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A comparative study of the effect of imdacloprid and dimethoate on soil enzyme

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Key words: Imidacloprid, dimethoate, *Drawida willsi,* soil enzyme, soil metabolism**.**

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Abstract

Soil metabolism seems to be one of the major tools to study the effect of agrochemicals (pesticides and fertilizers) on soil health. It is an appropriate indicator for highlighting the impact of land use management, soil quality monitoring and pollution. Several experiments were conducted to find out the effect of pesticides on soil metabolism. However few studies were conducted on Imidacloprid (neonicotinoid) and Dimethoate (organophosphate) which showed no concrete conclusion. So an attempt was undertaken to find out the toxicity of Imidacloprid and Dimethoate which are being used mostly in the Indian crop fields today due to its less toxicity. The study found an increase of dehydrogenase activity by 15.36% in recommended agricultural dose of imidacloprid treated soil and decreased by 36.25% in dimethoate treated soil after 15 days. The acid phosphatase activity was also increased by 24.37% in imidacloprid treated soil and decreased by 23.65% in dimethoate treated soil till 15 days. Similar trend was also found in alkaline phosphatase activity where there is an increase of 22.87% in imidacloprid treated soil and decreased by 31.77% in dimethoate treated soil. But the urease activity was less in soil treated with recommended agricultural doses of imidacloprid and dimethoate as compared to control soil. The present study indicates greater toxicity of dimethoate in comparison to Imidacloprid. So it is suggested to avoid dimethoate even at the recommended doses.

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Introduction

Soil consists of several bacteria and other micro-organism which decomposes living material (plants and animals) and converts them into nutrients which is called soil metabolism. During this biochemical process, bacteria and other micro-organisms release some enzymes (mostly urease, phosphates, dehydrogenases etc.) (Macfadyen, 1970). These enzymes are responsible for various decomposition and chemical transformations in the soil. They are of immense biological significance as they participate in the cycling of elements and can influence the availability of nutrients to plants. Their measurement can give the indications of the extent of the specific processes in soil and soil fertility (Mishra *et al.,* 1979).

Under normal agricultural practices, tremendous uses of agrochemicals are there in each year to boost crop production. These agrochemicals are applied either directly to the soil or transported from the treated crops (Hernadez-Soreano *et al.,* 2007) but they are imposing a treat to the soil environment (Zafar and Hasan, 1994 : Meuhlenberg *et al.,* 1995) killing the non-target beneficial microorganisms that are responsible for enhancement of soil fertility (Gundi *et al.,* 2007).

The effect of pesticides on soil microorganisms can be assessed following two ways-(a) directly by estimating the soil microbial population and biomass, and (b) indirectly by studying the soil metabolism through soil respiration and soil enzyme activities. Measurement of carbon dioxide evolution, enzyme activity and essential nutrients like carbon, nitrogen etc of soil are common method for measurement of soil metabolism and are regarded as an index of microbial dynamics (Skujins, 1978).

Microbial respiration and enzymatic activities were used as appropriate indicators for highlighting the impact of land use management, soil quality monitoring and pollution (Endo *et al.,* 1982; Baath, 1989; Anderson and Domsch, 1990; Dick, 1992, 1994; Brookes, 1995; Wardle and Ghani, 1995; Fernandes *et al.,* 2005; Sebiomo *et al.,* 2011). Some of the most studied enzymes in soil are urease, phosphates and dehydrogenases (Singh and Kumar, 2008). Urease is an important enzyme for N-economy of the soil (Tiwari *et al.,* 1989; Vaughan and Ord, 1991), while the level of phosphates activity affects the phosphorous mineralization in soil (Pang and Kolenko, 1986 : Nakas *et al.,* 1987). Finally dehydrogenases, a group of intracellular enzymes, have been widely used to measure catabolic activities in the soil and have shown to be correlated with microbial activity (Cochran *et al.,* 1989; Garcia *et al.,* 1994).

Several studies were conducted to find out the effects of pesticide on soil enzymes (Davies and Grreaves 1981; Mishra and Pradhan 1987; Tu 1988, 1993; Rangaswamy *et al.,* 1994; Panda and sahu, 2000; Min *et al.,* 2001; Omar and abdel-Sater, 2001; Sannino and Gianfreda, 2001; Klose and Tabatabai, 2002; Burrows and Edwards, 2004; Klose and Ajwa, 2004; Gundi *et al.,* 2005; Menon *et al.,* 2005; Stromberger *et al.,* 2005; Pampulha and Oliveria, 2006; Yu *et al.,* 2006; Bending *et al.,* 2007; Qian *et al.,* 2007; Wang *et al.,* 2007; Yang *et al.,* 2007). Most of these studies conclude that pesticides at higher doses inhibit enzymatic activities.

