ASSESSMENT OF INOCULATION TECHNIQUES AND EVALUATION OF FUNGICIDES AGAINST POTATO *Fusarium* DRY ROT

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ABSTRACT

Five methods of inoculating *Fusarium* spp., the causal organisms of potato dry rot, were assessed to find the most suitable technique to evaluate the effectiveness of fungicides against the disease. Depositing 0.2 mL of spore suspension (10^5 spores/mL) of *Fusarium* spp. into holes in the tubers was the best inoculation method resulting in 75% tuber infection at 12 days after inoculation. Seven fungicides were evaluated for their effectiveness in controlling the disease in stored potato tubers. Benlate at the rate of 3 tbsp/ 19 L water was most effective in minimizing tuber infection and lowering the rate of lesion development. Dithane M-45 and Captan were also effective in controlling the disease and are good alternatives for Benlate.

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KEYWORDS: *Fusarium* dry rot. Potato. Inoculation techniques. Evaluation. Fungicides.

INTRODUCTION

Fusarium dry rot is one of the most important diseases of stored potato tubers. Infected tubers initially show slightly recessed lesions which later expand with internal rot. Rotten tubers eventually shrivel and harden (International Potato Center, 1984). Leach (1985) reported that dry rot causes great losses to the potato industry.

Different fungicides may vary in their effectiveness in controlling dry rot. Hide and Cayley (1980) reported that the effectiveness of chemicals varied with the species of *Fusarium* causing potato dry rot. They reported that dry rot caused by *F. solani* var. *coeruleum* was controlled best by prochloraz, carbendazim and thiabendazole. Dry rot caused by *F. sulphureum*, on the other hand, was controlled best by prochloraz, sisthane, imazalil, thiabendazole and nuareniol.

A simple but effective method of inoculating the dry rot fungus needs to be identified before evaluating different fungicides against the disease. This work presents an appropriate inoculation technique for dry rot, the evaluation of different fungicides in controlling dry rot, and the possible interaction effect of chemicals and cultivars in reducing *Fusarium* dry rot infection.

MATERIALS AND METHODS

Inoculum Preparation

Pure culture isolate of *Fusarium* sp. was obtained from the Plant Pathology laboratory of the International Potato Center (CIP) at Mt. Banahaw, Dolores, Quezon. The fungus was allowed to mass grow and sporulate in a plated sweet potato dextrose nutrient agar (SPDNA) medium especially formulated for the fungus. The cultures were incubated until sporulation occurred which usually took 5 to 7 days.

Evaluation of Inoculation Techniques

Plugging holes in tubers with agar disc cultures. A hole (6 mm dia.) was punched in each tuber using a sterile cork borer. The same cork borer was used to cut an agar disc from plated culture of dry rot fungus. The cut agar disc was used to plug the hole in the tuber.

Inserting agar discs in slits made at the center of the cut tubers. The tubers were halved and agar discs (6 mm dia.) were inserted in between the cut portions.

Injecting spore suspension into intact tubers. A spore suspension with 105 spores/mL was used in inoculating the tubers. In this method, 0.1 mL of the spore suspension was injected into the tubers using a sterile microsyringe.

Dipping tubers with holes into spore suspension. A hole (5 mm deep) was made on each tuber using a sterile cork borer (6 mm dia.). The holed tubers were then dipped into the fungal spore suspension with a concentration of 10^5 spores/mL for one hour.

Depositing spore suspension into holes made in tubers. Holes (5 mm deep) were made on the tubers and filled with 0.2 mL of fungal spore suspension (10^5 spores/ mL) using a sterile microsyringe.

Tubers of Cosima, a potato cultivar susceptible to dry rot fungus, were used. The inoculated tubers were placed inside 10 x 14 cm clear plastic bags with moist cotton. They were incubated at room temperature for 12 days and the number of infected tubers was determined. Percent tuber infection was calculated. The treatments were arranged in the laboratory using the completely randomized design (CRD) with two replications.

Test Cultivars and Inoculation of the Pathogen

Eight potato cultivars of varying degrees of susceptibility to dry rot as reported by CIP researchers, namely: 65-ZA-5, 678011, LT-5. LT-1, Jemseg, Desiree, Cosima and Sequoia were used. Tubers that were relatively free from skin injuries were selected and disinfected with 2.5% calcium hypochlorite solution prior to inoculation.

