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Original Research Paper

Effect of Organophosphorus Pesticides on Enzyme Activities in Alluvial Soil (Typic Ustochrepts)

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ABSTRACT

Acephate, dimethoate and phosphamidon are organophosphate pesticides with high toxicity and may significantly affect soil microbial activities. However, the magnitude of this effect is unclear yet. The potential harmful effect of these pesticides on soil enzyme activity was accessed in the soil collected under controlled laboratory conditions. We examined the effect of recommended (RD) and double the recommended doses (2RD) of these pesticides on the soil enzymatic activities. The incubation study was carried out at 60% of maximum water holding capacity of the soil sample at 28±2°C for a period of 42 days. Our results indicated that high acephate, dimethoate and phosphamidon doses significantly affect enzymatic activities in the soil. Our results provide the first evidence that acephate, dimethoate and phosphamidon differentially affected the soil microbial community through inhibiting fungal and bacterial populations.

INTRODUCTION

The agriculture sector dominates the economic scenario of our country. The expanding global population and higher demand of food leads to increasing the sustainability of food production through intensive agriculture, attention of public health and proper utilization of natural resources. It is evident that land is a limiting factor in food production; therefore the improvement of agriculture with advanced techniques to meet this demand and keeping soil in its productive quality plays a dominant role for much of the today's productivity (Anderson 1978). Soil is a dynamic living system and consists of variety of micro and macro flora and fauna including a wide range of microorganisms. In soil, the sources of nutrients for the growth and development of plants and to maintain fertility status are inorganic minerals and organic matter (Das & Mukherjee 1994).

Microbes in soil are the most important agents that decompose organic materials to enable their recycling in the environment. Enzymes released through microbes and plants in soil are undoubtedly responsible for the decomposition and contribute to the total biological activity of the soil-plant environment under different states (Dick 1994). Natural and anthropogenic factors may affect directly or indirectly the activities of enzymes in soil. Among, anthropogenic factors, pesticides are of primary importance due to their continuous entry into soil environment (Topp 1997). Pesticides may enter the soil either by direct applications (agricultural practices) or indirect applications (accidental spillage, leaks at pesticide dump sites and discharge of waste from production facilities or urban pollution). Globally, about 3 $\times 10^9$ kg of pesticides is applied annually. The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminate the soil environment (Carriger et al. 2006). Concern of environmental contamination in current context, pesticide use has assumed great importance. The fate of the pesticides in the environment in respect of pest control efficacy, non-target organisms and offsite mobility has become a matter of environmental concern (Hafez & Thiemann 2003) potentially because of the adverse effects of pesticide on soil microorganisms which in turn may affect soil fertility as well as soil health. The organophosphorus group of pesticides is now gradually occupying important position amongst all. Organophosphorus pesticides exert their acute effects in insects and mammals by inhibiting acetylcholinesterase in the nervous system with subsequent accumulation of toxic levels of acetylcholine, which is a neurotransmitter. This occupies the largest group of pesticides used in India (Dureja & Gupta 2010).

Pesticides interact with soil organisms and their metabolic activities may alter the physiological and biochemical behaviour of soil microbes (Singh & Walker 2006). The impact of organophosphorus pesticides on soil enzyme activities has been studied less intensively. In recent years emphasis has been made on the application of pesticides which are environmentally safe and have multi-pronged function. Hence, looking into the previous gaps in the research related to effects of pesticides on soil health, in the present study, the authors investigated the impact of three organophosphorus pesticides *viz.,* acephate, dimethoate and phosphamidon on soil enzyme activities in the alluvial soil (Inceptisol).

MATERIALS AND METHODS

Source of pesticides and reagents: The technical grade organophosphorus insecticides: Acephate (94%), Dimethoate (91%) and Phosphamidon (96%) were obtained from United Phosphorus Limited, India.

