

VOLUME 8 ISSUE 2 2022

ISSN 2454-3055



**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India



ISSN: 2454-3055

Comparative Study of Physicochemical Properties and Enzymatic Status of Imidacloprid Treated Soils from Various Zones of Haryana, India

Yadav Poonam¹, Bhardwaj Monika¹, Vashishth Divya¹, Yadav Mansi¹, Kadyan Pooja¹, Kumar Manoj², Dalal Sunita² and Kataria Sudhir Kumar^{1*}

¹Department of Zoology, Maharshi Dayanand University, Rohtak, India

²Department of Biotechnology, Kurukshetra University, Kurukshetra, India

*Corresponding Author

Received: 25th June, 2022; Accepted: 1st August, 2022; Published online: 7th August, 2022

<https://doi.org/10.33745/ijzi.2022.v08i02.025>

Abstract: Imidacloprid is the most commonly used insecticide in Haryana that play an important role in pest management and hence improved crop yield production. This study was done to investigate the consequences of Imidacloprid on the activity of dehydrogenase and urease enzymes in soils of selected zones of Haryana. A 28 days experiment was carried out where sampling was done after 1st, 7th, 14th, 21st and 28th day of Imidacloprid application at different concentrations like T1 (1 mg/kg), T2 (2 mg/kg) and T3 (10 mg/kg). After analysing the physicochemical properties of soils of various zones the enzymatic activities were measured using colorimetric assays. Dehydrogenase activity was decreased initially after imidacloprid exposure but after 14 days a stimulation was observed in dehydrogenase activity at lower doses. The decrease was directly proportional to concentration of imidacloprid and indirectly proportional to time of incubation. Urease activity was inhibited by highest dose of imidacloprid after first day exposure. However, a stimulation in urease activity was observed after 14 days of imidacloprid treatment at all doses. Obtained results showed that the activity of dehydrogenase and urease were affected by concentration and treatment time of Imidacloprid significantly in soils of each selected zone of Haryana. The results provide a clue about the possible ecological effects of Imidacloprid.

Keywords: Pesticides, Imidacloprid, Soil, Dehydrogenase enzyme, Urease enzyme

Citation: Yadav Poonam, Bhardwaj Monika, Vashishth Divya, Yadav Mansi, Kadyan Pooja, Kumar Manoj, Dalal Sunita and Kataria Sudhir Kumar: Comparative study of physicochemical properties and enzymatic status of imidacloprid treated soils from various zones of Haryana, India. Intern. J. Zool. Invest. 8(2): 197-204, 2022.

<https://doi.org/10.33745/ijzi.2022.v08i02.025>



This is an Open Access Article licensed under a Creative Commons License: Attribution 4.0 International (CC-BY). It allows unrestricted use of articles in any medium, reproduction and distribution by providing adequate credit to the author (s) and the source of publication.

Introduction

Currently the whole world is facing two major challenges -- one is changing climatic conditions and the second one is population explosion. According to a report, it was predicted that estimated population of world will rise higher by a

billion till 2050 that demand more food production in limited resources. About 15-20% crop yield production is reduced by pests in India (Bhalerao and Puranik, 2007) that cause a huge loss. To fulfil the food requirements of increasing

population, use of pesticides has become a necessary evil. Consumption of pesticides is unevenly distributed in India i.e. southern parts consumes less pesticides as compared to northern parts due to variation in local climatic conditions and soil types (Zhang *et al.*, 2012). With persistent use, these pesticide gets accumulated in soil, cause soil pollution and impose a threat to soil microbiota. Soil is the top most layer of earth's crust, composed of rock particles, air, water, humus, organic and inorganic components, mixture of organic remains and microorganism. Soil composition may vary from place to place, generally soil is composed of about 90-95% abiotic components (air, water, rock particles, sand, silt, clay) and only 5-10% biotic component (fungi, bacteria, invertebrates, insects etc.). Haryana is making significant contribution for the food production and hence, known as food bowl of the country. About 80% area is classified as net sown area in Haryana hence, agriculture is the main driver of the state economy. Haryana is divided in four zones according to the cropping pattern and type of soil. These four zones are (A) Rohtak zone, (B) Hisar zone, (C) Kurukshetra zone, and (D) Mahendergarh zone. One zone soil differs from the other in their physicochemical properties and type of soil. Various pests like jassids, thrips, beetles, aphids and flies reduces the crop yield production that become a major issue of concern for the existing situation. To prevent reduction of crop yield due to these pests, pesticides are used at a large scale that leads to soil contamination.

