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EFFECT OF PESTICIDES ON MICROBIAL TRANSFORMATION OF SULPHUR IN SOIL

Pradip Kumar Giri[a1*], Mintu Saha[b], Murari Prasad Halder[b] and Debatosh Mukherjee[b]

[a]ICAR Niche Area of Excellence, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani- 741235, Nadia, West Bengal

[b]Department of Agricultural Chemistry & Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur- 741252, Nadia, West Bengal

[1*] Corresponding author, Email: pradipbckv@gmail.com

ABSTRACT : A pot study was conducted in the laboratory of Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India in the year 2007-2008 to investigate the effect of pesticides, on the microbial transformation of sulphur (S) in soil. Insecticide (Endosulfan), fungicide (Dithane M-45), herbicide (2,4-D) were added to the soil at their recommended doses, respectively and their effect on the proliferation and potentiality of thiosulphate oxiding bacteria, aryl sulphatase, available and total sulphur were investigated in soil. The results of the present investigation revealed that insecticide, endosulfan effectuated a significant detrimental effect on some microbiological, biochemical and chemical properties in soil whereas fungicide, dithane M-45 caused a significant detrimental influence during the later stages in spite of stimulating influence at early stages incubation period. However, herbicide, 2,4-D, brought about a beneficial influence on the microbiological, biochemical and chemical properties in soil. Among the pesticides used in the study, the performance of the herbicide was favorable in all respect.

Key words: pesticide, sulphur, soil microbiological, biochemical, chemical properties

INTRODUCTION

Sulphur (S) is an essential element for all biological systems and has been recognized as a major nutrient for optimal plant growth (Moris *et al.,* 1988). It is used by plant for synthesis of amino acid, biotin, thimate glutathione, co-enzyme–A, formation of chlorophyll, glucoside oils, disulphide and sulfyoryl groups and activation of sulphurylase. Now a days, S fertilization is an essential part of modern agriculture. It leads to increase the crude protein content of forages, oil content of oilseeds, persistence of legumes' stand and winter hardiness and drought tolerance of crop plants. It also improve quality of cereals, uniformity and quality of vegetables, control of some soil borne diseases and ultimately to higher yield.

Total S may indicate the total pool of available S in soil but it has got little value in describing short time availability of S in soil. Therefore, the element, to get available to plant, is readily metabolized in soil in a cyclic manner. There are four distinct processes organic compound decomposition, that is; microbial assimilation or immobilization of simple compounds of sulphur; oxidation of inorganic compounds such as sulphide (S^{2-}), thiosulphate ($S_2O_2^-$), sulphite (SO_3^{2-}), polythionates and elemental S; reduction of sulphate and other anions of sulphide. Apart from these abiotic factors, one of the major process to convert S in available form for plant uptake is microbial oxidation, mainly by thiosulphate oxidizing bacteria, of the unavailable element and reduced S to plant available SO_4^{2-} (Jensen *et al.,* 1995).

It is the fact that the intense external input oriented agriculture, which was part of Green Revolution Strategy of our country (India), has raised the pesticide consumption as high as 43584 ton (technical) in 2000-2001 from 2330 ton (technical) in 1950 to 2001. as high as 43584 tonne (technical) in 2000-01 from 2330 tonne (technical) in 1950. Pesticides after its application and subsequent degradation mostly accumulated within topsoil (0-10 cm) Harris and Sans, 1967), which is considered as the zone of maximum microbial activity. The pesticides, metabolized by several soil microorganisms, are involved in several transformation of nutrient elements like solubilization of phosphorus, mineralization of nitrogen as well as oxidation of sulphur (Shidhar *et al.,* 2000), through constitutive enzymes in rhizosphere and dissipated from soil slowly as the time progresses.

Therefore, it is necessary to investigate an intensive microbiological study for sulphur transformation in soil. i.e. the population of thiosulphate oxidizing bacteria, thiosulphate oxidizing potentiality and aryl sulphatage activity upon pesticide.

