JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Two Insecticides, Chlorpyrifos and Quinalphos, on Arginine Ammonification and Mineralizable Nitrogen in Two Tropical Soil Types

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Effects of seed treatments with chlorpyrifos [5 g of active ingredient (ai) kg^{-1} of seed] and quinalphos (6.25 g of ai kg^{-1} of seed) and standing crop treatments with chlorpyrifos (800 g of ai ha^{-1}) and quinalphos (1000 g of ai ha^{-1}) on arginine deamination and mineralizable nitrogen were monitored, in the sandy loam and loamy sand soils of two tropical semiarid fields, for three consecutive crop seasons. The arginine ammonification activity of rhizospheric microbes was inhibited after seed treatment with chlorpyrifos and quinalphos and their principal metabolites, 3,5,6-trichloro-2-pyridinol (TCP) and 3,5,6-trichloro-2-methoxypyridine (TMP) and 2-hydroxyquinoxaline and quinoxaline-2-thiol, respectively. Quinalphos produced transient inhibitions, whereas chlorpyrifos and its metabolites (TCP and TMP) exerted a greater inhibition in both loamy sand and sandy loam soils. Arginine ammonification by nonrhizospheric microbes was stimulated by standing crop treatments with both pesticides. In the loamy sand soil, the parent compounds stimulated rhizospheric N-mineralization, whereas the metabolites were inhibitory. However, nonrhizospheric N-mineralization was inhibited by both chlorpyrifos and quinalphos and stimulated by their metabolites. A higher magnitude of inhibition of arginine deamination in the loamy sand than in the sandy loam soil could be due to greater bioavailability of the pesticides in the former, resulting from lesser sorption of the pesticides due to alkalinity of the soil and its low content of clay and organic carbon. Although both pesticides affected mineralizable nitrogen, seed treatment with quinalphos and standing crop treatment with quinalphos and chlorpyrifos produced the most significant effects. The recommended doses of the pesticides not only efficiently controlled whitegrubs, which increased pod yields, but also left no residues in harvested kernels. They also caused no long-term inhibition of ammonification, which could have been of significant concern during the short crop period in semiarid areas where nitrogen determines plant productivity.

KEYWORDS: Chlorpyrifos; quinalphos; arginine deaminase; mineralizable nitrogen; semiarid; whitegrubs; ammonification

INTRODUCTION

Arginine deaminase catalyzes the mineralization of nitrogenous compounds in soil to release ammonium and nitrate, which are the principal sources of nitrogen for plants. As a function of nitrogen metabolism, arginine ammonification reflects the potential activity of microbes rather than the fluctuations of microbial populations (*1*) because it correlates strongly with the amidohydrolase or amidase activity of soil bacteria and fungi, the carbon content of soil (*2*, *3*), and microbial biomass and respiration as an index of oxygen consumption (*1*, *4*−*8*). The rate of ammonium production is inversely proportional to the C N^{-1} ratio of an amino acid and has been correlated to N mineralization in soils (*1*, *9*, *10*).

A blanket application of agrochemicals, namely, metolachlor, pendimethalin, chlorpyrifos, quinalphos, monocrotophos, phorate, carbofuran, thiram, benomyl, and carbaryl, for the protection of oilseeds from whitegrubs (*Holotrichia* and *Maladera* spp.) and other pests in the semiarid regions masks the properties of soils, which are important for crop production, impairs soil "health" (*11*−*15*), and affects the sustainability of agricultural systems (*16*−*18*). Soil enzymes are indicators of biological equilibrium (*8*), fertility (*11*, *17*−*19*), and changes in the biological status of soil due to pollution (*16*, *20*). Both processes of N-mineralization, namely, ammonification and nitrification, are sensitive to pesticides (*21*). In agronomical terms, however, the inhibition of ammonification more than nitrification is a

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10.1021/jf049502c CCC: $27.50
Published on Web 10/30/2004

limiting factor for agricultural productivity and determines the fertilizer regimen needed (*22*). A Coordinated Research Project (CRP) on Whitegrubs recommends the application of (a) chlorpyrifos and quinalphos at specific doses for the treatment of both monsoon-sown seeds and pre-monsoon-sown standing groundnut (*Arachis hypogaea* L.) crops and (b) a single application of urea (33 kg ha^{-1}) and superphosphate (60 kg ha^{-1}). The objectives of the CRP were to reduce the burden of pesticides in soils, to enhance both pest control efficacy and the yield of groundnut kernels having pesticide residues below the maximum residue limit (MRL), and to design further effective pest control measures (*23*) that would require a limited use of agrochemicals. Nitrogen is a limiting factor in plant productivity. However, data about the effects of agrochemicals on N-cycling in different semiarid soils are scant. Because arginine ammonification is an indicator of the metabolic status of soil microbes (in situ), which can be monitored without causing any changes in microbial number and activity during analyses (*7*), the effects of quinalphos and chlorpyrifos on mineralizable nitrogen and arginine ammonification were monitored in two semiarid fields having different types of soils and pesticide(s) usage.

