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Chemical Control of Mango Anthracnose in Malaysia. *In vitro* Fungitoxicity of Selected Chemicals

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RINGKASAN

Lima belas fungisida serta dua formulasi komersial insektisida dicrotophos, telah diuji fungitoksisitinya in vitro *ke atas patogen penyakit berpusar mangga,* Colletotrichum gloeosporioides. *Kumpulan-kumpulan benzimidazole, thiophanate dan dua fungisida daripada kumpulan N-trichloromethylthio telah didapati sungguh toksik kepada patogen tersebut dengan* ED_{50} *yang kurang dari 5 μg/ml. Kumpulan dithiocarbamate pula menunjukkan darjah toksik yang berlain-lainan dengan* ED_{50} *di antara 100 μg/ml ke 580 μg/ml. Kedua-dua fungisida chlorothalonil dan iprodione dari kumpulan fungisida pelbagai menunjukkan fungitoksisiti yang rendah, dan kedua-duanya langsung tidak fungitoksik. Persamaan dan perbezaan di antara dan sesama kumpulan-kumpulan fungsida selanjutnya dibincangkan berkaitan dengan struktur kimia, cara tindakan biokimia dan formulasi.*

SUMMARY

Fifteen fungicides and two commercial formulations of the insecticide, dicrotophos, were investigated for in vitro *fungitoxicity against mango anthracnose pathogen,* Colletotrichum gloeosporioides. *Among the fungicides tested, the pathogen was extremely sensitive to the thiophanate, the benzimidazoles and two of the N-trichloromethylthio group of fungitoxicants which exhibited* ED_{50} *of less than 5 μg/ml. The dithiocarbamates varied in their degree of toxicity, with* ED_{50} *ranging from 100 μg/ml to 580 μg/ml. The two miscellaneous fungicides, chlorothalonil and iprodione and the two insecticides showed extremely low and negligible fungitoxicity, repectively. Similarities and differences between and within groups of fungicides tested are further discussed in relation to their chemical structures, biochemical modes of actions and formulations.*

INTRODUCTION

Recent years have witnessed an upward trend in the cultivation of mango brought about by government sponsorship and the increase in the number of private enterprises growing the fruit in orchards; interest among urban home dwellers too, has contributed towards this trend. From an estimate of 1,118.8 ha in 1972 (Anon, 1972), the area under mango has increased to 2,453 ha in 1977 (Anon, 1977). Research on fruit orchard management and economic pest and disease management, however, has not kept pace with this increase. In the tropics, one of the major limiting factors to mango production is poor fruit set and low yields due to anthracnose disease caused by the fungus *Colletotrichum gloeosporioides* Penz. [*Glomerella cingulata* (Stonem.) Spauld & Schrenk]. This fungus causes leaf spot, leaf blight, peduncle blight, blossom blight, fruit stain, premature fruit abortion, preharvest and post harvest fruit rot in mangoes.

Where mango is grown commercially in countries such as Israel, India, Taiwan, the Philippines, South Africa and America (Florida and Hawaii), much is known about preharvest and post harvest control of mango anthracnose. In Malaysia, however, there is a paucity of literature on mango cultivation, particularly on pest and disease control of the fruit. There is a pressing need for more work to be done in these areas if the economic potential of mango is to be realized and sustained. As a start, research is currently being carried out to study the epidemiology and control of mango anthracnose. This paper reports the comparative *in vitro* fungitoxicity of selected chemicals currently being investigated in the greenhouse and field for the control of this disease.

MATERIAL AND METHODS

Fifteen fungicides and two insecticides as listed in Table 1 were screened against mango anthracnose pathogen *C. gloeosporioides*. The fungicides were tested at concentrations of 1.25, 2.5, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 μg/ml active ingredient, while the insecticides were tested at 160, 320, 640, 1280 and 2560 μl/l active ingredient. The chemicals were separately incorporated into molten Difco potato dextrose agar (PDA) in glass petri plates held at 45°C. On solidification each plate was inoculated with a 6 mm PDA disc taken from a week old culture of *C. gloeosporioides*. Each concentration level was assessed in four replicates and the experiment was repeated twice. Colony diameter growth of the test fungus was measured and compared with untreated checks (0 μg/ml) for calculating percentage inhibition. The log probit inhibition of growth was plotted against the log of the concentration of the chemical and the ED_{50}, which is the concentration required to inhibit growth by 50% (Finney, 1971), was then determined. ED_{50} values are preferred over the more extreme level of ED_{100} as the former can be estimated with a greater degree of precision than the latter (Finney, 1971). For convenience of comparative evaluation, compounds that inhibit growth by 50% at concentrations of less than 10μg/ml are considered as highly toxic, 10 – 100 μg/ml as toxic, 100 – 400 μg/ml as moderately toxic, 400 – 700 μg/ml as slightly toxic and more than 700 μg/ml as showing negligible toxicity.

