

Microbial phosphate solubility as influenced by pyrethroid insecticide in tea soil of the Himalayan Terai region of West Bengal

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ABSTRACT

An experiment was conducted under laboratory conditions to investigate the effect of four pyrethroid insecticides, viz. cypermethrin, deltamethrin, fenvalerate and permethrin at rates of 225, 200, 225 and 210 g a.i. ha⁻¹, respectively, on growth and activities of microorganisms, phosphate solubility and its availability in tea soil of West Bengal, India. Insecticides in general, had a deleterious effect on bacteria and actinomycetes, being the highest with fenvalerate (11%) followed by permethrin (10.3%) for bacteria and with permethrin (13.9%) for actinomycetes. Fungi propagules were highly increased with permethrin (51%) followed by fenvalerate (32.6%). Cypermethrin and deltamethrin had no significant effect on the proliferation of microorganisms, excepting fungi with deltamethrin. Phosphate solubilizing capacity of the soil was badly affected with fenvalerate (16.3%) and permethrin (15.2%). Application of insecticides had a meager positive effect on the retention of total phosphorus, while availability of soluble phosphorus remained unchanged in the treated soils.

Keywords: Pyrethroid insecticides, soil microorganisms, phosphate solubilization, available phosphorus, tea soil

Phosphorus is an important metabolic and structural constituent as well as energy transforming element for cellular metabolism of soil microflora and crop plants. It is second only to nitrogen as an essential nutrient required by plants and microorganisms, and occupies a critical position both in plant growth and in the biology of soil. Microorganisms bring about a number of transformations of the element such as altering the solubility of inorganic compounds of phosphorus and mineralizing organic compounds to soluble inorganic phosphates with the release of extracellular enzymes in soil (Alexander, 1977). The extracellular soil enzymes like phosphomonoesterases play an important role in solubilizing insoluble phosphates (Majumder and Das, 2016) and reflect soil biological health. Multidimensional effects including shifts in microbial counts and differential biochemical processes due to varied effects on soil enzymes have been reported (Singh and Singh, 2005) after application of insecticides in soil. By virtue of medium to low persistence, high effectiveness (Boucard *et al.*, 2008) and broad-spectrum efficacy (Palmquist *et al.*, 2012), synthetic pyrethroid insecticides are being widely used in India, somewhere in tea gardens of Himalayan *terai* (at the lower base) regions to combat insect pests. Therefore, effect of pyrethroid insecticides on the phosphate solubilizing microorganisms, biological transformation of insoluble phosphates in soil and ultimately on its availability to plants are of paramount significance so far as productivity of crops is concerned.

The objectives of the present study were to investigate the effect of four commonly used synthetic

pyrethroid insecticides viz., cypermethrin, deltamethrin, fenvalerate and permethrin (Fig.1) at their recommended field application rates on microbiological activities in relation to phosphorus solubilization and its availability in a tea garden soil of Himalayan *terai* region of West Bengal, India.

MATERIALS AND METHODS

An experiment has been conducted under laboratory conditions with Himalayan *terai* soil collected from the tea garden of Anandapur Tea Estate, Jalpaiguri district, West Bengal, India (26°85'86''N and 88°73'31'' E) by taking several thin slices from the surface soil layer (0-15 cm) by means of a spade as outlined by Jackson (2014). The composite soil samples were air dried at 30-35 °C in shade and passed through a 2 mm (4-8 mesh cm⁻¹) sieve. The processed soils were stored in a screw-cap jar and used for the experiment. The soil belongs to fluventic eutrochrepts (USDA 1975) having general characteristics: silt-loam in texture (sand 53%, silt 31% and clay 16%), water holding capacity 64.9%, pH (1:2.5 w/v) in water 4.6, EC 0.12 dS m⁻¹, CEC 3.64 cmol (p⁺) kg⁻¹, oxidizable organic C 7.57 g kg⁻¹, total N 0.87 g kg⁻¹, total P 294.1 mg kg⁻¹, available N 176.9 mg kg⁻¹, NaHCO₃ extractable P 29.9 mg kg⁻¹, C:N ratio 8.7 and C:P ratio 25.9. Four synthetic pyrethroid insecticides, viz. cypermethrin [10% emulsifiable concentrate (EC) - United Phosphorus Ltd], deltamethrin [11% EC - Bayer Crop Science Ltd], fenvalerate [20% EC - Rallis India Ltd] and permethrin [25% EC - Devidayal Agro Ltd], at recommended field application rates (225, 200, 225 and 210 g a.i. ha⁻¹, respectively), were mixed thoroughly with 2 kg of air-dried and sieved soil (≤ 2 mm) and were

placed in earthenware pots having a soil depth of 15 cm. Soil moisture was adjusted to 60 per cent of water holding capacity of the soil and maintained throughout the experimental periods. The pots were kept covered with black polyethylene sheets to avoid photodegradation of insecticides and evaporation loss of water from soil surface and incubated in the dark at 30 ± 1 °C for 60 days. There were three replications for each treatment.

