

Studies on the Methods for Testing the Effectiveness of Fungicides.
II. The Use of Pot Cultured Rice Plants for Testing Spray Materials
against *Helminthosporium Oryzae* Infection.*

By

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In evaluating fungicides, there leaves little doubt as to the significance of results obtained from the direct application of materials upon living plants. The test will be of general value if performed under controlled laboratory conditions, where maximum infection of plants is obtained. Following experiments were recorded from some of the available fungicides in their preventive action against the sesame leaf spot (*Helminthosporium oryzae*) outbreak upon pot cultured rice plants.

I. Materials and Methods

Thirteen fungicides were tested: detailed informations as to the source and ingredients of some fungicides and the fungus (*Helminthosporium oryzae*) are referred to the previous report.⁴⁾ Others are listed below:

| Product | Active ingredient | Manufacturer |
|------------------|-------------------|---------------|
| Copper arsenate | Cu 20% | Nippon Nōyaku |
| Organic copper | Cu 5% | Sanykō Nōyaku |
| Low copper | Cu - 3% | Ōshima Kagaku |
| Dry lime sulphur | | Nippon Nōyaku |

Two types of plants were used for the comparison, namely, the seedling and the more matured rice plants. For young rice seedlings, sterilized seeds were sown in galvanized iron pots 16 cm. in diameter and 21 cm. deep, and cultivated under paddy conditions. When the plants were from 15 to 20 cm. tall, the size usually attained after 3 to 4 weeks, they were ready for use in the experiments. Matured rice plants were also prepared in galvanized pots by transplanting two uniform seedling plants to a pot and allowed them to mature till just prior to the appearance of the inflorescence. On each plant at the time of the experiment, three fully expanded young blades were selected and the rest trimmed off.

Solutions of test fungicides were prepared as prescribed by the makers and were sprayed upon plants set on a rotating platform, using a pistol atomizer connected to

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- 4) Y. NISIKADO, T. NAKAYAMA, and Y. MIYAWAKI, Studies on the methods for testing the effectiveness. 1. The use of slide glasses as a medium for testing spray materials. Ber. d. Ōhara Inst. f. Landw. Forsch. IX: 3:317-328, 1951

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Table 1. Outbreak of Sesame Leaf Spot on Seedling and Matured Rice Plants as Affected by Spraying Various Concentrations of Different Fungicides.