MATERIALS AND METHODS

Soil analysis

A laboratory based pot culture experiment was conducted with a typically gangetic alluvium (Inceptisols) soil at the Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India during 2005-2006 Surface soil samples (0-15 cm depth) from monoculture (*Kharif* rice) cultivated field were collected, air dried, ground and passed through a 80 mesh sieve The soils were analyzed for pH (1:2.5 soil-to-water) (Jackson, 1973), texture (International pipette method) (Piper, 1966), organic carbon by 1N $K_2Cr_2O_7$ solution method (Walkley and Black, 1934), CEC (Cation Exchange Capacity) by 1N NH4OAc, pH 7.0 solution method (Hesse, 1971), total nitrogen by modified Kjeldahl method (Jackson, 1973), available P_2O_5 by ammonium molybdate extractable method (Olsen et al., 1954), available K_2O by 1N NH4OAc, pH 7.0 solution method (Hanway and Heidel, 1952) and available S turbidimetrically by 0.15% $CaCl_2$ extractable method as well as total sulphur by turbidimetrically using $BaCl_2$ and gelatin, after digesting the soil sample with di-acid mixture (perchloric acid and nitric acid 1: 2 v/v) mixture (Tabatabai, 1974) (Table 1).

Pot experiment

Small earthen pots were filled with 100 g of soil and adjusted to field capacity with deionized water. Each of the four pots were treated with no pesticides (control), with endosulfan 35% EC at 0.5 kg a.i. ha^{-1}, dithane M-45 at 1.52 kg a.i. ha^{-1} and 2,4–D 38% EC at 2.5 kg a.i. ha^{-1} as their general recommendation doses for the field crops with thrice replication, using a completely randomized block design through SPSS statistical software. Irrigation was provided as required, using deionized water. The pots were then incubated at 37^0 C ± 1 upto 90 days.

Sample collection and analysis

The samples were collected from the respective pots periodically (5^{th}, 10^{th}, 15^{th}, 30^{th}, 60^{th} and 90^{th} days after incubation) and analyzed for total sulphur (S) turbidimetrically using $BaCl_2$ and gelatin, after digesting the soil sample with di-acid mixture (perchloric acid and nitric acid 1: 2 v/v) mixture; and available S by 0.15% $CaCl_2$ (extractable) turbidimetrically as described by Tabatabai (1974). Thiosulphate oxidizing bacteria were measured by serial dilution technique and pour plate technique using thiosulphate oxidizing bacteria medium as described by Pramer and Schmidt (1965). Thiosulphate oxidizing potentiality of soil was calculated as the difference between the total S content of a sterilized and non-sterilized soil (Parker and Frisk, 1953). Aryl sulfatase activity was estimated on the basis of the determination of concentration of p-nitrophenol released after incubation of soil with p-nitrophenol sulphate for 1 hour at 37^0 C temperature and measured the p-nitrophenol concentration at 400 nm by spectrophotometer (Tabatabai and Bremner, 1970).

Table 1. Physico-chemical, biochemical and microbiological properties of experimental soil

Sl. No.	Parameters	Values
A.	Physico-chemical properties	
1.	Textural class	
a.	Coarse sand (%)	44.54
b.	Fine sand (%)	20.42
c.	Silt (%)	16.82
d.	Clay (%)	18.21
2.	pH	6.82
3.	Organic carbon (mg kg^{-1})	7080
4.	Total nitrogen (mg kg^{-1})	690
5.	Available nitrogen (mg kg^{-1})	88.10
6.	Available phosphorus (mg kg^{-1})	10.62
7.	Available potassium (mg kg^{-1})	66.28
8.	Available sulphur (mg kg^{-1})	6.76
9.	Total sulphur (mg kg^{-1})	104.22
B.	Biochemical properties	
10.	Aryl sulphatase activity (n kat 100 g^{-1})	2.89
C.	Microbiological properties	
11.	Thiosulphate oxidizing bacteria (CFU × 10^3 g^{-1} dry soil)	32.8
12.	Thiosulphate oxidizing potentiality (mg of thiosulphate oxidized / 19.3 mg reduced sulphur [75 mg $Na_2S_2O_3$] / 0.15 g sucrose / g soil)	0.33

