributed to the use of diseased rice seeds. KUROZAWA (1900), and HORI (1901) in their earlier reports on the description of the disease made comments of its occurrence on the grains. HARA (1918) reported that the diseased grains serve as an agent in spreading the disease. Beginning in 1916, the senior author has made studies on the disinfection of diseased rice seeds, on the treatment with hot water (NISIKADO and MIYAKE 1920), and on the treatment with copper sulphate (Do., 1922). NISIKADO'S *Helminthosporiose of Graminae* (1928) gives detailed accounts on the fungicidal value of various chemicals with reference to the concentration and length of treatment,

NISIKADO and MIYAKE (1927) further reported on the use of Uspulun. KURIBAYASHI (1929) described the overwintering of the fungus and the cause of the primary infection; and SUZUKI (1930) on the pathogenicity of the fungus to rice grains. ITO (1932) made a thorough study on the subject of seed disinfection for the control of several major rice diseases.

As a practical measure for the control of the disease, seed disinfection with formalin as recommended by ITO (1932) is widely being followed. Formalin and Uspulun, on account of their merits of effectiveness and simplicity in use, are the two most important fungicides used for this purpose in Japan. The present writers have taken up studies on the method of hot-water steeping as a possible substitute for the chemical methods.

II. Chemical Disinfection of Diseased Rice Grains.

With the purpose of comparing the effectiveness of hot-water steeping with that of chemical treatment following preliminary experiments were performed. The rice seeds used in this and the subsequent experiments were the product of 1940 which consisted of very high percentage of naturally infected grains. The result of each seed treatment was noted by observing the number of diseased seedlings appearing after sowing in sterilized sand in germinating dishes. The notes were taken usually at the end of 9 to 11 days after sowing in a room held constant at 25 degrees Centigrade. The various fungicides tested were: formalin, copper sulphate, mercuric chloride, Uspulun and Germisan. The concentration and the length of treatment were selected from the results of the previous workers, NISIKADO and MIYAKE (1921, 1927), ITO (1932) and others. They were as follows:

Formalin (Japanese Pharmaceutical Method)	1 Per cent. Solution	4 Hours
Copper Sulphate (Merck Product, Germany)	2 Per cent. Solution	24 Hours
Mercuric Chloride (Japanese Pharmaceutical Method)	1 : 1,000 Solution	1 Hours
Uspulun (I. G. Farbenindustrie, Germany)	1 : 400 Solution	3 Hours
Germisan (Saccharin Fabrik A. - G., Germany)	1 : 400 Solution	3 Hours

The results of the experiment are shown in table I. The treatment with 1 per cent. solution of formalin for 4 hours reduced the diseased seedlings from 52 per cent. of the untreated control to 6 per cent; similarly, with 1 : 400 solution of Uspulun for 3 hours the result was 10 per cent. It seems from these figures that a satisfactory control of the seedling blight can be obtained with formalin and Uspulun. Treatment in 2 per cent. solution of copper sulphate produced 16 per cent. diseased seedlings; 1 hour treatment in 1 : 1,000 mercuric chloride solution and 3 hours in 1 : 400 Germisan each with 30 and 33 per cent. respectively, show decreasing effectiveness.

By taking into consideration the results of the previous experiment, further tests were made with the same fungicides with varied concentration and length of treatment. Similar method was followed in observation.

Table 1.
 Chemical Disinfection of Rice Grains Affected with *Helminthosporium Oryzae*.
 (Preliminary experiment)
 Variety of rice: Yûsin, sp. gr. 1.18.
 Results at 11 days after sowing.

Chemicals & concentration	Hours of treatment	Number of seeds sown	Seed germination.		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
Formalin 0.1 %	4	100	98	98.0	6	6.0	92	92.0
Copper sulphate 2.0 %	24	100	99	99.0	16	16.0	83	83.0
Uspulun 0.25 %	3	100	100	100.0	10	10.0	90	90.0
Germisan 0.25 %	3	100	99	99.0	33	33.0	66	66.0
Mercuric chloride 0.1 %	1	100	98	98.0	30	30.0	68	68.0
Control		100	98	98.0	52	52.0	46	46.0

Table 2.
 Disinfection by Formalin of Rice Grains Affected with *Helminthosporium Oryzae*.
 Variety of rice: Yûsin, sp. gr. 1.18.
 Results at 11 days after sowing.

