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Effect of Fungicides on Growth of *Rhizobium japonicum In Vitro**

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Pesticide seed treatment is widely used to control insect damage and seed borne pathogens and to promote early plant emergence. A previous study (TU 1977) indicated that the fungicide thiram, singly or in combination with lindane and/or chlorpyrifos, significantly delayed growth of soybean plants and affected the activity of nitrogenase in the symbiotic fixation of nitrogen. An inhibitory effect of thiram on rhizobia was reported by many workers (HARTY and BYGOTT 1964, MILTHORPE 1945, RUHLOFF and BURTON 1951), but information on the bactericidal activities of fungicides on rhizobia is limited. The object of the present study was to determine if some protective fungicides are more specific than others in their bactericidal activities against Rhizobium japonicum, and, if so, to select the least toxic fungicides for further studies on soybean seed treatments.

MATERIALS AND METHODS

Growth inhibition of 13 selected fungicides (Table 1) commonly used for control of a wide range of plant pathogens were tested by the filter paper disc method against 3 strains of Rhizobium japonicum on yeast extract mannitol agar (YEMA). Strains 3I1b6 and 3I1b110 were kindly provided by Dr. D. F. Weber. RI16 was isolated previously from soybean root nodules (TU, 1977). Seeded medium for paper disc assays was prepared by adding 10 ml of the bacterial suspension containing $5x10^8$ cells/ml to 100 ml of the medium at 45°C before dispensing into petri-dishes. With the exception of dodine, folpet and Pyroxychlor, the required amounts of fungicides were dissolved in water and applied on 10-mm filter paper discs by micropipettes. Dodine, folpet and Pyroxychlor were dissolved in acetone and applied by a similar manner. Control discs with acetone or water only were dipped into acetone or water. The solvent was allowed to evaporate before placing the discs on the agar surface. Plates were incubated at 28°C for 7 days and then the zones of growth inhibition surrounding the discs were measured. As a basis for comparison, the inhibition zones from discs in mm were recorded. Each plate had four discs containing four levels of fungicide. There were five replications of each plate.

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TABLE 1

Effects of fungicides on growth of three species of Rhizobium japonicum by disc inhibition tests. Figures represent inhibition zones from disc in mm

Fungicide	Rhizobium japonicum strains												Remark
	3I1b6				3I1b110				RI16				
	Concentration of active ingredients (μg/ml medium)												
	5000	500	50	5	5000	500	50	5	5000	500	50	5	
Benomyl[a]	1.3ef*	0 g	0 d	0 c	1.6f	0 f	0 e	0 b	2.8e	1.8d	0.6d	0 c	
Captan	6.4c	4.6b	3.4b	0 c	6.6c	4.8b	2.0b	0 b	8.8c	6.4a	4.2b	2.2a	
Carbendazin[b]	0 g	0 g	0 d	0 c	0 g	0 f	0 e	0 b	0 f	0 f	0 e	0 c	
Chloroneb[c]	2.5e	0.8f	0 d	0 c	0 g	0 f	0 e	0 b	0 f	0 f	0 e	0 c	
Chlorothalonil[d]	4.8d	3.0d	1.2c	0.1b	2.6e	1.0e	0.1d	0 b	6.0d	6.0a	4.6b	1.8a	
Dodine[e]	4.2d	3.2cd	0.7c	0 c	3.6d	2.8c	0.8c	0 b	3.0e	2.0cd	0 e	0 c	S**
Fenaminosulf[f]	11.2a	4.0bc	0 d	0 c	10.2a	1.0e	0 e	0 b	14.0a	6.8a	0 e	0 c	
Folpet[g]	2.4e	2.0e	0.5c	0 c	3.0de	1.0e	0 e	0 b	3.7e	2.8bc	1.5c	0.5b	S
Metazoxolon[h]	0.4f	0 g	0 d	0 c	1.0f	0 f	0 e	0 b	3.6e	0.4e	0 e	0 c	
Pyroxychlor[i]	0 g	0 g	0 d	0 c	0 g	0 f	0 e	0 b	0 f	0 f	0 e	0 c	S
Quintozene[j]	0 g	0 g	0 d	0 c	0 g	0 f	0 e	0 b	0 f	0 f	0 e	0 c	
Thiram[k]	12.2a	10.6a	6.4a	2.5a	10.8a	8.2a	5.8a	1.7a	11.2a	6.6a	6.0a	2.2a	
Zineb[l]	9.6b	1.9e	0 d	0 c	7.4b	1.8d	0 e	0 b	8.4c	3.4b	1.4c	0 c	
Control	0 g	0 g	0 d	0 c	0 g	0 f	0 e	0 b	0 f	0 f	0 e	0 c	

* Values within each column indicated by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

**S = slightly soluble in water.

a = Benlate, b = MBC (Bas 3460F), c = Demosan 65W, d = Daconil 2787W75, e = Cyprex 96.9%, f = Dexon 70%WP, g = Phaltan 80%, h = Metazoxolon 50%, i = Pyroxychlor 8%, j = Terraclor 75%, k = Arathan 75, l = Dithane Z-78.