Soil samples were collected after 0 (1 h), 15, 30, 45 and 60 days following insecticide applications by taking 3-5 soil cores as described by Das and Mukherjee (2000). Soil moisture content was measured from the subsamples at each sampling day. The subsamples were immediately analyzed to determine microbial populations, their biochemical activities and chemical transformations. The subsamples were immediately analyzed to enumerate colony forming units (cfu) of bacteria, actinomycetes, fungi and phosphate-solubilizing microorganisms following serial dilution technique and pour plate method (Salle, 1973) in asparagine-mannitol agar (Thornton, 1922), dextrose-casein agar (Jensen, 1930), rose-bengal agar (Martin, 1950) and sucrose-tricalcium phosphate agar (Pikovskaia, 1948) media, respectively. The agar plates were incubated at 30 ± 1 °C for 7 days and after the incubation period, the cfu of bacteria, actinomycetes, fungi and phosphate-solubilizing microorganisms were counted following the method as described by Salle (1973). Soil samples were analyzed to estimate phosphate solubilizing capacities following the method as outlined by Debnath *et al.*, (1994) by incubating 1 g soil of each sample in 15 ml Pikovskaia's broth (1948) in culture tubes (15 cm length, 1.8 cm outer diameter) at 30 ± 1 °C for 15 days followed by estimation of soluble phosphorus (Olsen and Dean, 1982) in the broth. Soil samples were analyzed to estimate total phosphorus through digestion with perchloric acid and quantified colorimetrically following vanadomolybdophosphoric acid method (Kuo, 1996). Water soluble phosphorus in soil was extracted with Bray and Kurtz's No. 1 extract (Jackson, 2014) and quantified colorimetrically following the method as outlined by Murphy and Riley (1962).

The results were evaluated by analysis of variance (ANOVA) following factorial RBD (two- factors) and the statistical significance ($p < 0.05$) of difference between mean results within factors (pyrethroid insecticides and sampling days) was evaluated using Fisher's LSD method (Petersen, 1994). The results were also evaluated by analysis of linear relationship between the variables and the statistical significance ($p < 0.01$) of correlation co-efficient (r value) was evaluated using statistical package for social sciences, version 16.0 (SPSS 16.0).

RESULTS AND DISCUSSION

Application of synthetic pyrethroid insecticides, in general, had a deleterious effect on growth and proliferation of bacteria in soil (Table 1), and this effect was more pronounced with fenvalerate (11%) followed by permethrin (10.3%), compared to untreated control. It was also revealed that soils treated with cypermethrin and deltamethrin exerted an alternate rise and fall in the proliferation of bacteria during the experimental period and this was highly significant ($p < 0.05$) after 15 days of sampling. A similar trend was exhibited with fenvalerate up to 45 days of sampling, while application of permethrin recorded a drop of bacterial proliferation after 15 days of sampling followed by a gradual increase up to 45 days of sampling. In general, the population of bacteria in the insecticides treated soils was significantly ($p < 0.05$) increased after 15 and 45 days of sampling, indicating that the microbes could have been adopted to the toxic effect of the insecticides and subsequently utilized the detoxified compounds to derive carbon and other nutrients for their cellular metabolism (Sarnaik *et al.*, 2006). Similar observations were also reported earlier (Das and Mukherjee, 1994) with different insecticides in the soil system. Application of insecticides had differential effects on the proliferation of actinomycetes in soil (Table 1). As compared to untreated control, the colony forming units of actinomycetes were reduced with permethrin (13.9%) and deltamethrin (4.3%), whereas, the growth of the microorganism was increased with cypermethrin to the extent of 4.6 per cent. In general, the growth and proliferation of actinomycetes were significantly ($p < 0.05$) augmented up to 30 days, more so with cypermethrin and fenvalerate followed by a gradual decrease up to 60 days of sampling. This indicated that actinomycetes preferably utilized the applied insecticides including the detoxified metabolites for their cellular metabolism (Das and Mukherjee, 2000). As compared to untreated control, application of deltamethrin and fenvalerate significantly ($p < 0.05$) reduced the numbers of actinomycetes after 45 days of sampling manifesting toxic effect of the cited pyrethroid insecticides on the growth of actinomycetes in soil (Moorman, 1989). Similar toxic effect was exhibited with permethrin during the period of 15 to 45 days of sampling. The proliferation of fungi was highly induced due to the application pyrethroid insecticides in soil (Table 1), and the stimulation was highly significant ($p < 0.05$) when the soil was treated with permethrin, which augmented fungal propagules to the extent of 51 per cent, followed by fenvalerate (32.6%) and deltamethrin (31.8%), as compared to untreated control. Most of the insecticides highly stimulated the fungal colonies during the period of 15 to 30 days of sampling, followed by a

