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Comparative Invitro Sensitivity of Selected Chemicals on *Phytophthora palmivora* from Cocoa and Durian

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ABSTRAK

Lima sebatian sistemik yang baru iaitu metalaxyl, benalaxyl, cyprofuram, propamocarb dan fosetyl Al dan dua racun kulat pelindung biasa, etridiazol dan captafol telah dibandingkan dari segi kesan invitro terhadap asingan-asingan Phytophthora palmivora *daripada koko dan durian yang terpilih. Metalaxyl, etridiazol dan cyprofuram memberi perencatan yang tinggi kepada pertumbuhan miselium, sporangium dan pengeluaran klamidospora, percambahan terus sporangium dan perkembangan tiub cambahan kedua-dua asingan-asingan koko dan durian, sementara benalaxyl memberi perencatan yang sederhana. Etridiazol dan benalaxyl memberi halangan yang lebih terhadap percambahan zoospora tetapi metalaxyl dan cyprofuram secara perbandingan adalah tidak berkesan. Captafol juga memberi halangan tinggi terhadap pertumbuhan miselium, percambahan terus sporangium dan zoospora, tetapi kurang berkesan terhadap pengeluaran sporangium dan klamidospora. Propamocarb dan fosetyl Al secara perbandingan adalah paling tidak berkesan terhadap semua peringkat perkembangan asingan-asingan melainkan bagi percambahan zoospora asingan durian di mana fosetyl Al memberi perencatan yang tinggi.*

ABSTRACT

Five new systemic compounds viz. metalaxyl, benalayxl, cyprofuran, propamocarb and fosetyl Al and two standard protectants, etridiazole and captafol were compared for invitro effects on representative isolates of Phytophthora palmivora *from cocoa and durian. Metalaxyl, etridiazole and cyprofuram were highly inhibitory to mycelial growth, sporangium and chlamydospore production, direct sporangium germination and germ-tube development of both cocoa and durian isolates while benalaxyl was moderately inhibitory. Etridiazole and benalaxyl were more suppressive on zoospore germination but metalaxyl and cyprofuram were relatively ineffective. Captafol was also highly suppressive to mycelial growth, direct sporangium and zoospore germination but less so on sporangium and chlamydospore production. Propamocarb and fosetyl Al were comparatively the least effective against all the developmental stages of the isolates except in the case of zoospore germination of the durian isolate where the latter was highly inhibitory.*

INTRODUCTION

The ubiquitous parasitism of *Phytophthora palmivora* is reflected by the wide array of crops it attacks (Chee, 1969; 1974). Among the important agricultural crops attacked are cocoa and durian. On cocoa, the fungus causes black pod rot, decay of flower cushions and cherelles,

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stem canker, blight of shoots and chupon, dieback of budgrafted and hand pollinated hybrid seedlings, and root rot. On durian, the fungus causes root rot, patch canker, leaf blight and dieback of seedlings and trees and recently fruit rot (Lim and Chan, 1986).

Control measures adopted to control this fungus are mainly confined to cultural means or the use of non-systemic fungicides which have provided little and erratic success. With the advent of new systemic fungicides such as the acylalanines – metalaxyl (Urech, *et al.*, 1977), and furalaxyl (Wiertsema and Wissink, 1977) by Ciba-Geigy; benalaxyl by Farmoplant Montedison (Bergamuschi *et al.*, 1981) and butryolactones (derivatives of acylalanines) like milfuram by Chevron (Lukens *et al.*, 1978) and cyprofuram by Schering, A.G. (Schering, 1982); the carbamates – prothiocarb and propamocarb by Schering, A.G. (Pieroh *et al.*, 1978); cymoxanil, a cyanoacetamide oxime by E.I. dupont de Nemours (Denis, 1976) and the ethyl phosphites e.g. fosetyl Al (Williams *et al.*, 1977); a marked improvement in the chemical control of Oomycete diseases can now be realised. This paper reports on the comparative invitro effects of some of these new compounds and a few conventional protectants on the mycelial growth, production of sporangia and chlamydospores, sporangium and zoospore germination on two representative isolates of *P. palmivora* from cocoa and durian.

