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Studies the Effects of Imidacloprid on Enzymatic Activities in Clay Loam Soil

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ABSTRACT
In-vivo experiment was conducted for toxicity evaluation of Imidacloprid on soil enzyme activities (arylsulphatase, acid-phosphatase and dehydrogenase activities) in the treated soil under control condition for 60 days at different application rate 3.97µg/mL (FR), 7.94µg/mL (2FR) and 39.7µg/mL (10FR). Imidacloprid has significant toxic at 2FR and 10FR doses but not on the recommended field rate (FR). Acid phosphatase activity was stimulated at FR rate, whereas at higher dose (10FR) inhibited the activity. A significant drop of dehydrogenase activity was observed irrespective of doses at 30 days of application and after that the enzymatic activity slowly increased. Application of Imidacloprid at FR to agriculture soil of Tripura is not likely to show any detrimental effects on soil acid phosphatase and dehydrogenase activities but at higher elevated doses there appear some harmful effects which was also very much transient.

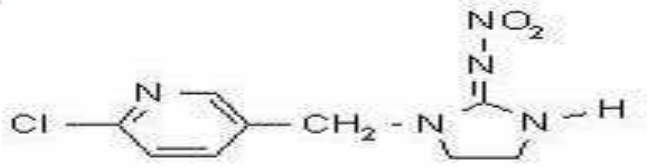
Keywords: *Arylsulphatase, Acid Phosphatase, Dehydrogenase, Enzyme activity, Imidacloprid, Degradation*

1. INTRODUCTION

In the present scenario the intensive use of agrochemicals, are among the most pressing issues concerning agriculture sustainability (1). The sustainable agriculture requires the maintenance or enhancement of soil quality (2). Fertilizers and pesticides tend to have long persistence in the soil so they are bound to affect the soil micoflora thereby disturbing soil health (3). Soil microbial communities produce extracellular enzymes to acquire energy and resources from complex soil environment (4). Soil enzymes are a group of enzyme whose usual inhabitants are the soil and the soil enzymatic activity assay is only one way to measure the ecosystem status of soils (5, 6) because the activity of soil enzymes control nutrient cycles (7). The sources of the enzyme in the soil are the microbial biomass as well as plant and animal residues (Fig. 1) (8). The soil enzyme activity could be made for evaluation the soil fertility, indicators of soil quality, sustainability and changes in biogeochemical function due to management or perturbations[9].

All the biochemical transformation in soil is related to the presence of enzymes (10). Soil enzymes increase the reaction rate at which plant residues decompose and release plant available nutrients [8]. Arylsulfatase are an important enzyme because of the common occurrence of sulphur in the soil and making it available to plants in an oxidation process and is typically widespread in soils (11). This enzyme hydrolyses organic sulphate (VI) esters ($R\text{-}O\text{-}SO_3^{2-}$) to phenols (R-OH) and inorganic sulphates (VI) (SO_4^{2-}) (12). Phosphatases are a broad class of phosphohydrolyase play a critical role in Phosphorus cycle. It catalyze the hydrolysis of organic phosphorous the hydrolysis of esters and anhydrides of phosphoric acid (13-14). The soil dehydrogenase as an biological indicators of overall soil microbial respiratory activities (15) which plays a significant role in the biological oxidation of soil organic matter by transforming protons and electrons from substrates to acceptors (16).

Imidacloprid [1-(6-chloro-3-pyridyl methyl)-N- nitro imidazolidin-2-ylidene-amine] is an extensively used systemic insecticide for crop protection due to its low soil persistence and insecticidal activity at low application rate with novel mode of action (17) used for the control of sucking insects, including rice hoppers, aphids, thrips and white files etc. It is also effective against termites and some species of cutting and chewing insects, such as rice weevil, and Colorado beetle, having no effect on nematodes or spider mite (18).



Stucture of Imidacloprid
(Empirical formula: $C_9H_{10}ClN_5O_2$)

Since Imidacloprid, a new generation insecticide widely used in our country and effect of this insecticide on the soil quality, especially enzymatic activities of our soil has not yet been undertaken so far, a laboratory incubation experiment was conducted to study the effect of Imidacloprid on some soil enzyme activities.

With the above perspective, an investigation has been undertaken in order to evaluate the effect of Imidacloprid on soil enzyme activity (arylsulphatase activity, acid phosphatase activity and dehydrogenase activity) in clay loam soil at laboratory conditions.

2. Materials and methods

2.1. Insecticides

A 17.8% soluble (liquid) concentrate (SL) formulation of Imidacloprid was obtained from Bayer Crop Science.

2.2. Collection of soil samples

Field-moist Red soil samples were collected from State Agricultural Research Centre, Arundhuti Nagar, Agartala, Tripura. The soil samples were randomly taken from the plough layered, i.e. 0-15 cm depth and brought to the laboratory in properly sealed and labeled polyethylene bags.

2.2.1. Processing of the soil samples

After collection of the soil, large lumps were broken, roots etc. were separated and the soil was divided into two portions. One part was thoroughly air-dried in the laboratory and subsequently, the soil was ground with a mortar-pestle and passed through a 2mm sieve for determining physico-chemical properties. Another part was sieved in field-moist condition (<2 mm) homogenized and kept in plastic sealed containers for 7 days in an incubator at 25°C for stabilization. An incubation studied was carried out at 60% Water Holding Capacity of soil, following pesticide application, and periodic changes of the arylsulphatase, acid-phosphatase and dehydrogenase activities were studied.

