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EFFECT OF PESTICIDES ON NITRIFICATION IN SOILS

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Abstract

A laboratory experiment was conducted to study the effects of two pesticides viz. diazinon (O,O-diethyl-O-[2 isopropyl-4-methylpyrimidyl-6] thiophosphate) and heptachlor (1, 4, 5, 6, 7, 8, 8-heptachloro- 3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene) on nitrification in soil. The two pesticides were applied at the recommended dose (0.5 and 0.8 mg active ingredient/ kg soil for diazinon and heptachlor respectively) and 200 and 400 mg active ingredient (a.i.)/kg soil in presence or absence of 200 mg N/kg as $(NH_4)_2SO_4$ and their effect on nitrification was studied over 28 days of incubation period. Two general effects of addition of pesticides on nitrification were apparent. At the recommended dose both the pesticides stimulated or did not affect the process. But at the higher doses both the pesticides inhibited the process. Degree of inhibition varied with the type of soils and type of pesticides. First order equations were used to calculate the maximum nitrification rate (K_{max}), duration of lag period (t'), period of the maximum nitrification (t) and duration of lag plus maximum nitrification period (ts). Nitrification rates were significantly affected by the application of pesticides at higher doses.

Introduction

Nitrification is one of the most sensitive soil microbial transformations. The unique nature of nitrifying organisms renders them susceptible to any change in the environment. Species diversity is narrow in case of nitrification process. Alexander[(1)] stated that the remarkable degree of sensitivity of the process to external influences was attributed in part to the great physiological similarity of the responsible species. As a result, modification in the environment often had a profound significance in governing the production of end product.

A number of studies have been made to examine the effect of different pesticides on nitrification. Outcome of such studies is so diverse in nature that it is difficult to make any general conclusion on the effect of pesticides on soil nitrification. Such diversity in results might be manifested either from diversity

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among dominant nitrifying population, difference in the experimental methodology and/or difference in edaphic factors like organic matter, pH, moisture holding capacity etc. Alexander[2] and Helling *et al.*[3] concluded that rather excessive quantities of chlorinated pesticides would be required to exert inhibitory effects of great consequence.

However, because of their persistent nature, certain alterations in the soil microflora might be maintained for extended period. Chandra[4] reported that dieldrin and heptachlor suppressed nitrification in heavy clay, loam and sandy loam soils up to eight weeks. On the other hand, Behera and Mishra[5] reported temporary inhibition of nitrification in a sandy loam soil with 25 ppm or higher concentration of insecticides. Verstraeten and Vlassak[6] also found lindane as a potential inhibitor of nitrification when applied at the rate of 1.5, 3, 15 and 30 ppm. Gaur and Misra[7] found lindane to distinctly inhibit nitrification at 10 and 100 ppm for three weeks. Tu[8] concluded that at recommended level there would not be lasting effects of insecticides on soil microorganisms and their activities to soil fertility. However, a different conclusion was drawn[9] who maintained that Malathion and Parathion might influence NH_4^+ or NH_4^+ forming fertilizers by regulating their rates of mineralization in soil. It is, therefore, important to know the effect of pesticides on an important microbial process, viz., nitrification. The effect of two selected pesticides on two soils were studied in the present study.

Materials and Methods

Two representative soils from Melandaha and Bhatpara series were used in the present experiment. Both the soils were collected from adjacent areas of Savar. A composite sample was collected to a depth of 15 cm in both the cases.

Before use, each sample was screened for debris, roots and other dead plant materials and sieved in a field moist condition to pass through a 2 mm sieve. Sub-samples were removed and stored at subzero temperature in sealed polyethylene bags for studies requiring field moist and freshly air-dried soil samples. The remainder of each of the samples was air-dried, crushed gently to pass a 2 mm screen, and stored at room temperature in a tightly sealed plastic container.