Table 1: Effect of pyrethroid insecticide on the proliferation of microorganisms in tea soil

Treatments	Conc ^a	Sampling days					Mean
		0 (1h)	15	30	45	60	
Number of total bacteria (cfu × 10⁵ g⁻¹)^b							
Control	0	109.0 ± 5.6 ^c	172.0 ± 8.6	165.5 ± 6.8	208.3 ± 7.6	181.5 ± 9.7	167.3
Cypermethrin	225	109.0 ± 5.6	187.0 ± 9.4	173.5 ± 9.5	210.3 ± 10.4	175.1 ± 8.5	171.0
Deltamethrin	200	109.0 ± 5.6	164.1 ± 7.2	143.7 ± 6.6	217.1 ± 8.4	154.1 ± 9.6	157.6
Fenvalerate	225	109.0 ± 5.6	159.6 ± 8.7	115.6 ± 8.8	165.3 ± 8.5	195.0 ± 7.5	148.9
Permethrin	210	109.0 ± 5.6	88.2 ± 6.5	146.0 ± 9.8	204.1 ± 10.1	202.6 ± 8.7	150.0
Mean	-	109.0	154.2	148.9	201.0	181.7	
LSD (0.05)		Treatments 25.5; Sampling date 25.5; Interaction 57.1					
Number of actinomycetes (cfu × 10⁵ g⁻¹)							
Control	0	78.0 ± 4.2	143.9 ± 8.8	162.5 ± 9.1	135.2 ± 5.7	93.8 ± 4.1	122.7
Cypermethrin	225	78.0 ± 4.2	148.8 ± 7.6	239.7 ± 9.8	105.7 ± 5.5	69.3 ± 5.8	128.3
Deltamethrin	200	78.0 ± 4.2	159.4 ± 7.4	194.8 ± 8.7	85.7 ± 5.2	69.1 ± 8.4	117.4
Fenvalerate	225	78.0 ± 4.2	141.6 ± 6.9	215.1 ± 8.4	92.3 ± 5.4	87.5 ± 6.3	122.9
Permethrin	210	78.0 ± 4.2	109.9 ± 5.8	126.4 ± 5.9	112.8 ± 7.8	101.3 ± 6.6	105.7
Mean	-	78.0	140.7	187.7	106.3	84.2	
LSD (0.05)		Treatments not significant; Sampling date 18.0; Interaction 40.3					
Number of fungi (cfu × 10³ g⁻¹)							
Control	0	33.5 ± 3.3	39.8 ± 3.2	32.8 ± 2.6	44.7 ± 2.6	47.8 ± 2.5	39.7
Cypermethrin	225	33.5 ± 3.3	55.0 ± 3.5	30.0 ± 3.4	39.5 ± 3.5	44.6 ± 2.3	40.5
Deltamethrin	200	33.5 ± 3.3	66.8 ± 4.1	46.0 ± 1.9	66.6 ± 3.8	48.9 ± 3.1	52.4
Fenvalerate	225	33.5 ± 3.3	60.6 ± 2.5	66.2 ± 3.8	54.0 ± 3.7	49.1 ± 1.4	52.7
Permethrin	210	33.5 ± 3.3	54.6 ± 2.5	98.8 ± 2.7	43.9 ± 3.7	69.0 ± 2.4	60.0
Mean	-	33.5	55.4	54.8	49.7	51.9	
LSD (0.05)		Treatments 13.5; Sampling date not significant; Interaction 30.9					

^a Conc: Concentration of insecticide (g a.i. ha⁻¹); ^b cfu: Colony forming units; ^c Mean ± SE

Table 2: Effect of pyrethroid insecticide on the activities of phosphate-solubilizing microorganisms in tea soil

