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Persistence of Quinalphos and Occurrence of Its Primary Metabolite in Soils

G. V. A. K. Babu,[1] B. R. Reddy,[1] G. Narasimha,[1] N. Sethunathar[2]

[1] Department of Microbiology, Sri Krishnadevaraya University, Anantapur 515 003, Andhra Pradesh, India
[2] Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack 753 006, Orissa, India

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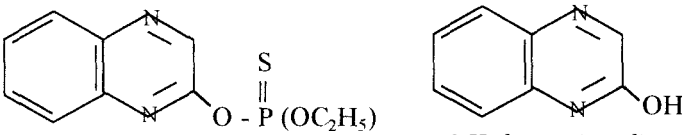
Quinalphos (*O,O*-diethyl *O*-quinoxalin-2-yl phosphorothioate) is one of widely used organophosphorus insecticides in Indian agriculture for control of incidence of pest attack over certain crops such as cotton, groundnut and rice (Reddy and Ghewande 1986; Jena *et al.* 1990; Armes *et al.* 1992). Quinalphos persisted for 125 days with half-life of 5 days in acid lime fruits (Awasti and Ahuja 1989). Build-up of tolerance to quinalphos in insects was attributed to extensive usage of insecticide (Armes *et al.* 1992). In spite of intensive usage of quinalphos, its fate and behaviour in the environment, in particular in soil is not clearly understood in comparison to other organophosphorus insecticides (Lal 1982). Only monitoring of the parent compound or insecticidal property figured in studies conducted on this insecticide in plants (Awasti and Ahuja 1989; Bodhade *et al.* 1987), cyanobacteria (Megharaj *et al.* 1987, 1988) and bacteria (Rangaswamy and Venkateswarlu 1992). Hence, we report the formation of the metabolite, 2-hydroxyquinoxaline during the degradation of quinalphos in soils under aerobic conditions in the present investigation.

MATERIALS AND METHODS

Quinalphos of technical grade (purity 99%) and of a commercial formulation. Ekalux (EC 25) and 2-hydroxyquinoxaline (purity 99.5%) obtained from Sandoz (India) Ltd. Bombay, were used in the present study and their structures are represented in Figure 1. All other chemicals used in this study were analytical reagent grade.



Quinalphos 2-Hydroxyquinoxaline

Figure 1. Structures of quinalphos and 2-Hydroxyquinoxaline

Two soils, a red sandy loam-sandy clay soil (pH 8.3, organic matter 0.83%, total nitrogen 0.046%) from groundnut field of Anantapur and a black clay soil (pH 7.2, organic matter 1.08%, total nitrogen 0.056%) from cotton field of Nandyal (a semi-arid zone of Andhra Pradesh, India), collected to a depth of 12 cm, were air-dried and seived through a 2-mm mesh screen before use.

Correspondence to: B. R. Reddy

For the determination of persistence of quinalphos, 10-gram portions of each soil were distributed into 2 sets of test tubes (150 X 15 mm) and were uniformly mixed with aqueous solution of commercial formulation of quinalphos (Ekalux) to yield final concentrations of 10 or 100 µg of active ingredient g^{-1} soil (ppm). After the addition of sterile distilled water to get 60% water-holding capacity (WHC), soil samples were incubated at room temperature (28 ± 4°C). At desired intervals, the parent compound quinalphos and its metabolites, if any, formed during degradation were separately extracted in duplicates from the first and second sets of soil samples, respectively and analysed in the manner as described earlier (Adhya *et al.* 1987).

In order to find out the extent of chemical *versus* biological degradation of quinalphos, 10-gram portions of soil samples moistened at 60% WHC, were sterilised by autoclaving at 121 °C for 1 hour for three consecutive days. Both sterile and non-sterile soils were treated with filter-sterilized aqueous commercial formulation of quinalphos at 10 µg g^{-1} soil and incubated at 28 ± 4°C. Residues in duplicate soil samples were analysed at regular intervals by gas chromatography (GC) after extraction.

