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Bioconcentration and Effects of Dieldrin, Dimethoate, and Permethrin on *Saccharomyces cerevisiae*

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Saccharomyces a yeast, widely distributed in nature has a unique capacity of brewing and baking. It is also a rich source of proteins and vitamins. Because of its rapid growth rate and resistance to foreign chemicals it has been used in toxicological studies (Lal and Lal 1987). Protoplast isolation and its interaction with the pesticides has been closely monitored in order to eliminate the barrier effect of cell wall, if any. How its naked cell behaves in the presence of toxic chemicals is an intriguing question. This paper describes the bioconcentration and effects of dieldrin, dimethoate and permethrin, each representing three major classes of insecticides, organochlorine, organophosphate and pyrethroid respectively, on Saccharomyces cerevisiae.

MATERIALS AND METHODS

All the three insecticides were obtained from Dr.Robert E.Thompson, NSI, U.S.A. Stock solutions of these insecticides were prepared in acetone. The stock cultures of yeast, S.cerevisiae obtained from Dr. S.Shivaji, Centre for Cellular and Molecular Biology, Hyderabad (India) were cultured in a liquid medium comprising of two solutions: Solution I contained 1% yeast extract (w/v) and solution II 30% glucose (w/v) in distilled water. These solutions were prepared, autoclaved separately and mixed in a proportion of 9:1 under aseptic conditions.

The preparation and maintenance of yeast protoplast (organisms without cell wall) culture requires : (1) Protoplast buffer containing 1M mannitol (pH 7.4), and (2) yeast-sorbitol (YS) medium prepared by adding 1M sorbitol to YG medium. Cultures of yeast were transferred to sterile tubes and the pellet was separated at 5000 rpm (2000xg) for ten minutes. The pellet was washed thrice with distilled water and finally suspended in 10 ml protoplast buffer. To this culture suspension, zymolase-60,000 activity (10 mg/g cells) was added and incubated at 30±1°C until the cell wall digestion is complete. The completion of cell wall digestion is indicated by the drop in absorbance monitored at A_{550} with LKB spectrophotometer (ultraspec-4050).

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Protoplasts, after formation, were separated by centrifugation (10 min x 5000 rpm) and resuspended in protoplast buffer. Centrifugation was repeated to remove cell debris. The pellet was resuspended in yeast-sorbitol medium.

Bioconcentration (uptake) of insecticides was studied for 12 hr following method of Fatichenti et al.(1983) and was calculated as the ratio of insecticide in the organism to the initial concentration added into the medium. The uptake of dieldrin and permethrin was studied at 0.1, 0.5 and 1.0 ug ml^{-1} while that of dimethoate at 1, 5 and 10 ug ml^{-1}. Saccharomyces was raised in 10 ml YG medium and treated with appropriate amounts of each of the three insecticides to obtain a required concentration. Sampling was done at an interval of 2 hr till 12 hr. After every 2 hr, whole cultures were centrifuged at 5000 rpm for 5 min. The pellet formed was washed thrice with distilled water to remove extracellular insecticide adhered to cell wall. It was mixed thoroughly in 12 ml acetone and subsequently evaporated to apparent dryness. The samples were reconstituted in a known quantity of hexane before subjecting to GLC. Cell dry weight was determined by centrifuging a defined volume of cell suspension (10 ml culture) in triplicate, washing the pellet and drying at 80°C for 12 hr.

Electron capture detector of packard-438 GLC coupled to CR-2A integrator was employed for the analysis of dieldrin and permethrin insecticides. Both these insecticides were analysed on glass columns (2m x 4mm, L x ID) packed respectively with 3% SE-30 and 3% XE-60 on 80/100 mesh gas chrom Q. The inlet, outlet and oven temeperatures were 220, 250 and 200°C respectively. Nitrogen at a flow rate of 40 ml/min was used as the carrier gas. Dimethoate was analysed by Flame photometer detector (FPD) using 4% SE-30+6% OV-210 column coated on 80/100 mesh gas chrom Q. The inlet, outlet and column temperatures were 220, 250 and 200°C respectively. Carrier gas (N_2) 15 ml/min and two external gases; H_2 105 ml/min and Air 145 ml/min were used.