Experimental Soil: Experimental soil was collected from Agricultural farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, sieved (2mm pore size) and air dried. Soil belongs to Typic Ustochrepts and is neutral in reaction (pH 7.2) with fine loamy texture. The physicochemical properties of the experimental soil were analysed (Table 1).

Experimental design: The experiment was conducted in a Completely Randomized Design (CRD) with five replications. The treatments were: (A) For Acephate: T_1- Control (C), T_2- Recommended dose (A_1), T_3- Double the recommended dose (A_2), (B) For Dimethoate: T_1- Control (C), T_2- Recommended dose (D_1), T_3- Double the recommended dose (D_2), (C) For Phosphamidon: T_1- Control (C), T_2- Recommended dose (P_1), T_3- Double the recommended dose (P_2). The recommended dose of the three pesticides acephate, dimethoate and phosphamidon were 584 g a.i. ha^{-1}, 600 g a.i. ha^{-1} and 500 g a.i. ha^{-1} (Sahu 2012). The experiment was conducted in a laboratory incubation method. The pesticides *viz.* acephate, dimethoate and phosphamidon were added (as mentioned in the experimental design) to soils in the form of water solution. Pesticide free soil served as control. The soil was moistened and maintained at field capacity (60% water holding capacity) by periodical addition of distilled water and incubated at 28±2°C for 42 days.

Soil sampling and analysis: Soil samples were analysed for determination of different soil enzymatic activities like cellulase, β-glucosidase, protease, phosphatase, arylsulphatase and dehydrogenase at 7, 14, 21 and 42 days. Cellulase activity was determined by Pancholy & Rice (1973) and Gianfreda et al. (1995), respectively. ?-glucosidase and protease were determined as per methods of Eivazi & Tabatabai (1988) and Rejsek et al. (2008), respectively. Alkaline phosphatase activity was analysed by measuring the *p*-nitrophenol (PNP) released by the method given by Tabatabai & Bremner (1969). Arylsulphatase was determined by using *p*-nitrophenol sulphate as substrate (Tabatabai & Bremner 1970 a). Dehydrogenase activity in the soil samples was measured by the triphenyl tetrazolium chloride method (Casida et al. 1964).

Statistical analysis: In laboratory experiments, the data were analysed as a completely randomized design (CRD) and the data subjected to analysis of variance (ANOVA) or Duncan's Multiple Range Test (DMRT) using statistical package for Social Sciences Version 16.0 programme. Data were compared with DMRT at $p \leq 0.05$.

RESULTS

Effect of organophosphorus pesticides on the soil enzyme activities: Results showed the changes in the enzymatic activities after organophosphorus pesticides application. The dehydrogenase activity in the soil samples treated with recommended and double the recommended dose of acephate, dimethoate and phosphamidon was recorded at 7, 14, 21 and 42 days of incubation. Dehydrogenase activity was found to decrease gradually up to three weeks and thereafter gradual increase was recorded at 42 days as compared to 21 days of incubation (Fig. 1a). Maximum decrease in the dehydrogenase activity was recorded in the soil samples treated with the double the recommended dose of phosphamidon followed by dimethoate and acephate (Fig. 1a).

Changes of phosphatase activities caused by different doses of acephate, dimethoate and phosphamidon are shown in Fig. 1b. Significant differences ($P > 0.05$) were observed for the activities applied at the recommended and double the recommended doses as compared with the control. In the first 14 days, the activity was found to increase gradually. Thereafter remarkable reduction was recorded on 21 and 42 days. Maximum reduction in the enzymatic activity was recorded in the soil sample treated with double the recommended dose of phosphamidon followed by dimethoate and acephate as compared to soil treated with the recommended dose of selected and control (Fig. 1b).

