An Overview of Persistence and Metabolism of Imidacloprid and Advances in its Estimation Techniques

Sahoo SK and Singh B

ABSTRACT

Imidacloprid is a widely used pesticide in agriculture for controlling sucking insect pests, as a seed dressing, for soil treatment and as a foliar treatment in a variety of crops and orchards. Imidacloprid undergoes extensive metabolism in plants and forms monohydroxy imidacloprid, imidacloprid guanidine, imidacloprid olefin and a monoglucoside of 6-chloropicolyl alcohol. The majority of toxicity studies have been focused on the parent compound, imidacloprid. The metabolites of imidacloprid viz. olefin and nitrosimine have greater insecticidal activity than the parent compound. The guanidine metabolite does not possess insecticidal properties, but has a higher mammalian toxicity than the parent compound. Despite the beneficial impacts of this pesticide in improving and stabilizing agricultural productivity by controlling obnoxious weeds, fungi and insects, these allochthonous organic chemicals are known to contaminate soil ecosystem and pose threat to the balanced equilibrium among various groups of microorganisms in soil, which play an important role in recycling plant nutrients. Although much information is available on bioefficacy of imidacloprid against various insect pests of different crops, there is not much information available on the residues, metabolism and persistence of imidacloprid in cotton and soil in a consolidated manner and hence the authors aim to present the data on persistence and metabolism of imidacloprid and advances in its estimation technique already published separately.

Introduction

Imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine) is the first commercially available representative of the neonicotinoid insecticides that was first registered for use and is presently one of the most available commercial pesticide (Fig.1). The history of the neonicotinoids could be traced back to the late 1970s, when chemists at Shell Chemical Company investigated the potential insecticidal properties of heterocyclic nitromethylenes (Schroeder and Flattum, 1984). The term “neonicotinoid” is denoted to distinguish these chemicals from the nicotinoids, with the neonicotinoids being more highly effective as insecticides and less toxic to vertebrate species. Representatives from these chemicals are also referred to as “chloronicotinyls” to emphasize the importance of the chlorine atom for insecticidal potency (Tomizawa and Yamamoto, 1993). The first registration of imidacloprid was achieved in France (1991) in sugar beet (Sur and Stork, 2003; Nauen et al., 1998). Since the discovery of imidacloprid, several other chemicals analogs with the 6-chloro-3-pyridylmethyl moiety have been developed for commercial usage (Kagabu et al., 1992).
Although it is now off patent, the primary manufacturer of this chemical is Bayer CropScience, (part of Bayer AG). It is sold under the trade names Kohinor, Admire, Advantage, Advocate, Gaucho, Mallet, Merit, Nuprid, Prothor, Turfthor, Confidor, Conguard, Hachikusan, Premise, Prothor, Provado, Intercept and Winner. Imidacloprid is a systemic pesticide with physical or chemical properties that allow residues to move into treated plants and then throughout the plant via xylem transport and trans laminar (between leaf surfaces) movement (Buchholz and Nauen, 2002).

**Properties of imidacloprid**

Imidacloprid is a colourless crystals with a slight but characteristics odour (Tomlin, 2006) with a molecular weight of 255.7 g mol⁻¹ and solubility of 0.61 g L⁻¹ at 20°C (Ware and Whitacre, 2004). The low vapour pressure of $1.0 \times 10^{-7}$ mmHg indicates that this insecticide is non-volatile. In addition, the low Henry’s law constant of $6.5 \times 10^{-11}$ atm-m³ per mole, indicates that it has low volatility from water. Therefore, it is unlikely to be dispersed in air over a large area from volatilization (Fossen, 2006).

![Fig. 1 Structure of imidacloprid (Tomlin, 2006)](image)

Degradation pathway for imidacloprid in soil conditions (Fig. 2) and plant (Fig. 3) was proposed by Miles (1993). The major photo-metabolites include imidacloprid desnitro, imidacloprid olefine, imidacloprid urea, and five minor metabolites. The end product of photodegradation is chloronicotinic acid (CNA).

