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Changes in fungal and bacterial populations in soil treated with two triorganotin(IV) compounds

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The effects of two triorganotin(IV) compounds, diphenylbutyltin bromide ($Ph_2BuSnBr$) and triphenyltin chloride·triphenylphosphine oxide ($Ph_3SnCl \cdot Ph_3PO$), on soil bacterial and fungal populations were compared with that of Thiram and the commercial triorganotin fungicide 'Brestan' (triphenyltin acetate, Ph_3SnOAc). Soil fungal populations were reduced most by Thiram, then by $Ph_3SnCl \cdot Ph_3PO$, $Ph_2BuSnBr$ and Ph_3SnOAc, in that order. Following the application of the compounds, there was a marked increase in the bacterial population in soil, the increase being greatest with Thiram and least with $Ph_3SnCl \cdot Ph_3PO$. The triorganotin(IV) compounds were less harmful to soil fungi than Thiram. In Thiram-treated soil, recolonization was slower than in soil treated with the triorganotin(IV) compounds. More species of fungi were tolerant to and persisted after application of the triorganotin(IV) compounds compared with Thiram. Among the fungi that were tolerant to the triorganotin(IV) compounds were cellulolytic species such as *Trichoderma*.

Keywords: Organotins, diphenylbutyltin bromide, triphenyltin chloride·triphenylphosphine oxide, soil bacteria, soil fungi

INTRODUCTION

It is now well established that pesticides not only affect the target organisms but also non-target organisms, many of which may be performing useful functions in soil or on aerial surfaces of plants. Pesticides used in plant protection ultimately reach the soil either directly as soil pesticides, as drifting sprays that settle, or (infrequently) as spillages. These pesticides may have secondary effects on plant growth because of their influence on the availability of plant nutrients in soil.[1–4] The changes in nutrient availability have been linked to an alteration in microbial population and activity resulting from pesticide application. Non-pathogenic soil micro-organisms are important in maintaining soil fertility and soil structure.

Very few pesticides are sufficiently specific to affect pathogens alone.[5] Kreutzer, in his review, concludes that while there is a need for more efficient and specific soil fungicides, a greater need exists for more information on the complex that is being treated – the soil and its microflora. The effects of pesticides on soil micro-organisms have been well reviewed.[7–12] A wide range of fungicides including Captan,[13–16] Verdasan,[16,17] Dichloran,[15,16] Milcol,[15] Triarimol[15] and Thiram[16] have been studied for their influence on soil microbial populations. There is no published report yet on the effects exerted by triorganotin(IV) compounds on soil micro-organisms although their agricultural applications are well documented.[18]

This has prompted the present study on the changes in soil fungal and bacterial populations observed over a period of 35 days following the application of two triorganotin(IV) compounds, viz. diphenylbutyltin bromide ($Ph_2BuSnBr$) and triphenyltin chloride·triphenylphosphine oxide ($Ph_3SnCl \cdot Ph_3PO$), both selected on the basis of their high *in-vitro* toxicity against pathogenic and saprophytic fungi.[19] Thiram 50 WP (tetramethylthiuram disulphide) and the commercial triorganotin fungicide 'Brestan' (triphenyltin acetate, Ph_3SnOAc) were used as controls. The soil plate method[20] was used to determine the succession

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patterns and to distinguish between tolerant and recolonizing species of fungi.

MATERIALS AND METHODS

Soil treatment

Soil in which black pepper (*Piper nigrum* L.) plants were previously cultivated was used for this experiment. The soil was air-dried overnight and sieved (<2 mm). The soil was classified as a sandy loam comprising of 97% sand (that fraction with particles >0.02 mm diam.) and 3% silt (that fraction with particles <0.02 mm diam.). The water holding capacity (WHC) of the soil as a whole was 30%, the total nitrogen content 0.14% and the total organic carbon content 21.7%.

Stock solutions (30 cm^3) of 10 mg cm^{-3} of triphenyltin acetate (Ph_2SnOAc), triphenyltin chloride·triphenylphosphine oxide ($Ph_3SnCl \cdot Ph_3PO$), diphenylbutyltin bromide ($Ph_2BuSnBr$) and Thiram 50 WP were prepared by dissolving 0.3 g (active ingredient; a.i.) of each compound in 10 cm^3 of acetone and adjusting to 30 cm^3 with sterile distilled water. Appropriate volumes of this stock solution were added to 600 g (oven-dry basis; o.d.b.) of soil to give 67 $\mu g\ g^{-1}$ a.i. Thiram, 10 $\mu g\ g^{-1}$ Ph_3SnOAc, 10, 50 and 250 $\mu g\ g^{-1}$ $Ph_3SnCl \cdot Ph_3PO$ and 10, 50 and 250 $\mu g\ g^{-1}$ $Ph_2BuSnBr$. A 1:2 acetone/sterile distilled water mixture (15 cm^3) was added to the control. The moisture content of the soil samples was adjusted to 60% of WHC with sterile distilled water. The soil samples were individually mixed thoroughly in a sterile enamel tray using a sterile spatula. Portions of 200 g (o.d.b.) of treated soil were weighed into sterile 250 cm^3 Erlenmeyer flasks and covered with parafilm. The flasks were arranged in a randomized complete block design and incubated at 27 ± 2°C in the dark for 35 days. Three replicates for every treatment were used. The moisture content of the soils was maintained at 60% of WHC throughout the experiment by adding sterile distilled water. Soil samples were removed from each flask on days 1, 7, 14, 21, 28 and 35 after treatment, for microbiological analyses. Soil samples from the three replicate flasks per treatment were bulked for the microbiological analyses.