Concentration of formalin	Hours of treatment	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
0.025 %	1	50	49	98.0	28	56.0	21	42.0
	4	50	50	100.0	23	46.0	27	54.0
	12	50	50	100.0	29	58.0	21	42.0
	24	50	50	100.0	32	64.0	18	36.0
0.1 %	1	50	48	96.0	25	50.0	23	46.0
	4	50	49	98.0	23	46.0	26	52.0
	12	50	48	96.0	27	54.0	21	42.0
	24	50	49	98.0	11	22.0	38	76.0
0.4 %	1	50	50	100.0	11	22.0	39	78.0
	4	50	49	98.0	2	4.0	47	94.0
	12	50	50	100.0	1	2.0	49	98.0
	24	50	50	100.0	2	4.0	48	96.0
1.6 %	1	50	50	100.0	0	0.0	50	100.0
	4	50	49	98.0	1	2.0	48	96.0
	12	50	49	98.0	1	2.0	48	96.0
	24	50	49	98.0	0	0.0	49	98.0
Control		100	99	99.0	45	45.0	54	54.0

A. Formalin.

The concentrations of the formalin solutions were 0.025, 0.1, 0.4, and 1.6 per cent.; and the lengths of treatment were 1, 4, 12, and 24 hours. The results after 11 days of growth are shown in Table 2.

Formalin at the concentrations of 0.025 and 0.1 per cent. were not effective in preventing the outbreak of the disease; only at 24 hours treatment in 0.1 per cent. the disease was reduced to 22 per cent. At the concentration of 0.4 per cent., a treatment longer than 4 hours reduced the amount of diseased plants to 4 per cent or less. It seems that this concentration is required to bring about its minimum effectiveness. At the higher concentration of 1.6 per cent., a treatment of one hour was sufficient, and this result approaches that shown by Iro (1932).

B. Copper Sulphate.

Copper sulphate as a seed disinfectant has long been known; NISIKADO (1928) immersed the dried rice grains in solutions of copper sulphate held at 20°C. and gave the following relation between concentration and length of treatment:

Relation of Concentration of Copper Sulphate and Length of Treatment.

Concentration in Per Cent.	25	6	1.5	0.4	0.1	0.025	0.006
Length of Treatment in Hour.	2	6	12	12	24	48	72

To have a lethal effect on the spores of *Helminthosporium Oryzae*, following relation of concentration and period of treatment was further noted:

Relation of Concentration of Copper Sulphate and Lethal Period
for the Spores of *Helminthosporium Oryzae*

Concentration, Mole.	$M/2^2$	$M/2^3$	$M/2^4$	$M/2^5 - 2^7$	$M/2^8 - 2^{11}$
Per Cent.	6.25	3.13	1.56	0.78 - 0.2	0.1 - 0.012
Lethal Period, Hour.	0.5 - 1	1 - 2	2 - 4	6 - 12	12 - 24

According to Iro (1932), copper sulphate had a lethal effect on the spores of *Helminthosporium Oryzae* at the concentration of 1.56 per cent. when treated for 48 hours.

The results obtained by the present writers using the naturally infected rice grains were as shown in Table 3.

Table 3 shows that at a concentration of as high as 8 per cent., a treatment for 24 hours produced 18 per cent. diseased seedlings; and after 48 hours, it was 10 per cent. These figures besides being not effective enough, concentrations as high as these would not be practical, and hence a use of copper sulphate for the rice seed disinfection to control the *Helminthosporium* disease would appear to be out of question.

Table 3.
Disinfection by Copper Sulphate of Rice Grains Affected with
Helminthosporium Oryzae.
Variety of rice: Yûsin, sp. gr. 1.18.
Results at 11 days after sowing.

Concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Hours of treat- ment	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
0.5 %	12	50	48	96.0	17	34.0	31	62.0
	24	50	48	96.0	12	24.0	36	72.0
	48	50	50	100.0	5	10.0	45	90.0
2.0 %	12	50	48	96.0	18	36.0	30	60.0
	24	50	50	100.0	11	22.0	39	78.0
	48	50	49	98.0	4	8.0	45	90.0
8.0 %	12	50	48	96.0	13	26.0	35	70.0
	24	50	50	100.0	9	18.0	41	82.0
	48	50	48	96.0	5	10.0	43	86.0
Control		100	98	98.0	60	60.0	38	38.0

Table 4.
Disinfection by Mercuric Chloride of Rice Grains Affected with
Helminthosporium Oryzae.
Variety of rice: Yûsin, sp. gr. 1.18.
Results at 11 days after sowing.

Concentration of HgCl_2	Hours of treat- ment	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
0.05 %	1	50	50	100.0	13	26.0	37	74.0
	3	50	50	100.0	12	24.0	38	76.0
	6	50	50	100.0	5	10.0	45	90.0
0.1 %	1	50	49	98.0	10	20.0	39	78.0
	3	50	50	100.0	7	14.0	43	86.0
	6	50	50	100.0	5	10.0	45	90.0
0.2 %	1	50	49	98.0	10	20.0	39	78.0
	3	50	50	100.0	4	8.0	46	92.0
	6	50	44	88.0	2	4.0	42	84.0
Control		100	99	99.0	45	45.0	54	54.0

C. Mercuric Chloride.

Mercuric chloride was tested by using the concentrations of 0.05, 0.1, and

0.2 per cent., with the lengths of treatment of 1, 3, and 6 hours. The results are shown in Table 4.

Mercuric chloride, according to Table 4, must have a minimum concentration of 0.2 per cent in order to produce its effectiveness as the seed disinfectant.