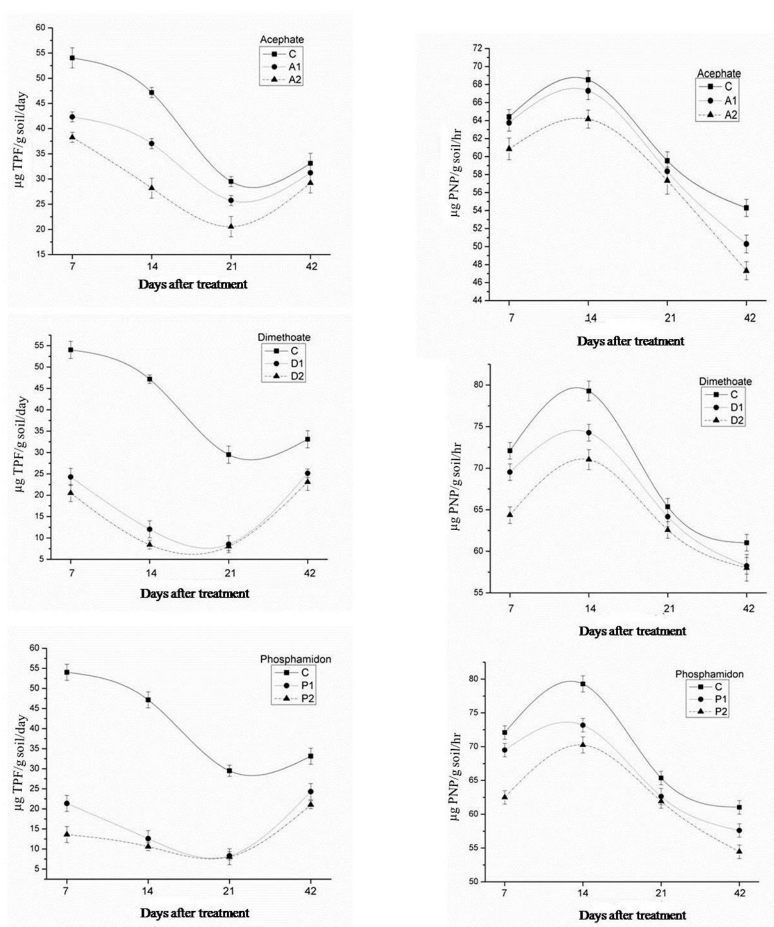
During the whole experiment, the cellulase activity in all the samples, including the control, fluctuated sharply, in a different manner (Fig. 1c). A tri-phasic reaction was recorded. In the initial 14 days, the enzyme activity of the samples decreased sharply and thereafter increased gradually up to 21 days. Again slight reduction in the enzymatic activity

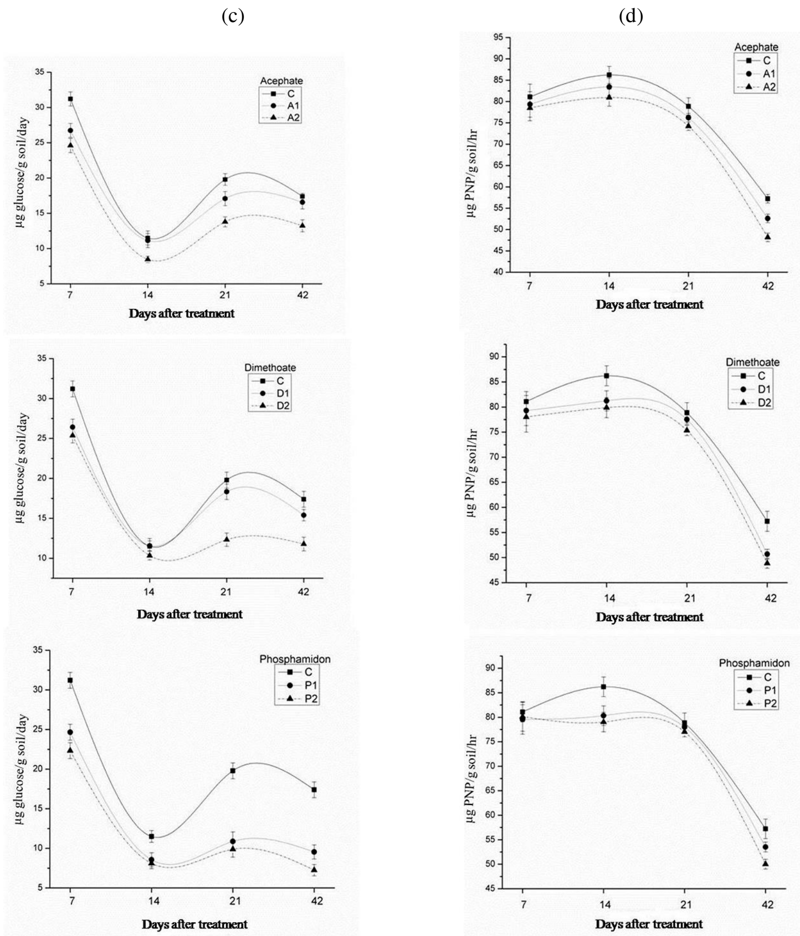
Table 1: Physicochemical characteristics of initial experimental soil.

