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Population and activities of microorganisms in rice soil as influenced by application of insecticides of different bio-degradability

A. CHAKRAVARTY, [1]S. MAJUMDER, [1]D. MUKHERJEE AND [1]D. SAHA

Department of Agricultural Biochemistry,[1] Department of Agricultural Chemistry and Soil Science
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur -741252,
Nadia, West Bengal, India

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ABSTRACT

A green house experiment was conducted to study the effect of different insecticides on population and activities of microorganisms in a typic ustiflavent soil. Results in general, revealed that insecticides BHC, Phosphomidon and Quinalphos exert beneficial effect on growth and activities of total bacteria, actimycetes, fungi, ammonifying and non-symbiotic nitrogen fixing bacteria as well as phosphate solubilizing organisms in the early stages whereas, Chlorpyriphos and Carbaryl showed positive influence at the later stages of the experiment. Nitrogen fixing and phosphate solubilizing power of soils are in accordance with the growth and activities of microorganisms in regard to different insecticides under study.

Keywords : Insecticides, microbial activities, microbial population, rice soil

India is the world's second largest producer (after China) of rice. The crop productivity (in 2013) in India is low for rice (3.66 t ha^{-1}) as compared to global averages of 4.53 t ha^{-1} (FAO, 2016) and needs to be increased to meet the food grain requirements of the growing population. Rice is also the major staple crop and plays a vital role in the food and livelihood security in India. It accounts for about 41 per cent of total food grain production and 55 per cent of cereal production in the country (Kishore *et al.*, 2017). The high yielding rice cultivars are susceptible to pest attack and thus require pesticide application.

High yielding varieties requires demand a variety of pesticides (including insecticides) to optimize crop production (Das and Mukherjee, 2000; Cycon *et al.* , 2006). However, continuous application of pesticides may result in soil pollution leading to influence the activities of soil microorganisms and, thereby, affecting soil fertility (Sturz and Kimpinski, 1999; Lo´pez *et al*., 2002).

The effects of pesticides on the soil microbes are of importance because many microbial functions are critical to crop production, soil sustainability, and environmental quality (Topp, 2003). Conflicting reports are available with regard to sensitivity of pesticides to microorganisms (Digrak and Kazanici, 2001; Ahtiainen *et al.*, 2003; Devare *et al*., 2007; Lo, 2010).

For the last few decades, organochlorine insecticides have been replaced by organophosphate and carbamate insecticides. Limited information is available on the effect of these pesticides on soil microbes and associated biological processes (Pandey and Singh, 2004). The investigation, was, therefore, conducted to monitor the proliferation of different important soil microorganisms and associated biological processes in a rice soil receiving pesticides of different biodegradability.

MATERIALS AND METHODS

The pot culture experiment was conducted with 1 kg 1 mm sieved air dry soil, collected from 0-15 cm depth of the 'C' block farm, BCKV, Kalyani, Nadia, West Bengal. Some of the relevant physical, chemical and physico-chemical properties of the soil used for the investigation are pH 6.9, WHC 40.8%, EC 0.4 dS m^{-1}, organic C 0.7 %, total N 0.006%, exchangeable NH_4^+ 81 mg kg^{-1}, soluble NO_3^- 40 mg kg^{-1}, available P 2.61 mg kg^{-1}, CEC 20.57 c mol (p^+) kg^{-1} and clay 36.8 % and the nomenclature of the soil according to USDA system is Typic Ustiflavent. The soils of the pots received a uniform application of fertilizers at 60 kg ha^{-1}N, 30 kg ha^{-1} P_2O_5 and 30 kg ha^{-1} K_2O as ammonium sulphate, single super phosphate and muriate of potash, respectively. One half of the amount of nitrogen with the whole amount of phosphorus and potassium were applied to pots just after collection of the initial soil. All the insecticides were applied as treatments at that time. 30-day old rice seedlings were transplanted in the pots. The other half of the nitrogen and the insecticides were applied on 30th day after transplanting of rice seedlings. Three pots were kept for each of the pesticide treatments. The pesticidal treatments were T_0 = Control (No insecticide application), T_1 = BHC 50% WP (Wettable Powder) @ 0.25% a.i. (active ingredient), T_2 = Phosphomidon 50 EC (Emulsifiable concentrate) @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i. BHC belongs to hydrocarbon group; Phosphomidon,Quinalphos and Chlorpyriphos belonging to organophosphate group where as Carbaryl belongs to carbamate group of pesticides. Each pot contains 1 kg soil and under the respective treatments received 115 mg BHC, 0.0003 ml Phosphomidon,

