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Effect of Three Insecticides on Growth Rates of Soil Fungi

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In the past there have been numerous papers written on the toxicity of heavy metals to fungi and the consequent effects on fungal growth rates (Babich et al. 1977, 1978, 1981, 1982; Thompson, et al., 1984). However, very little research has been conducted on the effects of insecticides on soil fungi: (Tu 1970, 1972.) Due to the importance of the soil fungi in the maintenance of soil fertility and because of the scant and superficial amount of research conducted on the effects of insecticides on the fungi, research is needed to determine whether various concentrations of insecticides adversely affect the soil fungi.

As the use of insecticides has increased, there has been a growing concern about the effects that these insecticides may have on soil processes and non-target organisms, such as soil fungi. Insecticides are classified into three major groups; carbamates, organochlorines, and organophosphates. One insecticide was selected from each of the three major groups for this study. The insecticides selected were carbofuran, (a carbamate), chlorpyrifos, (an organophosphate), and lindane,(an organochlorine). These insecticides are applied to plants in both agricultural and residental areas. The effects of these three insecticides (carbofuran, chlorpyrifos, and lindane) on the colony diameter growth rates of five common soil fungi (Aspergillus giganteus, Penicillium vermiculatum, Rhizopus stolonifer, Trichoderma reesei, and Trichoderma viride) were determined in this study.

MATERIAL AND METHODS

The poisoned-agar technique for assessing toxicity which was used in this study, involves the addition of aliquots of a stock solution of insecticide to specific volumes of agar medium. The differences in fungal mycelium growth, as measured by colony diameter on control and experimental plates, was used to determine the toxicities of the insecticides (Burrell 1980).

In this study, five common soil fungi species; (Aspergillus giganteus, Penicillium vermiculatum, Rhizopus stolonifer, Trichoderma reesei, and Trichoderma viride) were grown on an autoclaved synthetic medium: 10 g D-glucose, 2 g L-asparagine, 1 g $KH_2PO_4 \cdot 7H_2O$, 0.2 mg ferrous sulfate, 0.2 mg zinc nitrate, 0.1 mg manganese nitrate, 5 ug biotin, and 100 ug thiamine hydrochloride. The pH of the medium was adjusted to 6.5 with a 0.5 M KOH solution and the solution was diluted to 1.0 liter with distilled water. The final solution was then added to agar.

Soil fungi were obtained from the Agricultural Research Service Culture Collection. Stock cultures of each fungal species were maintained in an incubator, at a constant temperature of $25^{o}C \pm 2^{o}C$.

Insecticides used in this study (carbofuran, chlorpyrifos, lindane) were obtained from the U.S. Environmental Protection Agency. Stock solutions of each were made by dissolving the insecticide in a distilled petroleum ether-distilled acetone solvent (1:1 volume).

The solid medium was melted ($93^{o}C$) and 15 ml aliquots were poured into sterilized glass bottles. Specific volumes of the stock solution of the desired insecticide were added to the 15 mls of medium. The solvent had to be driven out of the medium because it affected the mycelial growth of the fungi. The medium was then placed in a $70^{o}C$ water bath for three minutes to insure that all the solvent was driven off and was then poured into petri dishes and allowed to solidify. Three replicate plates (both control and experimental) were used for each concentration of each insecticide. Fungi on control plates were grown on medium containing no insecticide.

A circular mycelial disc was obtained from the stock culture. The innoculum for each experiment was taken from a single culture plate. The stock cultures had been incubated for at least two days before being used. A sterilized metal cork borer (approximately five millimeters in diameter) was used to obtain a mycelial disc from the peripheral edge of the stock culture. This mycelial disc was then removed from the cork borer and placed, with the fungal growth up, in the center of the petri dish. The dish was then incubated in the dark (except for Trichoderma ssp. which were exposed to light) at a temperature of $25^{o}C \pm 2^{o}C$ The length of the incubation period was dependent on the fungus. Trichoderma reesei and T. viride were measured after two days, Rhizopus stolonifer after one or two days, Aspergillus giganteus after nine days, and Penicillium vermiculatum after ten days. The incubation times for the Trichoderma species and Rhizopus stolonifer were relatively short because the controls had reached the periphery of the petri dish within one to two days.

The diameter growth of the colony was measured by simply placing a ruler over the dish. Four different diameter measurements were taken for each plate and then averaged to give the reported diameter of the colony. It should be noted that the colony growth of all five fungi used in this study were circular and there were no major irregularities in colony shape. A T-test was performed to determine significant differences ($P<0.05$) in control versus experimental plates.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 list the concentrations of the insecticides used to determine the tolerance levels of the fungi. Because the fungi had different sensitivity levels to the insecticides, the insecticide concentrations studied varied for each fungus. This explains the gaps in concentrations in the tables. The initial inhibition of mycelial growth was narrowed down to a range of less than 10 ppm between nonsignificance and significance. However, the initial level of significance should not be taken as final. The initial level of inhibition may actually be between the nonsignificant concentration and the initially significant concentration (e.g. In Table 1, the initial inhibition level of chlorpyrifos to *P. vermiculatum* is designated as 43 ppm but initial inhibition actually occurs between 37 and 43 ppm.).

Table 1. Influence of chlorpyrifos on mycelial growth of fungi as compared to a control*

Fungus	1	6	12	18	25	31	37	43	50	75	100
P. vermiculatum	NS				NS		NS	S[a]	S[a]		S[a]
T. viride	NS				NS	S[b]	S[b]				S[b]
T. reesei [c]	S				S				S	S	S
R. stolonifer	NS	S	S		S				S		S
A. giganteus	NS		NS	S	S				S		S

* Control plates did not contain chlorpyrifos. T-test performed with $P=0.05$ being considered significant

NS Mycelial growth statistically not significantly inhibited as compared to control

S Mycelial growth statistically significantly inhibited as compared to control

a Colony color change from dark yellow to dark orange-brown

b Appearance of line-green spots dispersed throughout colony

c Eighty % of trial tests indicated significance for 1, 25, 50, and 75 ppm while 20 % of tests indicated insignificance for all four concentrations.

