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TOXICITY OF CHLORONITROBENZENES TO *FUSARIUM OXYSPORUM* AND *RHIZOCTONIA SOLANI* AS RELATED TO THEIR STRUCTURE

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The structure–activity relationship of several chlorinated nitrobenzenes was studied using two soilborne fungi, *Fusarium oxysporum* f. sp. *melonis* Snyder and Hansen and *Rhizoctonia solani* Kühn. Fungitoxicity increased with the increase in number of chlorine substituents and was also affected by the position of the halogens on the phenyl ring. A linear relationship was obtained when the fungitoxicity values (EC_{50}) of the compounds were plotted against their lipophilicity values calculated from octanol-water partition coefficient π. *R. solani* was much more sensitive than *F. oxysporum* to chloronitrobenzenes, particularly with respect to the pentachloro derivative.
KEY WORDS: *Fusarium*; pentachloronitrobenzene; *Rhizoctonia*; nitrobenzene; structure—activity relationship.

INTRODUCTION

The fungicidal activity of chloronitrobenzenes has been studied by several workers (1,4,5,7,11). The pentachloro (PCNB, quintozene) derivative has been used as a commercial fungicide against soilborne plant pathogens, such as *Rhizoctonia solani* and *Sclerotium rolfsii*, and the 2,3,5,6-tetrachloro (tecnazene) derivative has been applied against *Fusarium caeruleum* (1). Unlike *R. solani*, *S. rolfsii* and some species of *Penicillium*, which are highly sensitive to PCNB, *Fusarium* and *Pythium* species are remarkably tolerant to this fungicide (4,7,10). The tetrachloro analogs are active against

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Fusarium (1), but phytotoxicity has limited the commercial value of the 2,3,4,6- and the 2,3,4,5- tetrachloro compounds (1,4).

Determination of toxicity of chemicals to organisms is done by exposing the tested organism to the toxicant. A common method is to grow the test organism on a growth medium supplemented with the tested compound at different concentrations. Toxicity is expressed by the EC_{50} value, which represents the effective median concentration (8). Increase in the number of chlorine substituents leads to an increase in fungistatic activity of chloronitrobenzenes against *R. solani* and *Pythium ultimum* (4,9,12). This increase is in good correlation with the increase in the lipophilic values of these compounds, apparently due to easier penetration of a lipophilic compound through membrane barriers. The lipophilic nature of the compounds can be determined by the π constant, which represents the octanol-water partition coefficient of a given structure relative to that of the parent compound (5). A similar correlation was obtained in a structure-activity relationship study of chlorophenols using *R. solani* and *F. oxysporum* (2). In the present work the relationships among structure, physical properties and biological activity of various chlorinated nitrobenzenes were examined, using the soilborne phytopathogenic fungi *F. oxysporum* and *R. solani.*

MATERIALS AND METHODS

Chemicals

The chemicals: 2-chloronitrobenzene, 4-chloronitrobenzene, 2,3-dichloronitrobenzene, 2,4-dichloronitrobenzene, 3,4,6-trichloronitrobenzene, 4,5,6-trichloronitrobenzene, 2,3,4,5-tetrachloronitrobenzene, 2,3,5,6-tetrachloronitrobenzene, 2,3,4,5,6-pentachloronitrobenzene, purchased from Sigma (St. Louis, MO, USA), were of 95–98% purity. All other chemicals used in the experiments were of analytical grade.

Fungi

Fusarium oxysporum Schlecht. f. sp. *melonis* Snyder and Hansen and *Rhizoctonia solani* Kühn were initially isolated from diseased melon and strawberry plants, respectively. Both fungi were grown in the dark in an incubator at 27°C on Czapek-Dox agar medium containing 1 g K_2HPO_4, 2 g $NaNO_3$, 0.5 g KCl, 0.01 g $FeSO_4$, 0.5 g $MgSO_47H_2O$, 0.25 g chloramphenicol, 30 g sucrose, 20 g agar, and 1 *l* distilled water (3).

Toxicity assays

the toxicity assays were carried out essentially as described previously (2). The chloronitrobenzenes were dissolved in dimethylsulfoxide (DMSO), and 50 µl of the

tested toxicant solution was added to 15 ml of melted (50°C) Czapek-Dox agar medium, to give the final indicated concentration. This amount of DMSO (final concentration of 0.3%) had no observable effect on fungal growth. The range of the compound concentrations tested was between 10^{-5} and 10^{-3}M for the monochloro and the dichloro derivatives, between 10^{-6} and 10^{-5}M for the trichloro derivatives, and between 10^{-7} and 10^{-5}M for the tetrachloro derivatives and PCNB. Agar discs (4 mm) were removed from the periphery of a 5-day-old culture of *Fusarium* or a 3-day-old culture of *R. solani* on Czapek-Dox medium, and placed in the center of a petri dish containing the toxicant-supplemented medium. The plates were incubated in the dark at 27°C, until the fungal hyphae in the untreated controls were 5–6 mm from the edge of the plate.

