

Full Length Research Paper

Pesticides jiggling microbial transformation of phosphorus in soil

Mousumi Ghosh¹, Niladri Paul^{2*}, Suprakash Das¹, Prasanta Kumar Patra¹, Murari Prasad Halder¹ and Debatosh Mukherjee¹

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

²C/o Nepal Paul, Town Bordowali, Mantri Bari Road Extension, P.O. - Agartala, West Tripura. PIN - 799001, India.

Accepted 30 January, 2014

A laboratory based pot culture experiment was conducted to investigate the impact of pesticides [endosulfan, dithan-M-45 and 2,4-dichlorophenoxy acetic acid (2,4-D)] at their recommended doses on the changes in microbial biomass carbon; potentiality and proliferation of phosphorus solubilizing organisms; acid and alkaline phosphatase activities; total and available phosphorus in soil. The results of the investigation reveal that though each of the pesticides exerted deleterious effect on phosphorus solubilizing power of soil in the beginning, the overall impact was stimulatory rather than detrimental to the growth and activity of microorganisms associated with the phosphorus transformation in soil. Application of 2,4-D affected the highest level of microbial biomass carbon, growth and activity of phosphorus solubilizing microorganisms and phosphatase activity which was translated to the highest level of available phosphorus in soil. On the other hand, endosulfan brought about the least increment in the level of microbial biomass carbon; potentiality and proliferation of phosphorus solubilizing organisms; acid and alkaline phosphatase activities besides total and available phosphorus in soil. The results of the investigation reveal the safe use of each of the pesticides in the field for the eradication of pest, especially 2,4-D.

Key words: Pesticides, microbial biomass carbon, growth and activity of phosphorus solubilizing microorganisms, p-solubilizing power, phosphatases, total and available phosphate.

INTRODUCTION

Pesticides are chemicals that wipe out the biological agents responsible for the loss of economic crops. Under the umbrella of pesticides viz. insecticides, fungicides and herbicides are designed to control insects, fungi and weeds, respectively. The ultimate destination of pesticides applied directly or as spray on the foliage is the soil ecosystem comprising inanimate and living components. Soil ecosystem gets distributed by the introduction of pesticides.

Pesticides sometimes exert detrimental influence on the growth and activity of microorganisms involved in various biochemical transformations in soil which results

in microbial population decline (Yaduraju et al., 2006), but, some microorganisms, having the capacity to withstand the detrimental influence of pesticides, survive. The population of those organisms rises by deriving energy and nutrients from the dead cells generated by the action of pesticides (Giri et al., 2006).

On the other hand, there are pesticides exerting stimulation (Samanta et al., 2005) by serving as nutrient and energy sources for the microorganisms. So, the population density of pesticide utilizers increases so long as source of energy and nutrients are there with a concomitant reduction in the active ingredients of pesticide

residue (Debnath et al., 2002; Nongthombam et al., 2008). Phosphorus transformation in soil is very important, as it is one of the major essential nutrients. But, soil phosphorus is predominantly insoluble and so unavailable to plants. Plants only make use of 10 to 12 per cent of the applied phosphatic fertilizers. The rest become unavailable through biotic and abiotic process. The unavailable inorganic forms of phosphorus are tricalcium phosphate in addition to iron and aluminium phosphates depending upon soil pH (Hue et al., 1986; Khan et al., 2009). Although, there are microbes capable of solubilizing insoluble inorganic compounds in soil. Among them, the important ones are *Pseudomonas*, *Bacillus*, *Micrococcus*, *Mycobacterium*, *Penicillium*, *Fusarium* and *Aspergillus* (Gaur, 2006). They make a large portion of the insoluble inorganic phosphorus soluble and the quantities in excess of their nutritional demand become available for the utilization of plants. Many of them are also capable of mineralizing organic phosphorus compounds by elaborating the enzymes-phosphatases and phytases (Tarafdar et al., 2002; Aseri et al., 2009; Ramesh et al., 2011). Crops also bring out soil enzymes in order to meet their nutritional demand.