2.3. Application of Imidacloprid in soil

The application at recommended field rate (FR) (18), two times of recommended field rate (2FR), and ten times of recommended field rate (10FR), with control (untreated), was chosen to determine the influence of insecticide on soil enzyme activities. The application of 10FR was chosen to minimize the effect of adsorption of pesticide on the studied properties of soil and emphasize the side effects of pesticides on soil microorganisms. Also the 10FR dose is recommended in laboratory tests to assess the side effects of pesticide on soil microflora (19). Three stock solutions containing 3.97µg/mL (FR), 7.94µg/mL (2FR) and 39.7µg/mL (10FR) of Imidacloprid was prepared in distilled water and applied to 200 g of accurately weighed soil and mixed thoroughly with a glass rod and set for incubation. All the treatments were replicated thrice. Enzymatic activities were studied on 1, 3, 7, 15, 30, 45 and 60 days of incubation.

2.4. Determination of the Physico-chemical properties of soil

Enzyme activities depend on a series of abiotic factors, such as the soil temperature, soil water content, pH, etc. (20). To evaluation of the effect of Imidacloprid on soil enzyme activities physico-chemical properties of soil were also examined. Freshly procured soil samples were analyzed for the physical and chemical characteristics.

2.4.1. Moisture Content

The moisture content was determined by the protocol proposed by Mishra (1968) (21). A requisite quantity of soil was dried overnight at 105°C. The percent loss in weight was calculated.

2.4.2. Determination of maximum water holding capacity

Maximum water holding capacity was determined by Keen-Raczkowski Box method (22).

2.4.3. Determination of the mechanical composition of soil

Soil texture was determined by the International Pipette Method (23).

2.4.4. Determination of pH and electrical conductivity

Soil pH was determined by pH- meter (Systronics, 324) with 1:2.5 sample water suspension using a glass rod for intermittent stirring for half an hour. Electrical conductance of soil was measured in 1:5 soil-water suspensions by conductivity bridge (Systronics, 305) (24).

2.4.5. Determination of CEC

CEC of soil was measured by the ammonium-acetate method described by Sankaram 1966 (22).

2.4.6. Determination of Organic Carbon

Organic carbon was determined by wet potassium dichromate digestion, followed by titrimetric measurements of unreacted dichromate (25).

2.4.7. Determination of Total Nitrogen, Available Phosphorus and Potassium

Total nitrogen (N) was determined by Micro-Kjeldahl method (22) and available phosphorus (P) was determined by Bray's method (26). Available potassium (K) determined by available K in the sample was extracted with neutral (1 N) ammonium acetate solution (pH) (24). K concentration in the extract was obtained by the flame photometer after necessary setting and calibration of the instrument (Systronics Model No. 121).

2.5. Determination of Enzyme Activity

Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on temperature, pH, ionic strength and the proper concentrations of the essential components like substrates and enzymes (27). Pesticide impacts on soil enzymes, which are essential catalysts ruling the quality of soil life[7]. The main reactions are catalysed by the three enzymes (Fig. 2a, b and c) as,

Arylsulfatases	$R\text{-}SO_3^- + H_2O = ROH + H^+ SO_4^{2-}$
Phosphatase	phosphate ester + H_2O = ROH + PO_4^{3-}
Dehydrogenases	XH_2 + acceptor = X + acceptor H_2

2.5.1. Determination of Acid Phosphatase Activity

Acid phosphatase activity of s activity was determined according to the method described by Eivazi and Tabatabai (1977) (28). A 1g soil sample (<2 mm) was placed in a 50 mL conical flask. Then 0.25 mL of toluene, 4 mL of Modified Universal Buffer (MUB), pH 6.5 and 1 mL of *p*-nitrophenyl phosphate (PNP) solution (made in the same buffer) were added to it and was swirled for a few seconds to mix the contents. The flask was closed by a stopper and placed in an incubator at 37°C. After 1 hour, the stopper was removed and 1 mL of 0.5M $CaC1_2$ solution and 4 mL of 0.5M NaOH solutions were added to the flask. Then it was again swirled for a few seconds and filtered the soil suspension through Whatman 42 filter paper. The intensity of the yellow color of the filtrate was measured with the help of spectrophotometer with wavelength adjusted to 420nm. For control sample, the same procedure was followed, but 1 mL of PNP solution was added after addition of 1 mL 0.5M $CaC1_2$ and 4 mL of 0.5M NaOH solutions, immediately before filtration of soil suspension. The *p*-nitrophenol content of the filtrate was calculated by reference to a calibration graph

that plots standards containing 0, 10, 20, 30, 40 and 50 µg of *p*-nitophenol.

To prepare a standard curve, 1 mL of the standard *p*-nitorphenol solution was taken in a 100 mL volumetric flask and the volume was adjusted to 5 mL by addition of distilled water. After that 1 mL of 0.5M $CaC1_2$ solution and 4 mL of 0.5M NaOH solution were added to each flask and the resultant suspensions were filtered. The *p*-nitrophenol content of the filtrates was measured with the help of spectrophotometer with wavelength adjusted to 420 nm.

Calculation

Acid Phosphatase activity of soil (µg of p-NP/g of oven dry soil/hr. at 37°C) = [{conc. of p-NP in sample – conc. of p-NP in control) vol. of reagent added + moisture content of 1g soil)}] / (1 x oven dry weight of 1 g soil x 1 hr.)