RESULTS AND DISCUSSION

Total S

The results showed that application of endosulfan resulted in a significant elevation in the level of total S in soil over the control, indicating that the extent of mineralization of organic S compounds was slower due to the detrimental effect of insecticide on proliferation of microorganism, particularly, thiosulphate oxidizing bacteria as well as their potentiality (Table 2). On the contrary, the proliferation and potentiality of the thiosulphate oxidizing bacteria was higher in soil by the application of either dithane M- 45 (fungicide) or 2,4–D (herbicide) than that of control. The growth and activities of heterotrophic microorganism, in particular, thiosulphate oxidizing bacteria was enhanced that in turn mineralized the organic matter followed by volatilization of organic and inorganic S compound to derive energy and carbon for their cellular constituent resulting lower build up of total S in soil. The significant negative (-ve) correlation between the level of total S and thiosulphate oxidizing bacteria (r = -0.98) as well as their potentiality (r = -0.99), respectively also reflects the above statements (Table 5). However, among the pesticides 2, 4-D manipulates the greatest loss followed by dithane M- 45 and endosulfan. The results substantiate the earlier reports of Balamani *et al* (1995).

Table 2. Effect of pesticides on total sulphur content (mg kg^{-1}) in soil

Treatments	Days after incubation						Mean
	5th	10th	15th	30th	60th	90th	
Control	107.8	106.2	104.5	102.4	101.8	102.6	104.2
Endosulfan	108.9	107.1	105.2	102.8	103.7	104.8	105.4
Dithane M- 45	106.3	103.8	101.5	100.9	102.0	103.1	102.9
2, 4- D	105.1	102.9	102.1	99.6	97.7	98.2	100.9
Mean	107.02	105.0	103.3	101.4	101.3	102.2	
Source	Treatment		Days		Treatment × Days		
SEm (±)	0.274		0.335		0.670		
CD (0.05)	0.782		0.958		1.916		

Available S

The influence of endosulfan resulted a significant reduction of available S in soil whereas 2, 4 -D and dithane M- 45 resulted in significant rise in the level of available S over that of control (Figure 1). The significant positive (+ ve) correlations were also obtained between available S and thiosulphate oxidizing bacteria (r = 0.98) as well as their potentiality (r = 0.98), (Table 5) respectively. The results were in the line of Bezbaruah *et al*, 1990.

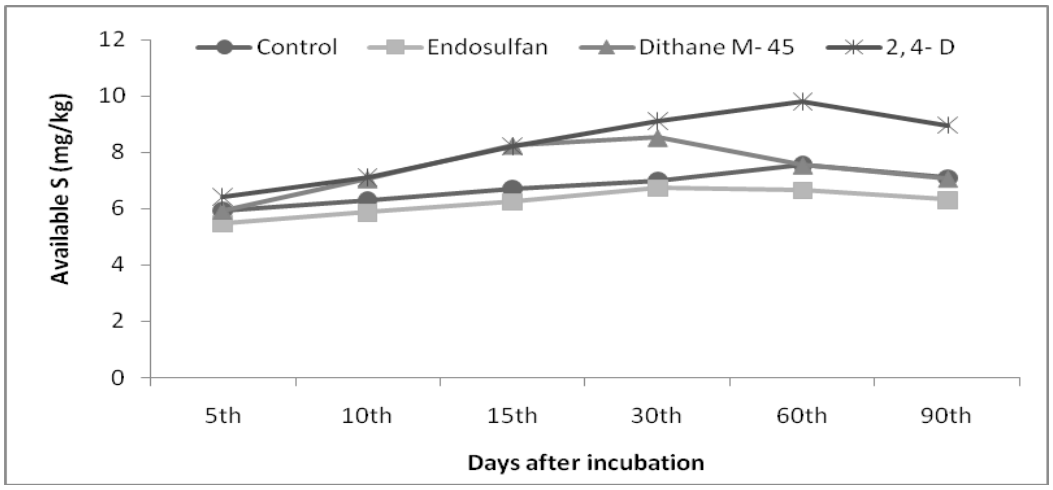

Figure 1 Effect of pesticides on available sulphur content (mg kg^{-1}) in soil at different days after incubation