D. Uspulun.

There are several reports on the use of Uspulun as a disinfectant for rice seeds; following experiment was performed to determine its effectiveness on the diseased rice grains. Treatment in each of the concentrations of 0.125, 0.25 and 0.5 per cent. for 3, 6, 12, and 24 hours resulted as shown in Table 5.

Table 5.
Disinfection by Uspulun of Rice Grains Affected with
Helminthosporium Oryzae.

Variety of rice: Yōsin, sp. gr. 1.18.
Results at 11 days after sowing.

Concentration of Uspulun	Hours of treat- ment	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
0.125 %	3	50	49	98.0	14	28.0	35	70.0
	6	50	50	100.0	9	18.0	41	82.0
	12	50	50	100.0	6	12.0	44	88.0
	24	50	49	98.0	5	10.0	44	88.0
0.25 %	3	50	50	100.0	6	12.0	44	88.0
	6	50	50	100.0	5	10.0	45	90.0
	12	50	49	98.0	6	12.0	43	86.0
	24	50	49	98.0	4	8.0	45	90.0
0.5 %	3	50	48	96.0	4	8.0	44	88.0
	6	50	49	98.0	4	8.0	45	90.0
	12	50	50	100.0	2	4.0	48	96.0
	24	50	50	100.0	2	4.0	48	96.0
Control		100	98	98.0	60	60.0	38	38.0

Table 5 shows that a treatment in Uspulun at a concentration of 0.125 per cent. or stronger for at least 12 hours resulted in reducing the number of diseased seedlings within the limit of 6 per cent. The result was in general similar to that of NISIKADO and MIYAKE (1927).

E. Germisan.

A further experiment with Germisan was performed. Following the identical

experimental method, the results attained by concentrations of 0.125, 0.25, and 0.5 per cent. for 3, 6, 12, and 24 hours' treatment were as shown in Table 6.

Table 6.
Disinfection by Germisan of Rice Grains Affected with
Helminthosporium Oryzae.

Variety of rice: Yûsin, sp. gr. 1.18.

Results at 11 days after sowing.

Concentration of Germisan	Hours of treat- ment	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
0.125 %	3	50	50	100.0	37	74.0	13	26.0
	6	50	50	100.0	31	62.0	19	38.0
	12	50	50	100.0	29	58.0	21	42.0
	24	50	49	98.0	25	50.0	24	48.0
0.25 %	3	50	50	100.0	33	66.0	17	34.0
	6	50	49	98.0	29	58.0	20	40.0
	12	50	50	100.0	27	54.0	23	46.0
	24	50	50	100.0	8	16.0	42	84.0
0.5 %	3	50	49	98.0	19	38.0	30	60.0
	6	50	49	98.0	19	38.0	31	62.0
	12	45	44	97.8	18	40.0	26	57.8
	24	50	48	96.0	6	12.0	42	84.0
Control		100	98	98.0	60	60.0	38	38.0

The effectiveness of this product was not as marked as Uspulun, although a treatment at a high concentration for a long period of time proved satisfactory. The low fungicidal effect of this preparation may have been due to the fact that it had been in stock since 1931.

Summarizing the results of seed treatment with various chemicals as given in Tables 1 to 6, materials such as copper sulphate, mercuric chloride or Germisan showed but slight effect in checking the development of seedling blight of rice caused by *Helminthosporium Oryzae*. Formalin and Uspulun on the other hand proved effective. A treatment in formalin solution for at least 4 hours in 0.4 per cent. solution or stronger yielded very high percentage of healthy seedlings, and had no injurious effect on the germination of the seeds. This result followed closely with that shown by ITO (1932). With Uspulun, a treatment in 0.125 per cent. solution for 12 hours or longer was sufficient in attaining its purpose of seed disinfection. High disinfecting property possessed by formalin and Uspulun in comparison with copper sulphate, mercuric chloride and Germisan is believed to be due to the greater penetrating power into the interior of the grain.

III. Simple Hot-Water Treatment of Rice Grains Affected with *Helminthosporium Oryzae*.

The preceding experiments with various chemicals such as formalin, mercuric chloride, Uspulun, copper sulphate, and Germisan showed that their effectiveness as seed disinfectants was influenced by the concentration and the length of treatment, as well as the penetrating power possessed by them. Hot-water treatment has long been known as one of the best methods for controlling seed borne diseases. The subsequent experiments were performed with the purpose of making a comparison of the effectiveness of this method with that of chemicals.