S.N.	Soil properties	Values	S.N.	Soil Properties	Values	S.N.	Soil Properties	Values
1.	Soil separates[a] (%)		9.	CEC[c] [cmol (P^+) kg^{-1}]		17.	Available macronutrients (kg ha^{-1})	
(i)	Coarse sand (0.2-2.0 mm)	13.38	(i)	Ca^{2+}	9.8	(i)	N	208.91
(ii)	Fine sand (0.02-0.2 mm)	16.12	(ii)	Mg^{2+}	2.8	(ii)	P	21.70
(iii)	Silt (0.002-0.02mm)	46.5	(iii)	Na^+	0.6	(iii)	K	151.79
(iv)	Clay (< 0.002 mm)	24.00	(iv)	K^+	0.51	(iv)	S	7.59
2.	Textural class	Silt loam	10.	Total CEC [cmol (P^+) kg^{-1}]	13.71	18.	Available micronutrients (mg kg^{-1})	
3.	pH[b]	7.2	11.	$CaCO_3$ (%)	6.23	(i)	Fe	12.31
4.	EC (dS m^{-1})	0.12	12.	Soil taxonomy	Typic Ustocrepts	(ii)	Mn	2.23
5.	OC (g kg^{-1})	4.6	13.	Water holding capacity (%)	46	(iii)	Cu	0.86
6.	OM (g kg^{-1})	7.93	14.	ESP (%)	4.37	(iv)	Zn	0.62
7.	Bulk density (Mg m^{-3})	1.23	15.	SAR	0.24	(v)	B	0.15
8.	Particle density (Mg m^{-3})	2.51	16.	ESR	0.045	(iv)	Mo	0.04

a-Hydrometer methods, b-Water:soil = 2.5:1, c-Method: 1M NH_4 acetate extractable cations with prewash, d-DTPA extractable, EC - Electrical conductivity, OC - organic carbon, OM - organic matter, CEC - cationexchange capacity

(a) (b)





was recorded at 42 days of incubation (Fig. 1c). In case of β-glucosidase activity, results showed similar pattern as recorded in case of phosphatase activity. There were no apparent differences in the enzyme activities between treated soil samples and the control. After that, however, the enzyme activities in samples treated with selected pesticides decreased by different degrees and the variation of activities was similar to the control sample (Fig. 1d). The highest reduction of β-glucosidase was recorded at 42 days of incubation as observed in case of phosphatase.

A unique bi-phasic pattern was recorded in case of protease activity across the treatment. In the first 21 days, gradual decrease in enzymatic activity was recorded in the soil sample treated with acephate. After 21 days a sudden increase was recorded in untreated soil (control) as compared to treated soil (Fig. 1e). More or less similar pattern was recorded in the soil treated with dimethoate (Fig. 1e). However, slightly different pattern was recorded in the soil treated with phosphamidon. On first 14 days, slight increase in the activity was recorded. Thereafter, it decreases sharply up to 21 days and sudden increase in the activity was observed as recorded in the case of acephate and dimethoate (Fig. 1e).