Growth inhibition was determined by the following formula, as described previously (2):

$$\% \text{ Inhibition} = 100 - \frac{R^2}{r^2} \times 100$$

where R and r represent the radius of fungus colony in the treated and control plates, respectively. The median effective concentration values for the toxicants (EC_{50}) were determined from computerized log concentration — probit regression lines (8). Lipophilicity of the compounds was defined using Hansch's partition coefficient π (5). The π values are the octanol:water partition coefficient values of each chloronitrobenzene compound compared with the coefficient of the parent nitrobenzene compound. These values were derived from the data of Fujita *et al.* (5). A correlation between fungitoxicity — using EC_{50} values — and lipophilicity was determined by means of regression analysis. All experiments were carried out in three replicates (plates) and repeated three times.

RESULTS

The level of fungitoxicity of various chloronitrobenzenes to *F. oxysporum* and *R. solani* is shown in Table 1. The inhibitory effect of these chemicals increased with the increase in the number of chlorine substituents, with the exception of PCNB for *Fusarium*. 2-Chloronitrobenzene and 4-chloronitrobenzene were least fungitoxic, whereas the tetrachloro and pentachloro derivatives were the most fungitoxic for *F. oxysporum* and *R. solani*, respectively. The chlorine position on the ring also affected fungitoxicity (especially to *R. solani*), which was more pronounced when the chlorine substitution was at the 3,4 rather than the 5,6 position. With both fungi the slopes of the regression lines increased with the increase in fungitoxicity.

Except for 4,5,6-trichloronitrobenzene, *R. solani* was more sensitive than *F. oxysporum* to all chloronitrobenzenes tested. The difference in sensitivity (EC_{50} values) between the two fungi was especially evident upon exposure to PCNB. *R. solani* was extremely sensitive to PCNB in comparison with *F. oxysporum*, which exhibited very high tolerance. Thus, the EC_{50} values for *F. oxysporum* could not be determined because of the flat slope in the plotted log-dose probit regression line. The linear regression lines (Fig. 1) show a significant negative correlation between the EC_{50} values and π values of the chloronitrobenzenes for both examined fungi.

TABLE 1

EC_{50}, SLOPE AND π VALUES OF CHLORONITROBENZENES FOR THE FUNGI *RHIZOCTONIA SOLANI* AND *FUSARIUM OXYSPORUM* F. SP. *MELONIS* [z,y]

Chloronitrobenzene ring substitution	*Fusarium*			*Rhizoctonia*			
	EC_{50} (log M)	*Slope*	r^2	*EC_{50} (log M)*	*Slope*	r^2	π[x]
2-chloro-	−3.56	0.017	0.9349	−4.05	0.061	0.7788	0.39
4-chloro-	−3.46	0.015	0.6771	−3.86	0.046	0.8469	0.54
2,3-dichloro-	−4.09	0.074	0.8980	−4.26	0.068	0.7848	1.01
2,4-dichloro-	−3.92	0.031	0.8902	−4.30	0.099	0.9502	0.94
4,5,6-trichloro-	−4.46	0.156	0.9326	−4.38	0.159	0.9846	1.55
3,4,6-trichloro-	−4.51	0.119	0.9826	−4.87	0.163	0.8599	1.55
2,3,5,6-tetrachloro-	−4.80	0.129	0.9117	−5.00	0.319	0.9211	2.02
2,3,4,5-tetrachloro-	−4.96	0.143	0.9184	−5.75	0.316	0.8237	2.16
2,3,4,5,6-pentachloro-[w]				−6.09	0.765	0.8289	2.56

[z]Figures represent average of three replicates. Experiments were repeated three times.

[y]EC_{50} values were calculated from computerized log concentration probit regression lines. Regression lines were significantly linear ($P = 0.01$). Slope indicates the slope of the regression line and r^2 represents the correlation of the regression analysis.

[x]π values are the octanol-water partition coefficients of the chloronitrobenzenes relative to those of nitrobenzene, and were derived from Hansch's formula (5).

[w]EC_{50}, slope and r^2 values of this compound for *Fusarium* could not be determined due to its very low fungitoxicity.

