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# Effect of Hot Water Treatment on the Control of Primary Seed Infection in Rice Caused by *Xanthomonas Oryzae*

*by*

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**Introduction :** Bacterial leaf blight caused by *Xanthomonas oryzae* (Uyeda and Ishiyama) was reported from India in 1951 from Khopoli area of Bombay. Sulaiman and Ahamed (1955) reported that this disease has caused consideable loss to paddy crop in several parts of Punjab, Uttar Pradesh, Bihar, Orissa, Madhya Pradesh, Maharasthra and Andhra Pradesh in recent years. In Tamil Nadu this disease appeared in the newly introduced high yielding variety Taichung Native 1 in a serious form. This disease was also noticed in some of the improved rice strains under cultivation in Tamil Nadu *viz.*, ADT 27, CO 25, CO 29, CO 30, CO 32 and BAM 3. Srivastava and Rao (1964) reported that seed-borne infection of *X. oryzae* can be eradicated to the extent of 95–100% by soaking infected paddy seeds for 12 hours at room temperature in aqueous solutions of Agrimycin + wettable ceresan (in concentrations of 0.025+0.05%) followed by hot water treatment at 52°–54°C for 30 minutes. With a view to assess the efficacy of hot water

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and chemical treatments alone and in combination in the control of primary infection of seed, studies were undertaken.

**Meterials and Methods:** ADT 27 paddy seeds showing a high percentage of bacterial infection (over 75%) were selected for the experiments and subjected to the following eight treatments. The thermal death point of the bacterium was reported to be 53°C (Elliot, 1951) and hence the temperature range of 51–55°C was tried.

1. Pre-soaking in cold water for 8 hours followed by hot water treatment at 54–55°C for (a) 10 minutes, (b) 20 minutes and (c) 30 minutes; 2. Soaking in 0.003% (27 ppm) streptocycline solution for 8 hours; 3. Soaking in 0.1% (1000 ppm) wet ceresan solution for 8 hours; 4. Pre-soaking in streptocycline solution for 8 hours followed by hot water treatment at 54–55°C for (a) 10 minutes, (b) 20 minutes and (c) 30 minutes; 5. Pre-soaking in wet ceresan solution for 8 hours followed by hot water treatment at 54–55°C for (a) 10 minutes, (b) 20 minutes and (c) 30 minutes; 6. Soaking in streptocycline and wet ceresan solution for 8 hours; 7. Pre-soaking in streptocycline and wet ceresan solution for 8 hours followed by hot water treatment at 54–55°C for (a) 10 minutes, (b) 20 minutes and (c) 30 minutes; 8. Control (Pre-soaking in cold water for 8 hours).

For each treatment 200 g. of seeds were taken and for assessing the effect of the treatment on bacteria one hundred seeds were divided into lots of 20 seeds each representing 5 replications. The treated seeds were air dried in shade and plated in nutrient agar medium in Petri-plates. Observations were recorded from 24 to 72 hours for the bacterial growth, Besides, the seeds treated with hot water were tested for their viability immediately after complete drying *i. e.* 10 days after actual treatment and also after one and three months of storage after treatment.

ADT 27 rice seeds were also subjected to hot water treatment at 58° and 60°C for 20 minutes both (1) with and (2) without pre-soaking in water for 8 hours prior to hot water treatment to study the effect of treatments on seed viability. The treated seeds were also sent to the Seed Testing Laboratory for their test certificate.

A method for hot water treatment under field conditions on the above lines was also devised.

**Results and Discussion:** Observations recorded on the effect of hot water treatment on the bacterium and germination of seeds are presented in Table 1.

TABLE 1

| S. No. | Treatments | | % of bacterial infection | % of germination |
|---|---|---|---|---|
| 1. | Pre-soaking in cold water for 8 hours followed by hot water treatment at 54°–55°C for | (a) 10 minutes | 17 | 94 |
| | | (b) 20 ,, | 4 | 96 |
| | | (c) 30 ,, | 1 | 96 |
| 2. | Soaking in streptocycline solution for 8 hours | | 56 | 98 |
| 3. | Soaking in wet ceresan solution for 8 hours | | 57 | 98 |
| 4. | Pre-soaking in streptocycline solution for 8 hours followed by hot water treatment at 54°–55°C for | (a) 10 minutes | 10 | 91 |
| | | (b) 20 ,, | 2 | 96 |
| | | (c) 30 ,, | 1 | 92 |
| 5. | Pre-soaking in ceresan wet solution for 8 hours followed by hot water treatment at 54°–55°C for | (a) 10 minutes | 24 | 93 |
| | | (b) 20 ,, | 6 | 93 |
| | | (c) 30 ,, | 2 | 95 |
| 6. | Soaking in streptocycline and wet ceresan solution for 8 hours | | 67 | 96 |
| 7. | Pre-soaking in streptocycline and wet ceresan solution for 8 hours followed by hot water treatment at 54°–55°C for | | | |
| | | (a) 10 minutes | 33 | 92 |
| | | (b) 20 ,, | Nil | 90 |
| | | (c) 30 ,, | Nil | 90 |
| 8. | Control (soaking in cold water for 8 hours) | | 85 | 97 |

The data were analysed statistically and summary of results are presented below.