MATERIALS AND METHODS

The systemic fungicides and protectants listed in Table 1 were tested for comparative invitro effects against two representative isolates of *Phytophthora palmivora,* PCl from cocoa and PDR from durian. For the studies, five-day-old vegetable juice agar (VJA) cultures were used and all experiments were carried out at 28 ± 1.5°C unless otherwise stated.

Effects on Mycelial Linear Growth

Suitable dilutions of each chemical (Table 1) were prepared separately with sterile distilled water and incorporated into standardized amounts of sterile molten Difco Corn Meal Agar (CMA) kept at 45°C to obtain the desired final concentrations of 0.01, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 and 50.0 μg/ml a.i. A six-mm inoculum

TABLE 1
Fungicides used in the toxicity studies on cocoa (PCI) and durian (PDR) isolates of *Phytophthora palmivora*

Product name	Trivial name	Chemical name
Ridomil 25 WP	Metalaxyl	N-(2, 6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester
Galben 25 WP	Benalaxyl	Methyl N-phenylacetyl-N-2, 6-xylyl-DL-alaninate
Aliette 80 WP	Fosetyl Al	Aluminium tris-ethyl phosphonate
Previcur-N 70 EC	Propamocarb	Propyl 3-(dimethylamino) propylcarbamate
Vinicur 20 WP	Cyprofuram	3-chloro-N-(2-oxoperhydro-3-furyl)-cyclopropane-carboxanilide
Difolatan 4F	Captafol	3a, 4, 7, 7a-tetrahydro-N-(1, 1, 2, 2, tetrachloroethane-sulphenyl phthalidamide)
Terrazole 25 EC	Etridiazole	5-ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole

plug taken from the advancing margin of the VJA colony of the test isolates was placed centrally on each plate after solidification. The seeded plates were incubated in the dark at 28 ± 1.5°C. Each chemical including the blank check agar which contained no chemical was tested in 4 replicates. Colony growth diameters of the fungus were measured at right angles regularly up to 7 days. Data at 7th day were subjected to probit analysis (Finney, 1971) where the probit inhibition of the fungal growth was plotted against the log concentration of the chemical. A regression line was calculated and the amount of fungicide required to inhibit 50% of the growth of the fungus (ED_{50} value) was determined.

Effects on Production of Zoosporangium and Chlamydospore

The procedure was similar to that used in mycelial growth studies except that VJA medium was used. The same chemicals were used at the following concentrations: 0.1, 1.0, 10.0, 100, 500 and 1,000 μg/ml. Blank check plates consisted of VJA minus the chemical. Test plates were incubated in the dark at 28 ± 1.5°C in 3 replicates.

After 7 days of incubation, colony measurements were made and a standardized amount of sterile distilled water was added into each plate. A bent glass rod was used to dislodge the spores and the suspension was filtered using muslin cloth to remove mycelial fragments. A Neubauer haemocytometer was used to determine the number of sporangia and chlamydospores produced. Mean spore count data were transformed using logarithmic transformation (log X + 1) (Gomez and Gomez, 1976) and were subjected to ANOVA statistical analysis.

Effects on Sporangial Direct Germination

A cellophane transfer technique was employed. Sterilized 20 mm × 20 mm cellophane (E.I. Dupont de Nemours & Co., PUDO 193) squares were laid on petri plates containing water agar (WA) which were incorporated with appropriate concentrations of the test fungicide. Drops of sporangial/fungicide solution prepared from equal volumes of double strength solution of the fungicide and spore suspension were placed on these cellophane squares making the final concentration similar to that in the sporangial production test. Control consisted of sterile distilled water plus the sporangial suspension. Petri plates were kept in the dark at 25 ± 1.2°C for 16 hrs after which cellophane squares were mounted on glass slides and stained with lactophenol blue. Slides were gently flamed to prevent further germination of the sporangia. Percent germination and length of germ-tube based on the first 200 sporangia encountered were determined. A sporangium was deemed germinated when the germ-tube exceeded the width of the spore.