Total numbers of fungi and bacteria were assessed using the soil dilution method.[20] The frequency of occurrence of fungi in the soils was determined using the soil plate method.[20]

Microbiological analysis of soil

(a) Dilution method for enumerating fungal colonies

For any one treatment, 5 g of fresh soil that had previously been bulked was transferred to sterile dilution bottles containing 50 cm^3 of sterile distilled water. The soil suspension was shaken vigorously at regular intervals for 10 min. Immediately following dispersion, a series of 10-fold dilutions of the suspension was made with sterile distilled water. Preliminary experiments indicated that 10^{-2} and 10^{-3} dilutions were ideal for the treated soil whereas 10^{-3} and 10^{-4} dilutions were suitable for the control, as 20–30 colonies were obtained per plate. These dilutions were subsequently used throughout the study to enumerate fluctuations in the fungal population. Aliquots (1 cm^3) of this suspension were transferred to each of five sterile plastic Petri dishes. About 15 cm^3 of cooled, molten corn meal agar (CMA) amended with 30 $\mu g\ cm^{-3}$ aureomycin was added to each dish. The dishes were swirled gently and allowed to set. The plates were incubated at 27 ± 2°C. The colonies that developed were counted after four days and subsequently at regular intervals until no new colonies were observed.

(b) Dilution method for enumerating bacterial colonies

The dilution method used for the isolation of fungi was employed but 10^{-5} dilution was necessary to obtain a bacterial count of between 30 and 100 colonies per plate. Aliquots (1 cm^3) of the soil suspension were transferred to each of five sterile plastic Petri dishes. About 15 cm^3 of cooled, molten nutrient agar was added to each dish. The dishes were swirled gently and allowed to set. The dishes were incubated at 27 ± 2°C. The colonies that developed were counted after three days and subsequently at regular intervals until no new colonies were encountered.

(c) Soil plate method for fungi (Warcup[21])

For any one treatment five soil samples were taken from the bulked sample using a sterile inoculating needle with a flattened tip and dispersed into a drop of sterile distilled water in each of five replicate sterile plastic Petri dishes. About 15 cm^3 of cooled, molten

CMA with 30 μg cm^{-3} of aureomycin mixed in it was added to each Petri dish. The dishes were swirled gently to disperse the soil particles in the medium. The dishes were incubated at 27 ± 2°C. The dishes were examined after four days and subsequently at regular intervals until no new species were encountered. Each fungus species isolated from a soil plate was enumerated as one isolate. The percentage frequency of occurrence of a species was calculated as:

$$\frac{\text{number of dishes in which the species was isolated}}{\text{number of dishes used}} \times 100\%$$

The percentage frequency of occurrence of a fungus species isolated from just one of the five dishes was therefore 20%.

RESULTS

(a) Soil dilution method: fungi

Compared with untreated control soil, the soils treated with $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ at 10, 50 and 250 μg g^{-1} significantly ($P = 0.05$) reduced the mean total number of fungal propagules per gram fresh soil at all sampling periods except day 1 (Figs 1 and 2). Thiram at 67 μg g^{-1} and Ph_3SnOAc at 10 μg g^{-1} showed results similar to those obtained with $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$. On day 1, however, soils treated with Ph_3SnOAc at 10 μg g^{-1} and $Ph_2BuSnBr$ at 250 μg g^{-1} showed a significantly ($P = 0.05$) higher fungal population than the untreated control soil (Table 1). In general, the fungal population was reduced most by Thiram, than by $Ph_3SnCl \cdot Ph_3PO$, $Ph_2BuSnBr$ and Ph_3SnOAc, in that order.

Analysis of variance for fungal population showed significant differences ($P = 0.05$) between compounds and between sampling days. The concentration of the compounds, however, did not cause any significant differences ($P = 0.05$) in fungal populations.

