

EFFECTS OF PESTICIDES ON UREASE ACTIVITY IN SOIL

S. M. IMAMUL HUQ*, R. SADIA AFROZE¹ AND RAQUIBUL AMIN²

*Department of Soil, Water and Environment, University of Dhaka
Dhaka-1000, Bangladesh*

Abstract

A pot culture experiment was conducted to study the effect of two pesticides *viz.* heptachlor (1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4,7-methanoindene) and diazinon [0,0-diethyl-(2-isopropyl-4-methyl pyrimidyl-6) thiophosphate] on the urease activity in a sandy loam (Melandaha series) and a silty clay loam (Bhatpara series) soil. Effects of these selected pesticides on urease activity were evaluated for up to 28 days of incubation. Three sets of each of the individual soil were treated with various doses of the pesticides; the recommended dose [0.8 and 0.5 mg active ingredient (a.i.) kg⁻¹ soil for heptachlor and diazinon, respectively], 100 and 400 mg a.i. kg⁻¹ soil. To the first set of treatments, no extraneous N was added to study the indigenous urease activity of both soils. Two and 100 ppm of N as urea were applied to the second and third set of experiments, respectively. Both the pesticides at any rate of application stimulated the enzyme activity significantly in all soils. Degree of stimulation varied with the type of soils. More stimulation was observed in Bhatpara soil than in Melandaha soil. The effectiveness of two pesticides to stimulate urease activity differed negligibly. The different concentrations of pesticides or incubation period did not show any effect on soil urease activity.

Key words: Pesticide, urease activity, soil

Introduction

Urease is the enzyme that catalyzes urea hydrolysis yielding ammonia and carbon dioxide. This is quite an important reaction in nitrogen metabolism. Urease activity in soil is an important part of the nitrogen cycle in ecology. It is also an important activity of some important pathogenic bacteria. Soil enzymes as catalysts are intimately concerned with the stability of soil organic nitrogen. The delicate balance between microbes and enzymatic activities, *viz.* urease, phosphatase, dehydrogenase is very important in nutrient release. The importance of urea as N fertilizer in world agriculture is well documented (Stangel 1984). A rapid hydrolysis of urea in soils due to enzyme urease leads to accumulation of ammonium N and increase in pH, which results in gaseous losses of ammonia (Moore and Russel 1972). A high concentration of ammonium N reduces nitrite

* Author for correspondence. ¹ BCSIR Laboratories, Dhaka, Bangladesh. ² IUCN Country Office, Dhaka, Bangladesh.

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oxidising bacteria resulting in accumulation of nitrite which may cause damage to germinating seedlings (Court *et al.* 1962). On the other hand, a slow rate of hydrolysis is likely to increase the leaching loss of urea with moisture due to its high solubility and non-interacting nature in soil solution (Viraj 1974). Due to the increased use of urea as a fertilizer in agricultural soil, soil urease level in conjunction with other tests has been used as one of the indices to lead to a better understanding of the fertility of soil ecosystem. Although many studies have been reported on non-target effects of pesticides in soil, the wide-spread use of urea as a nitrogen fertilizer has stimulated investigation on urease activity particularly when pesticides are applied to soils. Research of such order has not been undertaken in Bangladesh. No valid information on pesticidal toxicity could also be found. On the other hand, indiscriminate use of pesticides is wide-spread. Farmers here are ignorant about the risk of pesticide toxicity and are reluctant to follow the standard procedure of application. Farmers also have little or no knowledge at all about the consequences of the use of these noxious chemicals. Moreover, nitrogen is the most limiting element in soils of Bangladesh and is thought to be the most susceptible to microbial transformations among the major elements (Alexander 1977); any detrimental impact of pesticides in any parts of N transformation might result in a dire consequence for soil fertility. Keeping these in mind, the present investigation was undertaken to look deeply into the soil urease activity as affected by pesticide application.

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. 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 methods of extraction and subsequent analysis of different parameters have been described elsewhere (Imamul Huq *et al.* 2004) and the results are shown in Table 1. Two generic group of pesticides *viz.* the organophosphate and chlorinated hydrocarbon were used in the present experiment. These were diazinon (0,0-diethyl-0-[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 proposed by Tabataba and Bremner (1972) has been followed in the present investigation for urease activity.

