Environ Monit Assess (2014) 186:2281–2288
DOI 10.1007/s10661-013-3536-1

Persistence behaviour of imidacloprid and its metabolites in soil under sugarcane

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Received: 2 May 2013 / Accepted: 12 November 2013 / Published online: 26 November 2013

Abstract The persistence and metabolism of imidacloprid in soil under sugarcane were studied following application of imidacloprid at 20 and 80 g active ingredient (a.i.) ha^{-1}. Soil samples were collected at different time intervals (7, 15, 30, 45, 60 and 90 days after application), and the residues of imidacloprid and its metabolites (6-chloronicotinic acid, nitrosimine, imidacloprid-NTG, olefin, urea and 5-hydroxy) were quantified by high-performance liquid chromatography. In soil, the total imidacloprid residues were mainly constituted by the parent compound followed by 6-chloronicotinic acid, nitrosimine and imidacloprid-NTG metabolites. Maximum residues of imidacloprid and its metabolites were 4.29 and 7.81 mg kg^{-1} in soil samples collected 7 days after the application of imidacloprid at 20 and 80 g a.i. ha^{-1}, respectively. At both doses, these residues declined to below the detectable limit in soil after 90 days of application. Olefin, urea and 5-hydroxy metabolites were not detected in soil. Dissipation of total imidacloprid residues did not follow the first-order kinetics with a coefficient of determination value of 0.883 and 0.838 for the recommended dose and four times the recommended dose, respectively. The half-life ($T_{1/2}$) value of total imidacloprid was observed to be 10.64 and 10.10 days for the recommended dose and four times the recommended dose, respectively.

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Keywords Soil · Imidacloprid · Metabolism · HPLC · Residues

Introduction

Soil is the repository of all types of agricultural inputs including pesticides which directly or indirectly influence soil productivity and agroecosystem quality. Although most pesticide use is directed to the control of pests on above-ground plant parts, a large proportion of pesticides reach soil regardless of the method of application; thus, they act as a major environmental sink. Sprays applied on crop foliage do not always reach the target, and as much as 50 % of the pesticide applied on a crop reaches soil either as spray drift or runoff or from leaves fallen on soil (Khan 1980). It has also been reported that only less than 0.3 % of the pesticide reaches the target leaving the remaining 99.7 % in the environment (Pimentel 1995). The metabolic fate of a pesticide is dependent on environmental conditions, microbial community, plant species, pesticide characteristics and biological and chemical reactions (Van Eerd et al. 2003). Data on the rate of pesticide degradation are extremely important to predict the potential risk associated with exposure. Such studies are, therefore, the foundation for understanding the fate and behaviour of a pesticide and any subsequent risk assessment (Skidmore and Ambrus 2004). Imidacloprid could persist in soil depending on soil type, pH, use of organic fertilizers and presence or absence of ground cover (Rouchaud et al. 1996); the primary imidacloprid breakdown products in soil are imidacloprid urea,

6-hydroxynicotinic acid and 6-chloronicotinic acid, and the final product is CO_2 which is formed from 6-chloronicotinic acid (Scholz and Spieteller 1992; Bacey 2000) (Fig. 1).

Most of the previous studies on other crops regarding the fate of imidacloprid in soil focused only on the disappearance of the parent compound and did not take into account the reaction products or metabolites formed which is required for exact quantification of residues present in soil. Moreover, earlier reports indicated that only 2–7 % of the applied imidacloprid was taken up by plants after application to cotton seeds and the major fraction of the parent compound applied was metabolized in the soil (El-Hamady et al. 2008). Hence, in-depth studies are needed on the residues, metabolism, persistence and degradation of imidacloprid in sugarcane soil under Indian conditions. Therefore, the present study was planned to study the metabolism of imidacloprid in soil under sugarcane.