The rice seeds used in the experiment were the product of the Institute and the varieties consisted of Ehime Sinriki, Yōsin, Dokai Sinriki, and Asahi; all of which are widely grown locally. There was a high percentage of grains naturally infected with *Helminthosporium Oryzae*, and in order to unify germination, only those seeds that had a specific gravity of greater than 1.18 were selected. Dry seeds were treated by immersing for 10 minutes in hot-water, kept constant thermostatically at 50, 52, 54, 56, 58, and 60 degrees Centigrade, after which the sample seeds were immediately cooled by dipping into cold water. Seeds were sown in germinating plates by covering with sterilized sand. Notes on the number of germination and seedlings were taken after 8 or 11 days of incubation at 25 degrees. Results from all of the varieties are shown in Tables 7 to 11. Curves for Kissin and Yōsin are drawn from the figures in Table 8 and 9 and are show in Figure 1. There were high percentage of germination in all of the treated lots.

Results with the variety Ehime Sinriki show that a treatment in hot-water at 60 degrees for 10 minutes, although it had no effect on the germination, did not eradicate the disease completely as shown by 29 per cent. diseased seedlings compared to 60 per cent. of the untreated control.

Table 7.

Hot-Water Disinfection of Rice Grains (Variety Ehime Shinriki) Affected
with *Helminthosporium Oryzae*.

Treatment in hot-water: 10 minutes.

Results at 8 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	100	99	99.0	61	61.0	38	38.0
52	100	99	99.0	53	53.0	46	46.0
54	100	100	100.0	46	46.0	54	54.0
56	100	97	97.0	28	28.0	69	69.0
58	100	100	100.0	31	31.0	69	69.0
60	100	99	99.0	29	29.0	70	70.0
Control	100	98	98.0	60	60.0	38	38.0

In the variety Yûsin (Table 8 and Figure 1, Curve B) the germination was normal even by the treatment at 60 degrees, and the diseased seedlings were successively lessened from 70.2 per cent. of the untreated lot to 61.3 of 50 degrees, 39.2 of 52 degrees, 32.9 of 54 degrees, 15.0 of 56 degrees, 6.4 of 58 degrees and 3.6 of 60 degrees. It appears that a treatment in hot-water held at 58 or 60 degrees for 10 minutes should be satisfactory for rice seed disinfection.

Table 8.
Hot-Water Disinfection of Rice Grains (Variety Yûsin) Affected with
Helminthosporium Oryzae.

Treatment in hot-water: 10 minutes.
Results at 11 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	310	304	98.2	190	61.3	114	36.9
52	319	310	97.3	125	39.2	185	58.0
54	304	299	98.4	100	32.9	199	65.5
56	274	267	97.5	41	15.0	226	82.5
58	251	245	97.6	16	6.4	229	91.3
60	249	247	99.3	9	3.6	238	95.6
Control	302	300	99.4	212	70.2	88	29.2

Fig. 1.

Development of Diseased Seedlings from Rice Seeds Affected with *Helminthosporium Oryzae* after Treatment at Various Temperatures of Hot-Water.

A: Variety Kissin. B: Variety Yusin.

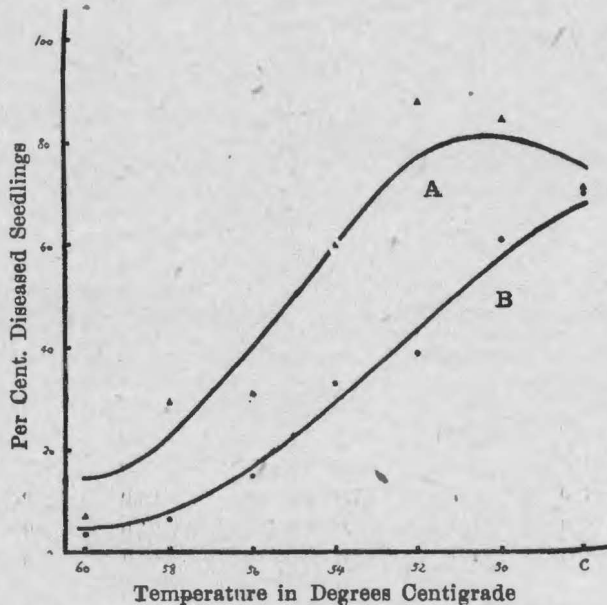


Table 9.
Hot-Water Disinfection of Rice Grains (Variety Kissin) Affected with
Helminthosporium Oryzae.
Treatment in hot-water: 10 minutes.
Results at 11 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Disea sedseedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	297	296	99.7	251	84.8	45	15.2
52	260	260	100.0	229	88.0	31	11.9
54	294	294	100.0	175	59.6	119	40.5
56	250	248	99.3	75	30.9	173	69.3
58	252	250	99.3	73	29.2	177	70.3
60	175	173	98.9	12	6.9	161	92.0
Control	191	190	99.5	135	71.1	55	28.8

Table 10.
Hot-Water Disinfection of Rice Grains (Variety Asahi) Affected with
Helminthosporium Oryzae.
Treatment in hot-water: 10 minutes.
Results at 11 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	253	238	94.0	100	39.6	138	54.6
52	270	250	92.6	71	26.3	179	66.3
54	270	247	91.5	52	19.2	195	72.3
56	256	225	88.0	39	15.2	186	72.7
58	219	203	92.7	20	9.1	183	83.6
60	202	201	99.5	3	1.5	197	97.5
Control	293	274	93.6	139	47.4	135	46.2

