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Compatibility of *Rhizobium japonicum* with Commercial Pesticides *in vitro**

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Rhizobial inoculant is commonly applied to seeds of legume crops to ensure effective nodulation and subsequent nitrogen fixation. This inoculant is often used in conjunction with fungicides and/or insecticides. In addition, rhizobia may become exposed to herbicides either present in the soil at planting time or later during the season. Most fungicides are inhibitory to rhizobia (AFIFI et al. 1969) but some are more toxic than others (STAPHORST and STRIJDOM 1976, TU 1980). Both adverse and innocuous effects of insecticides and herbicides on rhizobia have been reported (FAIZAH et al. 1980, SEKAR and BALASUBRAMANIAN 1979). However, an experimental herbicide and MCPA were found stimulatory to rhizobia in broth culture respectively by NELSON and HEDRICK (1976), and GILLBERG (1971). Discrepancies between reports may be due to concentration of the chemicals used and strains of the bacterium employed. Periodic evaluation of new pesticides for toxicity to Rhizobium japonicum may assist soybean growers in selection of compatible pesticides. The present investigation was concerned with relative compatibility of selected pesticides with different strains of R. japonicum in vitro.

MATERIALS AND METHODS

Ten strains of R. japonicum and 13 pesticides were evaluated. Strains 3Ilb6, 3Ilb110, 3Ilb122, 3Ilb136, 3Ilb142, 3Ilb143, and 3Ilb144 were received through courtesy of Dr. H. Keyser, U.S.D.A., Beltsville, MD. Strain LU1 was isolated in our laboratory from a nodule on a soybean root inoculated with peat inoculant kindly supplied by the Nitragin Co., Milwaukee, WI. Strains 10324 and 11927 were purchased from American Type Culture Collection (Rockville, MD).

Because growers use commercial formulations of pesticides, we used commercial formulations. The following pesticides were used in paper disc experiments: alachlor (Lasso), captafol (Difolatan), captan (Captan 50 WP), carbaryl (Sevin), carboxin (Vitavax), diazinon (Diazinon 50 W), fenaminsulf (Lesan), malathion (Malathion 55), mancozeb (Dithane M-45), metribuzin (Sencor), PCNB (Terraclor), thiram (Arasan 70-S), and trifluralin (Treflan). An amount equivalent to rates 0.5, 1, 2 and 5 times the recommended rate of each pesticide was suspended (wettable powders) or diluted (emulsifiable concentrates) in sterile distilled water.

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Filter paper discs (7 mm diameter) were soaked in prepared suspensions or emulsions. Plates of yeast extract-mannitol agar (YMA) (VINCENT 1970) were seeded with 0.1 ml of the inoculum (ca. 2 X 10^6 cells/ml harvested at an early logarithmic phase of growth) and spread uniformly with a bent glass rod. After air drying 30-45 min., four filter paper discs containing different concentrations of a pesticide were placed equidistant from each other. The lowest concentration was 25 μg/disc except for mancozeb which was 5 μg and PCNB, 250 μg. All calculations were based on active ingredient content of the pesticides. Seven replicate plates of each chemical were made. The diameter of the inhibition zone was measured after one week of incubation at 30 C (Fig. 1). The diameters of the inhibition zone produced at each concentration of fungicides were averaged for all strains to determine toxicity of a chemical. Also, these diameters for all fungicides at each concentration were combined to determine sensitivity of a strain. The data for insecticides and herbicides were similarly treated.

