A Rice Nematode Disease "Senchu Shingare Byo": IV. Prevention of the Present Disease

Yoshii, Hazime
Laboratory of Plant Pathology, Department of Agriculture, Kyushu University

Yamamoto, Shigeo
Laboratory of Plant Pathology, Department of Agriculture, Kyushu University

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A RICE NEMATODE DISEASE "SENCHÛ SINGARE BYÔ" [1]

IV. Prevention of the Present Disease [2]

HAZIME YOSHII AND SHIGEO YAMAMOTO

The most valid method to prevent the present disease may be the seed disinfection, with the precaution not to bring the pathogen in rice field after the commencement of seed-time. On the other hand, following methods may have some possibilities to control the severity of the disease, though the authors have not yet ample data on these subjects.

1) Improvement of manuring, 2) Modification of seed-time, 3) Application of resistant varieties.

Following experiments to destroy the nematode within seed were carried out for the last four years.

SPRAYING CHEMICAL AT THE EARING STAGE

The majority of the present nematode are situated on the outer side of the flower at the earing stage (Fukano, Yokoyama 1949), and enter the inner side of the glume at the flowering stage. While it was found that the nematode could be killed by a spray of nicotine sulphate (Inoue 1947).

Nicotine sulphate solution with soap, 1:500 in dilution, was sprayed to a half of a diseased plot, 400×30 cm. in size, on September 7th (the beginning of the earing stage), 10th and 17th in 1948. The other half of the plot was left for control.

The mature grains of these two blocks were divided into five parts each. From every part 10 grains were picked out at random,

[1] Contribution from the Laboratory of Plant Pathology, Kyushu University.

[2] I. Symptom and pathogenic nematode. Jour. Facult. Agr., Kyushu Univ., Vol. **9**, No. 3, 1950. II. Hibernation of *Aphelenchoides oryzae*. Ibid, Vol. **9**, No. 3, 1950. III. Infection course of the present disease. Ibid, Vol. **9**, No. 4, 1950.

and the approximate number of living nematodes was counted. Thus fifty counts were carried out for each of ten parts.

A simplified method was applied to obtain the total numbers of the living nematode. 50 measurements of each part were sorted into four classes with the number of nematodes (Table 1). Multiplied the number of each class by the class value, added up, thus the total sum of numbers of the living nematode of each part of two blocks were obtained (Table 2).

Table 1. Classification of the grain samples (each with ten grains) by the number of the nematodes.

Number of nematodes	Class	Class value
Above 20	A	20
6 to 20	B	10
1 to 5	C	3
0	D	0

Table 2. Efficacy of nicotine sulphate spray at the earing stage.

	Total living nematodes in sprayed block	Total living nematodes in not sprayed block
	60	621
	481	684
	155	684
	402	909
	306	701
Total	1404	3599
Mean	280.1	719.8

$D \gtrsim 334$ Difference between means significant at 5 per cent level.

While, the spray injury due to the chemical was tested and the results were represented in Table 3.

Table 3. Spray injury due to nicotine sulphate applied at the earing stage. Weight of 1000 grains.

	Nicotine sulphate 1:500 sprayed	Not sprayed (Check)
Total weight of 25 counts	(g) 537.07	(g) 555.68
Mean weight	21.483	22.227

$D \gtrsim 0.686$ Difference between means significant at 5 per cent level.

It is obvious from these data that nicotine sulphate spray at the earing stage will to some extent decrease the number of the present nematode in seed grain, though it is somewhat injurious to rice grain.

Seed Disinfection by Chemicals

a) Disinfection by chloropicrinc.

Inspite of their efforts the present authors could not found any concentration of chloropicrine which was not injurious to the vitality of seed, but was available to kill the nematodes in dormant state within hulls of the affected rice grains.

b) Disinfection by formaline.

Formaline solution (containing 35 per cent of formaldehyde) was diluted with water, 1 : 50. Into this were put the following treated rice grains affected by the nematode: 1) soaked in water for 24 hours, 2) soaked in water for 48 hours, and 3) not treated. After being immersed in formaline solution for one, two, and three hours respectively, the grains were drained and washed with water for 2 days. The efficacy of the formaline treatment was examined as usual by searching the living nematode within the treated grains after they were dried.[1)]

Data in Table 4 indicate that the nematode within seed grain

Table 4. Existence of the living nematode within seed grain treated with formaline solution, 1 : 50.