Table 11.
Hot-Water Disinfection of Rice Grains (Variety Dokai Sinriki) Affected
with *Helminthosporium Oryzae*.
Treatment in hot-water: 10 minutes.
Results at 11 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Diseased seedlings		Healthys eedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	315	153	48.7	41	13.1	112	35.4
52	267	125	46.9	10	3.8	115	43.1
54	288	149	51.7	10	3.6	139	48.3
56	292	158	54.1	14	4.8	144	49.4
58	272	110	40.5	0	0.0	110	40.5
60	241	74	30.7	0	0.0	74	30.7
Control	329	135	41.3	45	13.8	90	27.3

Similarly, the variety Kissin showed 6.9 per cent. diseased seedlings at 60 degrees, indicated the effectiveness of this method (Table 9 and Figure 1, curve A); and in the variety Asahi, it had a favorable effect with 1.5 per cent. diseased seedlings at 60 degrees. In the variety Dokai Sinriki a treatment in hot-water slightly increased the germination of the seeds, and showed the similar effect of disinfection at 58 and 60 degrees as the other varieties (Table 11).

A further experiment was conducted on the relation of temperature of hot-water and length of treatment on the efficacy of control of seedling blight of rice. The variety of rice seed was Kissin and the method of study was identical as those of the preceding experiments. The results are given on Table 12. The experiment showed that all temperatures between 50 and 60 degrees Centigrade had no effect of reducing the germination, nor were there any marked difference shown among the three different lengths of treatment of 5, 10 and 20 minutes. There was a general tendency of reducing the number of diseased seedlings as

Table 12.

Disinfection in Relation to the Temperature and the Length of Time Treatment in the Hot-Water Treatment of Rice Grains Affected with *Helminthosporium Oryzae*.

Variety of rice: Kissin, sp. gr. 1.18.

Results at 11 days after sowing.

Temperature of hot-water, °C	Treatment in minutes	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
			Number	Per cent.	Number	Per cent.	Number	Per cent.
50	5	100	100	100.0	59	59.0	41	41.0
	10	100	100	100.0	46	46.0	54	54.0
	20	100	95	95.0	37	37.0	63	63.0
52	5	100	97	97.0	47	47.0	53	53.0
	10	100	98	98.0	42	42.0	58	58.0
	20	100	96	96.0	29	29.0	71	71.0
54	5	100	94	94.0	40	40.0	60	60.0
	10	100	97	97.0	30	30.0	70	70.0
	20	100	98	98.0	28	28.0	72	72.0
56	5	100	95	95.0	33	33.0	62	62.0
	10	100	98	98.0	26	26.0	72	72.0
	20	100	96	96.0	22	22.0	74	74.0
58	5	100	98	98.0	28	28.0	70	70.0
	10	100	96	96.0	20	20.0	76	76.0
	20	100	95	95.0	19	19.0	76	76.0
60	5	100	95	95.0	26	26.0	69	69.0
	10	100	95	95.0	19	19.0	76	76.0
	20	100	98	98.0	15	15.0	83	83.0
Control		100	98	98.0	65	65.0	35	35.0

the length of treatment increased. Viewing from the results obtained, it seems that a treatment of 10 minutes was sufficient for all practical purposes and although our result did not show any detrimental effect by longer treatment, for the sake of safety a short treatment of 10 minutes should be recommended.

Summarizing the results of hot-water steeping of rice seed affected with *Helminthosporium Oryzae*, a treatment in hot-water of 58 to 60 degrees Centigrade for 10 minutes would prove effective in controlling the outbreak of seedling blight.

IV. Modified Hot-Water Treatment of Rice Seeds Affected with *Helminthosporium Oryzae*.

The simple hot-water steeping of rice seeds had been described, and the results obtained were quite favorable. A further study was made on the effectiveness of the modified hot-water treatment of rice seeds, and to find out if there is any advantage in the use of this method. The method differs from the simple hot-water treatment in the inclusion of preliminary soaking of seeds in cold water prior to immersion in hot water.

A. Temperature Factor in the Modified Hot-Water Treatment.

The experiment was conducted with the rice variety Ehime Sinriki collected in 1940, showing high percentage of diseased grains. The preliminary soaking in cold water consisted of 8 hours immersion in tap water held more or less constant at 20 degrees Centigrade. The pre-soaked seeds were held for 10 minutes in hot-water whose temperature was kept constant at two degrees interval between 50 and 60 degrees Centigrade. Seeds were then sown in germinating plates by

Table 13.

Disinfection by the Modified Hot-Water Treatment of Rice Grains Affected
with *Helminthosporium Oryzae*.

Variety of rice: Ehime Sinriki.

Treatment: 8 hours pre-soaking in cold water, followed by 10 minutes
immersion in hot-water.