The effect of these pesticides contamination in soil can be predicted better by measuring certain soil enzymes activity (Pandey and Singh, 2006; Flotch *et al.*, 2011). The activities of these enzymes are the sensitive indicator of the microbial response to natural and chemical stress. Enzyme activities in soil results either from the activities of accumulated enzymes excreted from dead cells or from activities of proliferating microorganisms. Soil enzymes analysis represent correlation with microbial activity, nutrient cycling, soil fertilization and degree of pollution in soil

(Kizilkaya *et al.*, 2004). Soil enzymes like dehydrogenase and urease are important biocatalyst and catalyse various biological reactions and play important role in increasing soil fertility (Bending *et al.*, 2006; Quian *et al.*, 2009; Wang *et al.*, 2010). Hence, both were selected in this study for assessing the effect of imidacloprid in soils of Haryana. Dehydrogenase is an intracellular oxidoreductase enzyme that transfer electrons and protons from substrate to acceptor and help in biological oxidation of organic matter present in soil (Seibomoet *et al.*, 2011). Urease enzyme hydrolyses urea into ammonia and carbondioxide gas. This ammonia forms ammonium carbonate with water that turns pH from 6.8 to 8.4 or acidic to alkaline. This enzyme is tightly bound to organic matter and mineral compounds present in soil. Interaction between abiotic and biotic factors are very complex and affected by various environmental factors.

Imidacloprid is a ring derivative neonicotinoid that is a fastest growing and highly used insecticide that gain 24% share of total market in 2008 (Jeshke *et al.*, 2011). Imidacloprid is used in so many ways such as-- to treat seeds (Zaller *et al.*, 2016), soil and foliar application (Karnar *et al.*, 2006; El-Nagger and Zidan, 2013). It acts agonistically on nAChRs of insects and specifically active on hemipteran pests species such as whiteflies, aphids and plant hoppers but it is also used to control some coleopteran and lepidopteran pest species (Nauen *et al.*, 2003). Persistence of Imidacloprid in soil was investigated by El-Hamady *et al.* (2008) and reported that after 7-21 days of application on cotton seeds only 1.8 to 6.8% of C¹⁴ labelled Imidacloprid was taken by plants.

In 2019-20 Imidacloprid production was 20 MT (Ministry of Chemicals and Fertilizers 2020-21) while consumption of Imidacloprid was 309 MT and increased to 372 MT in 2020-21 in India (<http://ppqs.gov.in/statistical-database?page=1>).

Imidacloprid was reported to alter the soil microbial and enzymatic activities in previous

studies. This study is an effort to evaluate whether Imidacloprid generous application by man affects soil health. To the best of our knowledge, this is the first study indicating the effect of imidacloprid pesticide on soil enzymes in Haryana soil.

Materials and Methods

Commercial formulation of Imidacloprid 17.8 SL (Bayer Crop Science Limited, India) was purchased from Rohtak local market. Various chemicals used for enzymatic activities were purchased from Central Drug House (CDH).

Collection of soil samples:

Soil samples were collected in sterile bags from non-contaminated places from different zones of Haryana i.e. Rohtak, Kurukshetra, Mahendergarh and Hisar. Collected soil was poured in sterile bags and brought to laboratory where the soil was first crushed, grinded and dried in air and then further analysed to determine various properties.

Physicochemical properties of soil:

Firstly, physicochemical properties of the soil were determined for its further analysis. pH determined by pH meter, water holding capacity by filter paper method, cation exchange capacity by barium chloride method (Gillman, 1979), total nitrogen content by Kjeldahl method (Kandeler, 1995), total organic carbon content by Kandeler method. Soil texture and presence of various micronutrients was analysed by soil testing kits.

