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Title FUNGITOXICITY OF SODIUM N-METHYLDITHIOCARBAMATE (VAPAM) AND ITS DECOMPOSITION PRODUCTS

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An assay for fungicidal activity of sodium N-methyldithiocarbamate (Na-MDC) and its decomposition products was developed in which there was a minimum influence due to the widely varying physical properties of the compounds (i. e. vapor pressure and water solubility). Decomposition of the toxicant during the tests was measured and held to a minimum. Tests were made against Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans. under aerobic and anaerobic conditions.

Under anaerobic conditions Na-MDC gave a bimodal dosage response (DR) curve. Vapam, a commercial preparation of Na-MDC, failed to give bimodal DR curve although it did produce a two phase curve. Under aerobic conditions both forms of NaMDC gave straight line DR curves.

Methyl isothiocyanate (MIT) produced identical DR curves under aerobic and anaerobic conditions, and thus, must have a

different mode of fungicidal action from Na-MDC. There was no decomposition of Na-MDC or MIT during the test period so toxicity differences must be due to the action of the chemicals per se.

N, N'-dimethylthiuram disulfide was highly fungitoxic and along with sodium trithiocarbonate, 2, 4-dimethyl-1, 2, 4-thiadiazolidine-3, 5-dithione and 4-methyl-5-methylimino-1, 2, 4-dithiazolidine-3-thione could account for the fungicidal effectiveness of Vapam. However, these compounds are not sufficiently volatile to explain the movement of the toxic principle from Vapam. Carbonyl sulfide, carbon disulfide, hydrogen sulfide, methylamine, N-N'-dimethylthiourea, and sulfur were not sufficiently fungicidal to account for the effectiveness of Vapam in plant disease control.

Young spores from an acid culture medium were less susceptible to MIT in buffer than in water. When older cells from a basic culture medium were used the buffer increased the susceptibility of the cells to MIT. Although MIT is probably the main toxic agent from Vapam, this study shows that other decomposition products may contribute to its overall fungicidal activity.

FUNGITOXICITY OF SODIUM N-METHYLDITHIOCARBAMATE (VAPAM) AND ITS DECOMPOSITION PRODUCTS

by

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FUNGITOXICITY OF SODIUM N-METHYLDITHIOCARBAMATE (VAPAM) AND ITS DECOMPOSITION PRODUCTS

INTRODUCTION

Sodium N-methyldithiocarbamate (Na-MDC) is a relatively non-selective soil fumigant marketed as Vapam and used to control soil borne plant pathogenic fungi, nematodes, insects and weed seeds. Fungitoxic action of Na-MDC is generally believed due to its decomposition to methyl isothiocyanate (MIT)(9, 17, 24), but additional products form either by decomposition of Na-MDC and MIT, or by reactions of their decomposition products (24). Although fungitoxicity of most known or potential decomposition products of Na-MDC has been investigated (Table 1), no attempt has been made in these studies to control decomposition of the toxicant during the assays. Comparisons of the fungitoxicity of these compounds from previous investigations may lead to invalid conclusions because all compounds have not been tested by a single method, and each of the several methods used favors compounds with particular physical properties (e.g. water solubility and vapor pressure).

In this investigation, an assay for fungicidal action was developed in which innate toxicity of Na-MDC and its decomposition products could be determined with minimum influence due to the widely varying water solubility and vapor pressure of the test compounds. The tests were made under conditions in which decomposition of the

Table 1. Sodium N-methyldithiocarbamate and its decomposition products.

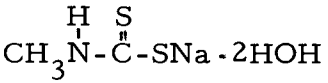
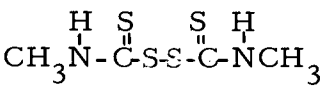
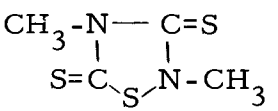
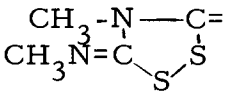
Chemical and abbreviation	Structure	Source of chemicals	Fungitoxicity literature citation
Sodium N-methyldithiocarbamate (Na-MDC)		Stauffer Chemical Co., Vapam (43.6% solution as the dihydrate or 34.1% active ingredient)	6, 7, 8, 9, 17, 24, 25
Methyl isothiocyanate (MIT)	$CH_3N{=}C{=}S$	Morton Chemical Co. (purified technical)	6, 7, 8, 9, 17, 23, 24, 25
N, N'-Dimethylthiuram disulfide (DMTD)		Fabriek van Chemische Producten Vondelingenplaat (Tridipam technical)	7, 8
2, 4-Dimethyl-1, 2, 4-thiadiazolidine-3, 5-dithione (DTD)		Stauffer Chemical Co. (experimental N1045)	21
4-Methyl-5-methylimino-1, 2, 4-dithiazolidine-3-thione (MMDT)		Stauffer Chemical Co. (experimental N1044)	21
Sodium trithiocarbonate (Na-TTC)	Na_2CS_3	Stauffer Chemical Co. (experimental N438)	

Table 1. (Continued)

Chemical and abbreviation	Structure	Source of chemicals	Fungitoxicity literature citation
Carbon disulfide	CS_2	J. T. Baker Chemical Co. (No. 9172, Reag. Grade)	2, 26
Carbonyl sulfide	COS	The Matheson Co., Inc.	16
Hydrogen sulfide	H_2S	The Matheson Co., Inc. (C. P. Grade)	2, 13, 14, 18, 22, 23
Methylamine	CH_3NH_2	Distillation Products Industries (40% in water, No. 527)	9, 23
N, N'-Dimethylthiourea (DMTU)	$CH_3\overset{H}{\underset{|}{N}}\text{-}\overset{S}{\overset{\|}{C}}\text{-}\overset{H}{\underset{|}{N}}CH_3$	Stauffer Chemical Co. (experimental N773)	8
Sulfur	S	Stauffer Chemical Co. Magnetic 6 Flowable Sulfur (50.7% aqueous dispersion)	13, 14, 25

toxicant was held to a minimum.

Knowledge of the relative toxicity of these compounds is necessary for an understanding of the fungitoxic mode of action of Na-MDC, and may ultimately aid in selecting soil conditions in which Vapam decomposition is guided most effectively toward production of highly fungicidal materials.

MATERIALS AND METHODS

Fungicidal action was measured using spores of strain R5-6 of Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans. grown in shake culture at 25 °C with 260 ft-c of artificial light on a 16-hour day. One liter of the liquid culture medium contained: 25 g glucose, 2.5 g KNO_3, 500 mg $MgSO_4$, 310 mg K_2HPO_4, 940 mg KH_2PO_4, 10 mg $ZnSO_4$, 10 mg $FeCl_3$, 1.25 mg H_2BO_3, 1 mg $CaCl_2$, 0.9 mg $MnCl_2$, 0.275 mg $CuCl_2$, and 0.01 mg MoO_3; the initial pH was 6.0. Spores were harvested during the log phase of growth (about 75 hrs. growth) by filtering through cheesecloth and then through Whatman No. 4 paper on a Buchner funnel. The spore suspension was centrifuged at 5860 × g for 10 minutes, the supernatant was discarded, and the spores were resuspended in sterile deionized water or 0.1 M phosphate buffer pH 5.8.