Treatments	Conc	Sampling days					Mean
		0 (1h)	15	30	45	60	
Number of phosphate-solubilizing microorganisms (cfu × 10⁵ g⁻¹)							
Control	0	83.5 ± 4.4	121.3 ± 6.9	159.5 ± 7.2	94.0 ± 5.6	123.7 ± 5.8	116.4
Cypermethrin	225	83.5 ± 4.4	154.8 ± 8.7	150.8 ± 6.9	67.0 ± 6.2	124.7 ± 4.4	116.2
Deltamethrin	200	83.5 ± 4.4	139.3 ± 6.2	150.2 ± 5.8	86.9 ± 6.2	115.7 ± 6.7	115.1
Fenvalerate	225	83.5 ± 4.4	140.2 ± 5.6	142.0 ± 6.5	69.1 ± 5.5	174.4 ± 7.6	121.8
Permethrin	210	83.5 ± 4.4	118.0 ± 7.1	178.1 ± 8.6	103.4 ± 7.8	150.7 ± 5.2	126.7
Mean	-	83.5	134.7	156.1	84.1	137.8	
LSD (0.05)	Treatments not significant; Sampling date 20.3; Interaction not significant						
Amount of phosphate solubilized (mg g⁻¹)							
Control	0	0.018 ± 0.002	0.020 ± 0.004	0.021 ± 0.002	0.017 ± 0.001	0.016 ± 0.003	0.018
Cypermethrin	225	0.018 ± 0.002	0.021 ± 0.002	0.016 ± 0.003	0.017 ± 0.003	0.015 ± 0.003	0.017
Deltamethrin	200	0.018 ± 0.002	0.023 ± 0.003	0.019 ± 0.004	0.015 ± 0.002	0.016 ± 0.001	0.018
Fenvalerate	225	0.018 ± 0.002	0.015 ± 0.001	0.018 ± 0.002	0.012 ± 0.002	0.014 ± 0.002	0.015
Permethrin	210	0.018 ± 0.002	0.016 ± 0.002	0.016 ± 0.002	0.015 ± 0.001	0.013 ± 0.002	0.016
Mean	-	0.018	0.019	0.018	0.015	0.015 ±	
LSD (0.05)	Treatments 0.001; Sampling days 0.001; Interaction 0.003						

Table 3: Effect of pyrethroid insecticide on the changes of phosphorus in tea soil

Treatments	Conc	Sampling days					Mean
		0 (1h)	15	30	45	60	
Amount of total P (mg kg⁻¹)							
Control	0	294.1 ± 5.2	256.9 ± 5.6	269.7 ± 7.2	295.7 ± 5.5	314.0 ± 8.2	286.1
Cypermethrin	225	294.1 ± 5.2	267.9 ± 6.5	279.0 ± 5.6	297.8 ± 8.2	329.2 ± 7.5	293.6
Deltamethrin	200	294.1 ± 5.2	271.7 ± 6.8	277.0 ± 4.5	288.1 ± 6.7	315.7 ± 5.4	289.7
Fenvalerate	225	294.1 ± 5.2	275.1 ± 7.6	276.9 ± 6.8	314.0 ± 7.4	338.1 ± 6.8	299.6
Permethrin	210	294.1 ± 5.2	277.1 ± 8.2	279.3 ± 7.8	308.5 ± 7.1	315.7 ± 7.8	294.9
Mean	-	294.1	269.7	276.4	300.8	322.5	
LSD (0.05) treatments not significant; sampling date 13.8; interaction not significant							
Amount of available P (mg kg⁻¹)							
Control	0	25.9 ± 2.3	25.9 ± 2.2	26.9 ± 2.5	27.2 ± 3.3	26.5 ± 1.9	26.5
Cypermethrin	225	25.9 ± 2.3	26.3 ± 2.3	26.5 ± 1.5	26.8 ± 3.2	26.4 ± 2.0	26.4
Deltamethrin	200	25.9 ± 2.3	26.2 ± 3.1	26.7 ± 1.6	26.5 ± 2.6	26.0 ± 1.5	26.3
Fenvalerate	225	25.9 ± 2.3	26.1 ± 1.6	26.6 ± 2.4	26.3 ± 1.6	26.3 ± 2.5	26.2
Permethrin	210	25.9 ± 2.3	26.7 ± 1.1	26.5 ± 3.2	26.3 ± 2.1	26.1 ± 2.6	26.3
Mean	-	25.9	26.2	26.6	26.6	26.3	
LSD (0.05) Treatments not significant; Sampling date 0.4; Interaction not significant							