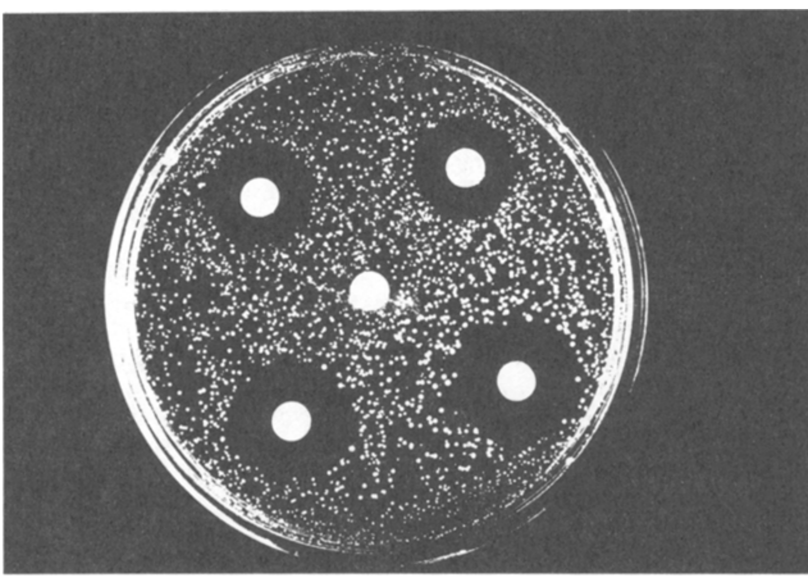

Figure 1. Growth inhibition of Rhizobium japonicum by a fungicide.

In addition to paper disc tests, 13 pesticides (Table 1) were tested against R. japonicum 3Ilb110 in broth culture. Inoculum from an early logarithmic growth phase in yeast extract-mannitol broth (YMB) was harvested by centrifugation (20,000 g) for 20 min. at 5-7 C, washed three times in Ringer's solution (COLLIN 1976), and resuspended in the same solution to obtain approximately 2 X 10^8 cells/ml. Each pesticide, except 2,4-DB, was suspended in water, and 5 ml of the suspension were added to 45 ml YMB contained in 250 ml culture flask to obtain the desired concentration. An appropriate quantity of ethanolic solution of 2,4-DB was pipetted into YMB to obtain the desired concentration. One milliliter of the inoculum suspension in Ringer's solution was added to YMB supplemented with pesticide. Control flasks received equal volumes of water or ethanol (in case of 2,4-DB) instead of a pesticide suspension or solution. Triplicate flasks of each chemical were made.

The flasks were incubated at 29 C on a rotary shaker (Environ-shaker 3597, Lab-Line) set at 110 rpm; viable numbers of rhizobia were determined at 0 and 48 hr of incubation by dilution plate count on YMA supplemented with Congo Red (to facilitate detection of possible contamination).

RESULTS AND DISCUSSION

Relative toxicity of seven fungicides against 10 strains of R. japonicum is presented in Fig. 2. Fenaminsulf and PCNB proved to be non-toxic to all strains even at the highest concentration tested. Based on diameter of inhibition zones, the fungicides in order of increasing toxicity were captan, captafol, thiram, mancozeb and carboxin. Similar variation in toxicity of different fungicides against different species of Rhizobium have been reported by others (STAPHORST and STRIJDOM 1976, TU 1980, FAIZAH et al. 1980). The inhibition zone around thiram treated discs was not complete in the sense that very light growth appeared within the inhibition zone. The inhibition zone in general increased proportionately with increasing concentration of the fungicide. Water solubility of a fungicide had little relation with level of toxicity; for example, mancozeb and PCNB both have identical solubility but the former produced large inhibition zones, and the latter did not produce any.

Different strains of R. japonicum differed in their sensitivity towards fungicides. In order of increasing sensitivity the strains were 3Ilb143, 3Ilb6, 3Ilb144, 3Ilb110, 10324, 3Ilb122, 3Ilb136, 11927, 3Ilb142 and LU1. FAIZAH et al. (1980), and KAPUSTA and ROUWENHORST (1973) found similar differences in sensitivity towards pesticides among different species and strains of the same species of Rhizobium. Strain LU1 was highly sensitive to thiram and carboxin but less sensitive to captan than several other strains, e.g. 3Ilb143, 3Ilb144.

Among three insecticides tested, diazinon was non-inhibitory (Fig. 2). Carbaryl produced an inhibition zone only at 250 µg/ml level, but malathion proved inhibitory at all four concentrations. Strain 3Ilb144 showed a high degree of tolerance to insecticides. TU (1977) found lindane, a chlorinated hydrocarbon, and chlorpyriphos, an organophosphate insecticide, inhibitory to R. japonicum . All five insecticides tested by DIATLOFF (1970) were inhibitory to four selected strains of Rhizobium sp.