Thiosulphate oxidizing bacteria

The 2,4-D and dithane M-45 a significant stimulation of thiosulphate oxidizing bacteria in soil as compared to that of control (Figure 2). The rise in the population of thiosulphate oxidizing bacteria was due to nutrient and energy sources from the pesticides in soil for their cell synthesis. Conversely endosulfan caused least proliferation of thiosulphate oxidizing bacteria in soil which was even significantly less than that of control due to the toxic effect of endosulfan and their activated compounds in soil. The reports were in the line with earlier investigation by Balamani *et al* (1995). Again in all the treatments, the population of thiosulphate oxidizing bacteria progressively increased from the 5^{th} to 60^{th} day of incubation which might be due to proto-cooperative or commensalic effect and thereafter their decrease was due to competition or amensalic effect.

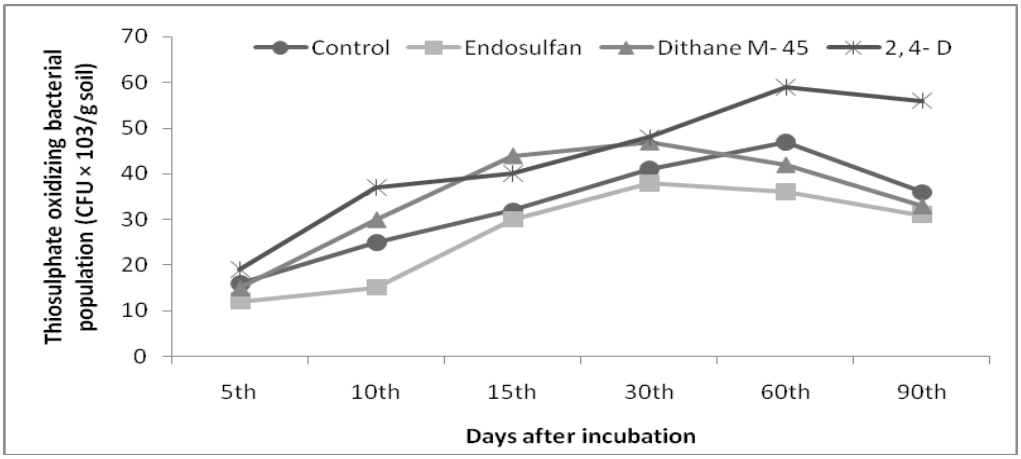

Figure 2 Effect of pesticides on the population of thiosulphate oxidizing bacteria (CFU × 10^3 g^{-1} dry soil) in soil at different days after incubation

Thiosulphate oxidizing potetiality

The endosulfan caused a significant depletion in the potentiality of total thiosulphate oxidizing bacteria as compared to the control (Table 3). The impact of endosulfan on thiosulphate oxidizing potentiality of soil was in accordance with the population of thiosulphate oxidizing bacteria in soil. The result, thus, confirms the report of Germida and Janzen (1993). On the other hand the influence of dithane M-45 and 2, 4-D caused a significant rise in thiosulphate oxidizing potentiality in soil as compared to that of control. The increase in thiosulphate oxidizing potentiality was due to higher proliferation of thiosulphate oxidizing bacteria in soil as dithane M-45 as well as 2, 4-D or their degradation product might furnish energy and nutrients to the thiosulphate oxidizers resulting higher proliferation in soil. As a consequence, there was a significant positive correlation (r = 0.99) in between the potentiality and proliferation of thiosulphate oxidizing bacteria in soil (Table 5). The results confirm the reports of Banerjee and Dey (2004).