Materials and methods

Insecticide

Reference standards of imidacloprid and its metabolites supplied by M/S Bayer Crop Science India Ltd., Mumbai, India, were as follows: imidacloprid (99.2 % purity), imidacloprid-nitrosimine (90.6 % purity), imidacloprid-olefin (97.9 % purity), imidacloprid-urea (99.4 % purity), imidacloprid-6-chloronicotinic acid (98.8 % purity), 5-hydroxy imidacloprid (96.8 %) and imidacloprid-NTG (99.0 % purity).

Reagents and chemicals

All the chemicals and reagents of high purity were procured from the local market. All the solvents used were of laboratory grade and were redistilled in all-glass apparatus before use. The suitability of all the solvents

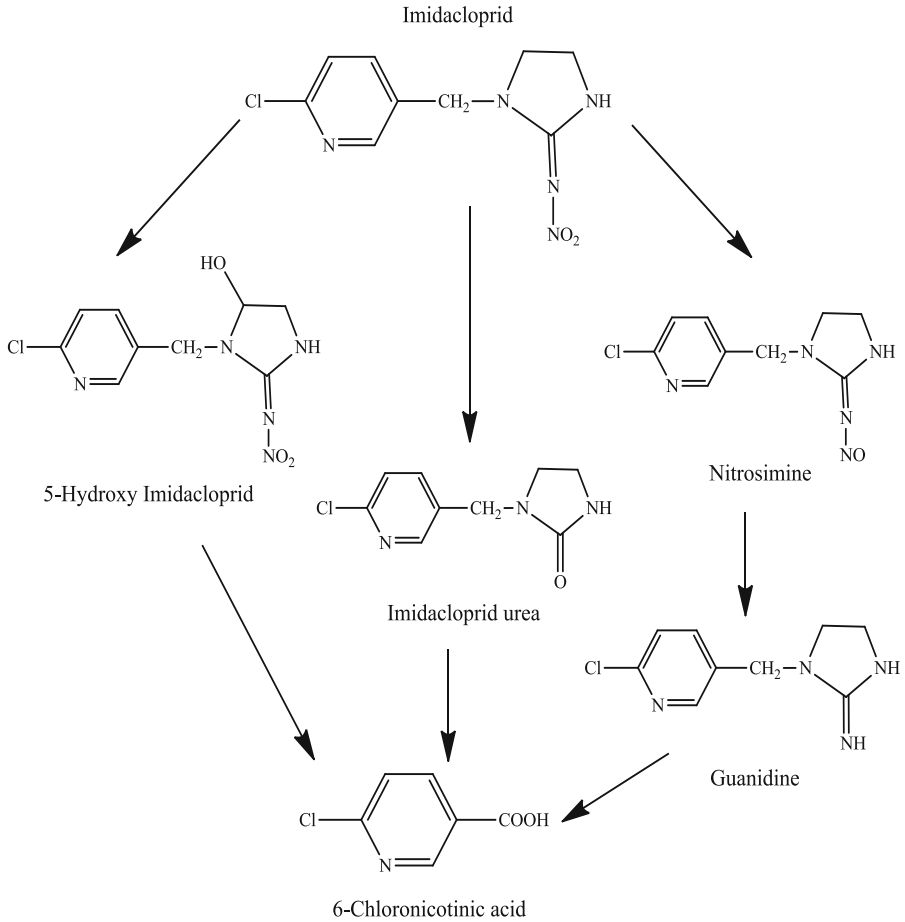

Fig. 1 The fate of imidacloprid and its main metabolites in soil. (Modified from Bacey 2000)

and other chemicals was ensured by running reagent blanks before actual analysis.

