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Quinalphos Persistence and Leaching Under Field Conditions and Effects of Residues on Dehydrogenase and Alkaline Phosphomonoesterases Activities in Soil

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Organophosphorus insecticides are considered priority pollutants due to their potential toxicity, mutagenicity, and carcinogenicity. Though they have shorter half-life, especially in the tropical conditions, their extensive and repeated usage often lead to build up of residues in the environment. Evidence of persistence and residue accumulation of organophosphate insecticides in soils have been reported (Miles et al. 1978; Racke and Coats 1988).

Quinalphos (*O,O*-Diethyl *O*-quinoxalin-2-yl phosphorothioate) is one of the widely used organophosphorus insecticide in Indian agriculture for control of pests on various crops such as cotton, groundnut and rice. Consequently there is a growing interest in its fate and behavior in the environment. Reports on quinalphos persistence in soil vary. Quinalphos persistence upto 40 days in soil has been reported (Gajbhiye et al. 1995; Babu et al. 1998). One of the major critical factors affecting the fate and behavior of insecticides in the environment, especially in soil ecosystems is microbial transformation.

Soil contains free enzymes, immobilized extracellular enzymes and enzymes within microbial cells. The various biochemical soil nutrient cycling processes are mediated by soil enzymes, which are derived from microorganisms, plant roots, and soil animals. These enzymes play an important role in the organic matter turnover and degradation of xenobiotics. Soil enzymes are therefore, useful in describing and understanding the ecosystem's quality and the interactions among subsystems and to assess the effects of various inputs on soil health (Burns 1982). Measurements of specific enzyme activities may be useful to assess soil biological activity, which might be used as a sensitive biological index of soil quality. Although numerous studies have been carried out on soil enzyme activities like dehydrogenase, arylsulfatase, urease, phosphatases, etc, little information is available to describe the effects of insecticides on soil enzyme activities especially under field conditions (Klein et al. 1985).

The present investigation was aimed to study quinalphos persistence and leaching under the field condition and effects of residues on dehydrogenase and alkaline phosphomonoesterases activities in the soil.

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MATERIALS AND METHODS

Quinalphos, 25% EC (Bayer's India Limited) for field treatment was bought from the local market whereas Technical quinalphos was gifted by Montari Industries Limited, Nehru Place, New Delhi, India.

Polyvinyl chloride cylinders (40cm l & 5 cm i.d.) open at both ends, were inserted in the field soil at the campus of University of Delhi, India (lat.29°N, long.77.3°E), two weeks prior to application, with about 3cm of the cylinder projecting above the soil surface to prevent the flow of run-off water onto the soil surface. Quinalphos (2.5 mg a.i) was dissolved in 10 ml acetone and spiked on to the surface of the soil of each column using a pipette in October 2003.

Two soil columns were removed for analysis at random at each sampling time. Soil from each column was divided into surface soil (0-15 cm) and sub-surface soil (15-30 cm). Soil from each depth was mixed; plant materials and other debris were removed and sieved through 4 mm mesh. For each sampling period, therefore, four samples were analyzed. Two columns making the zero-time samples were dug out two hours after the quinalphos treatment and the rest of the columns were removed after 1, 3, 7, 15, 30, 60, 90 and 180 days after the treatment. From each soil sample, triplicates sub-samples were analyzed for residue analysis. Soil samples were collected in polythene bags and stored at 4°C until analyzed.

Quinalphos was extracted from the soil (25 gm) using 50 ml hexane + acetone mixture (9:1 v/v). The samples were shaken for one hour at 200 rpm on an orbital mechanical shaker at and filtered under suction. The same soil was extracted again for two more times using 25 ml solvent each time and the filtrates were pooled. The extract was reduced to 5 ml using a rotary flash evaporator. Clean up of the sample was done using a glass column packed with 20 g each of silica gel (60-120 mesh) and sodium sulphate. Sample was transferred to the column and the column was eluted with 100 ml benzene. Collected solvent was dried up completely using rotary flash evaporator prior to Gas Liquid Chromatography analysis.