However few studies were conducted on Imidacloprid (neonicotinoid) (Yao *et al.,* 2006) and Dimethoate (organophosphate) which showed no concrete conclusion. So an attempt was undertaken to find out the toxicity of Imidacloprid and Dimethoate which are being used mostly in the Indian crop fields today due to its less toxicity.

Materials and methods

Pesticide

Imidacloprid, Victor 17.8% SL, Insecticide (India) Jammu and Roger 30% EC the trade name of dimethoate, (Rallis India Limited, Mumbai) and was used in the present work as the test chemicals. The chemical composition of imidacloprid is 1 (1 (6 chloro-2-pyridimyl) methyl-N-nitro-2-imidazolidinimene. The chemical composition of

dimethoate is 0, 0-dimethyl-S-(N methyl-carbamoylmethyl) di-thiophosphate.

Soil

The soil was collected from an upland non-irrigated paddy field, which had no record of input of agrochemicals (fertilizers and pesticides). The soil belongs to laterite type and of yellow colouration called latosols. By texture, the soil is of sandy loam type. The pH of the soil was 6.8. The soil was found to contain 2.7 g% of the total organic carbon and 0.22 g% nitrogen. The C: N ratio of the soil was 12.27. The soil was air dried and sieved before use.

Preparation of experimental sets

To study the effect on soil metabolism, three sets of plastic culture pots each with thirty five replicates and 2 kg of soil were kept for control and recommended doses of dimethoate and imidacloprid. The recommended agricultural dose (mg a.i./ kg) for dimethoate and imidacloprid (0.4 and 0.05 respectively) was prepared in dilution of water and sprayed on to the soil surface. After evaporation of the solvent, enough water was added and the treated soil was thoroughly mixed for even distribution of the pesticide. Only water was applied to prepare the controls. The experiment was maintained at 20±2g% soil moisture and 25±2°C soil temperature. Out of thirty five replicates of each set, five replicates were taken at 15 days intervals up to 105 days to assess the soil metabolic activities.

Enzyme activities

Dehydrogenase

Dehydrogenase activity was assayed by the reduction of 2, 3, 5 trinitro tetrazolium chloride (TTC) following the triphenyl formazan method of Casida *et al.* (1964). Each soil sample (2g) was treated with 0.1 g of $CaCO_3$ followed by 2 ml of 1% TTC (2 ml distilled water for control) and incubated for 24 hour at 37°C. The triphenyl formazan formed was extracted from the reaction mixture with methanol and assayed at 485nm in a spectrophotometer. The dehydrogenase activity was expressed in mg formazan /g soil/h.

Urease

Urease activity was measured according to Kaplan (1965). Soil sample (5g) was taken in a 50 ml volumetric flask mixed with 0.2 ml toluene. To it 9 ml of Tris-HCl buffer (pH9, 0.2 m) was added and the content was mixed thoroughly. Then 1 ml of 0.2 M urea (substrate solution) was added to it. A control was kept simultaneously with 1 ml of distilled water instead of substrate solution. After a little swirling, flasks were incubated at 37°C for a period of 2 hours. After 2 hours, KCl-$AgSO_4$ solution was added up to the mark (50 ml). A portion of the content was centrifuged and the supernatant was used for analysis. To 1 ml of supernatant, 1 ml of phenate and 1 ml of alkaline hypochloride solution was added. After 5 minutes, O.D. was measured at 625 nm. Ammonium chloride was used as standard. The value was expressed in terms of mg $NH4^+$/g dry soil/h.

Phosphatase

Soil phosphatase activity was assayed following the procedure of Tabatabai and Bremner (1969) using modified universal buffer (MUB) (Skujins *et al.,* 1962). Soil (1 g) sample was placed in a 10 ml capped test-tube. After oxidizing with 0.2 ml toluene and swirling the tube in capped condition, the content was incubated for 4 hours with 4 ml modified universal buffer (pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase) and 1.0 ml of 0.115M p-nitrophenyl – phosphatase disodium salt. The control contained no substrate. The reaction was stopped by adding 1.0 ml 0.5 M $CaCl_2$ and 5 ml of 0.5 M NaOH. The mixture was centrifuged and 1.0 ml of supernatant was taken. Absorbance was measured at 400 nm by spectrophometer using p-nitrophenal as standard. Soil phosphatase activities were expressed in terms of mg p-nitrophenol produced per g of dry soil per hour.

