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Efficacy of selected triorganotin(IV) compounds on leaves against *Phytophthora palmivora* (Butler) Butler isolated from black pepper and cocoa

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Several triorganotin(IV) compounds and Terrazole® 35 WP were screened for their *in vitro* antifungal activity against three isolates of *Phytophthora palmivora*. Two isolates (isolates Phy. 2 and Phy. 334) were obtained from black pepper (*Piper nigrum* L.) and one isolate (isolate Phy. 56) from cocoa leaves (*Cacao theobromae*). ED_{50} values for radial growth of the isolates ranged from 0.09 to 1,700 $\mu g\ cm^{-3}$ for the triorganotin(IV) compounds and from 3.46 to 1 227 000 $\mu g\ cm^{-3}$ for Terrazole®. Diphenylbutyltin bromide exhibited the highest antifungal activity against the three isolates of *P. palmivora* with ED_{50} values ranging from 0.30 to 0.73 $\mu g\ cm^{-3}$.

Diphenylbutyltin bromide was equally effective against a freshly isolated virulent culture of *P. palmivora* (isolate Phy. 346) from black pepper leaves in Sarawak, East Malaysia, yielding an ED_{50} value for radial growth of 0.87 $\mu g\ cm^{-3}$ and a probit-log concentration regression line slope value of 1.04. *In vitro* efficacy of diphenylbutyltin bromide against isolate Phy. 346 using detached healthy pepper leaves showed 40–75% infection of leaves at 100 $\mu g\ cm^{-3}$ and no infection at 500 $\mu g\ cm^{-3}$. Diphenylbutyltin bromide at 100 $\mu g\ cm^{-3}$, however, inhibited the diameter of lesion by 43.3–73.7% compared with the untreated controls. Black pepper leaves treated with Terrazole® at 778 $\mu g\ cm^{-3}$ exhibited 5.3–33.3% inhibition of lesion diameter compared with the untreated controls, where 90–100% of the leaves were infected. Concentrations of diphenylbutyltin bromide of 1000–2500 $\mu g\ cm^{-3}$ caused some injury lesions on the leaves. From the results obtained, it appears that diphenylbutyltin bromide could be used as a protective spray or drench against *P. palmivora* infection of black pepper at 100–500 $\mu g\ cm^{-3}$.

Keywords: Organotins, fungitoxicity, *Phytophthora*, pepper, cocoa

INTRODUCTION

Phytophthora palmivora parasitizes 51 genera in 29 families of seed-bearing plants.[1] The most serious *Phytophthora* diseases in Malaysia are foot rot in black pepper[2,3] and black pod rot in cocoa.[4] Black pepper is a crop of primary importance in Sarawak, East Malaysia, and losses of over 10% have been estimated to be due to foot rot disease.[5,6] Cocoa is one of the three important export crops of Malaysia after rubber and oil palm. Terrazole® has been recommended for the control of foot rot in black pepper,[7] whereas metalaxyl (Ridomil®), a systemic fungicide, is widely used for the control of black pod rot in cocoa.[8,9] Resistance to Ridomil®, however, has developed in *P. infestans* on potato[10–12] and on tomato.[13] Development of resistance to Ridomil® in *P. palmivora* too cannot be discounted and signs of Ridomil® resistance have already been observed in East Malaysia (Kueh Tiong-Kheng, personal communication). Hence, new fungicides are urgently needed for the effective control of *Phytophthora* diseases in black pepper and cocoa. The present study was carried out to evaluate the *in vitro* and *in vivo* efficacies, including the structure–activity relationships, of some new and known triorganotin(IV) compounds against *P. palmivora* isolates from black pepper and cocoa.

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MATERIALS AND METHODS

Culture isolates

The characteristics of the *P. palmivora* isolates used in this study are summarized in Table 1.