The two soils were found to be sandy loam and a silty clay loam after particle size analysis performed by hydrometer method[10] and subsequent textural class was determined by following Marshall's triangular co-ordinates as designed by USDA.[11] The water holding capacity of soils was found to be in the range 49.6 - 58.8%. pH of the soils was measured by a combined electrode pH meter at a soil water ratio of 1 : 2.5 as suggested by Jackson.[12] Soil organic carbon was determined by Walkley and Black's wet oxidation method as outlined by Jackson.[12] Soil organic matter was calculated by multiplying the per cent value of organic carbon with the Van Bemmelen factor, 1.724.[13] The total nitrogen content of the soils was determined by alkali distillation of the Kjeldhal digest with 40% NaOH, the NH_3 evolved being absorbed in 4% boric acid with mixed indicator and the absorbed NH_3 was estimated by back titration with H_2SO_4 as described by Jackson.[12] Available phosphorus was extracted by 0.5M $NaHCO_3$ and phosphorus content of the extracts was determined colorimetrically using ascorbic acid as the reductant. CEC of soil was determined using neutral 1*N* NH_4OAc method.[14]

Two generic group of pesticides *viz*. the organophosphate and chlorinated hydrocarbon, were used in the present experiment. These were diazinon (*O,O*-diethyl-*O*-[2 isopropyl-4-methylpyrimidyl-6] thiophosphate) and heptachlor (1, 4, 5, 6, 7, 8, 8-heptachloro- 3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene). The method used was identical to that described by Schmidt and Belser[15] with some modification. Soil samples amounting to 10 g (air-dry basis) was poured into plastic pots. One set of samples then received 1 ml of 2 mg N as $(NH_4)_2SO_4$. To another set of samples no amendment was added. Both the set of samples then received different doses of either diazinon or heptachlor. Diazinon was applied to soils in 1 ml of aqueous solution to achieve pesticide concentration of 0, 0.5, 100 and 400 mg a.i. (active ingredient)/kg soil. 1 ml of homogenized suspension of heptachlor was applied to second set of soils to achieve pesticide concentration of 0, 0.8, 100 and 400 mg a.i./kg soil. A control without pesticide was included in each case.

Sufficient water was added to bring the soils to 60% of its water holding capacity. The pots were then covered with parafilm. No restoration of moisture was required during incubation, since the polyethylene film was permeable to gases but not to water.[8] All the treatments were replicated two times and incubated in an incubator at 28°C for four weeks. At 1, 2, 3 and 4 weeks 10 g of duplicate samples were extracted with 100 ml of 2 *M* KCl and the levels of NO_3^- and NO_2^- were determined colorimetrically by salicylic-sulfuric acid method[16] and colorimetrically by Griess-Llosvay reagent, respectively. NO_3^- plus NO_2^- accumulation in excess of that in the control samples was used as the primary criterion for assessing nitrification of the applied ammonium.

In order to express the accumulation of NO_3^- with time (t) quantitatively in presence of pesticides the nonlinear regression described[17,18] was used to solve the Verhulst equation, $dN/dt = kN(a - N)$,[19] which upon integration gave a sigmoid curve:

$$NO_3^- = a/\{1 + [a/(NO_3^-)_0 - 1]\exp(-ak[t - t_0])\}$$

where NO_3^- is accumulated as NO_3^- - N (mg NO_3^- - N/kg) at specific time (t), a is the asymptotic value of NO_3^-, $[NO_3^-]_0$ is the value of NO_3^- at time zero(t_0), and k is a constant. The parameters a, k and $[NO_3^-]_0$ were calculated by the least squares fit of Eq. 1 to experimental data of NO_3^- vs. t. Maximum nitrification rate (K_{max}) was calculated as the maximum slope of Eq.1, at the inflection point (where $NO_3^- = a/2$):

$$k_{max} = ka^2/4 \quad [2]$$

as described.[17,18,20]

The delay or lag period (t'), according to the definition of Sabey *et al.*[20] was calculated as the value of t when the maximum slope was extrapolated to the initial value of NO_3^-,[17]

$$t' = (1/ak)\ln[a/(NO_3^-)_0 - 1] + [(NO_3^-)_0 - a/2]/k_{max} \quad [3]$$

The duration of the period of maximum nitrification (Δt) was calculated as described by,[18]

$$\Delta t = 2[a/2 - (NO_3)_0]/k_{max} \quad [4]$$

The termination of the maximum rate or duration of lag plus maximum nitrification period (t_s) was computed from t' and Δt as defined by.[18]

$$t_s = t' + \Delta t \quad [5]$$

The SPSS/PC + statistical software[21] was used to calculate k_{max}, t', Δt and t_s.

