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Decrease in Microbial Biomass Due to Pesticide Application/Residues in Soils Under Different Cropping Systems

M. N. Jha, S. K. Mishra

Department of Microbiology, Faculty of Basic Sciences and Humanities, Rajendra Agricultural University, Pusa, Bihar, Samastipur 848 125, India

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The use of pesticides for crop protection is an integral part of modern agriculture. Soil is the ultimate sink for pesticides as less than 1 per cent of the total applied pesticides generally gets to the target pests (Pimentel 1983). Generally, pesticide residues accumulate in the top 15 cm of soil which is also, the region of activity of most of the soil microorganisms ((Harris and Sans 1969; Alexander 1961). The evaluation of published data on the effect of the pesticide residues in soils on soil microorganisms by Domsch *et al.* (1983), it was concluded that nearly 89 per cent of the induced effects were either negligible delays in population growth and activities with only 2 per as critical delays. However, most of the reported research work was done on testing the side effects of single application of pesticides (Atlas *et al.* 1978; Greaves 1987; Das *et al.* 2003). Meager information is available in literature for more realistic situation where different active ingredients of pesticides are applied in sequence come together in the soil. Information on modifications of the stress condition due to pesticides residues on soil microorganism by change in the cropping system is also meager. Our aim therefore, was to measure the side effects of pesticides application on microbial population under different cropping systems. Farmer's brinjal growing fields under quick succession of various pesticides application were also evaluated in terms of microbial population and pesticide residues in/on brinjal fruit for consecutive two years.

MATERIALS AND METHODS

Experimental sites were located in North Bihar of India. The experimental plots include sites under four cropping systems viz. vegetable based, Rice-wheat, sugarcane based and pulse based; farmer's field under brinjal cultivation and research farm plots. The soil texture in general was predominantly silt loam with pH ranging from 8.0 to 8.6, EC 0.22 to 0.46 dSm^{-2}, organic carbon 0.31 to 0.56% and free $CaCO_3$ 32.0 to 48.0 per cent. The average rainfall was 1100 to 1300mm. April, May and June are the hottest months with the daily maximum temperature ranging between 35 to 39^{0}C. January is the coldest month of the year with an average maximum and minimum temperature of 22.8 and 7.4^{0}C, respectively. Highest consumption of pesticides was by vegetable growers. Weedicides were least preferred by farmers.

Correspondence to: M. N. Jha

Ten to twelve sub-samples of top soil (0-15cm) taken randomly with a special soil Sampling auger and combined to give one mixed sample of each plot taken for analysis. The soil samples were thoroughly mixed and allowed to air dry before sieving. The soil samples were stored in a freezer at -5^{0}C until analyzed.

Enumeration of microorganisms included viable counts for bacteria, fungi, actinomycetes, cyanobacteria, *Rhizobium* and *Azotobacter* and most probable number (MPN) for *Azospirillum, Nitrosomonas* and *Nitrobacter.* Viable microorganisms were counted from duplicate samples of each treatment. The samples were diluted serially (10 fold steps) in sterilized distilled water and transferred aseptically on to respective agar media as per microbial types. The inoculated Petri plates were incubated at 28 $\pm$ 2^{0}C for 72h for heterotrophs and in illuminated culture room at 30 $\pm$ 2^{0}C for 25d for autotrophic cyanobacteria.

The chemicals applied were typical for a plant protection systems associated with intensive cultivation of vegetables. It consisted of cypermethrin, chloropyriphos and endosulfan.

To evaluate the effect of insecticides on soil microorganisms and its residue in/on brinjal fruit, field experiment was conducted during kharif, 03 in silt loam calcareous soil at Rajendra Agricultural University, Pusa (Bihar), India. The soil in experimental plot had pH 8.5, EC 0.32 dSm^{-2}, OC 0.38% and free $CaCO_3$ 38.2%. The test variety of brinjal was Pusa Purple Long. The experimental lay out had RBD design and each treatment had three replications in 10m^2 plots. All pesticides were used at recommended level as foliar spray during the formation of marketable size of fruits.

Fifty g sub samples of soils were taken in a beaker and mixed with 0.5g activated charcoal, 0.5g florisil and 10g anhydrous sodium sulphate. The mixture was packed in a glass column and the pesticides were eluted with 200 mL of acetone: hexane mixture (2:8) for 4-5 hours. The elute was concentrated to about 1-2 mL and then final volume was made in hexane for analysis. A 2-3 µL extract was injected in a Nucon GC 5700 equipped with NI63 detector and a glass column (2m) packed with 1.5% OV-17 + 1.95% q F-1 on 80-100 mesh CHW (HP). The operating conditions were nitrogen carrier gas flow 30 mL min^{-1}, and oven injector and detector temperature were 205^{0}C, 220^{0}C and 270^{0}C respectively. Under this condition recovery was 85.6-98.4 per cent.