(b) Soil dilution method: bacteria

The effects of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ on the mean total number of bacteria in soil are shown in Figs 3 and 4 respectively. Unlike the effects of the compounds on fungal populations, the treatment of soils

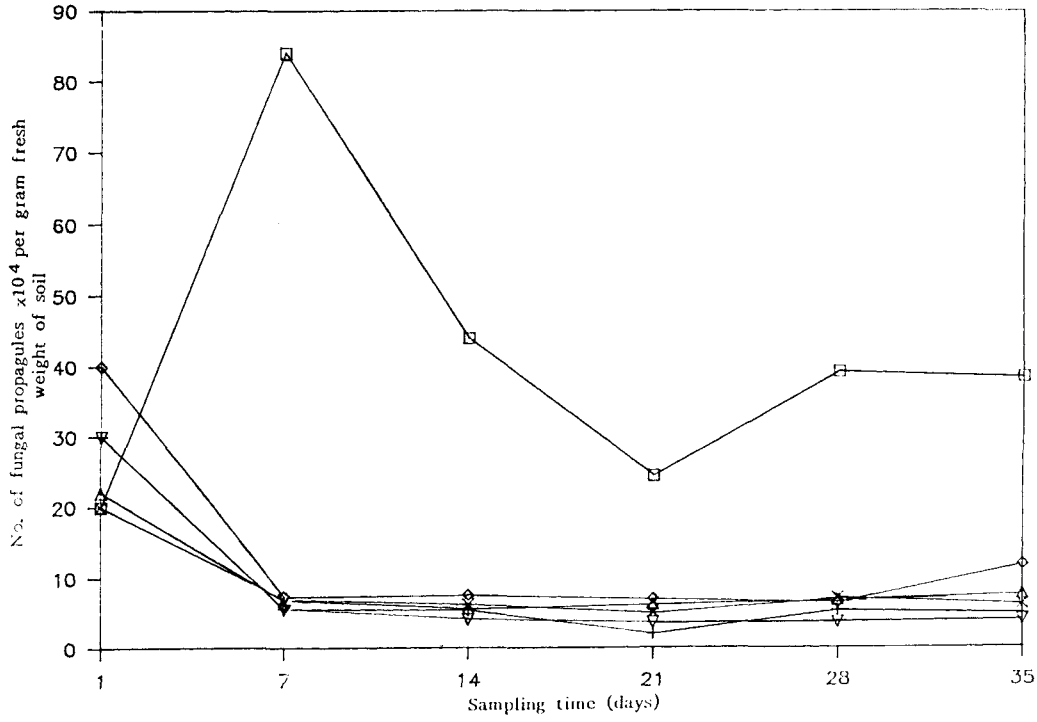

Figure 1 Effect of triphenyltin chloride·triphenylphosphine oxide (P4) on fungal numbers in soil: □, control; +, Thiram, 67 μg g^{-1} soil; ◇, triphenyltin acetate, 10 μg g^{-1} soil; △, P4, 10 μg g^{-1}; ×, P4, 50 μg g^{-1}; ▽, P4, 250 μg g^{-1}. SE (standard error of mean) = 2.06 × 10^4 fungal propagules g^{-1} fresh weight of soil.