The best technique in inoculating the dry rot fungus was used in the actual evaluation of fungicides against *Fusarium* dry rot.

Chemical Treatment

Seven fungicides which are locally available and commonly used by farmers were evaluated. They were applied as tuber dips 2 days after inoculation of the pathogen. The fungicides with their manufacturer's recommended rates were: 1) Benlate (methyl 1- butylcarbamoyl-2-benzimidazole carbamate) at 3 tbsp/19 L water; 2) Dithane M-45 (manganese zinc ethylene bisdithiocarbamate) at 4.5 tbsp/19 L water; 3) Captan (N-trichloromethyl thiocyclohex ene-1,2-dicarboximide) at 4.5 tbsp/16 L water; 4) Maneb (manga nese ethylene bisdithiocarbamate) at 4.5 tbsp/19 L water; 5) Vitigran blue (copper oxychloride) at 8 tbsp/19 L water; 6) Zineb (zinc ethylene bisdithiocarbamate) at 4 tbsp/19 L water; and 7) Difolatan (Captafol) at 4.5 tbsp/16 L water. The eighth treat ment was the control (no chemical treatment).

The tubers were air dried after chemical treatment and placed inside plastic bags. The experiment was laid out in a split plot design with three replications. The different cultivars served as the main plots and the different chemicals as the subplots. Eight potato cultivars and eight fungicides including the control were used. Six tubers per treatment per replicate were used. A total of 1,152 tubers was used for the whole experiment.

RESULTS AND DISCUSSION

Inoculation Techniques

Among the five inoculation methods tested, depositing 0.2 mL spore suspension into holes in the tubers yielded the highest percent tuber infection (Table 1). Plugging holes in tubers with agar disc cultures gave the second highest percent tuber infection followed by dipping tubers with holes in spore suspension. Inserting agar discs in slits at the center of cut tubers and injecting spore suspension into intact tubers gave the lowest percent tuber infection.

Among the inoculation methods used, two involved direct inoculation of fungal growth in the form of agar discs taken from plate culture and three involved inoculation using spore suspension. Inoculation using mycelial mat is sometimes resorted to because *Fusarium* dry rot fungus occasionally does not produce spores depending on the growth medium and conditions. Between the two methods involving mycelial mat inoculations, plugging holes in the tubers with agar disc cultures gave a higher percent tuber infection than inserting agar discs in slits at the center of the cut tuber. However, a disadvantage of these two methods is that the inoculum is not standardized. The latter method resulted to lower tuber infection probably because a large wounded surface area of the tuber was exposed which led to immediate drying of the wound. *Fusarium* dry rot fungus could not penetrate intact or suberized tissues (Nielsen, 1981).

Table 1. Percent tubers showing *Fusarium* dry rot lesions in the different inoculation techniques at 12 days after inoculation of the pathogen.

Inoculation Technique	Tuber Infection (%)[1]
Depositing spore suspension into holes made in tubers	75.0 a
Plugging holes in tubers with agar disc cultures	41.7 b
Dipping tubers with holes into spore suspension	33.3 bc
Inserting agar discs in slits made at center of cut tubers	16.7 d
Injecting spore suspension into intact tubers	16.7 d
C.V. (%)	20.3

[1] Average of six tubers per replicate per treatment. In a column, means followed by a common letter are not significantly different at 5% level, DMRT.

The use of fungal spore suspension in every inoculation is most ideal because the inoculum can be standardized. The inoculation method which resulted to the highest percent tuber infection in this experiment was depositing spore suspension into holes made in the tubers. Since this method also used spore suspension, it was considered ideal and the most effective thus, it was used in the actual evaluation of fungicides against *Fusarium* dry rot.

Chemical Control of Fusarium Dry Rot

The average number of tubers showing *Fusarium* dry rot lesions significantly varied among the different fungicide treatments at various time intervals (Table 2). Based on the average number of tubers infected at different observation periods, Benlate gave the best control. It was followed by Dithane, Captan and Maneb.

Using the average dry rot lesion diameter as the basis in fungi cide evaluation, Benlate was again the most effective because it obtained the smallest lesion diameter (Fig. 1). Aside from reducing the number of infected tubers, the fungicide also inhib ited lesion development in the test tubers. This was again followed by Dithane, Captan and Maneb.