All data were analyzed using the probit analysis to determine the ED 50 values.

Effects on Zoospore Germination

Coors porcelain plates with 12 depressions (112 mm × 87 mm; 5 mm deep) were used. The depressions were filled with 0.5 ml of double strength solution of each fungicide and a spore solution with 2,000 sporangia/ml was added giving the final test concentrations of 0.1, 1.0, 10.0, 100, 500 and 1,000 μg/ml a.i. Check treatments consisted of half ml of sterile distilled water added with another half ml of sporangium suspension of the isolate. Plates were incubated in the dark for 4 hrs at 6°C and then returned to room temperature (28 ± 1.5°C) to facilitate the release of spores. With the use of a sterilized pipette, drops of the mixed solution from the Coors porcelain plate were mounted on clean glass slides, stained with lactophenol and gently flamed with an alcohol lamp. Percent germination and length of germ-tube were determined based on the first 200 zoospores encountered under the ×40 magnification of the microscope. Data was then subjected to probit analysis.

RESULTS

Effects on Mycelial Linear Growth

The fungicides tested exhibited varying degrees of fungitoxicity on mycelial growth of

both isolates of *P. palmivora* from cocoa and durian (Table 2). Metalaxyl and captafol were the most effective with ED_{50} values of >0.1 µg/ml for both isolates. Etridiazole was also effective against the two isolates with ED_{50} value of 0.2 µg/ml. Mycelial growth of the durian isolate (PDR) appeared to be extremely sensitive to etridiazole with an ED_{50} value as low as 0.001 µg/ml. Of the four remaining fungicides tested, the order of fungitoxicity was cyprofuram, benalaxyl, fosetyl Al and propamocarb.

Effects on Sporangium Production

The invitro efficacy of fungicides against sporangium production of *P. palmivora* cocoa and durian isolates is presented in Table 3. Metalaxyl displayed its superiority over the rest of the fungicides tested. It significantly reduced sporangium production at 0.1 µg/ml and completely inhibited production of sporangia at 1.0 µg/ml. Etridiazole and cyprofuram ranked second best followed by benalaxyl. Captafol and fosetyl Al showed greater toxicity towards the cocoa isolate, completely inhibiting sporangium formation at 500 µg/ml. Adverse effects of propamocarb on both isolates were only observed at 1,000 µg/ml.

Effects on Chlamydospore Production

A similar trend was observed in chlamydospore production as in sporangium production (Table 4). Metalaxyl and etridiazole were the most effective followed by cyprofuram. The PDR isolate was, however, more sensitive to etridiazole compared to PCl, completely halting chlamydospore production of the former isolate at 0.1 µg/ml. Similarly, propamocarb displayed greater toxicity towards the durian isolate effecting 100% inhibition at 10 µg/ml. Fosetyl Al was only effective at 500 µg/ml.

Effects on Direct Germination of Sporangia

Metalaxyl, captafol and etridiazole had the most adverse effects on germination of sporangia of *P. palmivora* (Table 5). On the cocoa isolate, these fungicides had ED_{50} values of <0.1 µg/ml while an ED_{50} value of 0.8 µg/ml was recorded for the durian isolate. Captafol and etridiazole were, however, superior to metalaxyl in completely suppressing direct germination of sporangia of both isolates as indicated by the lower ED_{100} values. Cyprofuram was also effective against PDR in particular with an ED_{50} value of 0.08 µg/ml. In contrast, benalaxyl and fosetyl Al displayed more toxicity towards PCl.