TABLE 1

Chemicals tested for *in vitro* fungitoxicity against the mango anthracnose pathogen

Registered Trade Name	Percent Active Ingredient	Formulation	Common Name of Active Ingredient	Chemical Name
Benzimidazole Fungicide				
Benlate	50	W.P.	Benomyl	Methyl–1–(butylcarbamoyl) 2 benzimidazol carbamate
Bavistin	50	W.P.	Carbendazim	2–(methoxy-carbamoyl)–benzimidazol
Delsene X	80	W.P.	Carbendazim + Mancozeb	2–(methoxy-carbamoyl)–benzimidazol (6.2%) + Zinc manganese ethylene bisdithiocarbamate (73.8%)
Thiophanate Fungicide				
Topsin M	70	W.P.	Thiophanate M	1, 2, –di–(3-methoxycarbonyl-2-thiouredo) benzene.
N-Trichloromethylthio and related Fungicide				
Captan	50	W.P.	Captan	3a, 4, 7, 7a-tetrahydro-N-trichloromethane sulphenyl pthalimide.
Difolatan 4F	39	Liquid	Captafol	3a, 4, 7, 7a-tetrahydro-N-(1, 1, 2, 2, -tetrachloroethane-sulphenyl) pthalimide.
Zincofol	68.5	W.P.	Captafol + Metallic Copper Metallic Zinc	3a, 4, 7, 7a-tetrahydro-N-(1, 1, 2, 2, -tetrachloroethane-sulphenyl) pthalimide (50%) Cu^{2+} (12.5%) + Zn^{2+} (6.0%).
Dithiocarbamate Fungicide				
Antracol	70	W.P.	Propineb	Zinc propylene bisdithiocarbamate.
Manzate D	80	W.P.*	Maneb + Zinc Salt	Manganese ethylene bisdithiocarbamate + Zn^{2+}
Tricarbamix Special	70	W.P.	Maneb + Zineb Ferbam	Mn^{2+} (2.9%), Zn^{2+} (10.0%), Fe^{3+}(1.9%), dimethyldithiocarbamate (12.1%), ethylene bisdithiocarbamate (43.1%).
Trimanzone	86.2	W.P.	Maneb + Zineb + Ferbam	Mn^{2+} (13.5%), Zn^{2+} (3.4%), Fe^{3+} (1.5%), dimethyldithiocarbamate (8.7%), ethylenebisdithiocarbamate (59.1%).

(Table 1 continued)

Registered Trade Name	Percent Active Ingredient	Formulation	Common Name of Active Ingredient	Chemical Name
Trimiltox blue	47.0	W.P.	Mancozeb + Metallic Copper + Iron stimulant additive.	20% (Manganese ethylene bisdithiocarbamate + Zn^{2+}) + 21% metallic copper in the form of Copper oxychloride, Copper sulphate + Copper carbonate, 6% $Fe_4[(CN)_6]_3$.
Trimiltox yellow	41.0	W.P.	Mancozeb + Metallic Copper	20% (Manganese ethylene bisdithiocarbamate + Zn^{2+}) + 21% metallic copper in the form of Copper oxychloride, Copper sulphate, Copper carbonate.
Miscellaneous Fungicide				
Daconil	50	W.P.	Chlorothalonil	Tetrachloroisophthalonitrile.
Rovral	50	W.P.	Iprodione	Isopropylcarbamoyl-3(3.5-dichlorophenyl)-hydantoin.
Insecticide				
Bidrin	24	E.C.**	dicrotophos	3-(dimethoxyphosphinyloxyl)-N, N-dimethyl ciscrotonamide
Carbicron	24	E.C.	dicrotophos	3-(dimethoxyphosphinyloxyl)-N, N-dimethyl ciscrotonamide

* W.P. = wettable powder
** E.C. = emulsifiable concentrate

RESULTS

The ED_{50} values of the compounds tested against the mango anthracnose pathogen are shown in Table 2. The benzimidazole compounds of Benlate (benomyl) and Bavistin (carbendazim) and the thophanate, Topsin M were the most toxic to *C. gloeosporioides,* with ED_{50} values of less than 1 μg/ml. Delsene X, a mixture of only 6.2% carbendazim and 73.8% mancozeb (a dithiocarbamate), was also highly toxic with an ED_{50} of 4.5 μg/ml.