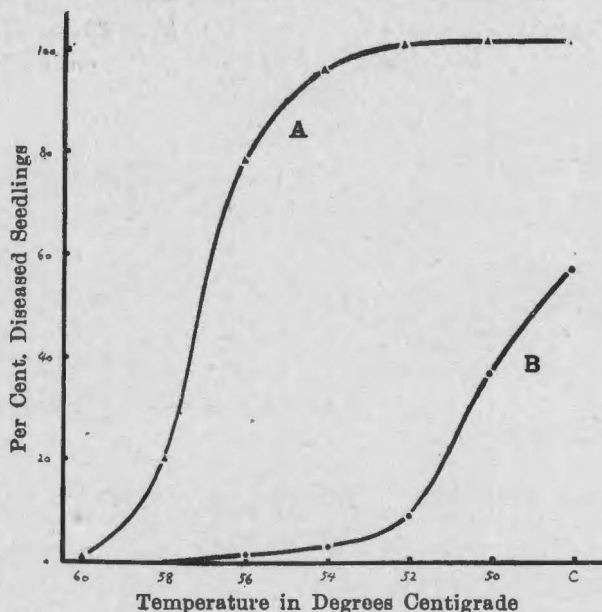
Results at 9 days after sowing.

Temperature of hot-water, °C.	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
50	212	210	99.2	78	36.8	132	62.3
52	200	199	99.5	18	9.0	181	90.5
54	211	201	95.3	6	2.8	195	92.5
56	202	143	70.8	3	1.5	140	69.3
58	222	45	20.3	0	0.0	45	20.2
60	222	2	0.9	0	0.0	2	0.9
Control	216	213	98.7	123	56.9	90	41.6

Fig. 2.

Relation of Temperature of Hot-Water to the Germination and the Development of Diseased Seedlings in the Modified Hot-Water Treatment of Rice Grains Affected with *Helminthosporium Oryzae*.

A: Germination. B: Diseased seedlings.



covering with the sterilized sand. Notes on the germination and the development of the blighted seedlings were taken at the end of 9 days. The results are given on Table 13 and Figure 2.

Results in Table 13 and Figure 2 show that the germination declined rapidly as the temperature increased. Namely, the untreated but pre-soaked seeds and those treated at 50 and 52 degrees displayed nearly perfect germination, but at 54 degrees the percentage dropped to 95.3, followed by 70.8 in 56 degrees, 20.3 in 58 degrees and finally 0.9 at 60 degrees. The critical temperature at which the germination begins to fall is therefore near 54 degrees. The disease rapidly declined with the increase in temperature. In the untreated group, the percentage of diseased seedlings was 56.9, but when the seeds were exposed to a temperature of 50 degrees for 10 minutes, it was reduced to 36.8; at 52 degrees, 9.0; at 54 degrees, 2.8 and at 56 degrees, 1.5. The highest percentage of healthy plants of 92.5 occurred at 54 degrees, followed by 90.2 of 52 degrees. At the other temperatures they fell to 60's or lower. It is evident from these results that in the modified hot-water treatment, a temperature of 52 to 54 degrees Centigrade for 10 minutes would suggest a most satisfactory disinfecting temperature.

B. The Factor of Length of Pre-soaking of Rice Seed on the Modified Hot-Water Treatment.

In order to determine the optimum period of pre-soaking, the following

experiment was performed. The pre-soaking periods were fixed at 0, 4, 8, 12, 24 and 48 hours. The treatment in hot-water was in all cases 10 minutes at 52 degrees Centigrade. The results obtained are shown in Table 14.

Table 14.

Disinfection in Relation to the Length of Time of Pre-soaking in the Modified Hot-Water Treatment of Rice Grains Affected with *Helminthosporium Oryzae*.

Variety of rice: Ehime Sinriki.

Treatment: 10 minutes at 52 degrees Centigrade.

Results at 9 days after sowing.

Hours of pre-soaking	Number of seeds sown	Seed germination		Diseased seedlings		Healthy seedlings	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
4	200	198	99.0	11	5.5	187	93.5
8	200	200	100.0	13	6.5	187	93.5
12	200	200	100.0	3	1.5	197	98.5
24	200	198	99.0	1	0.5	198	99.0
48	200	108	54.0	0	0.0	108	54.0
Control	200	196	98.0	49	24.5	147	73.5

According to Table 14, the germination of the seeds were maintained as high as 99 and 100 per cent. when the pre-soaking periods were shorter than 24 hours; but at 48 hours this was reduced to 54 per cent. The amount of diseased seedlings rapidly declined with the lengthening of the pre-soaking period, reaching its maximum effectiveness at 12 and 24 hours.

Summarizing the results of the modified hot-water treatment of rice seeds, for 10 minutes treatment in hot-water, the temperature of the hot-water should be kept between 52 and 54 degrees, and the period of pre-soaking in cold water from 12 to 24 hours.

V. Summary and Conclusion.

Rice seeds naturally infected with *Helminthosporium Oryzae*, were treated with various chemical fungicides to control the primary outbreak of Sesame-leaf-spot or 'Gomahagare' disease of the seedling stage.