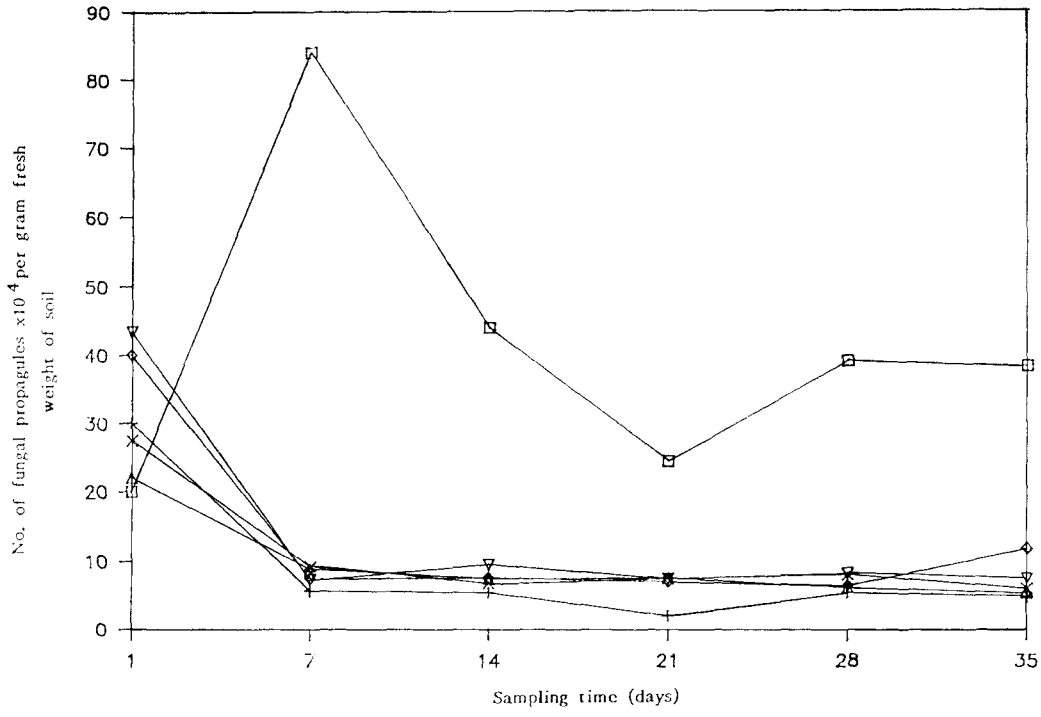

Figure 2 Effect of diphenylbutyltin bromide (M1) on fungal numbers in soil: □, control; +, Thiram, 67 μg g^{-1} soil; ◇, triphenyltin acetate, 10 μg g^{-1}; △, M1, 10 μg g^{-1} soil; ×, M1, 50 μg g^{-1} soil; ▽, M1, 250 μg g^{-1} soil. SE = 2.06 × 10^4 fungal propagules g^{-1} fresh weight of soil.

Table 1 Changes in mean number of fungal propagules per gram of fresh soil treated with selected triorganotin(IV) compounds (data expressed as % increase (+) or decrease (−) compared with untreated control)

Compound	Concn (μg g^{-1})	Sampling time (days)					
		1	7	14	21	28	35
Thiram 50WP	67.0	+ 50	− 93*	− 88*	− 92*	− 86*	−87*
Ph_3SnOAc	10.0	+100*	− 91*	− 83*	− 72*	− 84*	−69*
$Ph_3SnCl \cdot Ph_3PO$	10.0	+ 10	− 92*	− 87*	− 74*	− 83*	−80*
	50.0	0	− 92*	− 86*	− 79*	− 82*	−84*
	250.0	+ 50	− 93*	− 91*	− 85*	− 91*	−89*
$Ph_2BuSnBr$	10.0	+ 10	− 90*	− 83*	− 69*	− 84*	−86*
	50.0	+ 38	− 89*	− 85*	− 70*	− 80*	−85*
	250.0	+117*	− 91*	− 78*	− 70*	− 79*	−81*

*Significantly different from control at $P = 0.05$.

with these compounds at 50 and 250 μg g^{-1} significantly increased ($P = 0.05$) the bacterial populations in the treated soil compared with that in the untreated soil (Table 2). The flushes in bacterial numbers observed for the two compounds between days 1 and 14, and days 1 and 28, at 50 μg g^{-1} and 250 μg g^{-1}, respectively, subsequently declined to levels lower than those observed for untreated control.

Significant ($P = 0.05$) increases in bacterial populations compared with untreated control were observed