The herbicides alachlor and trifluralin inhibited growth of all strains, trifluralin being more toxic than alachlor (Fig. 2).

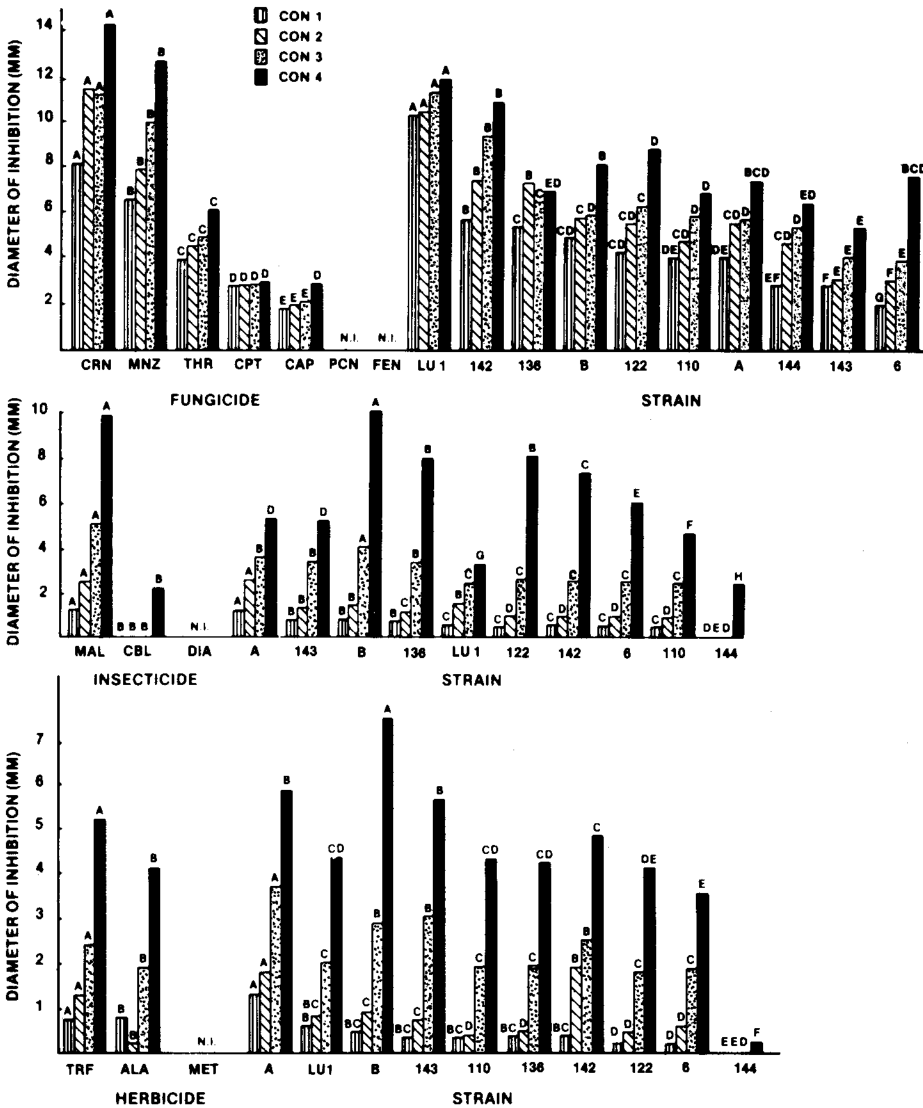

Figure 2. Evaluation of seven fungicides, three insecticides, and three herbicides at four concentrations for their toxicity to R. japonicum, and comparison between 10 strains of R. japonicum for their sensitivity to the fungicides, insecticides and herbicides. Within concentrations, values for the histogram with the same letters are not significantly different at 5% level by Duncan's New Multiple Range Test.
ALA = Alachlor, CPT = Captafol, CAP = Captan, CBL = Carbaryl, CRN = Carboxin, DIA = Diazinon, FEN = Fenaminsulf, MAL = Malathion, MET = Metribuzin, MNZ = Mancozeb, PCN = PCNB, THR = Thiram, TRF = Trifluralin.
A = ATCC 10324, B = ATCC 11927

Metribuzin was non-inhibitory. Strain 3I1b144 was highly tolerant to all herbicides tested. KAPUSTA and ROUWENHORST (1973) found chlorpropham, out of 13 herbicides tested, to be bacteriostatic at 15 μg/ml concentration.

