

# EFFICIENCY OF VERSATIC ACID AND ITS COPPER SALT IN INHIBITING THE SPORE GERMINATION OF *Memnoniella echinata*

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Studies on the inhibition of spores by spore germination technique, using *Memnoniella echinata* as a test organism, have revealed that copper versatate is more efficacious as a rot-proofing agent than versatic acid. Its efficiency in inhibiting spore germination is one-tenth that of copper naphthenate.

Copper and zinc naphthenates are used in large quantities as rot-proofing agents. Since naphthenic acids are in short supply and imported, it was of interest to ascertain the possibility of substituting them by readily available indigenous materials.

Versatic acid, a synthetic material developed by M/s Burmah Shell, is now available in commercial quantities. This acid and its copper salt have been investigated for their germination-inhibiting efficiency and compared with copper naphthenate.

## MATERIALS AND METHODS

*Versatic acid*—Versatic acid was obtained from M/s Burmah Shell & Co., New Delhi.

*Copper versatate*—The copper salt of versatic acid was obtained from Egham Laboratories, London. It contained 7.95 % copper by weight.

*Copper naphthenate*—Commercial copper naphthenate was obtained from the market. It contained 7.8% copper by weight.

*Memnoniella echinata* (Kanpur Culture No. 119)—The test organism used in the study was *Memnoniella echinata*. The slide germination technique<sup>1</sup> was employed for evaluating the percentage inhibition.

*Preparation of spore suspension*—The culture of the test organism was flooded with 2 ml of sterilised water and the surface was gently rubbed with a glass rod to detach the spores. The suspension was filtered through sterilised muslin cloth.

*Stimulant*—The spore suspension was diluted with an equal volume of germinating medium<sup>2</sup> which served as a stimulant. Nearly hundred percent germination of spores was observed in 18-20 hours.

*Slide germination technique*—Starting from a mother solution of the fungicide in an inert organic solvent (acetone), a series of dilutions were prepared. One ml of each diluted solution was transferred to a test tube containing 2 ml of a freshly prepared 5 per cent aqueous solution of gelatine. The test tube was then shaken to effect homogeneous dispersion of the fungicide. A measured quantity (0.3 ml) of the dispersion was then pipetted

to a microscopic slide and spread as uniformly as possible and allowed to dry in air. Three such slides were prepared for each dilution. Three individual drops of the spore suspension of the test organism were then pipetted on to each slide. The slides were then placed in Petri dishes over moist filter papers and incubated at  $30 \pm 2^\circ\text{C}$  for 18 hours. A control set for three replicates with spore suspension placed on plain glass slides was also prepared and incubated at  $30 \pm 2^\circ\text{C}$ . Slides were then removed and each drop was examined under magnification ( $\times 100$ ) for (i) total number of spores and (ii) number of spores whose germination had been inhibited. If the control showed less than 90 per cent germination in 18 hours, the experiment was repeated.

## RESULTS AND DISCUSSION

The results of percentage germination of spores of the fungus after 18 hours of incubation, using different concentrations of versatic acid, copper versatate and copper naphthenate are given in Table 1. It will be seen that the concentration of versatic acid required for complete inhibition of germination of spores of *Memnoniella echinata* is approximately ten times that of copper versatate, while the concentration of copper versatate is similarly ten times that of copper naphthenate.

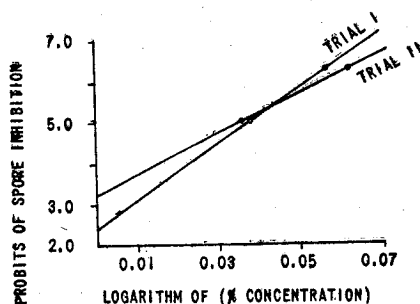


Fig. 1—Logarithm of (% concentration) Versatic Acid.