MATERIALS AND METHODS

Design of the Experiment. The trials were conducted for three successive crop seasons in the field: (a) plots of the Department of Zoology, University of Delhi (season 1) and (b) at the Agricultural Research Station (ARS), Jaipur (seasons 2 and 3). The groundnut seeds were sown (110 kg ha^{-1}) in late June−early July, and the pods were harvested by mid-November. Eight plots of average size 40 m^2 were used for the control (which had no pesticide treatments for the three seasons) and for each of the two types of treatments with the following pesticides: (1) quinalphos (*O*,*O*-diethyl *O*-quinoxalin-2-yl phosphorothioate), 25% emulsified concentrate (EC), 20% aqua flow (AF) of Sandoz India Ltd. and 25 EC (Tropical Agroecosystems Ltd.)' (2) chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloropyridyl)phosphorothioate], 20 EC of Searle Agrochemicals Ltd. and ring-labeled [^{14}C]chlorpyrifos (specific activity = 35.63 kBq mg^{-1}).

Treatments with Pesticides. The applications of the pesticides at the doses mentioned below were done per the methods recommended in the Technology Bulletin of ICAR (*24*).

1. [^{14}C]Chlorpyrifos. During season 1, 68 mg of active ingredient (ai) chlorpyrifos and 97.02 μCi of [^{14}C]chlorpyrifos in 600 μL of hexane/ethanol (2:1, v v^{-1}), dissolved in a total volume of 789 mL of water, was sprayed over the soil of an enclosed subplot.

2. Chlorpyrifos 20 EC. (a) Delhi. Sequential treatments for season 1 involved (i) presowing soil drenching with quinalphos 25 EC (1000 g of ai ha^{-1}) on day 0, (ii) sowing of chlorpyrifos-treated (5 g of ai kg^{-1} of seed) seeds on day 14, and (iii) standing crop treatment (SC) with chlorpyrifos (800 g of ai ha^{-1}) on day 40.

(b) Jaipur. For seasons 2 and 3, two treatments were done in separate plots: (i) the presowing seed treatment (ST) at the rate 5 g of ai kg^{-1} of seed and (ii) SC at the rate 800 g of ai ha^{-1}, done 14 days after ST.

3. Quinalphos. The trials carried out during seasons 2 and 3 involved (a) ST with (i) 25 EC (6.3 g of ai kg^{-1} of seed) for season 2 and (ii) 20 AF (5 g of ai kg^{-1} of seed) for season 3 and (b) SC done 14 days after ST with (i) 25 EC (1000 g of ai ha^{-1}) for season 2 and (ii) 20 AF (800 g of ai ha^{-1}) for season 3.

Sampling Procedure and Physicochemical Analyses. The estimation of pesticide residues and physicochemical characterization were done using three and five replicates, respectively, from a sample of 1 kg of soil per plot, which was collected using an auger from 0−20 cm depth. The soil was sieved (<2 mm) and dried overnight at ambient temperature and 20−60% relative humidity (RH). Five replicates of field-fresh soils were used for quantifying arginine deamination. Organic carbon (OC) (*25*), organic matter (OM) (*26*), mineralizable N (*27*), pH (*28*), mineralizable P (*29*), electrical conductivity, clay, silt and sand contents (*30*), and the water-holding capacity (*31*) were estimated. The chemicals used were of analytical reagent grade. The solvents were double distilled before use.

Arginine Deaminase Activity. Arginine deamination was quantified (*7*, modified) by incubating 5 g of soil for 3 h at 37 °C with 2 mL of L-arginine solution (11.5 M). The NH_4^+-N released was extracted with 18 mL of potassium chloride (2 M) and filtered. One milliliter of the filtrate was mixed with 3 mL of potassium chloride solution (2 M), 2 mL of sodium phenolate solution (0.12 M), 1 mL of sodium nitroprusside solution (0.17 mM), and 1 mL of sodium hypochlorite solution (0.005 M NaOCl in 0.125 M NaOH) and allowed to stand for development of color. The calibration curve was prepared using dilutions of NH_4Cl (working) standard (10 μg of NH_4^+-N mL^{-1}), which was processed similarly. The colorimetric determination as micrograms of NH_4^+-N per gram of dry matter pe hour was done using a UV−vis spectrophotometer (Shimadzu, Biospec-1601).