Instrumentation

High-performance liquid chromatography

A high-performance liquid chromatograph (HPLC; Model DGU-2045) equipped with a reverse-phase (RP) C_{18} column, photodiode array (PDA) detector and dual pump was supplied by M/S Shimadzu Corporation, Kyoto, Japan. The HPLC was equipped with a LC-20AT pump, CBM-20A system controller and Luna 5-μm C_{18} column (250×4.6 mm size, 5.20±0.30 μm particle size, 2.20±0.30 (90 %/10 %) particle distribution, 95±15 Å pore diameter, 430±40 m^2 g^{-1} surface area, <55.0 ppm metal content, 19.00±0.70 % total carbon and 3.25±0.50 μmol m^{-2} surface coverage). LC Solution software was used for instrument control and data acquisition and processing.

Rotary vacuum film evaporator

The rotary vacuum film evaporator (Model Heidolph Labrota 4002) was supplied by M/S Heidolph, Germany.

Stock solution

Stock solutions of imidacloprid, nitrosimine, olefin, urea, 6-chloronicotinic acid and 5-hydroxy (1 mg mL^{-1}) were prepared in HPLC-grade acetonitrile. The stock solution of imidacloprid-NTG (1 mg mL^{-1}) was prepared in HPLC-grade acetonitrile and HPLC-grade water in a ratio of 9:1 (*v*/*v*). The standard solutions (100, 10 and 1 μg mL^{-1}) were prepared from the stock solutions by serial dilutions with HPLC-grade acetonitrile. All standard solutions were stored below 4 °C before use.

Field experiment

Planting of the crop

Sugarcane (variety CoJ 88) was planted during the second week of February 2011 according to the recommended agronomic practices at University Seed Farm, Ladhowal, Punjab Agricultural University, Ludhiana (Anonymous 2011). Three replications of treatments, viz. control, recommended dose and four times the recommended dose, were arranged in randomized block design (RBD) with a total plot size of 500 m^2 for each treatment. The soil under the crop was of light texture with low organic matter. The other relevant properties of the soil were 0.315 % organic carbon, pH 8.1 and 0.34 dS m^{-1} EC.

Application of insecticide

Imidacloprid (Imidagold 17.8SL) was applied at the recommended dose (20 g active ingredient (a.i.) ha^{-1}; Anonymous 2011) and four times the recommended dose (80 g a.i. ha^{-1}) in the experimental plots at post-germination stage (45 days after planting). Soil samples were collected randomly from control and treated plots from each treatment 7, 15, 30, 45, 60 and 90 days after the application of insecticide. Soil samples were collected from 10 to 15 sites of each treated plot with the help of a tube auger at a depth of about 10–15 cm; the soil from each core was pooled and sieved, and extraneous matter, including stones/pebbles, were removed. After thorough mixing, a subsample of about 1 kg was taken from each pooled sample from each treatment plot and transported to the laboratory, and the moisture content of the soil was analyzed to express residues on a dry weight basis.

Extraction and cleanup

A gross soil sample of 1 kg, in three replications, was taken after thorough mixing of soil from four to five locations per plot. From each replication, a subsample of 250 g was drawn and then a representative 10-g sample of soil was dipped overnight into 100 mL acetonitrile/water (80:20 *v*/*v*) in an Erlenmeyer flask. The extract was filtered into a 1-L separatory funnel along with rinsing of acetonitrile. The filtrate in the separatory funnel was diluted with 600 mL saturated brine solution; the contents were partitioned three times with dichloromethane of 100, 80 and 50 mL, respectively, passed through anhydrous sodium sulphate and collected in a 500-mL beaker. Cleanup was done using activated charcoal (0.5 g) with continuous shaking in a shaker for 15 min. The clear extract so obtained was filtered through Whatman filter paper No. 1 with rinsing of acetonitrile and concentrated to near dryness using a rotary vacuum evaporator at <40 °C. The final volume was reconstituted to about 2.5 mL using HPLC-grade

acetonitrile. Before being injected into HPLC, the sample was further purified by filtering through a Millipore 0.45-μm filter.