2.5.2. Determination of Arylsulphatase Activity

Arylsulphatase activity was determined according to the method described by Tabatabai (1994).[29] A 1g soil sample (<2 mm) was placed in a 50 mL conical flask. Then 0.25 mL of toluene, 4 mL of acetate buffer (pH 5.8) and 1 mL of p-nitrophenyl sulfate (PNS) solution (made in the same buffer) were added to it and was swirled for a few seconds to mix the contents. The flask was stoppered and placed in an incubator at 37°C. After 1 hour, the stopper was removed and 1 mL of 0.5M of $CaC1_2$ solution and 4 mL of 0.5M NaOH solution were added to the flask. Then it was swirled for a few seconds and filtered the soil suspension through Whatman 42 filter paper. The intensity of the yellow color of the filter was measured with the help of spectrophotometer with wavelength adjusted to 420 nm. For control sample, the same procedure was followed, but 1 mL of PNS solution was added after addition of 1 mL 0.5 $CaCl_2$ and 4 mL of 0.5M NaOH solution, immediately before filtration of soil suspension. The *p*-nitrophenol content of the filtrate was calculated.

Calculation

Arylsulphatase activity of soil (µg of p-NS/g of oven dry soil/hr. at 37°C) = {(conc. of p-NS in sample – conc. of p-NS in control) x (total vol. of reagent added + moisture content of 1 g soil)} / (1 x oven dry weight of 1 g soil x 1 hr.)

2.5.3. Determination of Dehydrogenase Activity

Dehydrogenase activity was determined according to the method described by Nisha et al. (2006) [9]. 20g of wet soil and 0.2 g Calcium carbonate was mixed thoroughly & 3 parts of 3g mixed soil was taken in a test tube. 5 ml double distilled water and 1ml 3% of TPF solution were added and kept at 37°C for 24 hrs. The mixture was then extracted in portion with methanol & filtered through Whatman no I filter paper in 50 ml volumetric flask & the volume was made up to the mark with methanol in diffused light. Reading was taken at 185 nm in Spectrophotometer. A standard curve was made with triphenyl formazen (TPF) solution at concentration 0, 5, 10, 20 & 40 ppm and the unknown concentration of the dehydrogenase in soil was measured with respect to standard curve. All experiments were performed in triplicate.

Calculation

Dehydrogenase activity of soil (µg TPF/g of oven dry soil at 37°C) = Concentration of sample (µg/ml) x 50 % (3 x wt of 1g oven dry soil x 24hr)

3. Results and Discussion

3.1. Physico-chemical Properties of the soil

The physico-chemical properties of soils are summarized in Table 1. Physico-chemical properties of soil have profound influence on soil enzyme activities (30) and also depend on the degradation of imidacloprid (31). The study was shown that the texture of the soil was clay loam, acidic (pH-4.6) in nature and high soil organic carbon content (0.93%). The soil pH influences the rate of synthesis, release and stability of the enzyme. Soil enzymatic activities were significantly correlated with soil organic carbon (32) and soil pH (33). Degradation of Imidacloprid also pH dependent (34) as soil pH was acidic it's degraded faster and lowers the influence on enzymatic activities.

3.2. Laboratory Incubation Study

Effect of different rates (FR, 2FR and 10FR) of Imidacloprid applications on the dynamics of sulphatase, phosphatase, dehydrogenase activities was studied under controlled laboratory conditions (25-35°C, using buffer solution, 60% WHC). The temperature of incubation was maintained at the ambient temperature supported by the fact that maximum growths and activities of micro-organisms in soil occur at 25-35°C and 60-80% W.H.C (35).

3.2.1. Arylsulphatase activity in soil

Any indirect effect of agrochemicals on soil enzymes involves the action of agrochemicals on soil organisms which, in time, contribute to the accumulated enzyme activity. Table 2 and Fig. 3 were shown that at higher doses of Imidacloprid was toxic to arylsulphatase activity. At the initial stage of its application, arylsulphatase activity was deteriorate in the first month and after degradation of the insecticide, arylsulphatase activity slowly increases assuming less toxic of its metabolite. From the correlation between activity with treatment curve (Fig. 6) it was revealed that after application of Imidacloprid, arylsulphatase activity decreased significantly at higher treatments 2FR and 10FR as compared to control, indicating Imidacloprid has significant toxic effect at 2FR and 10FR doses. From the Table 2 it also indicates that insecticide has a detrimental effect on arylsulphatase activity.

There was an inhibiting effect on arylsulphatase activity observed up to 30-35 days and then slowly increases its indicates that the activity decreases with days may be due to the toxicity of Imidacloprid and then increases may due to its biodegradation. Over all, the inhibiting effect on the soil enzyme arylsulphatase activity by Imidacloprid and same results as reported by Omar et al. (2001)[14] the arylsulphatase activity fluctuated between promotion and inhibition, but inhibition was predominant[36] whereas Kalyani et al. (2010) (10) and El-Aswada and Badawy (37) reported that the endosulfan (insecticide) applied at elevated level (100ppm) increased significantly arylsulfatase activity.

3.2.2. Acid Phosphates Activity in Soil

Acid Phosphatase Activities after application of Imidacloprid was presented in tabulated form at table 3. From the Fig. 4 and Table 3 shown that after application of Imidacloprid, acid phosphates activity increases at the first 1-3 days, then gradually decrease up to 30 days and after that, the enzymatic activity slowly increases when pesticide becomes less toxic.