Pre-treatment of seed	Immersed in formaline for 1 hour	2 hours	3 hours
Soaked in water, 24h.	+	+	+
Soaked in water, 48 h.	+	+	+
Not soaked in water	+	+	+

+ indicates the existence of living nematode.

1) Ten to twenty unhulled-grains are stripped of their husks. Materials thus obtained are put in a test-tube, into which one cc. of distilled water is poured. Thus about 20 tubes are prepared for one lot. After being kept at 25°C. for 16 to 20 hours, the materials in each tube are poured into a watch-glass. The numbers of both moving (living) and immobile (dead) nematode are counted und a binocular microscope.

could not be killed with the formaline treatment, one of the standard disinfection methods for rice seed.

As for the field trial, the diseased seed treated with formaline solution,[1] was sown in a pot, in parallel with that treated by hot water[2] and with the check.

After 40 days from sowing, the seedlings of these three sources were transplanted in 4 pots respectively, 50 cm. in diam., as there were planted 25 seedlings per pot. At the time of the ear, the number of plants with bleached-tip symptom was counted. The results were given in Table 5.

Table 5. The efficacy of formaline treatment and of the modified hot water treatment for control of the present disease.

	Formaline treatment	Hot water treatment	Check
	0/25	0/25	6/25
	1/25	0/25	2/25
	3/25	0/24	0/25
	7/25	0/25	1/25
Total of converted values of per cents affected	19.55	0	17.67

Numerator represents the number of plants with the symptom. derminator represents the number of total plants examined.

$D \geqq 20.3$ Difference between any two of totals significant at 5 per cent level.

Although no significant differences were found within these cases in Table 5, it is apparent that the formaline treatment is invalid, while it is not hopeless in case of the hot water treatment.

[1] The seed was immersed in formaline of 1 : 50 in dilution for 3 hours, after being soaked in water for 2 days. At the end of 3 hours immersion in formaline, the seed was soaked again in water for 5 days.

[2] The seed was immersed in hot water at 52°C for 10 minutes, after being soaked in water for 24 hours at 18°C.

Modified Hot Water Treatment of Seed Grain

Nisikado and Nakayama (1942) reported that *Piricularia oryzae* and *Helminthosporium oryzae* on seed could be destroyed when rice seed was treated with a modified hot water treatment: immersed in hot water for 10 minutes at 52°C. after being soaked for 24 hours in water below 20°C. The present authors applied with success the modified hot water treatment to kill the present nematode in seed (Yoshii 1946).

As it was already pointed out (Nisikado, Nakayama 1942), the modified hot water method seems to be almost of the limit permissible to the vitality of rice seed. If the duration of seed soaking in water be prolonged to a certain extent, or if the temperature of hot water be over a certain limit, the germination of seed will at once be inhibited. With two years field trials the authors come to the conclusion that, at least in case of the present nematode disease, it is sufficient to disinfect the seed with hot water at 51°C. for 10 minutes after being soaked in water for 24 hours. The following are the studies carried out from 1944 to 1949 on some factors relating to the modified hot water treatment of seed rice to prevent the present disease.

a) Conditions sufficient to kill the dormant nematode.

The relation between hot water temperature and the vitality of the nematode (preliminary).

Affected grains (Variety Asahi) were divided into ten parts. Eight of them were immersed respectively in different hot water, ranging from 49° to 52°C., with intervals of one degree, for 5 and 10 minutes respectively, after the grains were soaked in water for 22 hours at 20°C. The ninth part was simply soaked in water for 22 hours, and the last one was reserved for control. 20 grains from each of these ten parts were picked out and the existence of the living nematode within them was examined, after the grains were dried up. The results obtained were given in Table 6.

Table 6 indicates that the modified hot water treatment is effective when hot water temperature is ranged above 50°C., provided that the grains were soaked in water for 22 hours at 20°C. previously and immersed in hot water for 5 to 10 minutes.

Table 6. Existence of the living nematode within rice grains treated with hot water methods of varied temperatures.