Estimation of residues

The estimation of imidacloprid residues was carried out using HPLC equipped with a PDA detector. Before use, the column was primed with several injections of a standard solution of imidacloprid till a consistent response was obtained. The other details of HPLC parameters used for estimation of residues of imidacloprid and its metabolites were as follows: mobile-phase acetonitrile/water (40:60 *v*/*v*), 0.30 mL min^{-1} pump flow, 1, 500 psi pressure and detector set at 270-nm wavelength. The sample injector was equipped with a 20-μL loop. For instrument control and data acquisition and processing, LC Solution software was used. Under these operating conditions, imidacloprid and its six metabolites were separated in a single run of 20 min with retention times of 5.7 min (6-chloronicotinic acid), 8.0 min (imidacloprid-NTG), 10.7 min (olefin), 11.3 min (nitrosimine), 11.8 min (urea), 13.1 min (5-hydroxy imidacloprid) and 17.2 min (imidacloprid). The compounds in the sample were identified and quantified by comparison of the retention time and peak heights of the sample chromatograms with those of the standards run under identical operating conditions.

Results and discussion

Efficiency of the method

In the present investigations, recovery experiments were carried out at different levels to establish the reliability and validity of the analytical method and to know the efficiency of the extraction and cleanup procedures. Soil samples (10 g each) from control plots of sugarcane were spiked at levels of 0.01, 0.10, 0.20, 0.50 and 1.00 mg kg^{-1}. These were extracted, cleaned and analyzed following the method already described. The control samples from untreated plots and reagent blanks were also processed in the same way so as to find out interferences, if any, due to the substrate and reagents, respectively. In soil samples, the mean percent recoveries of imidacloprid ranged from 83.20 to 99.20. In the case of metabolites, the mean percent recoveries at these fortification levels were 85.40 to 99.70 for 6-chloronicotinic acid, 80.20 to 96.00 for imidacloprid-NTG, 84.95 to 97.10 for nitrosimine, 81.30 to 96.80 for olefin, 87.80 to 95.90 for 5-hydroxy and 84.30 to 99.70 for urea metabolites. Also, the mean percent recoveries of imidacloprid and its metabolites were >80 % at all the dates of sampling. As the average recovery values were found to be more than 80 %, thus, the results have been presented as such without applying any correction factor. The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid and its metabolites in soil were found to be 0.01 and 0.003 mg kg^{-1}, respectively.

Persistence and metabolism of imidacloprid in soil

After the application of imidacloprid at 20 g a.i. ha^{-1}, total residues of imidacloprid and its metabolites were found to be 4.29 mg kg^{-1} in samples of soil collected after 7 days. These residues declined to 1.80, 1.10, 0.99 and 0.41 mg kg^{-1} in the samples collected after 15, 30, 45 and 60 days of application of the insecticide (Table 1). The total residues of imidacloprid and its metabolites in soil after 7 days of its application at 80 g a.i. ha^{-1} were found to be 7.81 mg kg^{-1}. The corresponding amount was 2.45, 1.60, 1.49 and 0.90 mg kg^{-1} in the samples collected after 15, 30, 45 and 60 days, respectively, after application. The residues further declined to below the detectable limit of 0.01 mg kg^{-1} in the samples collected 90 days after the application of imidacloprid at both doses (Table 2). While in the extract of soil samples from the control, no peaks of imidacloprid and its metabolites were detected.

It was found that the total imidacloprid residues were mainly constituted by the 6-chloronicotinic acid metabolite followed by the parent compound along with a small amount of imidacloprid-NTG and nitrosimine metabolites. The imidacloprid-NTG metabolite was detected till 45 days after treatment, but olefin, urea and 5-hydroxy metabolites were not detected. This study implies that after soil application, imidacloprid probably undergoes reduction to form nitrosimine and then imidacloprid-NTG metabolites which are then oxidized to form 6-chloronicotinic acid. Imidacloprid might also be metabolized directly to 6-chloronicotinic acid, and hence, comparatively higher amounts of this metabolite were detected in the study. The presence of 6-chloronicotinic acid in a significantly higher amount than other metabolites is in accordance with the study by Scholz and Spiteller (1992).