The pattern of lesion development from 4 to 20 days after chemical treatment is shown in Figure 2. The slope of each curve was analyzed using simple linear regression analysis between lesion diameter and observation time in days. Lesion diameter was considered the dependent variable while observation time was the independent variable. The computed slope of the regression line represents the steepness of increase in lesion diameter with reference to time. This slope also represents the rate of increase in dry rot lesion

Table 2. Average number of tubers showing *Fusarium* dry rot lesions in each fungicidal treatment of all cultivars at different time intervals.

Chemical	Average Number of Infected Tubers[1]				
	Days After Chemical Treatment				
	4	8	12	16	20
Benlate	0.8 b	1.3 f	1.8 f	2.2 f	2.4 f
Dithane	1.3 b	2.1 b-f	2.7 b-f	3.2 b-f	3.4 b-f
Captan	1.6 b	2.3 b-f	2.9 b-f	3.6 b-e	4.0 b-e
Maneb	1.6 b	2.8 b-e	3.3 b-e	4.1 bcd	4.1 bcd
V. Blue	2.5 ab	3.5 b	4.1 b	4.4 ab	4.4 bc
Zineb	2.3 ab	3.4 bc	3.9 bc	4.4 ab	4.6 ab
Difolatan	2.5 ab	3.3 bcd	3.5 bcd	4.3 bc	4.6 ab
Control	3.7a	5.1 a	5.6 a	5.8 a	5.9 a
C.V. (%)	38.6	22.6	17.8	16.6	14.4

[1]Average of six tubers per replicate per treatment. In a column, means followed by a common letter are not significantly different at 5% level, DMRT

diameter in tubers treated with the different fungicides. The slopes of the regression line or the rate of increase in lession diameter was designated b. The higher the b value, the greater is the increase in lession diameter with time, therefore the less effective is the treatment and vice versa. The b value of Benlate was the lowest which indicates its greater effectiveness than the other chemicals tested.

The efficacy of the different fungicides was not influenced by the cultivars used. No interaction was observed between the cultivars and chemicals in reducing the number of dry rot infected tubers and lesion diameter. The disease control patterns of the different fungicides therefore were more or less similar in all cultivars studied. Disease level in the different cultivars was also the same regardless of chemical treatment. Moreover, the cultivars did not differ in the average number of infected tubers (Table 3) and average lesion diameter (Table 4).

The effectiveness of Benlate was probably due to its systemic nature. It is one of the most commonly used fungicides to control fungal diseases including dry rot (Hide et al., 1969; Copeland and Logan, 1975).

Although relatively expensive, Benlate is still recommended for the control of *Fusarium* dry rot of potato tubers. It may still be profitable to use the fungicide for dry rot control because potato in the Philippines is expensive due to its high demand and its being highly selective in growth requirements. Potato could not be grown anywhere because it prefers the low temperature in the high-