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Table 1. Results of routine analyses of two soils used in the current study.

Soil series	Particle size distribution %			Textural class	CEC meq/100g	pH	Organic carbon (%)	Total N (%)	Available N (ppm)	C/N ratio	Maximum water holding capacity %
	Sand	Silt	Clay								
Melandaha soil	55.9	40.0	4.1	Sandy loam	6.98	6.7	0.7	0.93	23.31	0.75	57.11
Bhapara soil	10.5	55.5	34.1	Silty clay loam	14.42	6.3	0.94	1.3	23.31	0.72	53.89

Two hundred gram each of soil samples (air dry basis) was taken in plastic containers in three sets. To the first set of experiments no additional N was applied. The second set received 1ml of 2 mg N as urea (2 ppm in soil). To the third set, 1 ml of 100 mg N as urea (100 ppm in soil) was added. All the sets 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^{-1} soil. 1 ml of homogenized suspension of heptachlor was applied to soils to achieve pesticide concentration of 0, 0.8, 100 and 400 mg a.i. kg^{-1} soil. In each case, only urea solution was added in one control. In another control, soil samples were incubated under identical conditions without addition of urea and pesticides. Sufficient water was added to bring the soil moisture content to 60% of its water holding capacity. The containers were capped with parafilm. No restoration of moisture was required during incubation, since parafilm was permeable to gases but not to water. All the treatments were replicated three times and incubated in an incubator at 30°C for 4 weeks. Urease activity at '0' time and after 2 and 4 weeks of incubation was estimated by the method of Tabatabai and Bremner (1972). Five g soil (wet basis) from each container was undertaken in 50 ml volumetric flask, 0.2 ml of toluene and 9 ml of THAM buffer were added. After swirling the flask, 1 ml of 0.2 M urea solution was added. The flask was then stoppered and incubated at $37 \pm 1^{\circ}\text{C}$ for 5 hours after which approximately 35 ml of KCl - Ag_2SO_4 solution was added to it. The flask was swirled for a few seconds and allowed to stand until the contents have cooled to room temperature. The volume was made up to 50 ml by the addition of KCl - Ag_2SO_4 solution. The flask was stoppered and inverted several times to mix the contents. Controls were performed in each series to determine the amount of ammonium N already present in soil before incubation with urea for 5 hours. Urea solution was added in controls after the addition of KCl - Ag_2SO_4 solution.

To determine NH_4^+ -N in the resulting soil suspension, 10 ml aliquot of the suspension was pipetted into a 100 ml distillation flask and NH_4^+ -N released was determined by steam distillation of the aliquot with 0.2 g of MgO for 4 minutes. Urease activity was expressed as mg of ammonium N released per kg soil in 5 hours.

Results and Discussion

Tables 2, 4 and 6 show the results obtained with the Melandaha and Bhatpara soils when the effects of diazinon and heptachlor on urease activity were compared by the 5 hours incubation test. Data on urease activity in soil showed that both pesticides had marked stimulatory effect on the activity of this enzyme during the entire period of incubation. It was difficult to generalize about the effects of different concentrations of both the

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pesticides. But it was apparent that urease activity was stimulated very quickly immediately after the application of different concentrations of pesticides. At low concentration of added urea (2 ppm N), the stimulation of urease activity by either of the pesticides was comparatively less than that of at higher dose (100 ppm N). Difference in the concentrations of pesticides had a little influence on the stimulatory effect of urease activity. In some cases, the same magnitude of stimulation was observed after the application of different doses of pesticides. But stimulatory effect differed with types of soil. In Melandaha soil, the urease activity as affected by pesticides was not as high as in Bhatpara soil.

Table 2. Effects of diazinon and heptachlor on urease activity of native N pool.