Table 1 Residues of imidacloprid and its metabolites (mg kg^{-1}) in soil after its application at 20 g a.i. ha^{-1}

Days after treatment	Imidacloprid	Metabolites						Total residues	Dissipation (%)
		6-Chloronicotinic acid	Imidacloprid-NTG	Nitrosimine	Olefin	5-Hydroxy	Urea		
7	0.52±0.08	1.89±0.15	0.04±0.01	1.84±0.17	BDL	BDL	BDL	4.29±0.23	–
15	0.25±0.04	0.29±0.01	0.04±0.01	1.21±0.14	BDL	BDL	BDL	1.80±0.16	58.04
30	0.04±0.01	0.82±0.11	0.03±0.01	0.21±0.03	BDL	BDL	BDL	1.10±0.12	38.84
45	0.03±0.00	0.38±0.01	0.01±0.00	0.57±0.05	BDL	BDL	BDL	0.99±0.06	10.00
60	0.02±0.00	0.08±0.01	BDL	0.31±0.01	BDL	BDL	BDL	0.41±0.02	58.58
90	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	–

Values are expressed as mean±standard deviation of three replications

BDL below determination limit of 0.01 mg kg^{-1}

These results are in accordance with those of Krohn and Hellpointner (2002) who reported that imidacloprid is completely degradable in soil. Research had demonstrated that granule or liquid formulations did not affect imidacloprid's persistence or metabolism in soil (Sarkar et al. 2001). Similarly, Dharumarajan et al. (2009) reported the persistence of a combination-mix (β-cyfluthrin+imidacloprid) at 20 g a.i ha^{-1} on tomato. In their study, up to 50–55 % of imidacloprid was degraded in 3 days of application and the remaining residues dissipated in another 7 days. The results showed that imidacloprid followed biphasic dissipation, i.e. at a faster rate, during initial days followed by a slow and steady pace. This has also been observed in the present study, i.e. imidacloprid decreased at a faster rate initially but after 15 days a gradual declining trend was observed. The percent dissipation was also initially higher till 15 days when more than half of total residues were dissipated, but later on, the decrease was slow; however, after 60 days of application, again an increasing trend of dissipation was observed, and the residues were BDL after 90 days of application. The results are in conformity with an earlier report that after 2 months of crop period of sugar beet, the rates of imidacloprid biodegradation in soil increased significantly (Rouchaud et al. 1994). Moreover, these results accord the fact that plants support bioremediation by the uptake of dissolved pesticide by roots and translocation throughout body of plants (Sun et al. 2004) because it was observed that the initial total residues in sugarcane were higher than those observed in soil till 45 days of treatment (Sharma and Singh 2013) which could be ascribed to the uptake of imidacloprid from soil by the sugarcane plant. Earlier studies have also demonstrated that the levels of pesticides or their metabolites found in sugarcane, mango leaves and guinea grass were higher than those found in

Table 2 Residues of imidacloprid and its metabolites (mg kg^{-1}) in soil after its application at 80 g a.i. ha^{-1}

Days after treatment	Imidacloprid	Metabolites						Total residues	Dissipation (%)
		6-Chloronicotinic acid	Imidacloprid-NTG	Nitrosimine	Olefin	5-Hydroxy	Urea		
7	3.54±0.22	1.88±0.10	0.12±0.07	2.28±0.28	BDL	BDL	BDL	7.81±0.39	–
15	0.42±0.03	0.43±0.02	0.05±0.01	1.56±0.03	BDL	BDL	BDL	2.45±0.07	68.63
30	0.06±0.01	1.28±0.07	0.04±0.01	0.22±0.03	BDL	BDL	BDL	1.60±0.06	34.69
45	0.05±0.01	0.70±0.09	0.02±0.01	0.71±0.04	BDL	BDL	BDL	1.49±0.12	6.87
60	0.01±0.00	0.78±0.05	BDL	0.11±0.02	BDL	BDL	BDL	0.90±0.03	39.59
90	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	–