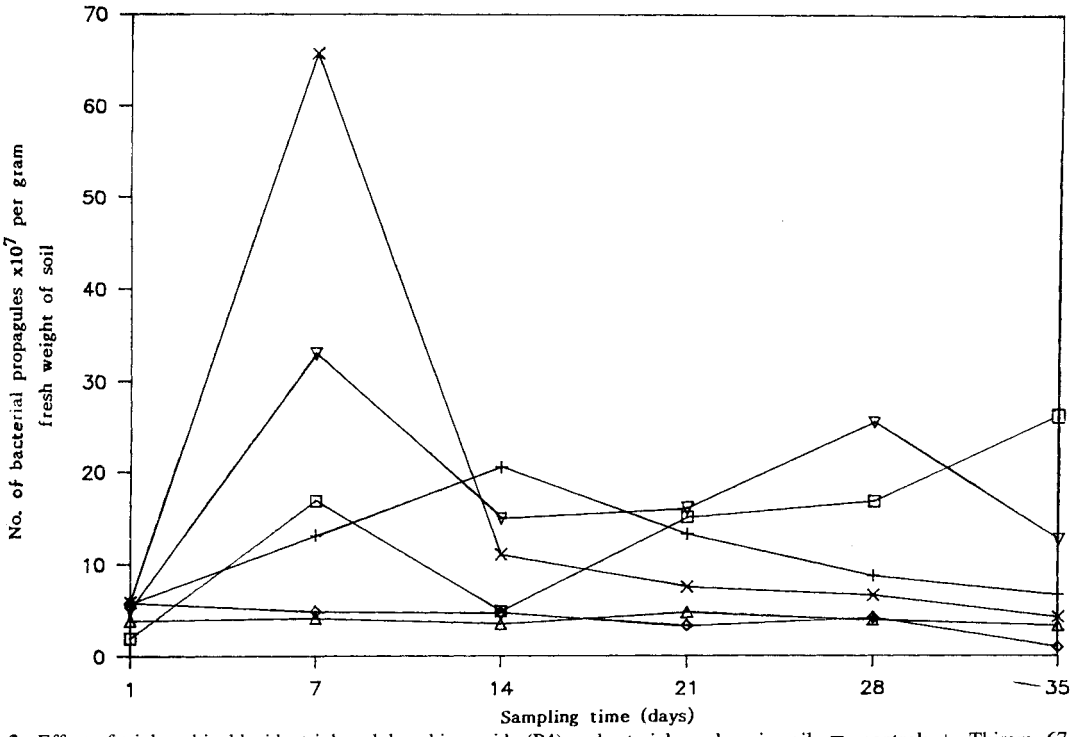

Figure 3 Effect of triphenyltinchloride·triphenylphosphine oxide (P4) on bacterial numbers in soil: □, control; +, Thiram, 67 μg g^{-1} soil; ◇, triphenyltin acetate, 10 μg g^{-1} soil; △, P4, 10 μg g^{-1} soil; ×, P4, 50 μg g^{-1} soil; ▽, P4, 250 μg g^{-1} soil. SE = 1.59 × 10^7 bacterial propagules g^{-1} fresh weight of soil.

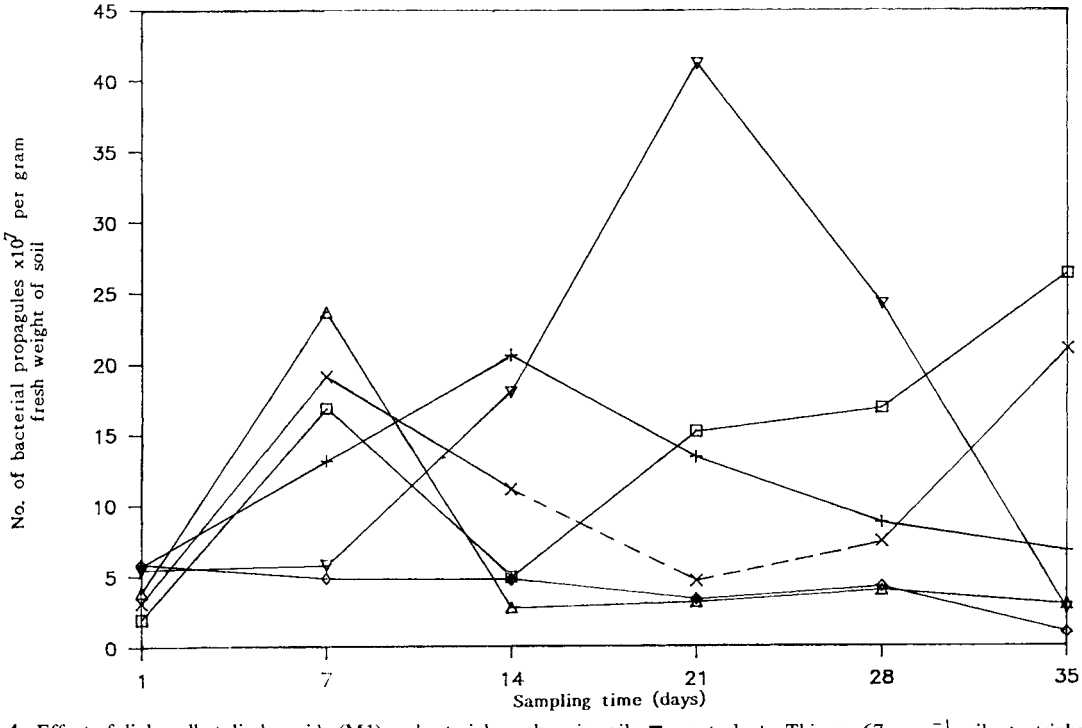

Figure 4 Effect of diphenylbutylin bromide (M1) on bacterial numbers in soil: □, control; +, Thiram, 67 μg g^{-1} soil; ◇, triphenyltin acetate, 10 μg g^{-1}; △, M1, 10 μg g^{-1} soil; ×, M1, 50 μg g^{-1} soil; ▽, M1, 250 μg g^{-1} soil. SE = 1.59 × 10^7 bacterial propagules g^{-1} fresh weight of soil.

Table 2 Changes in mean number of bacterial propagules per gram of fresh soil treated with selected triorganotin(IV) compounds (data expressed as % increase (+) or decrease (−) compared with untreated control)

Compound	Concn ($\mu g\ g^{-1}$)	Sampling time (days)					
		1	7	14	21	28	35
Thiram 50WP	67.0	+193*	− 22	+319*	− 12	− 48	−75*
Ph_3SnOAc	10.0	+197*	− 71	− 3	− 78	− 75	−96*
$Ph_3SnCl \cdot Ph_3PO$	10.0	+ 99	− 76	− 28	− 68	− 76	−87*
	50.0	+203*	+289*	+126	− 50	− 61	−84*
	250.0	+158*	+ 95	+206	+ 6	+ 51	−51
$Ph_3BuSnBr$	10.0	+ 98	+ 40	− 44	− 79	− 76	−89*
	50.0	+ 59	+ 13	+126	− 69	− 56	−20
	250.0	+178*	− 66	+266*	+170*	+ 43	−90*

*Significantly different from control at $P = 0.05$.

on day 14 for Thiram at 67 $\mu g\ g^{-1}$ (319%) and $Ph_2BuSnBr$ at 250 $\mu g\ g^{-1}$ (266%) and on days 1 and 7 for $Ph_3SnCl \cdot Ph_3PO$ at 50 $\mu g\ g^{-1}$ (203% and 289%, respectively) (Table 2).