Experimental design:

For this experiment soil of each zone divided and poured into 12 pots @ 2 kg soil per pot (three replicates and four imidacloprid doses) in a completely randomized design. Various concentrations taken are control, T1 (1 mg/kg soil), T2 (2 mg/kg soil) and T3 (10 mg/kg soil). Imidacloprid dissolved in water and poured drop wise to the pots then mixed thoroughly with the help of a sterile spatula. In control only water was mixed. 45% to 50% maximum water-holding capacity of the soil was maintained by periodically adding distilled water. Polypropylene sheets were

used to cover the pots and incubated in dark to prevent evaporation and photodegradation of imidacloprid. Appropriate soil samples were taken after 1, 7, 14, 21 and 28 days from three pots of each zone and each treatment. Soil dehydrogenase enzyme and urease enzyme assays were performed by following methods:

Soil Dehydrogenase enzyme activity (DHA):

Soil DHA was determined by the method developed by Casida *et al.* (1964) after 1, 7, 14 and 28 days in the soil samples. 5 g dried soil from each soil sample containing various concentration of imidacloprid (i.e. C, 1 mg/kg, 5 mg/kg, 10 mg/kg) was taken in 25 ml test tube. 3% triphenyl tetrazolium chloride (TTC) and 2.5 ml of distilled water was added in each tube and mixed thoroughly using a stirrer. The tubes were stoppered and incubated at 37 °C for 24 h. Methanol was used to extract triphenyl tetrazolium formazan (TPF) from this mixture. The absorbance was measured at 485 nm with a Shimadzu Spectrophotometer. Results were calculated using the standard curve that was prepared by taking final concentration of 10 to 100 µg TPF. DHA activity was expressed as µg TPF g⁻¹h⁻¹ (Alef, 1995).

Urease activity Measurement:

Urease activity was determined by adding urea (10% w/v) to wet soil (10 g) supplemented with 20 ml citrate buffer (pH 6.7) followed by incubation at 37 °C for 20 min. The mixture was diluted by adding distilled water and mixed thoroughly and filtered immediately. 3 ml filtrate was transferred to a 50 ml capacity flask in having 10 ml of distilled water, 4 ml of sodium phenolate (phenol 50%, NaOH 21.6%, 1:1) and 3 ml of sodium hypochlorite (0.9% active Cl₂) was added and left for 20 min. Then, ammonium ions concentration was determined in the reaction mixtures by measuring the blue colour intensity that was produced due to the reaction of ammonia with sodium chlorate and sodium phenolate. The optical absorbance was measured colorimetrically at 630 nm by a Shimadzu Spectrophotometer. The

Table 1: Physicochemical properties of soils of various zones of Haryana

Nutrients	Rohtak	Hisar	Kurukshetra	Mahendergarh
Soil Type	Sandy Loam	Sandy Loam	Coarse loam	Sandy
pH	7.89	7.39	8.01	7.49
Organic Carbon (%)	0.33	0.30	0.36	0.24
Electrical conductivity (1:2)	0.21	0.33	0.23	0.45
potassium (kg/ha)	155.00	127.00	265.00	167.00
Phosphate (kg/ha)	6.50	5.80	4.90	3.90
Sulphur (ppm)	101	115.00	131.00	98.5
Zinc (ppm)	0.94	0.80	0.97	0.63
Iron (ppm)	5.43	4.55	5.44	4.84
Manganese (ppm)	1.69	2.82	4.00	3.56
Copper (ppm)	1.00	0.41	0.68	0.79

activity of Urease was expressed in the form of $\text{mg NH}_4^+ \text{ kg}^{-1}\text{h}^{-1}$ (Gianfreda *et al.*, 1994).

Results and Discussion

Physicochemical properties of soil:

Various physicochemical properties and significant nutrients of soils of four zones of Haryana were studied to check effect of Imidacloprid treatment in agricultural soil of Haryana state. The results given in Table 1 clearly indicated variability of all parameters.

Dehydrogenase enzyme activity:

Highest activity of dehydrogenase (Fig. 1) was observed in untreated soil of each zone except soil D in which T1 and T2 doses have non-significant higher dehydrogenase activity after 21 and 28 days of imidacloprid treatment. Our results are in conformity with the results of Naumann (1970) who have reported that methyl parathion at 15 kg/ha stimulated the DHA but negative effects were observed at higher doses. At single dose malathion also accelerated DHA but inhibited at

double dose (Panda and Sahu, 2000). The dehydrogenase activity decreases significantly with the increasing concentration of imidacloprid after 1st and 7th days of treatment in each soil except soil C where no significant change was observed. After 14 days of treatment a significant increase in each soil was observed as compared to DHA activity after 1 day. Similarly, DHA activity was increased by 15.36% after 15 days when recommended dose of imidacloprid is applied (Bhattacharya and Sahu, 2013). After 21 and 28 days of treatment non-significant changes were observed. However, maximum change was observed in soil B and Soil D. But the T3 showed a significantly lower DHA activity as compared to control soil at all sampling time. But this trend was not observed in soil D. In this soil after 21 and 28 days of exposure no effect was observed in DHA activity with dose of imidacloprid.