In a typical experiment, a spore suspension containing sufficient cells to give 500,000/ml in the final test solution was added to 12 cc glass vials. The vials were sealed with serum caps and the contents were flushed for five minutes with air or O_2-free helium obtained by scrubbing helium with an acetic-chromous chloride solution by the method of Stone and Skavinski (19). An inert atmosphere was provided to retard oxidative decomposition of the toxicants during the assays.

Spore suspensions flushed with O_2-free helium contained less than one ppm dissolved O_2, while the air flushed counterpart had 7-8 ppm O_2. Oxygen concentrations were determined by purging the cell suspensions with helium and determining the O_2 present with a Beckman GC-2A gas chromatograph using the method of Swinnerton, Linnebom and Cheek (20). The gas chromatographic determination was standardized against the Winkler method for dissolved O_2 (1).

Fungicide solutions were prepared immediately before each experiment using solvents flushed with O_2-free helium. Dilutions were made in sealed containers and transfers were made with syringes to minimize re-oxygenation. Oxygen-free helium was injected into the stock solutions during removal of the liquid contents to prevent development of a vacuum.

The test chemicals were injected into the spore suspensions, and the serum caps were momentarily punctured to release any pressure within the vials, because pressure or vacuum could influence fungicidal activity.

The vials containing treated cells were kept at 25° C on a mixing device that tumbled the vials 18 times per minute. After two hours incubation, the spore suspensions were diluted and a 3 ml aliquot was placed in 30 ml of potato-dextrose-agar (PDA) at 41° C adjusted to pH 3.2 with lactic acid. The PDA was distributed

in three petri plates, and after four days incubation the number of colonies in each plate was counted to determine fungicidal effectiveness. Each experiment was suitably replicated and when applicable analyzed statistically.

Stability of the test compounds during the tests was determined by measuring appropriate strongly absorbing bands in the ultraviolet region with a Beckman DB recording spectrophotometer.

RESULTS AND DISCUSSION

Sodium N-methyldithiocarbamate

The fungicidal activity of Na-MDC and its commercial preparation, Vapam, was determined in air and helium under conditions in which there was very little if any decomposition of the toxicant. A highly purified sample of Na-MDC was obtained by concentrating Vapam to about one third its original volume under reduced pressure in a flash evaporator at 30° C. The concentrate was cooled, the Na-MDC crystals were collected on Whatman No. 1 filter paper, repeatedly washed with chloroform, and finally recrystallized from acetone with diethyl ether.

Stability of Na-MDC and Vapam during the fungicide tests was measured by comparing the U. V. absorption spectrum in the 200-340 mμ range at the beginning of the experiment with the spectrum obtained at the end of the assay period. Vapam and Na-MDC have strong absorption bands at 203, 250, and 282 mμ. Judging primarily from these bands, Na-MDC and Vapam did not decompose during any of the toxicity tests.

All experiments with Na-MDC and Vapam were carried out in 0.1 M phosphate buffer at pH 5.8. It would have been desirable to determine their fungicidal activity without buffer ions present but

this was impossible because addition of strong acid to adjust the normally basic dithiocarbamate solution caused decomposition of the toxicant. Addition of buffer to adjust the pH did not affect stability of the Na-MDC and Vapam probably because the buffer did not have as high a hydrogen ion concentration as the strong acid.

In air Na-MDC and Vapam have different dosage-response (DR) curves (Figs. 1 and 2). The LD_{50} concentration (i. e. the concentration giving 50% kill) of Na-MDC is higher than that of Vapam, but its LD_{95} is slightly lower. The different slopes of the DR curves for Na-MDC and Vapam suggest a different mode of action for these two preparations. In this case, the difference is probably due to the presence of fungitoxic compounds other than Na-MDC in Vapam.

Under anaerobic conditions, Na-MDC produced a bimodal DR curve (Fig. 1) characteristic of the dialkyl dithiocarbamates whose fungitoxic action may be associated with the formation of chelate complexes. The first peak in the bimodal DR curve probably results from the formation of a toxic 1:1 complex between the toxicant and certain heavy metal ions present either in the assay medium or the fungal spores themselves. As the concentration of the toxicant increases, a relatively insoluble and nontoxic 2:1 chelate may form which accounts for the depressed toxicity with increasing toxicant concentration. At still higher concentrations an excess of the toxicant itself may bring about complete inhibition (5).

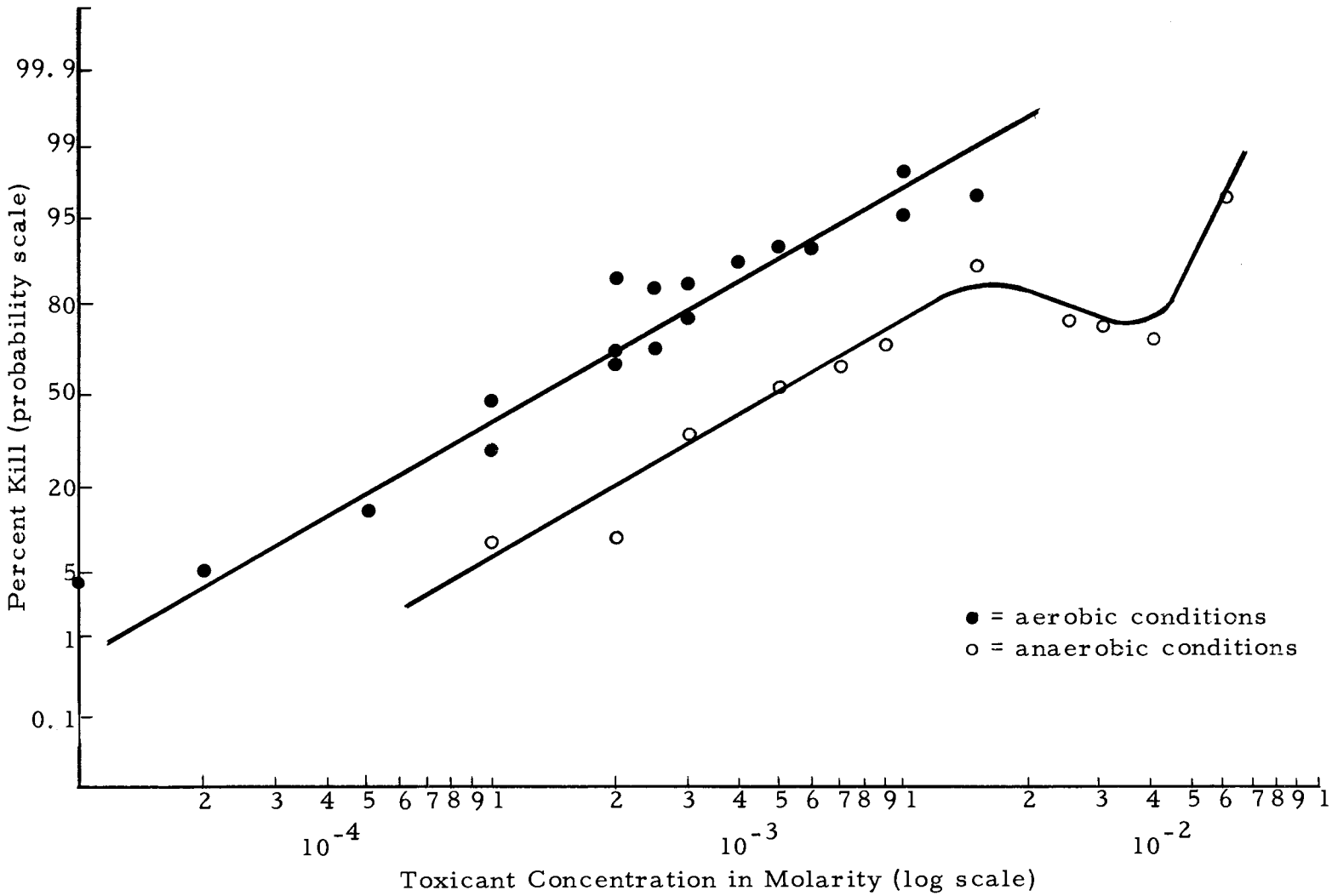


Figure 1. Fungicidal activity of Na-MDC on spores of Fusarium in pH 5.8 buffer under aerobic and anaerobic conditions.