Residue Analyses. *1. Extraction and Cleanup. (a) Chlorpyrifos.* Three replicate samples (50 g) of the two soils were Soxhlet-extracted with three 50 mL volumes of acetone/methanol (1:1, v v^{-1}) for 48 h, which gave a recovery of 98%. The volume of pooled extracts was restored to 150 mL prior to filtration through a Millipore funnel (Hydrosol stainless steel, 47 mm filter holder). The filtrate was concentrated to 2 mL and then diluted to 25 mL with saturated sodium chloride solution for partitioning with three changes of 25 mL of dichloromethane. The aqueous layer was discarded after re-extraction twice with 10 mL of dichloromethane. The pooled dichloromethane fractions were passed through anhydrous sodium sulfate, concentrated to 10 mL, and resuspended in 1 mL of methanol. Eight hundred microliters of this extract was loaded on C_{18} Sep-Pak Rp cartridges (Millipore, Waters Chromatography), which had been preconditioned at first with 8.5 mL of methanol and then 8.5 mL of water. The cartridge was eluted with acetonitrile/water (9:10, v v^{-1}) and dried. Subsequently, the analyte was eluted with 1 mL of methanol. The total recovery was 97 ± 1%.

(b) Quinalphos and Groundnut Seeds. The procedures for extraction and cleanup have been described (*32*).

2. Analyses of Insecticide Residues. The analyses were done using a high-pressure liquid chromatograph (HPLC, Shimadzu LC-4A) equipped with an SPD-2AS, UV detector set at 240 nm (0.8 AUFS) for the samples from Delhi and at 254 nm (0.16 AUFS) for the samples from Jaipur. The Whatman Partisil-10 ODS-2, C_{18}, reverse phase WC analytical column (4.6 × 250 mm) was used at 25 °C. The mobile phase was set at a flow rate of 0.5 mL min^{-1} for the analyses of samples from Delhi and at 1.0 mL min^{-1} for samples from Jaipur. Methanol was used as mobile phase for the analyses of samples from Delhi and for the detection of quinalphos in the samples from Jaipur. For the detection of chlopyrifos in the samples from Jaipur, hexane/dichloromethane/methanol/2-propanol (80:15:0.2:4.8) was used as the mobile phase. Before HPLC analyses, the cleaned up extracts were passed through 0.45 μm nylon (Alltech Associates) or PTFE (Whatman) syringe filters. Fifteen second fractions from HPLC analyses of ^{14}C treatments were assayed using a liquid scintillation counter (Packard, 2000 CA) with capacity for autoquench correction. The retention times were 6.2 min for quinalphos and 7.1 min for chlorpyrifos for samples from Delhi. For Jaipur, the retention times were 3.7 min for quinalphos, 3.09 min for quinoxaline-2 thiol, 5.4 min for 2-hydroxyquinoxaline, 2.5 min for chlorpyrifos, 4.4 min for 3,5,6-trichloro-2-pyridinol (TCP), and 2.9 min for 3,5,6-trichloro-2-methoxypyridine (TMP). The identification of the pesticides was done by comparison with the retention times of the standards and gas chromatography−mass spectrometry analyses (GC-MS Fisons make, model Tlio-1000) of the fractions eluted from HPLC analyses. The ionization was done using electron impact and the analyses by quadrapole. The standardization for MS analyses was done using peaks of heptachlor at 69, 219, 264, and 502 for maximum signal intensity.

Statistical Analyses. The data were checked for normality (Kolmogorov−Smimov test) and homogeneity of variance (Cochran's C) and then analyzed (*33*) by one-way classified analysis of variance (ANOVA) using PROC GLM of SAS (version 8.2). Significant differences in treatments were subjected to multiple comparisons, and the least significant difference (LSD) values ($P < 0.05$) were calculated when the effects of treatments were found to be significant. The mean

Table 1. Properties of the Soils of the Two Semiarid Regions

properties	Delhi	ARS, Jaipur
soil texture and type	sandy loam (a) sand, 59 ± 0.3% (b) silt, 26 ± 0.4% (c) clay, 15 ± 0.1% Typic Haplustepts	loamy sand (a) sand, 83 ± 1.2% (b) silt, 14 ± 0.4% (c) clay, 3.4 ± 1% Typic Ustipsamments
pH		
soil/H_2O (1:2.5)	7.7	8.2
soil/KCl (1:2.5)	7.4	7.9
conductivity (EC) in μS	464 ± 0.1 (28.2 °C)	338 ± 0.4 (26.9 °C)
organic carbon %	0.6 ± 0.13	0.3 ± 0.02
organic matter %	1. 02 ± 0.03	0.5 ± 0.1
WHC[a] (g of water per 100 g of dm)	24 ± 2.1	13 ± 1.6
mineralizable N, kg ha^{-1}	66 ± 0.2	147 ± 0.2
mineralizable P, kg ha^{-1}	46 ± 0.1	14 ± 1.1

[a] Water-holding capacity.