Analysis of variance for bacterial population showed significant differences ($P = 0.05$) between the concentrations of the compounds and between the sampling days. The interaction between concentration and sampling time also showed significant differences ($P = 0.05$) in bacterial populations. Contrary to the results obtained for analysis of variance for fungal populations, the compounds did not cause any significant differences ($P = 0.05$) in bacterial populations.

(c) Soil plate method

The highest number of fungus species in treated soils was observed on day 35 while in the untreated control the highest number was observed on day 28 (Table 3). After the application of Thiram at 67 $\mu g\ g^{-1}$ of soil, only five to nine species of fungi were isolated. After the application of Ph_3SnOAc at 10 $\mu g\ g^{-1}$ of

Table 3 Effect of selected triorganotin(IV) compounds on mean number of fungus species per plate

Compound	Concn ($\mu g\ g^{-1}$)	Sampling time (days)					
		1	7	14	21	28	35
Control	0	9	10	9	12	13	10
Thiram 50WP	67.0	8	7	7	5* $t = 3.33$	7* $t = 3.16$	9
Ph_3SnOAc	10.0	10	10	11	10	11	12
$Ph_3SnCl \cdot Ph_3PO$	10.0	10	9	9	9	9* $t = 2.11$	11
	50.0	10	7	10	11	12	13* $t = 2.5$
	250.0	7	5* $t = 2.38$	7	7* $t = 2.38$	8* $t = 2.63$	11
$Ph_3BuSnBr$	10.0	11	7	10	10	9* $t = 2.11$	12
	50.0	10	11	9	9	10	11
	250.0	8	6	8	10	9* $t = 2.11$	11

*Significantly different from control at $P = 0.05$.

soil and $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ at 10, 50 and 250 $\mu g\ g^{-1}$ of soil, however, five to 13 species of fungi were isolated. The mean numbers of fungus species per plate in soils treated with Ph_3SnOAc at 10 $\mu g\ g^{-1}$, $Ph_3SnCl \cdot Ph_3PO$ at 50 $\mu g\ g^{-1}$ and $Ph_2BuSnBr$ at 50 $\mu g\ g^{-1}$ were not statistically lower than that obtained in the untreated control. The highest mean number of fungus species per plate in the treated soils was obtained with Ph_3SnOAc at 10 $\mu g\ g^{-1}$ followed, in decreasing order, by compounds $Ph_3SnCl \cdot Ph_3PO$ at 10 $\mu g\ g^{-1}$, $Ph_2BuSnBr$ at 50 $\mu g\ g^{-1}$, $Ph_3SnCl \cdot Ph_3PO$ at 250 $\mu g\ g^{-1}$ and Thiram at 67 $\mu g\ g^{-1}$. This implies that the triorganotin(IV) compounds, Ph_3SnOAc, $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, were less harmful to soil fungi compared with Thiram.

A total of 51 taxa of fungi were isolated from the control and treated soil. The most commonly isolated fungi from treated and untreated soils were *Absidia glauca, Aspergillus* spp., *Cunninghamella echinulata, Fusarium* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and *Syncephalastrum racemosum. Aspergillus fumigatus, Fusarium* spp. and *Rhizopus* spp., which were common in untreated control soil, were, however, absent from soil treated with Thiram. *Penicillium* spp. which were common in untreated control soils and soil treated with $Ph_3SnCl \cdot Ph_3PO$ at 10 and 50 $\mu g\ g^{-1}$ were, however, isolated in very low frequencies from 0 to 40% in soil treated with $Ph_3SnCl \cdot Ph_3PO$ at 250 $\mu g\ g^{-1}$, indicating the effect of dosage of $Ph_3SnCl \cdot Ph_3PO$ on *Penicillium* spp. Similarly, *Rhizopus* spp. were affected by the dosage of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ and were isolated at frequencies of 0–40% a 250 $\mu g\ g^{-1}$ after day 1.

Based on the response of the fungi to Thiram and triorganotin(IV) compounds, the soil fungi isolated were divided into four groups, namely:

(1) *Tolerant fungi:* fungi isolated througout the incubation period in untreated and treated soils.
(2) *Non-tolerant fungi:* fungi isolated in untreated control soil but not in treated soils.
(3) *Recolonizing fungi:* fungi isolated on day 1 in treated soils, but absent or isolated in low frequencies (20–40%) on days 7–21 and later isolated in high frequencies (60–100%) on days 28–35.
(4) *Infrequently isolated fungi:* fungi isolated in low frequencies (20–40%) in untreated and/or treated soils sporadically during the incubation period.