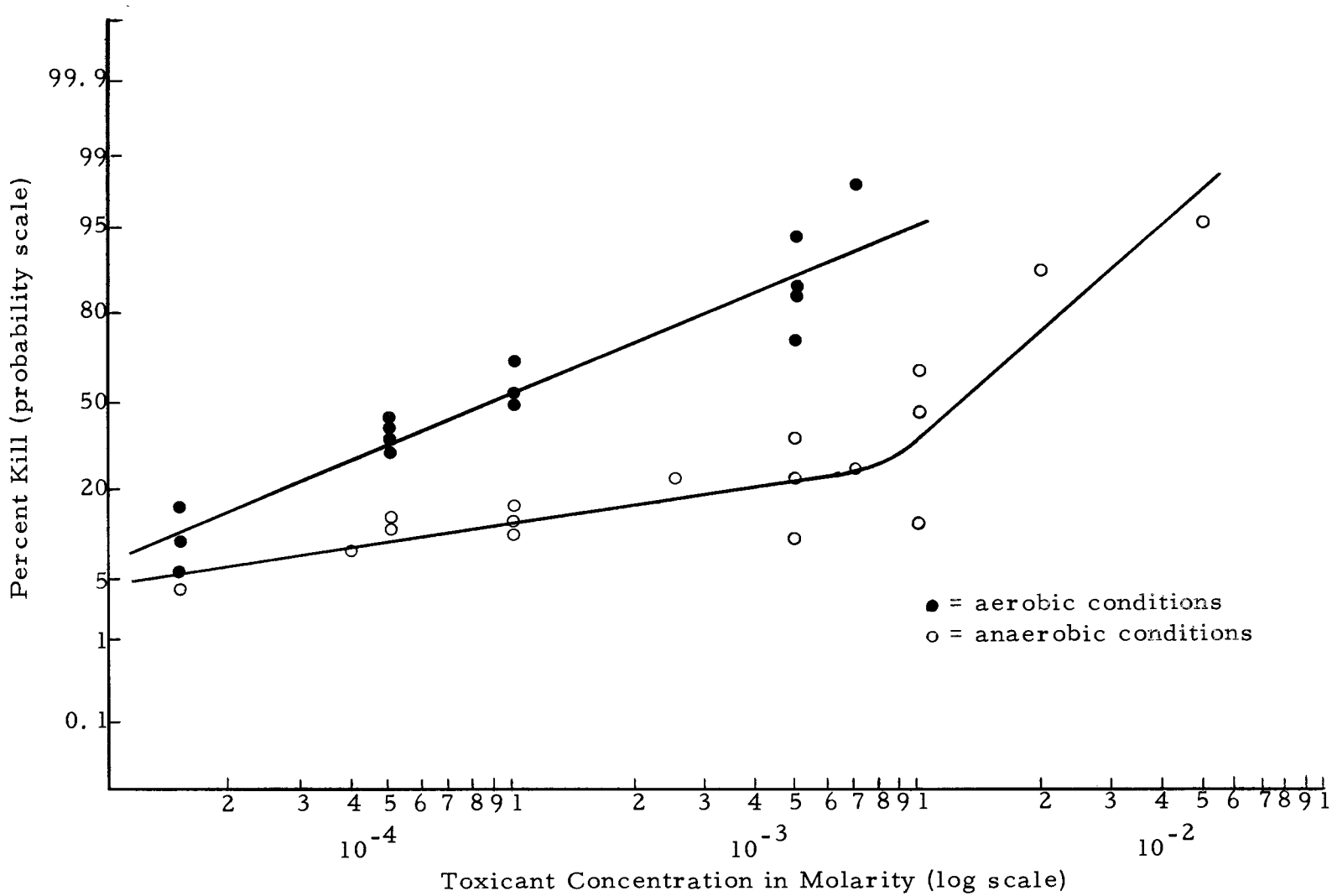


Figure 2. Fungicidal activity of Vapam on spores of Fusarium in pH 5.8 buffer under aerobic and anaerobic conditions.

This inversion effect (i. e. bimodal dosage-response) has been reported on two occasions for monoalkyl dithiocarbamates (4, 27), but little credence was placed on these reports (22). Recently it was discovered that the inversion effect of certain monoalkyl dithiocarbamates may be due to production of oxidative decomposition products of the original toxicants (3).

In the case of Na-MDC under anaerobic conditions, it is doubtful that sufficient decomposition of the toxicant could have occurred to account for the toxicity obtained. Thus, unless decomposition occurred within the fungal cells, which is a distinct possibility, the inversion effect obtained with Na-MDC is due to the dithiocarbamate itself. Additional studies will be required to determine the exact cause of the inversion phenomenon with Na-MDC.

Vapam under anaerobic conditions failed to give a typical bimodal DR curve, although it showed two modes of fungicidal activity (Fig. 2). The heterogeneous composition of Vapam may account for its failure to behave like Na-MDC. The different mode of action and reduced toxicity of Na-MDC and Vapam under helium probably is a reflection of changes in the metabolism of Fusarium under anaerobic conditions.

Methyl isothiocyanate

The innate fungitoxicity of MIT was determined under air and

helium in deionized water and phosphate buffer. In water the initial pH of the assay mixture containing MIT was about 5. 5 and by the end of the assay it had increased to about 6. 0. The pH shift was independent of MIT because the untreated cell suspension made a similar pH change.

Stability of MIT during assays was checked by measuring the broad U. V. absorption band of this compound at about 245 mμ. Determinations before and after the assays indicated that MIT remained stable during the tests.

The DR curves for MIT in air and helium were the same whether in buffer or water (Figs. 3 and 4). MIT must therefore have a different mode of fungicidal action than Na-MDC, which was quite different in its fungicidal action under aerobic and anaerobic conditions.

Wedding and Kendrick (25) and Goksøyr (6) suggested on the basis of some physiological experiments that MIT and Na-MDC have a different mode of fungitoxic action. Because precautions were not taken in their experiments to prevent decomposition of these compounds, and conditions were such that breakdown probably occurred, their suggestions can not be taken as fact. Nevertheless, the present results substantiate their contentions.