There exists conflicting reports regarding the effects of pesticides on the growth and potentiality of phosphorus solubilizing microorganisms in soil. Das et al. (2012) found a significant enhancement in the potentiality and growth of phosphate solubilizing microorganisms by the application of pesticides in soil. On the other hand, there are reports about the detrimental influence of pesticides on the growth and performance of phosphorus solubilizers (Das et al., 1998). Bibliographical antecedence speaks about the differential influence of pesticides on the mineralization of organic phosphorus in soil. In this connection, Brown and Lean (1995) found the negative aspect of pesticides on the mineralization of organic phosphorus in soil, while Das and Mukherjee (1994) observed the positive aspect, with an increase in extractable phosphorus in soil. Chemo-organotropic microorganisms through enzymes mediate the process of organic phosphorus mineralization. The contribution of acid and alkaline phosphatase is worth mentioning in this respect. However, pesticides exert differential influence on acid and alkaline phosphatase activity in soil. A group of workers advocate positive effect (Tu, 1992; Rahmansyah et al., 2009). On the contrary, others put forward negative effect (Abul et al., 2002; Jastrzebska, 2011). The above revelation reflects a great diversity in phosphorus transformation under the influence of pesticides in soil. Hence, an attempt was made to investigate the effect of pesticides on some chemical, biochemical and microbiological properties in relation to phosphorus transformations in soil.

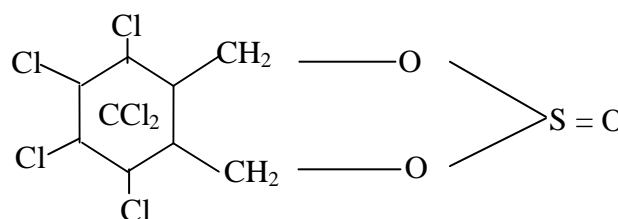
MATERIALS AND METHODS

The investigation was carried out with earthen pots in the laboratory

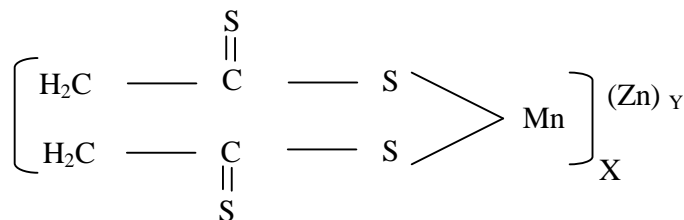
of the Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India.

Description of pesticides

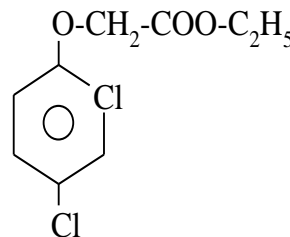
Endosulfan (Insecticide): IUPAC name- 1,2,3,4,7,7-hexachlorobicyclo(2,2,1)-heptane(2)-bis (oxymethylene)- 5,6 sulphite. Molecular name- $C_9H_6O_3Cl_6S$.



Dithane M - 45 (Fungicide): IUPAC name- Manganese-zinc, double salt of N-N-ethylene bis dithiocarbamic acid.



2, 4-D ethyl ester (Herbicide): IUPAC name- 2,4-dichlorophenoxy acetic acid. Molecular formula: $C_{10}H_{12}Cl_2O_3$.



Collection of soil

Typical gangetic alluvium (inceptisol) soil was collected from the University Instructional Farm. Surface soil samples (0-15 cm depth) gathered from monoculture (Kharif rice) cultivated field were air dried and passed through 80 mesh sieve. The physico-chemical and microbiological properties of soil are delineated hereunder in Table 1.

Experimental

100 g of soil was thoroughly mixed with urea, single super phosphate and muriate of potash at the rate of 100 Kg N, 50 Kg P_2O_5 and 50 kg K_2O ha^{-1} , respectively and placed in small earthen pots. Three pots were kept as such to be considered as triplicate control (without any pesticide). In the rest pots, each of the three pesticides endosulfan (35 EC) at 2.0 kg a.i. ha^{-1} ; dithane-M 45 at 1.5 kg a.i. ha^{-1} and 2,4-D (38 EC) at 1.0 kg a.i. ha^{-1} were again

Table 1. Physico-chemical and microbiological properties of soil.