Table 2. Arginine Ammonification as Micrograms of N per Gram of Dry Weight of Soil per Hour for Season 1

days after treatment	pesticide untreated (control)[a]	sequential pesticide treatments[a]	[^{14}C]chlorpyrifos-treated area[a]	LSD[b] at 1% (LSD at 5%)
0	11.4 ± 1.13	12.0 ± 1.0	11.0 ± 0.61	NS[c]
7	12.3 ± 0.4	13.2 ± 1.0	12.0 ± 0.5	NS
14	12.0 ± 1.1 a,1	9.0 ± 0.4 b,2	12.1 ± 1.0 a,1	2.43 (1.6)
21	13.6 ± 1.0	10.5 ± 0.4	12.6 ± 2.6	NS
42	6.6 ± 0.6 1	5.5 ± 0.5 2	6.0 ± 0.11 1,2	(1.0)
62	10.6 ± 2.7	9.7 ± 0.6	10.4 ± 2.6	NS
125	11.8 ± 1.4	13.4 ± 1.0	11.2 ± 1.0	NS
142	12.2 ± 2.0	12.8 ± 1.2	12.5 ± 1.3	NS
160	11.2 ± 1.0	11.2 ± 1.2	12.0 ± 1.1	NS

[a] Letters are for significance at $P < 0.01$. Numbers are for significance at $P < 0.05$. Means with the same letter/number are not significantly different. [b] Least significant difference. [c] Not significant at $P < 0.05$.

square errors of the data of seasons 2 and 3 for ST (SC), 1 (−8), 7 (−7), 14 (0), 16 (2), 75 (61), and 90 (76), were nonhomogeneous. The figures in parentheses represent the days after standing crop treatment. Therefore, these data were transformed (Aitkens transformation) by dividing each of the observations by the square root of the corresponding mean square root of the number of replicates (*34*, *35*). The mean squares obtained from the two errors for each set of dates were used for testing the homogeneity of error variances. PROC CORR of SAS (version 8.2) was used for obtaining correlations between arginine deamination and mineralizable N and testing their significance ($P < 0.05$).

RESULTS AND DISCUSSION

The properties of the two soils are given in **Table 1**. Mineralizable N was estimated as 66 kg ha^{-1} in the coarse sandy loamy, mixed, hyperthermic, Typic Haplustepts soil of Delhi and as 147 kg ha^{-1} in the loamy sandy, Typic Ustipsamments soil of Jaipur. Significant differences were observed in the pattern of pesticide dissipation, arginine deamination (**Tables 2**−**4**), and mineralizable nitrogen (**Figure 1**). For season 1, the average pod yield per plot was 0.23 kg from control and 3.01 kg from plots treated with pesticides. For seasons 2 and 3, the average yields per control plot were 0.5 and 0.25 kg, respectively. With chlorpyrifos, the average yields per plot were 4.8 kg with ST and 4.6 kg with SC and for quinalphos they were 4.7 kg for ST and 4.3 kg for SC. The principal metabolites detected for chlorpyrifos were TCP and TMP (**Figure 2**), and for quinalphos they were 2-hydroxyquinoxaline and quinoxaline-2-thiol (**Figure 3**; *32*). Chlorpyrifos and quinalphos had half-lives ($t_{1/2}$) (*36*) of 29.3 and 3.6 days in the sandy loam soil and 12.5 and 6 days in the loamy sand soil, respectively. For all three seasons, no pesticide residues were detected in groundnut kernels analyzed 24 h postharvest.

Effects on Arginine Deamination. For season 1 (**Figure 2**), soil drenching with quinalphos (0.2 μg g^{-1} of soil) and SC with [^{14}C]chlorpyrifos (0.04 μg g^{-1} of soil) had no significant impact on arginine deamination, whereas both ST and SC with chlorpyrifos caused significant inhibitions by day 14 ($P < 0.01$) and day 42 ($P < 0.05$) (**Table 2**).