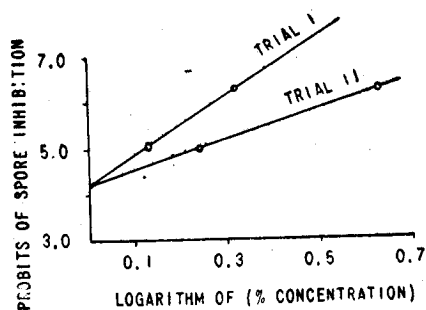


Fig. 2—Logarithm of (% concentration) Copper Versatate.

In Figs. 1—3, the log of percentage concentration of fungicides is plotted against percentage inhibition of germination expressed as probit.<sup>3</sup> The regression line is subsequently fitted. The regression line enables the determination of fungicide concentration required for 50 per cent or 90 per cent inhibition of germination as required. Fiducial limits of  $LD_{50}$  and  $LD_{90}$  are also determined<sup>4</sup>. The probit line and fiducial limits are given in Table 2.

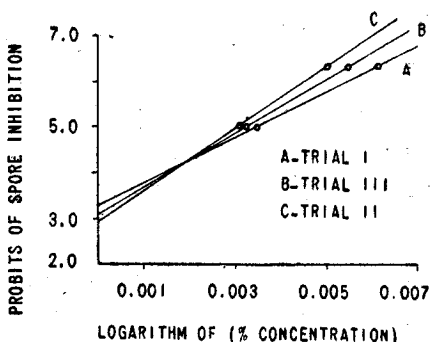


Fig. 3—Logarithm of (% concentration) Copper Naphthenate.

TABLE 1  
PERCENTAGE GERMINATION OF SPORES OF *Memnoniella echinata*

Copper naphthenate		Copper versatate		Versatic acid	
Conc. expressed as % copper	% germination (average of three trials)	Conc. expressed as % copper	% germination (average of two trials)	% Conc. (%)	% germination (average of two trials)
0.0020	90.8	0.020	85.8	0.04	100.0
0.0025	74.9	0.025	80.2	0.05	96.2
0.0030	62.3	0.030	75.8	0.06	95.0
0.0035	55.0	0.035	55.5	0.07	87.0
0.0040	41.7	0.040	47.0	0.08	67.2
0.0045	20.3	0.045	36.1	0.10	51.5
0.0050	4.8	0.050	19.0	0.15	45.4
0.0060	Nil	0.055	14.3	0.20	27.3
Control (w/o fungicide)	100	0.060	Nil	0.25	23.7
		Control (w/o fungicide)	100	0.30	9.1
				0.35	8.8
				0.40	6.4
				0.50	Nil
				Control (w/o fungicide)	100

TABLE 2  
TOXICITY OF COPPER NAPHTHENATE, COPPER VERSATATE AND VERSATIC ACID

Probit line	$x^2$ value	$LD_{50}$	Fiducial limits	$LD_{90}$	Fiducial limits
Copper naphthenate					
$Y=5.31588 X+18.00958$	17.03 (5)*	0.0035669	0.0030695; 0.0041535	0.0062199	0.0041484; 0.0093264
$Y=6.18748 X+15.43782$	7.69 (4)	0.0031724	0.0029784; 0.003379	0.0051111	0.0045063; 0.0057969
$Y=5.85785 X+19.49587$	9.51 (5)	0.0033527	0.0031904; 0.0035231	0.0055486	0.0050076; 0.0061480
Copper versatate					
$Y=7.53466 X+15.67557$	71.15 (4)*	0.038294	0.016229; 0.090362	0.056654	0.032250; 0.099525
$Y=5.56466 X+12.99208$	7.66 (8)	0.036625	0.035041; 0.038281	0.062243	0.056877; 0.068115
Versatic acid					
$Y=3.26012 X+7.88282$	20.07 (9)*	0.130536	0.11149; 0.15284	0.322738	0.24443; 0.42614
$Y=3.09894 X+7.89158$	3.68 (6)	0.245245	0.21619; 0.27187	0.635565	0.50596; 0.79836

(The figures within brackets give the degrees of freedom; significant).

## CONCLUSION

Statistical analysis of data on inhibition of spore germination by slide germination technique show that copper versatate is toxic to fungi. Its potency is one-tenth of copper naphthenate.

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