A RAPID METHOD FOR THE ASSESSMENT OF WATER SOLUBLE AND INSOLUBLE FUNGICIDES AND THE RESULTS OF EXAMINATION OF CERTAIN FUNGICIDES

by

M. G. Chatterjee, S. K. Ranganathan, B. B. L. Saxena and S. R. Sengupta

Defence Research Laboratory (Stores), Kanpur

ABSTRACT

A rapid and simple method for assessing water soluble fungicides based on the principles of fungicide's ability to inhibit the germination of fungal spores has been described. The relationship between concentration of fungicide and the extent of inhibition of germination of spores is transformed to the probit plane so that a quantitative appreciation of the fungicide is obtained. *Aspergillus niger and Memnoniella echinata* are used as test organisms. With slight change in procedure, the method is applicable for water-insoluble fungicides also.

Introduction

The need for a rapid method of screening fungicides intended to protect a wide variety of Service stores against attack by saprophytic fungi during adverse conditions of storage and use has been felt. In this context, the method recommended by the American Phytopathological Society[1,2,3] for the evaluation of fungicides against plant pathogens based on the fungicide's ability to inhibit the germination of spores appeared promising, but since the method as described involved the use of special equipment, an attempt has been made to simplify the technique so that it can be readily practised. Details are presented in this paper. While every effort was made to have an identical technique for the evaluation of both water-soluble and water-insoluble fungicides this was not found possible. A slight change in procedure has been found necessary for water insoluble fungicides.

Experimental Procedure

Test organisms—From the stock collection of fungi[4] in this laboratory, isolated from deteriorated Service stores, *Aspergillus Niger* was selected as the test organism. This organism has been found to be of very common incidence on a large number of materials. Where protection of cellulosic materials is concerned *Memnoniella Echinata* is the test organism of choice.

Preparation of Spore Suspension—Juice from fresh oranges was squeezed through a muslin cloth. The juice diluted with water nine times was filtered

through Seitz filter and collected aseptically in sterilised tubes. Spore suspensions of the test organisms were prepared in such orange juice from cultures 7-8 days old. The density of each spore suspension was adjusted with the help of hæmacytometer so as to have 50,000 spores/ml.

Procedure for evaluation of fungicides soluble in water—From standard solution of the fungicide in distilled water, a series of dilutions are prepared[5]. From each dilution, one ml. is transferred to a sterile test tube to which is then added one ml. of the spore suspension of the test organism. The mixture is shaken and three individual drops are pipetted on to each of three microscope glass slides. In a similar manner, a control set of three slides is prepared from the spore suspension alone not containing the fungicide. Both the assay and control sets are placed inside Petri dishes over moist filter papers and incubated simultaneously at $30\pm2°C$ for 18 hours. The slides are then removed and each drop examined under magnification ($\times100$) for (*a*) total number of spores in the field and (*b*) the number of spores whose germination has been inhibited. The percentage inhibition in the assay set is calculated. Under the conditions of the experiment the percentage germination in the control set should not be less than 90 per cent.; otherwise the experiment is repeated. It is ensured that five to eight concentrations causing inhibition ranging from about 30 per cent. to 80 per cent. are investigated.

