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Effect of HCH and Fenvalerate on Growth and Distribution of Microorganisms in Relation to Persistence of the Insecticides in the Rhizosphere Soils of Wetland Rice

A. C. Das,[1] A. Chakravarty,[2] P. Sukul,[2] D. Mukherjee[1]

[1] Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741 252, India
[2] Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252, India

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Insecticides are frequently applied to crop fields to combat insect pests for better crop production. After application, a large portion of insecticides accumulates in the top soil layer (0-10 cm) where maximum microbiological activities occur. As microorganisms are scavengers in soil and possess physiological variability, they degrade a great variety of chemical substances including the insecticides. Some insecticides are degraded by soil microorganisms to derive energy and nutrients (Bhuyan et al. 1993) with the resultant increase in the population of insecticide-degrading organisms. On the other hand, there are some insecticides that exert deleterious effect on microorganisms (Moorman 1989; Martinez-Toledo et al. 1992). Therefore, no definite conclusion can be made on the effect of insecticides on microorganisms and their activities in soil since different groups of insecticides exhibit manifold variations in toxicity (Simon-Sylvestre and Fournier 1979). The present experiment has been conducted to investigate the effect of HCH (1, 2, 3, 4, 5, 6 - hexachlorocyclohexane) and fenvalerate [(*RS*)-cyano-(3-phenoxyphenyl)(*RS*) methyl- 4 - chloro - α - (1- methylethyl) benzeneacetate] at their recommended field rates, on the population and distribution of bacteria, actinomycetes and fungi as well as the persistence of the insecticides in the rhizosphere soils of wetland rice.

MATERIALS AND METHODS

An experiment has been conducted in microplots (4m x 4m) with three replications following randomized block design (RBD). The soil belongs to Typic Fluvaquent (USDA 1975) having the general characteristics: sand 52.8%, silt 33.8%, clay 13.4%, water holding capacity 56.9%, pH (1:2.5 w/v) in water 7.7, electrical conductivity 0.35 dS m^{-1}, cation exchange capacity 16.3 cmol (p^+) kg^{-1}, organic C 8.2 g kg^{-1}, total N 0.8 g kg^{-1}, C : N ratio 10.2, exchangeable NH_4^+ 50.4 mg kg^{-1}, soluble NO_3^- 22.2 mg $kg^{-1}$and available P 4.3 mg kg^{-1}. A fertilizer dose of 50, 22 and 42 kg ha^{-1} of N, P and K as urea, single superphosphate and muriate of potash respectively, was mixed thoroughly with soil during land preparation. Thirty-day old seedlings of rice (*Oryza sativa* L. variety IR-50) were transplanted, two seedlings per hill, with a spacing of 15 cm by 20 cm hill to row. Another 50 kg ha^{-1} of N as urea was applied as top dressing 21

Correspondence to: A. C. Das

days after transplanting. After 30 days of transplanting, HCH (50WP) and fenvalerate (20EC) were applied at rates of 7.5 and 0.35 kg a.i. ha^{-1} respectively, to the crop by mixing with 600 Lha^{-1} of distilled water. The crop was cultivated following usual agronomic practices.

Rhizosphere soils of rice were collected after 0 (1 hour), 5, 10, 15, 30, 45 and 60 days of application of insecticides from each plot at random by uprooting the plants and were analyzed immediately to enumerate the colony forming units (cfu) of bacteria, actinomycetes and fungi following serial dilution technique and pour plate method (Salle 1973) in asparagine-mannitol agar (Thornton 1922), dextrose-casein agar (Jensen 1930) and rose-bengal agar (Martin 1950) media, respectively. After counting, ten isolates of each type of microorganisms were selected at random from the agar plates of each treatment and were identified to generic level following the guide of Skerman (1967) as modified by Bowie et al. (1969) for bacteria, the key of Skerman (1975) for actinomycetes and procedure of Gilman (1957) for fungi.