Quantification and identification of quinalphos residues was done using Gas Liquid Chromatography. Soil extracts were redissolved in 3 ml ethyl acetate and analyzed by Gas Liquid Chromatography. Shimadzu chromatograph model GC-17 AAF, equipped with ^{63}Ni Electron capture detector (ECD) was used. Nitrogen was used as carrier gas. The column specification and other operating parameters employed in the quantification of quinalphos are given in Table 1. Two microlitre of sample was injected into the GLC with a 10 µL Hamilton micro syringe. Retention time for quinalphos under these conditions was 12.1 min. Extraction efficiency was obtained by fortifying 50 g untreated oven dry soil with 0.01 mg g^{-} of quinalphos. The fortified samples were extracted and analyzed by GLC. Extraction recovery of 90% for quinalphos was obtained in the present study.

Table1. Column specifications and operating conditions of Shimadzu Chromatograph model GC-17 AAF, V3.

Column	Capillary column (ID-DP10.25UM), 30m x 0.25i.d mm
Column Temperature	200°C
Injector Port Temperature	250°C
Detector Temperature	300°C
Carrier Gas	Nitrogen
Carrier Gas Flow Rate	20 ml min^{-1}
Split Ratio	20
Injection Volume	2 µL

Soil was analyzed for physico-chemical parameters using standard methods. Soil pH was estimated using saturated soil paste (1:2.5 :: soil:water). Organic carbon was estimated by method of Walkley and Black (1934).

Dehydrogenase activity in the experimental soil samples was determined by method of Lenhard (1956). This method is based on the reduction of 2,3,5-triphenyl-tetrazolium chloride (TTC) to triphenyl formazan (TPF) by microbial action. Field-moist soil samples (3 g) in triplicates were incubated with 1ml glucose (30 mg / L) solution, 4.5 ml of 0.5% TTC solution in 0.1 M tris buffer (pH 7.8) and 3.5 ml of deionised water at 37°C for 24 hr. TPF produced was extracted with 10 ml of acetone and estimated photometrically at 485 nm against the blank . Total DHA was expressed as µg TPF g^{-1} dry soil 24 hr^{-1}.

Alkaline Phosphosphomonoesterase activity was determined by method of Margesin and Schinner (1994). Soil samples (1 g) in triplicates were incubated with 0.25 ml toluene, 4.0 ml modified universal buffer (pH 11.0) and 1 ml p-nitrophenol phosphate solution (115 mM) at 37°C for 1 hr. The p-nitrophenol released was extracted with 1.0 ml of 0.5 M $CaCl_2$ solution and 4.0 ml of 0.5 M NaOH. p-nitrophenol produced was estimated photometrically at 400 nm. Results were calculated as µg p-nitrophenol g^{-1} dry soil.

All statistical analyses were performed using SAS statistical software (Statistical Analysis Software Inc. 1990). Analysis of variance (ANOVA) and least significant difference (LSD) were used to test the significant difference at 5% ($p<0.05$).

RESULTS AND DISCUSSION

Soil used in the present investigation was sandy loam (71% sand, 21% silt and 8% clay) with pH 7.02, organic matter 0.9% and 9.8% water holding capacity.

Persistence of insecticide in soil is dependent on its chemical properties, soil properties as well as on the prevailing environmental conditions. The summation of various reactions affects movement and dissipations of these chemicals. Some insecticides and/ or their dissipated products accumulate in soil and leach in ground water causing adverse effects.

In the present investigation, quinalphos persistence, dissipation and its effects on soil dehydrogenase activity and alkaline phosphomonoesterases activity was studied under field conditions using soil columns. Quinalphos residues in the surface soil (0-15 cm) of soil column are given in Table 2. Two hrs after the treatment, 0.62 $\pm$ 0.003 µg quinalphos g^{-1} d. wt. soil were detected in the soil which reduced to 0.57 $\pm$ 0.002 µg g^{-1} d. wt. soil in 24 hours (Table 2). Only 76.05% quinalphos residues remained after 3 days (Fig. 1). After 15 days 57.1% quinalphos residues were detected which further reduced to 19.8 % in 90 days (Fig. 1). No quinalphos residues were detected after 180 days of the treatment. Quinalphos dissipation was linear in the present study and followed first order kinetics with upto 80% dissipation observed in 90 days. Low levels of quinalphos residues have been observed both in soil and on crop by many workers under field conditions (Gajbhiye et al. 1995; Batnagar and Gupta 1998). Disappearance of 87-90% quinalphos residues after seed treatment and 98% after field treatment within 75 days of the treatment was also documented by Pandey and Singh (2004).