Table 2. Influence of carbofuran on mycelial growth of fungi as compared to a control*.

Fungus	Carbon concentration (ppm) 1	6	12	25	50	62	68	75	87	93	100	106	112	125	150
P. vermiculatum	NS	S[a]	S[a]	S[a]	S[a]						S[a]				
T. viride	ST				NS	NS	NS	S			S				
T. reesei	NS				NS	NS	NS	S			S				
R. stolonifer	NS				NS			NS	NS	NS	S				
A. giganteus	NS										NS	S	S	S	S

* Control plates did not contain carbofuran
T-test performed with $P<0.05$ being considered significant

NS Mycelial growth statistically not significantly inhibited as compared to control

S Mycelial growth statistically significantly inhibited as compared to control

ST Mycelial growth statistically significantly stimulated as compared to control

a Colony color change from dark yellow to dark orange-brown

Lindane was consistently the most toxic of the insecticides as shown in Table 4. The initial level of lindane at which statistically significant inhibition of the mycelial growth of all five soil fungi occurred was 6 ppm. This indicates that lindane was indiscriminate in its effect on the soil fungi in this study. The mycelial growth of R. stolonifer was stimulated at 1 ppm lindane and inhibited at 6 ppm lindane (Table 4). The high toxicity at low levels of lindane is expected since lindane is a member of the organochlorine group of insecticides which has been regarded as very stable, very toxic, and very resistant to biological degradation.

Lindane production has been banned due to its high and indiscriminate toxicity. However, this pesticide is still commercially available and large stock supplies of it are prevalent.

Table 3. Influence of lindane on mycelial growth of fungi as compared to a control*

Fungus	Lindane Concentration (ppm) 1	6	12	25	50	100
P. vermiculatum	NS	S	S	S	S	S
T. viride	NS	S	S	S	S	S
T. reesei	NS	S	S	S	S	S
R. stolonifer	ST	S	S	S	S	S
A. giganteus	NS	S	S	S	S	S

* Control plates did not contain lindane
T-test performed with $P<0.05$ being considered significant
NS Mycelial growth statistically not significantly inhibited as compared to control
S Mycelial growth statistically significantly inhibited as compared to control
ST Mycelial growth statistically significantly stimulated as compared to control

Table 4. The lowest concentration of insecticide at which fungal growth inhibition was detected

Fungus	Insecticide concentration (ppm) Lindane	Chlorpyrifos	Carbofuran
P. vermiculatum	6	43	6
T. viride	6	31	75
T. reesei	6	1 - 75	75
R. stolonifer	6	6	100
A. giganteus	6	18	106

With respect to chlorpyrifos, R. stolonifer was found to be the least resistant (initial inhibition occurring at 6 ppm). The most resistant fungus was P. vermiculatum with an incipient inhibition at 43 ppm (Table 4).

Overall, carbofuran was found the least toxic of the three insecticides (Table 4). A. giganteus was resistant to carbofuran up to a concentration of 100 ppm but was significantly inhibited at 106 ppm. P. vermiculatum was the least resistant of the fungi. Its growth was initially inhibited at the concentration of 6 ppm carbofuran.

T. reesei was the only fungal species which presented a problem in determining the tolerance level (Table 1). This problem occurred when T. reesei was exposed to chlorpyrifos. Five trials each at concentrations of 1, 25, 50, and 75 ppm of chlorpyrifos, were run on T. reesei to determine the toxicity level. Eighty percent of the trials showed a significant inhibition of mycelial growth for all four concentrations, while 20% of the trials showed no significance at the four concentrations. Because the initial concentration of inhibition could not be positively determined, a concentration range of 1-75 ppm was designated as the tolerance level (Table 4).

The mycelial growth of T. Viride and R. stolonifer was stimulated at 1 ppm of carbofuran and lindane respectively (Tables 2 & 3). This stimulation was probably due to the ability of these species to metabolize the insecticides at these low concentrations. Two species of the fungi studied were characterized by visual changes associated with mycelial growth inhibition (Tables 1 & 2). T. Viride showed lime-green spots dispersed throughout the colony when exposed to chlorpyrifos. These spots were noted at 31 ppm of chlorpyrifos (the initial level at which inhibition was significant). The spots were also consistently present at concentrations above 31 ppm. Color change is sometimes an indicator of metal complexation. Metals are often essential in the production of enzymes. The nutrient medium used to grow the fungi contained several metal ions (iron, zinc, manganese, etc.), and since chlorpyrifos has a thiophosphate group which includes a sulfur atom, these lime-green spots may be associated with a metal complexation reaction involving the sulfur atom of chlorpyrifos. If a metal in the medium was involved in a complexation reaction it might not be available to the fungus for enzyme production and the fungus might not be able to metabolize or tolerate the insecticide, resulting in growth inhibition. The presence of the spots coincided with the initial level of mycelial inhibition, which may indicate that a complexation reaction was occurring.

P. vermiculatum also displayed a characteristic colony color change associated with growth inhibition. Exposure of the species to 43 ppm chlorpyrifos (incipient inhibition) and 6 ppm carbofuran (incipient inhibition) resulted in a change from dark

yellow to a very dark orange-brown color which was present for all concentrations above the concentration of initial inhibition (Tables 1 & 2). This may be a result of the conversion of the insecticide to a metabolite or a complexation reaction.

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