

## Attendant Changes in the Microflora of Rice Field Soil as Influenced by the Application of Granular Insecticides

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### ABSTRACT

The influence of six granular insecticides, *v/z.* diazinon, cytolane, Carbofuran, carbaryl+lindane, quinalphos and Dursban on the quantitative changes in the microbial population of rice field soils was studied. When applied at recommended doses (1.5 kg a.i./ha) there was no deleterious effect on the fungal population due to the insecticides. Among the different insecticides, there was no significant difference in respect of their influence on the microbial population. The same inference held true with actinomycetes. However, the bacterial population underwent a significant fall due to the insecticidal application. When the activities of soil enzymes like phosphatase,  $\beta$ -glucosidase and invertase were determined following the insecticidal application,  $\beta$ -glucosidase alone was significantly inhibited and other enzymes recorded no change.

### INTRODUCTION

Soil being the repository of pesticides at one stage or other, is greatly influenced by the toxic biocides. Nevertheless exhaustive studies have been made on the effect of these chemicals on the soil microorganisms and their activities, our understanding of the interactions in soil ecosystem is far from complete. While one group of investigators claim the stimulation of microorganisms due to a chemical, the other group looks up the very same chemical to be of inhibitory nature (Harries, 1972). Again, under water-logged soil condition, such studies are very limited. The present study aims at gathering information on the nature of the changes in the microorganisms when the soils applied with recommended levels of insecticides.

### MATERIALS AND METHODS

A field trial was laid out with rice variety IR. 20. Immediately after transplantation, the insecticides, *v/z.* diazinon, cytolane, carbofuran, carbaryl + lindane, quinalphos and Dursban were applied at 1.5 kg a.i./ha level. Representative soil samples from 0-15 cm depth were collected in polyethylene bags from each treatment at desired intervals. The samples were immediately analysed for microbial population following the standard dilution plate technique on appropriate agar media. Soil samples obtained from an untreated plot of IR 20 variety served as control.

Fresh soil samples were used for all enzyme measurements. The excess moisture in the samples was swiftly

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removed by blotting between folds of filter paper and analysed for enzyme activities. The methods of enzyme analysis were essentially the same as detailed by Skujins (1967).

*Phosphatase* (E. C. 3. 1. 3) : The assay system consisted of 3.0 g of soil in 5 ml of 0.1 M phosphate buffer (pH 7.0) with 1.0 ml of one per cent disodium mono phenylphosphate as substrate and a drop of toluene. The tubes were incubated at 30°C for 24 hr with occasional shaking. The reaction was arrested at the stipulated time by the addition of 1 ml of 25 per cent trichloro acetic acid. The clear supernatant obtained through centrifugation at 2,100 x g was analysed for the quantity of phenol released by the methods of Bray and Thorpe (1954).

*Invertase* (E. C. 3. 2. 1. 26) : The assay system consisted of 3.0 g of soil in 5.0 ml of 0.1 M acetate buffer

(pH 5.2) and 1 ml of one per cent solution of sucrose and a drop of toluene. At the end of the incubation period, the amount of reducing sugars liberated was determined in an aliquot of the clarified reaction mixture by the methods of Inman (1965).

*β-Glucosidase* (E. C. 3. 2. 1. 20) : The reaction mixture contained 3.0 g of soil in 5.0 ml of acetate buffer (pH. 6.0) with 1 ml of one per cent solution of D - Salicin and a drop of toluene. At the end of the reaction time the amount of reducing sugars released into the reaction mixture was calculated by the colorimetric methods described by Inman (1965).

## EXPERIMENTAL RESULTS

**Effect of different insecticides on the microbial population of wet land soil :** The data revealed that due to the application of different

TABLE I. Effect of granular insecticides on the microflora of water logged soil

Treatment	Bacterial count (x 10 <sup>6</sup> /g of soil)				Fungal population (x10 <sup>4</sup> /g of soil)				Actinomycete population (x 10 <sup>5</sup> /g of soil)			
	1st day	7th day	15th day	30th day	1st day	7th day	15th day	30th day	1st day	7th day	15th day	30th day
Diazinon	15.01	11.31	0.90	1.95	8.80	26.50	1.30	3.50	4.40	3.50	0.90	10.00
Cyrolane	17.93	3.33	2.16	1.87	3.40	3.00	1.80	1.80	3.40	3.00	1.80	1.80
Carbofuran Carbaryl+	16.74	18.25	1.59	0.49	4.80	33.30	0.70	2.10	2.60	4.40	5.20	8.80
lindane	11.43	7.07	3.62	0.90	4.60	15.50	5.00	2.40	3.80	3.40	15.00	7.40
Quinalphos	11.24	2.92	2.34	1.32	5.30	4.60	0.20	1.70	4.90	3.10	3.10	3.80
Dursban	19.83	14.54	2.33	1.64	5.90	9.80	1.50	4.70	3.40	4.10	6.30	5.20
Control	6.61	12.23	3.16	1.50	9.30	44.70	3.30	6.30	2.75	0.64	1.49	0.74
Stages :	S. E. = 1.43, C.D. = 4.25				S. E. = 3.99				S. E. = 1.05			
Treatment :	S. E. = 1.89				S. E. = 5.29				S. E. = 1.39			