Although the slopes of the DR curves for MIT and Na-MDC in air are the same, it does not necessarily follow that

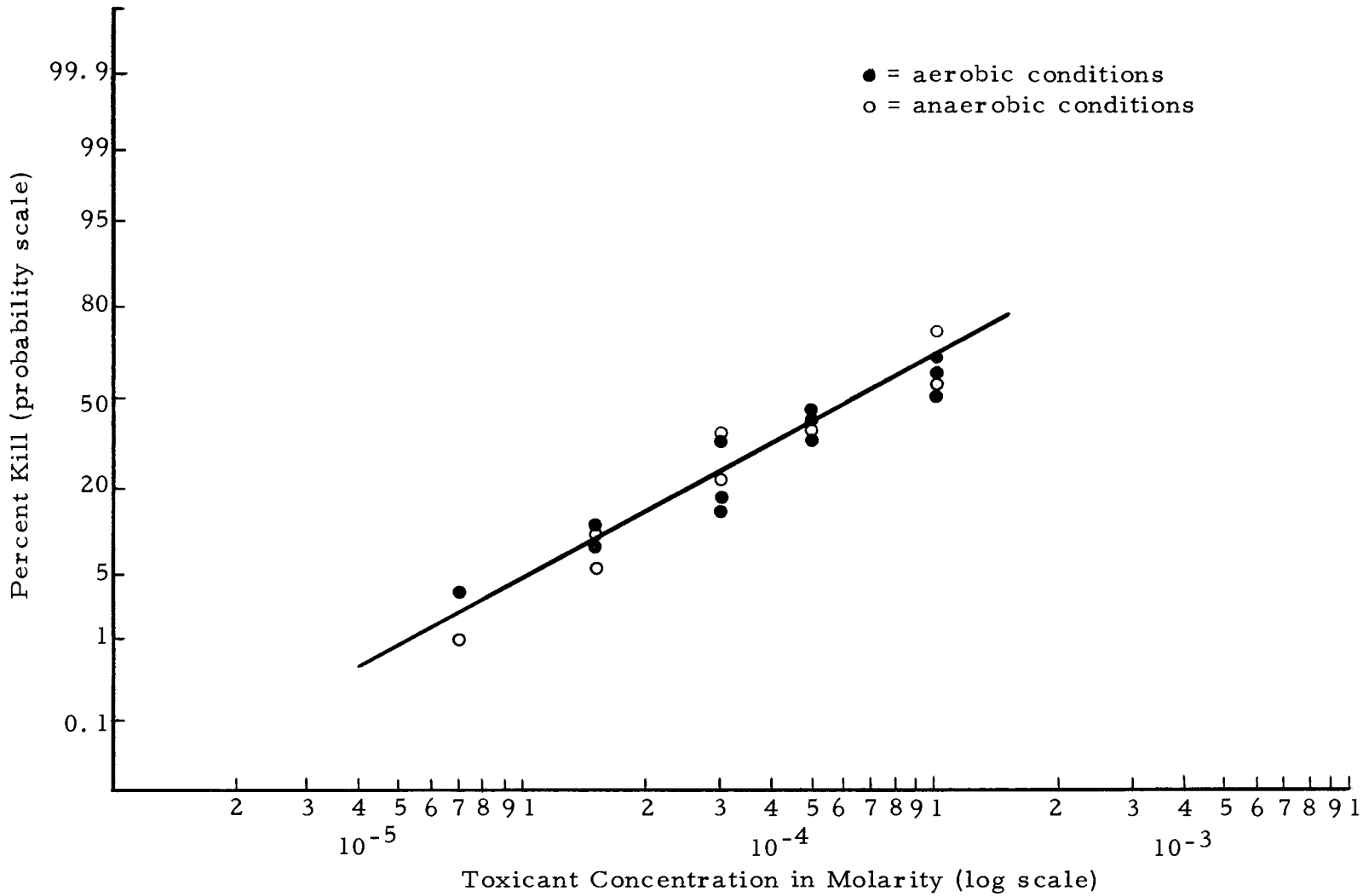


Figure 3. Fungicidal activity of MIT on spores of Fusarium in pH 5.8 buffer under aerobic and anaerobic conditions.

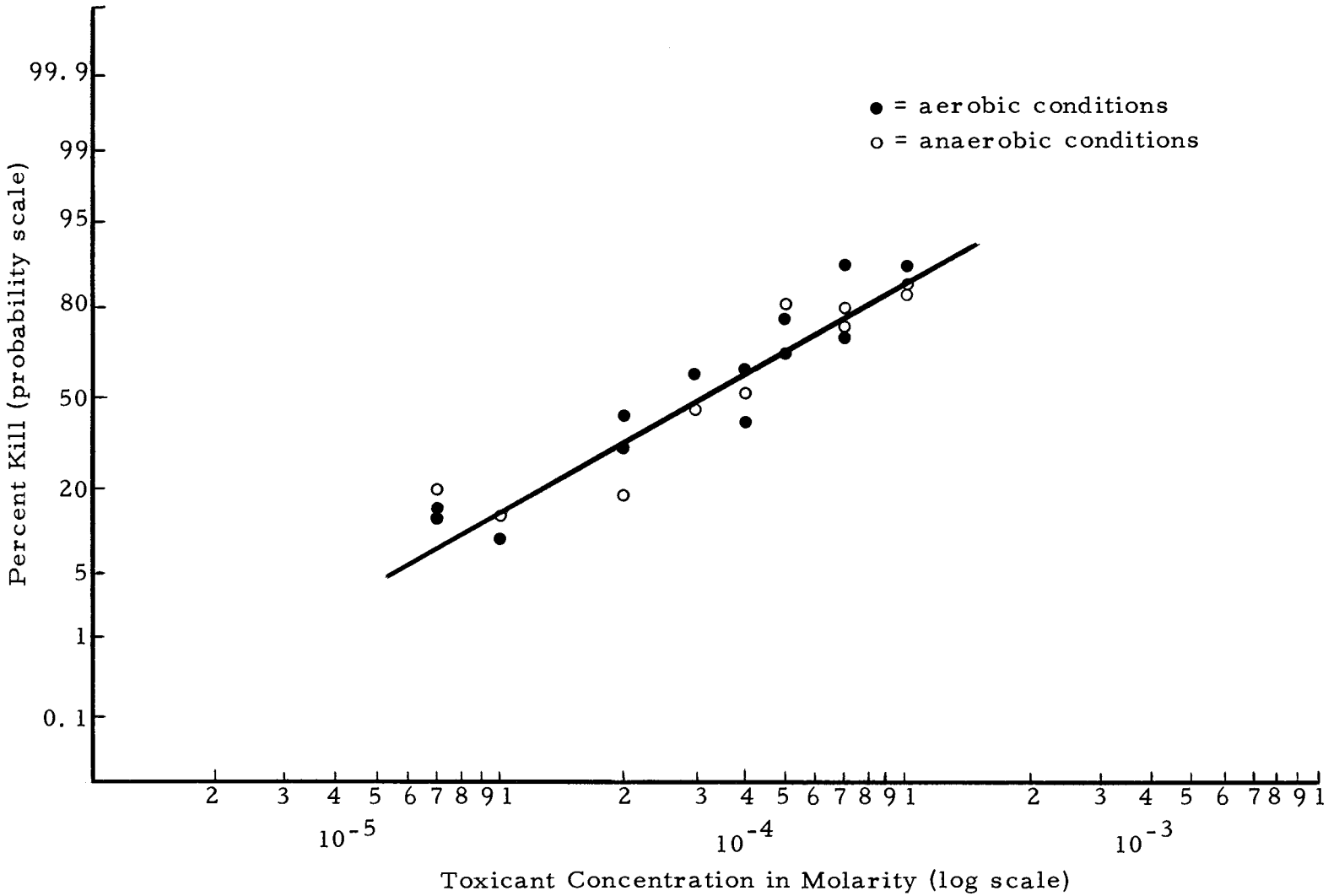


Figure 4. Fungicidal activity of MIT on the spores of Fusarium in deionized water under aerobic and anaerobic conditions.

the modes of fungicidal action for these compounds are necessarily similar.