Values are expressed as mean±standard deviation of three replications

BDL below determination limit of 0.01 mg kg^{-1}

soil because in some species of plants, biota uptake exceeds the adsorption capacity of soil particles, thus allowing plants to accumulate more pesticides than those found in soil in which they are grown (Zacharia et al. 2010). It has also been shown that the degradation of imidacloprid was continuous though not rapid. Further, earlier metabolism studies have shown that imidacloprid is thoroughly metabolized, finally leading to the formation of carbon dioxide (Krohn and Hellpointner 2002). The absence of any major metabolite accounting for more than 10 % of the applied radioactivity indicated that the first reaction step determined its overall rate of degradation. Subsequent degradation of the metabolites occurred more rapidly than imidacloprid; therefore, significant residue levels of metabolites did not accumulate in soil (Krohn and Hellpointner 2002). However, higher dissipation of imidacloprid was reported in soil under brinjal by Mandal et al. (2010) who found that soil samples collected after 15 days did not reveal the presence of imidacloprid. Similarly, Romeh et al. (2009) studied dissipation of imidacloprid in tomato crop and soil and reported that average initial deposits of imidacloprid in the soil were observed to be 1.39 mg kg^{-1} dissipating to 0.640 mg kg^{-1} 14 days after spraying. Donnarumma et al. (2011) studied the persistence of imidacloprid in soil under maize and found that the residues of imidacloprid were as high as 0.65 mg kg^{-1} 30 days after sowing, and these declined to nearly half in the next 15 days and decreased to 0.05 and 0.01 mg kg^{-1} 80 and 130 days after sowing.

Studies on residue dynamics of imidacloprid in rice and field environment conducted by Wu et al. (2004) showed that the half-life of imidacloprid in soil was 12.1–24.1 days. Similar findings were reported by Dikshit et al. (2005) on cotton and rice crops that residues of imidacloprid were non-detectable in soil samples at harvest. In another study after application of imidacloprid as seed treatment (5 and 10 g a.i. kg^{-1} seed) and foliar spray (20 and 40 g a.i. ha^{-1}) at 50 % pod formation stage on mustard, no residues were detected in soil (Dikshit et al. 2005; Gopal et al. 2002). The present studies are also in agreement with that of Scholz and Spiteller (1992) who found that imidacloprid degradation was more rapid in soils with cover crops than in bare soils. The decrease in amount of residues in the current investigations might be due to the uptake by the sugarcane plant and a concomitant metabolism in soil.

In another bioefficacy study conducted by the authors, it was revealed that the effectiveness of imidacloprid to control the early shoot borer of sugarcane (*Chilo infuscatellus* Snellen) remained till 45 days after treatment (Sharma and Singh 2012). This control provided by imidacloprid could be attributed to the residues of the parent compound and toxic nitrosimine metabolite formed in soil till 60 days after application. Thus, it could be concluded that during the peak activity period, imidacloprid residues in soil along with the translocated residues within the plant gave effective control of the pest. It has been earlier reported that an outstanding insecticidal activity and long-term crop protection shown by imidacloprid may be attributable to the combined action of the parent compound and olefin and nitrosimine derivatives, which have greater potency than imidacloprid (Nauen et al. 1998).