The effects of insecticides on the growth of normal yeast were studied by inoculating (0.1 ml) 12 hr old yeast culture into 10 ml YG medium. Simultaneously, appropriate quantities of each insecticide was transferred to obtain a final concentration of 1, 10, 50 and 100 ug ml^{-1}. Controls were treated with equivalent amount of acetone. Growth was followed at regular intervals of 2 hr upto 12 hr by measuring the optical density (OD) at 550 nm (Pringle and More 1975). Protoplasts were subcultured in YS medium incubated for 8 hr so that the initial OD was between 0.3 to 0.5. The cultures, were then treated with each of the three insecticides to give a final concentration of 10, 50 and 100 ug ml^{-1}.

RESULTS AND DISCUSSION

Saccharomyces accumulated low to high amounts of different insecticides depending upon the type of insecticide, exposure period, growth phase and metabolic state of the organisms. Dieldrin

was rapidly accumulated and was maximum (602.5) ug/g dry wt.) after 2 hr at 1 ppm. Incubation for longer periods invariably showed a rapid decrease of dieldrin concentration in the cells. The ability of yeast to bioconcentrate dieldrin was comparatively high with increasing concentrations in the culture medium (Table 1). The uptake of dimethoate, compared to dieldrin was slightly slow and less. The yeast took 6 hr to accumulate the highest concentration of 330 ug/g dry wt. from a medium containing 1 ug ml^{-1} dimethoate. Subsequently, the insecticide concentration showed a rapid decline and after 12 hr, 6 ug g^{-1} dry wt. dimethoate was left in the cells. The bioconcentration of dimethoate was inversely proportional to the treatment level. For instance, after 2 hr, a concentration of 116, 25 and 19 ug g^{-1} was observed with 1, 5 and 10 ug ml^{-1} respectively. This ability to accumulate insecticides is common to a variety of fungi and many species have been recognized as biomagnifiers of different insecticides (Ko and Lockwood 1968; Boush and Batterton 1972). The amounts of dieldrin accumulated by yeast were comparable to those reported in literature (Lal 1984). Removal of lindane from the medium by the cells of yeast, two bacteria and an alga ranged from 29 to 57% (Mac Rae 1985).

At present permethrin is being evaluated for its environmental impact. However, no information is available on its uptake and bioconcentration by microorganisms. The accumulation of permethrin was surprisingly quite high notwithstanding its easily degradable nature. Accumulation of permethrin ranged from 9 to 504 ug g^{-1} dry wt. The bioconcentration of permethrin was inversely proportional to the treatment level and exposure period and was highest of the other two insecticides which ranged from 11 to 2550 ug g^{-1} dry wt. Fenvalerate was also accumulated in algae at levels 477 to 993 times that found in treatment water (OhKawa et al.1980). The concentration of permethrin rapidly decreased with increase in biomass. The yeast caused complete degradation of pyrethroid insecticides, deltamethrin and permethrin (Fatichenti et al.1983;1984). Evidently, yeast in the present study also caused degradation of permethrin resulting in low amount in the cells.

The water solubility of dieldrin (0.186 mg L^{-1}) was lowest, followed by permethrin (0.25 mg L^{-1}) and dimethoate (25 g L^{-1}). Generally bioconcentration of pesticides by microorganisms shows an inverse relation to their water solubility. However no such relation was observed in the present study which may be due to the conversion of the pesticide to water soluble metabolites.

Although different insecticides were accumulated to different levels, the rate of accumulation was very rapid in the beginning, maximum amounts were accumulated within 2-6 hr and decreased with increase in biomass. The pattern of accumulation was almost similar for all insecticides. Certain bacteria, yeast and algae rapidly accumulated Thimet and then excreted it out slowly (Ahmed and Casida 1958). Similarly accumulation of malathion by bacteria was much

rapid than fungus, Aspergillus oryzae (Paris et al.1975). However, malathion concentration declined subsequently which was attributed to its metabolism by bacteria. The amounts of dimethoate picked-up by yeast was very rapid in the beginning, afterwards, a decline in its concentration at all treatment levels was evident. However, no metabolic products could be identified in the present investigation.

The yeast, Saccharomyces cerevisiae was quite resistant to most pesticides and even at high concentrations the effect was transient only (Voerman and Tammes 1969; Karenlampi et al. 1982; Lal and Lal 1987). The growth of yeast was inhibited maximum by 52% at 100 ug ml^{-1} dieldrin (Table 2). Permethrin also produced almost similar maximum inhibition of 58%. But the toxicity of dimethoate towards the growth of normal yeast was less and a maximum inhibition of only 31% was observed with 100 ug ml^{-1} after 8 hr. Many organophosphorus insecticides like fenitrothion and chlorpyrifos inhibited the growth at all treatment levels (Lal and Lal 1987). The inhibition of growth ranged from 3 to 42 and 25 to 78% in cultures of Saccharomyces treated with fenitrothion and chlorpyrifos, respectively. However, toxicity did not last long and after 12 hr almost complete recovery was achieved.