Pesticides	Dose (ppm)	Incubation time (days) and mg of $\text{NH}_4^+\text{-N}$ released per kg soil in 5 hours					
		Melandaha soil			Bhatpara soil		
		0	14	28	0	14	28
Diazinon	0	18.45	18.45	18.45	54.75	54.75	54.75
	0.5	139.8	130.5	147.6	280.85	304.25	273.8
	100	130.5	139.8	147.6	331.95	341.15	301.15
	400	139.8	139.8	156.85	341.15	341.15	319.4
Heptachlor	0.8	130.45	129.15	119.95	301.15	365.05	273.3
	100	111.85	110.7	110.7	319.4	355.9	273.3
	400	111.85	129.15	110.7	328.55	346.8	264.65

Under control condition i.e. without the application of these pesticides, the experiment showed different result for two different soils. In Melandaha soil, the urease activity was increased by the application of every nitrogen dosage and application of different nitrogen concentration produced the same amount (27.95 mg/kg). Whereas in Bhatpara soil, it was decreased. Under control condition, the highest urease activity was estimated from Bhatpara soil at its native nitrogen pool (54.74 mg/kg).

Effect of diazinon and heptachlor on urease activity of native N pool: Effects of diazinon and heptachlor on the Melandaha and Bhatpara soil in the absence of urea are summarized in Table 2. From Table 2, it is clear that diazinon was more active in stimulating urease activity than heptachlor. Again the stimulatory effect of pesticides differed with types of soil. In Bhatpara soil, there was a higher urease activity than in Melandaha soil, and under control condition (when no pesticide was applied) urease activity remained constant throughout the entire incubation period in both soils. The amount of urease activity as affected by different doses of both the pesticides was more in

Bhatpara than in Melandaha soil. In Melandaha soil, the tendency of urease activity was increasing up to last incubation period by higher doses of diazinon. At recommended dose, the urease activity was slightly decreased at 14 days of incubation. But in Bhatpara soil, the tendency was first increasing (up to 14 days) and then decreasing at the last i.e. 28 days of incubation time by each dose of pesticides. In Melandaha soil, urease activity was highest at 400 ppm diazinon at 28 days of incubation (156.85 mg of $\text{NH}_4^+\text{-N/kg}$ soil). Whereas in Bhatpara soil, the highest was estimated at recommended dose of heptachlor at 14 days of incubation time (365 mg/kg).

Table 3 shows the results of different statistical analyses used to see the significance of the effects of pesticides on urease activity of native N pool. From Table 3, the 't' values, as observed, are highly significant both at 5 and 1% levels for diazinon in two soils. But for heptachlor in Bhatpara soil, the 't' value is significant only at 5% level. This result suggests that when these pesticides were applied the urease activity of soils in their native N pool was increased significantly. But the effectiveness was differed with the type of pesticides and type of soils. The ANOVA table shows that the calculated values of 'F' are much lower than that of the tabulated values. So, there was no effect of incubation time for either of the pesticides on urease activity in both soils. Also, χ^2 test shows the same result. The insignificant 'p' values are observed from Pearson χ^2 test. Thus, it can be said that there is no difference in the effectiveness of different rates of either of the pesticides on urease activity.

Table 3. Results from different statistical analyses used to see the significance of the effects of pesticides on urease activity of native N pool.

Name of test statistics	Pesticides	Calculated values for soils of tabulated value			
		Melandaha	Bhatpara	0.05	0.01
't'	Diazinon	24.42** (2 df)	24.48** (2 df)	4.303	9.925
	Heptachlor	32.7** (2 df)	9.5* (2 df)		
'F'	Diazinon	0.2775	0.4711	199.5	4999.5
	Heptachlor	0.1503	0.2563		
' χ^2 ' (Pearson)	Diazinon	0.2381 (9 df)	0.21331 (9 df)	16.9	21.7
	Heptachlor	0.21331 (9 df)	0.21331 (9 df)		