Logarithmic dissipation plots of quinalphos in the experimental setup can be described by the following equation:

$$y = - ax + b$$

where *a* and *b* denote slope and intercept values of the dissipation line, respectively, 'y' is the concentration (µg g^{-1}) of quinalphos in soil and 'x' is the post- application time in days. The equation describing the dissipation of quinalphos is:

$$y = -0.0101x + 1.97 \ (r^2 = 0.96)$$

Half-life calculated from regression equation in 0-15 cm soil was 30 days (Fig. 1). The terminal residues of organophosphorus insecticides are derived largely by hydrolysis. Quinalphos is known to be susceptible to hydrolysis. In soil, rapid degradation of quinalphos under aerobic conditions with a DT_{50} of about three weeks to quinoxalin-2-ol has been observed (Schmidt 1972). Further breakdown to polar metabolites and CO_2 was also documented (Schmidt 1972). In another study, quinalphos degradation in soil with half-life of 13-15 days was documented (Babu et al. 1998). The metabolite 2-hydroxyquinoxaline was identified and quantified and could be detected in soil after complete dissipation of parent compound at the end of 20 days (Babu et al. 1998). Quinalphos photodegradation, on exposure to natural sunlight with half-life of 30 days in distilled water has also been documented (Dureja et al. 1988). In general, organophosphorus insecticides

Table 2. Quinalphos residues and leaching in soil columns

Time (days)	Quinalphos residues in soil (μg g^{-1} d.wt. soil) ± S.D	
	Top soil (0-15cm)	Sub soil (15-30cm)
0	0.62 ± 0.003	*ND
1	0.57 ± 0.002	*ND
3	0.47 ± 0.003	0.0006 ± 0.0001
7	0.39 ± 0.003	0.0007 ± 0.00011
15	0.35 ± 0.003	0.0016 ± 0.00034
30	0.27 ± 0.003	0.0019 ± 0.00031
60	0.20 ± 0.004	0.0250 ± 0.0013
90	0.12 ± 0.002	0.0030 ± 0.00022
180	*ND	*ND

* ND – Not detectable

have been observed to be less persistent with a half-life ranging from one to three weeks (Meher and Sethi 1992; Suri and Joia 1996).

Significant insecticide losses occur due to leaching to subsurface soil. Losses are even more in the case of water-soluble insecticides with low adsorption on soil. Excessive leaching of insecticide poses a threat to the ground water. As most of the insecticides undergo transformations in soil, sometimes the aqueous solubility of metabolite / transformed compound may exceed the solubility of the parent compound. Leaching of quinalphos to subsoil (15-30 cm) was observed from 3rd day onwards (Table 2). Quinalphos residue in 15-30 cm soil on 3rd day was 0.092% of the initial residues in upper soil layer. Leaching upto 0.025 ± 0.0013 μg quinalphos g^{-1} d. wt. soil was observed in 60 days. No detectable residues were observed after 180 days of application in the subsoil samples. Quinalphos leaching to subsurface soil was also observed by Awasthi et al. (1984). The determination of soil leaching rates is important because the rate of leaching indicates how long a chemical is retained in the soil, where it is subjected to degradation and dissipation. It also allows an estimation of the potential of the insecticides for contamination of ground water.

Soil enzymes are very useful in describing and making predictions about an ecosystem's function, quality and interaction among subsystems. The most use of soil enzyme perhaps, is to assess the effects of various inputs on the relative soil health. In the present work, dehydrogenase activity ranged from 6.02 to 10.22 μg TPF g^{-1} d. wt. soil 24 hr^{-1} and 4.15 to 8.73 μg TPF g^{-1} d. wt. soil 24 hr^{-1} in the control and quinalphos treated soils respectively (Fig. 2). On the zero day of treatment the activity in the treated soil was 7.72 μg TPF g^{-1} d. wt. soil 24 hr^{-1},