In order to determine persistence of quinalphos in soils with preexposure to the same insecticide, soil samples were treated with aqueous solution of quinalphos in staggered manner as earlier studied by Rajagopal *et al.* (1983). Tubes (150 X 15 mm) containing 10-gram portions of each soil were divided into 3 sets. Tubes of the first set recieved quinalphos at 10 µg g^{-1} soil whereas other two sets recieved only sterile distilled water. After ten days of incubation at room temperature quinalphos was added to tubes of the first and second sets at the same concentration whereas the third set recieved only sterile distilled water. At this stage, soil samples of first, second and third sets recieved 2.1 and 0 applications of quinalphos. At the end of another 10 days of incubation, the final application of quinalphos at 10 µg g^{-1} soil was made to soil samples of all 3 sets. After incubation at room temperature (28 ± 4°C) with maintenance of 60% WHC, residues in duplicate soil samples at regular intervals were extracted and analysed as mentioned earlier.

For extraction of quinalphos residues for GC analysis, 10 mL distilled water was added to the first set of soil samples and contents were transferred to 250-mL Erlenmeyer flask with 25 mL of acetone and shaken on a rotatory shaker. After 2 hours of equilibration with acetone, 10 mL of hexane was added and contents shaken for 1 hour. Then all the contents in the flask were transferred to 100 mL volumetric flask followed by the addition of 2% solution of Na_2SO_4 to make the volume upto 100 mL. Residues of quinalphos in the hexane fraction were quantified by GC after suitable dilutions with hexane.

Quinalphos residues extracted in hexane were analysed in a Varian 3600 Gas chromatograph fitted with a phosphorus-specific flame photometric detector. The spiral glass column (2 mm OD, 2 m in length) was packed with 1.5% OV- I7 and 1.95% OV-210 on chromosorb W-HP, 80/100. The operating conditions were as follows: argon (carrier gas) flow, 30 mL min^{-1}; hydrogen flow, 30 mL min^{-1}; air flow, 300 mL min^{-1}; injector temperature, 220°C; column temperature, 210°C; and detector temperature, 230°C. Under these conditions the retention time of quinalphos was 3.1 min. The recovery of parent insecticide, quinalphos from soils by the extraction and analytical procedures employed was 95 ± 5%.

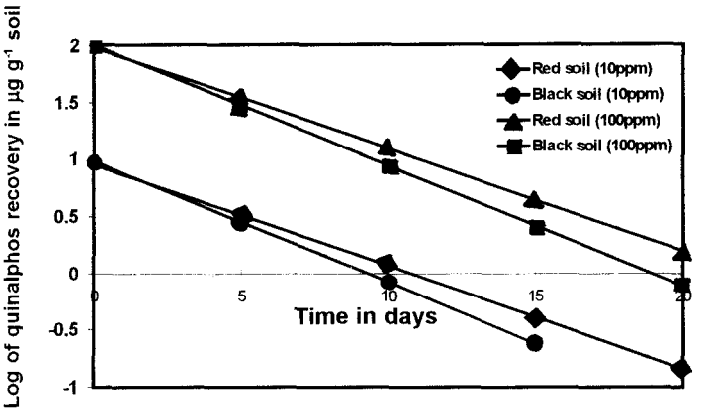


Figure 2. Kinetics of dissipation of quinalphos in soils at concentrations of 10 and 100 µg g^{-1} soil

For the identification and estimation of any metabolite formed during degradation from parent insecticide, duplicate soil samples of another set were transferred into 250-mL Erlenmeyer flask and 10 mL distilled water was added. The residues were extracted by shaking the soil samples for 2 hours with 25, 20 and 15 mL portions of chloroform-diethyl ether (1:1) each in succession. After each extraction chloroform-diethyl ether fractions were pooled into 100 mL beaker, allowed to evaporate at room temperature, and residues were redissolved in methanol for further separation by thin-layer chromatography (TLC).