Samples of brinjal fruits (250-500 g) were collected randomly from each replication at 0 (1h), 1, 3, 5, 10 and 15 days after insecticide spraying. The samples were cut into pieces, mixed thoroughly and a representative sample of 50g in each case was taken for residue analysis according to the procedure described by Kole et al. (2002).

RESULTS AND DISCUSSION

All the thirty two soil samples of different cropping systems analyzed were found

Table 1. Organochlorine insecticide residue (μg g^{-1} soil) under different cropping System.

Cropping System	Pesticide	Number of samples		Range of Residues
		Analysed	Contaminated	
Vegetables	Σ HCH	8	7 **(87.5)**	ND-0.074 (0.046)
	Σ DDT	8	8 **(100)**	0.031-0.131 (0.065)
	Σ Endosulfan	8	8 **(100)**	0.004-0.0069 (0.030)
Rice-wheat	Σ HCH	8	7 **(87.5)**	ND-0.054 (0.030)
	Σ DDT	8	8 **(100)**	0.008-0.162 (0.040)
	Σ Endosulfan	8	7 **(87.5)**	ND-0.027 (0.013)
Sugarcane	Σ HCH	8	7 **(87.5)**	ND-0.049 (0.027)
	Σ DDT	8	8 **(100)**	0.008-0.058 (0.030)
	Σ Endosulfan	8	6 **(75.0)**	ND-0.032 (0.014)
Pulse	Σ HCH	8	6 **(75.0)**	ND-0.019 (0.009)
	Σ DDT	8	8 **(100)**	0.004-0.101 (0.026)
	Σ Endosulfan	8	5 **(62.5)**	ND-0.038 (0.0106)

Figures in parenthesis indicate mean value.
Figure in bold indicate per cent contamination.

To be contaminated with HCH or DDT or endosulfan or all three (Table 1). The DDT residues were present as o, p^1 DDT, p, p^1-DDT and p, p^1 DDE in all the samples. The contamination with endosulfan was 62.5 to 100 per cent, highest being for vegetable soils. Endosulfan consisted of alpha, beta-isomer and endosulfan sulphate. Residues of HCH were detected in 75 to 87.5 per cent samples and consisted of alpha, beta and gamma-isomer δ-HCH was not detected in any sample.

The average concentration of total HCH was 0.046, 0.030, 0.027 and 0.009 μg g^{-1} in vegetables, rice-wheat, sugarcane and pulse growing soils. Total DDT concentration was more than total HCH in all cropping systems. Endosulfan had least value. Its concentration for vegetable, rice-wheat, sugarcane and pulse field soils was 0.030, 0.013, 0.014 and 0.009 μg g^{-1}, respectively.

Pesticide residues in soil had definite influence on microbial population (Table 2). Vegetable soils had least microbial population but highest pesticides residue (0.14

μg g^{-1}). The population varied from 64 million to 16 thousands g^{-1} soil, highest being for bacteria. Decrease in microbial population in vegetable soils were 10-20, 10-15, 1-2, 2-4, 2-3, 1-2 and 1-2 fold for bacteria, fungi, actinomycetes,

Table 2. Microbial biomass in soils under different cropping systems.

Cropping system	Range of microbial population			
Organism	Vegetable based	Rice-wheat	Sugarcane based	Pulse based
Bacteria $X10^7$	1.9-12.8 (6.4)	14-136 (94)	9-140 (78.0)	89-153 (119)
Fungi $X10^4$	1.0-14.6 (4.0)	16-82 (45)	19-115 (58.7)	26-92 (66.0)
Actinomycetes $X10^4$	12-40 (23.4)	21-35 (27.4)	18-54 (29.5)	27-58 (40.6)
Cyanobacteria $X10^3$	3-34 (16.25)	43-82 (58.6)	13-52 (34.6)	24-63 (44.4)
Rhizobium $X10^3$	23-56 (43.1)	41-125 (83.0)	41-111 (81.4)	98-212 (115.6)
Azotobacter $X10^3$	28-93 (50.6)	41-148 (95.4)	47-131 (85.9)	88-180 (91.1)
Azospirillum $X10^3$	11-92 (27)	14-122 (36)	28-160 (66)	14-92 (41)

* Figures in parenthesis indicate mean values.

cyanobacteria, *Rhizobium, Azotobacter* and *Azospirillum,* respectively than other cropping system soils. Highest microbial population (12 billion to 40 thousands) recorded in pulse growing soils had least pesticides residue (0.046μg g^{-1}). Actinomycetes had least effect of pesticides residue. Besides pesticide residues, different cropping systems had an influence on diazotrophic population. Domination of *Rhizobium* in pulse soil, *Azospirillum* in sugarcane soils and cyanobacteria in rice-wheat soils irrespective of pesticide residues level suggest the influence of cropping systems. Decrease in microbial population can not be attributed only to HCH, DDT and endosulfan pesticide residues. Other pesticide residues might have their presence in the observed soil samples. This is further evident from the fact that some of the vegetable growers applied 8-10 types of pesticides in their crop in one growing season. Thus, the observations indicate a trend. Different crop cultures had different side effect was also observed by Marsh and Davies, 1981.