C. gloeosporioides was also highly sensitive to two of the N-trichloromethylthio related compounds, Difolatan and Zincofol. Difolatan had an ED_{50} of 3μl/l and the latter 4 μg/ml. However Captan was less fungitoxic with an ED_{50} of 70 μg/ml. Antracol (propineb) with an ED_{50} of 50 μg/ml was comparatively the more toxic of the dithiocarbamates tested. Special combination products of mancozeb with other dithiocarbamates or other metallic salts *viz.* Trimanzone, Tricarbamix special, Trimiltox yellow and Trimilox blue were more fungitoxic (ED_{50} of 100 – 380 μg/ml) than the fungitoxicant mancozeb (ED_{50} 580 μg/ml) itself.

The miscellaneous fungicides, Daconil (chlorothalonil) and Rovral (iprodione) exhibited very low toxicity to the anthracnose pathogen ($ED_{50} > 400$ μg/ml). The pathogen was insensitive to the insecticides, Bidrin and Carbicron (ED_{50} values > 2000 μl/1).

DISCUSSION

The striking similarities in fungitoxic response of the benzimidazoles and thiophanate-methyl could be attributed to similarities in biochemical mode of action. In water, both benomyl and thiophanate-methyl are rapidly decomposed to methyl benzimidazol-2-yl carbamate (MBC) (Clemons and Sisler, 1969). MBC has been reported to primarily inhibit mitosis in sensitive fungi, while inhibition of DNA synthesis is a secondary effect (Hammerschalg and Sisler, 1973). Kaars-Sijpesteijn (1972) suggested that the toxic principle in MBC is the benzimidazol moeity of the molecule. It appears that *C. gloeosprioides* is extremely sensitive to compounds which possess this toxic moeity. Benlate (benomyl) has been reported to give good field control of mango anthracnose pathogen when used singularly or in combinations with other fungicides (McMillan, 1973; Zauberman *et al,* 1976).

TABLE 2

In vitro toxicity of chemicals to *Colletotrichum gloeosporioides* based on the concentration required to inhibit growth by 50% (ED_{50})

Chemical tested	ED_{50} µg/ml (µl/1*)
BENZIMIDAZOLE	
Benlate	<1
Bavistin	<1
Delsene X	4.5
THIOPHANATE	
Topsin M	<1
N-TRICHLOROMETHYLTHIO GROUP	
Captan	70
Difolatan	3*
Zincofol	4
DITHIOCARBAMATE	
Antracol	50
Manzate D	580
Tricarbamix Special	100
Trimanzone	200
Trimiltox Blue	380
Trimiltox Yellow	120
MISCELLANEOUS FUNGICIDE	
Daconil	570
Rovral	700
INSECTICIDE	
BIDRIN	>2000*
CARBICRON	>2000*

Differences in fungitoxic response between compounds of similar or closely related groups could be due to differences in chemical structure and formulation as can be seen in the case of the greater fungipotency of Difolatan and Zincofol over Captan. This group of N-trichloromethylthio related compounds has an unspecific mode of action inhibiting SH groups of enzymes (Lukens, 1971) as well as enzymes not containing the SH groups such as amino groups (Siegel, 1971). Captan has given good anthracnose control when used alone or in combination with other fungicides (Aragaki and Goto, 1950; Aragaki and Ishii, 1960; Zauberman *et al,* 1976) and preliminary observation trials (Lim, unpublished data) have indicated that Difolatan can effectively control anthracnose.

As a group, the dithiocarbamates were relatively less toxic to *C. gloeosporioides* as compared to the benzimidazoles, thiophanate and Captan groups of compounds. Like the Captan family of compounds, the dithiocarbamates have multisite, unspecifically acting modes of action and the toxic principle was found to be a degradation product, most probably methyl isothiocyanate or ethylenethiuram disulfide (Lukens, 1971). The higher fungitoxicity of premixed formulations of dithiocarbamates over the single fungitoxicant product probably resulted from an enhancement in fungipotency brought about by synergistic effects between the various chemical compounds in the formulation. Differences in formulation of active ingredients and adjuvants could also account for the varying degree of *in vitro* fungitoxicity to *C. gloeosporioides* among the special premixed products. Many of the dithiocarbamates used alone or in combination with other fungicides have given varying degrees of success in the control of mango anthracnose (Aragaki and Goto, 1958; Aragaki and Ishii, 1960; Brodrick, 1971; Conover, 1965; Mora and Vaquez, 1969; and Tandon and Singh, 1968).