The mercuric chloride, copper sulphate, and Germisan were proved weak disinfectants at the concentrations and lengths of treatment used in this experiment.

Formalin and Uspulun were relatively strong disinfectants. Formalin at the concentrations of 0.4 to 1.0 treated for 4 hours or longer decreased the development of the disease to a marked degree. Results with Uspulun were comparable to those of formalin.

In the simple hot-water treatment, steeping temperatures of between 53 and 60 degrees Centigrade were found to be effective.

Modified hot-water treatment of 12 to 24 hours pre-soaking in cold water of 20°C. followed by 10 minutes immersion in hot-water held at 52 to 54°C. gave

good disinfection. Pre-soaking in cold water for longer than 24 hours or treating at higher than 54°C. decreased the germination markedly.

From the results of this investigation, the simple as well as the modified hot-water treatments of rice seeds affected with *Helminthosporium Oryzae*, proved satisfactory substitutes for the usual formalin and Uspulun methods in controlling the primary outbreak or seedling blight in the nursery.

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PLATE XIV.

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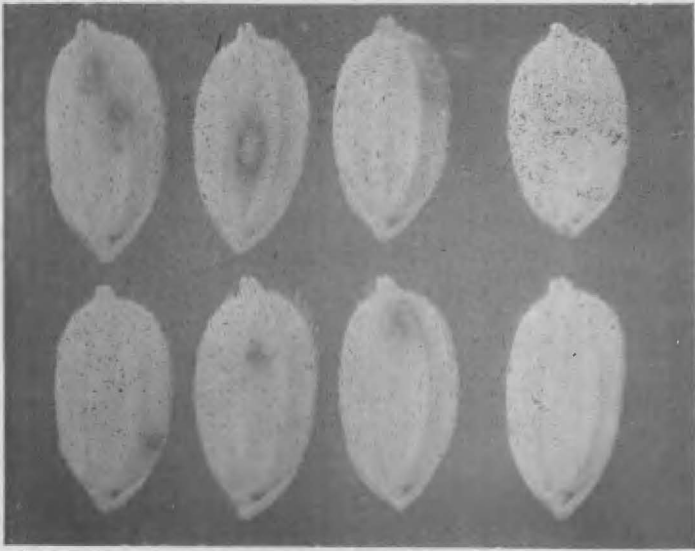


Fig. 2.

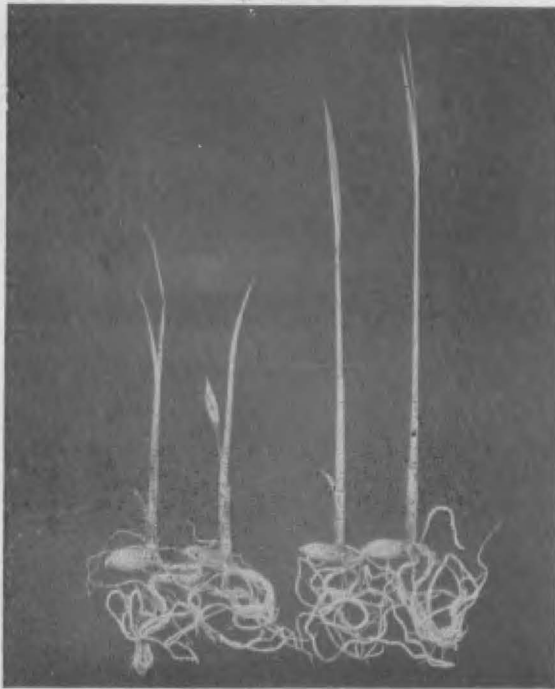


PLATE XV.

Fig. 3.