A plant protection system consisting of 9-10 pesticides treatment in brinjal crops by the farmers during 2001 and 2002 was investigated for its side effects on soilmicroorganisms (Table 3). Cypermethrin and endosulfan was more frequently used than other pesticides viz., chloropyriphos, monocrotophos, quinlophos, imidachlopreid, triazophos, deltamethrin, thidiocarb, prophenophos, acephate, carbosulfan and thiomethoxam. Weedicides and fungicides were not applied during the course of investigation. These pesticides were applied in September and October during cultivation cycle.

Table 3. Microbial count (% of control) of pesticide burdened farmer's field soil.

	Date of observation	10.09.01 (23.09.02)	25.09.01 (08.10.02)	10.10.01 (23.10.02)	25.10.01 (08.11.02)	09.11.01	24.11.01	09.12.01	24.12.01
Sl. No.	Duration (days)	15	30	45	60	75	90	105	120
	Control	100	100	100	100	100	100	100	100
1.	Bacteria	85.9 (77.3)	46.0 (60.5)	49.3 (51.7)	20.4 (46.8)	56.9	72.4	54.1	65.2
2.	Fungi	33.3 (52.5)	6.5 (41.3)	23.6 (28.4)	8.1 (24.2)	41.3	48.7	37.4	68.6
3.	Actinomycetes	87.2 (68.8)	49.6 (47.2)	54.5 (42.6)	42.7 (39.3)	67.8	80.9	63.2	88.5
4.	Rhizobium	85.8 (81.6)	40.5 (63.4)	61.3 (49.7)	39.7 (45.5)	52.4	64.2	48.5	62.3
5.	Azotobacter	66.8 (64.5)	46.4 (55.1)	64.6 (51.5)	36.2 (48.3)	47.5	55.4	42.3	68.4
6.	Azospirillum	44.3	35.8	49.2	33.5	43.6	52.5	48.9	64.4
7.	Cyanobacteria	56.6 (67.6)	39.4 (51.0)	59.3 (43.8)	45.2 (37.9)	72.7	73.3	67.5	76.5
8.	Nitrifying	90	67.4	69.7	43.6	50.7	66.5	52.8	68.3
9.	Denitrifying	65.7	47.2	45.2	31.4	45.3	46.6	34.9	58.2

First year 02.09.01 - Chloropyriphos; 11.09.01 - Monocrotophos; 15.09.01 - Cypermethrin; 19.09.01 - Quinolphos, Imidochlopreid; 23.09.01 – Triazophos, Deltamethrin; 25.09.01 - Endosulfan; 07.10.01 - Cypermethrin, Imidochlopreid; 11.10.01 - Larwin, Thiodicarb; 13.10.01 - Endosulfan; 14.10.01 - Cypermethrin, Prophenophos ; 23.10.01 - Cypermethrin; 29.11.01 - Cypermethrin.

Second year - 08.09.02 - Monocrotophos, Acephate; 12.09.02 - Cypermethrin, Quinolphos; 19.09.02 - Chloropyriphos; 01.10.02 - Monocrotophos, Quinolphos; 04.10.02 - Cypermethrin, Acephate; 06.10.02 - Endosulfan, Prophenophos; 11.10.02 - Carbosulfan; 15.10.02 - Cypermethrin, Thiomethoxam; 18.10.02 - Endosulfan; 23.10.02 - Endosulfan; 24.10.02 - Thiomethoxam.

Figures in parenthesis indicate the value of 2nd year observation of same plot and same test crop (Brinjal).

Rapid succession of pesticide applications drastically reduced the microbial population. Recovery trend was found for all microbes. Microbial population was in the range of 33.3 to 90 per cent as compared to that in control at 15d of observation having only chloropyriphos application. The population was further decreased enormously on 30th d with the application of six types of pesticides between 15 and 30th day. The population range was 6.5 to 67.4 per cent, lowest being for fungi. A recovery trend was recorded on 45th day with an increase in the population of all types of microbes than just previous observation. However, there was again a strong inhibition of microbial population on 60th day with an application of four types of pesticides. In subsequent days of observation, same trend of recovery and inhibition depending on pesticides application was recorded. There was a strong recovery at the end of observation period as evident from the higher population of fungi, actinomycetes, *Azotobacter* and cyanobacteria as Ist observation. However, in the entire period of observations, none of the microbes were able to reach the value of control level. This might be due to the execution of pesticide application by the farmers before the disappearance of side effects. The trend was similar in 2002 but the side effects were comparatively less than in 2001.