*Summary of Results*

| Treatments | Mean % (Transformed value) |
|---|---|
| 1 | 1.23 |
| 2 | 3.41 |
| 3 | 3.44 |
| 4 | 1.07 |
| 5 | 1.46 |
| 6 | 3.73 |
| 7 | 1.34 |
| 8 | 4.18 |

Conclusion: $\overline{4,\ \underline{1,\ 7,\ 5,}\ 2,\ 3,\ 6,\ 8}$ SE: 0.18 CD: 0.36

a) Comparison of treatments 1, 4, 5 and 7.

Conclusion: $\overline{4,\ \underline{1,\ 7,\ 5}}$ SE: 0.12 CD: 0.24

b) Comparison of treatments 2, 3, 6 and 8.

Conclusion: $\overline{2,\ 3,\ 6,\ 8}$ SE: 0.22 CD: 0.36

c). Comparison of (a) *Vs.* (b) treatments:

| | | | | | | |
|---|---|---|---|---|---|---|
| Conclusion: | 1, 8 | 1, 6 | 1, 3 | 1, 2 | SE: 0.18 | CD: 0.36 |
| | 4, 8 | 4, 6 | 4, 3 | 4, 2 | | |
| | 5, 8 | 5, 6 | 5, 3 | 5, 2 | | |
| | 7, 8 | 7, 6 | 7, 3 | 7, 2 | | |

d). Comparison of periods within treatments:

| | | | | |
|---|---|---|---|---|
| Treatment 1 | hot water | 10 min. | 20 min. | 30 min. |
| | | 1.83 | 1.04 | 0.81 |
| Conclusion: | 30, 20, 10 | | SE: 0.22 | CD: 0.44 |
| Treatment 4 | Streptocycline and hot water | 10 min. | 20 min. | 30 min. |
| | | 1.51 | 0.88 | 0.81 |
| Conclusion: | 30, 20, 10 | | SE: 0.22 | CD: 0.44 |
| Treatment 5 | Wet ceresan and hot water | 10 min. | 20 min. | 30 min. |
| | | 2.23 | 1.25 | 0.91 |
| Conclusion: | 30, 20, 10 | | SE: 0.22 | CD: 0.44 |
| Treatment 7 | Streptocycline and wet ceresan and hot water | 10 min. | 20 min. | 30 min. |
| | | 2.60 | 0.71 | 0.71 |
| Conclusion: | 30, 20, 10 | | SE: 0,22 | CD: 0.44 |

The results revealed that all the treatmenis were better than the control. Treatments 1, 4, 5 and 7 with hot water alone and in combination with chemicals *viz.*, streptocycline and ceresan wet were significantly superior to treatments with chemicals alone *viz.*, 2, 3, 6 and 8. Pre-soaking of seeds in streptocycline solution for 8 hours followed by hot water (treatment 4) was significantly superior to other treatments but was on par with treatment 1 (pre-soaking in cold water for 8 hours followed by hot water treatment). In respect of time of exposure, all these treatments were less effective at 10 minutes and their efficacy was greatly increased at 20 and 30 minutes. Seed treatment with hot water alone or in combination with chemicals at 54° – 55°C for 20 – 30 minutes was significantly superior to 10 minutes treatment. Pre-soaking the seeds in cold water for 8 hours followed by hot water treatment at 54° – 55°C for 20 – 30 minutes was effective for the eradication of seed-borne infection of bacterial leaf blight.

*Viability of the hot water-treated seeds:* (a) The hot water-treated seeds were tested for their viability, one month after treatment and the data are presented in Table 2.

TABLE 2. *Germination of rice seeds one month after treatment.*

| Treatments | % of germination |
|---|---|
| Pre-soaking in cold water for 8 hours followed by Hot water treatment at 54° – 55°C for 10 minutes | 94 |
| " " 20 " | 92 |
| " " 30 " | 90 |
| Control | 97 |

The germination of the seeds was found to be good one month after treatment in all the treatments tried.

(b) The treated rice seeds were dried both in sun and shade and stored in cloth bags for a period of 3 months and their viability was tested and is given in Table 3.

TABLE 3. *Germination of rice seeds treated in hot water at 54°–55°c after 3 months of storage*

| Time of exposure of the seeds in hot water at 54°–55°C | % of germination after 3 months of storage | |
|---|---|---|
| | Sun dried | Shade dried |
| 10 minutes | 92 | 90 |
| 20 ,, | 38 | 30 |
| 30 ,, | 32 | 30 |
| Control (untreated) | 100 | 90 |

The germination of the seeds subjected to hot water treatment at 54°–55°C for 20 and 30 minutes was very poor after 3 months of storage.

*Effect of hot water treatment of rice seeds at 58° and 60°C:* The germination of rice seeds treated at 58° and 60°C for 20 minutes are given in Table 4.