** Significant at both 5 and 1% level; * Significant at 5% level.

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Effect of diazinon and heptachlor on urease activity of 2 ppm N as urea: Effects of diazinon and heptachlor on the Melandaha and Bhatpara soil in presence of 2 ppm N as urea are summarized in Table 4. From Table 4 it is clear that the stimulation was higher in Bhatpara soil than that of Melandaha soil. Under control condition, in Melandaha soil, the urease activity was higher than that of native N pool throughout the incubation period. At zero concentration of pesticides, in Melandaha soil, 27.95 mg/kg urease activity (expressed as mg of NH_4^+ -N released/kg soil in 5 hrs) was produced at '0' time and remained unchanged throughout the whole incubation period. On the other hand, urease activity of native N pool was observed to be as 18.45 mg/kg during the entire incubation period. When 2 ppm N as urea was applied in Melandaha soil, the stimulation by diazinon was decreased in most cases. On the contrary, the stimulation by heptachlor was increased or in some cases it remained the same. More or less the same tendency was observed in Bhatpara soil. The differences between the urease activity of native N and of amended 2 ppm N by the application of pesticides were found to be negligible in Melandaha soil (Table 4). On the other hand, the urease activity of native N was lower than that of added 2 ppm N as urea in Bhatpara soil.

Table 4. Effects of diazinon and heptachlor on urease activity in soils treated with 2 ppm N as urea.

Pesticides	Dose (ppm)	Incubation time (days) and mg of NH_4^+ -N released per kg soil in 5 hours					
		Melandaha soil			Bhatpara soil		
		0	14	28	0	14	28
Diazinon	0	27.95	27.95	27.65	46.1	46.1	45.65
	0.5	139.8	130.5	119.95	359.6	341.15	273.3
	100	130.5	121.15	129.15	313.5	313.45	319.4
	400	130.5	130.5	138.25	350.35	350.35	310.3
Heptachlor	0.8	111.85	129.15	119.95	337.65	365.05	273.3
	100	121.15	138.4	110.7	337.65	383.3	228.15
	400	102.5	138.4	110.7	337.65	365.05	228.15

When heptachlor was applied at recommended dose, 100 and 400 mg a.i. kg^{-1} , the initial sharp stimulation was followed by a slight increase up to 14 days of incubation period and then by a decrease at the last incubation time in both soils. In case of diazinon, it was observed that the initial increase of urease activity had a declining tendency up to last incubation time at recommended dose in both soils. But at the dose of 100 ppm, the initial rise was followed by an increase up to 14 days and then it was decreased in both soils. At

the highest dose i.e. at 400 ppm a different trend was observed in both soils. The initial stimulation remained steady up to 14 days of observation time and then at the final observation it was slightly increasing.

Results obtained from different statistical analyses used to see the significance of the effects of pesticides on urease activity in soils having 2 ppm N applied as urea are shown in Table 5. The 't' statistic suggests that both diazinon and heptachlor significantly affected the urease activity in Melandaha soil. But in Bhatpara soil, the 't' values were significant only at 5 % level. This indicates that, both the pesticides are effective in stimulating urease activity in both the soils. But the effectiveness differed with type of soils. The 'F' test indicates that the incubation period had no effect on the urease activity of the soils having 2 ppm added N. From ' χ^2 ' test it can be said that the effect of different rates of the pesticides on urease activity is insignificant.

Table 5. Results from different statistical analyses used to see the significance of the effects of pesticides on urease activity of 2 ppm N as urea.

Name of test statistics	Pesticides	Calculated values for soils of		Tabulated value	
		Melandaha	Bhatpara	0.05	0.01
't'	Diazinon	33.55** (2 df)	07.03* (2 df)	4.303	9.925
	Heptachlor	18.49** (2 df)	6.91* (2 df)		
'F' (ANOVA table)	Diazinon	0.2511	0.3066	199.5	4999.5
	Heptachlor	0.1522	0.2502		
' χ^2 ' (Pearson)	Diazinon	0.21331 (9 df)	0.21331 (9 df)	16.9	21.7
	Heptachlor	0.21331 (9 df)	0.21331 (9 df)		

** Significant both at 5 and 1% level; * Significant at 5% level.

Effect of diazinon and heptachlor on urease activity of soils having added 100 ppm N as urea: The effects of diazinon and heptachlor on Melandaha and Bhatpara soil in presence of 100 ppm N as urea are summarized in Table 6. From Table 6, it is apparent that in both soils at zero time of incubation the amount of urease activity was the same at all doses of these pesticides. In presence of the pesticides, there was a stimulation of the urease activity in both the soils. However, the incubation period appears to have no effect on the stimulation although the doses of pesticide application seem to have some positive effect.