| Fungicide | Seedling | | Matured plant | |
|------------------|---------------|--------------|---------------|--------------|
| | Concentration | Average mark | Concentration | Average mark |
| Bordeaux mixture | 1.0 % | 0.44 | 0.8 % | 0.87 |
| | 0.5 | 0.69 | 0.4 | 0.87 |
| | 0.25 | 0.79 | 0.2 | 0.87 |
| | 0.125 | 0.98 | 0.05 | 0.96 |
| San Bordeaux | — | — | 0.8 | 1.02 |
| | — | — | 0.4 | 1.09 |
| | — | — | 0.2 | 1.13 |
| | — | — | 0.05 | 1.19 |
| Ōdo | 1.0 | 1.19 | 0.8 | 0.88 |
| | 0.5 | 1.39 | 0.4 | 1.17 |
| | 0.25 | 1.20 | 0.2 | 1.13 |
| | 0.125 | 1.61 | 0.05 | 1.29 |
| Kassel Bordeaux | 1.0 | 0.86 | 0.8 | 1.14 |
| | 0.5 | 0.92 | 0.4 | 1.23 |
| | 0.25 | 1.08 | 0.2 | 1.43 |
| | 0.125 | 1.12 | 0.05 | 1.49 |
| Kupoid | 1.0 | 1.11 | 0.8 | 0.85 |
| | 0.5 | 1.07 | 0.4 | 1.06 |
| | 0.25 | 1.45 | 0.2 | 1.54 |
| | 0.125 | 1.67 | 0.05 | 1.73 |
| Copper arsenate | 1.0 | 1.37 | — | — |
| | 0.5 | 1.82 | — | — |
| | 0.25 | 1.96 | — | — |
| | 0.125 | 2.22 | — | — |
| Organic copper | 1.0 | 0.90 | — | — |
| | 0.5 | 0.89 | — | — |
| | 0.25 | 1.10 | — | — |
| | 0.125 | 1.15 | — | — |
| Low copper | 1.0 | 0.92 | — | — |
| | 0.5 | 1.14 | — | — |
| | 0.25 | 1.40 | — | — |
| | 0.125 | 1.91 | — | — |
| Colloidinoid | 1.0 | 2.31 | 0.8 | 1.16 |
| | 0.5 | 2.50 | 0.4 | 1.32 |
| | 0.25 | 2.60 | 0.1 | 1.99 |
| | 0.125 | 2.63 | — | — |
| Soid | 1.0 | 2.19 | 0.8 | 1.57 |
| | 0.5 | 2.49 | 0.4 | 1.89 |
| | 0.25 | 2.45 | 0.1 | 1.98 |
| | 0.125 | 3.43 | — | — |
| Larvacide | 1.0 | 2.49 | 0.8 | 2.07 |
| | 0.5 | 2.10 | 0.4 | 1.85 |
| | 0.25 | 2.28 | 0.1 | 2.00 |
| | 0.125 | 2.24 | — | — |
| Lime sulfur* | 1.0 | 2.28 | 0.8 | 1.31 |
| | 0.5 | 2.82 | 0.4 | 2.04 |
| | 0.25 | 3.05 | 0.1 | 2.18 |
| | 0.125 | 3.93 | — | — |
| Dry Lime sulfur | 1.0 | 3.07 | 0.8 | 1.68 |
| | 0.5 | 3.26 | 0.4 | 2.03 |
| | 0.25 | 3.25 | 0.1 | 2.39 |
| | 0.125 | 2.99 | — | — |

* The concentration in Baume degrees.

a motor driven air compressor. All plants were sprayed for 30 seconds at 12 rotations.

Suspensions of inoculating fungus spores were prepared by lightly agitating the surface of the culture in rice straw decoction agar slants with a small amount of sterile water. The concentrated suspension was then filtered through a layer of cheese cloth and diluted to a concentration giving approximately 30 spores to a field of a microscope magnified to $\times 200$. The inoculation was made by using similar equipments as was for spraying fungicides.

In recording the degrees of infection occurring on the seedling plants, each pot was taken as a whole and the result expressed *in toto*. Standards with values ranging from 0 to 4, the higher mark indicating a greater infection, were selected from the treated group. There were from 4 to 5 persons matching each pot to the set standards, and their values were averaged to be used as the final mark for that pot. On the matured plant, values of 0 to 5 were considered for each of three blades taking into account the number of leaf spot as well as the size of them. Whenever a value appears to fall between the two standards, an appropriate fraction was used to denote the intermediate position. To facilitate accuracy in marking individual blade, standards of dried preserved leaves with different degrees of leaf spot were used, as a guide.

II. Experimental results

Leaf spot development as affected by spraying various concentrations of different fungicides is summarized in Table 1. A glance down the columns with average mark for the matured and seedling plant groups show that the Bordeaux mixture stands out with the least outbreak. Other copper containing fungicides followed, but with small differences among them. Colloidinoid and other non-copper fungicides, on the other hand, did not show as high a preventive action. The tendency of difference due to concentration is also clearly brought out.

A statistical analyses of Table 1 is given in Table 2. We find on copper fungicides, the variation due to fungicide, concentration and replication highly significant in both matured and seedling plants, with the exception of the blade position on matured plants. Non-copper fungicides on the other hand, showed significant variance of the above items only on matured plants. It is probable from this, that there are still other factors contributing toward inconsistency of results from non-copper fungicides when tested on seedling rice plants.

A consistency of results of experiments between matured and seedling plants, compared on basis of 0.03 and 0.05 per cents copper content, for Bordeaux mixture, Ôdo, Kassei Bordeaux and Kupoid, are shown in Table 3. The copper content having been referred to the analysis shown in the first report. The table shows the Chi square values for each pair of fungicide treatment highly significant, ranging from 0.80 upward.