The methanolic fraction was spotted along with authentic compounds, 2-hydroxyquinoxaline and quinalphos on 300-µm-thick silica gel-G plates. The plates were developed for a distance of 15 cm with hexane-chloroform-methanol (7:2:1) and air-dried. After drying, the plates were observed under UV light for localization of the parent compound and its metabolite. The silica gel of the samples alongside to the authentic compound, 2-hydroxyquinoxaline were scraped off carefully, transferred to centrifuge tubes and 5 mL of ethanol was added. After shaking for 1 min, tubes were centrifuged at 4000 rpm for 15 min and the absorbance of the supernatant was measured at 308 nm in a dual-beam Shimadzu UV-Visible spectrophotometer (model 1601). The quantity of metabolite, 2-hydroxyquinoxaline, was calculated by comparing the absorbance values with a standard curve prepared with the authentic compound. The recovery of 2-hydroxyquinoxaline from soil samples by extraction and analytical procedures employed was 72 ± 3%.

RESULTS AND DISCUSSION

The amount of quinalphos recovered from these soils were plotted on a log scale against time of incubation (Frost and Pearson 1961). The dissipation pattern of quinalphos followed a first-order kinetics as the plots yielded straight lines (Figure 2) based on equation $C=C_o e^{-kt}$, where C is the concentration of insecticide remaining in the soil after time t, C_o is the initial concentration and k is the first-order kinetic constant. The degradation pattern of quinalphos from these plots was characterized by half-lives ($t_{1/2}$) and rate constants (k). The dissipation pattern of quinalphos in these soils at lower and higher concentrations were similar as indicated by half-life and rate constants computed from the plots. The rate constant and half-life for disappearance of quinalphos in black cotton soil and red sandy loam-sandy clay soil

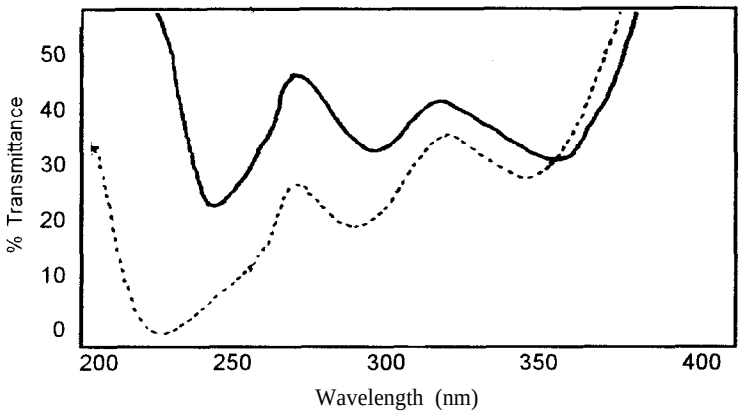


Figure 3. Ultraviolet spectrum of a metabolite formed from the degradation of quinalphos, (----) authentic 2-hydroxyquinoxaline, (---) metabolite

under aerobic conditions were 0.0533 and 0.0462 day^{-1} and 13 and 15 days, respectively. Besides the parent compound, a flourescing metabolite on TLC plate was detected. This metabolite had an Rf value corresponding to that of 2-hydroxyquinoxaline. and the spectrum of this metabolite matched with that of an authentic compound, 2-hydroxyquinoxaline (Figure 3) with an absorption maximum at 308 nm. This metabolite identified as 2-hydroxyquinoxaline, was quantified by UV-spectrophotometry. Formation of 2-hydroxyquinoxaline to the tune of 7 $\mu g\ g^{-1}$ soil within 10 days from quinalphos added to soils at 10 ppm level was observed (Table 1). The metabolite was still present in significant amounts in soils in spite of complete degradation of the parent compound at the end of 20 days. Higher amounts of 2-hydroxyquinoxaline were recovered from soils fortified with quinalphos at 100 ppm.

Generally, organophosphorus insecticides are rapidly degraded with less persistence in soils (Lal 1982). For instance, parathion disappeared from soils within 14 days after its application and submergence of soils enhanced the degradation of parathion (Sethunathan and Yoshida 1973). A comparative study conducted by Racke and Coats (1988) indicated relative persistence of organophosphorus

Table 1. Formation of 2-hydroxyquinosnline[a] from quinalphos - treated soils.