Rhizosphere soils were also analyzed for the presence of insecticidal residues by drawing samples at different sampling days and extracting the soils as outlined by Das and Mukherjee (2000). HCH and fenvalerate residues were estimated by gas-liquid chromatography (GLC) using 5890A model of Hewlett-Packard (HP) gas chromatograph coupled with 3392A (HP) integrator and equipped with Ni^{63} electron capture detector and a glass column (180 cm x 2 mm) packed with 3% OV-17 on 80 to 100 mesh chromosorb-W (HP). The operating temperatures of injector, column and detector were maintained for HCH at 200 °C, 160 °C and 300 °C, and for fenvalerate at 275 °C, 225 °C and 275 °C respectively. The flow rate of carrier gas (N_2) was adjusted to 37.5 ml min^{-1} for HCH and 70 ml min^{-1} for fenvalerate. The recovery rates of fortified controls of HCH and fenvalerate were 90% and 98%, respectively. From the residue values, the half-life ($T_{1/2}$) of each insecticide in the rhizosphere soils was calculated following the method of Hoskins (1961).

RESULTS AND DISCUSSION

Application of HCH and fenvalerate induced the proliferation of bacteria, actinomycetes and fungi in the rhizosphere soils of rice (Table 1) and the stimulation was more pronounced with HCH than fenvalerate. This indicated that these microorganisms were able to use the insecticides and/or their degraded products for growth and metabolism (Alexander 1978; Bhuyan et al. 1992). It was also recorded that microbial population was highly induced during 30^{th} to 45^{th} days when the crop was at the stage of flowering. This indicated that at this stage, the crop roots released maximum amount of exudates (Rovira 1969) which were preferably utilized by the enhanced microbes resulting in greater microbial population in the rhizosphere soils. The earlier workers (El-Shahaat et al. 1987; Bhuyan et al. 1993) also reported similar observations. Regarding the distribution of individual microorganisms in the rhizosphere soils, it was revealed that HCH

Table 1. Effect of insecticides on the population of microorganisms in the rhizosphere soils of rice.

Sampling days	Treatments		
	Control	HCH	Fenvalerate
	Number of bacteria (cfu x 10^5 g^{-1} soil)		
0 (1 hr)	114.6 ± 6.4	117.6 ± 6.8	122.9 ± 5.4
5	143.5 ± 3.6	152.4 ± 8.2	163.2 ± 6.8
10	150.1 ± 6.4	160.1 ± 5.4	166.1 ± 4.2
15	158.3 ± 8.3	215.6 ± 8.1	188.7 ± 7.3
30	166.6 ± 9.3	237.2 ± 9.1	190.6 ± 9.1
45	140.8 ± 5.8	193.4 ± 6.1	144.7 ± 4.9
60	125.3 ± 7.3	153.7 ± 8.1	136.2 ± 6.5
Mean	142.7	175.7	158.9
	Number of actinomycetes (cfu x 10^5 g^{-1} soil)		
0 (1 hr)	87.5 ± 4.9	94.8 ± 2.2	90.9 ± 4.4
5	104.9 ± 7.2	134.2 ± 8.3	114.2 ± 3.1
10	112.5 ± 5.1	138.8 ± 7.1	147.7 ± 5.6
15	125.1 ± 5.5	169.6 ± 5.4	151.5 ± 9.4
30	137.5 ± 7.1	191.1 ± 4.7	166.2 ± 9.6
45	137.5 ± 4.4	164.4 ± 7.8	174.8 ± 9.7
60	112.1 ± 2.2	125.4 ± 5.4	125.3 ± 8.1
Mean	116.7	145.4	138.6
	Number of fungi (cfu x 10^4 g^{-1} soil)		
0 (1 hr)	14.1 ± 2.7	14.4 ± 3.8	12.6 ± 2.2
5	14.1 ± 1.4	21.3 ± 2.9	15.3 ± 2.7
10	15.2 ± 2.4	25.5 ± 3.5	16.3 ± 2.1
15	15.8 ± 2.6	46.7 ± 5.5	20.6 ± 3.3
30	19.4 ± 2.5	39.1 ± 3.8	27.6 ± 3.4
45	10.9 ± 2.2	22.1 ± 3.4	18.4 ± 2.3
60	7.7 ± 2.6	19.1 ± 3.6	11.9 ± 2.5
Mean	13.8	26.8	17.5