TABLE 2
Effects of chemicals on mycelial growth of *Phytophthora palmivora* cocoa (PCI) and durian (PDR) isolates

Chemical	ED_{50} Value (µg/ml)	
	PCI	PDR
Metalaxyl	0.02	0.01
Benalaxyl	13.01	4.98
Fosetyl Al	20.31	20.34
Propamocarb	30.70	39.81
Cyprofuram	3.09	1.78
Captafol	0.01	0.09
Etriadiazole	0.21	0.001

TABLE 3
Effects of fungicides on sporangium formation of *Phytophthora palmivora* cocoa (PCI) and durian (PDR) isolates

Chemical	Isolate	Mean sporangial count* ($\times 5 \times 10^4$)						
		Chemical concentration (μg/ml)						
		0	0.1	1	10	100	500	1000
Metalaxyl	PCI	4.23 a**	0.77 b	0 c	0 c	0 c	0 c	0 c
	PDR	3.83 a**	0.80 b	0 c	0 c	0 c	0 c	0 c
Benalaxyl	PCI	1.50 a	1.07 a	1.07 a	0.43 b	0.43 b	0 c	0 c
	PDR	5.60 a	2.47 b	1.67 c	0.30 d	0.13 de	0 e	0 e
Fosetyl Al	PCI	0.87 a	1.77 a	0.90 a	0.77 a	0.83 ab	0.07 bc	0 c
	PDR	8.60 a	8.53 a	7.90 a	9.10 a	1.60 b	0 c	0 c
Propamocarb	PCI	2.09 a	1.17 ab	0.83 b	0.07 c	0.13 c	0.07 c	0 c
	PDR	1.73 ab	2.20 a	0.97 abc	0.27 c	0.57 abc	0.13 bc	0 c
Cyprofuram	PCI	5.43 a	5.57 a	1.47 b	0 c	0 c	0 c	0 c
	PDR	4.73 a	0.83 b	0.13 c	0.13 c	0 c	0 c	0 c
Captafol	PCI	3.23 a	2.83 a	1.23 b	0.57 b	0.33 bc	0 c	0 c
	PDR	1.83 a	1.30 a	0.43 b	0.27 b	0.67 b	0 e	0 e
Etridiazole	PCI	3.53 a	2.13 b	0.83 c	0 d	0 d	0 d	0 c
	PDR	2.50 a	0.23 b	0.13 b	0 b	0 b	0 b	0 b

*Each figure is an average of 3 replicates.

**Figures followed by the same letter in each row are not significantly different at P = 0.05 using transformed data.

Propamocarb was the least effective among the fungicides tested.

The same pattern was also recorded for the germ-tube development of sporangia of both isolates (Table 5). Etridiazole, captafol and metalaxyl were more effective in reducing the germ-tube length in comparison with the other fungicides tested. Benalaxyl, fosetyl Al and cyprofuram were intermediate in their effects while propamocarb remained comparatively ineffective.

Effects on Zoospore Germination

Among the fungicides tested, captafol showed the most toxic effect on zoospore germination of both isolates (Table 6). Captafol at 1.0 μg/ml and < 0.1 μg/ml produced 100% and 50% inhibition of zoospore germination of the two isolates, respectively. Although higher amounts of fosetyl Al was needed against the cocoa isolate, it displayed greater toxicity against zoospore germination of the durian isolate with an ED_{50} value of 0.29 μg/ml. Benalaxyl and etridiazole were moderate in action whereas metalaxyl, propamocarb and cyprofuram were only effective at higher concentrations.

On zoospore germ tube development, a similar trend was observed, with captafol maintaining its leading efficacy on the two isolates (Table 6). Captafol at 0.1 μg/ml resulted in 50% reduction in length of germ-tube of both isolates. The rest of the chemicals required higher dosages before they could bring about the same effect. Metalaxyl, in particular, exhibited less effects against the zoospores compared to its high toxicity against sporangium and clamydospore production, and sporangium germination of the isolates.