From the Fig. 6, it was revealed that a variation of acid phosphatase activity was observed for FR, 2FR and 10FR compared with the control one. A significant difference in acid phosphatase activity was observed for FR as compared to control, this indicates that at the recommended dose of insecticide Imidacloprid stimulates the acid phosphatase activity, whereas at the higher dose, especially 10FR inhibited the acid phosphatase activity. From the Fig. 4 it was observed that enzymatic activity initially increases 1-3 days after that some inhibiting effects observe which is also very much transient. After some incubation periods, it has been found that the activity of acid phosphatase activity increases. Omar et al. (2001) reported that the effect of soil treatment with the pesticides on acid phosphatase was promotive at field application rates after some incubation periods (14) but the enzyme activity was delayed at the higher application doses (36). Vavoulidou et al. (2009) reported that no effect on phosphatase activities at 10 times the recommended doses of the cadusaphos (38).

3.2.3. Dehydrogenase Activity in Soil

Results of Dehydrogenase activity on following application of Imidacloprid have been given Table 4. From the above Fig.5 & Table 4, it is found that dehydrogenase activity decreases gradually up to 30-35days after application of Imidacloprid and after that, the enzymatic activity slowly increases as also control value decreases with time.

From Fig. 5 it is observed that dehydrogenase activity was decreased for all the treatments, viz., FR, 2FR and 10FR as compared with the control. It was revealed that higher inhibiting enzymatic activity for 10FR dose compared with the control.

The correlation between the enzyme activity and the treatment of the insecticide is shown in Fig. 6 indicating a detrimental effect on the soil enzyme activity after Imidacloprid application. Application of Imidacloprid led to the significant drop of dehydrogenase activity irrespective of doses. At the initial stage with the application of pesticide dehydrogenase activity was gradually decreased upto 30-35 days, after that the activity was slowly increased. This might be due to degradation of Imidacloprid and increase in microbial populations with the capability of utilizing the pesticide formulation as a carbon source. Sebimo et al. (2011) (16) and Dzantor and Felsot (39) were found same results in sandy loam soil. Chen et al. (2001) also reported that application of captan at same pH dehydrogenase activity decreased at a heavier rate (40).

Pesticide degradation or decomposition is generally a positive phenomenon. Most of the degradation by-products (6-hydroxynicotinic acid, Imidacloprid urea, 6-chloronicotinic acid) of Imidacloprid [41] are biologically inactive, less toxic, and harmless. In the field studied after few days Imidacloprid application at FR dose (3.97µg/mL) was less influence to soil enzymatic activities as the Imidacloprid degraded gradually (field dissipation half-life: 26.5 to 229 days) (42). Mahapatra et al., 2017 reported that Imidacloprid dissipation was 100%, 90.45% and 76.85% from FR, 2RD and 10RD treatments respectively in sandy clay loam soil after 30 days of pesticide application (43). These results also support our results. But at higher does (mainly 10 FR: 39.7 µg/mL) shown any detrimental effect to soil enzymatic activities.

4. Conclusion

It is possible to say that without proper soil enzymes system, soil life processes will be disturbed. As soil enzymes are very sensitive to the agricultural practices, pH of the soil, nutrients, inhibitors and weather conditions the present work was analyzed to establish the relationship, if any, between the effects of Imidacloprid on soil arylsulphatase, acid phosphatase and dehydrogenease activities.

This study confirmed that application of Imidacloprid to Tripura agriculture soil at recommended doses not showed any detrimental effect on soil arylsulphatase, acid phosphatase whereas it was toxic to dehydrogenease. At higher elevated doses, some transient harmful effects were observed. At a later stage of incubation, the activities of different enzymes were going to increase irrespective of the dose of Imidacloprid its indicating Imidacloprid metabolite are less toxic to soil enzymes.

Conflicts of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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References

[1] Tiziano, G, David P, Maurizio GP (2011) Is There a Need for a More Sustainable Agriculture?. Crit Rev Plant Sci 30: 6–23.

[2] Das B, Chakrabarti K (2013) Assessment of community level physiological profiles and molecular diversity of soil bacteria under different cropping systems. Tur J Agric For 37: 468–474.

[3] Pratibha P, Shachi S (2016) Impact of Fertilizers and Pesticides on Soil Microflora in Agriculture. Sustain Agric Rev 19: 331-361.

[4] Dong WY, Zhang XY, Liu XY, Fu XL, Chen FS, Wang HM, Sun XM, Wen XF (2015) Responses of soil microbial communities and enzyme activities to nitrogen and phosphorus additions in Chinese fir plantations of subtropical China. Biogeosci 12: 5537–5546.

[5] Utobo EB, Tewari L (2014) Soil Enzymes as Bioindicators of Soil Ecosystem Status. Appl. Ecol. Environ. Res 13: 147-169.

[6] Ramanamma P, Meghana D, Rangaswamy V (2017) Effect of the fungicide dimethomorph at different application rates on enzyme activities in Groundnut (arachis hypogaea l.) Soils. Int J Adv Res Ideas Innov Techn 3(3).

[7] Riah W, Laval K, Ajzenberg EL, Mougin C, Latour X, Gattin IT (2014) Effects of pesticides on soil enzymes: a review. Environ. Chemi Lett 12: 257–273.

[8] USDA, NRCS (2010) Soil Quality Indicators., (https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=nrcs142p2_053139&ext=pdf) Accessed on 25th January 2019.

[9] Nisha K, Shahi DK, Sharma A (2006) Effect of endosulfon and monocrotophos on soil enzymes in acid soil of Ranchi. Pestol 15: 42–44.