TABLE 4. *Effect of hot water treatment of rice seeds at 58° and 60° on seed germination after drying*

| Treatments | % germination of seeds | |
|---|---|---|
| | On moist filter paper | Result from S.T:L, Coimbatore |
| Pre-soaking in cold water for 8 hours and hot water treatment at 58° for 20 minutes | 0 | 0 |
| Pre-soaking in cold water for 8 hours and hot water treatment at 60°C for 20 minutes | 0 | 0 |
| No pre-soaking in cold water and hot water treatment at 60°C for 20 minutes | 94 | 75 |
| Control | 99 | 92 |

Seeds soaked in water for 8 hours prior to hot water treatment were completely killed at 58° and 60°C for 20 minutes. The germination was better in the seeds which were not pre-soaked in water prior to the treatment indicating that the heat does not penetrate into the inner tissues of the dry rice kernal at this temperature.

*Hot water treatment under field conditions:*

| | |
|---|---|
| Quantity of seed/acre | 20 kg |
| Boiling water | 1 part |
| Cold water | 1½ part |

The above ratio gives a temperature of 55°C under the prevailing conditions at Coimbatore (The boiling point of water at Coimbatore is 98°C).

*Procedure: Stage I.* Soak the seed in 4½ buckets (*i. e.*, 72 litres) of cold water for 8 to 10 hours (pre-soaking of seeds before hot water treatment is an essential pre-requisite).

*Stage II.* Take water in a drum which can hold 3 buckets, (*i.e.*, 48 litres) of water and boil it. In another container keep one bucket of water (16 litres) and boil it separately. The latter is intended for adding in small quantities to maintain the temperature range of 54°–55°C for 20 to 30 minutes.

*Stage III.* When the 3 buckets of water reach the boiling point, have it poured into the drum containing the soaked seeds. Stir well at the same time using a strong bamboo stick to ensure uniform distribution of the temperature. Read the temperature with a thermometer. It reads 55°C under the existing conditions at Coimbatore and remains at 55°C for 7 to 10 minutes. (This may vary from place to place depending on the boiling point. So the ratio of cold water and boiling water can be arrived at by prior trials).

The hot water treatment is to be given at 54°–55°C for 20 to 30 minutes. When the temperature falls down to 54°C from 55°C add 1125 ml. of boiling water (*i. e.* ¼ gallon or 2 pints) to the drum and the temperature will raise by 1°C. Thus the temperature range of 54° – 55°C can be maintained for 20 to 30 minutes by adding the above mentioned quantity of boiling water (*i. e.* ¼th gallon) twice or thrice.

*Stage IV.* After treatment, drain the water and spread the seed for complete drying in shade or sun. This seed can be used even after a month after treatment.

**Discussion :** The results have shown that treatment of infected rice seeds in hot water at 54° – 55°C for 20 – 30 minutes after pre-soaking has greatly reduced the seed-borne infection of the bacterium. This is in agreement with the findings of Sindha and Nene (1967). It was further observed that treatment at this temperature for 10 minutes is not effective. Pre-soaking of seeds for 8 hours in cold water, in streptocycline (27 ppm) and in wet ceresan (1000 ppm) individually and followed by hot water treatment as well as streptocycline plus wet ceresan treatment for 8 hours followed by hot water treatment were all found to be effective in reducing bacterial infection. This is in agreement with the findings of Srivastava and Rao (1964). But among these treatments pre-soaking of seeds in streptocycline solution followed by hot water treatment was on par with treatment (1) *viz.*, pre-soaking of seeds in cold water alone for 8 hours followed by hot water treatment. Therefore the increased efficacy due to the treatment with chemicals plus hot water is

not sufficient enough as to make a general recommendation for large scale application.

The germination of seeds subjected to hot water treatment at 54° – 55°C for 20 – 30 minutes was found to range from 90 – 94% up to one month after treatment but their viability was reduced to 30% at the end of 3 months. The seeds which were pre-soaked for 8 hours in cold water and subjected to hot water treatment at 58° and 60°C for 20 minutes resulted in complete failure of germination.

**Summary:** The effect of hot water treatment alone or in combination with chemical treatments on the control of seed-borne infection of bacterial blight organism in rice seeds was studied in detail. Pre-soaking of seeds for 8 hours in cold water and followed by hot water treatment at 54° – 55° C for 20 – 30 minutes was found to be effective in reducing the bacterial infection. Hot water treatment of seed at 54° – 55°C for 10 minutes duration was not found to be effective. Pre-soaking of seeds for 8 hours in cold water, in streptocycline (27 ppm) and in wet ceresan (1000 ppm) individually and followed by hot water treatment as well as steptocycline plus wet ceresan treatment for 8 hours followed by hot water treatment were all found to be effective in reducing bacterial infection. But among these treatments pre-soaking of seed in streptocycline followed by hot water was significantly superior to other treatments. The germination of seeds subjected to hot water treatment at 54° – 55°C for 20 – 30 minutes was found to range from 30 – 38% at the end of 3 months. The viability of the seed was completely lost at 58°C for 20 minutes.

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