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Effect of Imidacloprid on the Microbial Biodiversity of Soils of Selected Zones of Haryana, India

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Abstract: Imidacloprid is one of the most frequently used insecticide in Haryana having a pivotal role in pest management and improvement in crop yield production. This study was performed to investigate the consequences of Imidacloprid on bacterial, actinomycetes and fugal population in soils of selected zones of Haryana, India. The experiment was conducted for 28 days and the samples were taken after 1st, 7th, 14th, 21st and 28th day of Imidacloprid application at various concentrations like T1(1mg/kg), T2 (2mg/kg) and T3(10mg/kg) doses. The number of CFU (colony forming unit) of microbes were grown on appropriate media using serial dilution and spread plate method. The results obtained showed a negative effect of Imidacloprid at T1 and T2 doses in first two weeks of incubation but non-significant effect in last two weeks of incubation on soil microbial number. Imidacloprid stimulated number of heterotrophic bacteria and fungi at T3 with time but a negative effect of N_2-fixing and phosphate solubilizing bacteria was observed at each dose and time point in each soil sample. Obtained results showed that these soil microbes 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: Imidacloprid, Neonicotinoid, Soil, Microbes, Haryana, Bacteria, Actinomycetes, Fugal population

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Introduction

Haryana also called food bowl of country, constitute 1.5% area of India and contributes 15% of its agricultural production. In Haryana total arable land area is 86% of which 96% is cultivated using wide crop diversity and modern agricultural practices. Soil is the upper layer of earth containing organic matter, minerals, mixture of gases, clay, rock particles as well as living organisms that together support life. Soil is also called as backbone of agriculture and "Skin of the Earth" is a vital alive system. It is a complex, highly heterogenous and dynamic system which inhabits a large number of alive organisms that play a crucial function for the ecosystem (Gardi

and Jeffery, 2009). Soil is of various types like alluvial, black, red and yellow soil, laterite soil, arid soil, mountain and forest soil and desert soil. In Haryana mainly loamy, sandy, coarse loamy and coarse sandy soil is found where out of every 100 hectares 3/4 is cultivated. The production of food grains per hectare is much higher in Haryana than in any other state of the country. Numerous threats such as soil erosion, soil compaction, soil contamination with agrochemicals, water logging, desertification, desalinization, loss of soil biodiversity etc. damages soil health and diminishes soil productivity. Overuse of pesticides to kill pests alter the biodiversity as well as enzyme activities in terms of nitrogen fixation, nitrogen transformation, nutrient release, organic matter decomposition and their availability that lead to changes in overall soil fertility (Khan and Scullion, 2000; Bending *et al*., 2007; Xia *et al*., 2011; Punitha *et al*., 2012). Most dominating organisms found in soil are fungi, actinomycetes, heterotrophic bacteria, arthropods, nematodes and earthworm (Jeffery *et al*., 2010). These perform positive or negative interactions with each other as well as with their inanimate counterpart existing in the soil and make it lively, well growing medium. They use dead organic matter of soil as food and transform them to simpler molecules that will improve nutrient level of the soil hence, maintains the soil fertility, control water balance, reduce soil pollution and normalize chemical, physical and biological properties of soil(Lavelle *et al*., 2006; Jeffery *et al*., 2010).

Present study is mainly concentrated on a neonicotinoid insecticide named Imidacloprid, which is the second most used agricultural chemical globally. It is a ring derivative that is popularly sold in many different countries of the world after its launching by Bayer Crop Science and highly used insecticide that gain 24% share of total market in 2008 (Jeschke *et al*., 2011). It is commercially sold under various brand names (i.e. Admire, Confidor, Gaucho, Winner, Prothor and Premise) and used for agriculture, horticulture and domestic pest control (Jeschke *et al*., 2011). It is used in more than 140 crops and the most admirable insecticide due to its systemic mode of action and selective targeting of insect's central nervous system. It is used in so many ways such as to treat seeds, soil and foliar application. Imidacloprid mainly destroy crop insects like aphids, thrips, beetles, Jassids and plant hoppers, termites, white flies, fleas etc. Various other ways like physical and mechanical control, biological control and integrated pest management and also used to overcome the pest population but major dependency is on chemical control methods (Pretty, 2018).

Production of Imidacloprid in 2019-20 was 20 MT (Ministry of Chemicals and Fertilizers 2020-21). Consumption of Imidacloprid was 309 M.T. in 2019-20 and 372 M.T. in 2020-21 in India (http://ppqs.gov.in/statistical-database?page=1). Soil microbiota are the first to show changes in their number, type and community structure, due to the direct and indirect impact of pesticides applied to crops. Overuse of Imidacloprid negatively interact with both the biotic and abiotic components of environment (Sharma *et al*., 2020). Thus, Imidacloprid shows a dual function: toxic on non-target organisms and beneficial for crop protection and production.

This study is an effort to analyse Imidacloprid effects on soil health. To our knowledge, this is the first study indicating the effect of imidacloprid pesticide on microbes in Haryana soil.

Materials and Methods

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

Collection of soil samples:

For this study four different zones of Haryana i.e. Rohtak, Hisar, Kurukshetra and Mahendergarh were chosen for soil collection. The sampling place was chosen which was neither contaminated with any pesticide from last four years nor added with fertilizers. The soil was collected in sterile bags at

a depth of 0-15 cm after removing upper layer of soil. Large aggregates of soil were crushed by hand and were further reduced by grinding.