Table 3 Effect of pesticides on the population of thiosulphate oxidizing potentiality (mg of thiosulphate oxidized / 19.3 mg reduced sulphur [75 mg $Na_2S_2O_3$] / 0.15 g sucrose / 1 g soil) in soil

Treatments	Days after incubation						Mean
	5th	10th	15th	30th	60th	90th	
Control	0.22	0.26	0.29	0.37	0.43	0.38	0.33
Endosulfan	0.17	0.23	0.27	0.33	0.29	0.26	0.26
Dithane M- 45	0.22	0.29	0.42	0.45	0.40	0.35	0.36
2, 4- D	0.25	0.31	0.36	0.49	0.57	0.53	0.42
Mean	0.22	0.27	0.34	0.41	0.42	0.38	
Source	Treatment		Days		Treatment × Days		
SEm (±)	0.004		0.004		0.009		
CD (0.05)	0.010		0.013		0.025		

Aryl sulphatase activity

Endosulfan exerted a significant decreased in aryl sulphatase activity over that of control. On the otherhand, dithane M-45, and 2, 4-D exerted significant enhancing influence on aryal sulphate activity in soil. So, effect of pesticides on aryl sulphatase activity was accordance with the proliferation of thiosulphate oxidizing bacteria in soil (Table 4). However, a significant positive correlation (r = 0.99) found between the aryl sulphatase activity and thiosulphate oxidizing bacteria which reveals thiosulphate oxidizing bacteria elaborate the enzyme aryl sulphatase and so mineralize organic S compounds in soil (Table 5). The results confirm the report of Przybulezwska *et al.* (2004).

Table 4 Effect of pesticides on aryl sulphatase activity (n kat 100 g^{-1}) in soil at different days after incubation

Treatments	Days after incubation						Mean
	5th	10th	15th	30th	60th	90th	
Control	2.34	2.52	2.81	3.07	3.46	3.16	2.89
Endosulfan	1.86	2.14	2.42	2.91	2.79	2.64	2.46
Dithane M- 45	2.36	3.01	3.39	3.41	3.14	2.97	3.05
2, 4- D	2.51	3.12	3.21	3.68	3.94	3.86	3.39
Mean	2.27	2.69	2.96	3.27	3.33	3.16	
Source	Treatment		Days		Treatment × Days		
SEm (±)	0.003		0.004		0.008		
CD (0.05)	0.010		0.012		0.024		

Table- 5. Correlation values studied among the parameters

Relationship between	Correlation coefficient (r)
Available sulphur *vs* total sulphur	-0.99
Available sulphur *vs* aryl sulfatage activity	0.97
Available sulphur *vs* thiosulphate oxidizing potentiality	0.98
Available sulphur *vs* thiosulphate oxidizing bacteria	0.98
Total sulphur *vs* aryl sulfatage activity	-0.96
Total sulphur *vs* thiosulphate oxidizing potentiality	-0.97
Total sulphur *vs* thiosulphate oxidizing bacteria	-0.99
Aryl sulfatage activity *vs* thiosulphate oxidizing potentiality	0.99
Aryl sulfatage activity *vs* thiosulphate oxidizing bacteria	0.98
Thiosulphate oxidizing bacteria *vs* thiosulphate oxidizing potentiality	0.99

*table value for 'r' = 0.95 at 5% level for 2 degrees of freedom

CONCLUSION

The Green Revolution of India primarily based on high value inputs like pesticides and fertilizers. The application of these inputs especially pesticides, may create a great problem on beneficial soil micro-organisms and microbial transformation of several primary and secondary nutrients. In our pot culture study, it was observed that the pesticides like endosulfan, dithane M-45 served a detrimental effect on transformation of S, whereas 2,4-D created a favorable beneficial effect on S transformation in soil environment. Although India produces 233 million tonnes (2009-10) of food grains per year at present, but our foodgrain production was stagnant from the last decades (Agriculture Survey by Hindu, 2010). But the foodgrain requirement of India by 2025 would be 320 million tons (high demand scenario) and 308 million tons (low demand scenario) (Kumar et al, 2005). So it is the right time to aware about the all agricultural inputs to use them properly and appropriately to maintain and sustain soil quality and health resulting sustainable agriculture in India.

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