Table 1 Isolates of *Phytophthora* used

Phytophthora palmivora isolates	Sporangial size on V-8 juice agar ($\mu m \times \mu m$)	Host
Phy. 2	20–40 × 59–88	Black pepper
Phy. 56	34–49 × 34–49	Cocoa
Phy. 334	(No sporangia formed in culture)	Black pepper
Phy. 346	25–39 μm × 49–59	Black pepper

Triorganotin(IV) compounds

A total of 11 triorganotin(IV) compounds and Terrazole® 35 WP were evaluated for their *in vitro* antifungal activity against the three isolates of *P. palmivora*, as shown in Table 2. Stock solutions of the compounds were prepared using analytical-grade acetone (10^4 μg cm^{-3}) and these were serially diluted for the tests using distilled water.

In vitro evaluation

Known volumes of V-8 juice agar (V8-JA) were autoclaved and cooled to approximately 40°C. Stock solutions of the triorganotin(IV) compounds and Terrazole® 35 WP were freshly prepared and each aseptically added to the cooled agar to give toxicant concentrations of 0.1, 1.0, 5.0, 10.0, 25.0, 50.0 and 100.0 μg cm^{-3}, respectively. The mixtures were shaken thoroughly and 15-cm^3 aliquots were poured into a series of 9-cm sterile plastic Petri dishes. Each dish was inoculated at its centre with a 4-mm diameter disc cut from the vegetative growing margins of four-day-old *P. palmivora* isolates Phy. 2, Phy. 56 and Phy. 334 maintained on V8-JA. Cultures seeded on plain V8-JA served as control. Three replicate plates per concentration for each test compound were used for every isolate. The plates were incubated at 27 ± 2°C for four–seven days. When the cultures on the control plates showed near maximum growth, the colony diameters were measured at right angles to each other for all treatments. Percentage inhibition of growth compared to the control was calculated from the mean diameter of colonies minus the diameter of the inoculum disc. A probit-log concentration analysis[14] was carried out to determine the ED_{50} values (i.e. concentrations causing a 50% reduction in growth

Table 2 Comparison of ED_{50} values (μg cm^{-3}) of triorganotin(IV) compounds and Terrazole 35 WP for radial growth of three isolates of *Phytophthora palmivora* (V8-JA; pH=6.9; 29±2°C)

Compound code	Compound	Fungus[b] Isolate Phy. 2	Isolate Phy. 56	Isolate Phy. 334
B1	$Bu_3SnOCOC_6H_4CO_2$—sucrose	0.47	1.07	1.04
P1	Ph_3SnOAc	0.46	5.47	25
P2	$Ph_3SnCl \cdot Ph_3PO$	1.46	1.18	62
P3	$(Ph_3Sn)_2S$	0.43	0.3	1700
P4	$Ph_3SnOCOR$ (R = 3-indolyl)	0.32	0.28	44
P5	Ph_3SnLH[a]	nd	nd	63
M1	$Ph_2BuSnBr$	0.30	0.35	0.73
M2	$(p\text{-}MeC_6H_4)Ph_2SnOAc$	0.64	0.18	120
M3	$(p\text{-}ClC_6H_4)_2(p\text{-}MeC_6H_4)SnCl$	nd	1.14	nd
M4	$(2\text{-}C_4H_3S)(p\text{-}MeC_6H_4)_2SnCl$	2.20	1.14	nd
M5	$(p\text{-}MeOC_6H_4)Ph_2SnBr$	0.69	0.90	nd
—	Terrazole® 35 WP	3.46	6.09	1 227 000

[a] LH = 2-ethylamino-4-hydroxy-5-(n-butyl)-6-methylpyrimidine. [b] Abrreviation: nd = not determined.

measured in $\mu g\ cm^{-3}$) for the various compounds against each of the *P. palmivora* isolates.