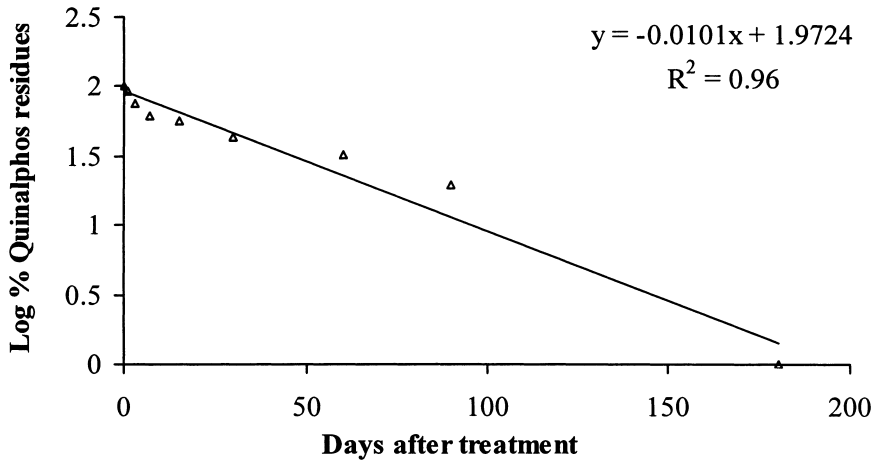

Figure 1. Log percentage dissipation of quinalphos residues in soil under field conditions.

which was 5.6 % lower than the control. Upto 30.3% inhibition ($p< 0.05$) in DHA was observed after 15 days. Maximum inhibition upto 35.5% ($p< 0.05$) was observed in DHA after 30 days of treatment compared to control soil. However, DHA in both the columns become similar within 90 days of treatment. This temporary inhibition in dehydrogenase activity in the quinalphos treated soil when compared to the control soil may be due to adaptation of soil microbes to counter the effect of chemical stress in hostile conditions. Similar observations were made by Megharaj et al (1998). The environmental factors and the availability of oxygen also influence the dehydrogenase activity. Few studies related to the effect of insecticides on soil enzymes have been documented in literature (Schuster and Schröder 1990; Tu, 1995; Pozo et al. 1995). Most of the results are from studies conducted under laboratory controlled conditions and results vary with the insecticide and the enzyme studied (Malkomes and Wohler 1983; Tu 1988, 1995). Decrease in DHA, after insecticide treatment followed by recovery in 14 days was observed (Pozo et al. 1995) whereas no effect of insecticides on enzyme activities including DHA has also been documented under laboratory conditions (Tu 1980, 1988, 1995).

Phosphomonoesterase catalyse the hydrolysis of organic phosphomonoester to organic phosphorus, which could be utilized by plants as nutrient. It is very likely that alkaline phosphatases in soil are mainly derived from microorganisms adaptive to the soil environment (Dick and Tabatabai 1983). Alkaline phosphomonoesterase activity in the present study, varied from 84.77 to126.29 μg pNP g^{-1} d. wt. soil hr^{-1} and 84.89 to 159.67 μg pNP g^{-1} d. wt. soil hr^{-1} in the control and quinalphos treated soil respectively (Fig. 3). The enzyme activity in treated soil, after 4 hrs of exposure to quinalphos was 0.56% less than the control

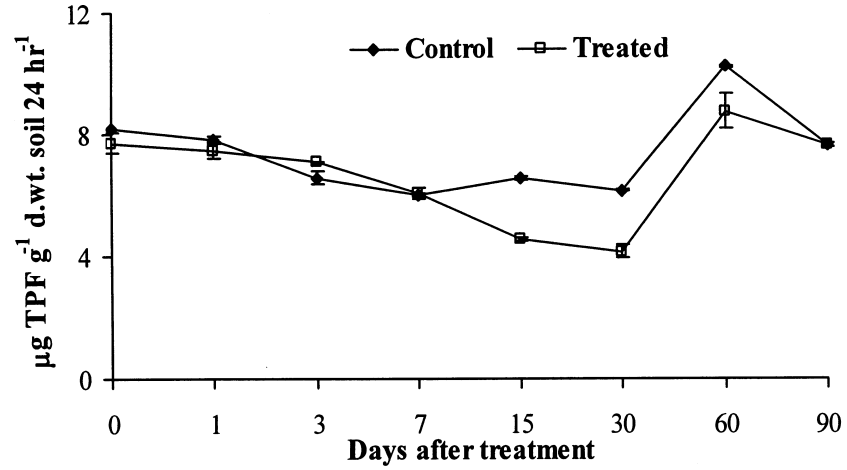

Figure 2. Dehydrogenase activity in control and quinalphos treated soils.