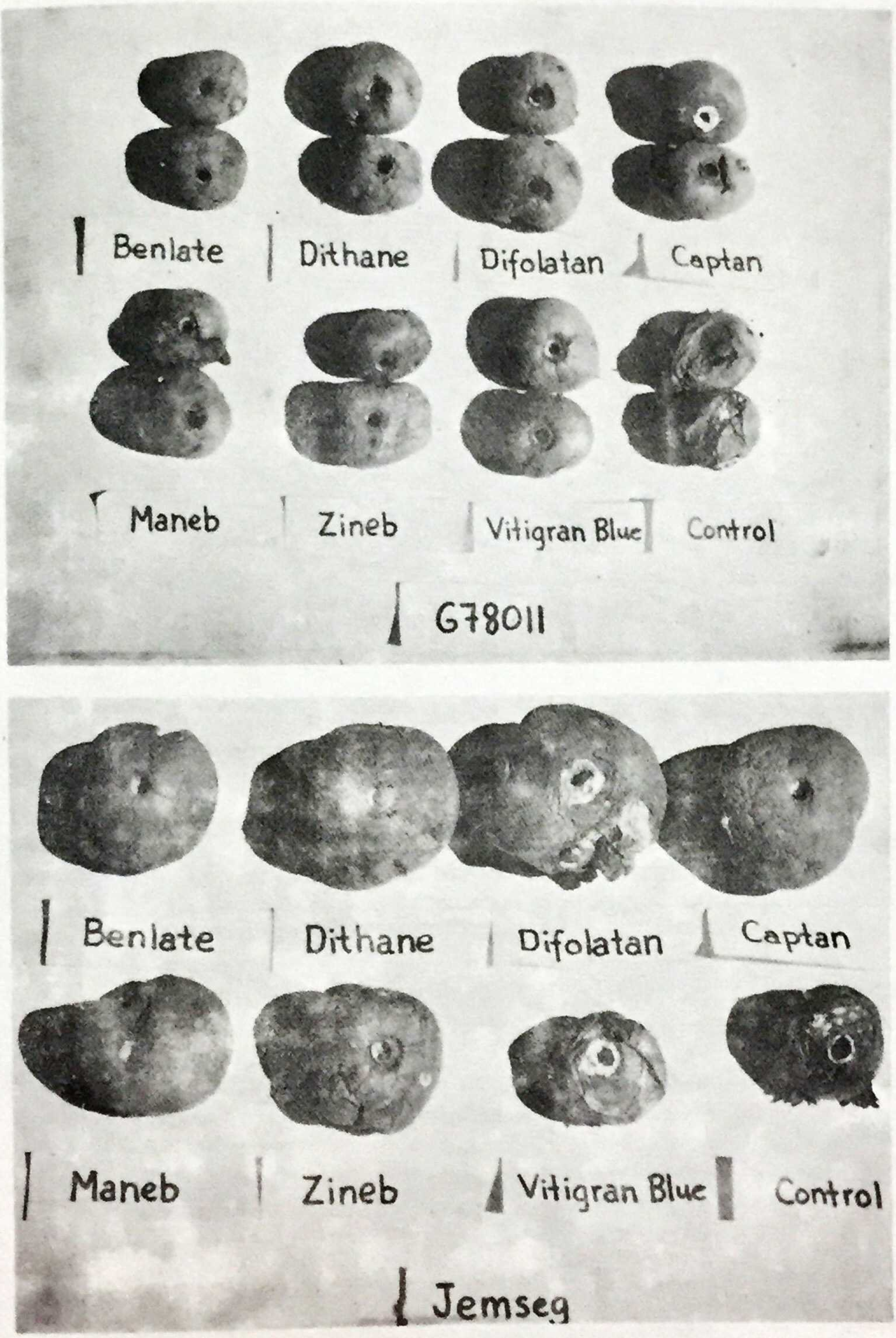

Figure 1. Effect of the different fungicidal treatments on the diameter of *Fusarium* dry rot lesions in cv. 678011 and cv. Jemseg tubers.