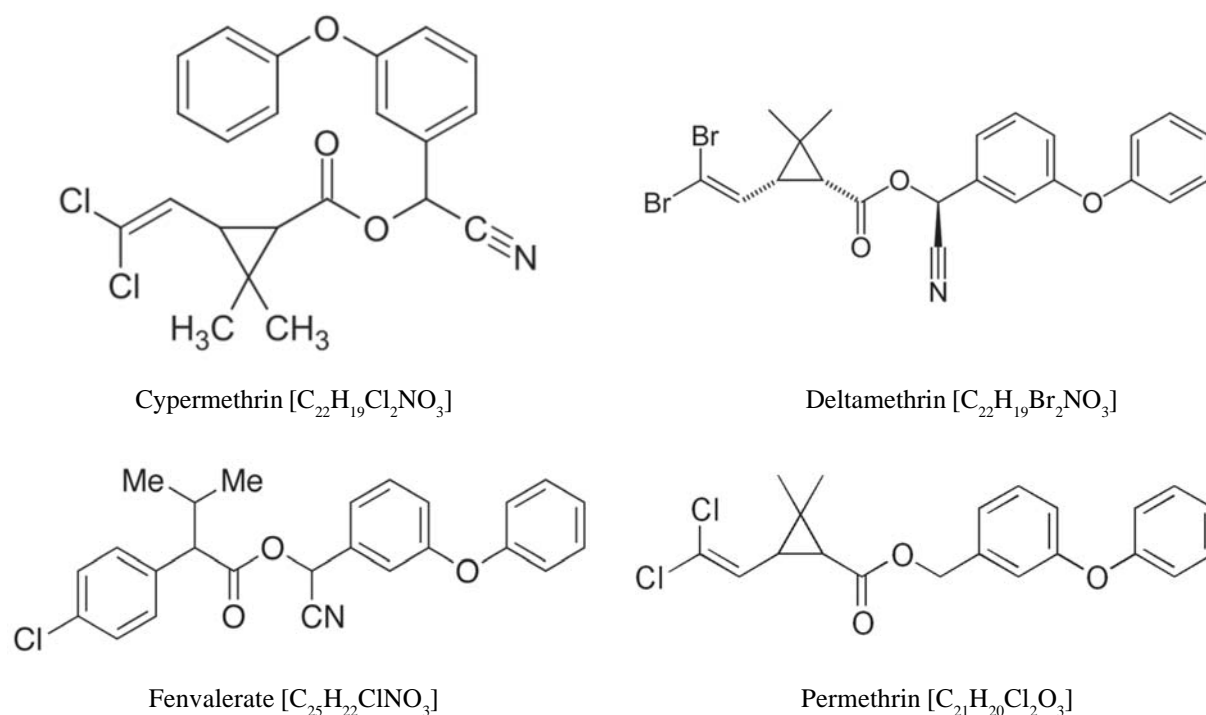


Fig. 1: The chemical structures of the insecticides used for the experiment

gradual decrease up to the end of the experiment. The significant ($p < 0.05$) rise in the fungal colonies with permethrin after 30 days of sampling indicated preferential utilization of the insecticide residues for their cellular metabolism (Chu, 2010). Similar trend was also recorded with deltamethrin and fenvalerate from 30 to 45 days of sampling.

Phosphate solubilizing microorganisms were not significantly ($p < 0.05$) induced due to the incorporation of pyrethroid insecticides in soil (Table 2). As compared to untreated control, there was a stimulation of phosphate solubilizing microorganisms under permethrin (8.9%) followed by fenvalerate (4.7%), while the incitement of the microbial propagules with cypermethrin and deltamethrin was *at par* with the untreated control. Similar differential influences of applied insecticides towards growth and proliferation of phosphate solubilizing microorganisms were also reported earlier (Boucard *et al.*, 2008). It was also revealed that application of insecticides in general, augmented the numbers of phosphate solubilizers incessantly up to 30 days followed by an alternate fall and rise up to the end of the experiment. This manifested that the cited microorganisms utilized the insecticide residues for their growth and metabolism in soil (Singh and Ghoshal, 2010). Incidentally, proliferation of phosphate solubilizing microorganisms was positively correlated