Statistical analyses of the data were made as per Snedecor and Cochran (1967). Statistical package SPSS (version 10) was also used to compute the data.

Result

Dehydrogenase activity

After 15 days, the dehydrogenase activity was increased by 15.36% in recommended agricultural dose of imidacloprid treated soil and decreased by 36.25% in dimethoate treated soiled. However, this increase and decrease in dehydrogenase activity was not consistent throughout the experimental period (Table-1 and Figure-1). Two-way ANOVA showed that the dehydrogenase activity of the soil was significantly affected by the doses of pesticides as well as days interval (F1= 9.18, F2= 3.97 p≤0.05). Significant difference in the dehydrogenase activity of control and the experimental sets was noticed up to 75 days (F≥5.92, p≤0.05) by one-way ANOVA. LSD test, however, showed significant difference (p≤0.05) between the control and imidacloprid sets up to 30 days, between the control and dimethoate treated soils up to 75 days and between the experimental sets up to 15 days. This test thus showed greater toxicity of dimethoate in comparison to imidacloprid (Table-1).

Table 1. Dehydrogenase activity (mg formazon/g dry soil/h±SD) following application of recommended agricultural doses of pesticides to soil.

Days	Control	imidacloprid	dimethoate	One-way ANOVA (F)	LSD (p<0.05)	Two-way ANOVA
0	41.70±3.1	41.76±3.1	40.56±3.1	_	_	_
15	58.53±4.35a	67.52±1.96b (15.36%)	37.31±1.8c (-36.25%)	111.12*	4.54	F1=9.18* F2=3.97*
30	59.02±2.57a	66.1±2.83b (11.99%)	41.01±2.22b (-30.52%)	102.77*	3.93	_
45	62.91±2.43a	65.18±1.8a (3.61%)	46.68±3.82b (-25.80%)	51.55*	4.33	_
60	59.22±2.12a	60.98±0.52a (2.97%)	49.44±5.24b (-16.51%)	14.32*	5.06	_
75	56.23±1.36a	57.53±0.62a (2.31%)	52.18±3.68b (-7.20%)	5.92*	3.54	_
90	43.71±1.19	43.91±0.72 (0.48%)	41.68±3.13 (-4.62%)	1.56	3.05	_
105	40.94±1.06	40.77±0.66 (-0.42%)	40.88±3.12 (-0.15%)	0.01	2.99	_

* p<0.05, F1=between pesticides, F2= between days
Values in the same row with different alphabets are significantly different by LSD.

Urease activity

Urease activity of the soil in recommended agricultural doses of imidacloprid and dimethoate treated soil was less as compared to control soil throughout the experimental period. Maximum decrease of 25.52 and 52.86% was recorded after 15 days in imidacloprid and dimethoate treated soil respectively (Table-2 and Figure-2). Two-way ANOVA showed significant difference in the urease activity of the soil with respect to doses of pesticides and days intervals (F1= 9.25, F2= 3.48 p≤0.05). Significant difference between control and experimental soils was observed up to 45 days (F≥5.01, p<0.05) by One-way ANOVA test and insignificant thereafter. LSD test demonstrated significant difference between control and experimental soils as well as between imidacloprid and dimethoate treated soils up to 15 days and between control and dimethoate treated soils up to 45 days (p<0.05) (Table-2).

Table 2. Urease activity (mg NH_4+/ g dry soil/ h±SD) following application of recommended agricultural doses of pesticides to soil.

Days	**Control**	**imidacloprid**	**dimethate**	**One-way ANOVA (F)**	**LSD (p<0.05)**	**Two-way ANOVA**
0	10.71±3.2	10.70±3.2	10.69±3.2	_	_	_
15	11.56±3.13a	8.61±2.13b (-25.52%)	5.45±1.61c (-52.85%)	15.14*	2.42	F1=9.25*
30	12.24±2.06a	10.22±0.53a (-16.50%)	6.92±2.4b (-43.46%)	8.29*	2.87	F2=3.48*
45	13.01±1.98a	12.14±0.61a (-6.69%)	9.36±2.13b (-28.06%)	5.01*	2.63	_
60	12.76±1.72a	12.33±0.72a (-3.37%)	10.81±2.4a (-15.28%)	1.21	2.86	_
75	12.51±1.56	12.22±0.77 (-2.32%)	11.24±2.52 (-10.15%)	0.53	2.81	_
90	11.79±1.25	11.52±0.80 (-2.29%)	11.38±3.13 (-3.48%)	0.28	1.21	_
105	11.01±1.13	11.02±0.72 (0.09%)	10.75±1.79 (-2.36%)	0.06	1.99	_

* p<0.05, F1=between pesticides, F2= between days
Values in the same row with different alphabets are significantly different by LSD.