Soaked in water, 22 h.	Treated with hot water	1	2	3	4	5
Soaked	49°C. 5 m.	−	−	−	−	+
ditto	49°C. 10 m.	−	−	−	−	−
ditto	50°C. 5 m.	−	−	−	−	−
ditto	50°C. 10 m.	−	−	−	−	−
ditto	51°C. 5 m.	−	−	−	−	−
ditto	51°C. 10 m.	−	−	−	−	−
ditto	52°C. 5 m.	−	−	−	−	−
ditto	52°C. 10 m.	−	−	−	−	−
ditto	Without hot water	+	+	+	−	+
Not treated (Check)		+	+	+	+	+

+ indicates the existence of living nematode.

Temperature variation of hot water in connection with the efficacy of the modified hot water treatment for the prevention of the present disease (Field trial).

It often occurs in rice plots that the bleached-tip symptoms could not be found on the leaves of the affected plants, especially when they were planted in small pots, though the bleached-tip is the main symptom of the disease. As it was shown in Table 5, the field trials of the present disease were often hindered by this phenomenon to obtain the reliable results, until it was reported by Fukano and Yokoyama (1947) that numbers of the nematodes would be found on the ear of the affected plant even when no bleached-tip symptoms had been observed, and that it was possible to verify the efficacy of a treatment by searching the nematode on the ear of the treated plants. This is an experiment in which the efficacy of the hot water treatment is tested by the method written above.

The diseased grains were immersed in hot water at 49°, 50°, and 51°C. respectively for 5 to 10 minutes, after being soaked in water at 21°C. for 20 hours. For control, not treated grains were used. All of them were sown respectively in small pots, 18 cm. in diam., filled with disinfected soil. After grown up to a certain height, 64 seedlings from each of five sources (cf. Table 7) were transplanted in four pots, 30 × 30 cm. in size, as there were 16 plants per pot. At the flowering stage, the ears of main culms of rice

plants of every pot were collected and each of them was washed separately in a dish with 5 to 10 cc. of water. The presence of the nematode within washed water was examined under a microscope. The results were given in Table 7.

Table 7. Temperature of hot water in modified hot water treatment and its efficacy to prevent the nematode disease.a)

	49°C. 10 m.	50°C. 5 m.	50°C. 10 m.	51°C. 5 m.	Check
	10 b) / 16	5 / 16	0 / 15	0 / 16	8 / 16
	2 / 16	3 / 16	0 / 9	0 / 16	16 / 16
	14 / 16	0 / 16	0 / 16	0 / 16	12 / 16
	0 / 16	0 / 16	0 / 13	0 / 12	11 / 14
Total of converted values of per cents affected	142.24	60.58	0	0	257.44

a) Grains were soaked in water before the treatment for 20 hours at 21°C.
b) Numerator indicates the number of plants with nematode, denominator indicates the number of total plants examined.

$D \geqq 108.65$ Difference between any two of totals significant at 5 per cent level.

It is evident from data in Table 7 that both cases, 50°C. 10 minutes and 51°C. 5 minutes are effective, while the case 49°C. 10 minutes is inferior to them.

Necessity of the combined application of two procedures, water soaking of seed and hot water immersion, as a seed treatment to destroy the present nematode.

Modified hot water treatment is a method somewhat complicated to apply in practice. This is an experiment which explains the necessity of the combined use of two procedures, soaking seed in water and immersing seed in hot water, as a method to kill the present nematode within seed, when hot water temperature is placed about 50°C.

Affected rice grains (Variety Asahi) were divided into four

lots. The grains of each lot were treated as follows:—

1) Soaked in water for 20 hours at 5°, 15°, 20°, 25°, and 30°C. respectively ..... Immersed in hot water at 51°C. for 7 minutes.
2) ..... ditto ..... Not immersed in hot water.
3) Not soaked in water ..... Immersed in hot water at 51°C. for 7 minutes.
4) ..... ditto ..... Not immersed in hot water.

The existence of the living nematode was examined as usual at 20 grains. The results were summarized in Table 8.

Table 8. Necessity of the combined application of two procedures, seed soaking in water and hot water immersion (51°C., 7 m.), in hot water treatment.