Soil characteristic	Status
Type of soil	Alluvial
Soil taxonomy	Typic <i>haplustepts</i>
Coarse sand (%)	44.5
Fine sand (%)	20.4
Silt (%)	16.8
Clay (%)	18.2
Soil p ^H (1: 2.5 w/v) in water	6.8
Organic carbon (g kg ⁻¹)	7.1
Total nitrogen (g kg ⁻¹)	0.7
Available phosphorus (mg kg ⁻¹)	11.7
Available potassium (mg kg ⁻¹)	44.0
Microbial biomass carbon (µg g ⁻¹)	138.2
Phosphorus solubilizers (cfu × 10 ⁵ g ⁻¹ dry soil)	15.2
Total Phosphorus (Kg P ₂ O ₅ ha ⁻¹)	536.7
Phosphate solubilizing power (mg 15 mg ⁻¹ insoluble P)	0.4
Acid phosphatase activity (nKat 100 g ⁻¹ soil)	7.4
Alkaline phosphatase activity (nKat 100 g ⁻¹ soil)	13.5

blended separately at three replications. The moisture level of soil for each pot was adjusted to 50% of water holding capacity of soil by compensating the loss in weight with deionized water on every alternate day. Thereafter, each pot was incubated at 37 ± 1°C for 90 days.

Sample collection and analysis

Following the method of Das et al. (2012), soil samples were collected from each of the respective pot on the 5th, 10th, 15th, 30th, 60th and 90th days of incubation. Soil moisture was measured immediately before the analysis of microbiological, biochemical and chemical transformations.

Microbial biomass carbon was determined by chloroform fumigation following the method of Joergensen (1995). There were two sets of moist soil- one set in conical flasks fitted with a stopper and the other set in beakers. The beakers containing soils were subjected to chloroform fumigation till the chloroform boiled in vacuum desiccator for two minutes. The desiccator was then incubated at 25°C for 24 h. Both the fumigated and non-fumigated soils were extracted with 0.5M K₂SO₄ and the extracts were analysed by potassium dichromate oxidation method (Jenkinson and Powlson, 1976). The difference in the status of organic carbon between the fumigated and non-fumigated soils, divided by a calibration factor (K_{ec} = 0.38) represented the measure of microbial biomass carbon in soil which was expressed as µg microbial biomass C g⁻¹ dry soil.

Colony forming units (cfu) of phosphorus solubilizing microorganisms were enumerated by serial dilution and pour plate technique (Pramer and Schmidt, 1965) in Pikovskaia medium (1948). Phosphorus solubilizing power was determined by the estimation of soluble P in 15ml of sucrose tri-calcium phosphate broth containing 1% sucrose after incubation of 1 gm soil in culture tubes at 30±1°C for 15 days (Das and Mukherjee, 2000). Acid and alkaline phosphatase activities were determined following the procedure of Tabatabai and Bremner (1969).

Total phosphorus in soil was extracted by Bowman (1988) and estimated following modified ascorbic acid method (Dick and Tabatabai, 1977). Water-soluble phosphorus in soil was extracted

in sodium bicarbonate (Jackson, 1973) and estimated through Olsen and Dean (1982).

Statistical analysis

The results were adjudicated by analysis of variance (ANOVA), and the statistical significance (P=0.05) of difference between means within factors (pesticides and incubation time) was determined by using completely randomized design following the method of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Microbial biomass carbon (MBC)

Application of pesticides- endosulfan, dithane M-45 and 2,4-D- resulted in a significant boost in the level of microbial biomass carbon over that of untreated control in soil from 5th to 90th days of incubation (Table 2). The extent of increment, however, differed depending on the type of pesticide. The herbicide 2,4-D generated MBC to the highest level (El-Ghamry et al., 2001) ranging from 2.7 to 5.9% over that of untreated control; while the minimum increment varying from 0.6 to 2.6% was brought about by endosulfan. However, dithane M-45 affected the increment ranging from 0.4 to 3.2% over that of control. The hike in MBC might be due to the utilization of pesticides as energy and nutrient sources by microbial community for MBC synthesis (Kumar et al., 2012). In addition, the pesticides could impose resistant microbial community through mutation (Daly et al., 1998) to spread their generations at the expense of dead susceptible organisms through energy and nutrient sources for additional microbial biomass carbon synthesis.

Table 2. Effect of pesticides on microbial biomass carbon ($\mu\text{g g}^{-1}$ soil) content of the soil during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	130.43±0.02	123.43±0.03	121.63±0.04	105.68±0.0058	99.28±0.01	95.79±0.02	112.71
Endosulfan	132.91±0.0058	124.08±0.0058	123.69±0.01	108.48±0.0058	100.29±0.01	97.63±0.01	114.52
Dithane-M-45	134.62±0.01	126.08±0.04	124.15±0.02	108.98±0.03	99.78±0.0058	96.24±0.0058	114.98
2,4-D	138.15±0.0058	128.63±0.0058	124.86±0.5777	113.6±0.05	103.79±0.1	100.11±0.02	118.19
Mean	134.03	125.57	123.58	109.19	100.78	97.44	

Anova (p value at 0.05): Treatment (Tr) 0.082, Days (D) 0.099, Interaction (Tr X D) 0.199. Mean of three replicates \pm SD.