Phosphatase activity

Acid phosphatase activity

Compared to control soil, after a period of 15 days the acid phosphatase activity was increased by 24.37% in imidacloprid treated soil and decreased by 23.65% in dimethoate treated soil (Table-3 and Figure-3). Significant difference was observed up to 45 days between control and the experimental sets as well as between the two experimental sets by LSD test ($p<0.05$). Significant difference ($p<0.05$) between control and dimethoate treated soil was further found up to 60 days indicating the greater toxicity of dimethoate. One-way ANOVA showed significant difference between control and experimental sets up to 60 days ($F\geq4.78$, $p<0.05$) and insignificant thereafter. Two-way ANOVA showed that the acid phosphatase activity of the soil was affected by the doses only ($F1=7.01$, $p<0.05$) (Table-3).

Table 3. Acid phosphatase activity (mg p-nitrophenyl phosphate/ g dry soil/ h±SD) following application of recommended agricultural doses of pesticides to soil.

Days	Control	imidacloprid	dimethate	One-way ANOVA(F)	LSD (p<0.05)	Two-way ANOVA
0	18.89±1.4	18.9±1.4	18.91±1.4	_	_	_
15	20.68±1.32a	25.72±0.92b (24.37%)	15.79±2.12c (-23.65%)	42.88*	2.36	F1=7.01* F2=0.316
30	21.42±1.22a	25.12±0.80b (17.27%)	18.46±1.91c (-13.82%)	25.95*	2.02	_
45	22.09±1.13a	24.14±0.72b (9.28%)	19.59±1.79c (-11.31%)	12.47*	1.99	_
60	21.86±1.19a	22.36±0.60a (2.29%)	19.89±1.82b (-9.01%)	4.78*	1.95	_
75	21.22±1.25a	21.42±0.53a (0.94%)	20.11±1.82a (-5.23%)	1.17	2.01	_
90	20.73±1.32	20.83±0.41 (0.48%)	20.24±1.72 (-2.36%)	0.25	1.96	_
105	20.34±1.48	20.34±0.36 (0%)	20.33±1.82 (-0.05%)	0	0	_

* $p<0.05$, F1=between pesticides, F2= between days.

Alkaline phosphatase activity

Alkaline phosphatase activity of the soil treated with both the pesticides (imidacloprid and dimethoate) followed the same trend as that of acid phosphatase activity of soil. After 15 days, alkaline phosphatase activity was increased by 22.87% in imidacloprid treated soil and decreased by 31.77% in dimethoate treated soil (Table-4 and Figure-4). Significant difference between control and experimental sets was observed up to 45 days ($F\geq5.69$, $p<0.05$) by one-way ANOVA test and up to 30 days by LSD test ($p<0.05$,) Two-way ANOVA showed that the alkaline phosphatase activity of the soil was affected by the doses of pesticides only ($F1=7.02$, $p<0.05$) (Table-4).

Table 4. Alkaline phosphatase activity (mg p-nitrophenyl phosphate/ g dry soil/ h±SD) following application of recommended agricultural doses of pesticides to soil.

Days	Control	imidacloprid	dimethoate	One-way ANOVA (F)	LSD (p<0.05)	Two-way ANOVA
0	14.21±2.1	14.22±2.1	14.2±2.1	_	_	_
15	15.74±1.91a	19.34±0.43b (22.87%)	10.74±2.05c (-31.77%)	27.95*	2.52	F1=7.02* F2=0.269
30	16.36±1.8a	19.16±0.44b (17.11%)	13.11±1.91c (-19.87%)	15.56*	2.37	_
45	16.74±1.72	17.94±0.61a (7.17%)	14.52±1.8a (-13.26%)	5.69*	2.26	_
60	16.26±1.56	16.76±0.71 (3.08%)	14.74±1.67 (-9.34%)	2.3	2.13	_
75	15.94±1.48	16.14±0.80 (1.25%)	14.98±1.51 (-6.02%)	1.02	1.89	_
90	15.92±1.36	15.67±0.94 (-1.57%)	15.01±1.48 (-5.72%)	0.54	1.98	_
105	15.11±1.25	15.11±1.09 (0%)	15.12±1.32 (0.07%)	0	0	_

* $p<0.05$, F1=between pesticides, F2= between days

Values in the same row with different alphabets are significantly different by LSD.