The reduced toxicity of MIT in buffer can not be due to pH because the spores in deionized water had a pH similar to that of the buffered suspension. Competition between the toxicant and buffer ions for reactive sites on the fungal cell membranes may account for the reduction.

N, N'-Dimethylthiuram disulfide

Purified DMTD was obtained from commercial preparations (Table 1) by recrystallization from chloroform and petroleum ether (b.p. 30-75° C). DMTD is relatively water insoluble and therefore stock solutions were prepared in ethanol (100%). The ethanolic solution (0.2 ml) was added to 9.8 ml of a Fusarium spore suspension in vials. A similar amount of ethanol was added to the controls.

Fungicidal activity of DMTD was determined in buffer and deionized water under air and helium. In water the pH of the assay mixture was in the range 5.5-6.0, and as with MIT the pH change during the assay was due to the fungal cells and not the toxicant.

Purified DMTD is so unstable that within the first few minutes of the fungicide tests it began to decompose and within two hours it was completely gone. However, if DMTD is accumulated by Fusarium spores within seconds as some fungicides are (15), then the

results obtained here may be a true indication of the innate fungicidal activity of DMTD.

Buffer reduced the toxicity of DMTD (Table 2), but the slopes of its DR curves in buffer under air and helium (Fig. 5) was exactly like those obtained in deionized water.

Like Na-MDC, DMTD is less toxic under anaerobic conditions than in air (Fig. 5), but unlike Na-MDC, the DR curve obtained in helium was the same as the one in air. The DR curve for DMTD was like those obtained for Na-MDC and MIT, but DMTD is considerably more toxic than either Na-MDC or MIT (Table 2). Similar results with these compounds have been obtained by others (7, 8). If small amounts of DMTD were formed during decomposition of Vapam this could in part account for the effectiveness of Vapam as a fungicide.

2, 4-N'-Dimethyl-1, 2, 4-thiadiazolidine-3, 5-dithione

Purified DTD was obtained by dissolving a crude sample in hot ethanol, cooling and collecting the crystals on filter paper. DTD is extremely insoluble in water and thus it was added to the spore suspensions in 100% ethanol as was DMTD. Unfortunately only low concentrations of DTD (i.e. below 6×10^{-4}M) could be accurately tested for fungicidal activity because DTD precipitated in the assay mixture at higher concentrations.

Stability of DTD was determined during the assays by

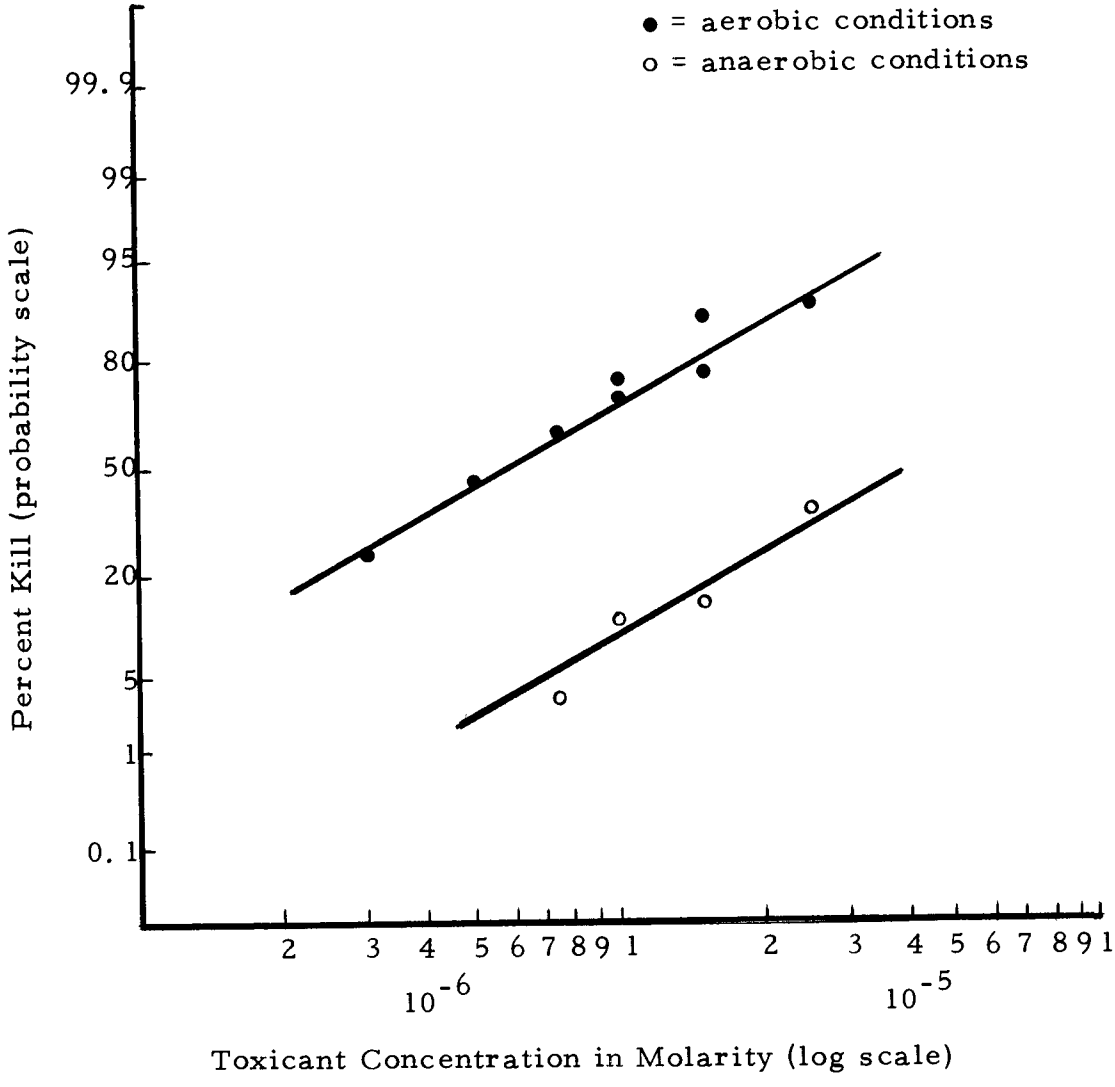


Figure 5. Fungicidal activity of DMTD on spores of Fusarium in deionized water under aerobic and anaerobic conditions.

Table 2. Concentration of toxicant required for 50% kill (LD_{50}) of Fusarium spores under aerobic and anaerobic conditions in 0.1 M phosphate buffer pH 5.8 unless otherwise indicated.