[10] Zhang L, Chen W, Burger M, Yang L, Gong P, Wu Z (2015) Changes in Soil Carbon and Enzyme Activity As a Result of Different Long-Term Fertilization Regimes in a Greenhouse Field,. PLoS One 10(2): e0118371.

[11] Kalyani SS, Sharma J, Dureja P, Singh S, Lata (2010) Influence of endosulfan on microbial biomass and soil enzymatic activities of a tropical Alfisol. Bull. Environ. Contam. Toxicol 84: 351–356.

[12] Elsgaard L, Vinther F (2004) Modelling of the temperature response of arylsulfatase activity. J. Plant Nutrit. Soil Sci 167: 196–201.

[13] Schmidt AJ, Weary M (1962) the distribution of alkaline phosphatase in the regenerating forelimb of the adult newt, Diemictylus viridescens (Triturus v.). J Exp Zool 150: 69–81.

[14] Omar SA Abdel-Sater MA (2001) Microbial populations and enzyme activities in soil treated with pesticides. Water Air. Soil Poll 127: 49–63.

[15] Salazar S, Sanchez L, Alvarez J, Valverde A, Galindo P, Igual J, Peix A, SantaRegina I (2011) Correlation Among Soil Enzyme Activities Under Different Forest System Management Practices. Ecol Engin 37: 1123-1131.

[16] Sebiomo A, Ogundero VW, Bankole SA (2011) Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. African J Biotechn 10: 770–778.

[17] Adak T, Kumar J, Dey D, Shakil NA, Walia S (2012) Residue and bio-efficacy evaluation of controlled release formulations of imidacloprid against pests in soybean (Glycine max). J Environ Sci Health B 47: 226–231.

[18] CIBRC, (2018) Major Uses of Pesticides (Registered under the Insecticides Act, 1968) (UPTO- 30.06.2016), (http://cibrc.nic.in/mupi2012.doc) Accessed on 25th January 2019.

[19] Sommerville L (1987) Perspective on side effect testing. In: L. Sommerville, M.P. Greaves (eds), Pesticide Effects On Soil Microflora. Taylor & Francis 5–13.

[20] Alster CJ, German DP, Lu Y, Allison SD (2013) Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol. Biochem. 64: 68–79.

[21] Mishra R (1968) Ecology Work Book, Oxford IBH. New Delhi. India.

[22] Sankaram AA (1966) laboratory manual for Agricultural Chemistry., Asia Publishing House, Calcutta, India 120–122.

[23] Piper CS (1966) Soil and plant analysis. Hans Publisher. Bombay.

[24] Jackson ML. (1967) Soil Chemical analysis. Prentice Hall of India, Pvt. Ltd., New Delhi, pp. 498.

[25] Walkley A Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37: 29–38.

[26] Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus in soils. Soil Sci 59: 39–45.

[27] Bisswanger H (2014) Enzyme assays. Perspect Sci1 1: 41–55.

[28] Eivazi F Tabatabai MA (1997) Phosphatases in soils. Soil Biol Biochem 9: 167–172.

[29] Tabatabai MA (1994) Soil enzymes. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties (Eds R.W. Weaver, S.H. Mickelson). SSSA Book Series 5: 814–818.

[30] Kumari JA, Rao PC, Padmaja G, Madhavi M (2017) Effect of Physico-Chemical Properties on Soil Enzyme Acid Phosphatase Activity of Some Soils in Vegetable Growing Soils of Ranga Reddy District of Telangana State, India. Int J Curr Microbiol Appl Sci 6: 3496–3503.

[31] Samnani P Vishwakarma K (2013) Pandey SY Persistence study of Imidacloprid in different soils under laboratory conditions. Int J Environ Sci 4(2).

[32] Zhao Q, Tang J, Li Z, Yang W, Duan Y (2018) The Influence of Soil Physico-Chemical Properties and Enzyme Activities on Soil Quality of Saline-Alkali Agroecosystems in Western Jilin Province, China. Sustainability 10: 1529.

[33] Wan Z, Wu J (2005) Study progress on factors affecting soil enzyme activity. J Northwest Sci Tech Univer Agric Forest 33: 87–91.

[34] Thuyet DQ, Watanabe H, Ok J (2013) Effect of pH on the degradation of imidacloprid and fipronil in paddy water. J Pestic Sci 38: 223–227.

[35] Alexander M (1977) Introduction to Soil Microbiology. Wiley-Eastern Ltd. New Delhi. 163–173.

[36] Kertesz MA, Mirleau P (2004) The role of soil microbes in plant sulphur nutrition. J Exp Bot 55: 1939–1945.

[37] El-Aswada AF, Badawy MEI (2015) Inhibition kinetics of acid and alkaline phosphatases by atrazine and methomyl pesticides. J Environ Sci Health B 50: 484–491.

[38] Vavoulidou E, Avramides E, Wood M, Lolos P (2009) Response of soil quality indicators to the pesticide cadusaphos. Commun Soil Sci Plant Anal

[39] Dzantor EK Felsot A (1991) Microbial response to large concentrations of herbicides in soil. Environ. Toxicol Chem 10: 649–655.

[40] Chen SK, Edwards CA, Subler S (2001) Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol Biochem 33: 1971–1980.

[41] Scholz K, Spiteller M (1992) Influence of groundcover on the degradation of ^{14}C-imidacloprid in soil. Brighton Crop protection Conference – Pest and Diseases 883–888.

[42] Kollman W, Segaw R (1995) Interim Report of the Pesticide Chemistry Database. Environmental Hazards Assessment Program.