Physicochemical properties of soil:

Soil used for this experiment were analysed first for its chemical and physical properties. pH meter was used for analysing soil pH. For calculating water holding capacity filter paper method was used. Cation exchange capacity (CEC) was determined using barium chloride method (Gillman, 1979). Soil texture and content of micronutrients were determined by Hi-media test kits. For determining organic carbon content (C_{org}) methodology of Kandeler was used with some modifications in which oxidation of dichromate in presence of concentrated sulfuric acid was calculated (Kandeler, 1995). The total nitrogen content (N_{tot}) was calculated using Kjeldahl method (Kandeler, 1995).

Experimental design:

Various treatments taken in this study were control, T1 (1 mg Imidacloprid/kg soil), T2 (2 mg Imidacloprid/kg soil) and T3 (10 mg Imidacloprid/kg soil). This experiment was done in a Completely Randomized Design (CRD) where triplicates of each treatment was performed. For this, soil of each zone was divided into four portions of equal weight and 12 pots were filled for soil of each zone (4 doses of Imidacloprid x 3 replication) @ 2kg of soil per pot. Imidacloprid was dissolved in water and poured drop wise then mixed thoroughly with the help of a sterile spatula. In control only water was mixed. The moisture in soil was also maintained during the whole experimental period by adding distilled water periodically, maintaining 45% to 50% maximum water-holding capacity of the soil. The pots were incubated in the dark and covered with polypropylene sheets to prevent the evaporation of water and photodegradation of Imidacloprid. All pots were incubated at 20 ± 2 C for 28 days. Three pots of each zone for each treatment were taken after 1, 7, 14, 21 and 28 days, respectively. Firstly, the soil in the pot was thoroughly mixed and appropriate amounts were taken for determining microbe number according to the described methods.

Enumeration of soil microbes:

The effect of various Imidacloprid concentrations on soils was determined in triplicates after 1, 7, 14, 21 and 28 days of incubation on microbial populations by standard dilution technique. After serial dilutions the appropriate amount were plated on the plates already prepared with preferred media for each type of microbe. For estimating the total number of heterotrophic bacteria, Tryptic Soy Agar (TSA) plates were used and incubated at 27 C for 24 h. To estimate actinomycetes, isolation agar was used and plates were incubated at 30C for 3 days. Likewise, Ashby's Mannitol Phosphate Agar was used for N_2 fixing bacteria and the samples were incubated for 14 days in nitrogen atmosphere. For fungal counts plates were incubated on Jensen's media for 5 days at 25 C and Pikovskaya's media was used for phosphate solubilizing bacteria. Bacterial, fungal and actinomycetes colonies were counted by plating 0.1 ml of suitable dilution on separate plate using spread plate method and incubated at suitable temperature according to the type of microbe. Colony forming units per g of soil (cfu/g) was counted using a colony counter and CFU/g of dry soil was calculated by following equation:

CFU/g soil = No. of colonies counted/Volume plated in ml × Dilution factor

Statistical analysis:

The results are presented as mean ± standard deviation (SD) of three replications. Two-way analysis of variance (ANOVA) was used to determine the interaction between the treatment doses and incubation time in soils of each zone. Post hoc Tuckey's test was used to measure the statistical significance (P<0.05) of measured data.

Results

The results of the present study indicated that Imidacloprid affects the total number of nitrogen fixing bacteria, heterotrophic bacteria, phosphate

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

Nutrients	Rohtak	Hisar	Kurukshetra	Mahendergarh
pH	7.9	7.40	8.00	7.50
Soil Type	Sandy Loam	Sandy Loam	Coarse loam	Sandy
Electrical Conductivity (1:2)	0.21	0.32	0.22	0.44
Organic Carbon (%)	0.33	0.30	0.36	0.24
Phosphorus (kg/ha)	6.50	5.80	4.90	3.90
Potassium (kg/ha)	154.00	126.00	264.00	166.00
Zinc (ppm)	0.94	0.80	0.97	0.63
Sulphur (ppm)	102	114.00	130.00	98.00
Manganese (ppm)	1.69	2.82	4.00	3.55
Iron (ppm)	5.43	4.54	5.43	4.84
Copper (ppm)	1.00	0.41	0.69	0.78

ppm= parts per million

solubilizing bacteria, fungi and actinomycetes present in the soil. The study showed dose, duration and soil type dependent correlation of microbial growth with Imidacloprid.

Physicochemical properties of soil:

Various physicochemical properties and composition of significant nutrients of soils of four zones of Haryana were studied to check effect of treatment in agricultural soil of Haryana state. The results given in Table1 indicate variability of all parameters.

Microbial Analysis:

Imidacloprid caused significant change in soil microorganisms count in different class of microbes studied.

Heterotrophic bacteria:

These type of bacteria helps in decomposition of dead and decaying remnants of plants and animals. These help in transformation of organic forms into inorganic one and hence increase soil fertility, nutrient cycling. The number of Heterotrophic bacteria declined significantly in soil of all four zones as shown in Figure 1. There was decline in bacterial count in A, B, C soils up to day 14 of Imidacloprid treatment in a dose dependent manner. In soil D, there was no significant fall in bacterial count. On 21stday all four soils showed almost similar microbial count as of control. There seems a decline in toxicity of Imidacloprid. Another interesting observation was seen on 28th days, a significant increase in bacterial number was observed in treated soil samples as compared to control. The results showed a strong stimulatory effect of Imidacloprid on Heterotrophic bacteria. ANOVA showed a significant effect ($P<0.05$) of dose and time of exposure of Imidacloprid on total number of heterotrophic bacteria but non-significant for soil type.