Incubation (days)	Quinalphos application			
	Black clay soil		Red sandy loam soil	
	10ppm	100 ppm	10 ppm	100 ppm
10	6.4 ± 1.0	41.37 ± 2.8	7.6 ± 1.0	26.42 ± 2.5
20	5.4 ± 0.6	37.14 ± 1.8	8.8 ± 0.8	33.71 ± 3.9

[a]Results ($\mu g\ g^{-1}$ soil) are means of duplicates ± deviation

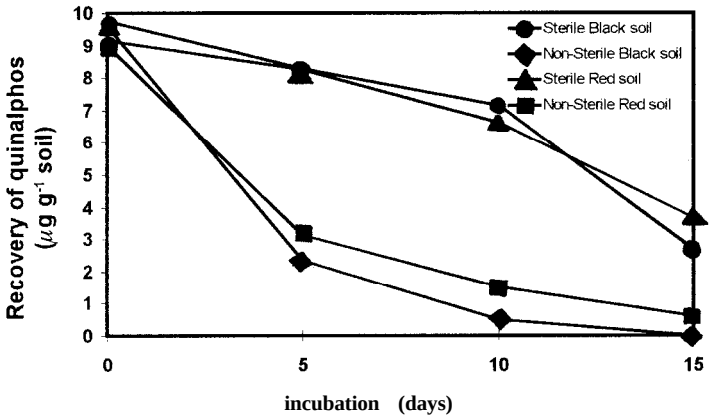


Figure 4. Recovery of quinalphos from sterile and non-sterile soils at 10 µg g^{-1} soil

insecticides in the following descending order: isofenphos>fonophos>chlorpyrifos> ethoprop >> fenofos>phoret. Rapid degradation of organophosphorus insecticides such as parathion, methyl parathion and fenitrothion resulted in their faster disappearance from alluvial soils under both flooded and non-flooded conditions with shorter half-lives (about 2 weeks) (Adhya *et al.* 1987). Like other organophosphorus insecticides, quinalphos persisted for 2 weeks in soils under aerobic conditions in the present investigation. This insecticide was short-lived with 5-days of half-life even in horticultural fruits (Awasti and Ahuja 1989).

Earlier studies with this insecticide in soils (Megharaj *et al.* 1988) or pure cultures of bacteria (Rangaswamy and Venkateswarlu 1992) determined only the parent compound or insecticidal property associated with the parent compound (Armes *et al.* 1992). In a recent study on metabolism of quinalphos by algae (Megharaj *et al.* 1957), two unidentified metabolites were reported. Formation of 2-hydroxyquinoxaline as a primary metabolite in the metabolism of quinalphos in the present study is probably the first report to our knowledge. Hydrolysis constitutes major means of degradation of organophosphorus insecticides in terrestrial, aqueous ecosystem and in pure culture studies (Lal 1982). Like other organophosphorus insecticides, quinalphos underwent hydrolysis in soils in the present study as clearly evident from formation of 2-hydroxyquinoxaline.

Though the disappearance of quinalphos was faster in non-sterile soils than in sterile soils, the amount of quinalphos dissipated even from sterile soils was considerable (Figure 4). Sixtyfive to seventy per cent of initially added quinalphos recovered from sterile soils as compared to 5-15% from non-sterile soils at 10-day interval. The organophosphorus insecticides are more susceptible to chemical hydrolysis at alkaline and neutral pH (Faust and Gomaa 1972). The degradation of quinalphos in sterile soils could be due to chemical means because of soils with slightly alkaline conditions used in this study and prevailing of neutral conditions during incubation. Similarly, more rapid degradation of organophosphorus insecticides including quinalphos from non-sterile soils and aqueous systems than from corresponding sterile systems was reported (Miles *et al.* 1979; Adhya *et al.* 1987; Megharaj *et al.* 1988).