The response of arylsulphatase to selected pesticides was noteworthy (Fig. 1f). The enzyme activity in the soil treated with these pesticides followed the similar pattern as recorded in case of phosphatase and was close to that of their control on experimental day (Fig. 1f).

DISCUSSION

Microbial activities are the prime factor affecting the degradation and persistance of the organophosphorus insecticides in agricultural soil (Adhya et al. 1981, Singh et al. 2013). The possibility that these insecticides may have a negative effect on soil microflora and their biological activities could be of considerable importance. In this context, our results show that acephate, dimethoate and phosphamidon significantly affect enzyme activities in the soil. Apparently certain group of soil microbes can tolerate high amounts of the organophosphorus insecticides without significant modifi-

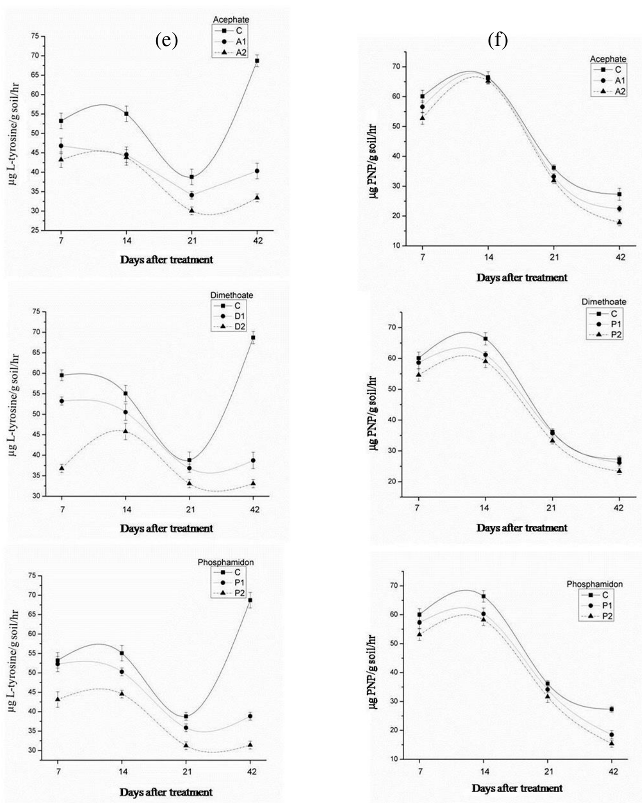


Fig. 1: Effect of organophosphorus pesticides, acephate, dimethoate and phosphamidon on soil enzymatic activities under controlled laboratory condition. (1a) dehydrogenase activity, (1b) phosphatase activity, (1c) cellulase activity, (1d) β-glucosidase activity, (1e) protease activity, (1f) arylsulphatase activity. C represents control (untreated), A1 represents recommended dose of acephate, A2 represents double the recommended dose of acephate, D1 represents recommended dose of dimethoate, D2 represents double the recommended dose of dimethoate, P1 represents recommended dose of phosphamidon, P2 represents double the recommended dose of phosphamidon. Data are mean (n = 5) and vertical bar lines represents standard error of mean.

cation to their biological activities. Similar results have been reported for other organophosphorus insecticides (Martinez-Toledo et al. 1992, Dick 1997, Singh et al. 2013, Sannino & Gianfreda 2001).