The fungi in the four groups are shown in Table 4. *Absidia glauca, Aspergillus* ex sp. 1, *Cunninghamella echinulata, Mortierella* spp., *Mucor* spp., *Penicillium* spp. and *Syncephalastrum racemosum* were isolated at high frequencies (60–100%) throughout the incubation period in both the untreated and treated soils. *Aspergillus fumigatus* was isolated in high frequencies

Table 4 Grouping of fungi[a] based on their response to Thiram and triorganotin(IV) compounds added to soil

Tolerant species	Non-tolerant species	Recolonizing species	Infrequently isolated species
Absidia glauca	*Cephalosporium* spp.[b]	*Aspergillus flavus*	*Aspergillus clavatus*
Aspergillus fumigatus		*Aspergillus niger*	*Aspergillus giganteus*
		Aspergillus restrictus	*Aspergillus sparsus*
Aspergillus ex sp. 1		*Aspergillus terreus*	*Cephaliophora tropica*
		Chaetomium cochliodes	*Ceph. irregularis*
Cunninghamella echinulata		*Fusarium* ex sp. 1	*Chaetomium globosum*
		Rhizopus spp.[b]	*Circinella linderi*
Mortierella spp.[b]		*Trichoderma hamatum*	*Cylindrocarpon* spp.[b]
Mucor spp.[b]			*Fusarium moniliforme*
Penicillium spp.[b]			*Geotrichum* spp.[b]
Syncephalastrum racemosum			*Gliocladium deliquescens*
			Gongronella butleri
Trichoderma viride			*Humicola grisea*
			Paecilomyces spp.[b]
			Papulaspora spp.[b]
			Taloromyces spp.[b]

[a]Fungi are arranged in alphabetical order in each group. [b]Aggregated; species not distinguished.

throughout the incubation period with all the treatments except Thiram. *Trichoderma viride* was moderately tolerant to Thiram and the triorganotin(IV) compounds. *Cephalosporium* spp. was not tolerant to any of the fungicide treatments.

Aspergillus restrictus recolonized all treated soils except that treated with $Ph_2BuSnBr$ at 50 $\mu g\ g^{-1}$ and 250 $\mu g\ g^{-1}$ and with the compound $Ph_3SnCl \cdot Ph_3PO$ at 250 $\mu g\ g^{-1}$. *Aspergillus terreus* and *Fusarium* ex sp. 1 recolonized soils treated with triorganotin(IV) compounds only. Recolonization of the fungus species listed in Table 4 occurred within one to three weeks after the application of the triorganotin(IV) compounds. In Thiram-treated soil, recolonization was generally slow. *Aspergillus terreus, Fusarium* ex sp. 1 and *Rhizopus* spp., which were recolonized within two to four weeks in triorganotin(IV)-treated soils, were completely inhibited in Thiram-treated soils. *Cladosporium* ex sp. 1, *Curvularia clavata, Curvularia eragrostidis, Curvularia lunata, Stachybotys* ex sp. 1, *Trichocladium asperum* and *Verticillium* spp. were isolated on one or two occasions only throughout the incubation period in treated soils, indicating their very scanty presence in the soil used in this study.

DISCUSSION

Fungicides, while selectively inhibiting some soil fungi, either increase or have no harmful effects on soil bacterial population.[11] Captan at 9 kg ha^{-1}, Thiram at 6.7 or 13.4 kg ha^{-1} and Quintozene at 5.6 or 11.2 kg ha^{-1} were shown to increase heterotrophic soil bacteria significantly.[2] Dubey and Rodriguez[22] also showed that Dyrene and Maneb at 1.5, 6.0, 24 and 96 kg ha^{-1} increased bacterial population for a period of 10 months. In this study, the triorganotin(IV) compounds $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, especially at concentrations of 50 and 250 $\mu g\ g^{-1}$, decreased the soil fungal population but caused a significant increase in the soil bacterial population. A decrease in soil fungal population thus resulted in a corresponding increase in soil bacterial population. The stimulatory effect on bacterial population could have resulted from increased availability of organic substrates (in the form of dead fungal biomass) for bacterial growth. Furthermore, the antifungal activity of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ in soil may have reduced competition for available space as well as nutrients, oxygen and water.