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The stimulation was greater in Bhatpara soil than that of Melandaha soil. In Bhatpara soil, as high as 396.45 mg/kg urease activity was estimated at initial incubation period when diazinon was applied as compared to 139.8 mg/kg in Melandaha soil.

Table 6. Effects of diazinon and heptachlor on urease activity in soils treated with 100 ppm N as urea.

Pesticides	Dose (ppm)	Incubation time (days) and mg of $\text{NH}_4^+\text{-N}$ released per kg soil in 5 hours					
		Melandaha soil			Bhatpara soil		
		0	14	28	0	14	28
	0	27.5	27.5	27.5	36.9	36.5	36.5
Diazinon	0.5	139.8	129.15	92.25	396.45	328.55	255.55
	100	139.8	119.95	110.70	396.45	328.55	255.55
	400	139.8	119.95	119.95	396.45	319.4	264.65
Heptachlor	0.8	139.8	119.95	92.25	378.0	301.15	237.3
	100	139.8	129.15	92.25	378.0	282.9	209.9
	400	139.85	138.4	110.7	378.0	301.15	219.05

In Melandaha soil, application of higher dose of substrate (100 ppm N as urea) did not affect the urease activity when no pesticides were applied. The urease activity (expressed as mg of $\text{NH}_4^+\text{-N}$ released in 5 hrs per kg soil.) was estimated to be 27.5 mg/kg, which was in proximity to that of 2 ppm added N as urea. A different trend was observed in Bhatpara soil. The urease activity was 46.1 mg/kg whereas the value was 54.75 mg/kg when no urea was added.

In Melandaha soil, the initial increase of urease activity was followed by a subsequent gradual decrease after the application of all doses of both the pesticides except for the maximum dose (400 ppm) of diazinon. In that case, the initial increase was followed by a gradual decrease at 14 and after 28 days of incubation this amount remained static. At recommended dose of diazinon in that soil, the initial rise was followed by a gradual decrease after 14 days of incubation time and sharp decrease was observed at the final observation period. In Bhatpara soil, the initial stimulation was also followed by a decrease during the subsequent incubation periods.

The 't' test from Table 7 shows that calculated 't' values are significant at 5% level. That is why it can be said that the pesticides have significant effect on urease activity in soils treated with 100 ppm N. The calculated 'F' values are much smaller than the tabulated values. Therefore, incubation time has no effect on urease activity in both soils after the

application of pesticides. The values of ' χ^2 ' tests are also found insignificant. So, it can be said that the effects of different rates of pesticides on urease activity at 100 ppm added N as urea are insignificant in both soils.

Table 7. Results from different statistical analyses used to see the significance of the effects of pesticides on urease activity of 100 ppm N as urea.

Name of test statistics	Pesticides	Calculated values for soils of		Tabulated value	
		Melandaha	Bhatpara	0.05	0.01
't'	Diazinon	6.4* (2 df)	7.18* (2 df)	4.303	9.925
	Heptachlor	6.5* (2 df)	6.62* (2 df)		
'F' (ANOVA table)	Diazinon	0.2511	0.2406	199.5	4999.5
	Heptachlor	0.3028	0.2126		
' χ^2 ' (Pearson)	Diazinon	0.21331 (9 df)	0.23810 (9 df)	16.9	21.7
	Heptachlor	0.21331 (9 df)	0.21331 (9 df)		

* Significant at 5% level.

It has already been stated earlier that knowledge of the action of pesticides on soil enzymes is considerably scarce. Again, reports on the impact of pesticides on soil enzymes show considerable diversity depending upon type of pesticide used, rate and mode of application, climatic factors, composition of soil, and organic matter content (Tu 1981 Endo *et al.* 1982). Therefore, direct comparisons of our results with those reported in the literature are rather difficult. The findings in the present study are that, there is a stimulation of soil enzymatic activity after the applications of pesticides. Stimulatory effects are often difficult to categorize. Some researchers refer to the rapid colonization of pesticide treated soils by such antagonistic fungi as *Aspergillus*, *Penicillium* and *Trichoderma* as 'stimulatory', when in actuality it may be the result of partial sterilization, their greater tolerance to the toxicant and a characteristic rapid growth rate in the absence of competing species (Kreutzer 1965). The stimulatory effect of pesticide on soil urease activity is in agreement with the study of Sannino and Gianfreda (2001) who observed the stimulation of urease activity by paraquat, atrazine and carbaryl in twenty

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two soils. In another investigation by Gianfreda *et al.* (1994) with four pesticides, paraquat, carbaryl and glyphosate enhanced the urease activity of soils and soil extracts.