TABLE 4
Effects of chemicals on chlamydospore production of *Phytophthora palmivora* cocoa (PCI) and durian (PDR) isolates

Chemical	Isolate	Mean chlamydospore count* ($\times 5 \times 10^4$)						
		Chemical concentration (μg/ml)						
		0	0.1	1	10	100	500	1000
Metalaxyl	PCI	1.37 a**	0.63 b	0 c	0 c	0 c	0 c	0 c
	PDR	0.20 a**	0.10 b	0 b	0 b	0 b	0 b	0 b
Benalaxyl	PCI	1.83 a	0.53 b	0.30 b	0.23 b	0 c	0 c	0 c
	PDR	0.60 a	0.50 a	0.17 b	0 b	0 b	0 b	0 b
Fosetyl Al	PCI	0.67 a	0.20 a	0.57 a	0.50 a	0.43 a	0 a	0 a
	PDR	0.77 ab	1.03 a	0.90 a	1.07 a	0.43 ab	0 b	0 b
Propamocarb	PCI	1.20 a	1.67 a	1.30 a	0.17 b	0.30 b	0.23 b	0 b
	PDR	0.37 a	0.33 a	0.27 a	0 a	0 a	0 a	0 a
Cyprofuram	PCI	1.77 a	1.07 b	0.30 c	0 d	0 d	0 d	0 d
	PDR	0.33 a	0.20 ab	0 b	0 b	0. b	0 b	0 b
Captafol	PCI	0.77 abc	1.57 a	1.00 ab	0.20 c	0.43 bc	0 d	0 d
	PDR	0.27 ab	0.17 b	0.43 a	0.07 b	0.23 ab	0 c	0 c
Etridiazole	PCI	0.83 a	0.07 b	0 b	0 b	0 b	0 b	0 b
	PDR	0.27 a	0 b	0 b	0 b	0 b	0 b	0 b

*Each figure is an average of 3 replicates.

**Figures followed by the same letter in each row are not significantly different at P = 0.05 using transformed data.

TABLE 5
Invitro effects of fungicides on direct germination of sporangia of *Phytophthora palmivora* isolates from cocoa (PCI) and Durian (PDR)

Chemical	ED_{50} value for germination* (μg/ml)		ED_{100} value for germination* (μg/ml)		ED_{50} tor germ tube length** (μg/ml)	
	PCI	PDR	PCI	PDR	PCI	PDR
Metalaxyl	0.05	0.68	1000	500	0.29	0.74
Benalaxyl	0.53	>1000	>1000	>1000	20.59	486.05
Fosetyl Al	>10<100	>1000	100	>1000	>10<100	>500<1000
Propamocarb	>10000	>1000	>1000	>1000	>1000	>1000
Cyprofuram	3.92	0.08	>1000	500	28.1	<100<500
Captafol	<0.1	>0.1<1	1	10	<0.1	<0.1<1
Etridiazole	<0.1	>0.1<1	1	100	<0.1	0.72

*Based on the first 200 zoosporangia per replicate encountered.

**Based on the actual number of germinated zoosporangia.

TABLE 6
Effects of fungicides on zoospore germination of *Phytophthora palmivora* cocoa (PCI) and durian (PDR) isolates after 4 hours of incubation

Chemical	ED_{50} value for germination* (μg/ml)		ED_{100} value for germination* (μg/ml)		ED_{50} value for germ-tube length** (μg/ml)	
	PCI	PDR	PCI	PDR	PCI	PDR
Metalaxyl	>100<500	>100<500	500	1000	>100<500	>100<500
Benalaxyl	10	6.92	>1000	>1000	7.56	281.84
Fosetyl Al	>100<500	0.29	500	1000	55.69	1.70
Propamocarb	100	277.21	500	>1000	137.29	85.60
Cyprofuram	>500<1000	79.22	1000	1000	125.89	60.23
Captafol	<0.10	< 0.10	1	1	0.10	0.10
Etridiazole	10.00	69.64	1000	500	1.98	69.64

*Based on the first 200 zoospores per replicate encountered.