Results and Discussion

Effects of diazinon and heptachlor on the Melandaha and Bhatpara soil in the absence of amendment are summarized in Table 1. From Table 1, it is clear that diazinon was more detrimental to nitrification than heptachlor. Again, the suppressive effect of pesticides differed with types of soil. Bhatpara soil was prompt to buffer the deleterious effect of either of the pesticides. Effect of Diazinon and heptachlor on the Melandaha and Bhatpara soil in presence of amendment are summarized in Table 2. Melandaha soil produced a total of 152.3 mg NO_3^- - N/kg soil at zero concentration of diazinon after 28 days of incubation. Only 64.6% of added 100 mg N/kg soil as $(NH_4)_2SO_4$ was transformed into NO_3 - N. Bhatpara soil yielded a total of 169.3 mg NO_3^- - N/kg soil in the control sample at the end of 28 days of incubation. Nitrification of the added N at 28 days amounted to 67.7%. Both the soils thus showed good nitrifying potential. From Table 2, it is apparent that with increasing concentrations of pesticides rate of accumulation of NO_3^--N gradually decreased as the inhibition increased.

Table 1. Effect of diazinon and heptachlor on nitrification of native N- pool.

Pesticide	Dose (mg/kg)	Incubation time (days) and mg NO_3^- - N/kg soil produced.									
		Melandaha					Bhatpara				
		0	7	14	21	28	0	7	14	21	28
	0	4.6a†	10.2a	18.3c	21.6a	23.1ab	9.8	15.0a	23.8b	30.2b	34.0a
Diazinon	0.5	4.6a	9.1a	18.2	22.0a	23.4b	9.8	14.5a	23.8b	31.5b	35.5a
	100.0	4.6a	5.9a	9.8a	16.6a	18.9ab	9.8	11.1a	18.4ab	25.8ab	29.9a
	400.0	4.6a	6.2a	11.8ab	16.6a	18.9a	9.8	104a	11.1a	22.6a	29.5a
Heptachlor	0.8	4.6a	9.9a	19.0c	21.6a	23.7b	9.8	14.4a	24.6b	31.8b	34.7a
	100.0	4.6a	8.3a	15.2bc	17.3a	19.5ab	9.8	12.9a	21.4ab	27.3ab	31.6a
	400.0	4.6a	7.2a	13.4ab	17.3a	19.4ab	9.8	12.3a	20.4ab	25.9ab	32.3a

†Within a given column, mean values accompanied by the same letter are not significantly different at 5% level.

Table 2. Effect of diazinon and heptachlor on nitrification of added 200 mg N as $(NH_4)_2SO_4$.

Pesticide	Dose (mg/kg)	Incubation time (days) and mg NO_3^--N/kg soil produced									
		Melandaha					Bhatpara				
		0	7	14	21	28	0	7	14	21	28
Diazinon	0	4.6	48e†	114.3c	144.7c	152.3d	9.8	65.2e	124.5c	158.6d	169.3c
	0.5	4.6	42.2de	111.5c	141.8c	153.7d	9.8	60.0de	122.8c	156.8d	170.0c
	100	4.6	26.1ab	50.7a	85.3a	110.2a	9.8	39.4ab	81.1a	117.4ab	146.8a
	400	4.6	21.8a	56.0a	89.5a	115.6ab	9.8	34.8a	75.4a	109.7ad	142.9a
Heptachlor	0.8	4.6	46.2de	115.2c	143.6c	156.2d	9.8	63.3e	130.5c	161.2	171.1c
	100	4.6	37.2cd	92.0b	117.3b	133.0c	9.8	51.4cd	104.7b	136.2c	158.4b
	400	4.6	32.2bc	81.6b	111.7b	126.0bc	9.8	48.2bc	96.9b	124.0bc	152.0ab

†Within a given column, mean values accompanied by the same letter are not significantly different at 5% level.