Mean of three replications

insecticides, there was no significant alteration in the population of microflora (Table I). In general there was a decline of bacterial population which lingered upto 30 days and more reduction was observed on 30th day. The fungal population did not undergo any significant change due to the different insecticides; after 7 days of application of insecticides, the population of fungi increased due to carbofuran application (Table II). The different insecticidal treatments have not also significantly altered the actinomycete flora; increased following the application of diazinon, carbaryl+lindane and carbofuran.

**Effect of different insecticides on the activity of enzymes:** The phosphatase activity remained unaltered due to the insecticides. The activity

of the enzyme significantly decreased on the 80th day of observation and the decrease was preceded by increased activity on 7th and 15th day (Table II). In general, invertase activity decreased significantly with sampling time. The last sampling done at 30th day recorded the least invertase activity. However, there was no significant effect on the invertase activity by different insecticides. Among the different insecticides, Dursban inhibited  $\beta$ -glucosidase maximally and Cytrolane had the least inhibitory activity. Also the sampling time had significant effect on the enzyme activity. The 7th day sampling recorded maximum activity and the least enzyme activity was recorded on the 30th day (Table II).

## DISCUSSION

None of the six granular insecti-

TABLE II. The activity of soil enzymes as influenced by different granular insecticides in water logged soil

Treatment	Phosphatase ( $\mu$ g of phenol released/g of soil)				Invertase ( $\mu$ g of glucose/g of soil)				$\beta$ -glucosidase ( $\mu$ g of catechol released /g of soil)			
	1st day	7th day	15th day	30th day	1st day	7th day	15th day	30th day	1st day	7th day	15th day	30th day
Diazinon	174	245	208	158	247	180	113	110	113	156	135	88
Cytrolane	149	195	166	149	270	214	108	98	130	124	140	108
Carbofuran	158	266	166	166	301	259	88	225	106	81	79	40
Carbaryl+lindane	187	261	278	114	281	304	108	81	87	127	90	22
Quinalphos	228	274	199	107	258	168	83	45	86	90	63	23
Dursban	215	216	191	122	315	171	90	34	81	117	90	36
Control	166	270	282	216	315	173	149	125	119	248	198	109
Stages:	C. D. 39.54				C.D=46.35				C.D.=26.88			
Treatment	S.E.=17.61				S.E.=20.64				C.D.=35.58			

Mean of two replications

cides exerted any deleterious effect on the microflora, when they were applied at recommended doses. Diazinon and carbaryl+lindane imparted a general stimulatory effect on the actinomycete flora indicating that the insecticides left no deleterious effect on the soil microorganisms at the doses used. Recent studies (Sreenivasulu and Rangaswami, 1973) have shown that certain organophosphorus insecticides increased the microbial counts of the soil as has been observed in the present study with actinomycetes. One of the sound arguments presented for such increase in microbial number due to the pesticide molecules is that in soil environment the microbial demand for assimilable carbon would always exceed the supply, these pesticides offer themselves as energy sources for the microorganisms (Kearney *et al.*, 1967). When applied at higher levels these insecticides disturb the beneficial microorganisms in soil; however, normal field dosages have not been shown to create imbalances sufficient to result in significant reduction of soil fertility (Matsumura Boush, 1971). Moreover, the organophosphorus insecticides hardly remain effective in soil for a long time (Tu, 1970).

Unlike the varied types of enzymes studied *in vitro*, the soil enzymes are generally resistant to inactivation by various inhibitory agents, or they exist in a certain physical and chemical association with the soil particles that render the protein molecules more stable. The present study

revealed that the soil enzymes like amylase, invertase and phosphatase were not affected by the insecticides while  $\beta$ -glucosidase alone exhibited a decline. It has been conceded that degradation of biocides is much faster in anaerobic environments than in well aerated soils (Sethunathan and Mc Rae, 1969; Yoshida, 1975). Therefore the insecticides under anaerobic conditions of the rice field soils undergo rapid degradation.

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