Table 3. Effect of pesticides on the number of phosphate solubilizing micro-organisms in the soil during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	32 \pm 1	49 \pm 3	73 \pm 0.58	109 \pm 1	46 \pm 0.58	35 \pm 0.58	57.56
Endosulfan	37 \pm 1	55 \pm 4	78 \pm 3	113 \pm 0.58	49 \pm 1	41 \pm 5	62.1
Dithane-M-45	55 \pm 3	62 \pm 1.01	84 \pm 3	119 \pm 0.58	56 \pm 1	48 \pm 0.58	70.78
2,4-D	62 \pm 0.58	74 \pm 3	98 \pm 3	128 \pm 3	62 \pm 0.58	48 \pm 7	78.78
Mean	46.83	60	83.33	117.25	53.42	43.25	

Anova (p value at 0.05): Treatment (Tr) 2.005, Days(D) 1.680, interaction (Tr X D) 4.113. Means of three replications \pm SD.

P-solubilizing micro-organisms

The chronological enhancing influence of 2,4-D as well as dithane M-45 and to a great extent endosulfan on phosphorus solubilizing microorganisms was almost similar to that of MBC and justifies that phosphorus solubilizers are fractional constituents of microbial community activity involved in biochemical transformation of phosphorus in spite of the influence of pesticides. Interestingly, there was a positive correlation ($r = 0.955$) between MBC and phosphorus solubilizing microorganisms in soil (Table 3). Although, the intensity of pesticidal impact on phosphorus solubilizing microorganisms was much higher than MBC.

Moreover, there was a rhythmic periodicity in the increment of microbial community during the incubation period. 2,4-D and dithane M-45 caused gradual declination in significant ($p = 0.05$) rise from 93.8 to 17.4% and 71.9 to 9.2%, respectively during initial 30 days of incubation. Then, the population density of phosphorus solubilizers increased progressively from 21.7 to 37.1% and 34.8 to 37.1% by the application of herbicide and fungicide respectively in last two successive stages of sampling. This proves that phosphorus solubilizers were zymogenous (Winogradsky, 1924) organisms flourished by the availability of organic substrates and they decline gradually by reduction of the substrate; while the progressive increment was due to multiplication of the

mutant ones (Kalyanasundaram and Kabita, 2012) or resistant ones propagated through the provision of energy and nutrient sources from the dead susceptible cells (Giri et al., 2006). The change was more pronounced with the insecticide - endosulfan- in bringing about a gradual decline in significant rise from 15.6% on the 5th day to 6.9% on the 15th day. Then, there was significant rise of P solubilizers by 17.1% over control on the 90th day following non-significant influence from 30th to 60th day of incubation.

Acid-alkaline phosphatase activities

Acid phosphatase activity remained non-significant by the application of each pesticide in soil up to 10 days of incubation (Table 4). On the other hand, endosulfan had non-significant influence on alkaline phosphatase activity from the beginning up to 30 days of incubation (Table 5). The non-significant influence pointed out the stationary phase or adaptive phase for the elaboration of the phosphatase enzyme. Then concurrent with the growth of phosphorus solubilizing microorganisms, pesticides, in general, and the herbicide, in particular, resulted in a significant boost in acid phosphatase activity over that of control in soil from 15th to 90th day of incubation; while dithane M-45 and 2,4-D caused a significant rise in alkaline phosphatase activity from 5th to 90th day of

Table 4. Effect of pesticides on the activity of acid phosphatase enzyme in the soil (nKat) during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	7.90 ± 0.02	8.12 ± 0.026	9.09 ± 0.0057	9.92 ± 0.015	10.98 ± 0.06	11.20 ± 0.006	9.54
Endosulfan	7.90 ± 0.006	8.12 ± 0.01	9.22 ± 0.095	9.99 ± 0.0152	11.41 ± 0.01	11.78 ± 0.175	9.74
Dithane-M-45	7.91 ± 0.006	8.13 ± 0.015	9.41 ± 0.025	10.00 ± 0.021	11.01 ± 0.04	11.31 ± 0.502	9.63
2,4-D	7.92 ± 0.015	8.15 ± 0.055	9.5 ± 0.006	10.93 ± 0.072	12.92 ± 0.01	13.16 ± 0.01	10.43
Mean	7.91	8.13	9.3	10.21	11.58	11.86	

Anova (p value at 0.05): Treatment (Tr) 0.037, Days (D) 0.031, Interaction (Tr X D) 0.074. Mean of three replicates ± SD.