[43] Mahapatra B, Adak T, Patil NKB, Pandi GP, Gowda GB, Jambhulkar NN, Yadav MK, Panneerselvam P, Kumar U, Munda S, Jena M (2017) Imidacloprid application changes microbial dynamics and enzymes in rice soil. Ecotoxicol. Environ. Safety144: 123–130.

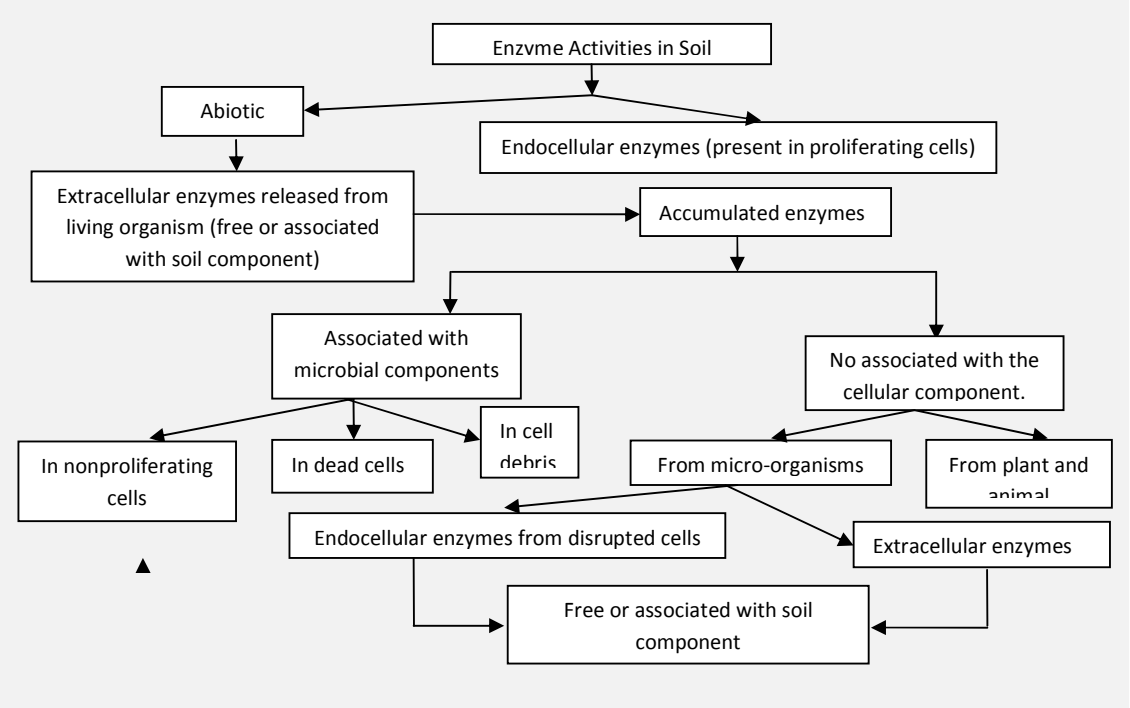

Figure1. Enzyme Activities in Soil

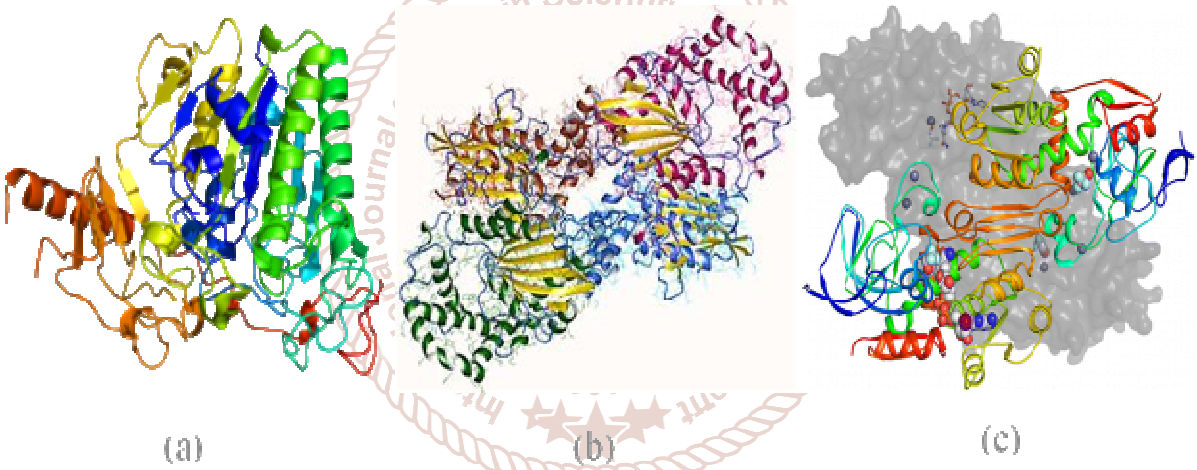

Figure 2. Soil enzymes (a) arylsulphatase (b) acid phosphatase and (c) dehydrogenase