**Based on the actual number of germinated zoospores.

DISCUSSION

A distinct variation in the invitro efficacy of selected fungicides on the cocoa and durian isolates of *P. palmivora* was observed. A differential response was noted among the acyalanines — metalaxyl, benalaxyl, and the acyalanine derivative (butryolactones) — cyprofuram, although all these were reported to share similar basic properties (Schwinn, 1983). Metalaxyl was the most active in inhibiting the various stages of development except zoospore germination of both cocoa and durian isolates followed by cyprofuram. The low ED_{50} values of below 1 μg/ml recorded for metalaxyl on mycelial growth, sporangium and chlamydospore formation and germination were also reported for other *P. palmivora* isolates from cocoa (Tey and Wood, 1983), orchids (Lim and Nio, 1983) and other *Phytophthora* spp. (Staub and Young, 1980; Farih *et al.*, 1981; Coffey *et al.*, 1984). Benalaxyl was more active in suppressing zoospore germination of the isolates than metalaxyl or cyprofuram. The low invitro fungitoxicity of metalaxyl on zoospore germination was also observed for *P. parasitica* (Farih, *et al.*, 1981), *P. cinnamomi* and *P. citricola* (Coffey, *et al.*, 1984).

The cocoa isolate of *P. palmivora* appeared to be more sensitive to fosetyl Al during sporangium germination whereas the durian isolate appeared to be more sensitive during zoospore germination. However, the overall invitro activity of fosetyl Al is relatively low. The relatively weak invitro toxicity of fosetyl Al was also reported by Farih, *et al.* (1981); Lim and Nio (1983), Schwinn, (1983) and Tey and Wood (1983). Compared to its breakdown product, phosphorus acid (Hai, *et al.*, 1979), fosetyl Al was less fungipotent invitro. However, recently Fenn and Coffey (1984) reported that in low phosphate medium, fosetyl Al exhibited much higher antifungal activity on *P. cinnamomi.*

The relatively low invitro efficacy of propamocarb (Previcur-N 70 EC) against all the developmental stages of *P. palmivora* cocoa and durian isolates suggest that it may not be active against this particular species of *Phytophthora*. Negligible activity of propamocarb hydrochloride was similarly obtained invitro against *P. palmivora* cocoa isolate by Tey and Wood (1983). The invitro toxicity of propamocarb might be similar to its analogue prothiocarb which was reported to exhibit full efficacy only at neutral pH of the substrate and against some

species of *Phytophthora* only at temperatures around 10°C (Kerkenaar and Kaars Sijpesteijin, 1977).

Etridiazole, which was introduced in 1969 for controlling diseases caused by Oomycetes, demonstrated a strong invitro efficacy against almost all the developmental stages of the two test isolates, comparable to that of metalaxyl. Both etridiazole and metalaxyl were comparatively less inhibitory to zoospore germination. The high invitro activity of etridiazole against other *Phytophthora* spp. was also reported by Grant and Chew, (1981).

Captafol, another non-systemic fungicide, demonstrated a very strong invitro efficacy against almost all the development stages of *P. palmivora* from cocoa and durian. Its high activity was comparable to metalaxyl against mycelial growth, sporangium germination and germ-tube extension of both isolates. Unlike metalaxyl, captafol was highly toxic to zoospore germination as well as its germ-tube development. The excellent invitro activity of captafol has also been reported against mycelial growth, production of sporangia and zoospore of *P. palmivora* from cocoa (Tey and Wood, 1983). Mycelial growth of *P. palmivora* from orchid was similarly affected by captafol (Lim and Nio, 1983).

Taking into consideration the varying efficacy of the fungicides reported here on the different developmental stages of *P. palmivora* as well as their strengths and weaknesses, integrating their use would result in a more efficacious control. Besides, such a strategy can help to counteract the development of resistant populations of *Phytophthora* spp. when only one systemic fungicide is used continuously (Schwinn, 1983).

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