Fungicides are designed to kill undesirable fungi. Fungicides can, however, influence the growth of non-pathogenic fungi and antagonists of pathogenic fungi.[13,23–26] As a general rule, fungicides immediately suppress all species to some extent. Recovery by re-invasion, germination of protected or resistant spores, or mutation may be relatively rapid or slow depending on the prevailing soil conditions and persistence of the fungicides.[11] In this study, recovery of the fungal population to that observed on day 1 was not achieved until day 35 in soils treated with both Thiram and the triorganotin(IV) compounds separately, indicating the persistence of the compounds. Bioassay of the persistence of fungitoxicity in soil for $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ indicated the presence of 8.3 and 5.1 $\mu g\ g^{-1}$ respectively on day 29 after treatment of soil with 50 $\mu g\ g^{-1}$ of soil for each compound (this journal in press). The sampling period has thus to be extended to determine the time taken for the fungal population to return to the population size before the application of the triorganotin(IV) compounds. Besides the antifungal effects of the triorganotin(IV) compounds, the decrease in fungal population may also be influenced by increased production of fungistatic components such as ammonia[27,28] and ethylene[29,30] in the soil.

The spectrum of species in the soil after treatment may be altered and persist for long periods. The rapid increase in numbers of a particular species or group of fungi may be due to alterations in competition for substrate material and changes in end-product metabolism. Dexon included in potato dextrose agar at rates of up to 300 ppm had no effect on *Mortierella* spp.[31] Quintozene at rates up to 20 kg ha^{-1} in glucose-amended soils increased *Fusarium* spp.[11] Ko and Lockwood[32] showed Quintozene accumulation in *Rhizoctonia rolani* to reach 300 $\mu g\ g^{-1}$ of moist mycelium, indicating the resistance of *R. solani* to Quintozene. This would subsequently affect the decomposition of the cell-material upon death of the mycelium.

Zygomycetes such as *Absidia glauca, Mucor* spp., *Mortierella* spp., *Cunninghamella echinulata* and *Syncephalastrum racemosum* were tolerant to compounds $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ at 10.0, 50.0 and 250.0 $\mu g\ g^{-1}$ as well as to Thiram at 67 $\mu g\ g^{-1}$ and to Ph_3SnOAc at 10 $\mu g\ g^{-1}$. *Rhizopus* spp. which were tolerant to the triorganotin(IV) compounds, however, were completely inhibited by Thiram. Conversely other Zygomycetes such as *Mucor, Rhizopus* and *Mortierella* spp. showed sensitivity to Thiram,

Nabam, Captan, Metham-sodium, Dazomet, allyl alcohol and mercury compounds.[33] *Trichoderma viride* appears to be uniformly resistant to many fungicides whereas species of *Fusarium* are generally sensitive.[11] *Trichoderma viride* was also resistant to the triorganotin(IV) compounds whereas *Fusarium* ex sp. 1 was able to recolonize the triorganotin(IV)-treated soil after an initial period of suppression. Saxena,[34] Moubasher and Mazen[35] and Kuthubutheen and Pugh[16] also showed that *Trichoderma* spp. dominate fungicide-treated and fumigated soils. The dominance of this genus may be due to its ability to utilize ammonium-nitrogen which is present in large amounts in treated soils or to resistance to the fungistatic influence of ammonia in soils.[27]

Pugh and Williams[17] and Williams[36] found that cellulose decomposers were more sensitive to Verdasan (an organomercury fungicide; active ingredient probably phenylmercuric acetate) than were non-cellulose decomposers. On the other hand, Wainwright and Pugh[15] found that cellulose decomposers were the major recolonizers of soils treated with Captan, Dichloran, Milcol and Triarimol. Similarly, in triorganotin(IV)-treated soils the cellulose decomposers, i.e. *Trichoderma*, *Aspergillus* and *Chaetomium*, were either tolerant or recolonizing species. Our preliminary studies also show that cellulose activity in soil was not affected after the application of the triorganotin(IV) compounds $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$. Furthermore, recolonization by fungi was generally faster in triorganotin(IV)-treated soils than in the Thiram-treated soils, indicating the shorter persistence of the triorganotin(IV) compounds in soil compared to Thiram.

Although the treatment of soil with the triorganotin(IV) compounds reduced its fungal population, the spectrum of fungal species in soil was not greatly altered to affect adversely the soil fertility. The two triorganotin(IV) compounds, $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, can be used for the control of plant pathogenic fungi at concentrations of 50–250 $\mu g\ g^{-1}$ without adversely affecting the non-target soil microorganisms which help to maintain the soil fertility.

CONCLUSIONS

Soil fungal population is reduced more by Thiram than by $Ph_3SnCl \cdot Ph_3PO$, $Ph_2BuSnBr$ and Ph_3SnOAc, and in that order. Following the application of Thiram and the triorganotin(IV) compounds there is a flush in bacterial population in soil and the increase is greatest with Thiram and least with $Ph_3SnCl \cdot Ph_3PO$. The triorganotin(IV) compounds, $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, are less harmful to soil fungi than is Thiram.

More species of fungi (5–13 spp.) persist in soil following the application of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ than in soil treated with Thiram (5–9 spp.).

Among the fungi resistant to the triorganotin(IV) compounds are fungi known to be strongly cellulolytic in soil, i.e. *Trichoderma* spp.

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