Course of nitrification in the two soils: The first order equation of Verhulst[19] as described[17,18] was used to describe the course of nitrification in the two soils treated with pesticides. Experimental data was fitted to the following equation:

$$NO_3^- = a/\{1 + (a/[NO_3^-]_0 - 1) \exp(-ak[t \; t_0])\}$$

where a and $[NO_3^-]_0$ are asymptotic and initial values of NO_3^-, respectively, k is a constant, and t_0 is the initial time, which equals to zero. The parameter a, k, and $[NO_3^-]_0$ were calculated by the least squares fit of the equation to the experimental data of NO_3^- vs. t. Calculated a, k, and $[NO_3^-]_0$ values were then fitted to other equations to derive maximal nitrification rate (K_{max}), lag period (t'), duration of the maximum nitrification period (Δt) and termination of the maximum rate phase (t_s).

The model did not fit the nitrification process when no amendment was used. Standard error in calculating asymptotic value, a, was large. Therefore, calculated parameters K_{max} and extension of t, Δt and t_s in unammended soils are not reliable and will not be further mentioned . However, the model fits the nitrification process when 200 mg N/kg was used as amendment. Calculated parameters for both the experiments are presented in Table 3 and 4.

Table 3. Calculated nitrification parameters† of native N pool.

Soil and pesticide	Dose (mg/kg)	K_{max} (mg/kg/days)	t' (days)	Δt	t_s
Melandaha					
	0	1.1	1.3	13.5	14.8
Diazinon	0.5	1.2	1.6	14.5	16.1
	200	0.7	2.4	22.0	24.4
	400	0.7	7.9	45.8	53.7
Heptachlor	0.8	1.2	1.4	13.6	14.9
	200	0.8	1.1	14.5	15.5
	400	0.7	1.6	18.3	19.9
Bhatpara					
	0	1.1	1.0	18.4	19.4
Diazinon	0..8	1.2	1.4	20.3	21.6
	200	0.9	2.6	30.8	33.4
	400	11. 6	62.9	88.2	151.1
Heptachlor	0.5	1.2	1.1	17.8	18.9
	200	0.9	1.4	22.5	23.9
	400	0.9	2.9	32.9	35.8

†K_{max} = maximal nitrification period; t' = lag period; Δt = duration of maximum nitrification period; t_s = duration of lag plus maximum nitrification period.

The results (Tables 1 and 2) indicated that the two soils under study had good nitrifying capacity. 64.6 and 67.7% of the added NH_4^+-N were oxidized in the control samples of Melandaha and Bhatpara soil, respectively while in the pesticide treated samples inhibition took place.

When K_{max} (Table 5) of the two soils were considered, it was found that Bhatpara soil had slightly lower maximum nitrification rate (K_{max}) than Melandaha soil. K_{max}, as derived graphically by Sabey *et al.*[20] from sigmoid curves, when NH_4^+ concentration is not rate limiting, is expected to depend on soil properties. Conditions of the experiment, aeration, moisture and temperature, were constant for the two soils. pH of the two soils was also within the optimum range for nitrification. Therefore, variation in K_{max} can be considered to be due to the difference in the inherent capacity of the soils to nitrify added NH_4^+.

Table 4. Calculated nitrification parameters[†] of added $(NH_4)_2SO_4$.

Soil and pesticide	Dose (mg /g)	K_{max} (mg/kg/days)	t' (days)	Δt	t_s
Melandaha					
	0	10.7	3.7	12.6	16.2
Diazinon	0.5	10.7	4.1	12.8	16.9
	200	5.1	5.6	21.6	27.3
	400	5.7	5.8	19.7	25.6
Heptachlor	0.8	10.8	3.8	12.7	16.5
	200	8.3	3.9	13.9	17.8
	400	7.6	4.3	14.8	19.0
Bhatpara					
	0	10.2	2.7	13.5	16.2
Diazinon	0.5	10.2	3.0	13.9	16.8
	200	6.6	4.2	19.9	24.1
	400	6.3	4.7	21.3	26.0
Heptachlor	0.8	11.1	2.9	12.9	15.7
	200	8.2	3.1	15.8	18.9
	400	7.2	3.1	17.1	20.3

[†]K_{max} = maximal nitrification period; t' = lag period; Δt = duration of maximum nitrification period; ts = duration of lag plus maximum nitrification period.