Email: 1991sayan@gmail.com

0.00048 ml Quinalphos, 0.00015 ml Chlorpyriphos and 1.2 mg Carbaryl from their stock solutions. Respective stock mixtures were taken, mixed with 150 ml distilled water and then thoroughly mixed with 1 kg soil of each pot. The moisture content of the soil in each pot was maintained at 60 per cent WHC throughout the period of experiment, by compensating the loss in weight with distilled water on every alternate day. Rhizosphere soil samples were collected (Das and Sett, 2003) on 0th day (before application basal fertilizer and insecticides), 15th, 30th, 45th and 60th day after transplanting corresponding to panicle initiation stage, flowering stage, grain filling stage and harvesting stage (maturity) of rice respectively.

Total nitrogen content of the soils was estimated following the method of Stevenson (1996). The soils were incubated on agar plates containing appropriate media following seial dilution and pour plate technique (Pramer and Schmidt, 1965) to get viable count of total bacteria (Thronton, 1922), actinomycetes (Jensen , 1930 b), Fungi (Martin, 1950), ammonifying bacteria (Allen, 1957), non-symbiotic nitrogen fixing bacteria (Jensen, 1930 a) and phosphate solubilizing microbes (Pikovskaya, 1948), Non-symbiotic nitrogen fixing and phosphate solubilizing power were estimated as followed by Das and Mukherjee (1994).

RESULTS AND DISCUSSION

Results in table 1 revealed that total N content of the control soils increased upto 30 days and declined thereafter. The soil receiving BHC, Phosphomidon and Quinalphos followed more or less similar trend of results. After an initial decline upto 30 days, total nitrogen content of soils in the series with carbaryl and chlorpyriphos was restored at the initial level at the later stages. On an average comprising all stages, the soils under quinalphos, BHC and phosphomidon contained significantly higher amount of total N compared to control, while those under chlorpyriphos and Carbaryl contained lower amount without significant difference. Comparatively higher amount of accumulation of total N in Quinalphos, BHC and Phosphomidon treated soil over control is primarily due to the greater fixation of N (Table 4) as a consequence of higher proliferation of nitrogen fixing bacteria (Table 3). An overall decrease in the nitrogen content in Chlorpyriphos and Carbaryl treated systems depicts that nitrogen fixing power of the soil was not always indicative of its nitrogen content, or vice versa (Das and Mukherjee, 1998a.). Incidentally, the influence of insecticides on the total N content of the soils had an overall significant direct relationship with the population of nitrogen fixing bacteria and nitrogen fixing power of the soils (Table 1).

Total number of bacteria, actinomycetes and fungi in general, were found to increase up to 30 days and then showed a decreasing trend upto the last stage of incubation in control as well as in BHC, Phosphomidon and Quinalphos treated systems (Table 2). The present result is at par with the recent works of Merlin *et al.* (2016) and Supreeth *et al.* (2016) who reported that application of organochlorine and organophosphate pesticides increased both total bacteria and total actinomycetes count in soil. On the other hand, in Chlorpyriphos and Carbaryl treated systems, total number of bacteria, actinomycetes and fungi increased throughout the period of investigation. The numbers are in general, comparatively less over control particularly on 30th and 45th day and more on 60th day of incubation. However, an exactly opposite trend of results was observed in BHC, Phosphomidon and Quinalphos treated systems (Table 2). Decomposition products of Chlorpyriphos and Carbaryl had beneficial effect which was evidenced from the strong stimulation on growth of organisms in the later stages of the investigations. In fact, Carbaryl on degradation produce ammonia and formic acid (Stadnyk *et al*., 1971) which might serve as nitrogen and carbon source to the micro organisms resulting in higher population. Phosphomidon and Quinalphos were stimulatory right from the beginning supporting the earlier reports that microorganisms were able to utilize some organophosphate insecticides as the sole source of phosphorus (Rosenberg and Alexander, 1979) in addition to utilizing the chemical as such or the degraded products as energy and carbon source. BHC was also found to be inciting from the starting, sustaining the earlier findings (Das and Mukherjee., 1998b.). However, the enhancing effect of BHC, Phosphomidon and Quinalphos inexplicably did not last long as evidenced from the data at later stages probably due to their exhaustion.