Seed soaking in water for 20 h.	Hot water immersion 51°C. 7 m.	Temperature of water at seed soaking, °C. 5	15	20	25	30
Soaked	Treated	0	0	0	0	0
ditto	Not treated	5	5	5	3	4
Not soaked	Treated	5	5	5	5	5
ditto	Not treated	5	5	5	5	5

Numerical figure in the table represents the number of cases per five replicates, in which living nematode was found.

Table of analysis of variance:—

		SS	DF	V	F
Presence or absence of seed soaking	(W)	7.84	1	7.84	313**
Presence or absence of hot water immersion	(H)	4.84	1	4.84	193**
Temperature of water at seed soaking	(C)	0.16	4	0.04	1.60
Interaction	(W×H)	4.84	1	4.84	193**
〃	(W×C)	0.16	4	0.04	1.60
〃	(H×C)	0.16	4	0.04	1.60
〃	(W×H×C)	0.16	4	0.04	1.60
Error		2.00	80	0.025	
Total		20.16	99		

Data in Table 8 indicate that, both factors, soaking seed in water and hot water immersion of seed, are effective to destroy the present nematode, and that the efficacy of the treatment is much increased when these two factors are combined. While no significant differences were observed within the effects of temperatures of water in which rice seed was soaked.

Effect of the duration of seed soaking and temperature of water on the efficacy of the modified hot water treatment.

Affected rice grains (Variety Asahi) were soaked in water for 4, 8, 16, and 20 hours respectively at each of following water temperatures: 5°, 15°, 20°, 25°, and 30°C. After being soaked in water all of them were immersed in hot water at 51°C. for 7 minutes. At the end of 7 minutes immersion they were drained, put into cold water for a while, and then dried.

20 grains were taken from each of these treated, and were examined of the existence of the living nematode after usual manner. The examination ended with five replicates. The results were given in Table 9.

Table 9. Duration of seed soaking and temperature of water in relation to the efficacy of the modified hot water treatment.

Duration of seed soaking	——Temperature of water, °C.——					Total
	5	15	20	25	30	
(hours)						
4	2	3	1	2	2	10
8	0	1	0	0	0	1
16	0	0	0	0	0	0
20	0	0	0	0	0	0

Numerical figure in the table represents the number of cases per five replicates, in which living nematode was found.

$D \geqq 5.28$ Difference between any two of totals significant at 1 per cent level.

Data in Table 9 indicate that the efficacy of the hot water treatment is dependent on the duration of seed soaking, and that only the case, soaking in water for 4 hours, is insufficient. As to the effect of temperature of cold water, no significant result was again obtained from this experiment.

Summarizing the experimental results, it is noted that the modified hot water treatment is effective to kill the nematode within rice grain, when applied with the following standards :—

1) Soaking seed in water for 8 to 20 hours, followed by,
2) Immersing seed in hot water at 51°C (with a range from 50° to 52°C.) for 7 minutes (with a range from 5 to 10 minutes).

b) Modified hot water treatment and the germinating power of seed grain.

Following experiments were carried out to find some bases on the modified hot water treatment practically not injurious to the vitality of rice grain but effective to kill the nematode. For all of the experiments unhulled-grains, Variety Asahi, were used which were well dried, and the water content was less than 12 per cent. Before use, they were immersed in water and the scums were driven away.

Loss of vitality of seed grain when treated with modified hot water treatments of varied factors.

36 varieties of modified hot water treatment will be obtained by the combinations of the following three factors: duration of seed soaking in water, temperature of water for seed soaking, and hot water temperature, when each of them varies as follows :—

Duration of seed soaking in water :	24, 48, 72 hours.
Temperature of water for seed soaking :	5–7°, 15°, 20°, 25°C.
Temperature of hot water :	51°, 52°, 53°C.

For the present study 144 cases were planned derived from above 36 combinations with four replicates. The duration of hot water immersion was fixed to 10 minutes.

20 grains treated with each of these 144 cases were put into a germinator with wet sand. After 5 days at 25°C. the germinative capacity was examined. The results were given in Table 10.

Data in Table 10 indicate that,

1. Within the durations of seed soaking in water, 24, 48, and 72 hours, the first is preferable; when it is prolonged, increase of ill-effect on the vitality of grain will result.

Table 10. Loss of vitality of seed grain treated with modified hot water methods of varied combinations of duration of seed soaking, water temperature for seed soaking, and hot water temperature.