Chemical	LD_{50} concentration (molarity)	
	Aerobic conditions	Anaerobic conditions
DMTD	6×10^{-6}	4×10^{-5}
DMTD, water	6×10^{-6}	2×10^{-5}
Na-TTC [1]	4×10^{-4}	4×10^{-4}
Na-TTC [2]	6×10^{-4}	6×10^{-4}
MIT	7×10^{-4}	7×10^{-4}
MIT, water	3×10^{-4}	3×10^{-4}
DTD [3]	8×10^{-4}	1×10^{-3}
DTD, water [3]	8×10^{-4}	1×10^{-3}
Vapam	8×10^{-4}	1×10^{-2}
Na-MDC	1×10^{-3}	5×10^{-3}
MMDT [3]	1×10^{-3}	1×10^{-3}
MMDT, water [3]	6×10^{-4}	8×10^{-4}
CS_2	1×10^{-3}	2×10^{-3}
COS	2×10^{-3}	6×10^{-3}
H_2S	4×10^{-3}	3×10^{-2}
MA	$>1 \times 10^{-2}$	$>1 \times 10^{-2}$
DMTU	5×10^{-1}	5×10^{-1}
Sulfur	>1	>1

[1] Molarity based on Na-TTC containing four waters of hydration.

[2] Molarity based on Na-TTC containing one water of hydration.

[3] Extrapolated LD_{50} concentration.

measuring its strong absorption band at 290 mμ and the weaker bands at 245 and 265 mμ. Comparison of determinations before and after the assays indicated that DTD was stable during these tests.

Only tentative DR curves for DTD can be drawn (Fig. 6) because of insufficient data on this compound at the higher concentrations. Nevertheless, DTD was similarly toxic in water and buffer, but less effective in helium than in air. Extrapolated LD_{50} concentrations for DTD suggest that this compound is a potentially effective decomposition product of Vapam (Table 2).

4-Methyl-5-methylimino-1, 2, 4-dithiazolidine-3-thione

Purified MMDT, a compound closely related to DTD, was tested for fungicidal activity similarly to DTD. The absorption spectrum between 200 and 340 mμ was used to indicate its stability. The original single absorption band at 275 mμ disappeared during the assay period and the three bands typical of DTD developed. Conversion of MMDT to DTD, which was complete in two hours, occurred only in the presence of the spore suspension, and did not occur in ethanol or deionized water, in which MMDT was stable for at least 23 hours.

As might be expected the tentative DR curves for MMDT (Fig. 7) are similar to those obtained for DTD, but MMDT is slightly more toxic than DTD (Table 2). Thorne (21) has shown DR curves for

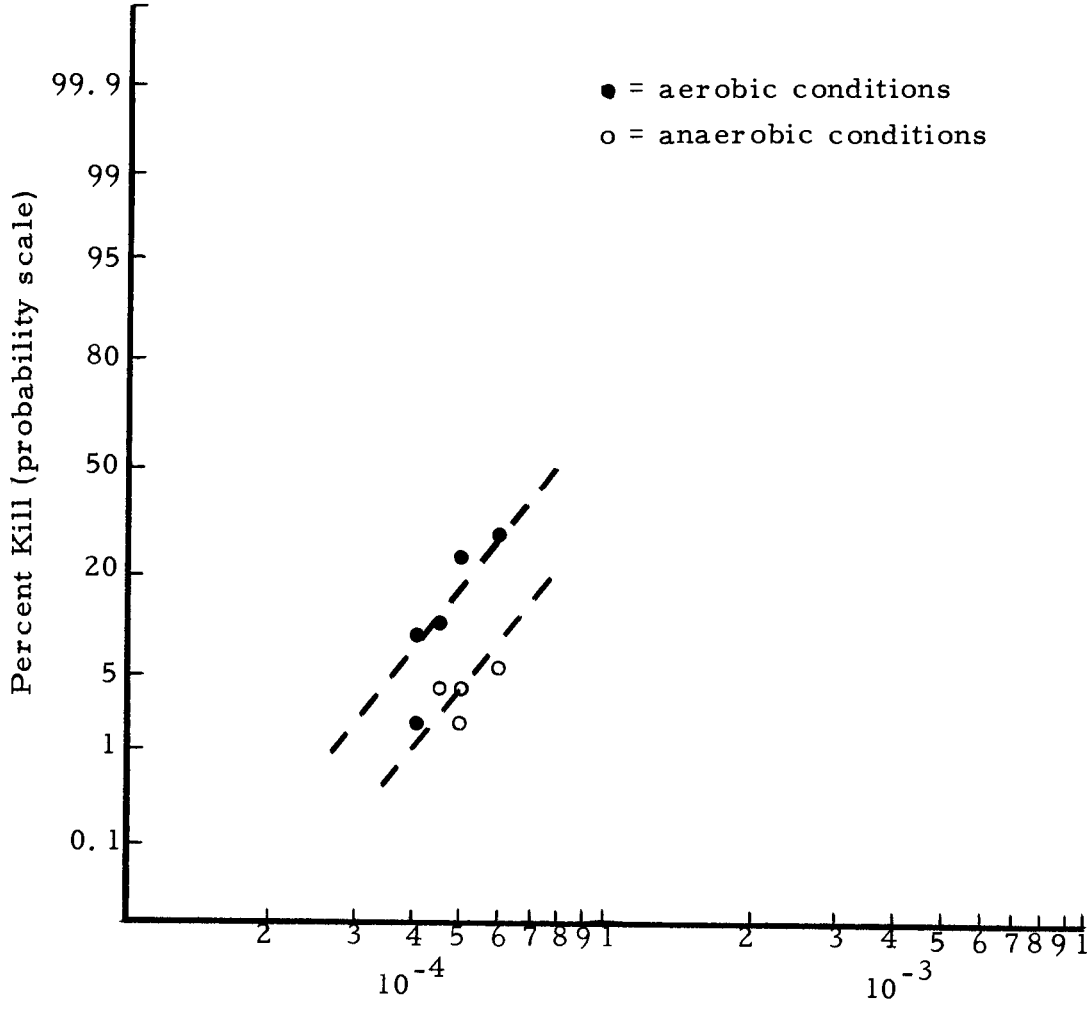


Figure 6. Fungicidal activity of DTD on spores of Fusarium in deionized water under aerobic and anaerobic conditions.