The present findings of stimulation of soil urease activity as affected by pesticides have also been attributed to the destruction of a part of soil population and release of mineral N from the decomposing tissues, in accordance with the reports from Jenkinson and Powlson (1970). Mineral N also is released from N-containing pesticides during their decomposition (Goring and Laskowski 1982). This may be the reason for increased mineralization in presence of diazinon which is a N-containing chemical. Pettit *et al.* (1976) have suggested that fluctuations or changes in soil enzyme activities after pesticide application may be due to the release of intracellular enzymes on the death and lysis of microorganisms. In Bhatpara soil, the native urease activity was higher in comparison to that of Melandaha soil, which may be due to its higher organic carbon content (0.94 %). The other two soil characteristics such as heavier texture and relatively low pH (6.3) may also have been partly responsible for the same in this soil. The reason may be that active urease is in the adsorbed form on clay and humus and also due to the presence of ureolytic organisms because of their slight difference in pH. It is in agreement with the report on the measurement of urease activity of nine different soils (Viraj *et al.* 1978). The presence of diazinon and heptachlor increased urease activity of both soils. This may be due to the enhancement of activity of ammonifiers or decreased activity of nitrifiers (Tu 1970). The ammonifiers are involved in the ammonification process which is an enzymatic process and in which the N of nitrogenous organic substances is liberated as NH_3 . The result from an earlier investigation on nitrification as affected by diazinon and heptachlor showed that at higher doses (100 and 400 ppm) both the pesticides inhibited nitrification process (Imamul Huq *et al.* 2004). The results from the present experiment show that urease activity is enhanced in the presence of diazinon and heptachlor and this effect continued till the end of the observations. From these results, we opine in agreement with earlier report on baygon treated soil that both the processes i.e. the enhancement of the activity of ammonifiers and the suppression of nitrifiers result in increased ammonia formation (Gupta *et al.* 1975). Another general observation is that ammonification or nitrogen mineralization is less sensitive to pesticides than is nitrification (Kreutzer 1963, Martin 1966). Often the ammonifying activity of the heterotrophic bacteria, actinomycetes and resistant saprophytic fungi is little affected or actually even increases after pesticide treatment (Kreutzer 1963, Martin 1966). Consequently, depending on the soil pH, rather large concentrations of NH_3 or NH_4^+ can accumulate following fumigation or fungicidal treatment. In Bhatpara soil, all doses of both the pesticides increased urease activity of native N pool up to first 14 days of

incubation, which decreased subsequently. The increase might be due to greater mineralization of organic matter and the decrease at later stages, due to volatilization and immobilization (Jana *et al.* 1988). The degree of stimulation of urease activity also varied with pesticides in some cases. The answer might lie in the classification and mode of action of pesticides. Heptachlor is a chlorinated hydrocarbon insecticide. It has weak insecticidal properties (Ramulu 1979). But heptachlor like other cyclodines, is generally recognized as the most persistent organic insecticides. On the other hand, diazinon is an organophosphorous insecticide. Organophosphorous insecticides are potent inhibitor of acetyl cholinesterase. Though it is less persistent, it has wider killing spectrum (Ramulu 1979). The present experiment showed that after the application of two concentrations of N, the urease activity was increased in Melandaha soil and decreased in Bhatpara soil.

It needs to be mentioned here that the doses used in the experiment are not likely to occur under field condition. Moreover, heptachlor has been banned, so its use by the farmers is also not likely. But prolong use of pesticides might result in accumulation in certain amount to cause harm. Kearney *et al.* (1965) stated the possibility of zone accumulation of pesticides at 100 ppm or higher due to uneven distribution of pesticides through 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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