	T_1				T_2				T_3			
	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4
H_1	0	0	1	1	0	0	0	5	1	0	11	10
	0	1	2	0	2	1	1	10	1	1	13	10
	1	0	1	0	0	0	1	12	1	1	11	11
	0	1	0	2	0	1	2	11	2	0	16	10
H_2	1	1	0	0	2	0	3	17	0	2	15	13
	0	1	4	1	1	2	1	17	1	2	12	14
	0	3	1	0	1	4	2	16	0	0	17	16
	0	0	2	0	2	1	1	14	2	2	18	18
H_3	2	2	3	5	2	4	9	18	9	8	18	19
	4	2	7	4	5	3	4	19	11	8	18	20
	4	1	3	4	6	4	4	19	11	7	16	20
	0	3	7	5	3	3	10	18	10	8	17	20

T_1 T_2 T_3, each indicates the duration of seed soaking: 24, 48, 72 hours.
C_1 C_2 C_3 C_4, each indicates the temperature of water for seed soaking: 5~7, 15, 20, 25 (°C.).
H_1 H_2 H_3, each indicates the temperature of hot water: 51, 52, 53 (°C.).
Figure in the table represents the number not germinated per 20 grains.

Table of analysis of variance:—

		SS	DF	V	F
Duration of seed soaking	(T)	1434.0	2	717.0	358.50**
Water temperature for seed soaking	(C)	1749.4	3	583.1	291.55**
Hot water temperature	(H)	697.6	2	348.8	174.40**
Interaction	(T×C)	1222.8	6	203.8	101.90**
〃	(T×H)	99.3	4	24.8	12.40**
〃	(C×H)	61.7	6	10.3	5.15**
〃	(T×C×H)	107.7	12	9.8	4.90**
Error		215.5	108	2.0	
Total		5588.0	143		

Total sum of numbers not germinated grain for each series (T, C, H), and the differences between any two of totals of each series significant at 5 per cent level are given below (D_T, D_C, D_H).

T_1 T_2 T_3: 80 261 451; $D_T \geqq 27.7$
C_1 C_2 C_3 C_4: 85 77 251 379; $D_C \geqq 24$
H_1 H_2 H_3: 155 230 407; $D_H \geqq 27.7$

2. Within the temperatures of water for seed soaking, 5−7°, 15°, 20°, and 25°C., the former two are excellent, while ill-effect on the vitality of grain will be observed, when it becomes higher than 20°C.

3. Within the hot water temperatures, 51°, 52°, and 53°C., 51°C. is preferable; when it becomes higher the worse effect on the vitality of grain will be the result.

4. The interaction within any two or three of these three factors is multiplicative, and the loss of vitality of every seed will be the result when worse factors are combined.

Effect of variation of the duration of hot water immersion and of the hot water temperature on the germinating power of rice grain, in the modified hot water treatment.

Rice grains soaked in water for 20 hours at a temperature ranging from 18° to 20°C., were treated with hot water at each of three sorts of temperatures, 51°, 52°, and 53°C., for 5, 10, and 15 minutes respectively. After being treated the grains were placed on the germinators with wet sand at 25°C. For control, grains not treated with hot water were used. The germinative capacity was tested after four days. The results obtained with four replicates were given in Table 11.

Table 11. Effect of hot water temperature and of duration of hot water immersion on the germinating power of seed grain.

	Control (minutes)			51°C. (minutes)			52°C. (minutes)			53°C. (minutes)		
	5	10	15	5	10	15	5	10	15	5	10	15
	0	1	1	0	3	0	0	1	1	1	1	7
	0	0	0	0	0	1	0	1	1	1	1	2
	0	0	0	0	0	1	0	0	1	0	3	6
	1	0	0	0	1	0	0	0	0	0	0	9
Total	1	1	1	0	4	2	0	2	3	2	5	24

Numerical figure in the table represents the number not germinated per 20 grains.

6, 5, 31 ..... Totals for 51°, 52°, 53°C.

2, 11, 29 ..... Totals for 5, 10, 15 minutes (Without Control).

$D \geqq 12.3$ Difference between any two of totals of each series significant at 5 per cent level.