All these above discussed experiment did not reveal the information about the effect of individual pesticides and their safety level for soil microorganisms. Thus, an experiment was conducted with brinjal crop exposed to foliar spraying of recommended levels of endosulfan, cypermethrin and chloropyriphos during the formation of marketable size fruits. Cypermethrin had least effect on microbial population (Table 4). Chloropyriphos had major effect. Initially (day5), microbial population decreased in all the treatments. Such losses of microbial population were short lived. The value reached to the control level at the end of the observation period (day25) in most of the cases. Bacterial population was almost unaffected in cypermethrin receiving plots. There was a strong inhibition of cyanobacterial population. The said organism failed to recover at pre-application level in chloropyriphos and endosulfan receiving plots. Fungi were next to cyanobacteria in sensitivity towards applied pesticides. The short lived effect observed in present investigation might be due to less availability of pesticides on soil in spraying application. Lots of pesticides might have accumulated on plant canopy. Such short lived effect of different pesticides were also reported by Schuster and Shroder (1990); Das *et al.* (2003).

Cypermethrin residues in/on brinjal fruits showed rapid reduction at different intervals. The initial deposits of 0.23 $\mu g\ g^{-1}$ was reduced to 0.18, 0.13, 0.08and 0.02 $\mu g\ g^{-1}$ causing a loss of 21.7, 43.5, 65.2 and 91.3 per cent on 1, 3, 5 and 10 days respectively. The chloropyriphos residue on day 0 was 3.67 $\mu g\ g^{-1}$ which was declined to 1.24, 0.52, 0.115, and 0.024 causing a dissipation of 66.3, 85.9, 96.9 and 99.3 per sent on 1, 3, 5 and 10 days, respectively. Similarly the endosulfan deposit of 4.8 ppm (1h / 0d) was reduced to 3.7, 2.3, 1.7, 0.63 and 0.28 $\mu g\ g^{-1}$ on day 1, 3, 5, 10 and 15 respectively. The observed dissipation of endosulfan was 23.0 (1d), 52.1 (3d), 64.9 (5d), 86.9 (10d) and 94.2 (15d) per cent. Residues of cypermethrin and chloropyriphos were below MRL even after 1d of application.

Table 4. Microbial population in insecticides treated brinjal field and pesticide residues

Treatment	Dose kg ai ha^{-1}	Days after spraying	Bacteria X 10^6	Fungi X 10^3	Actino-mycetes X 10^4	Rhizo-bium X 10^3	Azoto-bacter X 10^4	Azo-spirillum X 10^4	Cyano-bacteria X 10^3	Pesticide residues (ppm)	Dissi-pation rate
Cypermethrin	0.005	0	226	38	73	19	188	15	26	0.23	-
		1	-	-	-	-	-	-	-	0.18	21.7
		3	-	-	-	-	-	-	-	0.13	43.5
		5	234	24	68	8	81	11	18	0.08	65.2
		10	-	-	-	-	-	-	-	0.02	91.3
		15	239	35	74	17	178	18	29	BDL	-
		25	237	39	76	22	189	24	31	-	-
Choropyriphos	0.5	0	235	41	73	16	191	24	28	3.67	-
		1	-	-	-	-	-	-	-	1.24	66.3
		3	-	-	-	-	-	-	-	0.52	85.9
		5	217	18	65	7	72	9	19	0.115	96.9
		10	-	-	-	-	-	-	-	0.024	99.3
		15	239	27	73	12	156	17	14	BDL	-
		25	243	38	76	26	187	21	19	-	-
Endosulfan	0.5	0	222	34	70	21	191	25	28	4.8	-
		1	-	-	-	-	-	-	-	3.7	23.0
		3	-	-	-	-	-	-	-	2.3	52.1
		5	187	19	61	12	89	8	14	1.7	64.9
		10	-	-	-	-	-	-	-	0.63	86.9
		15	206	26	68	15	164	13	20	0.28	94.2
		25	231	33	72	28	190	23	24	-	-

However, endosulfan residue reached below MRL on day 5 suggesting a suitable waiting period need to be followed before marketing. Our findings corroborate with the finding of Kole et al. in the minimum waiting period for cypermethrin but differ with respect to endosulfan and chloropyriphos in the minimum waiting period.

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