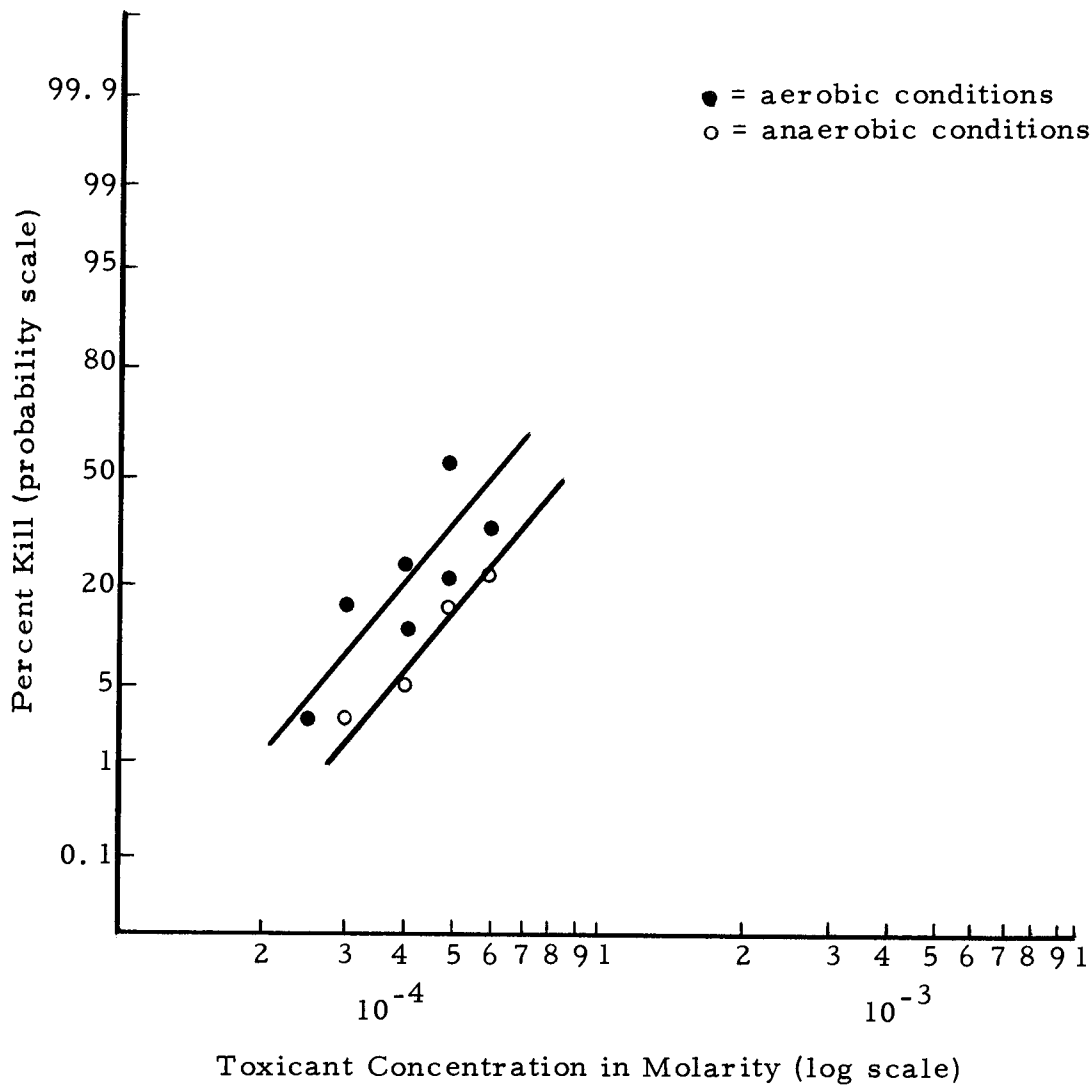


Figure 7. Fungicidal activity of MMDT on spores of Fusarium in deionized water under aerobic and anaerobic conditions.

these compounds in a spore germination assay. MMDT was slightly more effective than DTD and if his curves are superimposed on each other their identity can be noted.

Sodium trithiocarbonate

Fungicidal activity of Na-TTC was determined in buffer using a preparation of unknown degree of hydration. Because several hydrates of Na-TTC exist, it was impossible to accurately determine the molarity in the fungicide assays. The calculations were based, however, on the highest degree of hydration (12) and for comparative purposes, calculations were made with the lowest degree of hydration (Table 2). As judged by absorption spectra, Na-TTC decomposed rapidly following initiation of the fungicide tests, probably mostly to CS_2(11).

Na-TTC is similarly toxic to Fusarium under aerobic and anaerobic conditions (Table 2). Regardless of the degree of hydration assumed for Na-TTC, this compound is more fungitoxic than Na-MDC, and may therefore be considered a potentially effective decomposition product of Vapam.

Carbon Disulfide

Fungicidal activity of CS_2 against Fusarium spores was determined by adding the compound to the cells in ethanol in a manner

similar to that done with other compounds of limited water solubility. In phosphate buffer under aerobic conditions, CS_2 was moderately fungicidal but less effective under anaerobic conditions (Table 2). CS_2 was less toxic than Vapam and several of its highly effective decomposition products. Similar studies with other dithiocarbamates indicate that CS_2 has insufficient toxicity to explain the effectiveness of dithiocarbamates (2, 10, 18, 26).

Carbonyl Sulfide

A solution of COS for toxicity assays was prepared by dissolving the gas in water and adding this solution to the Fusarium spore suspensions in buffer.

Fungicidal activity of COS was slightly lower than that of Na-MDC, and was less under anaerobic conditions than in air (Table 2). COS vapor is toxic to the mycelium of Pythium irregulare in closed containers (16), and was suggested as an effective decomposition product of nabam.

Hydrogen Sulfide

A stock solution of H_2S was prepared for the fungicide assays by saturating water at 22° C with the gas. Fungicidal activity of H_2S was lower in helium than in air, and this compound was considerably less effective than Vapam (Table 2). These results agree

with the conclusion that H_2S is too weak a toxicant to explain the general effectiveness of the dithiocarbamate fungicides (22).

Methylamine

In the standard fungicide assay, methylamine was relatively nontoxic (Table 2), and probably does not contribute directly to the fungicidal action of Vapam. It may, however, enter into reactions with other decomposition products (24).

N, N'-Dimethylthiourea

DMTU was relatively ineffective against Fusarium under aerobic and anaerobic conditions in the standard fungicide assay (Table 2). Klöpping (8) measured the fungitoxicity of DMTU against four fungi and similarly found that DMTU was considerably less effective than Na-MDC as a fungicide.

Elemental Sulfur

An aqueous suspension of finely divided sulfur was evaluated for fungicidal activity under air and helium in phosphate buffer. Toxicity of sulfur in this assay was extremely low (Table 2). Similar results were obtained by Miller, McCallan and Weed (14) using a spore germination assay in which cells were treated for 24 hours in sealed vials.

Sulfur, per se is probably of little importance as a toxicant from the decomposition of Vapam. This conclusion agrees with the results of Wedding and Kendrick (25) who obtained 100% survival of Rhizoctonia solani following a two hour treatment with sulfur at concentrations that with Na-MDC and MIT gave complete inhibition.

Influence of Cell Source on Fungitoxicity of MIT

The Fusarium spores used throughout this investigation were collected after about 75 hours growth when the pH of the growth medium had dropped to around 4.5 (Fig. 8). If older cells were used for the assays (i.e. about 120 hours when the pH was 7.0-7.5), the toxicity of MIT, for example, was greatly influenced.

Phosphate buffer in the assay medium reduced the effectiveness of MIT in air and helium (Table 3) as it did with most other compounds (Table 2). When older cells from a basic medium were used, however, buffer increased the fungicidal activity of MIT (Table 3). The influence of buffer was particularly evident under aerobic conditions, but was also present under anaerobic conditions.

The significant degree to which the source of cells and composition of the assay mixture can influence the results of fungicide assays indicates the caution which must be exercised in comparing results from tests by different methods.