Table 2. Degradation of quinalphos in black clay soil pre-treated with quinalphos

Incubation (days)	Residues[a] recovered in µg g^{-1} from soil pre-treated with quinalphos[b] of					
	2 applications		1 application		0 application	
	Quinalphos	2-Hydroxy-quinoxaline	Quinalphos	2-Hydroxy-quinoxaline	Quinalphos	2-Hydroxy-quinoxaline
0	11.60 ± 0.7	-	10.50 ± 0.6	-	9.00 ± 0.5	-
5	6.01 ± 0.3	-	4.56 ± 0.4	-	3.10 ± 0.3	-
10	1.75 ± 0.1	17.70 ± 1.4	1.10 ± 0.1	12.55 ± 2.3	0.85 ± 0.0	6.1± 0.8
15	0.50 ± 0.0	-	0.42 ± 0.0	-	0.25 ± 0.0	-
20	0.15 ± 0.0	15.55 ± 0.5	0.10 ± 0.2	15.10 ± 0.9	0.07 ± 0.0	5.2 ± 1.3

[a]Results (µg g^{-1}soil) are means of duplicates ± deviation
[b]In each application quinalphos added to soil at 10 µg g^{-1} soil
- not determined

The degradation rates of quinalphos were similar in both soils with/without preexposure (Tables 2 and 3). The amounts of quinalphos recovered at the end of 10-day interval after the final application of insecticide from both soils with/without pretreatment fell within range from 1 to 3 ppm. Even at the end of 20-day interval after the fina1 application of quinalphos, both soils with pretreatment registered formation of 15-20 ppm of 2-hydroxyquinoxaline from quinalphos in comparison to only 5-8 ppm in soils without pretreatment.

In the present study, repeated applications of quinalphos to soils failed to accelerate the degradation rate under laboratory conditions. As a result, quinalphos persisted to the same extent in soils with and without preexposure. But recovery of 2-hydroxyquinoxaline in higher amounts from soils with repeated applications may probably be due to accumulation of the product formed from previous application of its parent compound to soils because of its longer persistence. Similarly, chlorpyrifos was resistant to enhanced biodegradation in soils with repeated applications (Racke

Table 3. Degradation of quinalphos in red sandy loam-sandy clay soil pre-treated with quinalphos

Incubation (days)	Residues[a] recovered in µg g^{-1} from soil pre-treated with quinalphos[b] of					
	2 applications		1 application		0 application	
	Quinalphos	2-Hydroxy-quinoxaline	Quinalphos	2-Hydroxy-quinoxaline	Quinalphos	2-Hydroxy-quinoxaline
0	11.70 ± 0.5	-	12.90 ± 0.1	-	9.00 ± 0.8	-
5	5.60 ± 0.1	-	5.50 ± 0.1	-	3.10 ± 0.1	-
10	2.70 ± 0.1	20.95 ± 1.3	2.20 ± 0.1	13.00 ± 0.8	1.50 ± 0.0	7.0± 0.5
15	0.80 ± 0.1	-	0.50 ± 0.1	-	0.60 ± 0.0	-
20	nd	22.95 ± 1.3	nd	17.30 ± 0.9	0.20 ± 0.0	8.2 ± 1.0

[a]Results (µg g^{-1}soil) are means of duplicates ± deviation
[b]In each application qninalphos added to soil at 10 µg g^{-1} soil
- not determined
nd not detected

and Coats 1990). This observation was well in agreement with the results of present study. In contrast, the persistence of certain organophosphorus insecticides in soils is inversely related to degradation ofrespective insectcide which in turn, is dependent on enrichment ofinsecticide-degrading microorganisms in soils with repeated applications. For instance, diazinon could not be detected 3 days after its application to paddy field, which had recieved earlier 3 applications of the same pesticide at 20 kg ha^{-1} at 20-day interval due to the development ofbiological factors (Sethunathan and Pathak 1972). Repeated applications of parathion to soils under flooded conditions reduced its residence time in soils from 25 days to 7 days (Barik *et al.* 1979). Similarly, enhanced degradation of isofenphos (Racke and Coats 1987), fonofos (Racke and Coats 1988), methyl parathion and fenitrothion (Misra *et al.* 1992) and fenamiphos sulfoxide and fenamiphos sulfone (Chung and Ou 1996) occurred in soils with a long history of usage of the respective insecticide.

It can be concluded from this laboratory study that quinalphos persisted in soils with a half-life of 2 weeks and was hydrolysed to 2-hydroxyquinoxaline in soils and was not susceptible to enhanced biodegradation with repeated applications to soils.

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