Fungal population:

Fungi are useful soil microbes that degrade lignin and other organic matter to simple forms so that

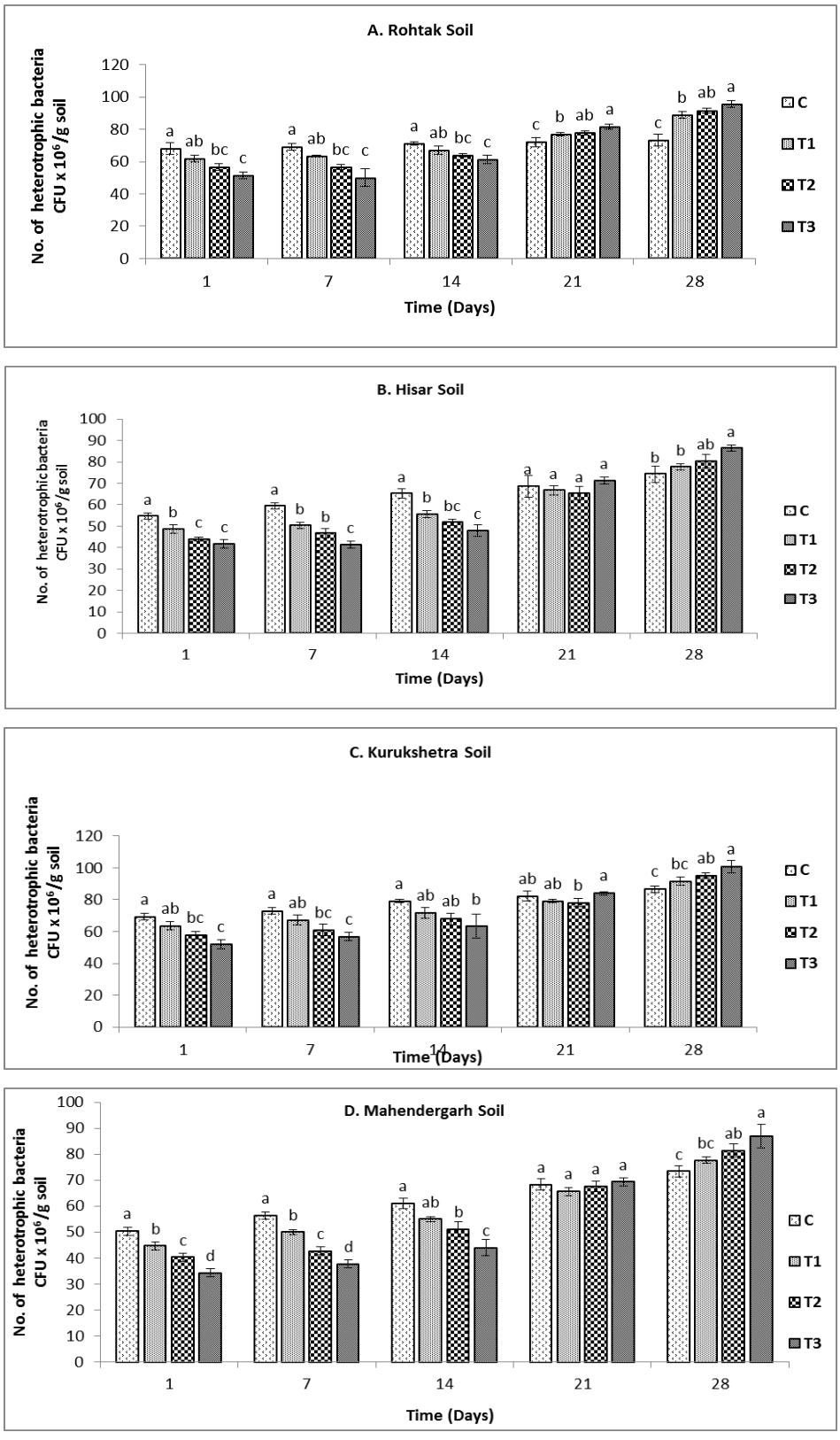

Fig.1: Number of heterotrophic bacteria (CFU-colony forming units) in soil of different zones of Haryana) treated with Imidacloprid (C: control; T1: 1 mg Imidacloprid/kg soil; T2: 2 mg Imidacloprid/kg soil; T3:10 mg Imidacloprid/kg soil). Mean ± S.D. of three replicates are represented by the bars of the graph. A significant statistical differences ($P<0.05$) between Imidacloprid dose and exposure time were analyzed using post hoc Tuckey test and represented using different letters (similar letters shows statistically insignificant while different letters show statistically significant with each other).

other microbes utilize them for their growth and energy. Imidacloprid application changes the fungal population size. Imidacloprid treatment after 1 day significantly decreased the fungal number only at T3 dose as shown in Figure 2. However, this decrease was observed at T1 dose in soil D. A significant decrease in fungal number was observed in A and B soil after each sampling time in a dose dependent manner. But no pattern was observed in B and D soil after 14, 21 and 28 days. No reduction in fungal number was observed after 28 days of incubation in soil D and after 7 and 14 days in soil B.

Nitrogen-fixing Bacteria:

These bacteria boost soil with inorganic N containing compounds and enhance soil fertility. The N_2-fixing bacteria showed great sensitivity to Imidacloprid application. 1 day after Imidacloprid application the negative effect was found dose dependently in bacterial count. Highest reduction was found in the soil D. However, a non-significant effect was observed in soil B at T1 and T2 dose after 1 day of incubation as shown in Figure 3. The negative effect was also observed in the sample taken at next time period in soil treated with control and T3 doses whileT1 and T2 doses showed a non-significant decrease. In soil D more than 50% decrease in bacterial number was observed at T3 dose after 7 days of exposure. In next sampling time T1 and T2 doses showed a non-significant decrease in these bacteria but a significant inhibitory effect was observed at T3 dose at each sampling time as compared to control.