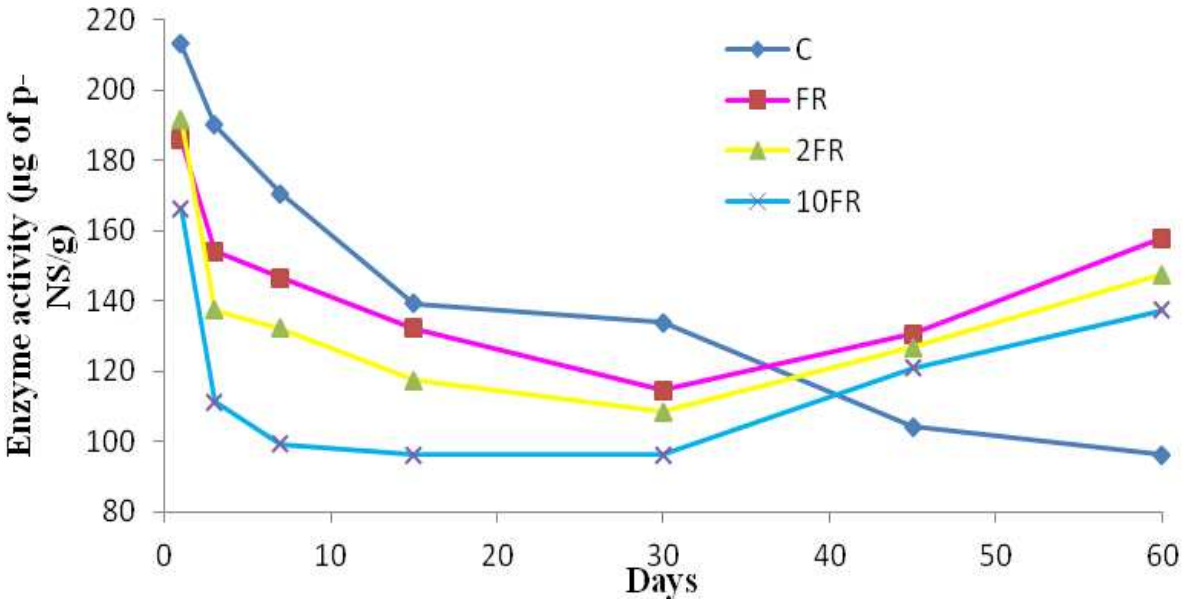

Figure3. Variation of Arylsulphatase activity (µg of p-NS/g) with the incubation period (Days)

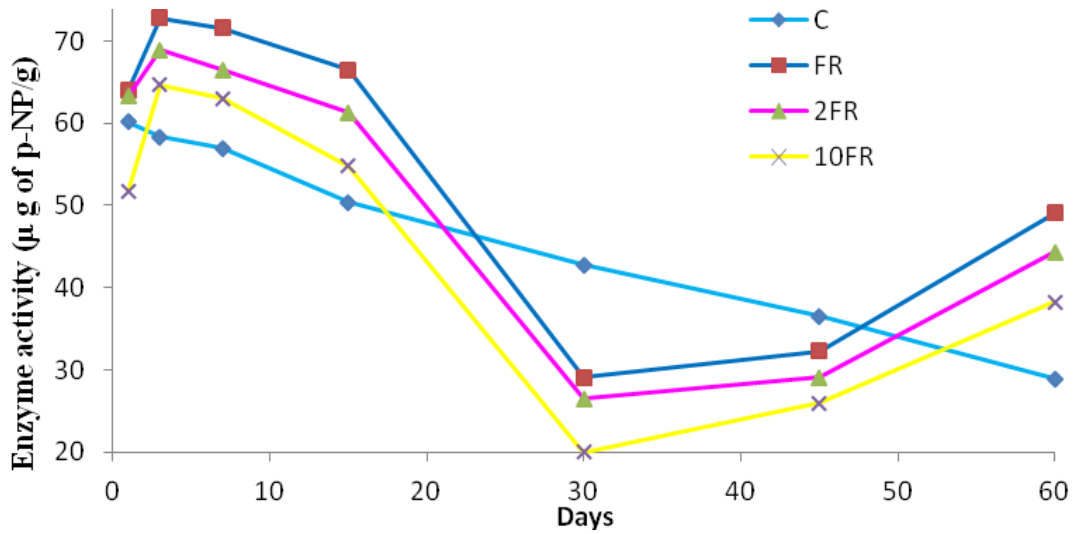

Figure4. Variation of Arylsulphatase activity (µg of p-NP/g) with the incubation period (Days)