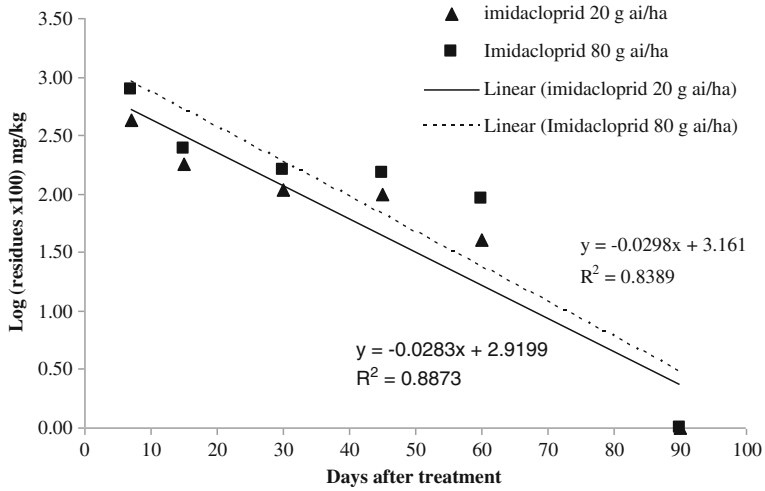

Fig. 2 Dissipation curves of total imidacloprid residues on sugarcane soil

Degradation dynamics of total imidacloprid residues in soil

The degradation kinetics of imidacloprid and its metabolites in sugarcane soil were determined by plotting the residue concentration against time, and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves. Confirmation of the order of kinetics was further made graphically from the linearity of the plots of log$C$ against time (Fig. 2). It was observed that total imidacloprid residues did not follow the first-order kinetics. However, these residues followed the pseudo-first-order kinetics with a coefficient of determination (R^2) value of 0.8873 and 0.8389 for the recommended dose and four times the recommended dose, respectively, and the corresponding value of the standard error was 0.35 and 0.45, respectively. The regression equation for the first dose of imidacloprid was $y=-0.0283x+2.9199$, and for imidacloprid at 80 g a.i. ha^{-1}, the equation was $y=-0.0298x+3.161$ with half-life values of approximately 10 days.

The persistence of an insecticide is generally expressed in terms of half-life ($T_{1/2}$) or DT_{50}, i.e. the time of disappearance of the pesticide to 50 % of its initial concentration. The $T_{1/2}$ value is usually defined as the time required for half of the given quantity of a material to dissipate (Gunthur and Blinn 1955). The $T_{1/2}$ of imidacloprid calculated as per Hoskins (1961) was observed to be 10.64 and 10.10 days for imidacloprid applied at 20 and 80 g a.i. ha^{-1}, respectively. The present results are in accordance with those of Scholz and Spiteller (1992) who reported that imidacloprid degradation was more rapid in soils with cover crops than in bare soils, with a $T_{1/2}$ of 48 and 190 days, respectively. However, variation still exists in the literature on reported dissipation times and half-lives and hence the potential for accumulation of imidacloprid in soil.

Conclusions

The total imidacloprid residues in soil resulting from its application at 20 and 80 g ha^{-1} on 45-day-old sugarcane plants constituted of the parent compound and its three metabolites, i.e. 6-chloronicotinic acid, imidacloprid-NTG and nitrosimine. Other metabolites, viz. imidacloprid olefin, urea and 5-hydroxy, were not found in a detectable range at any stage of sampling up to 90 days after treatment. The residues of imidacloprid remained at low levels in soil till 60 days after application, but total residues dissipated to below detectable limits in soil in 90 days. These residues along with metabolites declined to below the detectable limit in the samples of soil collected 90 days after the application of imidacloprid made at the recommended dose and four times the recommended dose. These residues followed pseudo-first-order kinetics with a coefficient of determination (R^2) value of 0.8873 and 0.8389 for the recommended dose and four times the recommended dose, respectively. The half-life of imidacloprid was calculated to be 10.64 and 10.10 days for imidacloprid applied at 20 and 80 g a.i. ha^{-1}, respectively. This decline in the level of residues in soil during the current investigations might be due to the uptake by the sugarcane plant and a concomitant metabolism in soil.

Acknowledgments The authors are thankful to the Professor and Head of the Department of Entomology, PAU, Ludhiana, for providing the necessary research facilities.

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