Means ± SD, cfu = colony forming unit

stimulated the growth of *Bacillus* and *Corynebacterium* but did not have any effect on the proportion of *Pseudomonas* and also on most of the predominant genera of actinomycetes and fungi (Table 2). Fenvalerate on the other hand, augmented the proportion of *Bacillus*, *Erysipelothrix* and *Escherichia* under bacteria, *Micromonospora* under actinomycete and *Aspergillus* and *Fusarium* under fungi but retarded the growth of bacteria like *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Proteus* and *Klebsiella* and fungi like *Penicillium* and *Rhizopus* in the rhizosphere soils. Similarly, the proliferation of bacteria such as *Staphylococcus*, *Serratia* and *Klebsiella* and fungi such as *Penicillium* and *Rhizopus* was impaired due to the application of HCH in soil. This clearly pointed out that all the microorganisms were not able to utilize the insecticides and their degraded products for their growth and development. Rather some of them were affected by the toxicity of the insecticidal residues in the rhizosphere soils of rice.

The dissipation both the insecticides in the rhizosphere soil varied (Table 3). The rate of dissipation was higher for fenvalerate as compared to HCH, depicting the half-life ($T_{1/2}$) 7.4 and 13.0 days, respectively. This was also in agreement with the earlier report (Das and Mukherjee 2000) that recorded the $T_{1/2}$ of the cited insecticides as 5.0 and 17.7 days, respectively in Typic Orchragualf soil. This indicated that the soil microorganisms degraded fenvalerate more favourably as compared to HCH. The long persistence of HCH as also investigated by earlier workers (Samuel et al. 1988; Kawano et al. 1992) could be attributed to its resistance to biodegradation (Kahlon et al. 1990) and/or its ability to form recalcitrant molecules in soil (Edwards 1966). The degradation of fenvalerate was very rapid during early days and insecticidal residues could not be detected after 45 days of application in soil. HCH persisted in soil up to 3.8% even after 60 days of application.

Table 2. Effect of insecticides on the distribution of predominant genera of microorganisms in the rhizosphere soils of rice.

Microorganisms	Treatments		
	Control	HCH	Fenvalerate
Bacteria			
Bacillus sp.	22	25	30
Pseudomonas sp.	18	18	13
Staphylococcus sp.	8	4	5
Micrococcus sp.	6	6	4
Corynebacterium sp.	2	6	1
Serratia sp.	3	0	2
Sarcina sp.	2	3	3
Erysipelothrix sp.	2	1	4
Escherichia sp.	0	2	3
Flavobacterium sp.	0	1	1
Proteus sp.	2	1	0
Xanthomonas sp.	1	1	2
Klebsiella sp.	3	0	1
Diplococcus sp.	1	2	1
Actinomycetes			
Streptomyces sp.	41	42	40
Nocardia sp.	26	25	25
Micromonospora sp.	3	3	5
Fungi			
Penicillium sp.	28	25	23
Aspergillus sp.	15	17	20
Fusarium sp.	12	14	16
Humicola sp.	6	8	7
Trichoderma sp.	3	3	2
Rhizopus sp.	5	2	1
Phytophthora sp.	1	1	1

Table 3. Persistence of the insecticides in the rhizosphere soils of rice.

Sampling days	Residues (mg kg^{-1} soil)	
	HCH	Fenvalerate
0 (1hr)	3.48 ± 0.04	0.16 ± 0.002
5	2.12 ± 0.03	0.12 ± 0.001
10	1.22 ± 0.04	0.09 ± 0.001
15	1.01 ± 0.03	0.07 ± 0.001
30	0.54 ± 0.03	0.01 ± 0.001
45	0.20 ± 0.03	ND
60	0.13 ± 0.03	ND
DL	0.01	0.002
$T_{½}$ (days)	13.0	7.4
r	- 0.987	- 0.976

Means ± SD, ND = not detected, DL = detection limit, $T_{½}$ = half-life, r = correlation coefficient

The results of the present investigation thus point out that application of HCH and fenvalerate at their recommended field rates in general, stimulate the growth and distribution of the predominant genera of bacteria, actinomycetes and fungi which in turn, degrade the insecticidal residues very rapidly resulting less persistence of the insecticides in the rhizosphere soils of wetland rice.

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