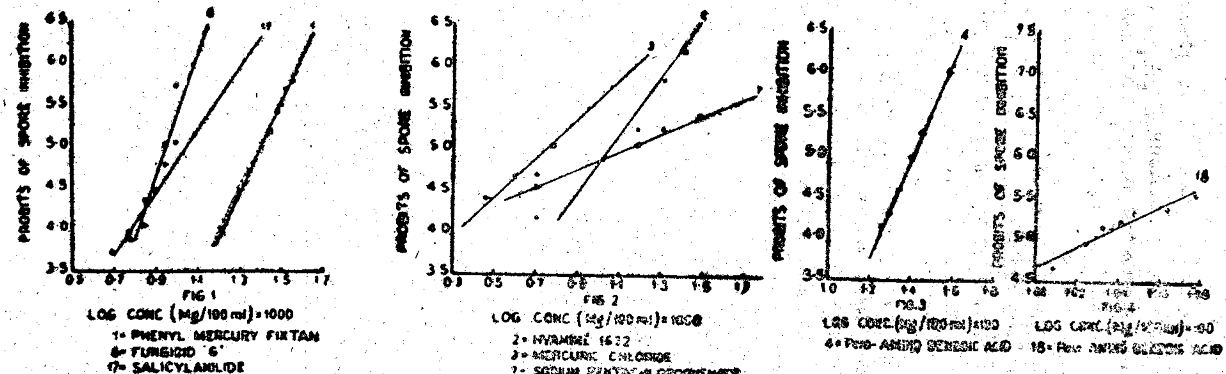
Procedure for evaluation of fungicides insoluble in water—A known quantity of the fungicide is dissolved in an organic solvent such as acetone[6] and a series of dilutions are prepared as in the case of water soluble fungicides—One ml. of each dilution is then transferred to separate test tubes containing 2 ml. of a freshly prepared 5·0 per cent. aqueous solution of gelatine (as used for photographic purposes). The test tube is shaken to effect homogeneous dispersion of the fungicide. 0·3 ml. of the dispersion is then pipetted on to a microscope glass slide and spread as uniformly as possible and allowed to dry in air. Three such slides are prepared for each dilution. Three individual drops of the spore suspension are then pipetted on to each slide. The slides are then placed in Petri dishes over moist filter papers and are then incubated at $30\pm2°C$ for 18 hours. A control set of three replicates in which spore suspension is placed on plain glass slides is also prepared and incubated along with the assay sets. The procedure for subsequent examination is the same as in the case of water soluble fungicides.

Results

The results obtained are graphically represented plotting the logarithm of percentage concentration of the fungicides against percentage inhibition of germination expressed as probits[7]. The regression line is subsequently fitted. The regression line enables one to determine the concentration of fungicide required for 50 per cent. or 90 per cent. inhibition of germination as may be required for the purpose of comparison of efficiency of different fungicides. Fiducial limits of LC50 and LC90 are also determined[8].

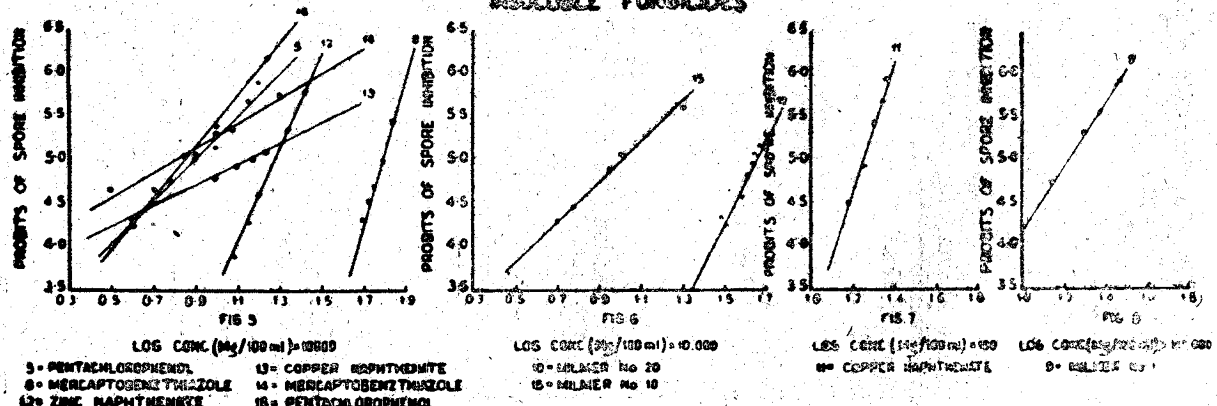
A number of water soluble as well as insoluble fnngicides was examined according to the above method. The results are given in Tables 1 and 2 and graphically presented in figures 1 to 4, and 5 to 8.