Pathogenicity study

Freshly detached black pepper leaves were surface-sterilized and lightly pricked in the centre with a sterilized inoculating needle. Agar discs (diameter 4 mm) from seven-day-old *P. palmivora* isolates Phy. 2, Phy. 56. Phy. 334 and Phy. 346 maintained on V8-JA in Petri dishes, which were previously chilled (at 10°C) to induce zoospore production, were cut out using a sterilized cork-borer. A disc was then placed over the wounded part on the leaf and the inoculated sites were covered with small pieces of moist sterile cotton wool. The inoculated leaves were then kept in humid chambers at 27 ± 2°C. Sterile V8-JA discs, 4 mm diameter placed over the wound were used as control.

In vivo evaluation of diphenylbutyltin bromide against isolate Phy. 346

Young and old leaves were picked from 18-month-old black pepper (*Piper nigrum* L. var. Kuching) plants with their petioles intact and immediately immersed in tap water. The leaves were washed thoroughly in tap water and surface-sterilized with 1% v/v Chlorox (sodium hypochlorite 5.25%, inert ingredients 94.75%) for 5 min. The leaves were then rinsed three times in sterile distilled water. The petioles were covered with sterile moist cotton wool and laid in 15 cm × 15 cm × 3.5 cm plastic humidity boxes lined with sterilized and moistened botting paper 0.5 mm thick. Two leaves were placed, some distance apart, in each box.

Concentrations of 50, 100, 500, 1000 and 2500 $\mu g\ cm^{-3}$ of diphenylbutyltin bromide were prepared with distilled water using a 10 000 $\mu g\ cm^{-3}$ stock solution in acetone. For each concentration, ten leaves were sprayed on the underside until run-off using a Shandon Laboratory Spray Gun No. 2046. A 1:4 (v/v) mixture of acetone and distilled water served as control. The commerically recommended concentration of 778 $\mu g\ cm^{-3}$ a.i. (active ingredient) Terrazole® 35 WP was used as a comparative control. Half an hour after spraying, one set of leaves was inoculated at the centre with a 4 mm-disc cut out from a previously chilled (at 10°C) well-sporulating 10-day old isolate of Phy. 346 culture on V8-JA. The disc was covered with a thin layer of sterile absorbent cotton wool and moistened with two drops of sterile distilled water. The leaves were incubated at 27 ± 2°C under alternating cycles of 12 h light and 12 h dark for six days. The blotting paper in the humidity boxes was moistened each day. Another set of leaves was inoculated one day after spraying with diphenylbutyltin bromide. Both experiments were performed in duplicate. The number of leaves infected was recorded each day. The diameters of the lesions as seen from the upper side of the leaves were measured at right angles to each other six days after inoculation.

RESULTS

In vitro efficacy of triorganotin(IV) compounds against *Phytophthora palmivora* isolates

The ED_{50} values for radial growth are given in Table 2. Isolates Phy. 2 and Phy. 56 were sensitive to all the triorganotin(IV) compounds with ED_{50} values ranging from 0.18 $\mu g\ cm^{-3}$ to 5.47 $\mu g\ cm^{-3}$. The non-sporulating isolate Phy. 334 was able to tolerate all the triorganotin(IV) compounds except diphenylbutyltin bromide (ED_{50}=0.73 $\mu g\ cm^{-3}$) which showed high activity. Terrazole® was not effective against isolate Phy. 334 (ED_{50} = 1 227 000 $\mu g\ cm^{-3}$). Diphenylbutyltin bromide showed the highest toxicity to all three *P. palmivora* isolates (Table 2), being effective at even lower concentrations than phthalic acid (tributylstannyl)(sucrose) ester (compound B1).

Pathogenicity study

Isolates Phy. 2, Phy. 56 and Phy. 334 were not pathogenic to the black pepper leaves. Isolate Phy. 346 caused lesions with frimbriate margins three days after surface inoculation on the underside of the leaves. A mean diameter of lesion of 3.0 cm was observed after six days of incubation.

In vitro efficacy of diphenylbutyltin bromide against isolate Phy. 346

An ED_{50} value of 0.87 $\mu g\ cm^{-3}$ was obtained for diphenylbutyltin bromide against Phy. 346. Thus, diphenylbutyltin bromide is effective against all four *P. palmivora* isolates at concentrations ranging from 0.30 $\mu g\ cm^{-3}$ to 0.87 $\mu g\ cm^{-3}$.