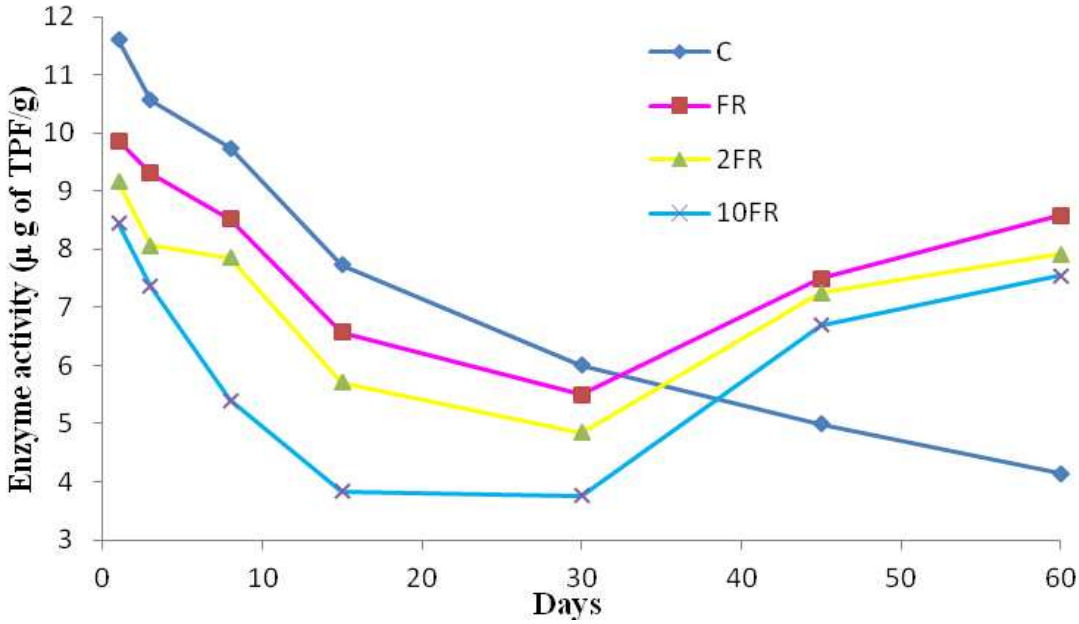

Figure5. Variation of Dehydrogenase activity (µg of TPF/g) with the incubation period (Days)

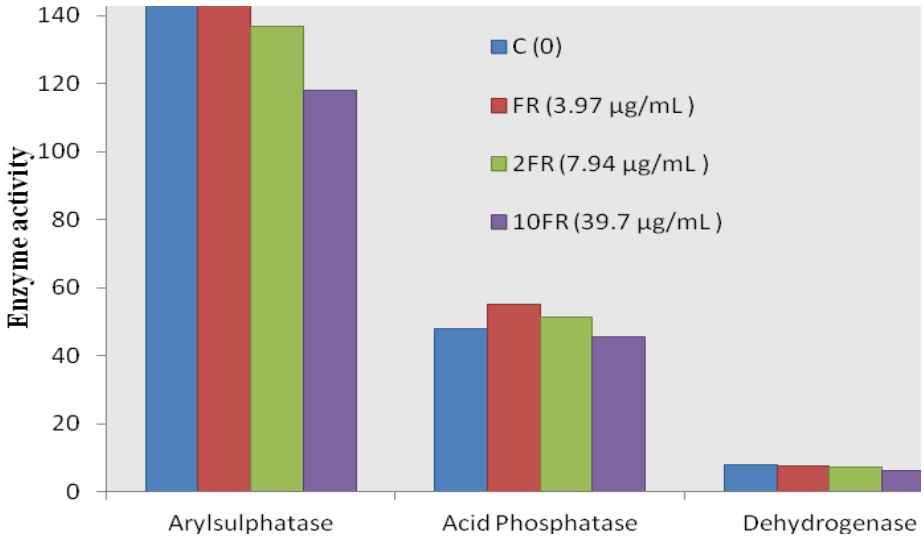

Figure6. Correlation between the (a) Arylsulphatase (b) Acid Phosphatase and (c) Dehydrogenase activity with the Treatment

Table1. Results of physico-chemical properties of soil

Sl. No.	Properties	Results
1.	pH (in water)	4.6
2.	EC (dSm)	0.41
3.	CEC (Cmolp+kg+)	28.26
4.	Soil Organic Carbon (%)	0.93
5.	Maximum water holding Capacity (%)	22.2
6.	Moisture Content (%)	58.3
7.	Texture analysis (%)	Sand (47) Silt (24) Clay (32)
8.	Texture	Clay loam
9.	Total Nitrogen (g/kg)	4.21
10.	Available K (mg/kg)	89
11.	Available P (mg/kg)	47

Table2. Arylsulphatase activity (µg of p-NS/g of oven dry soil/hr. at 37°C) with Period of Incubation and Treatments

	Arylsulphatase activity with period of incubation (Days)							
Treatment	1	3	7	15	30	45	60	Mean
C	213.11	190.04	170.51	139.32	133.9	104.17	96.14	149.60
FR	185.82	154.01	146.47	132.21	114.54	130.52	157.9	145.92
2FR	191.7	137.32	132.14	117.32	108.47	126.72	147.41	137.01
10FR	166.21	111.14	99.21	96.15	96.22	120.8	137.31	118.15
Mean	189.21	148.13	137.08	121.25	113.28	120.55	134.19	

Table3. Acid Phosphatase activity (µg of p-NP/g of oven dry soil/hr.at 37°C) with Period of Incubation and Treatments

	Acid Phosphatase activity with period of incubation (Days)							
Treatment	1	3	7	15	30	45	60	Mean
C	60.2	58.3	57	50.4	42.8	36.5	28.9	47.73
FR	64	72.8	71.6	66.5	29.1	32.3	49.1	55.06
2FR	63.3	68.9	66.5	61.3	26.5	29.1	44.3	51.41
10FR	51.7	64.7	63	54.8	20	26	38.3	45.50
Mean	59.80	66.18	64.53	58.25	29.60	30.98	40.15	

Table4. Dehydrogenase activity (µg of TPF/g of oven dry soil/hr. at 37°C) with Period of Incubation and Treatments

	Dehydrogenase activity with period of incubation (Days)							
Treatment	1	3	8	15	30	45	60	Mean
C	11.6	10.57	9.74	7.73	6	4.99	4.14	7.82
FR	9.86	9.31	8.52	6.57	5.5	7.5	8.58	7.62
2FR	9.16	8.06	7.85	5.71	4.85	7.25	7.91	7.26
10FR	8.45	7.36	5.4	3.83	3.76	6.7	7.55	6.15
Mean	9.77	8.83	7.88	5.96	5.03	6.61	7.05	