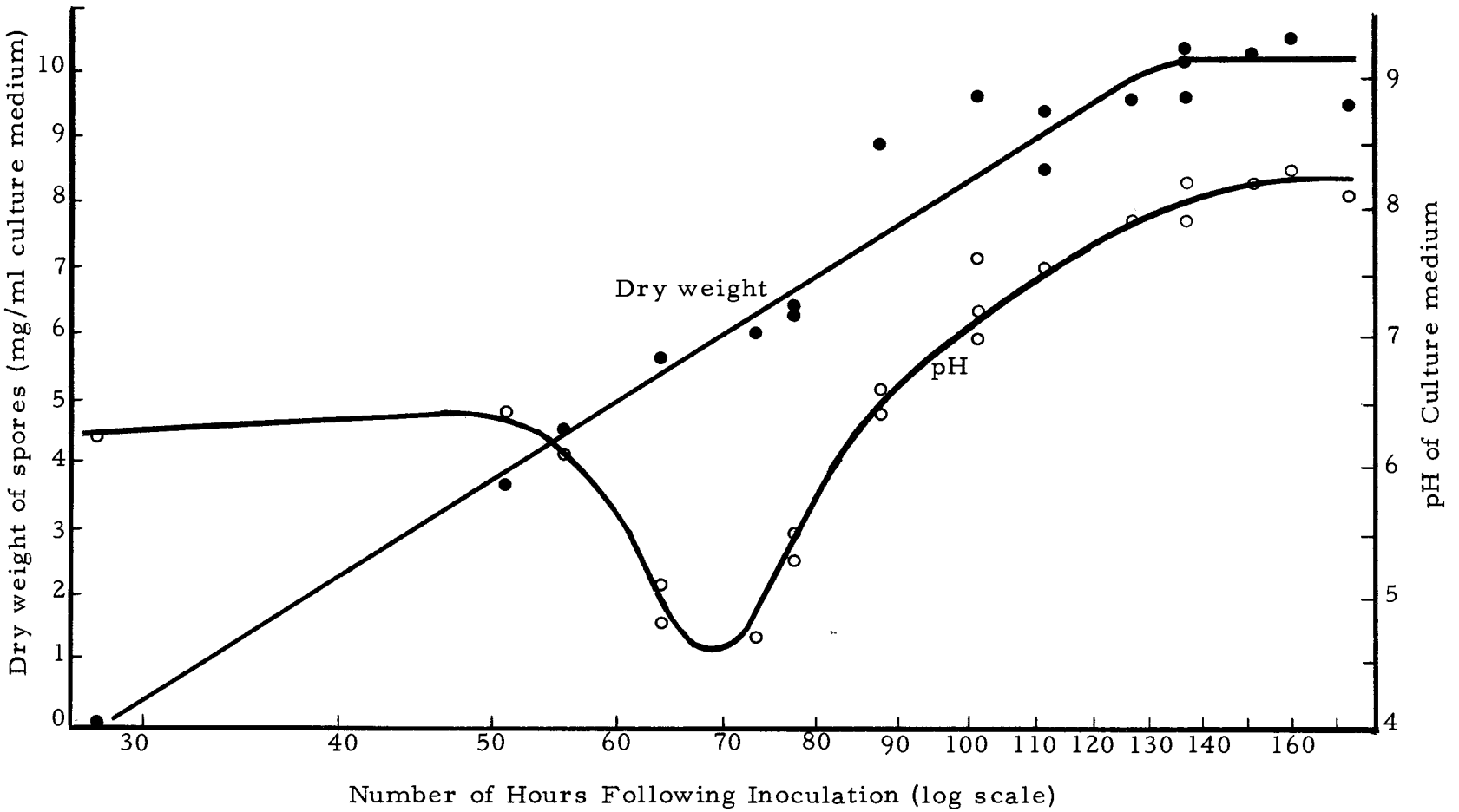


Figure 8. Dry weight of Fusarium and pH of the shake culture medium.

Table 3. The influence of phosphate buffer (0.1 M, pH 5.8) on the fungicidal activity of MIT (5×10^{-4} M) under aerobic and anaerobic conditions against Fusarium spores from acid and basic culture medium.

	Percent kill			
	Spores from acid culture conditions assayed in water and buffer		Spores from basic culture conditions assayed in water and buffer	
Assay conditions	Water	Buffer	Water	Buffer
Aerobic	65	54	46	71
Anaerobic	73	57	39	53

GENERAL DISCUSSION

Testing the fungicidal activity of Na-MDC and its decomposition products by a standard method has allowed valid comparisons of the action of these chemicals. In previous studies the fungitoxicity of some of these compounds has been determined but a variety of conditions were used and direct comparisons between compounds may have led to erroneous conclusions.

Most of the previous tests were carried out under conditions where decomposition of the toxicant could have taken place during the assay. In the present study, stability of the compounds during the assays was determined; in this manner it was known if the compound remained in its pure state or if it decomposed.

In many of the earlier studies only fungistatic effects of these compounds was determined. In the present study fungicidal action, which is probably a more important criterion for a soil fumigant, was measured. Although LD_{50} concentrations were used to compare the effectiveness of these compounds (Table 2), their toxicity ranking was not greatly altered by comparing them at their LD_{95} concentrations.

DMTD, the most fungicidal compound tested, was considerably more effective than Na-MDC. Klöpping (8) and van der Kerk and Klöpping (7) obtained similar results with four different fungi. They

suggested that MIT and Na-MDC belong in different toxicity groups based on their fungitoxic spectrum against these fungi although they considered the toxic action of these compounds similar. DMTD was placed in a different group based on its spectrum. The similarity of the antifungal spectrum of Na-MDC and MIT, however, may have been due to decomposition of Na-MDC during Klöpping's experiments (8).

The results obtained in the present study indicate a difference in the mode of action of Na-MDC and MIT (i. e. under anaerobic conditions). Similarly, Wedding and Kendrick (25) showed that glucose metabolism was differentially inhibited by MIT and Na-MDC, thus indicating a different mode of action. However, because Na-MDC probably decomposed during their experiments, the differences between Na-MDC and MIT might have been due to other products in the Na-MDC preparation. Consequently differences in effects on glucose metabolism may not be correlated with the differences obtained here under anaerobic conditions.

DMTD, Na-TTC, DTD and MMDT are fungitoxic enough to account for the effectiveness of Vapam. It is doubtful, however, if any of these compounds account for the primary toxic action of Vapam under normal conditions in the soil. These compounds do not possess sufficient volatility to explain the rapid diffusion of the toxic principle from Vapam. MIT, however, is sufficiently volatile

and has been recovered from soil treated with Vapam (17, 24). Nevertheless, small quantities of the other highly toxic decomposition products may account for some control of soil borne plant parasites and weed seeds by Vapam.

SUMMARY

1. An assay for fungicidal activity of Na-MDC and its decomposition products was developed in which there was a minimum influence due to the widely varying physical properties of the compounds, and their decomposition during the assay was measured and held to a minimum.
2. Under anaerobic conditions, Na-MDC gave a bimodal DR curve. Vapam, a commercial preparation of Na-MDC, failed to give a bimodal DR curve although it did produce a two phase curve. Under aerobic conditions both forms of Na-MDC gave straight line DR curves.
3. MIT produced identical DR curves under aerobic and anaerobic conditions, and thus, must have a different mode of fungicidal action from Na-MDC.
4. DMTD was highly fungitoxic and along with Na-TTC, DTD and NMDC could account for the fungicidal effectiveness of Vapam. However, these compounds are not sufficiently volatile to account for the movement of the toxic principle from Vapam.
5. H_2S, COS, CS_2, DMTU, and methylamine were not sufficiently fungicidal to account for the effectiveness of Vapam in plant disease control.
6. Young spores from an acid culture medium were less susceptible to MIT in buffer than in water. When older cells from a basic medium were used the buffer significantly increased their susceptibility to MIT.

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