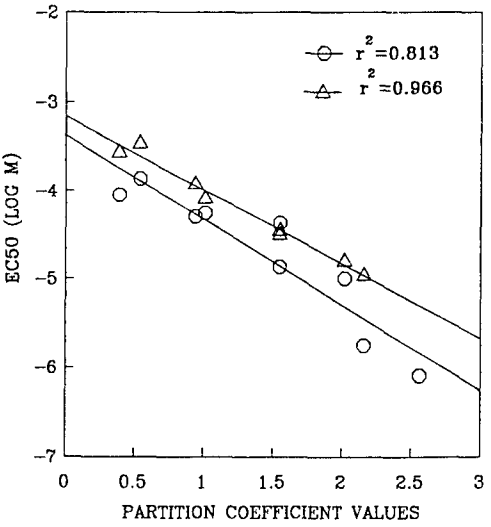

Fig. 1. The relationship between EC_{50} values of chloronitrobenzenes and their lipophilic parameters. The π values were calculated according to the partition in octanol:water of the chloronitrobenzenes relative to that of nitrobenzenes. Figures represent the average of three replicates. Experiments were repeated three times. Regression lines were significant (P = 0.05). *Fusarium oxysporum* (triangles); *Rhizoctonia solani* (circles).

DISCUSSION

A decrease in water solubility of chloronitrobenzenes as a result of an increase in the number of chlorine substitutions was shown by Eckert for *Rhizoctonia* and *Pythium* (4). A similar pattern with substituted chlorophenols was demonstrated by Cohen *et al.* (2) for *Fusarium* and *Rhizoctonia*. PCNB was an exception to this rule as regards *Fusarium* in the present work. In this study and a previous, similar, study carried out with chlorophenols (2), we showed for the same two fungi a linear correlation between the lipophilic properties of the toxicants, and their toxicity. Apparently, penetration of these chemicals through the fungal cell wall and cell membrane is a major factor determining their toxicity. Since *Fusarium* is very tolerant to PCNB but sensitive to the tetrachloro derivatives, it is likely that the mode of action of these two compounds is different. This is presumably related to cell barriers and to the physico-chemical nature of the toxicants which determine penetration and, thus, fungitoxicity. In general, *R. solani* is more sensitive to chloronitrobenzenes (Table 1, Fig. 1) and to chlorophenols (2) than is *F. oxysporum*. This difference in sensitivity is highly evident with PCNB.

Better knowledge of the structure–activity relationships of toxicants is another tool for revealing their mode of action.

REFERENCES

1. Brook, M. (1952) Differences in the biological activity of 2,3,5,6-tetrachloronitrobenzene and its isomers. *Nature, Lond.* **170**:1022.
2. Cohen, E., Gamliel, A. and Katan, J. (1988) The fungitoxicity of chlorophenols to the pathogenic fungi, *Fusarium oxysporum* and *Rhizoctonia solani*: A structure–activity relationship study. *Pestic. Sci.* **24**:139–146.
3. Collins, C.H. (1964) Microbiological Methods. Butterworths, London. pp. 86–110.
4. Eckert, J.W. (1962) Fungistatic and phytotoxic properties of some derivatives of chloronitrobenzene. *Phytopathology* **52**:642–649.
5. Fujyta, T., Iwasa, J. and Hansch, C. (1964) A new substituent constant π derived from partition coefficients. *J. Am. chem. Soc.* **86**:5175–5180.
6. Georgopoulos, S.G. (1963) Tolerance to chlorinated nitrobenzenes in *Hypomyces solani* f. *cucurbitae* and its mode of inheritance. *Phytopathology* **53**:1086–1093.
7. Katan, J. and Lockwood, J.L. (1970) Effect of pentachloronitrobenzene on colonization of alfalfa residues by fungi and streptomycetes in soil. *Phytopathology* **60**:1578–1582.
8. Klaassen, C.D. (1986) Principles of toxicology. *In*: Klaassen, C.D., Amdur, M.O. and Doull, J. [Eds.] Toxicology, the Basic Science of Poisons. 3rd ed. Macmillan Publ. Co., New York, NY. pp. 11–32.
9. Lukens, R.J. (1971) Chemistry of Fungicidal Action. Springer-Verlag, New York, NY. pp. 76–89.
10. Nakanishi, T. and Oku, H. (1969) Metabolism and accumulation of pentachloronitrobenzene by phytopathogenic fungi in relation to selective toxicity. *Phytopathology* **59**:1761–1762.
11. Reavill, M.J. (1954) Effect of certain chloronitrobenzenes on germination, growth and sporulation of some fungi. *Ann. appl. Biol.* **41**:448–460.
12. Richardson, L.T. (1968) Selective vapor phase activity of chloronitro- and chlorobenzenes in soil. *Phytopathology* **58**:316–322.