For seasons 2 and 3, ST with chlorpyrifos (0.12 μg g^{-1} of soil) and quinalphos (0.2 μg g^{-1} of soil) caused prolonged inhibition despite a significant ($P < 0.05$) initial stimulation in enzyme activity during season 3 (**Tables 4** and **5**). This indicated inhibition of ammonification activity of rhizospheric microbes by both the pesticides and their metabolites, because the complete degradation of parent compounds by day 16 in season 2 and by day 14 in season 3 reduced the inhibitions, and levels similar to control were attained by day 45 when their metabolites dissipated completely (**Figure 2**). For season 2, chlorpyrifos and its metabolites (TCP and TMP) produced more prolonged inhibitions (of 32% even by day 30) and were therefore more inhibitory than quinalphos or its metabolite quinoxaline 2-thiol (**Figure 3**; *32*). The percent increase/decrease mentioned is based on the respective control for the day. Significant fluctuations observed in season 3 on day 60 after ST with chlorpyrifos resulted from an erroneous mixing of a batch of soil samples with subsamples from a heavily manured plot of another field,

Table 3. Arginine Ammonification as Micrograms of N per Gram of Dry Weight of Soil per Hour for Season 2

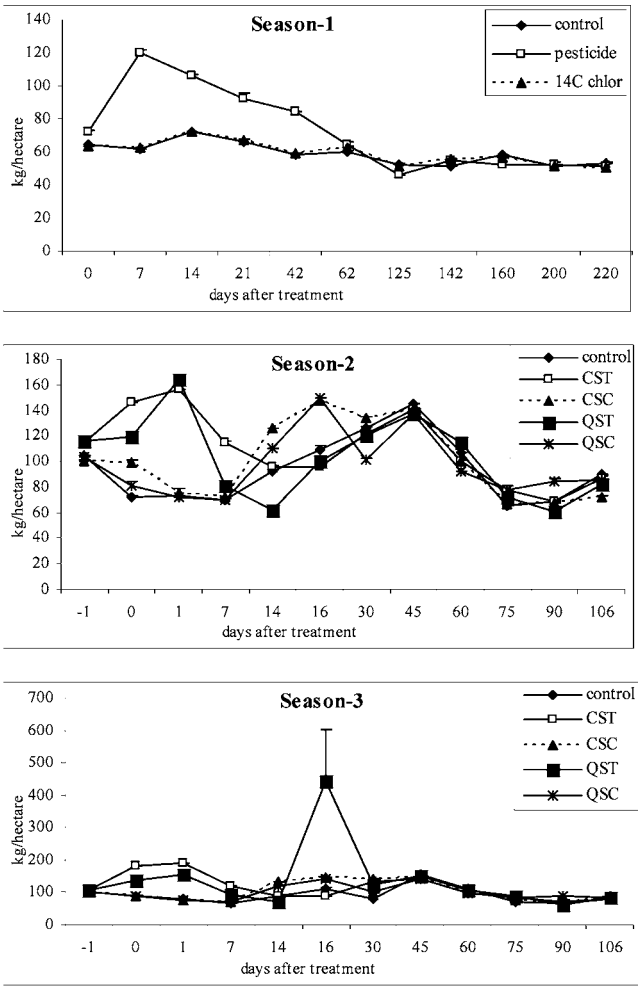
days after treatment[a]			chlorpyrifos[b]		quinalphos[b]		LSD[e] at 1% (LSD at 5%)
ST[c]	SC[d]	control	ST	SC	ST	SC	
–1	–10	17.5 ± 0.9	16.6 ± 1.21	16.6 ± 1.5	16.6 ± 0.1	17.0 ± 0.01	NS[f]
0	–9	16.6 ± 1.53	16.7 ± 1.3	16.7 ± 0.3	16.1 ± 0.4	16.8 ± 0.7	NS
1	–8	16.5 ± 0.4 a,1	15.5 ± 0.5 b,2	16.4 ± 0.4 a,1	15.04 ± 0.1 b,2	16.6 ± 0.2 a,1	0.9 (0.6)
7	–7	16.0 ± 0.6 1	16.0 ± 0.4 1	16.3 ± 0.1 1	15.0 ± 0.81 2	16.2 1 ± 0.114 2	(0.9)
14	0	16.2 ± 0.2	17.6 ± 1.4	17.4 ± 1.0	18.0 ± 1.307	17.6 ± 1.1	NS
16	2	7.8 ± 0.2 b,c,2	8.4 ± 0.4 a,1	8.3 ± 0.22 a,b,1	8.6 ± 0.1 a,1	7.3 ± 0.1 c,3	0.6 (0.4)
30	16	12.5 ± 1.0 b,2	8.6 ± 0.1 c,d,3	7.8 ± 1.23 d,3	11.0 ± 2.0 b,c,2	17.3 ± 0. 2 a,1	2.9 (2.02)
45	31	15.7 ± 0.7	16.0 ± 1.0	16.1 ± 0.1	16.2 ± 0.1	17.0 ± 1.0	NS
60	46	15.6 ± 0.7	15.4 ± 0.6	16.3 ± 0.7	17.04 ± 1.2	16.4 ± 1.01	NS
75	61	16.4 ± 0.8	16.7 ± 7.6	16.6 ± 0.5	17.03 ± 0.03	16.6 ± 1.2	NS
90	76	16.4 ± 2.0	16.4 ± 7.8	16.6 ± 0.6	17.0 ± 0.14	16.5 ± 0.5	NS
100	106	16.6 ± 1.4	16.8 ± 1.0	16.8 ± 1.7	17.0 ± 0.22	16.8 ± 1.4	NS