Phosphate solubilizing bacteria:

These are a very important type of soil bacteria that solubilise inorganic phosphate from insoluble forms hence associated with plant phosphate nutrition. Phosphate solubilizing bacteria showed a strong inhibitory effect in their number with Imidacloprid application both with the dose and time. However, T1 dose of Imidacloprid did not show any significant inhibition in number of phosphate solubilizing bacteria with time in soil of each zone as shown in Figure 4. The number of phosphate solubilizing bacteria in control soil and T1 treated soil were not significantly different in each soil type. However, the T3 dose showed a significant decrease in number of phosphate solubilizing bacteria at each sampling time in soils of each zone. We can say that the control soil of each zone harbour the maximum number of phosphate solubilizing bacteria which gets reduced with dose and time of exposure of Imidacloprid.

Actinomycetes:

These are the microorganisms that share the characteristics of both bacteria and fungi. These produced a volatile compound geosmin, enhance mobility and solubility of phosphate and iron, accelerate plant growth by supporting plant growth agents, protect plants from some dreadful pathogens. A reduction in number of actinomycetes was observed with Imidacloprid treatment and the highest reduction was observed in T3 followed by T2 as shown in Figure 5. With time of exposure no significant pattern in number of actinomycetes was observed. T1 treatment showed an increase in actinomycetes number as compared to control in soil A and soil B where non-significantly increased number was found as compared to control.

Discussion

Imidacloprid showed different pattern of soil microbes count depending upon the dose, time of exposure and soil type. In this study a significant increase in population of heterotrophic bacteria with time and dose of Imidacloprid was observed in soil of each zone. Similar results were reported by Monkiedje *et al*. (2002) and Das *et al*. (2005). The reason behind this might be utilization of Imidacloprid as an energy source like carbon, nitrogen and other nutrients by some microbes (Bhuyan *et al*., 1993). The increase may also be due to degradation of some biodegradable bacteria present in the soil (Cycon *et al*., 2013). Similar stimulatory results were also shown by other pesticides like fenvalerate (Das and

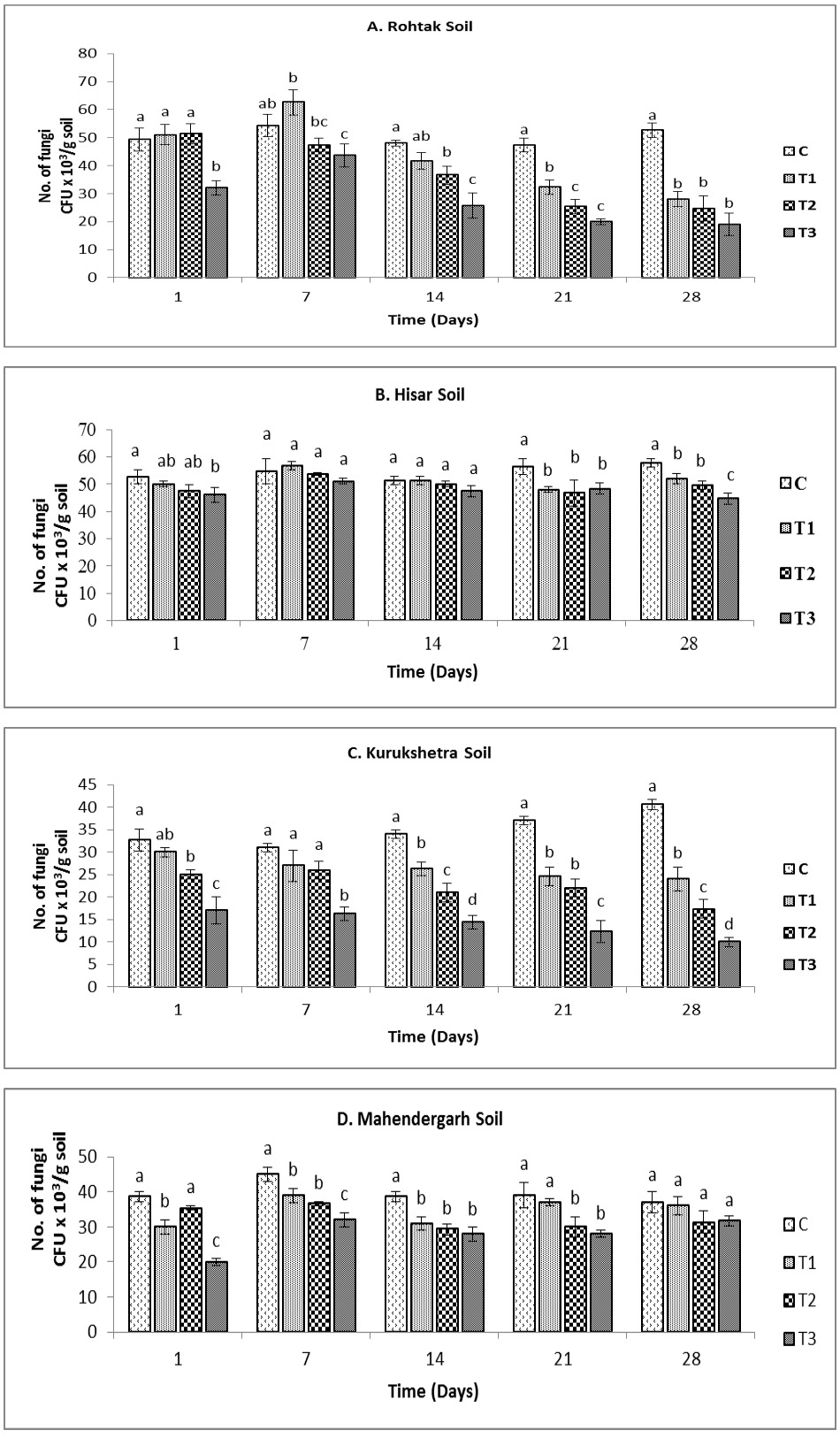

Fig. 2: Number of Fungi (CFU-colony forming units) in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg Imidacloprid/kg soil; T2: 2 mg Imidacloprid/kg soil; T3:10 mg Imidacloprid/kg soil). Mean ± S.D. of three replicates are represented by the bars of the graph. A significant statistical differences ($P<0.05$) between Imidacloprid dose and exposure time were analyzed using post hoc Tuckey test and represented using different letters (similar letters shows statistically insignificant while different letters show statistically significant with each other).