An anomalous feature of Melandaha soil was observed both in short term and long term incubation experiment. Inhibition caused by 200 mg a.i. of diazinon /kg soil was greater than that caused by 400 mg a.i. of diazinon/kg soil. Generally the reverse should have occurred. The reason behind the phenomenon remained unexplained.

Except for these anomalous features of diazinon in Melandaha soil, inhibition followed the usual trend. Inhibition increased with increasing concentrations. Two general effects are observed with increasing concentrations of pesticides: at the recommended dose little or very low inhibition was observed and with higher concentration nitrate production was suppressed.

The degree of inhibition also varied with types of pesticides. Diazinon was found in all cases to be more toxic to nitrification than heptachlor. The answer might lie in the classification and mode of action of the pesticides. Heptachlor is a chlorinated hydrocarbon insecticide. It has weak insecticidal properties.[22] But heptachlor like other cyclodines, are generally recognized as the most persistent organic insecticides. Epoxidation is the major mechanism of microbial metabolism. The epoxidation product of heptachlor is also insecticidal and thus its biological activities are prolonged in soil.[23] Winely and San Clemente[24] suggested that heptachlor inhibits nitrite oxidase by depressing *Nitrobacter* growth rather than by a specific effect on the nitrite oxidase system. Organophosphorus insecticides like diazinon are potent inhibitor of acetyl cholinesterase. Though it is relatively less persistent, it has wider inhibitory spectrum.[22] Nishihara[25] demonstrated that diazinon was potent inhibitor of NH_4^+ and NO_2^- oxidation. Variations in results observed in the present study are thus mainly due to the difference to the mode of action of the two pesticides. However, the effects of soil properties could also be there.

Table 5. Reduction in K_{max} and extension of t, Δt and t_s resulting from addition of diazinon and heptachlor.

Soil and pesticide	Dose (mg/kg)	K_{max} (mg/kg/days)	t' (days)	Δt (d)	ts
Melandaha					
	Control	0.0	0	0	0
Diazinon	0.5	-0.2	0.4	0.2	0.7
	200	52.5	1.9	9	11.1
	400	46.5	1.7	7.1	9.4
Heptachlor	0.5	-0.9	0.2	0.1	0.3
	200	22.9	1.0	1.3	1.6
	400	29.5	0.6	2.2	2.8
Bhatpara					
	Control	0.0	0	0	0
Diazinon	0.5	0.1	0.3	0.4	0.6
	200	35.0	1.5	6.4	7.9
	400	38.2	2	7.8	9.8
Heptachlor	0.5	-8.8	0.2	-0.6	-0.5
	200	19.6	0.4	2.3	2.7
	400	29.3	0.4	3.6	4.1

*K_{max} = maximal nitrification period; t' = lag period; Δt = duration of maximum nitrification period; t_s = duration of lag plus maximum nitrification period.

The effect of various insecticides, particularly the persistent chlorinated hydrocarbons, on specific groups of soil microorganisms, including those responsible for nitrification, have been reviewed.[2,3] They concluded that rather excessive quantities would be required to exert inhibitory effects of significant consequence. Jaiswal,[26] Narain and Datta[27] and Shaw and Robinson[28] reported that application rate of more than 100 kg/ha of heptachlor was needed to inhibit the nitrification rate by 25%. Verstraeten and Vlassak[6] found that heptachlor had very mild or no inhibition to nitrification at the rate of 10 ppm. Reports about other chlorinated hydrocarbon insecticides revealed similar results. Aldrin was reported by several workers as harmless.[29-31] However, there are also some contradictory reports. Chandra[4] reported that dieldrin and heptachlor suppressed nitrification in a heavy clay, loam and sandy loam soils up to eight weeks. Eno and Everaenlt[32] reported that heptachlor and especially lindane depressed nitrification. However, reports on inhibition by organophosphate insecticides are not so bleak. Tu[8] reported that malathion significantly inhibited nitrification in a sandy loam soil for two weeks. Sahrawat[9] reported that malathion and parathion at the rate of 10 and 50 μg/g inhibited nitrification of urea-N and $(NH_4)_2SO_4$-N.