[a] Days with minus signs are the days before the first pesticide treatment. [b] Letters are for significance at $P < 0.01$. Numbers are for significance at $P < 0.05$. Means with the same letter/number are not significantly different. [c] Seed treatment. [d] Standing crop treatment. [e] Least significant difference. [f] Not significant at $P < 0.05$.

Table 4. Arginine Ammonification as Micrograms of N per Gram of Dry Weight of Soil) per Hour for Season 3

days after treatment[a]			chlorpyrifos[b]		quinalphos[b]		LSD[e] at 1%
ST[c]	SC[d]	control	ST	SC	ST	SC	(LSD at 5%)
–1	–10	16.1 ± 0.4	16.02 ± 0.01	16.0 ± 1.3	16.2 ± 0.02	16.0 ± 0.2	NS[f]
0	–9	16.7 ± 0.32 3	19.7 ± 2.8 1	16.2 ± 0.3 3	19.01 ± 0.4 1, 2	16.8 ± 0.01 2,3	(2.4)
1	–8	17.0 ± 0.3 a,1	12.6 ± 0.3 b,2	16.1 ± 1.8 a,1	12.3 ± 0.7 b,2	16.6 ± 0.1 a,1	2.23 (1.6)
7	–7	14.3 ± 0.4	13.0 ± 1.4	14.4 ± 0.4	15.5 ± 2.2	14.4 ± 1.8	NS
14	0	16.2 ± 1.0	17.2 ± 1.7	17.0 ± 0.7	17.1 ± 0.5	17.5 ± 1.0	NS
16	2	11.6 ± 0.6 b,3	12.4 ± 0.5 b,3	17.8 ± 0.3 a,2	12.0 ± 1.0 b,3	19.0 ± 0.3 a,1	1.52 (1.10)
30	16	13.0 ± 0.03 b,3	14.7 ± 0.02 b,3	19.3 ± 2.0 a,1,2	17.6 ± 0.24 a,2	20.02 ± 1.5 a,1	2.80 (2.0)
45	31	14.8 ± 0.60	15.0 ± 0.6	15.43 ± 0.5	15.1 ± 0.3	15.0 ± 0.3	NS
60	46	16.0 ± 0.22 2	19.0 ± 0.1 1	15.8 ± 0.2 2	16.6 ± 2.01 2	15.4 ± 1.31 2	(2.0)
75	61	16.7 ± 0.33	16.5 ± 0.5	17.0 ± 0.41	17.51± 0.7	16.34 ± 0.31	NS
90	76	17.0 ± 0.2	17.0 ± 0.6	16.7 ± 1.0	17.0 ± 0.4	16.62 ± 1.0	NS
100	106	15.7 ± 1.11	16.8 ± 1.72	16.7 ± 0.8	17.0 ± 1.14	16.8 ± 1.0	NS

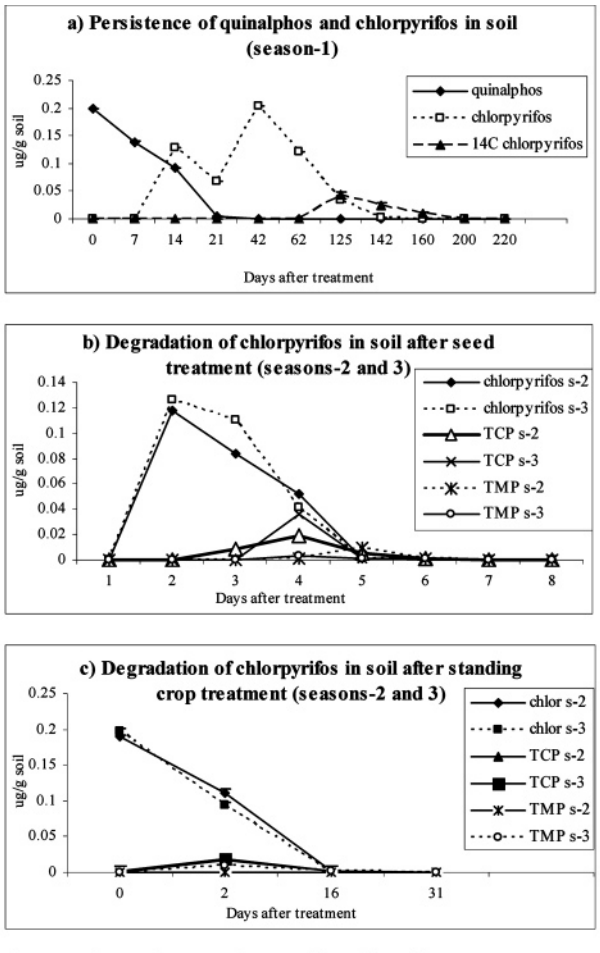
[a] Days with minus signs are days before the first pesticide treatment. [b] Letters are for significance at $P < 0.01$. Numbers are for significance at $P < 0.05$. Means with the same letter/number are not significantly different. [c] Seed treatment. [d] Standing crop treatment. [e] Least significant difference. [f] Not significant at $P < 0.05$.