The population of ammonifying bacteria, non-symbiotic nitrogen fixing bacteria and phosphate solubilizing organisms in soils significantly changed with treatment of different insecticides as well as with days of incubation (Table 3). A significant increase in the number of ammonifying bacteria was obtained in the series under Quinalphos, BHC and Phosphomidon over control after 15-30 days. The results are at par with Das and Mukherjee, 1999. However, under Carbaryl and Chlorpyriphos treated systems, the increase in number were continued upto 60th day of incubation. Considering all the stages, Quinalphos was the most conducive followed by the BHC and Phosphomidon as compared to control while Carbaryl and Chlorpyriphos had reverse effect (Singh *et al*., 2015). Among all insecticides, organophosphate pesticides particularly Quinalphos exerted an enhancing influence on ammonifying bacteria

Table 1: Effect of different pesticides on the changes in total N (%) in rhizosphere soil

Treatments	Days after transplanting of rice seedlings					Mean
	0	15	30	45	60	
T_0	0.065	0.066	0.068	0.065	0.062	0.065
T_1	0.066	0.068	0.070	0.068	0.068	0.068
T_2	0.066	0.067	0.070	0.070	0.067	0.068
T_3	0s.065	0.070	0.071	0.070	0.069	0.069
T_4	0.065	0.063	0.063	0.064	0.064	0.064
T_5	0.065	0.063	0.063	0.065	0.065	0.064
Mean	0.065	0.066	0.067	0.067	0.066	

Statistical Analysis

	Pesticides	Others
LSD (0.05)	**0.0027**	**NS**
LSD (0.05)	**0.0037**	**NS**

Notes : NS = Not significant

T_0 = Control, T_1 = BHC 50% WP @ 0.25% a.i., T_2 = Phosphomidon 50 EC @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i.

Table 2: Effect of different pesticides on changes in population of total number of bacteria ($\times 10^5$), actinomycetes ($\times 10^4$) and fungi ($\times 10^3$) in gm^{-1} of rhizospheric soils

Treatments	Days after transplanting of rice seedlings														
	0			15			30			45			60		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
T_0	74	36	28	119	41	48	127	52	30	118	54	42	110	48	36
T_1	76	34	38	152	72	58	176	79	64	136	49	26	100	40	22
T_2	75	38	29	153	85	56	175	85	68	131	35	36	105	36	24
T_3	74	37	27	125	68	52	148	81	54	124	29	21	109	35	18
T_4	77	36	30	80	38	30	83	44	31	114	50	52	134	67	58
T_5	77	35	32	91	40	32	92	46	32	106	55	58	125	69	64

Statistical Analysis

	Pesticides (P)			Stages (S)			Interaction (P × S)		
	A	B	C	A	B	C	A	B	C
LSD (0.05)	**4.12**	**4.93**	**4.27**	**3.76**	**4.50**	**3.90**	**9.22**	**11.02**	**9.74**
LSD (0.01)	**5.55**	**6.64**	**5.75**	**5.07**	**6.06**	**5.25**	**12.41**	**14.84**	**12.31**

Notes : A = Total bacteria, B = Total Actinomycetes, C= Total Fungi

T_0 = Control, T_1 = BHC 50% WP @ 0.25% a.i., T_2 = Phosphomidon 50 EC @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i.

throughout the experiment. Irrespective of treatments, in general, higher number of nitrogen fixing bacteria was recorded after 30 days followed by those after 15, 45 and 60 days (Table 3). The variation in number after 15 and 45 days was, however not significant considering all stages. Phosphomidon was the most conducive for nitrogen fixing organisms followed by Quinalphos and BHC with no significant differences among themselves. Chlorpyriphos was found to be the most recessive followed by Carbaryl. Like total bacteria, BHC exerted a stimulatory effect on the proliferation of nitrogen fixing bacteria upto 30 days. The beneficial effect of organophosphate insecticides (Phosphomidon and Quinalphos) is observed upto 30 days. The supressing effect of these pesticides at the later stage of experiment might be due to the formation of some activated products, as regard to toxicity (Alexander, 1978). In general, the number of phosphate solubilizers during a period of 60 days, was significantly increased with Carbaryl and Chlorpyriphos as compared to control.