The toxic effect of DDT on Saccharomyces was evident only at 100 ug ml^{-1} while 10 ug ml^{-1} was stimulatory (Lal and Lal 1987). The maximum inhibition of cell number in yeast by DDT was quite low (33% compared to permethrin 58%). It has been suggested that the toxicity of pesticide depends upon the chemical structure. A PCB-mixture with one to three chlorine atoms is quite toxic to Colpidium (Dive et al. 1976). Both permethrin and DDT are chlorinated insecticides. The toxicity of these insecticides may be due to the presence of two or three chlorine atoms in their molecules.

Dieldrin was more toxic towards the growth of normal yeast than protoplast culture (Table 2). Similar effects of endosulfan on the yeast cells Rhodotorula gracilis were observed by Srivastava and Mishra (1981). The effect on protoplast was slightly less and an inhibition of 49% was observed with 10 ug ml^{-1} dieldrin. Dimethoate upto 10 ug ml^{-1} was not toxic towards the growth of normal as well as protoplast cultures of yeast. However 50 and 100 ug ml^{-1} substantially inhibited the cell number, the inhibition being slightly less pronounced in normal cultures compared to protoplast. The growth of yeast protoplast treated with dieldrin, dimethoate and permethrin was inhibited maximum by 49, 31 and 41% respectively. Protoplast compared to normal cultures were less susceptible to chlorpyrifos (Lal and Lal 1987). However, DDT and fenitrothion, although very toxic to protoplasts, the toxicity was transient in nature. Similarly permethrin was less toxic to the growth of normal protoplast, which remained unaffected or showed stimulation upto 50 ug ml^{-1} and only at 100 ug ml^{-1} appreciable inhibition was noticed. It is evident from the present finding that toxicity of insecticides is more

Table 1 . Bioconcentration of insecticides by Saccharomyces cerevisiae for 12 hr

	Initial concentration in the medium (ug/ml)								
	Dieldrin			Dimethoate			Permethrin		
Exposure time (hr)	0.1	0.5	1	1	5	10	0.1	0.5	1.0
2	*181.7 ±9.5	305.3 ±55.5	602.5 ±27.5	117.5 ±10.2	25.1 ±2.6	18.6 ±2.3	2550.5 ±396.2	422.4 ±52.7	171.1 ±34.5
4	157.2 ±3.5	92.4 ±7.4	189.7 ±12.0	132.1 ±12.1	118.8 ±15.5	75.9 ±9.1	2040.0 ±63.3	450.1 ±75.7	504.2 ±95.2
6	162.5 ±6.8	38.6 ±5.7	49.8 ±4.4	330.4 ±78.0	44.5 ±6.0	5.4 ±0.7	489.4 ±23.1	61.3 ±3.7	126.9 ±32.7
8	22.8 ±1.61	91.3 ±1.0	54.0 ±11.1	56.9 ±3.9	10.0 ±0.6	4.2 ±0.6	189.5 ±36.8	27.3 ±0.2	35.6 ±2.8
10	108.9 ±9.5	52.2 ±2.8	143.3 ±8.9	20.0 ±4.5	14.7 ±2.1	2.5 ±0.2	136.0 ±39.0	21.1 ±0.1	23.0 ±1.6
12	236.5 ±10.3	52.0 ±1.1	75.6 ±13.8	5.9 ±1.2	1.4 ±0.2	0.5 ±0.01	93.8 ±6.9	20.3 ±1.1	11.0 ±3.4

* Mean of triplicate (± standard deviation)

Table 2. Comparison of the effects of dieldrin, dimethoate and permethrin on the growth (a) normal and (b) protoplast cultures of Saccharomyces cerevisiae

Percent (+) increase/(-) decrease over the control				
	Insecticide treatment in ug/ml			
Insecticides	1	10	50	100
(a) Normal culture				
Dieldrin	-11	-24	-28	-52
Dimethoate	-8 +3	-13 +1	-21	-30
Permethrin	-40	+2 -58	+64 -41	+16 -50
(b) Protoplast culture				
Dieldrin		+11 -23	+18 -25	-49
Dimethoate		-21	-28	-31
Permethrin		+24 -13	+38 -20	+8 -41

pronounced on normal yeast than protoplast.

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