ST = seed treatment SC = standing crop treatment Q = quinalphos

C = chlorpyrifos

Figure 1. Mineralizable nitrogen in soil (kg ha^{-1}).



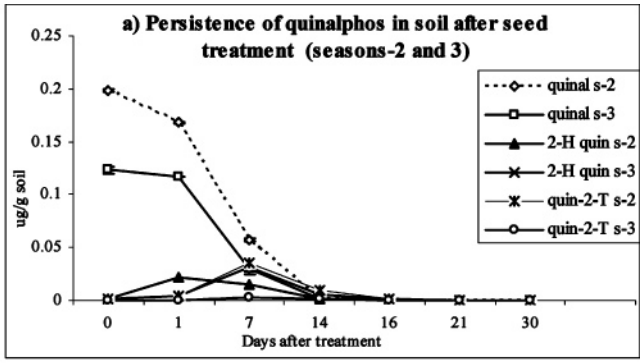
s-2 =season-2 s-3 = season-3 chlor = chlorpyrifos

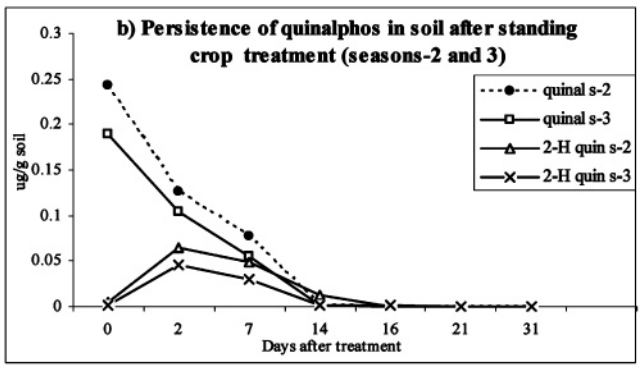
TCP = 3, 5, 6 – trichloro-2- pyridinol TMP = 3, 5, 6 – trichloro-2-methoxy pyridine

Figure 2. Persistence of chlorpyrifos in soil.

which was observed to have high levels of enzyme activity (*37*). For season 2, SC with chlorpyrifos caused prolonged inhibition, whereas SC with quinalphos stimulated arginine deamination. For season 3, the behavior differed, as both chlorpyrifos and quinalphos were stimulatory even 48 h after SC treatment, indicating utilization of both the parents and metabolites as substrates by nonrhizospheric microbes. For seasons 2 and 3, parity with control levels was attained on complete dissipation of the pesticides by day 31.

Effects on Mineralizable N. ST with quinalphos increased mineralizable N in the sandy loam (season 1), whereas both ST and SC with chlorpyrifos were inhibitory (**Figure 1**). The inhibition may be due to the higher concentration of chlorpyrifos in soil due to repeated inputs. In the loamy sand soil (seasons 2 and 3), inhibition followed a short stimulation after ST with both pesticides, whereas SC with both pesticides caused delayed





s-2 =season-2 s-3 = season-3 quinal = quinalphos

2-H quin = 2-hydroxy quinoxaline quin -2-T = quinoxaline-2-thiol

Figure 3. Persistence of quinalphos in soil.

Table 5. Correlation between Arginine Ammonification and Mineralizable Nitrogen in the Control and Pesticide-Treated Soils

Season 1

control	sequential treatments with pesticides	[^{14}C] chlorpyrifos treated	overall value for season 1
0.24	–0.22	0.2	–0.11
*0.24[a]	*0.3	*0.34	*0.35

Seasons 2 and 3

control		chlorpyrifos				quinalphos			
		s-2		s-3		s-2		s-3	
s-2	s-3	ST	SC	ST	SC	ST	SC	ST	SC
–0.4	–0.42	–0.03	–0.6	–0.12	0.4	–0.25	–0.6	–0.32	0.3
*0.03	*0.01	*0.9	*0.0001	*0.5	*0.02	*0.14	*0.0003	*0.1	*0.12
overall value for season 2		–0.34				overall value for season 3		–0.1	
		*0.0001						*0.32	