Since imidacloprid has a low vapor pressure ($1.0 \times 10^{-7}$ mmHg); it normally does not volatilize readily. Imidacloprid undergoes extensive metabolism in plants and forms monohydroxy imidacloprid, imidacloprid guanidine, imidacloprid olefin and a monogluicoside of 6-chloropicolyl alcohol (Miles, 1993). The majority of toxicity studies have been focused on the parent compound, imidacloprid. The metabolites of imidacloprid viz. olefin and nitrosimine have greater insecticidal activity than the parent compound (Nauen et al., 1998). The guanidine metabolite does not possess insecticidal properties, but has a higher mammalian toxicity than the parent compound (Tomizawa and Casida, 1999).

![Fig. 2 Fate of imidacloprid in soil and its main metabolites (Scholz and Spiteller, 1992 and Krohn and Hellpointer, 2002)](image)

**Novel mode of action of imidacloprid**

The mode of action of the nicotinoid was described by Schroeder and Flattum (1984) as it acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system. It is a systemic insecticide that translocates rapidly through plant tissues following
groups including birds. The blood-brain barrier in vertebrates blocks access of imidacloprid to the central nervous system, reducing its toxicity (Matsuda et al., 1998; Tomizawa and Casida, 2005).

**Analytical methodology for determination of imidacloprid residues**

Summarized results of advances in analytical methods for estimation of imidacloprid and its metabolites adopted by various workers are presented in Table 1. Different substrates have been extracted and cleaned up by employing different techniques depending upon the availability of requisite facilities prevailing to carryout the study for estimation of imidacloprid residues. It was possible to achieve limit of quantification (LOQ) of 0.05 mg kg$^{-1}$ for most of substrates like honey bees, pollens, grapes leaves, grape berries, cardamom, tomato and mango whereas LOQ of 0.01 mg kg$^{-1}$ was achieved with substrates like brinjal, cabbage, mustard, rice straw, potato, onion and soil. Most of the methodology use acetonitrile as extraction solvent followed by liquid-liquid partitioning and analysed by reversed phase High Performance Liquid Chromatography (HPLC). The λ – max for most of the analytical procedure was 270.

**Residues and metabolism of imidacloprid in various crops**

In a study conducted by Westwood et al. (1998) imidacloprid was applied to pelleted seeds of sugar beet which were grown in pots of field soil. Leaves, roots and soil were analysed for the distribution of parent compound and its metabolites at intervals up to 97 days after planting. The first sampling of leaves of sugar beet conducted after 21 days of application revealed the presence of parent compound only and its concentration averaged 15.2 µg g$^{-1}$ fresh weights. The concentration of parent compound in the leaves had fallen to an average of 0.5 µg g$^{-1}$ at 97 days after sowing; the metabolites and parent compound in the leaves then represented respectively 44.5 per cent and 4.5 per cent of the total applied radioactivity.
Table 1 Summarized results of advances in analytical methods for estimation of imidacloprid and its metabolites adopted by various workers