Table of analysis of variance for 51, 52, 53°C.:—

	SS	DF	V	F
Duration of immersion	31.5	2	15.8	10.53**
Temperature of hot water	36.2	2	18.1	12.67**
Interaction	42.8	4	10.7	7.13**
Error	40.5	27	1.5	
Total	151.0	35		

Data in Table 11 indicate that in case of the modified hot water treatment, both factors, the duration of hot water immersion and the temperature of hot water have a decided effect on the vitality of seed grain. Within the durations of immersion in hot water examined, 5 and 10 minutes are preferable while 15 minutes is inferior. Within the temperatures of hot water examined, only 53°C. is the worse. The effect of these two factors, the duration of immersion and the temperature of hot water, on the vitality of seed grains are multiplicative.

In case of practical apply of the modified hot water treatment of rice seed to prevent the nematode disease, it is preferable to place the upper limit of hot water temperature at 52°C., and the limit of duration of immersion in hot water at 10 minutes.

Retardation of germination of seed treated with the modified hot water treatment.

As indicated in the last experiment, the germinating power of seed grain will not be injured when it is treated with the modified hot water treatment: soaked in water at 18° to 20°C. for 20 hours then immersed in hot water at 51° to 52°C. for 10 minutes. By experience, however, it seems probable that the seedling from the treated grain is somewhat retarded in its growth. This is an experiment to see whether the retardation of growth of the treated grain is true or not.

20 grains soaked in water for 22 hours at 20°C., were treated at 52°C. for 10 minutes. After the treatment they were placed on a germinator with wet sand. After four days at 25°C., each of them was measured of the length of radicule and coleoptile. Two groups of 20 grains each, soaked only in water for 22 hours, were used for control. One of them was measured after three days at

25°C., while the other after four days at 25°C. The results were summarized in Table 12.

Table 12. Retardation of germination of seed treated with the modified hot water treatment (Mean values of 20 measurements).

Treatment.....	Modified hot water treatment a)	Water-soaked (Check 1) b)	Water-soaked (Check 2) b)
Duration in germinator	4 days at 25°C.	3 days at 25°C.	4 days at 25°C
	(mm)	(mm)	(mm)
Radicule length	23.25	22.60	34.25
Coleoptile length	7.60	5.35	10.80

a) Grains soaked in water at 20°C. for 22 hours, immersed in hot water at 52°C. for 10 minutes.

b) Grains soaked in water at 20°C. for 22 hours.

$D \geqq 1.95$ Difference between any two of radicule lengths significant at 5 per cent level.

$D \geqq 2.37$ Difference between any two of coleoptile lengths significant at 5 per cent level.

Data in Table 12 indicate that the grain treated with the modified hot water treatment: soaked in water at 20°C. for 22 hours, then treated with hot water at 52°C. for 10 minutes, is retarded by one day growth.

Best season for the modified hot water treatment.

All of the preceding experiments were given to the treatment applied just before the seed-time. Following trials were undertaken to see the efficacy to kill the nematode and the ill-effect of the treatment to the grain when applied long before the seed-time.

(i) Vitality of the nematode and the germinating power of seed grain, when treated long before the seed-time with the hot water method; the case in which hot water temperature was kept at 51°C. for 7 minutes.

Both healthy and affected grains were treated with the hot water method at every month from January to April. The temperatures of water in which the grains were soaked for 18 to 24 hours, varied under room temperature. The grains were equally immersed in hot water at 51°C. for 7 minutes. After being treated,

they were dried for several days and preserved until the seed-time (June 23rd). The checks were treated at June 22nd.

On June 23rd, the healthy grains of all five lots were placed on the germinators with wet sand, at 25°C. for three days. While the diseased grains were arranged to find the living nematode as usual. The results obtained were given in Table 13.

Table 13. Vitality of the nematode and the germinating power of seed grains treated with the hot water treatment at varied seasons before the seed time.

Date of treatment	Seed soaking in water		Hot water immersion		Existence of living nema.	Germinability[a]	
						Not germinated grains	Total grains
	(°C.)	(hours)	(°C.)	(hours)			
Jan. 26	6~10	24	51	7	—[b]	8	266
Feb. 19	12	24	51	7	—	9	276
March 23	10~12	18	51	7	—	5	371
April 29	12~16	18	51	7	—	6	304
June 23	20~21	18	51	7	—	6	245

a) Examined after 3 days at 25°C.
b) 100 grains were examined.