Table 5. Effect of pesticides on the activity of alkaline phosphatase enzyme in the soil (nKat) during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	11.64 ± 0.06	11.82 ± 0.006	12.03 ± 0.015	13.91 ± 0.006	16.41 ± 0.006	17.03 ± 0.006	13.81
Endosulfan	11.88 ± 0.06	12.03 ± 0.006	12.24 ± 0.1	14.12 ± 0.01	17.00 ± 0.012	17.43 ± 0.04	14.12
Dithane-M-45	12.04 ± 0.05	12.53 ± 0.513	13.68 ± 0.02	16.43 ± 0.01	18.47 ± 0.05	19.87 ± 0.017	15.5
2,4-D	12.3 ± 0.006	12.8 ± 0.01	14.5 ± 0.01	16.39 ± 0.006	18.31 ± 0.01	19.21 ± 0.006	15.59
Mean	11.97	12.3	13.11	15.21	17.55	18.39	

Anova (p value at 0.05): Treatment (Tr) 0.102, Days (D) 0.125, Interaction (Tr X D) 0.253. Mean of three replicates ± SD.

incubation and endosulfan only on the 60th day of incubation. This may unfold the truth that phosphorus solubilizing microorganisms not only solubilize insoluble inorganic phosphorus compounds but also mineralize organic phosphorus through acid and alkaline phosphatase activities (Khan et al., 2009). However, 2,4-D caused a progressive increment from 4.5% on the 15th day to 17.7% on the 60th day and then 17.5% on the 90th day as compared to that of control. The corresponding figures for endosulfan on the 15th and then from 60th to 90th day were 1.4% and 3.9 to 5.2% respectively while those for dithane M-45 were 3.5% and 0.8 to 1.0%, respectively. In the case of alkaline phosphatase activity, 2,4-D resulted in a significant increase ($p = 0.05$) from 5.7% on the 5th day to 20.5% on the 15th day; then a gradual reduction up to 11.6% on the 60th day followed by 12.8% increase on the 90th day over that of untreated control.

On the other hand, dithane M-45 resulted in a significant progressive rise from 3.4% on the 5th day to 18.1% on the 30th day; then a diminution by 12.6% on the 60th day followed by an increase of 16.7% on the 90th day. However, endosulfan resulted in a significant increment of 3.6% in alkaline phosphates activity only on the 60th day as compared to that of control.

Phosphorus solubilizing power

Each of the pesticides induced an initial significant detrimental influence on the potentiality of phosphorus

solubilizing microorganisms in soil up to 10th day of incubation as compared to that of control (Koley and Dey, 1989). The ferocity of pesticidal impact on phosphorus solubilizing power, though declined from 5th to 10th day, was most fierce (55.3 to 45.3%) in endosulfan followed by dithane M-45 (48.9 to 41.5%) and 2,4-D (40.4 to 32.1%) respectively as compared to that of untreated control (Table 6). The impaired effect was due to greater utilization of solubilized insoluble phosphate by phosphate solubilizers than that of their capacity of solubilization of tricalcium phosphate. This is also an indication of higher induction on multiplication than the efficiency of phosphorus solubilizers by the pesticides in soil (Das et al., 1998).

However, the harmful effect did not last long and similar to the proliferation of P-solubilizing microorganisms, application of the herbicide and fungicide exerted significant stimulation on phosphorus solubilizing capacity of soil from 15th to 90th day of incubation which, in turns, gradually reduced from 110% and 80 to 9.1 and 10.2%, respectively, as compared to that of untreated control in soil (Table 6). Endosulfan also significantly enhanced the efficiency of phosphorus solubilizing organisms by 43.3 and 8.6% on the 15th and 60th day, respectively over that of control (Kukreja et al., 2010). A cursory glance at the results reveal that though pesticides brought about gruesome influence in the beginning on phosphorus solubilizing power in soil, the horror subsided very soon and in accordance with the chronological sequence of phosphate solubilizers. 2,4-D augmented the efficiency of