Non-significant effect in DHA were observed due to various doses of imidacloprid. Highest concentration of imidacloprid reported to show lowest DHA followed by T2 and highest in

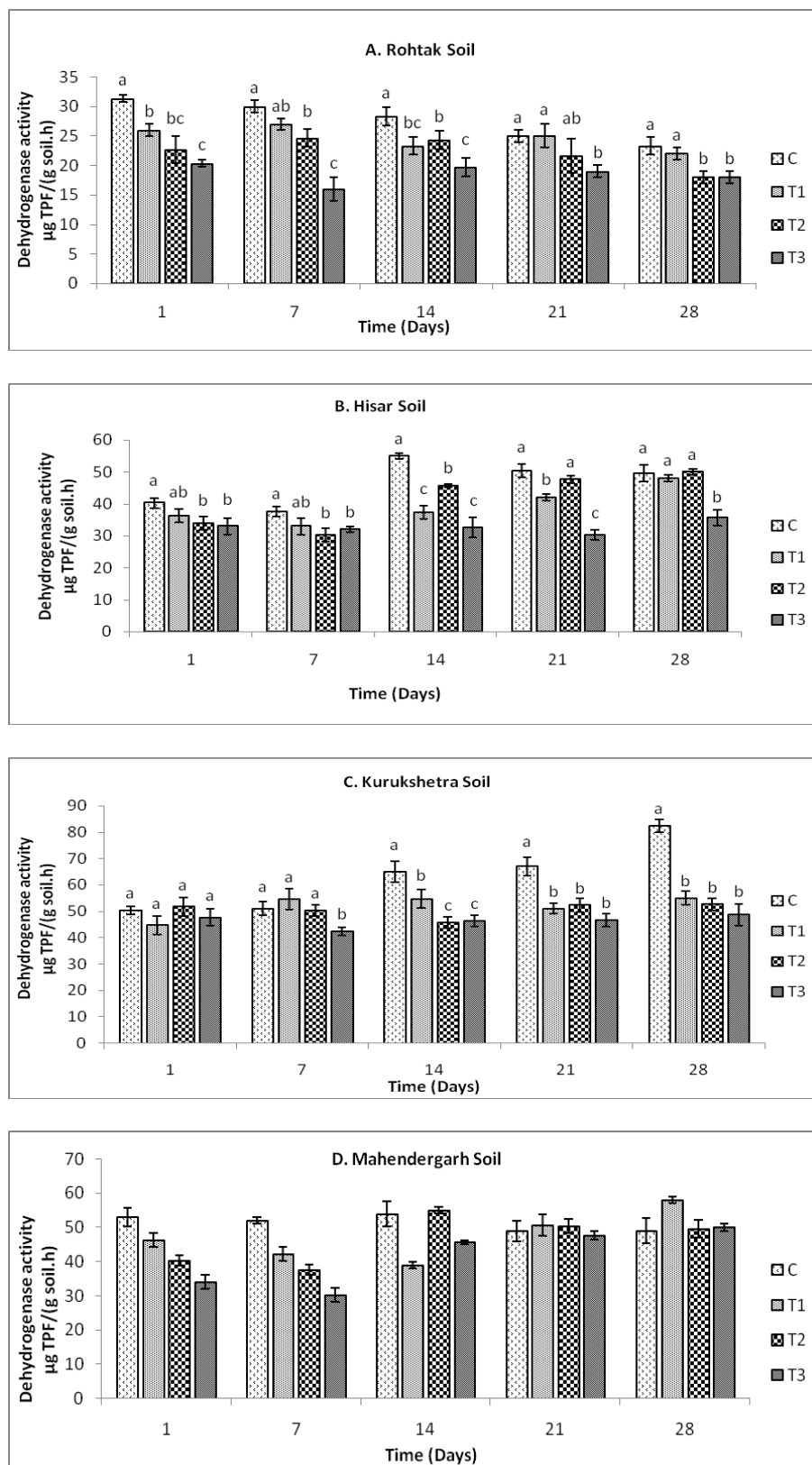


Fig. 1: Dehydrogenase enzyme activity [$\mu\text{g TPF}/(\text{g soil.h})$] in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg/kg soil; T2: 2 mg/kg soil; T3: 10 mg/kg soil). Each value represents Mean $\pm$ S.D. of three replicates. A significant statistical differences ($P < 0.05$) between Imidacloprid dose and exposure time were analysed using post hoc Tuckey test and represented using different letters (Similar letters show statistically insignificant while different letters show statistically significant with each other).