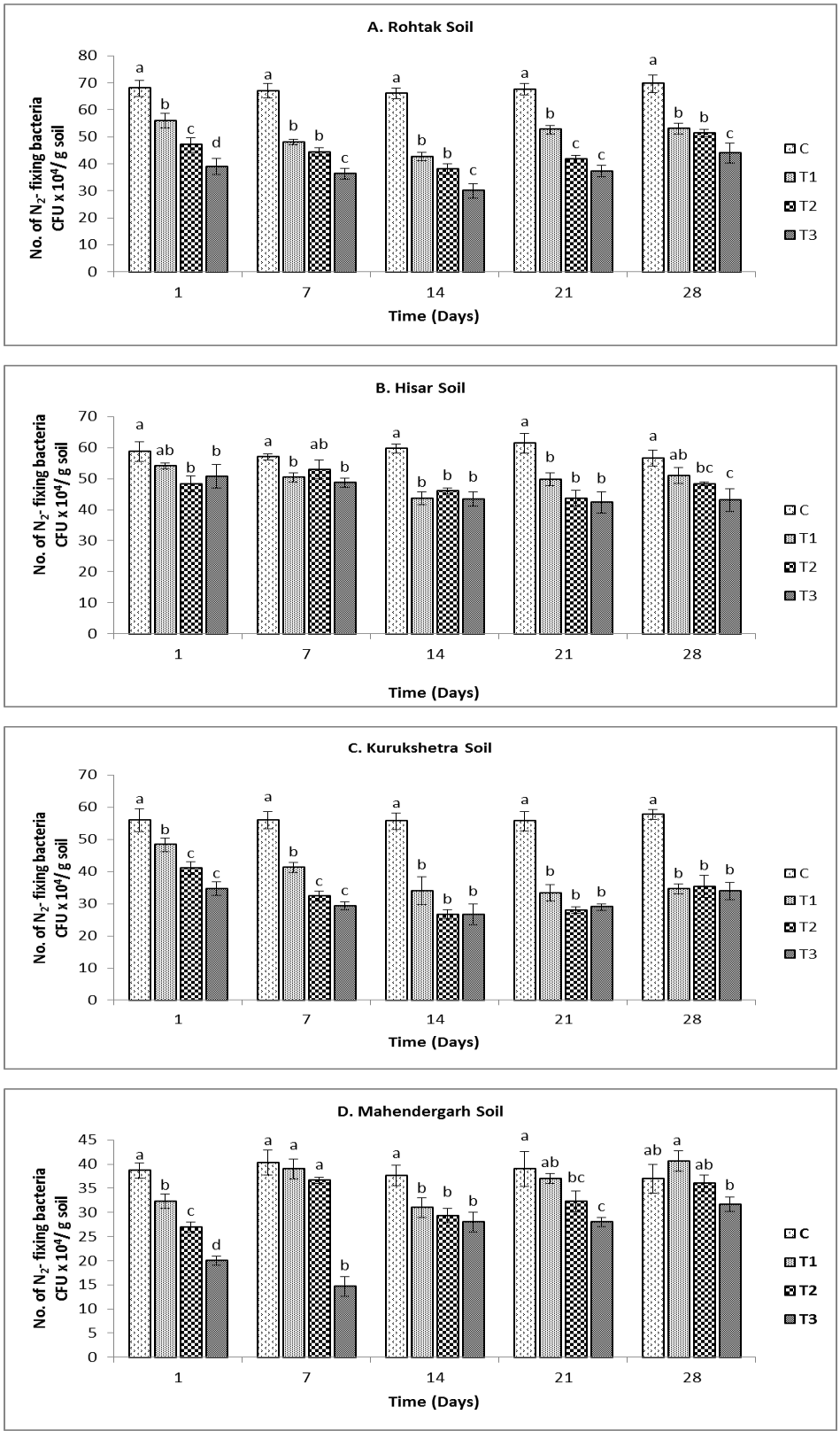

Fig. 3. Number of N_2-fixing bacteria (CFU-colony forming units) in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg Imidacloprid/kg soil; T2: 2 mg Imidacloprid/kg soil; T3:10 mg Imidacloprid/kg soil). Means ± S.D. of three replicates are represented by the bars of the graph. A significant statistical differences ($P<0.05$) between Imidacloprid dose and exposure time were analyzed using post hoc Tuckey test and represented using different letters (similar letters shows statistically insignificant while different letters show statistically significant with each other).