In the miscellaneous group, Daconil (chlorothalonil), exhibited very low *in vitro* toxicity to the anthracnose pathogen, though its fungitoxic response is similar to the dithiocarbamates in that it inhibits cell thiols (Vincent and Sisler, 1968). However, Daconil has been used singularly and in combinations with other chemicals as in-the-bloom sprays for control of anthracnose (Conover, 1965). Rovral, representing a relatively novel family of fungicides, the hydantoin, appears to be rather ineffective *in vitro* against the mango anthracnose pathogen. Rovral was found to exhibit higher fungipotency towards basidiomycetous pathogens especially *Ganoderma psuedoferreum* and *Rigidoporus lignosus* (Lim and Varghese, 1979). Though it was developed in 1970 by Rhone-Poulenc of France its biochemical mode of fungitoxicity has yet to be elucidated.

Unsurprisingly, the two commercial formulations of dicrotophos, Bidrin and Carbicron, showed negligible toxicity towards *C. gloeosporioides.* They were included in this investigation as they are frequently used in triple "cocktail" combinations together with mancozeb and a foliar fertiliser for controlling the anthracnose pathogen in the Serdang campus. Possible synergism between dicrotophos and other fungitoxicants in such triple mixtures had been observed and will be dealt with in a subsequent paper.

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REFERENCES

ANON (1972): Acreage of miscellaneous crops for Peninsular Malaysia. *Ministry of Agriculture and Cooperatives*. Kuala Lumpur, Malaysia.

ANON (1977): Area of miscellaneous crops for Peninsular Malaysia. *Ministry of Agriculture*. Kuala Lumpur, Malaysia.

ARAGAKI, M. and GOTO, S. (1958): Mango anthracnose control in Hawaii. *Plant Disease Reptr.* **42:** 474–475.

ARAGAKI, M. and ISHII, M. (1960): Fungicidal control of mango anthracnose. *Plant Disease Reptr.* **44:** 318 – 323.

BRODRICK, H. T. (1971): Mango diseases. *Farming in S. Africa* **47:** 29 – 32.

CLEMONS, G. P. and SISLER, H. D. (1969): Formation of a fungitoxic derivative from benlate. *Phytopathology* **59:** 705.

CONOVER, R. A. (1965): Results of recent experiments or the control of mango anthracnose. *Proc. Fla. Sta. Hort. Soc.* **78:** 364 – 369.

FINNEY, D. J. (1971): Probit analysis. (3rd ed.). Cambridge, London, New York and Melbourne. Cambridge Univ. Press.

HAMMERSCHLAG, R. S. and SISLER, H. D. (1972): Differential action of benomyl and methyl-2-benzimidazole carbamate (MBC) in *Saccharomyces pastorianus*. *Pestic. Biochem. Physiol.* **3:** 42 – 54.

KAARS-SIJPESTEIJN, A. (1972): "Effects on fungal pathogen" in *Systemic fungicides*. R. W. Marsh (Ed.) London. Longman.

LIM, T. K. and VARGHESE, G. (1979): Fungitoxicity of some new compounds against selected Malaysian plant pathogens. *In vitro* studies: Paper presented at the Second Malaysian Microbiology Symposium. Kuala Lumpur. 19 – 20 Nov, 1979.

LUKENS, R. J. (1971): *Chemistry of fungicidal action*. Berlin and New York. Springer-Verlag.

MCMILLAN, R. T. (1973): Control of anthracnose and powdery mildew of mango with systemic and non-systemic fungicides. *Trop. Agric.* **50:** 245 – 248.

MORA, R. F. and VAQUEZ, R. M. (1969): Fungicides trial to prevent anthracnose *(Glomerella cingalata)* on mango. *Agriculture Tec. Mexico* **11:** 479 – 482.

SIEGEL, M. R. (1971): Reactions of the fungicide folpet (N-Trichloromethythro-phthalimide) with a nonthiol protein. *Pestic. Biochem. Physiol.* **1:** 234 – 240.

TANDON, J. N. and SINGH, B. B. (1968): Control of mango anthracnose by fungicides. *Indian Phytopathol.* **21(2):** 212 – 216.

VINCENT, P. G. and SISLER, H. D. (1968): Mechanism of antifungal action of 2, 4, 5, 6-tetrachloro-isophthalonitrile. *Physiol. Plant.* **21:** 1249 – 1264.

ZAUBERMAN, G.; SCHIFFMAN, N. M. and YONKO, M. (1976): Control of fungi causing decay of mango fruits during storage. *Special Publication of the Agric. Res. Org. Israel.* **65:** 105 – 106.

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