$F_0 = 0.750$ Homogeneity test within per cents of grains not germinated. F_0 is smaller than F value ($n_1=4$, $n_2=\infty$) at 5 per cent level.

Data in Table 13 indicate that when the temperature of hot water was kept at 51°C. and the duration of hot water immersion at 7 minutes, no significant differences are found within the germinabilities of grains treated at varied seasons, and that the nematode is dead in every treatments.

(ii) Germinating power of seed grains and the growth of seedlings at the fourth-leaf-stage, when seed grains were treated long before the seed-time with the hot water method; the case in which the hot water temperature was kept at 52°C. for 10 minutes.

Seed grains, treated with the hot water method (soaked in water for 18 to 20 hours then treated with hot water at 52°C. for 10 minutes) on Feb. 3rd, April 2nd, and May 23rd respectively, were placed on each of three germinators with wet sand, on May 24th at 25°C. After being kept for six days the germinating

power was examined, counting the number not germinated per 20 grains. The trials were ended with eight replicates. The results were given in Table 14.

Table 14. Inhibition of germinability of seed grains treated with the modified hot water treatment at varied seasons before the seed-time; the case in which hot water temperature was kept at 52°C. for 10 minutes. a)

Date of treatment	Feb. 3rd	April 2nd	May 23rd
Seed soaking	15°C. 18 h.	12°C. 18 h.	20°C. 20 h.
Hot water immersion	52°C. 10 m.	52°C. 10 m.	52°C. 10 m.
	1 b)	0	1
	2	1	1
	0	0	0
	1	0	0
	2	2	0
	0	0	0
	0	0	0
	2	0	0
Total	8	3	2

a) Examined after 6 days at 25°C.

b) Numerical figure in the table represents the number not germinated per 20 grains.

$D = 6.14$ Difference between any two of totals significant at 5 per cent level.

From data in Table 14, no significant differences could be found between any two of these three treatments. While a decided difference of growth of the seedlings from the grains treated on Feb. 3rd is observed, when compared with others at the fourth-leaf-stage (Table 15).

It is evident from these experiments that when the hot water temperature is lower and the duration of immersion is shorter (e.g. 51°C. 7 minutes), the ill-effect of the seed treatment to the germination will not occur even though treated five months before the seed-time. While the retardation of growth of seedlings will be observed when treated with the factors somewhat higher (e.g. 52°C. 10 minutes), at the season three months before the seed-time. It may also be probable, though not experimented, that the ill-effect on the growth of rice will result, when the factors, that

sometimes cause ill-effect on the germinating power of rice grains, such as, the temperature of water for seed soaking and the duration of soaking, are combined with the prolonged period, from the treatment to the seed-time.

Table 15. Growth of seedling from the grains treated with the modified hot water treatment at varied seasons before the seed-time; the case in which hot water temperature was kept at 52°C. for 10 minutes.[a)]

Date of treatment	Feb. 3rd	April 2nd	May 23rd
Seed soaking	15°C. 18 h.	12°C. 18 h.	20°C. 20 h.
Hot water immersion	52°C. 10 m.	52°C. 10 m.	52°C. 10 m.
	(cm)	(cm)	(cm)
Total height of 50 plants	586.6	665.6	684.6
Mean	11.73	13.31	13.69

a) Measured at the fourth-leaf-stage.

$D \geqq 0.786$ Difference between any two of means significant at 5 per cent level.

To apply the modified hot water treatment in practice for the disinfection at the season before the seed-time, it may be preferable to settle the day of disinfection not exceeding 60 days before the sowing time, even when the seed be treated at 51°C. for 7 minutes.

SUMMARY

Experiments on the seed disinfection method have been carried out.

A modified hot water treatment of seed, "Soaking in cold water below 20°C. for 16 to 20 hours, then, immersing in hot water at 51°C. (with a range from 50° to 52°C.) for 7 minutes (with a range from 5 to 10 minutes)" is found to be a safe and effective method to prevent the present disease.

Considerations on the season in which it is best to apply the modified hot water treatment are given.

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