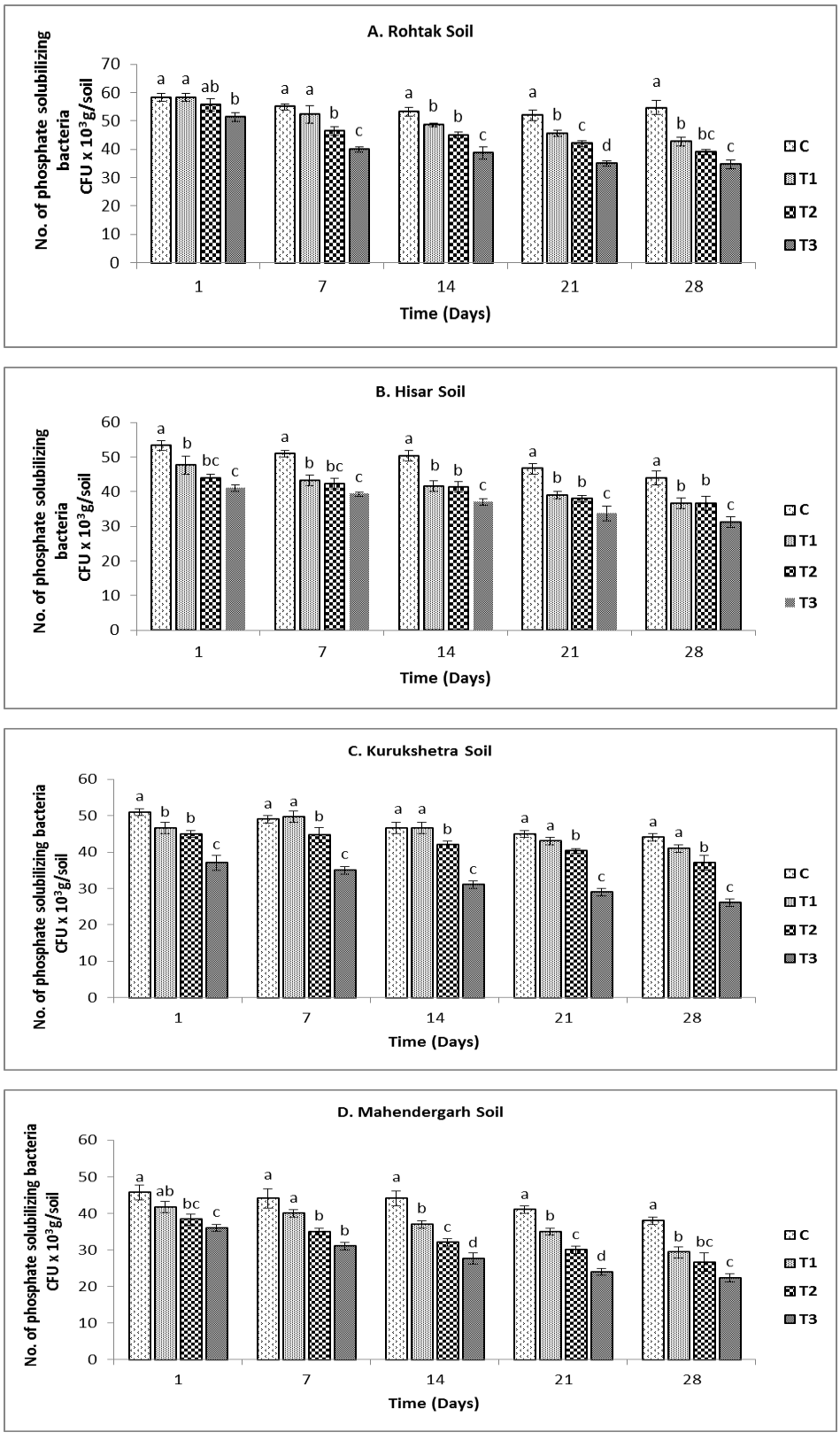

Fig. 4: Number of phosphate solubilizing bacteria (CFU-colony forming units) in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg Imidacloprid/kg soil; T2: 2 mg Imidacloprid/kg soil; T3:10 mg Imidacloprid/kg soil). Mean ± S.D. of three replicates are represented by the bars of the graph. A significant statistical differences ($P<0.05$) between Imidacloprid dose and exposure time were analyzed using post hoc Tuckey test and represented using different letters (similar letters shows statistically insignificant while different letters show statistically significant with each other).