Five fungicides, three insecticides and five herbicides were tested against R. japonicum 3I1b110 for compatibility in broth culture. Table 1 summarizes the results. The results from paper disc test were comparable with those from broth culture; fenaminsulf and PCNB were non-inhibitory by both methods. Captan, carboxin and thiram inhibited growth of the bacterium to varying degrees. Among the three fungicides in disc experiment, carboxin produced the largest inhibition zone followed by thiram and captan. In broth culture the fungicides in order of increasing toxicity were thiram, carboxin and captan. This apparent discrepancy between results obtained by broth culture and paper disc methods may be explained by the fact that the action of thiram was bacteriostatic and that of captan and carboxin at a higher dose was bacteriocidal. This assumption is supported by our observation of an incomplete nature of the inhibition zone induced by thiram on agar plates. Again, water solubility of a fungicide was not related with its degree of toxicity.

Malathion inhibited growth of R. japonicum both in agar plate and broth culture. Toxaphene and acephate were non-inhibitory in broth culture. KAPUSTA and ROUWENHORST (1973) noted inhibitory effect of disulfoton and carbaryl in broth culture.

Table 1. Survival of R. japonicum 3I1b110 in YMB after 50 hr incubation with varying concentrations of different pesticides

Pesticide	Number of surviving rhizobia (Log_{10})					
	Concentration of pesticide (μg/ml)					
	0	5	10	25	50	250
Acephate (Orthene)	6.6	N.D.*	N.D.	6.3	6.3	6.2
Alachlor (Lasso)	7.2	7.3	7.2	7.1	N.D.	N.D.
Captan (Captan 50 wp)	7.3	N.D.	N.D.	7.0	5.6	0.0
Carboxin (Vitavax)	7.3	N.D.	N.D.	6.9	6.6	5.5
2,4-DB	7.2	7.2	7.1	6.6	N.D.	N.D.
Fenaminsulf (Lesan)	6.4	N.D.	N.D.	6.6	6.5	6.2
Glyphosate (Roundup)	7.2	7.1	7.1	7.1	N.D.	N.D.
Malathion (Malathion 55)	6.6	N.D.	N.D.	6.3	N.D.	4.1
Metribuzin (Sencor)	7.2	7.4	7.3	7.3	N.D.	N.D.
PCNB (Terraclor)	6.4	N.D.	N.D.	6.4	6.6	6.8
Thiram (Arasan)	7.3	N.D.	N.D.	7.0	6.8	6.6
Toxaphene	6.6	N.D.	N.D.	6.4	6.5	6.2
Trifluralin (Treflan)	7.2	7.3	7.2	7.2	N.D.	N.D.

* Not Determined

Of the five herbicides tested only 2,4-DB at 25 μg/ml level reduced growth. GILLBERG (1971) reported that dinoseb and MCPA were inhibitory to most strains of *R. leguminosarum* and *R. trifolii*, but one strain of *R. meliloti* was stimulated by MCPA at 50-200 μg/ml.

The broth culture method of assay of pesticide toxicity appears to be more sensitive than the paper disc method. It is apparent that different pesticides differ in their toxicity to nodule bacteria. It is inappropriate to extrapolate these results to field conditions, where harmful effect of the pesticide is likely to be minimized by several soil factors. The *in vitro* tests indicate relative toxicity of pesticides to nodule bacteria. It is safer to use a chemical with minimal toxicity to rhizobia.

Our results indicated that different strains of *R. japonicum* differ in their sensitivity towards different pesticides. Therefore, strain selection for high tolerance to pesticides in the preparation of commercial inoculant should be of practical interest to soybean growers.

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