Table 3: Effect of different pesticides on changes in population of total number of ammonifying bacteria (× 10^4), Non-symbiotic nitrogen fixing bacteria (×10^4) and phosphate solubilizing microorganisms (× 10^5) in gm^{-1} of rhizospheric soils

Treatments	Days after transplanting of rice seedlings														
	0			15			30			45			60		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
T_0	41	28	31	72	46	58	60	45	55	57	45	52	54	39	47
T_1	38	28	33	93	60	76	84	80	78	48	40	58	50	26	24
T_2	37	31	33	91	68	68	92	83	74	47	52	49	45	24	24
T_3	40	29	31	102	65	66	109	79	62	86	44	44	61	18	20
T_4	41	28	33	42	29	35	46	30	38	52	39	79	65	41	81
T_5	40	28	33	44	27	36	44	27	77	50	48	93	68	55	76

Statistical Analysis

	Pesticides (P)			Stages (S)			Interaction (P × S)		
	A	B	C	A	B	C	A	B	C
LSD (0.05)	**6.68**	**5.26**	**4.67**	**6.10**	**4.80**	**4.26**	**14.94**	**11.77**	**10.45**
LSD (0.01)	**8.99**	**7.09**	**6.29**	**8.21**	**6.47**	**5.74**	**20.12**	**15.85**	**14.07**

Notes : A = Ammonifying Bacteria, B = Non-symbiotic nitrogen fixing bacteria, C = Phosphate solubilizing microorganisms ; T_0 = Control, T_1 = BHC 50% WP @ 0.25% a.i., T_2 = Phosphomidon 50 EC @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i.

Table 4: Effect of different pesticides on changes in non-symbiotic nitrogen fixing (mg/g sucrose/g soil) and phosphate solubilizing {mg per15 mg insoluble p [75 mg $Ca_3(PO_4)$] g $soil^{-1}$ 0.15g^{-1} sucrose} power of the rhizosphere soil

Treatments	Days after transplanting of rice seedlings									
	0		15		30		45		60	
	A	B	A	B	A	B	A	B	A	B
T_0	7.85	0.091	9.16	0.104	9.64	0.101	9.14	0.100	8.28	0.092
T_1	7.91	0.094	10.35	0.117	11.32	0.116	9.57	0.085	8.62	0.068
T_2	7.95	0.090	11.03	0.110	11.08	0.108	9.55	0.077	8.23	0.060
T_3	7.86	0.093	10.22	0.0126	10.36	0.122	9.39	0.087	8.87	0.061
T_4	7.84	0.091	7.84	0.090	7.98	0.098	9.45	0.142	10.12	0.161
T_5	7.85	0.094	7.88	0.095	7.93	0.125	9.24	0.152	10.54	0.149

Statistical Analysis

	Nitrogen fixing power		Phosphate solubilizing power	
	LSD (0.05)	LSD (0.01)	LSD (0.05)	LSD (0.01)
Treatment (T)	**0.158**	**0.210**	**0.0047**	**0.0063**
Incubation (I)	**0.091**	**0.121**	**0.0027**	**0.0036**
Days (D)	**0.144**	**0.192**	**0.0043**	**0.0057**
T × D	**0.353**	**0.469**	**0.1060**	**0.1410**
D × I	**0.204**	**0.271**	**NS**	**NS**
T × I × D	**0.499**	**0.664**	**NS**	**NS**

Notes : A = Nitrogen fixing power of soil (Mean value of 10^{th} and 15^{th} day of incubation), B = Phosphate solubilizing power of soil (Mean value of 10^{th} and 15^{th} day of incubation), NS = Not Significant
T_0 = Control, T_1 = BHC 50% WP @ 0.25% a.i., T_2 = Phosphomidon 50 EC @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i.