WATER SOLUBLE FUNGICIDES



1.2.3.4.6.7.17 TESTED AGAINST ASPERGILLUS NIGER. 18 TESTED AGAINST MEMNONIELLA ECHINATA

INSOLUBLE FUNGICIDES



8,9,10,11,15,16 TESTED AGAINST ASPERGILLUS NIGER 5,12,13,14 TESTED AGAINST MEMNONIELLA ECHINATA

Acknowledgements

The authors desire to thank Dr. T.S. Subramanian, Chief Superintendent and Mr. J. J. Bagchi, Superintendent of Development for their interest in this work. They are grateful to Shri S.S. Nigam for help in the preparation of the paper.

References

1. Anon, *Phytopathology*, **33**, 627, 1949.
2. Anon, *Phytopathology*, **37**, 354, 1947.
3. Horsfall, J.G. *et al.*, *Phytopathology*, **30**, 546, 1940.
4. Anon, Directory of collection of Micro-organisms and list of species maintained in India (1953).
5. Parker Rhodes, A.F., *Ann. Appl. Biol.*, **28**, 398, 1941.
6. Koopmans, M.J., *Philips Tech. Rev.*, **17**, 222, 1956.
7. Bliss, C.I., *Ann. Appl. Biol.*, **22**, 307, 1935.
8. Pradhan, S., Jotwani, M.G. and Rai, B.K., *Ind. J. Entomol.*, **18**, 315, 1956.

TABLE 1

Toxicity of certain water soluble fungicides

Serial No.	Name of fungicide	Test Organism	Heterogeneity	Regression equation	Standard error of *b* (Regression coefficient	Percentage concentration required for 50 per cent. inhibition of spores (Lc 50) (mg/100ml)	Percentage concentration required for 90 per cent. inhibition of spores (Lc 90) (mg/100ml)	Fiducial limits of Lc 50	Fiducial limits of Lc 90
1	SodiumPentachlorophenate.	*Aspergillus niger.*	$\chi^2(3)=0\cdot7012$	$Y=5\cdot10+1\cdot07(X-1\cdot23)$	$107\pm\cdot223$	0·00211	0·02159	0·00219 0·00202	0·02567 0·01816
2	Phenyl mercury Fixtan	,,	$\chi^2(3)=6\cdot4017$	$Y=5\cdot28+6\cdot0(X-1\cdot46)$	$6\cdot0\pm\cdot800$	0·02590	0·04235	0·02827 0·02377	0·05064 0·03542
3	Mercuric Chloride ..	,,	$\chi^2(3)=3\cdot4167$	$Y=4\cdot34+2\cdot37(X-68)$	$2\cdot37\pm\cdot451$	0·00056	0·00194	0·00068 0·00046	0·00462 0·00081
4	Salicylanilide (in dilute ammonia).	,,	$\chi^2(3)=30\cdot6124$	$Y=4\cdot30+3\cdot7(X-\cdot87)$	$3\cdot7\pm\cdot594$	0·01146	0·02544	0·02585 0·00503	0·19851 0·00326
5	*Para*-Amino benzoic acid.	,,	$\chi^2(3)=2\cdot0022$	$Y=4\cdot90+5\cdot9(X-1\cdot40)$	$5\cdot9\pm\cdot572$	0·26116	0·43033	0·28203 0·24182	0·51689 0·35892
6	*Para*-Amino benzoic acid.	*Memnoniella echinata.*	$\chi^2(5)=38\cdot677$	$Y=5\cdot13+11\cdot75(X-1\cdot04)$	$11\cdot75\pm2\cdot225$	0·10688	0·13740	0·10524 0·09913	0·18780 0·10053
7	Hyamine*	*Aspergillus niger.*	$\chi^2(3)=4\cdot2436$	$Y=5\cdot22+2\cdot82(X-1\cdot11)$	$2\cdot82\pm\cdot271$	0·00108	0·00306	0·00128 0·00091	0·00418 0·00225
8	Fungicide G** ..	,,	$\chi^2(3)=11\cdot3396$	$Y=4\cdot7+7\cdot5(X-\cdot91)$	$7\cdot5\pm\cdot830$	0·00891	0·01318	0·01246 0·00637	0·01877 0·00929

* Active ingredient 1, 1, 3, 3, Tetra methyl butyl—p—phenoxy ethoxy dimethyl benzyl ammonium chloride.