RESULTS AND DISCUSSION

The strains differed in their sensitivity to some fungicides, particularly with benomyl, captan, chloroneb, chlorothalonil, dodine, fenaminosulf, folpet, Metazoxolon and zineb (Table 1). Strain RI16 showed greater inhibition by captan, chlorothalonil and folpet at all levels, by benomyl and zineb at 50 µg/ml and higher, and by fenaminosulf and Metazoxolon at 500 and 5000 µg/ml.

The results indicated that fungicides carbendazin, Pyroxychlor and quintozene were the least toxic and thiram the most toxic chemical followed closely by captan, zineb and then by chlorothalonil, dodine, folpet and benomyl. The toxic effect of fenaminosulf at 5000 µg/ml was equal to that of thiram to strains 3I1b6 and 3I1b110 and was greater to RI16. Strain RI16 was more susceptible to benomyl than 3I1b6 and 3I1b110. The most resistant strain tested was 3I1b110, which was affected by all concentrations of thiram. A selective action in which one fungicide is more toxic than others was found. The marked differences in toxicity between seed protecting chemicals observed in culture work seem to indicate that some materials are safer to use in conjunction with a legume inoculant than others.

With the exception of dodine, folpet and Pyroxychlor, formulated chemicals were used. They dissolved readily in water and diffused easily through the medium. Not all

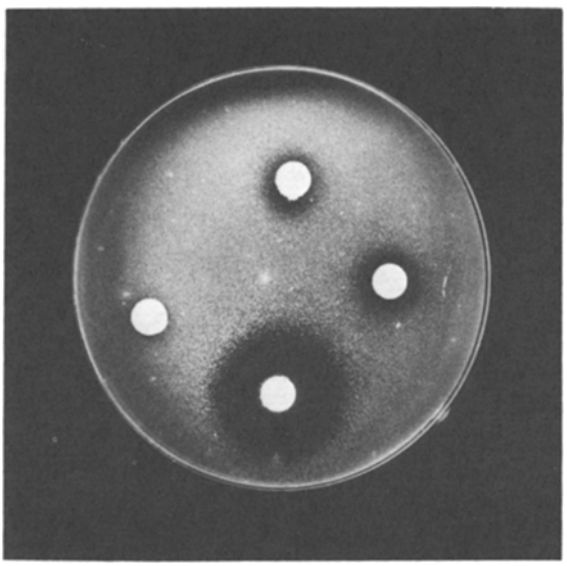

Figure 1. Bacteriostatic effect of a fungicide on Rhizobium japonicum.

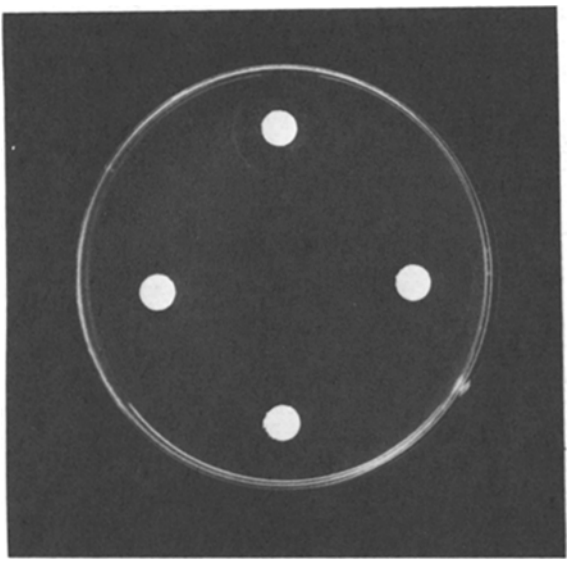

Figure 2. A polymodal effect of a fungicide on Rhizobium japonicum 3I1b110.

growth inhibition zones with fungicides represent bactericidal action. The determined effect found in disc test can also be due to bacteriostatic action on bacterial growth or an influence on the mechanism of bacterial cell metabolism in adaptation to the fungicides. With some fungicides there was a fading of the zone of inhibition (Fig. 1) which indicates bacteriostatic inhibition then regrowth as the bacteria adapt to the fungicide. The size of the inhibition zone in culture may not give a true measure of toxicity to rhizobia since the test depends not only on the sensitivity of the test organism but also on tha concentration of the chemical and its ease of diffusion through the agar medium. The size of this zone was independent on the chemicals and on their concentrations and so differed from the other fungicides tested.

One effect of a fungicide on growth of strain 3I1b110 is shown in Fig. 2. It is interesting to note that the rhizobia were stimulated at the periphery of the toxic zones. This was especially noticeable when thiram was used. This inversion or polymodal effect is similar to results obtained in investigations with fungicides by many workers (KAARS SIJPESTEYN et al. 1956, 1957, MONTGOMERY and SHAW 1943, RICHARDSON and THORN 1967).

The above results are from laboratory experiments and they should be related to field condition with caution since other environmental factors could also have an effect. The fungicidal effect in the disc test is expected to be much greater than that which would be found in soil.

The disc test is quick and exact and allows repeated determinations and measurements on the same samples. The

method reported seems a useful supplement to other methods used to determine the effects of pesticides on soil microorganisms.

In view of the results obtained, strains of rhizobia appear to differ in their ability to tolerate fungicides. It should be feasible to select less toxic compounds, ie benomyl, carbendazin, Metazoxolon and quintozene for further study as seed treatments and to quantitatively test their interaction with inoculants on soybean plant growth and nitrogen fixation.

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