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Matrix/substrates</th>
<th>Extraction</th>
<th>Cleanup</th>
<th>Analysis</th>
<th>LOD (µg g⁻¹)</th>
<th>LOQ (µg g⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>Acetonitrile</td>
<td>Passed through anhydrous sodium sulphate</td>
<td>Reversed phase HPLC with UV variable detector at 270 nm</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Sarkar et al. (1999)</td>
</tr>
<tr>
<td>2.</td>
<td>Eggplant, cabbage and mustard</td>
<td>Extracted with acetone and partitioned into dichloromethane</td>
<td>Column clean up packed with sodium sulphate and neutral alumina and eluted with hexane and acetone</td>
<td>Reversed phase HPLC with UV detector at 270 nm</td>
<td>0.003</td>
<td>0.01</td>
<td>Mukherjee and Gopal (2000)</td>
</tr>
<tr>
<td>3.</td>
<td>Soil</td>
<td>Acidic mixture of acetone: water (2:8) by dipping and shaking</td>
<td>No clean up</td>
<td>Reversed phase HPLC with UV-Visible detector at 270 nm</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Gupta et al. (2002)</td>
</tr>
<tr>
<td>4.</td>
<td>Honeybees, Pollen</td>
<td>Dichloromethane</td>
<td>Chromatography column containing florisil and eluted with ethyl acetate-n-hexane, 80:20 (v/v) and then acetonitrile.</td>
<td>Reversed phase HPLC with UV detector at 270 nm</td>
<td>Not reported</td>
<td>0.05</td>
<td>Rossi et al. (2005)</td>
</tr>
<tr>
<td>5.</td>
<td>Potato and onion</td>
<td>dichloromethane</td>
<td>High Performance Liquid Chromatography (HPLC) with Diode-array detector at 270 nm</td>
<td>0.0075 for potato and 0.006 for onion</td>
<td>0.015 for potato and 0.012 for onion</td>
<td>Mandic et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Rice straw</td>
<td>Acetonitrile: water (80:20 v/v) followed by liquid-liquid partitioning</td>
<td>Column chromatography</td>
<td>Reversed-phase HPLC with Diode-array detector at 270 nm</td>
<td>Not reported</td>
<td>0.015</td>
<td>Prasad et al. (2007)</td>
</tr>
<tr>
<td>7.</td>
<td>Grape leaves, Grape berries and soil</td>
<td>Acetonitrile followed by liquid-liquid partitioning</td>
<td>Treated with activated charcoal</td>
<td>HPLC with Diode-array detector at 268 nm</td>
<td>0.017</td>
<td>0.05</td>
<td>Arora et al. (2009)</td>
</tr>
<tr>
<td>8.</td>
<td>Cardamum</td>
<td>Acetonitrile followed by liquid liquid partitioning</td>
<td>Column chromatography</td>
<td>HPLC at 270 nm</td>
<td>Not reported</td>
<td>0.05</td>
<td>Kumar et al. (2009)</td>
</tr>
<tr>
<td>9.</td>
<td>Tomato</td>
<td>Acetone followed</td>
<td>Column</td>
<td>Reversed-</td>
<td>Not reported</td>
<td>0.05</td>
<td>Dharumaraj</td>
</tr>
</tbody>
</table>
by liquid liquid partitioning chromatography phase HPLC equipped with UV-VIS detector at 270 nm reported

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Matrix/substrates</th>
<th>Dose</th>
<th>Half life (days)</th>
<th>Days taken to reach below the determination level</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.</td>
<td>Cotton seed cake</td>
<td>Acetone redissolved in acetonitrile</td>
<td>-</td>
<td>5.0</td>
<td>Mohan et al. (2010)</td>
</tr>
<tr>
<td>11.</td>
<td>Water and soil</td>
<td>Water samples were filtered through 0.2 µm syringe filters. For soil samples, sownicated with acetonitrile.</td>
<td>LC-ESI-MS/MS with MRM scan mode</td>
<td>0.0003 for water and 0.0005 for soil</td>
<td>Thuyet et al. (2010)</td>
</tr>
<tr>
<td>12.</td>
<td>Brinjal and soil</td>
<td>Acetonitrile followed by liquid-liquid partitioning</td>
<td>HPLC with Diode-array detector at 268 nm</td>
<td>0.003</td>
<td>Mandal et al. (2010)</td>
</tr>
<tr>
<td>13.</td>
<td>Mango</td>
<td>Extracted with acetonitrile and partitioned with mixture of hexane + ethyl acetate</td>
<td>Column packed with florisil and eluted with acetonitrile</td>
<td>Not reported</td>
<td>Mohapatra et al. (2012)</td>
</tr>
<tr>
<td>14.</td>
<td>Wheat</td>
<td>Acetonitrile: methanol (1:1), dichloromethane</td>
<td>HPLC equipped with UV-VIS detector</td>
<td>0.01</td>
<td>Iqbal et al. (2012)</td>
</tr>
</tbody>
</table>

Table 2: Summarized results of persistence of imidacloprid and its metabolites in different matrixes
In the root at 97 days, parent imidacloprid and its metabolites together accounted for 0.1 per cent of applied activity, while in the soil there was 23 per cent of parent compound and 4 per cent as metabolites. The persistence and metabolism of imidacloprid in sugarcane leaves and juice were studied following application of imidacloprid @ 20 and 80 g a.i. ha$^{-1}$. The residues were mainly constituted by the parent compound, imidacloprid followed by 6-chloronicotinic acid metabolite in sugarcane leaves. The residues declined to below the detectable limit in the leaves after 90 days of application with the half life values of 9.68 and 8.14 days of imidacloprid @ 20 and 80 g a.i. ha$^{-1}$ respectively. The residues of imidacloprid and its metabolites were not detected in samples of sugarcane juice (Sharma and Singh, 2014). Summarized results of residues of imidacloprid and its metabolites in various crops were given in Table 2.