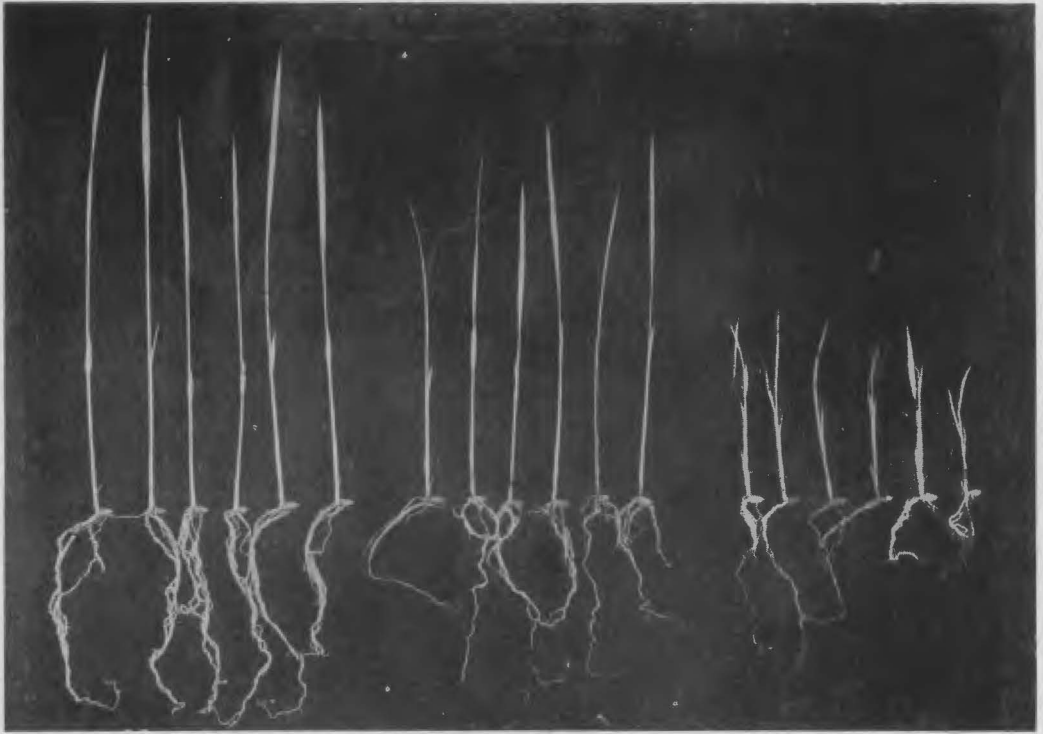
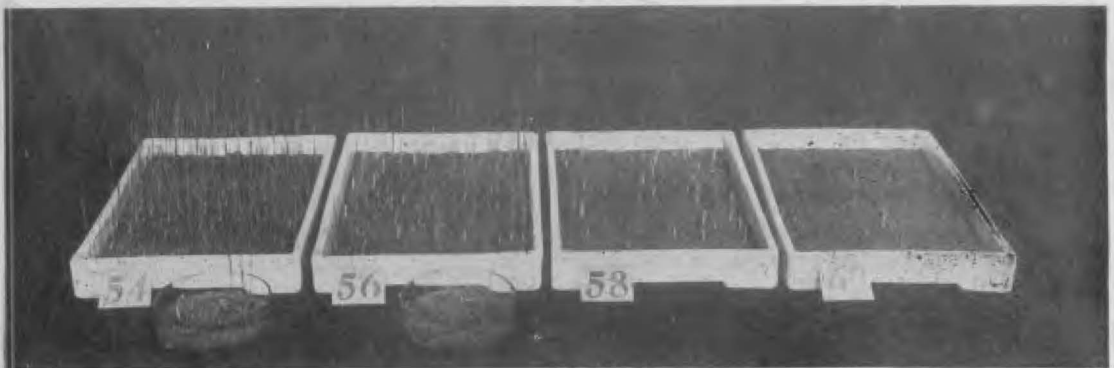
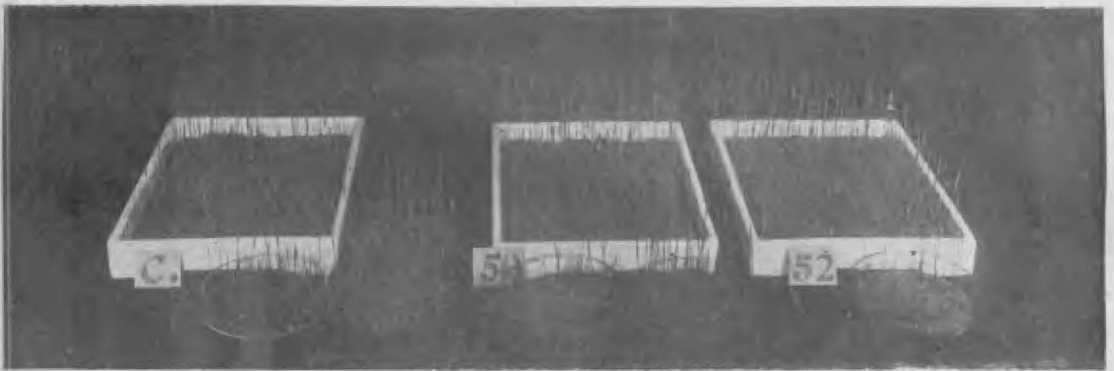


Fig. 4.



Explanation of Plates.

Plate XIV.

- Fig. 1.** Rice grains affected with *Helminthosporium Oryzae*. Six grains at the left show various size of lesions on the hulls, and the 2 grains at the right are the unaffected controls.
- Fig. 2.** Rice seedlings grown from the diseased grains. Two plants at the left are heavily diseased.

Plate XV.

- Fig. 3.** Comparative size of rice seedlings affected with *Helminthosporium Oryzae*. Left, healthy plants; center, plants slightly diseased at the primary leaf sheath; right, severely diseased plants.
- Fig. 4.** Result of disinfection by the modified hot-water treatment of rice grains affected with *Helminthosporium Oryzae*. The diseased seedlings are shown in the Petri dishes removed from the respective germinating plates.
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