($r = 0.470$, $p < 0.01$) with numbers of actinomycetes, indicating that actinomycetes preferentially utilized pyrethroid insecticide residues in the tea soils. Similar observations were also depicted earlier (Hefnawy *et al.*, 2012). The proliferation of phosphate solubilizing microorganisms in the soils treated with permethrin and fenvalerate was not concomitant to their efficiency (Table 2), rather these insecticides exerted a significant ($p < 0.05$) deleterious effect on the phosphate solubilizing capacity, more so with fenvalerate (16.3%) compared to permethrin (15.2%). This sustained the findings of Krishnamurthy and Alagawadi (1994) who pointed out that proliferation of phosphate solubilizing microorganisms was not the indicative to the solubilization of insoluble phosphates in soil. It was also revealed that as compared to untreated control, phosphate solubilizing capacity was significantly ($p < 0.05$) decreased with cypermethrin after 30 days, with fenvalerate after 15 and 45 days, and with permethrin up to 45 days of sampling. Similar observations were also reported earlier (Sarnaik *et al.*, 2006) with different agrochemicals in soil. Incidentally, phosphate solubilizing capacity was positively correlated with the proliferation of actinomycetes ($r = 0.304$, $p < 0.05$) and this confirmed the active participation of actinomycetes in the solubilization of insoluble phosphates in soils treated with pyrethroid insecticides.

Application of synthetic pyrethroid insecticides in general, did not have any significant ($p < 0.05$) influence on the content of total phosphorus in soil (Table 3). It was also revealed that there was a significant ($p < 0.05$) drop of total phosphorus content in the insecticides treated soils after 15 days of sampling followed by an incessant rise of total phosphorus up to the end of the experiment. This sustained the findings of earlier workers (Das and Dey, 2014) who pointed out that the enhanced microbial biomass retained more phosphorus in their cells due to greater utilization of different agrochemicals as well as their degraded products in soil (Nongthombam *et al.*, 2009). As compared to untreated control, maximum stimulation of total phosphorus was recorded with fenvalerate (4.7%) followed by permethrin (3.1%). The results also pointed out that there was a progressive rise in the accumulation of total phosphorus in the insecticides treated soils. This indicated that the simultaneous stimulation of microorganisms enhanced the utilization of insecticide residues for their cellular metabolism leading to greater retention of total phosphorus in soil (Shan *et al.*, 2006). The availability of phosphorus was negatively affected due to the application of pyrethroid insecticides in soil and this effect was not statistically significant ($p < 0.05$), although the microbial biomass of phosphate solubilizing microorganisms was increased to some extent due to application of fenvalerate and permethrin (Table 2), compared to untreated control. This indicated that proliferation of phosphate solubilizing microorganisms was not always reflected in the mobilization of available phosphorus in soil (Yu *et al.*, 2006). In addition to this, the acidic nature of the soil (pH 4.6), where some of the soluble phosphorus was immobilized chemically in the form of insoluble aluminum and iron polyhydroxy phosphates (Tiwari, 2009), resulted lesser availability of soluble phosphorus in the treated soils. It was also revealed that maximum release of available phosphorus was recorded with cypermethrin after 45 days, with deltamethrin and fenvalerate after 30 days and with permethrin after 15 days of sampling. This result clearly indicated greater utilization of applied insecticides as well as their degraded metabolites by the microorganisms for their cellular metabolism resulting in greater release of available phosphorus in soil. Similar observations were also reported earlier by Das and Dey (2014).

The results of the present investigation thus pointed out that application of synthetic pyrethroid insecticides exerted differential influences on growth and activities of microorganisms in relation to solubilization and availability of phosphorus in tea soil. Application of insecticides in general, had a deleterious effect on the proliferation of bacteria and actinomycetes in soil,

suppression being the highest with fenvalerate (11%) followed by permethrin (10.3%) for bacteria and with permethrin (13.9%) for actinomycetes. The numbers of fungi propagules were highly increased with permethrin (51%) followed by fenvalerate (32.6%) and deltamethrin (31.8%), while permethrin stimulated the growth of phosphate solubilizing microorganisms to the extent of 8.9%. Incorporation of cypermethrin did not exert any significant effect on the proliferation of microorganisms in soil. It was also revealed that phosphate solubilizing capacity of the soil was badly affected due to the application of fenvalerate (16.3%) and permethrin (15.2%). Application of synthetic pyrethroid insecticides had a meager positive effect on the retention of total phosphorus, while the availability of soluble phosphorus remained unchanged in the treated soils. The overall effect of synthetic pyrethroid insecticides on the proliferation of microorganisms and phosphate solubility can not be generalized; rather in most cases the effects were harmful to microbial activities in soil.

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