**Fate of imidacloprid in soil**

The fate of imidacloprid in the soil is highly sensitive to soil composition and sources of organic carbon. The sorption level of imidacloprid is also affected by soil properties such as organic carbon and minerals. Organic fertilizers, such as chicken and cow manure increased the pesticide adsorption to the organic matter and increased its half-life. Half-lives ranged from 40 days when no organic fertilizers were used to 124 days when cow manure was used (Rouchaud et al., 1996). As the organic carbon levels and laminar silicate clay content in the soil increase, the potential for imidacloprid to leach decreases (Cox et al., 1997). In contrast, imidacloprid adsorption in a calcareous soil was found to decrease with addition of organic carbon obtained from past and tannic acid (Flores-Cespedes et al., 2002). Field and laboratory studies have determined that imidacloprid adsorption to soil particles increases as the concentration of insecticide decreases (Oi 1999; Kamble and Saran, 2005). The persistence of imidacloprid from two formulations (Confidor 200 SL and Gaucho 700 WS), and its metabolism in three different soils of West Bengal, India was studied following application of 0.5 kg a.i. ha$^{-1}$ and 1.0 kg a.i. ha$^{-1}$.

Dissipation of imidacloprid in soil followed first-order kinetics and DT$_{50}$ values ranged from 28.7 to 47.8 days. The shortest half-lives (28.7 and 35.8 days) were observed in the lateritic soil for both liquid and powder formulations. The formation of two metabolites of imidacloprid, imidacloprid-urea and imidacloprid-olefin, was first detected on 30th day of degradation at 28°C in all three soils (Sarkar et al., 2001). The study was carried out as a pot culture experiment under laboratory conditions using Gaucho formulation containing $^{14}$C-labelled imidacloprid. Results also revealed that only 1.8-6.8 per cent of the applied $^{14}$C was taken up by the plants and fluctuated within the test period. $^{14}$C levels were higher in plants grown in autoclaved soil than those in unsterilized soils and the radioactivity tended to accumulate in the cotton leaves. Most of the radioactivity in the soil extracts was identified as unchanged $^{14}$C-imidacloprid (El Hamady et al. 2008).

Imidacloprid sorption of the treated soils was studied by Ping et al. (2010) at three pH levels (4.5, 6.0 and 7.5) and two temperatures (15 and 25°C). When soil solution pH was 6, the amount of adsorbed imidacloprid was enhanced with increasing exogenous HA (humic acid) and decreased with increasing quantity of exogenous FA (fulvic acid). Adsorption of imidacloprid in the FA treatment at 5.0 and 10.0 g kg$^{-1}$ was lower than the controls (untreated soil or treatment with HAs at 0 g kg$^{-1}$) when the soil solution pH was 6.0. However, adsorption of imidacloprid in the HA treatment was higher than the controls. Imidacloprid adsorption was usually higher under lower pH and/or lower temperature at same conditions. Thus, exogenous HA could be used to control the mobility of soil pesticide under appropriate conditions to decrease pesticide pollution diffusion and probably increase effectiveness of pesticides. Samnani et al., (2013) reported that the dissipation of imidacloprid was found to be faster in sandy loam soil than that of clay soil following application @ 1.0, 2.0 and 4.0 μg g$^{-1}$ fortification levels with reference standard of imidacloprid under laboratory conditions.

The persistence and metabolism studies of imidacloprid in sugarcane field soil by Sharma and Singh (2013) following
20 and 80 g a.i. ha\(^{-1}\) imidacloprid showed the total imidacloprid residues were mainly constituted by the parent compound, imidacloprid followed by metabolites like 6-chloronicotinic acid, nitrosimine and nitroguanidine. These residues were declined to below the detectable limit in soil after 90 days of application in both the doses. 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.

References


Chromatographia 61:189-195


Sahoo and Singh (2014)