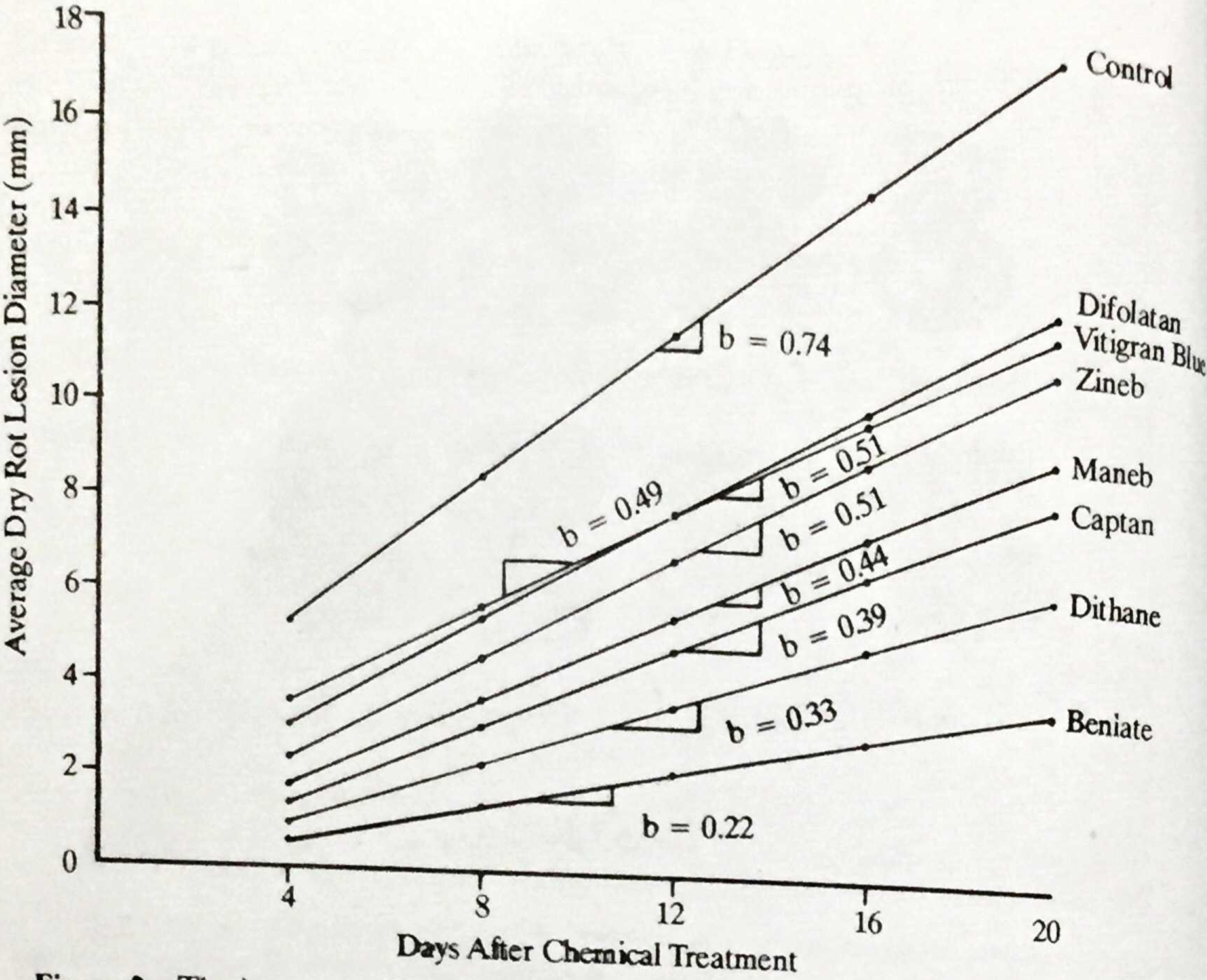

Figure 2. The increase of dry rot lesion diameter with time after the different fungicidal treatments showing the computed rate of increase in lesion diameter (b).

Table 3. Average number of tubers showing *Fusarium* dry rot lesions in each potato cultivar at different time intervals.

Cultivar	Average Number of Infected Tubers[1]				
	Days after Chemical Treatment				
	4	8	12	16	20
65-ZA-5	1.0	1.9	2.8	3.4	3.6
678011	1.5	2.6	3.0	3.6	3.9
LT-5	2.3	3.2	3.6	3.9	4.1
LT-1	2.1	2.9	3.5	3.9	4.2
Jemseg	2.1	2.9	3.9	4.3	4.4
Desiree	2.3	3.4	3.6	4.2	4.4
Cosima	2.4	3.1	3.7	4.2	4.4
Sequoia	2.6	3.6	4.0	4.4	4.5
C.V. (%)	47.2	27.1	20.1	16.9	16.8

[1]Average of six tubers per replicate per treatment. In a column, means are not significantly different.

Table 4. Average dry rot lesion diameter and rate of increase in lesion diameter (b) in each potato cultivar at different time intervals.

Cultivar	Average Lesion Diameter[1]					
	Days after Chemical Treatment					
	4	8	12	16	20	b[2]
65-ZA-5	1.3	2.4	3.8	5.1	5.9	0.31
678011	1.7	3.2	4.6	6.4	7.9	0.40
LT-5	2.8	4.8	6.4	8.2	10.2	0.47
LT-1	2.6	4.2	5.9	7.7	9.6	0.45
Jemseg	2.6	4.3	6.2	8.5	10.3	0.50
Desiree	2.9	5.0	6.0	9.0	11.3	0.53
Cosima	3.0	4.9	6.7	8.8	10.9	0.51
Sequoia	3.3	5.5	7.4	9.3	11.1	0.50
C.V. (%)	44.8	30.7	30.3	24.1	23.1	24.0

[1] Average of six tubers per replicate per treatment. In a column, means are not significantly different.

[2] Computed slope by linearly regressing lesion diameter and observation time.

lands. Thus, present potato production is restricted to the difficult, often inaccessible, steep mountain terrain in the Philippines where climatic conditions favor its growth (Vander Zaag et al., 1983).

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