Discussion

Several studies were conducted on the impact of pesticides belonging to the group of organocholine, organophosphate, carbamate, neonicotinoid etc. on soil enzymatic activities. Naumann (1970, 1972) reported a stimulation of dehydrogense activity with methyl parathion, an organophosphate at 15 kg/ha, whereas at higher doses (150 – 300 kg/ha) complete inhibition was observed. Chendrayan and Sethunathan (1980) in a laboratory incubation study showed that benomyl (fungicide), B.H.C. (organocholine) and carbaryl (carbamate) are most effective in inhibiting dehydrogenase activities. But Tu (1980) found no inhibitory effect on dehydrogenase activity with five pyrethroid insecticides in a sandy loam soil. Yao *et al.* (2006) reported that acetamiprid, a neonicotinoid, at normal field dose could not impose any serious threat to soil enzymes. In our experiment, dehydrogenase activity was initially enhanced by 15.36% following application of recommended dose of imidacloprid and lessened by 36.25% following application of recommended dose of dimethoate to soil and the activity stabilized after 75 days in both the doses of pesticides. This is in agreement with the findings of Panda and Sahu (2000) and Singh and Singh (2005). Panda and Sahu (2000) reported that at single agricultural dose of malathion, (organophosphate) dehydrogenase activity was accelerated and inhibited at double agricultural dose. Singh and Singh (2005) reported that in imidacloprid seed treated field, dehydrogenase activity was increased between 15 and 60 days after showing. Our experiment further indicated that the dimethoate is more toxic compared to imidacloprid.

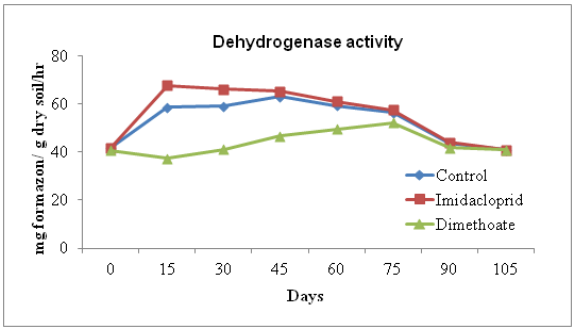

Fig. 1. Dehydrogenase activity following application of recommended agricultural doses of pesticides to soil.

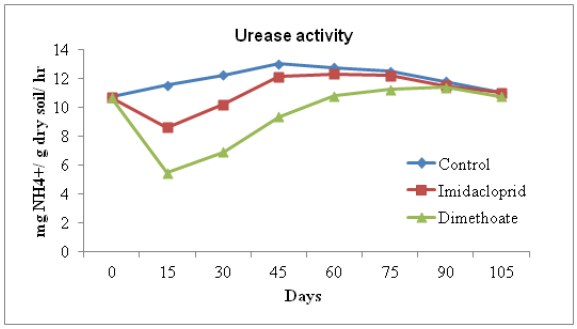

Fig. 2. Urease activity following application of recommended agricultural doses of pesticides to soil.

Urease is responsible for nitrogen mineralization (Kandeler and Eder, 1993; Kandeler *et al.*, 1999; Tu, 1981). Effect of pesticides on urease activity has been reported by many workers. Some reports are available on the effect of organochlorine pesticides, herbicides, fungicides on the soil urease activity. Tsirkov (1970) reported inhibition of urease activity in meadow soils by organocholine pesticide heptachlor, while lindane and dieldrin increased it. While studying the herbicides, Zubets (1973) reported that urease is the only enzyme affected by simazine and atrazine. Voets *et al.* (1974) showed that urease activity was reduced by 50% or more when atrazine had been applied at normal field dose. Marsh (1980) in his study showed that the lowest concentration of asulam (herbicides) at normal field application is unlikely to have effects on agronomic practice. Karanth *et al.* (1975) found that urease was stimulated at 20 ppm of dexon, a fungicide, but this enzyme was inhibited at 100-200 ppm concentration. Lethbridge and Burns (1976) reported that inhibition of urease enzyme occurs by organophosphorus pesticide at higher concentration. Wainwright (1978) stated that the pesticides with exception of fumigants have little deleterious influence on soil processes when applied at field rate. Tu (1981, 1995) reported inhibition of urease activity after terbufos (organophosphate), amitraz, (formamidine insecticides) tebupirimphos and aztec (organophosphate and pyrethroid) application. Martikainen *et al.* (1998) had reported that dimethoate and bemomyl (carbamate) had affected soil microorganism but their influence on nutrient