Table 6. Effect of pesticides on phosphate solubilising capacity of the soil (mg/15 g insoluble P per g soil per 0.15g sucrose) during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	0.047 ± 0.0006	0.053 ± 0.004	0.03 ± 0.0006	0.07 ± 0.03	0.081 ± 0.002	0.088 ± 0.005	0.062
Endosulfan	0.021 ± 0.01	0.029 ± 0.002	0.043 ± 0.0006	0.07 ± 0.006	0.088 ± 0.01	0.089 ± 0.0006	0.057
Dithane-M-45	0.024 ± 0.003	0.031 ± 0.0006	0.054 ± 0.0006	0.082 ± 0.0006	0.094 ± 0.003	0.097 ± 0.002	0.064
2,4-D	0.028 ± 0.002	0.036 ± 0.005	0.063 ± 0.007	0.088 ± 0.02	0.092 ± 0.001	0.096 ± 0.0006	0.067
Mean	0.03	0.037	0.048	0.077	0.089	0.093	

Anova (p value at 0.05): Treatment (Tr) 0.0028, Days (D) 0.0028, Interaction (Tr X D) 0.0057. Mean of three replicates ± SD.

Table 7. Effect of pesticides on the level of total phosphorus in soil (Kg P₂O₅ ha⁻¹).

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	534.00 ± 2.65	530.67 ± 0.58	529.67 ± 1.15	528.00 ± 1.0	525.33 ± 1.53	523.67 ± 5.69	528.56
Endosulfan	535.33 ± 0.58	532.67 ± 0.58	530.67 ± 1.15	529.00 ± 0.01	526.00 ± 1.0	522.33 ± 1.15	529.33
Dithane-M-45	536.00 ± 4.0	533.00 ± 3.0	530.67 ± 0.58	529.67 ± 0.58	526.67 ± 1.53	524.33 ± 0.58	530.06
2,4-D	536.67 ± 0.58	534.00 ± 1.0	532.33 ± 3.21	530.67 ± 0.58	527.33 ± 2.08	524.67 ± 1.53	530.94

Anova (p value at 0.05): Treatment (Tr) 1.339, days (D) 1.637, interaction (Tr X D) NS. Mean of three replicates ± SD.

Table 8. Effect of pesticides on the available phosphorus content (mg.kg⁻¹) in the soil during different days of incubation.

Treatment	Days of incubation						Mean
	5	10	15	30	60	90	
Control	72.34 ± 0.07	81.3 ± 0.006	89.25 ± 0.01	103.53 ± 0.021	98.61 ± 0.051	71.43 ± 0.012	86.08
Endosulfan	74.39 ± 0.053	82.6 ± 0.006	90.45 ± 0.006	105.51 ± 0.10	99.72 ± 0.08	74.36 ± 0.04	87.84
Dithane-M-45	75.1 ± 0	83.11 ± 0.01	92.12 ± 0.02	112.66 ± 0.36	100.83 ± 0.125	75.00 ± 0.017	89.80
2,4-D	76.33 ± 0.006	85.12 ± 0.04	94.23 ± 0.006	116.07 ± 0.071	102.11 ± 0.12	78.13 ± 0.006	92.00
Mean	74.54	83.03	91.15	109.44	100.32	74.73	

Anova (p value at 0.05): Treatment (Tr) 0.060, days (D) 0.073, interaction (Tr X D) 0.148. Mean of three replicates ± SD.

phosphorus solubilizers to the highest extent followed by dithane M-45 and endosulfan, respectively.

Total and available phosphorus

Application of insecticide, fungicide and herbicide did not render any significant influence on the level of total phosphorus in soil as compared to that of control nor was there significant difference among the pesticides at any stage of sampling (Table 7). The results thus reflect virtually negligible loss of total phosphorus by biotic or abiotic means. However, each of the pesticides resulted in a significant enhancement in the level of available phosphorus over that of control from the 5th to 90th day of incubation in soil (Table 8). 2,4-D caused significant (p = 0.05) increase ranging from 4.7% to 10.0% in between 5th

to 90th day of incubation over that of control. The corresponding figures for dithane M-45 and endosulfan were 2.2 to 6.8% and 1.1 to 4.1%, respectively. Using pesticides as nutrient and energy source (Paul et al., 2010), the higher growth and activity of P-solubilizing micro-organisms as well as greater acid and alkaline phosphatase activity affected greater solubilization of insoluble inorganic phosphate compounds as well as mineralization of organic P to a larger extent. This in turn was translated to the enhancement in P availability of soil (Das and Debnath, 2006; Das et al., 2012). Even the microbial phosphorus may provide addendum to P availability in soil after mineralization of dead zymogenous organisms (Tiessen et al., 2011).