In vivo efficacy of diphenylbutyltin bromide against isolate Phy. 346

Tables 3 and 4 show results obtained for percentage infection of leaves surface-inoculated with Phy. 346 isolate 0.5 h and 24 h, respectively, after spraying with diphenylbutyltin bromide. On both occasions, more that 80% of the untreated leaves and leaves sprayed with 778 $\mu g\ cm^{-3}$ a.i. Terrazole® 35 WP were infected. Percentage infection was lower when isolate Phy. 346 was inoculated 24 h after fungicide application than when inoculated 0.5 h after fungicidce application. Leaves sprayed with 500 $\mu g\ cm^{-3}$ of diphenylbutyltin bromide suspension were not infected on both occasions.

Table 3 Percentage infection of black pepper leaves when surface-inoculated[a] with isolate Phy. 346

	Treatment:				
	Untreated	Terrazole® 35 WP	Diphenylbutyltin bromide		
	Concn ($\mu g\ cm^{-3}$)				
Day	0	778	50	100	500
1	0	0	0	0	0
2	0	0	0	0	0
3	20	10	10	40	0
4	90	100	100	50	0
5	100	100	100	70	0
6	100	100	100	75	0

[a] 0.5 h after fungicide application.

Table 4 Percentage infection of black pepper leaves when surface-inoculated[a] with isolate Phy. 346

	Treatment:				
	Untreated	Terrazole® 35 WP	Diphenylbutyltin bromide		
	Concn ($\mu g\ cm^{-3}$)				
Day	0	778	50	100	500
1	0	0	0	0	0
2	0	0	0	0	0
3	30	90	0	10	0
4	50	90	0	30	0
5	60	90	10	30	0
6	80	90	30	40	0

[a] 24 h after fungicide application.

The mean diameter of lesions and the percentage inhibition of lesion diameters six days after inoculation for leaves inoculated 0.5 h and 24 h after fungicide application are given in Tables 5 and 6 respectively. The mean diameter of lesions on leaves sprayed with diphenylbutyltin bromide at 100 $\mu g\ cm^{-3}$ and above was lower than that for the untreated leaves and leaves sprayed with Terrazole®. Control of infection was better when leaves were sprayed 24 h before inoculation than when sprayed just prior to inoculation. At concentrations of 1000 $\mu g\ cm^{-3}$ and 2500 $\mu g\ cm^{-3}$ of diphenylbutyltin bromide, browning of leaf veins was observed indicating a certain degree of phytotoxic effect.

DISCUSSION

As a structural class, the triorganotin(IV) compounds, R_3SnX, are well established as biocides.[15,16] The influence on toxicity in these compounds is governed largely by the nature of the organic R group compared with the anionic X residue, although in certain species-dependent cases, which include both fungi and insects, the X group is known to exert a strong influence.[17–19] In the present study, the triorganotin compounds, although somewhat randomly chosen, revealed substantially stronger fungicidal activities than Terrazole® and also discriminatory activities against the isolates of *P. palmivora* studied. As indicated by the results in Table 2, the fast growing, non-sporulating isolate Phy. 334 was generally less susceptible than the other isolates but responded more markedly to structural variations in the carbon-bonded moieties and X groups on tin. Thus, replacement of the acetate group in Ph_3SnOAc by the plant growth hormonal moiety,[20] indolyl-3-acetate, resulted in an almost two-fold reduction in activity towards Phy. 334, but improved activities were noted in the cases involving Phy. 2 and Phy. 56. Among the triphenyltins, $(Ph_3Sn)_2S$ (compound P2) was the least active (ED_{50} 1700 $\mu g\ cm^{-3}$) towards Phy. 334. The formally pentaco-ordinated triphenyltin compound, $Ph_3SnCl \cdot Ph_3PO$, was generally less fungitoxic than either P1 or P4 and, surprisingly, was also less active than P2 towards Phy. 2 and Phy. 56 (Table 2).