dynamics was negligible. Nasreen *et al.* (2012) found that there was a decrease in urease activity after 24h of incubation which continued up to 20 days following application of profenophos, an organophosphate. Ingram *et al.* (2005) in their study have found that there is no significant positive or negative effect of Imidacloprid on urease activity at the field concentration. In the present investigation, urease activity of the soil was decreased to maximum after 15 days of application of recommended agricultural doses imidacloprid (25.52%) and dimethoate (52.86%) to soil and this inhibition was significantly continued up to 45 days ($F \geq 5.01$, $p < 0.05$) for both the pesticides (Lethbridge and Burns, 1976).

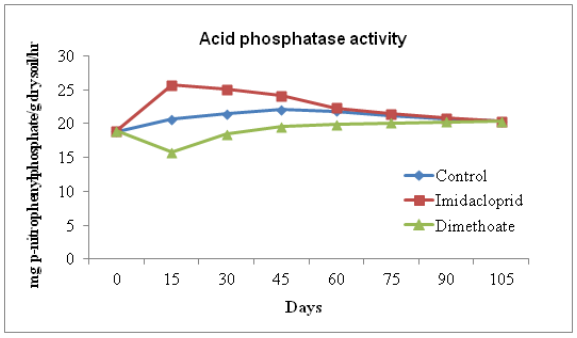

Fig. 3. Acid phophatase activity following application of recommended agricultural doses of pesticides to soil.

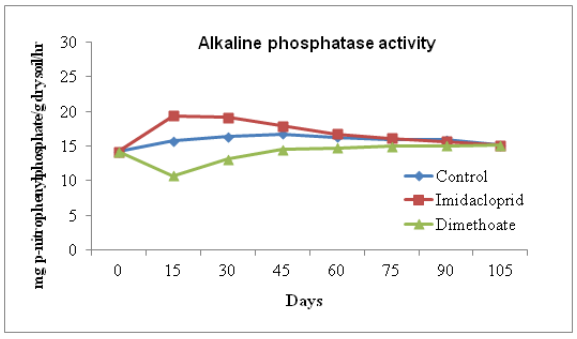

Fig. 4. Alkaline phosphatase activity following application of recommended agricultural doses of pesticides to soil.

Phosphatase is either inhibited or stimulated or has no effect on pesticide application. Positive significant correlation between phosphatase activity and microbial populations have been reported (Aliev and Gadzhiev, 1973; Tarafdar *et al.*, 1989) and yet some earlier reports (Ramirez-Martinez and Mc Laren, 1966; Roizin and Egarov, 1972; Beck, 1974) reveals insignificant correlation. Domsch (1970) commented on the sensitivity of phosphatase activity to different pesticides, especially organophosphorus insecticides. Inhibitory effect of different pesticides on soil phosphatase activity has been reported by Anderson and Drew (1976). Abdel'Yussif *et al.* (1976) got both positive and negative effect of diazinon and carbathion, organophosphorus insecticides, on soil phosphatase activity. Tu (1995) found that imidacloprid and other insecticides are affecting phosphatase for one week only. In the present study, acid and alkaline phosphatase activities were increased following application of imidacloprid at the recommended agricultural dose to the soil, whereas both the phosphatase activities were decreased following application of recommended dose of dimethoate to soil. Acid phosphatase activity was significantly affected up to 60 days following application of recommended agricultural dose of both the pesticides to soil and alkaline phosphatase activity was significantly affected up to 30 days following application of recommended agricultural dose of both the pesticides to soil.

The dehydrogenase and phosphatase activity was increased in imidacloprid treated soil and decreased in dimethoate treated soil at recommended agricultural dose. Urease activity was reduced in case of both the pesticide. The present study indicates that dimethoate is more toxic than imidacloprid. If enzyme activities are reduced then beneficial microorganisms would get decreased imparting the soil fertility. At recommended doses imidacloprid is increasing the dehydrogenase and phosphatase activities but at higher doses, it may decrease it as studied by other authors. So it would be better to avoid dimethoate even at the recommended doses.

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