Each of the pesticide- 2,4-D, dithane M-45 and endosulfan- at their field recommended doses caused a significant acceleration in solubilization and mineraliza-

tion of insoluble inorganic and organic P compounds in spite of detrimental influence on the potentiality of P-solubilizing micro-organisms in the beginning. As a consequence, there was higher retention of available P in soil. So, all of the pesticides are not only safe but also rejuvenator of mobile P in soil ecosystem. Among the pesticides, 2,4-D performance is preeminent in all virtues.

REFERENCES

Abul K, Mukherjee AK, Kalam A (2002). Effects of hexaconazole, tropiconazole, profenofos and pretilachlor on soil phosphatase and dehydrogenase activities. *Pestic. Res. J.* 14:337-342.

Aseri GK, Jain N, Tarafdar JC (2009). Hydrolysis of Organic Phosphate Forms by Phosphatases and Phytase Producing Fungi of Arid and Semi Arid Soils of India. *Am. Eurasian J. Agric. Environ. Sci.* 5:564-570.

Bowman RA (1988). A rapid method to determine total phosphorus in soils. *Soil Sci. Soc. Am. J.* 52:1301-1304.

Brown LS, Lean DRS (1995). Toxicity of selected pesticides to lake phytoplankton measured using photosynthetic inhibition compared to maximal uptake rates of phosphate and ammonium. *Environ. Toxicol. Chem.* 14:93-98.

Daly H, Doyen JT, Purcell AH III (1998). Introduction to insect biology and diversity, 2nd edition. Oxford University Press. New York.

Das AC, Debnath A (2006). Effect of systemic herbicides on N₂ fixing and phosphate solubilizing microorganisms in relation to availability of nitrogen and phosphorus in paddy soils of West Bengal. *Chemosphere* 65:1082-1086.

Das AC, Mukherjee D (1994). Effect of insecticides on the availability of nutrients, nitrogen fixing and phosphate solubility in the rhizosphere soil of rice. *Biol. Fertil. Soils* 18:37-41.

Das AC, Mukherjee D (2000). Influence of insecticides on microbial transformation of nitrogen and phosphorus in Typic Orchragualf soil. *J. Agric. Food Chem.* 48:3728-3732.

Das AC, Nayek H, Chakravarty A (2012). Soil application of dinitroaniline and arylphenoxy propionic herbicides influences the activities of phosphate solubilising micro-organisms in soil. *Environ. Monit. Assess.* 184:7453-7459.

Das AC, Saha SK, Chakravarty A, Mukherjee D (1998). Effect of Insecticides on the microbial populations and their biochemical activities as influenced by organic matter in a Typic fluvaquent soil. *Indian Agric.* 42:1-12.

Debnath A, Das AC, Mukherjee D (2002). Rhizosphere effect of herbicides on nitrogen fixing bacteria in relation to availability of nitrogen in rice soil. *J. Indian Soc. Soil Sci.* 50:463-466.

Dick WA, Tabatabai MA (1977). Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. *J. Environ. Qual.* 6:82-85.

El-Ghamry AM, Huang CY, Xu JM (2001). Combined effect of two sulfonylurea herbicides on soil microbial biomass and N mineralization. *J. Environ. Sci.* 13:311-317.

Gaur AC (2006). Biofertilizers in Sustainable Agriculture. Indian Council of Agricultural Research. New Delhi.

Giri PK, Samanta A, Das S, Ghosh T, Halder M, Mukherjee D (2006). Persistence of pesticides on the proliferation and potentialities of nitrogen fixing micro-organisms in soil. *J. Interac.* 10:349 - 353.

Gomez KA, Gomez AA (1984). Statistical procedures for Agricultural Research. John Wiley and sons. New York.

Hue NV, Craddock GR, Adams F (1986). Effect of organic acids on aluminium toxicity in subsoils. *J. Soil Sci. Soc. Am.* 50:28-34.

Jackson ML (1973). Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Jastrzebska E (2011). The effect of Chloropyrifos and Teflubenzuron on the enzymatic activities of soil. *Pol. J. Environ. Stress* 20:903-910.