The mixed triorganotin compound, $Ph_2BuSnBr$, showed maximal activity towards all three isolates of *P. palmivora* compared with the symmetrical Bu_3Sn and Ph_3Sn compounds.

Table 5 Mean diameter of lesions and percentage inhibition of lesion diameters six days after inoculation[a]

	Treatment:				
	Untreated	Terrazole® 35 WP	Diphenylbutyltin bromide		
	Concn ($\mu g\ cm^{-3}$)				
Day	0	778	50	100	500
mean diameter of lesions (mm ± SD)	3.0 ± 1.1	2.0 ± 0.7	3.8 ± 0.8	1.7 ± 1.5	0.0 ± 0.0
Mean diameter of lesions (% of control)	—	66.7	126.7	56.7	0
Reduction (−) or increase (+) over control (%)	—	(−)33.3	(+)26.7	(−)43.3	(−)100

[a] Isolate Phy. 346 was inoculated 0.5 h after fungicide application.

Table 6 Mean diameter of lesions and percentage inhibition of lesion diameters six days after inoculation[a]

	Treatment:				
	Untreated	Terrazole® 35 WP	Diphenylbutyltin bromide		
	Concn ($\mu g\ cm^{-3}$)				
Day	0	778	50	100	500
mean diameter of lesions (mm ± SD)	1.9 ± 1.3	1.8 ± 0.8	0.1 ± 0.3	0.5 ± 0.6	0.0 ± 0.0
Percentage inhibition of lesion diameter (%)	—	5.3	94.7	73.7	100

[a] Isolate Phy. 346 was inoculated 24 h after fungicide application.

Placement of electron-donating substituents in the phenyl ring tended to lower the activity as borne out by the data on (p-MeC_6H_4)Ph_2SnOAc and (p-$OMeC_6H_4$)Ph_2SnBr, compared with the unsubstituted cases. Currently, investigations are being carried out to explore this aspect more fully, including the effects introduced by electron-withdrawing substituents in the phenyl rings.

The *in vivo* efficacy of diphenylbutyltin bromide against isolate Phy. 346 was found to be far superior to that of Terrazole® 35 WP. Control of *P. palmivora* infection on black pepper leaves was achieved at concentrations in the range 100–500 $\mu g\ cm^{-3}$. Concentrations of diphenylbutyltin bromide above 500 $\mu g\ cm^{-3}$ proved to be phytotoxic as evidenced by the injury lesions of leaves sustained at 1000 and 2500 $\mu g\ cm^{-3}$ levels. The injury lesions are probably the consequence[21] of reduced availability of reaction sites as a result of polymerization of the diphenylbutyltin bromide molecules.

The *in vitro* and *in vivo* efficacies of diphenylbutyltin bromide indicate that it is evidently a promising candidate compound as a protectant fungicide to control *P. palmivora* from black pepper. Field studies, however, are required before any recommendations can be made on the large-scale use and dosage of diphenylbutyltin bromide or other diphenylbutyltin derivatives.

CONCLUSIONS

Of the several triorganotin(IV) compounds screened for *in vitro* antifungal activity against four isolates of

Phytophthora palmivora, diphenylbutyltin bromide exhibits the highest antifungal activity with ED_{50} values ranging from 0.30 to 0.87 $\mu g\ cm^{-3}$. With diphenylbutyltin bromide at a spray concentration of 500 $\mu g\ cm^{-3}$, no symptoms of infection develop on healthy pepper leaves inoculated with a freshly isolated virulent strain of *P. palmivora* (Phy. 346). Concentrations of diphenylbutyltin bromide above 100 $\mu g\ cm^{-3}$ cause injury lesions on pepper leaves. Diphenylbutyltin bromide at 100–500 $\mu g\ cm^{-3}$ could therefore be used as a protective spray or drench against *P. palmivora* infection of black pepper.

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