Combined Data of Seasons 2 and 3

	chlorpyrifos		quinalphos		
control	ST	SC	ST	SC	overall value
–0.4	–0.05	–0.25	–0.3	–0.25	–0.21
*0.001	*0.7	*0.03	*0.03	*0.03	*0.0001

[a] An asterisk denotes the probability level of significance of the correlation coefficient. The figure given above it is the Pearson correlation coefficient (*r*).

increases. This indicated that in the loamy sand soil, the parent compounds were stimulatory to N-mineralization by rhizospheric microbes, but the increasing concentrations of metabolites were inhibitory, whereas for the nonrhizospheric microbial N-mineralization, the parent compounds inhibited and the metabolites were stimulatory and may be utilized as substrates. Levels similar to control plots were attained only after the complete dissipation of the pesticides (**Figures 2** and **3**). The correlation between arginine deamination and available N content of control plots was not significantly positive for season 1, but for seasons 2 and 3, it was inverse and significant ($P < 0.05$). The data from seasons 1−3 showed an inverse and significant correlation to N from plots with pesticide treatments, except for ST with chlorpyrifos for (the combined data of) seasons 2 and 3 (**Table 5**). A high N content, implying the energy source might not be limiting, may have caused low ammonification (*7*). As reported in other studies (*1*, *9*, *10*), it was observed that the correlation of ammonium production to the N ratio of an amino acid, although significant, was not close. This indicated that N-turnover was not effected merely by a simplistic linear relationship with the microbial deaminase activity but could also be influenced by other factors that have been investigated, namely, rhizodeposition (*38*, *39*) and intermediary pool sizes of mineralizable N (*40*).

Variations in arginine deamination and mineralizable N in control soils may be attributed to seasonal changes and nutritional factors (*41*−*44*). Slightly higher values for ammonification observed in seasons 2 and 3 could be due to the reported greater enzyme production in alkaline soils (*45*). A higher magnitude of impact of pesticide treatments on deamination and N-mineralization in seasons 2 and 3 than during the season 1 trial (**Figure 1**) might have resulted from a lower sorption (and hence greater bioavailability) of pesticides due to higher alkalinity and the lower clay, organic matter, and organic C content in the loamy sand than in the sandy loam (**Table 1**; *46*, *47*). The inhibitory effects (*18*, *37*, *48*) of the pesticides and their metabolites or their utilization are known to cause retention of mineralizable N in the rhizosphere (*49*, *50*) and a flush of nutrient (especially NH_3) production. This may be responsible for an increase in the biomass activity and pod yield. The more prolonged inhibition and slower recovery of deamination observed after treatments with chlopyrifos in comparison to quinalphos may be due to the reported inhibition of denitrification of nitrate (*51*, *52*) by the halogen substituents on the aromatic ring of chlorpyrifos, which is known to cause impairment of activities of ammonium oxidizers (*53*). However, the toxicity may have been reduced due to the presence of organic matter, soil moisture (*54*), because the crop was sown during the monsoon season, and the complete dissipation of the pesticides (*32*, *55*−*57*; **Figure 2**).

The efficacy of the recommended doses and the heterogeneity of microbial populations were reflected by the realization of greater yield from the pesticide-treated plots in comparison to control plots, the absence of pesticide residues in the harvested groundnut kernels, and the recovery of deamination to control levels after the removal of inhibition due to complete dissipation of both the pesticides and perhaps as a consequence of a fall in NH_3 levels with the onset of nitrification. Pesticides are known to immobilize NH_4^+-N, in the form of reduced NH_3, which in alkali soils may prove to be injurious to plants (*50*, *51*). Smaller inhibitions resulting from the pesticide treatments, although not deleterious, might still be significant in semiarid cropping areas where ammonium salts are used as nitrogen fertilizers or if N-cycling and availability to the growing crop during the short crop cycle (60−75 days) are reduced to levels known to affect plant productivity (*58*). It is therefore suggested that if the need for pest control is still paramount, the problem might be overcome by modifying fertilizer practice to synchronize supplies of N with periods of maximum uptake by crops (*59*).

ACKNOWLEDGMENT

We are grateful to Dr. Prem Dureja, Division of Agricultural Chemicals, IARI, New Delhi, and the International Atomic Energy Agency, Vienna, Austria, for providing pesticide standards and [^{14}C]chlorpyrifos and to Drs. Jagatram and S. K. Mahapatra of the NBSSLUP, New Delhi, for classifying the two soils.

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Received for review March 25, 2004. Revised manuscript received July 23, 2004. Accepted August 5, 2004.

JF049502C