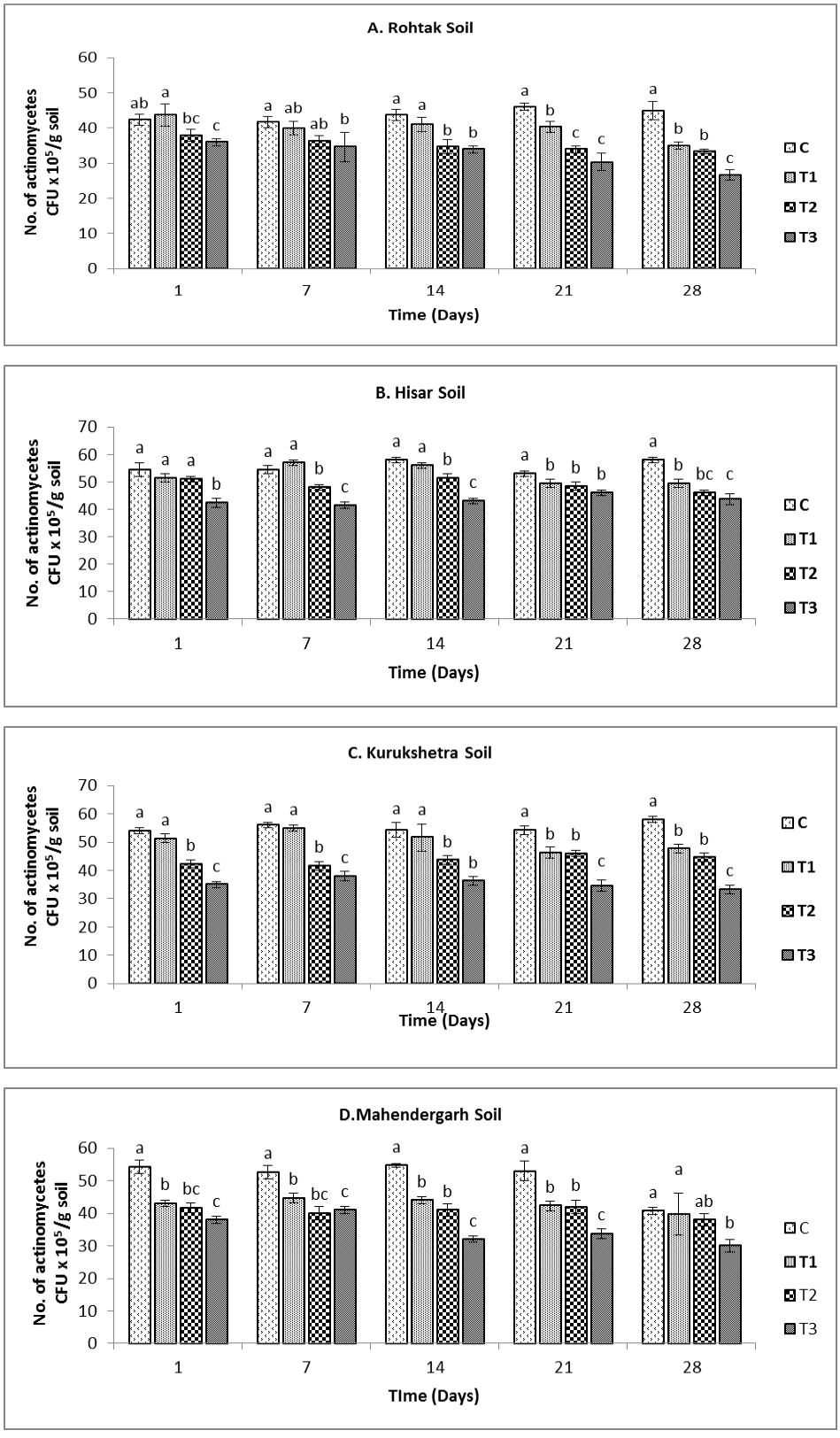

Fig. 5: Number of actinomycetes (CFU-colony forming units) in soil of different zones of Haryana treated with Imidacloprid (C: control; T1: 1 mg Imidacloprid/kg soil; T2: 2 mg Imidacloprid/kg soil; T3:10 mg Imidacloprid/kg soil). Mean ± S.D. of three replicates are represented by the bars of the graph. A significant statistical differences ($P<0.05$) between Imidacloprid dose and exposure time were analyzed using post hoc Tuckey test and represented using different letters (similar letters shows statistically insignificant while different letters show statistically significant with each other).

Mukhrjee, 2000) and hexachlorocyclohexane (HCH), phorate and teflubenzuron (Cycon *et al*., 2012).

We have found that Imidacloprid showed a strong inhibitory effect on fungal population in initial days of Imidacloprid exposure at highest dose. Similar to our results Imidacloprid showed insignificant effect on fungal number (Devashree *et al*., 2014; Zhang *et al*., 2015). Contrary to our results stimulation in fungal number was observed with the application of insecticide diazinon, phorate, linuron (Das *et al*., 2005; Cycon and Piotrowska-Seget, 2007). A fall in proliferation of fungi was observed in initial days of Imidacloprid exposure followed by a progressive rise in fungal number in soil of Mahendergarh and Hisar zone. This indicated that with time fungi might be adopted to utilize the Imidacloprid metabolites for its nutrients.N_2-fixing bacteria were found to be highly sensitive to this insecticide. Similar effect of Imidacloprid on these bacteria was reported by Cycon and Piotrowska-Seget (2015). These microbes are also considered as sensitive indicators of the environmental threat in soil with respect to raised concentration of pesticide and other agro-chemicals.

Actinomycetes at higher doses of Imidacloprid showed adverse effects but at lower dose of Imidacloprid a synergistic effect was observed. Stimulation of actinomycetes was also observed in a black clay soil at lower doses of endosulfan and profenofos (Nasreen *et al*., 2015), whereas inhibition was observed at higher doses. In a sandy loam soil, Metalxyl also showed a significant stimulation in actinomycetes number at 0.5 ppm incubated for 4 weeks (Shetty and Magu, 2000).

Phosphate solubilizing bacteria is reported to be very much susceptible towards Imidacloprid exposure. Phosphate solubilizing bacteria showed strong inhibition in their number at highest dose of Imidacloprid during the whole experimental time period. Similar results were observed by Das and Mukherjee (2000) and Wani *et al*. (2005). Chlorpyrifos was reported to show maximum decrease when applied as foliar spray (Saranaik *et al*., 2006).

Imidacloprid showed a variable effect on soil microflora where some microbes use Imidacloprid as an energy source and increase their multiplication, other might show a reduction in their number due to their more sensitivity towards this insecticide (Bollag and Liu, 1990). Some other microbes utilized the nutrients released form dead bacteria and increase their number but this change may decrease the biodiversity of microbes and their community structure (Cycon *et al*., 2013).

Any change in microbial community may alter availability of nutrients and their cycling. These changes negatively affect ecosystem activity and stability. Behaviour of Imidacloprid in soil depends upon a number of biotic and abiotic factors. So, it becomes very tough to measure the overall effect of Imidacloprid on soil microbiota and hence soil ecosystem. The present study just gave an idea of the health hazard caused by Imidacloprid on soil microbes in selected zones of Haryana. Comparative studies of number of microbes in soils of selected zones of Haryana in response to Imidacloprid is just to analyse Imidacloprid effect with soil type and time of exposure. It gave a warning of the cross effects of pesticides on soil microbial activity. Although in plate count method we can estimate only the culturable microflora, still it is a convenient method for evaluation of variable microbial response to this insecticide.