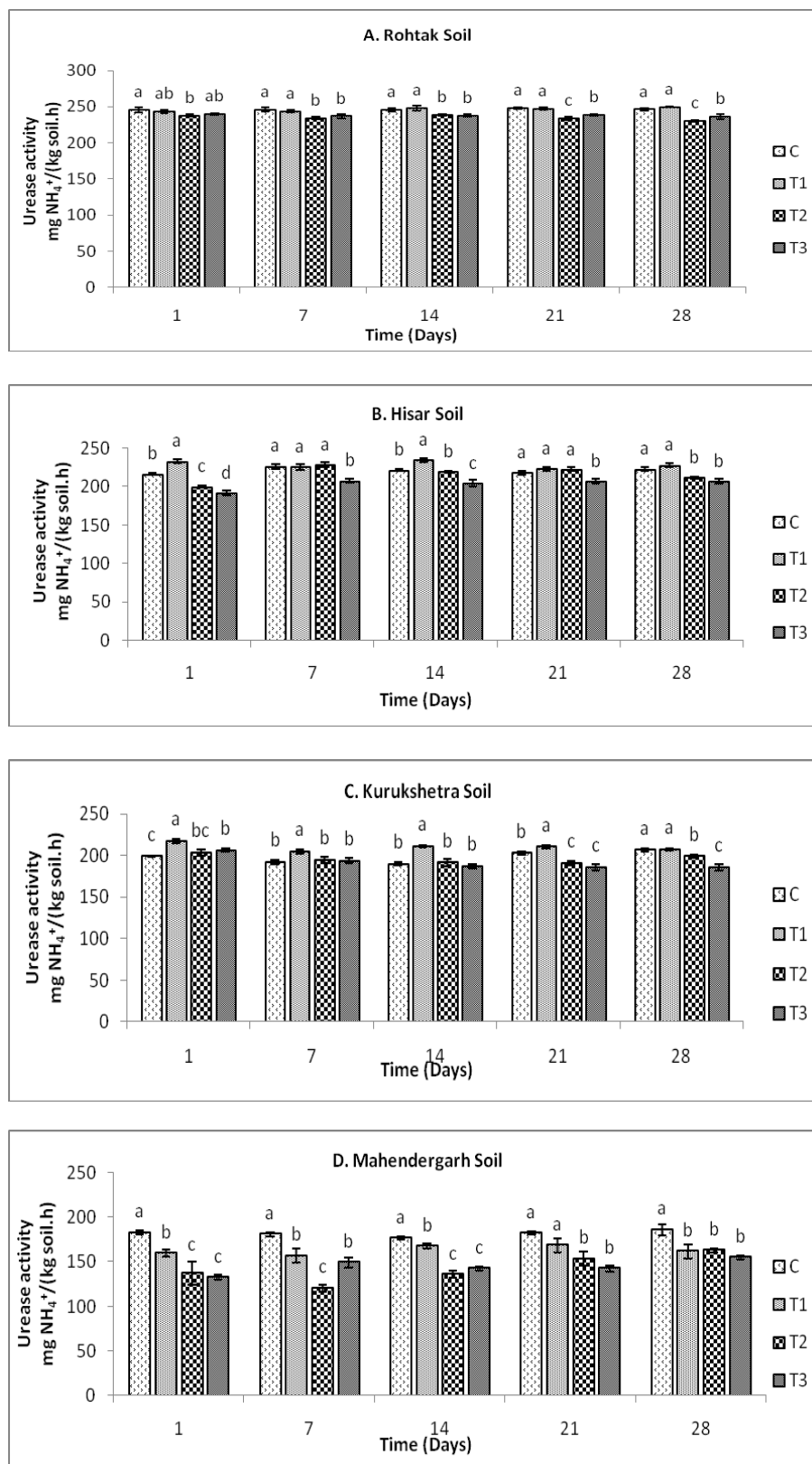


Fig. 2: Activity of Urease enzyme (mg NH₄⁺/ kg dry soil) in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg/kg soil; T2: 2 mg/kg soil; T3:10 mg/kg soil). Each value represents Mean $\pm$ S.D. of three replicates. A significant statistical differences (P<0.05) between Imidacloprid dose and exposure time were analysed using post hoc Tuckey test and represented using different letters (Similar letters show statistically insignificant while different letters show statistically significant with each other).