With reference to fungicides, it can be concluded that for preventing sesame leaf spot of rice, Bordeaux mixture at a concentration of 0.05 per cent will suffice; and under certain cases, further dilution is possible. Other copper preparations re-

quire a much higher concentrations of between 0.5 and 1.0 per cents for securing a similar result.

Table 2 Mean Squares in Analyses of Variance of Fungicides on Matured and Seedling Rice Plants.

| Source of variation | Copper fungicides | | | | Non-copper fungicides | | | |
|---------------------|-------------------|----------|-----------|----------|-----------------------|----------|-----------|----------|
| | Matured plants | | Seedlings | | Matured plants | | Seedlings | |
| | D. f. | Mean sq. | D. f. | Mean sq. | D. f. | Mean sq. | D. f. | Mean sq. |
| Fungicide | 3 | 1.405** | 6 | 2.525** | 3 | 2.054** | 4 | 1.427 |
| Concentration | 3 | 0.551** | 3 | 1.945** | 2 | 3.953** | 2 | 0.141 |
| Blade | 2 | 0.004 | — | — | 2 | 1.501** | — | — |
| Replication | 3 | 4.122** | 4 | 1.160** | 3 | 1.966** | 3 | 1.170 |
| Error | 180 | 0.0794 | 126 | 0.0855 | 133 | 0.1735 | 50 | 1.2030 |

** F value of mean square exceed tabular 1% value.

Table 3. Comparison of Leaf Spot Outbreak on Matured and Seedling Rice Plants Sprayed with Fungicides at Concentrations of 0.05 and 0.03 Per Cent Pure Copper.

| Fungicide | 0.05 per cent | | | 0.03 per cent | | |
|------------------|---------------|----------|----------------|---------------|----------|----------------|
| | Matured plant | Seedling | X ² | Matured plant | Seedling | X ² |
| Bordeaux mixture | 0.87 | 0.72 | 0.0142** | 0.91 | 0.79 | 0.0084** |
| Ōdo | 1.30 | 1.20 | 0.0460* | 1.30 | 1.50 | 0.0150** |
| Kassei Bordeaux | 1.18 | 0.86 | 0.0502* | 1.27 | 0.91 | 0.0594* |
| Kupoid | 1.16 | 1.45 | 0.0321* | 1.34 | 1.63 | 0.0282* |

*, ** P values of Chi square exceed 0.80 and 0.90, respectively.

III. Discussion

From the authors' experience and results obtained for testing the effectiveness of fungicides on pot cultured rice plants against sesame leaf spot, it was found that the seedling plants are near equally suitable for experimental purposes. One of the outstanding advantages of the seedling plants is that the plants can be prepared for use in a comparative short time. In certain instances where additional replication of tests is required, tops of the plant can be clipped and reuse the new growth that will soon appear.

The tests have shown that Bordeaux mixture exhibited a remarkable protective action against the leaf spot. It appears that for copper sensitive diseases, Bordeaux mixture should be used as the standard upon which other preparations should be compared, and the indices thus obtained should show the immediate potentialities of the materials being tested. There should also accompany further studies considering such effects as the aging upon the plant, particularly under various meteorological conditions that the plants are normally subjected to during growth in the field.

VI. Summary

1. Seedling and matured rice plants were cultured in galvanized iron pots to be used for determining the effectiveness of various fungicides against the sesame leaf spot caused by *Helminthosporium oryzae*. It was found that both types of plants were equally suitable, but from the practical point of view, the seedling plants were more desired.
 2. Outstanding performance was observed on Bordeaux mixture. Its effective concentration was 0.25 per cent and less. Other copper preparations followed but required a much higher concentrations of 0.5 per cent and upward, and showed but slight difference among them.
 3. There was a close consistency of results of experiments between seedling and matured plants. Further application of the methods was discussed
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