** Active ingredient 2, 2—Dihydroxy 5,5,—dichloro—diphenylmethane.

TABLE 2

Toxicity of certain water insoluble fungicides.

Serial No.	Name of fungicide	Test Organism	Heterogeneity	Regression equation	Standard error of b (Regression coefficient	Percentage concentration required for 50 per cent. inhibition of spores (Lc 50) (mg/100ml)	Percentage concentration required for 90 per cent. inhibition of spores (Lc 90) (mg/100ml)	Fiducial limits of Lc 50	Fiducial limits of Lc 90
1	Penta chlorophenol ..	*Aspergillus niger.*	$\chi^2(4)=1\cdot5718$	$Y=4\cdot901+2\cdot98(X-\cdot82)$	$2\cdot98\pm0\cdot219$	0·00708	0·01907	0·00302 0·00626	0·02445 0·01487
2	Vulcafor Daw† ..	„	$\chi^2(3)=3\cdot4793$	$Y=4\cdot73+8\cdot8(X-1\cdot75)$	$8\cdot8\pm1\cdot205$	0·06035	0·8439	0·06390 0·05701	0·10144 0·07021
3	Milmer No. 1* ..	„	$\chi^2(4)=4\cdot8710$	$Y=5\cdot29+7\cdot6(X-1\cdot29)$	$7\cdot6\pm1\cdot053$	0·000178	0·00026	0·00024 0·00017	0·00029 0·00023
4	Copper naphthenate ..	„	$\chi^2(5)=1\cdot7546$	$Y=5\cdot15+3\cdot7(X-1\cdot27)$	$3\cdot7\pm0\cdot361$	0·20440	0·37653	0·22223 0·18802	0·45698 0·31024
5	Milmer No. 10* ..	„	$\chi^2(4)=13\cdot6524$	$Y=4\cdot74+2\cdot36(X-0\cdot9)$	$2\cdot36\pm0\cdot073$	0·00102	0·00357	0·00113 0·00093	0·00461 0·00277
6	Milmer No. 20* ..	„	$\chi^2(4)=37\cdot300$	$Y=4\cdot3+4\cdot9(X-1\cdot5)$	$4\cdot9\pm0\cdot205$	0·00439	0·00802	0·00505 0·00382	0·01128 0·00570
7	Copper naphthenate ..	*Memnoniella echinata.*	$\chi^2(4)=1\cdot4734$	$Y=4\cdot84+1\cdot195(X-1\cdot02)$	$1\cdot195\pm0\cdot901$	0·01425	0·16842	0·02010 0·01011	1·08268 0·02620
8	Pentachlorophenol ..	„	$\chi^2(5)=3\cdot0345$	$Y=5\cdot13+2\cdot47(X-\cdot95)$	$2\cdot47\pm0\cdot259$	0·00789	0·02607	0·00893 0·00782	0·03569 0·01905
9	Zinc naphthenate ..	„	$\chi^2(3)=0\cdot2469$	$Y=4\cdot82+5\cdot42(X-1\cdot24)$	$5\cdot42\pm0\cdot512$	0·01875	0·03220	0·02044 0·01722	0·03967 0·2635
10	Vulcafor Daw† ..	„	$\chi^2(5)=0\cdot8960$	$Y=5\cdot32+1\cdot29(X-1\cdot03)$	$1\cdot29\pm0\cdot178$	0·00605	0·05962	0·00821 0·00459	0·11413 0·03115

† Active ingredient—Mercapto benzothiazole.

* Active ingredient—Copper—8—hydroxyquinolinolate