Conclusion

Change in soil microbial properties due to a pesticide give a brief idea about non-target toxicity of that pesticide. Soil microbes get disturbed due to extraneous application of pesticides that in turn changes nutrient cycling. Imidacloprid negatively affected the 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 microbes. Results of this study showed that Imidacloprid at lower doses not caused a significant harm to the soil microbes but when applied frequently to higher doses it hinder the regeneration of microbial fauna. It is the first study that examine the effects of Imidacloprid in bacterial and fungal number in soil of different zones of Haryana in our knowledge. 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

Bending GD, Rodríguez-Cruz MS and Lincoln SD. (2007) Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69(1): 82-88.

Bhuyan S, Sreedharan B, Adhya TK and Sethunathan N. (1993) Enhanced biodegradation of (γ-HCH) in HCH (commercial) acclimatized flooded soil: Factors effecting its development and persistence. Pestic Sci. 38: 450-463.

Bollag JM and Liu SY. (1990) Biological transformation processes of pesticides. In: Pesticides in the soil environment: processes, impacts and modelling, (ed.) Cheng H.H., SSA Book Series.

Cycon M and Piotrowska-Seget Z. (2007) Effect of selected pesticides on soil microflora involved in organic matter and nitrogen transformations: pot experiment. Pol J Ecol. 55(2): 207-220.

Cycoń M and Piotrowska-Seget Z. (2015) Biochemical and microbial soil functioning after application of the insecticide imidacloprid. J Environ Manage. 27: 147-158.

Cycoń M, Markowicz A, Borymski S, Wójcik M and Piotrowska-Seget Z. (2013) Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. J Environ Manage. 131: 55-65.

Cycoń M, Wójcik M, Borymski S and Piotrowska-Seget Z. (2012) A broad-spectrum analysis of the effects of teflubenzuron exposure on the biochemical activities and microbial community structure of soil. J Environ Manage. 108: 27-35.

Das AC and Mukherjee D. (2000) Influence of insecticides on microbial transformation of nitrogen and phosphorus in typic orchragualf soil. J Agric Food Chem. 48(8): 3728-3732.

Das AC, Chakravarty A, Sen G, Sukul P and Mukherjee D. (2005) A comparative study on the dissipation and microbial metabolism of organophosphate and carbamate insecticides in orchaqualf and fluvaquent soils of West Bengal. Chemosphere 58(5): 579-584.

Devashree Y, Dutta BK, Paul SB and Choudhury S. (2014) Effect of imidacloprid on the soil and rhizosphere microflora of tea agro-ecosystem. Glob J Biotechnol Biochem. 9: 35-40.

Gardi C and Jeffery S. (2009) Soil biodiversity. Brussels: EUR-OP.

Gillman GP. (1979) A proposed method for the measurement of exchange properties of highly weathered soils. Soil Res. 17(1): 129-139.

https://chemicals.nic.in/sites/default/files/Annual_Report_2021.pdf Ministry of chemicals and fertilizers Annual Report 2020-21.

Jeffery S, Gardi C, Jones A, Montanarella L, Marmo L, Miko L and Van der Putten HRSG. (2010) European atlas of soil biodiversity. European Commission. Publications of the European Union, Luxemburg.

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.

Khan M and Scullion J. (2000) Effect of soil on microbial responses to metal contamination. Environ Poll. 110(1): 115-125.

Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P and Rossi JP. (2006) Soil invertebrates and ecosystem services. Eur J Soil Biol. 42: 3-15.

Monkiedje A, Ilori MO and Spiteller M. (2002) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol Biochem. 34(12): 1939-1948.

Nasreen C, Jaffer Mohiddin G, Srinivasulu M, Manjunatha B and Rangaswamy V. (2015) Interaction effects of insecticides on microbial populations and dehydrogenase activity in groundnut (*Arachis*

hypogea L.) planted black clay soil. Int J Curr Microbiol Appl Sci. 4(2): 135-146.

Pretty J. (2018) Intensification for redesigned and sustainable agricultural systems. Science 362: 6417.

Punitha BC, Hanumantharaju TH, Jayaprakash R and Shilpashree V. (2012) Acetamiprid impact on urease and phosphatase activity in selected soils of southern Karnataka. Int J Basic Appl Chem Sci. 2(1): 1-6.

Sarnaik SS, Kanekar PP, Raut VM, Taware SP, Chavan KS and Bhadbhade BJ. (2006) Effect of application of different pesticides to soybean on the soil microflora. J Environ Biol. 37(2): 423-426.

Sharma RK, Singh P, Setia A and Sharma AK. (2020) Insecticides and ovarian functions. Environ. Mol Mutagen. 61(3): 369-392.

Shetty PK and Magu SP. (1998) In vitro effect of pesticides on carbon dioxide evolution and dehydrogenase activities in soil. J Environ Biol. 19: 141-144.

Wani PA, Zaidi A, Khan AA and Khan MS. (2005) Effect of phorate on phosphate solubilization and indole acetic acid releasing potentials of rhizospheric microorganisms. Plant Protect Sci. 13(1): 139-144.

Xia X, Zhao M, Wang H and Ma H. (2011) Influence of butachlor on soil enzymes and microbial growth. J Food Agric Environ. 9(2): 753-756.

Zhang Q, Xue C and Wang C. (2015) Effects of imidacloprid on soil microbial communities in different saline soils. Environ Sci Poll Res Int. 22(24): 19667-19675.