untreated soil. Initially DHA was decreased up to 7 days and after 14 days it started to increase with time in each soil. Similar results were obtained in a study done by Wang *et al.* (2014). The DHA activity at RD and 2RD doses increased non-significantly with time. This suggests that DHA is correlated with microbial population and sensitive to Imidacloprid and other insecticides (Shetty and Magu, 1998; Singh and Singh, 2005).

Urease Enzyme Activity:

Compared to control soil urease activity was significantly inhibited by highest dose (T3) of imidacloprid only after 1st day of exposure. Similar results were obtained by Wang *et al.* (2014) who have reported that urease activity was decreased by 21.7% and 30.5% in imidacloprid and acetamiprid treated soils, respectively. We found stimulation of urease activity after 14 days of treatment at all the doses of imidacloprid in each soil (Fig. 2). Similar to our study a 35% decrease in urease activity was observed in first 15 days and then a stimulation in urease activity was observed in the urease activity in Imidacloprid treated soil (Singh and Kumar, 2008).

No significant changes were observed in the control and T1 doses as well as in T2 and T3 doses in each soil at each time point (Fig. 2). However, a slight decrease in urease activity was observed at higher doses of Imidacloprid (T2 and T3) as compared to control and T1 doses. Similar results were observed by Mohiddin *et al.* (2011) in urease activity. In comparison to other soils, a significant decrease was observed even after 7th and 14th days at T2 and T3 dosage in soil D and this was the maximum decrease as observed in this study in urease activity. Bhattacharya and Sahu (2013) also reported similar results where 25.52% decrease was observed in urease activity at recommended agricultural dose of imidacloprid. These results indicated that urease activity was temporarily inhibited by Imidacloprid application but urease activity would be recovered once the imidacloprid gets degraded.

Conclusion

At lower recommended field rate doses imidacloprid increased the dehydrogenase but at higher doses, it may decreased. Urease significantly inhibited at lower doses after day 1. But at next time stimulation in urease was observed at T2 and T3 doses. Change in soil enzymatic activities due to a pesticide gave a brief idea about non-target toxicity of that pesticide. Soil enzymatic activities get disturbed due to extraneous application of pesticides that in turn changes nutrient cycling. Imidacloprid negatively affected the soil enzymes and hence, soil microbes for short term at lower doses, but at higher doses these changes remained persistent in soil and decreases the soil fertility. However, the interaction between time of exposure, dose of Imidacloprid and soil physicochemical properties were very complex. Many factors help in the modification of the reaction of Imidacloprid with these enzymes. Results of this study showed that Imidacloprid at lower doses not cause significant harm to these soil enzymes but when applied frequently to higher doses it hinders the regeneration of microbial fauna and hence, these enzymes. To the best of our knowledge it is the first study that examine the effects of Imidacloprid on these soil enzymes in soil of different zones of Haryana. From this study it is concluded that Imidacloprid is somehow safer than other insecticide if used at lower dose and instruction for its application in agriculture are followed.

References

- Alef K and Nannipieri P. (1995) Methods in applied soil microbiology and biochemistry. Academic Press, ISBN 978-0-12-513840-6.
- Bending GD, Lincoln SD and Edmondson RN. (2006) Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. *Environ Poll.* 139(2): 279-287.
- Bhalerao TS and Puranik PR. (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. *Int Biodeterioration Biodegrad.* 59(4): 315-321.