NO_2^--N was never found to exceed 1 mg/kg throughout the experiment that contradicted the observation of Winely and San Clemente[24] that several pesticides - Chlordane, CIPC, eptam, heptachlor and lindane, depressed *Nitrobacter* growth. Verstraeten and Vlassak[6] observed NO_2^--N accumulation far exceeding the level of 10 ppm with lindane when applied at the rate of 30 ppm. No such observation, however, was noted with organophosphorus insecticides. Sahrawat[9] observed that malathion and parathion did not appreciably affect the conversion of NO_2^--N to NO_3^--N. Tu[8] did not report any such accumulation with diazinon.

The depressive effect caused by higher doses was more severe at the first one or two weeks in both substrate limiting and non-limiting conditions. The depressive effect lessened thereafter rapidly. The decreased inhibitive effect of the pesticides after 14 - 21 days indicated that either the pesticides underwent transformation and detoxification in the soil during incubation or that the nitrifiers had adapted to the pesticides. Findings of several other workers are in conformity to the findings of the current experiment with organophosphate insecticides.[8,33] Such gradual recovery after initial suppression was also observed with more toxic and persistent fungicides.[9,34]

The depressive effect of the pesticides depended also on the N source. The depressive effect was more severe in the amended soil than where no N amendment was used. Difference in inhibition was also observed for differences in soils' physico-chemical properties. Melandaha soil which was lighter than Bhatpara soil exhibited more inhibition. It happened because of inherent soil properties of the two soils. The bioactivity, movement and persistence of pesticides in soil depend in large part on interaction of the pesticide molecule with the soil adsorption complex. Adsorption reduces the concentration in the soil solution, thus removing part of the pesticides from the field of potent action.(3,35) Both inorganic and organic surfaces constitute the soil adsorption complex. Insecticidal activity of organophosphates has been reported to be related to the soil texture and organic matter content of soil.(36) Studies(38,39) showed that organic matter is the major sorbing component of the applied nitrification inhibitors. Bhatpara soil contained more clay and organic matter. As it possessed higher adsorption sites than Melandaha soil, it is likely that it neutralized the effect of pesticides more effectively.

Recommended dose of either of the pesticides did not produce any appreciable inhibition and in some instances stimulated nitrification. The stimulation might have resulted from the killing of a portion of the soil microflora. However, these low concentrations of pesticides did not affect the nitrifying bacteria, which were able to utilize the newly available substrate. Such partial sterilization would bring about changes in the many complex interactions that are likely to upset the dynamic equilibrium of the soil microflora and thus affect soil fertility. From the nitrification model used in the current study, it was seen that diazinon lengthened the maximum nitrification period to about 11 days in Melandaha soil. Question arises as to what effect the resulting ammonium-nitrogen accumulation would have on plant growth. On relatively infertile well-drained soils delayed oxidation of ammonium fertilizers can prevent losses by leaching and denitrification when irrigation and rainfall is excessive, and this could increase nitrogen availability for such crops as corn and small grains during their early stages of growth. Under soil conditions where significant losses of fertilizer nitrogen are not likely to occur, delayed nitrification should not adversely influence nitrogen uptake unless there is a specific response to ammonium versus nitrate nutrition.(34,40) However, if the opinions(41,42) that most plant species grow better when supplied with nitrate than with ammonium salts is assumed true, growth may be hindered when ammonium accumulates due to partial sterilization. A number of crops, including tomato (*Lycopersicon esculentum* Mill.), potatoes (*Solanum tuberosum* L.), and tobacco (*Nicotina tabacam* L.), are highly sensitive to excess NH_4^+ nutrition.(43)

It needs to be mentioned here that the doses used in the experiment are not likely to occur under field condition. But prolong use of pesticides might result in accumulation in certain amount to cause inhibition. Kearney *et al.*(44) stated the possibility of zone accumulation of pesticides at 100 ppm or higher due to uneven distribution of pesticide through the plow depth. This is particularly important in our country where farmers lack basic training and equipment for proper use of these toxic agrochemicals. Besides, insecticidal resistance by agricultural pests led farmers to use pesticides repeatedly and also to choose more persistent chemicals. All of these might lead to zonal or widespread ecological disaster.

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