soil. A transient depression of enzyme activity in the treated soil was noticed in the initial days of treatment till 7 days when compared to control soil. Maximum inhibition of 11.66% ($p<0.05$) in quinalphos treated soil compared to control was observed in 1 day sample, however, this lasted for very short period and recovery in alkaline phosphomonoesterase activity in treated soil was observed from 15th day onwards of soil treatment when compared to control.

The transient inhibition in the alkaline phosphomonoesterase activity in the initial days of the application could be due to the toxic effects of quinalphos which was neutralized after the concentration of this insecticide lowered in the soil. Similar inhibition in phosphomonoesterase activity was also reported by Tu (1995). Many workers have evaluated the impact of different insecticides and other agro-chemical products, on the soil phosphomonoesterase activity (Ismail et al. 1996). The activity of phosphomonoesterases is strongly influenced not only by pH and temperature but also by the organic matter content, soil moisture and anaerobiosis. Due to these effects, phosphomonoesterase activity in soil varies with the season (Sparling et al. 1986; Pulford and Tabatabai 1988; Restin et al. 1988). Very significant correlations were established between the activities of phosphomonoesterases and those of catalase, invertase, urease, protease, C and N biomass (Djordjevic 1998).

The present data thus indicates temporary inhibition on both dehydrogenase and alkaline phosphomonoesterase activity. However, recovery was observed in both enzyme activities within few days / weeks of soil treatment. One of the factors responsible for the fluctuation in the enzymes activities following insecticide application may be due to the differences in the toxicity of the parent compound and its metabolites. Other factors like pH, soil moisture, organic matter content, variation in climatic factors etc also influence the soil metabolic activity. And of

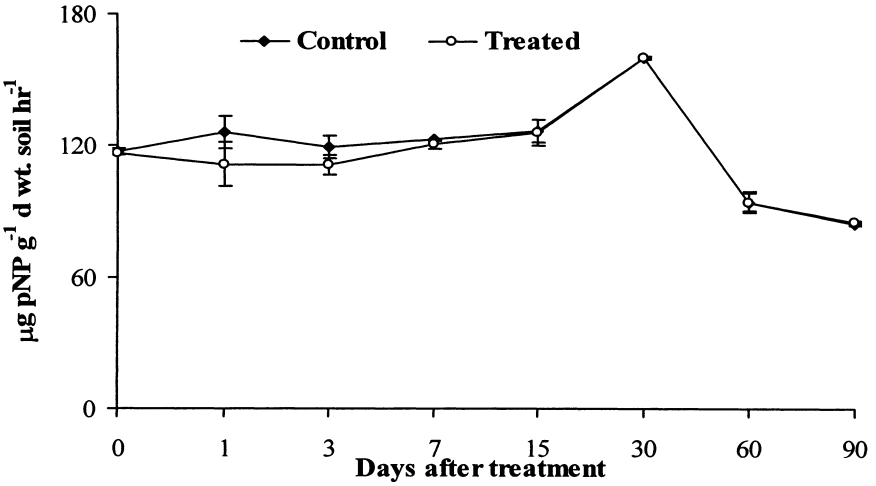

Figure 3. Alkaline phosphomonoesterases activity in control and quinalphos treated soils

course, soil enzyme activities are also affected by the persistence, concentration and bioavailability of the chemical and its mode of inhibition. The results of this study, therefore, indicate that quinalphos is moderately persistent with half-life of 30 days. It is capable of leaching to subsurface soil and therefore, poses a problem of groundwater contamination if present in large concentration. Among the soil enzyme studied, dehydrogenase was more sensitive to quinalphos residues compared to alkaline phosphomonoesterases and the effects observed were only temporary.

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