Jenkinson DS, Powlson DS (1976). The effects of biocidal treatments on metabolism in soil. Fumigation with chloroform. *Soil Biol. Biochem.* 8:167-177.

Joergensen RG (1995). Microbial biomass. In: methods in applied microbiology and biochemistry (Alef, K and Nannipieri, P., eds). Academic press.

Kalyanasundaram D, Kavitha S (2012). Effect of Butachlor on the Microbial Population of Direct Sown Rice. *World Academy of Science, Engineering and Technology* 69:853-855.

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed R (2009). Phosphorus Solubilizing acteria: Occurrence, Mechanisms and their Role in Crop Production. *J. Agric. Biol. Sci.* 1:48-58.

Koley SC, Dey BK (1989). Effect of aromatic amine herbicides on microbial population and phosphate solubilizing power of the rhizosphere soil of groundnut, *Indian Agric.* 33:1-8.

Kukreja GP, Bhute SS, Mangate SA, Dhawale MN (2010). Exploring the Potential of Species as Phosphate Solubilizer, Plant Growth Promoter, Biocontrol Agent and Pesticide Degrader. *Asian J. Exp. Biol. Sci.* 40-44.

Kumar A, Nayak AK, Shukla AK, Panda BB, Raja R, Shahid M, Tripathi R, Mohanty S, Rath PC (2012). Microbial Biomass and Carbon Mineralization in Agricultural Soils as Affected by Pesticide Addition. *Bull. Environ. Contam. Toxic.* 88:538-542.

Nongthombam S, Nayek H, Das AC (2008). Effect of anilofos and pendimethalin herbicides on N₂-fixing and phosphate solubilizing microorganisms in relation to availability of nitrogen and phosphorus in a Typic Haplustep soil. *J. Crop Weed* 4:1-6.

Olsen SR, Dean LA (1982). Phosphorus. In C.A Black, D.P. Evans, J.L. White, L.E. Ensminger, F.E. Clark, & R.C. Diahnauer (Eds.) *Methods of soil analysis.* Madison: American Society of Agronomy. pp. 1035-1049.

Paul N, Ghosh M, Das DK, Sur P, Mukherjee D (2010). Pesticidal impact on DTPA extractable Iron vis-à-vis actinomycetes in soil. *India Agric.* 54(1&2):83-86

Pikovskaia RI (1948). Mobilization of phosphates in soil in connection with the vital activation of some microbial species. *Microbial Biol.* 17: 362-370.

Pramer D, Schmidt EL (1965). *Experimental Soil microbiology*, Burgess Publishing Co. Minneapolis. 5, Minn.

Rahmansyah M, Antonius S, Sulistinah N (2009). Phosphatase and urease instability caused by pesticides present in soil improved by grounded rice straw. *ARPN J. Agric. Biol. Sci.* 4:56-62.

Ramesh A, Sharma SK, Joshi OP, Khan IR (2011). Phytase, Phosphatase Activity and P-Nutrition of Soybean as Influenced by Inoculation of Bacillus. *Ind. J. Microbiol.* 51:94-99.

Samanta A, Ghosh S, Halder M, Mukherjee D, Ghosh RK (2005). Influence of herbicides on microorganisms and transformation of iron in rice rhizosphere soil. *J. Inter.* 9:528-532.

Tabatabai MA, Bremner JM (1969). Use of P-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1:301-307.

Tarafdar JC, Yadav RS, Niwas R (2002). Relative efficiency of fungal intra- and extracellular phosphatase and phytase. *J. Plant Nutr. Soil Sci.* 165:17-19.

Tiessen H, Ballester MV, Salcedo I (2011) Phosphorus and global change. In: Bunemann EK, Oberson A, Frossard E (eds) *Phosphorus in action: biological processes in soil phosphorus cycling.* *Soil Biol.* 26:9-18.

Tu CM (1992). Effect of three newer pesticides on microbial and enzymatic activities of soil. *Bull. Environ. Contam. Toxic.* 49:120-128.

Winogradsky S (1924). Sur la microflora autochtone de la terre arable. *Compt. Rend. Acad. Sci.* 178:1236-1239.

Yaduraju NT (2006). Herbicide resistant crops in weed management. In: *The Extended Summaries, Golden Jubilee National Symposium on Conservation Agriculture and Environment.* Oct., 26-28, Banaras Hindu University, Banaras. pp. 297-98.