Table 5: Significant relationship among different parameter of soils comprising all the stages in pot culture experiment with regression equations

Relationship between	Value of Correlation	Regression equation
No. of total bacteria (x) and N fixing bacteria (y)	0.923	0.493x - 12.805
No. of total bacteria (x) and actinomycetes (y)	0.936	0.228x - 25.117
No. of total bacteria (x) and non symbiotic N-fixing power (y)	0.976	0.031x - 5.648
No. of total bacteria (x) and total N content (y)	0.873	0.0015x + 0.497
No. of ammonifying bacteria (x) and total N content (y)	0.906	0.0018x - 0.556
No. of N-fixing bacteria (y) and actinomycetes (y)	0.830	0.379x + 34.705
No. of N-fixing bacteria (x) and average N fixing power (y)	0.937	0.0548x + 6.742
No. of N-fixing bacteria (x) and total N content (y)	0.955	0.003x + 0.535
No. of actinomycetes (x) and average N-fixing power (y)	0.902	0.116 x + 3.2
Average non-symbiotic N-fixing power (x) and total N content (y)	0.917	0.495 + 0.213

Notes : Table value of R^2 at 5% level = 0.811 and at 1% level = 0.917
T_0 = Control, T_1 = BHC 50% WP @ 0.25% a.i., T_2 = Phosphomidon 50 EC @ 0.05% a.i., T_3 = Quinalphos 25 EC @ 0.04% a.i., T_4 = Chlorpyriphos 100 EC @ 0.05% a.i., T_5 = Carbaryl 50% WP @ 0.2% a.i.

The overall fixation of nitrogen by the soils was highest after 30 days followed by that after 15, 45 and 60 days (Table 4). The variation between the amounts of nitrogen fixed after 15 days and after 45 was not significant. On an average, irrespective of stages, the soils under Phosphomidon, BHC and Quinalphos fixed significantly lower amount, as compared to control (Das and Mukherjee, 1994). The influence of the insecticides on the nitrogen fixation power of the soil (Table 4) was concurrent with the proliferation non-symbiotic nitrogen fixing bacteria (Table 3) therein. Incidentally there was an overall significant positive correlation between the number of non-symbiotic nitrogen fixing bacteria and nitrogen fixing power of soil (Table 5).

The stimulatory effect of the chlorinated insecticide, BHC; organophosphate pesticides Chlorpyriphos, Phosphomidon and Quinalphos at the earlier stage and carbamate pesticide Carbaryl at the later period finds support of earlier works by Nayek and Rao (1980). Irrespective of treatments, solubilization of phosphate by the soils was highest after 30 days followed by that after 45, 15 and 60 days. But the variation between the amount after 45 and 15 days was not significant. Again, irrespective of stages, the soils under Carbaryl and Chlorpyriphos solubilized significantly higher amount and those under Phosphomidon solubilized significantly lower amount as compared to control. Like nitrogen fixation, the influence of insecticides on the phosphate solubilizing power of soils (Table 4) was in accordance with the proliferation of phosphate solubilizing microorganisms (Table 3) under respective insecticides. Phosphate solubilizing power of the soils under Chlorpyriphos and Carbaryl was always concurrent with the proliferation of phosphate solubilizing microbes. The decrease in the phosphate solubilizing power after 30 days in the series under the above insecticides in contrast with the increase in the proliferation of phosphate solubilizers points out that the growth of phosphate solubilizers is not always indicative of that of their efficiency. The proliferation of total bacteria had a direct relationship with that of nitrogen fixing bacteria, actinomycetes, total nitrogen content and the amount of nitrogen fixed in soil (Table 5). A significant positive correlation was obtained between the preponderance of ammonifying bacteria and total nitrogen content; that of nitrogen fixing bacteria and actinomycetes, total nitrogen content and amount of nitrogen fixed. All the above relationships were linear, as were shown the regression equations in table 5.

Insecticides of different formulations behaved differently towards growth and activitities of different microorganisms in the soils under controlled laboratory conditions. At normal field rates and with short term application, the effects were generally more limited and often stimulatory. Although insecticides BHC, Phosphomidon and Quinalphos had more determining effects at the later stage; Chlorpyriphos and Carbaryl behaved differently for some days but did not pose any serious threat in relation to the growth and activities of soil microflora and consequently to the maintenance of soil health and fertility.

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