Our results of enzymatic activities further showed that various components of the soil microbial community differentially responded to these selected pesticides. The dehydrogenase activity was highest at the initial phase and decreased drastically up to their lowest levels on the 21st day as compared to control and thereafter gradual increase was recorded at 42 days as compared to 21 days of incubation in inceptisol (Fig. 1a). The reduction of the activities showed maximum in the soils at 14 days of incubation (19.75-82.16%). Probably at this stage, organophosphorus pesticides are generally degraded. Thus, the cumulative effects of the parent compound as well as transformed products of pesticides suppressed the activities of the dehydrogenase system. After degradation of pesticides, microbial activities again recover and dehydrogenase activity increase gradually (Fig. 1a). Aerobic soil metabolism is the main degradation process for acephate (United State Environmental Protection Agency Report 2001). It was observed that half-lives are less than 2 days under expected use conditions, producing the intermediate degradate methamidophos which is metabolized by soil microorganisms to carbon dioxide and microbial biomass (half-lives<10 days). Whereas phosphamidon is stable in neutral and acid media but is hydrolysed by alkali; half-life at 23°C is 13.8 days at pH 7 and 2.2 days at pH 10 (FAO & WHO report 1969). However, dimethoate is highly mobile, relatively non-persistent organophosphate insecticide. The primary route of dissipation is microbially-mediated hydrolytic and oxidative degradation in aerobic soil, particularly under moist conditions, with a half-life of 2.2 days. It hydrolyses very slowly in sterile buffered solutions at pH 5 and 7 (156 and 68 days, respectively), but hydrolyses rapidly to desmethyl dimethoate and dimethylthiophosphoric acid with a half-life of 4.4 days at pH 9. The anaerobic half-life was found to be approximately 22 days, with the major non-volatile degradate being desmethyl dimethoate (United State Environmental Protection Agency Report 2006).

Similar to dehydrogenase, other soil enzymes *viz.* cellulase and protease appear in bi and triphasic condition (Fig. 1). This may occur due to changes in the soil microbial metabolisms and organic content in the soil. It was depicted from our result that enzyme activities again recover after 14 and 21 days of treatment. These findings coincided with United State Environmental Protection Agency Report (US EPA 2001, 2006) and FAO/WHO Report (1969). The acephate, dimethoate and phosphamidon inhibited the phosphatase, β-glucosidase and arylsulphatase activities in the soils in a different manner at both the recommended and double the recommended doses of pesticides (Fig. 1). This may occur due to the various reactions going on inside the soil system. Initially the activities of these enzymes increased at 14 days of treatment and decreased sharply at 21 and 42 days. The possibility that intermediate compounds may act as substrate for enzymes and/or less toxic in the early stage to soil microflora and their biological activities (Sahu 2012). The transformation product of acephate and dimethoate are N-methamidophos and transisomer of dimethoate, which are more toxic than parent compounds; whereas phosphamidon persists for long period in acidic to neutral soil. Thus, toxic effect of the transformation products of acephate, dimethoate and persisted phosphamidon reduced the enzymes activity even up to 42 days (US EPA 2001, 2006). Dehydrogenase and other enzymatic activities are present only in viable cells and they are the useful indicator of overall microbial activity in soil (Trevors 1984, Sahu 2012). The increase in soil enzymatic activities possibly indicated active metabolism of the compound by microbes either for use as nutrient source or for the detoxification of this compound (Burns 1982). Pesticide hydrolysis by microbial enzymes can serve as a detoxification mechanism that can govern pesticide resistance and in turn determine the rate of pesticide biodegradation in the environment (Kalyani et al. 2010). The influence of pesticide in soil is generally a long term process and is closely related to soil characteristics. Hence, for obtaining a more realistic picture long-term field study is required (Sahu 2012, Singh et al. 2013).

CONCLUSION

The study describes the multifarious effects of organophosphorus pesticides, especially acephate, dimethoate and phosphamidon on soil enzyme activity. These pesticides significantly decreased enzyme activities and various other attributes in soil as well. Further, it was noted that phosphamidon was found to be more potential in reducing enzymatic activities in the soil even at RD and 2RD as compared to acephate and dimethoate. Results suggest that application of these pesticides in higher doses not only decrease enzymatic activities but also decrease microbial activities and suppress the physicochemical and biological properties of soil. Thus, it proves to be an excellent cause as well as toxic agents for overall deterioration of soil and environment.

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