- Bhattacharya A and Sahu SK. (2013) A comparative study of the effect of imidacloprid and dimethoate on soil enzyme. *Int J Biosci.* 3(1): 172-182.
- Casida Jr LE, Klein DA and Santoro T. (1964) Soil dehydrogenase activity. *Soil Sci.* 98(6): 371-376.
- El-Hamady SE, Kubiak R and Derbalah AS. (2008) Fate of imidacloprid in soil and plant after application to cotton seeds. *Chemosphere* 71(11): 2173-2179.
- El-Naggar JB and Zidan NEHA. (2013) Field evaluation of imidacloprid and thiamethoxam against sucking insects and their side effects on soil fauna. *J Plant Prot Res.* 53(4): 375-387.
- Floch C, Chevremont AC, Joanico K, Capowicz Y and Criquet S. (2011) Indicators of pesticide contamination: Soil enzyme compared to functional diversity of bacterial communities via Biolog® Ecoplates. *Eur J Soil Biol.* 47(4): 256-263.
- Gianfreda L, Sannino F, Ortega N and Nannipieri P. (1994) Activity of free and immobilized urease in soil: effects of pesticides. *Soil Biol Biochem.* 26(6): 777-784.
- Gillman GP. (1979) A proposed method for the measurement of exchange properties of highly weathered soils. *Soil Res.* 17: 129-139.
- https://chemicals.nic.in/sites/default/files/Annual_Report_2021.pdf (1):129-139.
- <http://ppqs.gov.in/statistical-database?page=1>.
- Jeschke P, Nauen R, Schindler M and Elbert A. (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem.* 59(7): 2897-2908.
- Kandeler E. (1995) Organic matter by wet combustion. In: *Methods Soil Biol*, (eds.) Schinner F, Ohlinger R., Kandeler E. and Margesin R., Springer-Verlag Berlin Heidelberg New York, pp. 397-398.
- Kanrar B, Ghosh T, Pramanik SK, Dutta S, Bhattacharyya A and Dhuri AV. (2006) Degradation dynamics and persistence of imidacloprid in a rice ecosystem under West Bengal climatic conditions. *Bull Environ Contam Toxicol.* 77(5): 631-637.
- Kızılkaya R, Aşkın T, Bayraklı B and Sağlam M. (2004) Microbiological characteristics of soils contaminated with heavy metals. *Eur J Soil Biol.* 40(2): 95-102.
- Mohiddin GJ, Srinivasulu M, Madakka M, Subramanyam K and Rangaswamy V. (2011) Influence of selected insecticides on enzyme activities in groundnut (*Arachis hypogaea* L.) soils. *Dynamic Soil Dynamic Plant* 1(5): 65-69.
- Naumann K. (1970) Dynamics of the soil microflora following application of insecticides. IV. Investigations on the effect of parathion-methyl on soil respiration and dehydrogenase activity. *Zentralbl Bakteriell Parasite Infektionskr Hyg.* 125: 119-133.
- Panda S and Sahu SK. (2000) Respiration and enzyme activities of soil following application of an organophosphorous insecticides. *Int J Ecol Environ Sci.* 26: 75-82.
- Pandey S and Singh DK. (2006) Soil dehydrogenase, phosphomonoesterase and arginine deaminase activities in an insecticide treated groundnut (*Arachis hypogaea* L.) field. *Chemosphere* 63(5): 869-880.
- Qian K, Tang T, Shi T, Wang F, Li J and Cao Y. (2009) Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3, 5-dinitrobenzotrifluoride. *Anal Chim Acta* 635(2): 222-226.
- Sebiomo A, Ogundero VW and Bankole SA. (2011) Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *Afr J Biotechnol.* 10(5): 770-778.
- Shetty PK and Magu SP. (1998) In vitro effect of pesticides on carbon dioxide evolution and dehydrogenase activities in soil. *J Environ Biol* 19(2):141-144.
- Singh DK and Kumar S. (2008) Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. *Chemosphere* 71(3): 412-418.
- Singh J and Singh DK. (2005) Dehydrogenase and phosphomonoesterase activities in groundnut (*Arachis hypogaea* L.) field after diazinon, imidacloprid and lindane treatments. *Chemosphere* 60(1): 32-42.
- Wang F, Yao J, Chen H, Chen K, Trebše P and Zaray, G. (2010) Comparative toxicity of chlorpyrifos and its oxon derivatives to soil microbial activity by combined methods. *Chemosphere* 78(3): 319-326.
- Wang F, Yao J, Chen H, Yi Z and Choi MM. (2014) Influence of short-time imidacloprid and acetamiprid application on soil microbial metabolic activity and enzymatic activity. *Environ Sci Poll Res.* 21(17): 10129-10138.
- Zaller JG, König N, Tiefenbacher A, Muraoka Y, Querner P, Ratzenböck A and Koller R. (2016) Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. *BMC Ecol* 16(1): 1-11.
- Zhang QC, Shamsi IH, Xu DT, Wang G.H, Lin XY, Jilani G and Chaudhry AN. (2012) Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Appl Soil* 57: 1-8.