Bull Environ Contam Toxicol (2009) 82:239–242
DOI 10.1007/s00128-008-9554-y

Persistence of Imidacloprid on Grape Leaves, Grape Berries and Soil

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Received: 18 March 2008 / Accepted: 27 August 2008 / Published online: 12 September 2008

Abstract Residues of imidacloprid were estimated in grape leaves, grape berries and soil following four applications of Confidor 200SL at 400 and 800 mL ha^{-1} using 1,000 L water. The average initial deposits of imidacloprid on grape leaves were found to be 10.01 and 16.97 mg kg^{-1} at single and double dosages, respectively. These residues of imidacloprid dissipated to be the extract of 98.8% and 97.0%, respectively, at single and double dosages in 15 days, with half-life period of 2.35 and 2.97 days. Residues of imidacloprid in grape berries at harvest time were observed to below determination limit of 0.05 mg kg^{-1} at single dose and 0.06 mg kg^{-1} at double dose. However, acceptable daily intake (ADI) of imidacloprid is 0.06 mg kg^{-1} body weight day^{-1}, which means an adult of 60 kg and a child of 10 kg can safely tolerate intake of 3,600 and 600 μg imidacloprid, respectively, without any appreciable risk to their life. Assuming consumption of 200 g grape berries contaminated at 0.06 mg kg^{-1}, it will lead to an intake of only 12 μg of imidacloprid, which is quite safe for a child as well as for an adult. Hence, the use of imidacloprid on grape crop seems to be toxicologically acceptable.

Keywords Imidacloprid · Grape leaves · Grape berries · Soil · Residues · MRL

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Imidacloprid 1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine, is a systemic chloronicotinyl insecticide with soil, seed and foliar uses for the control of sucking pests. It is widely used in vineyards for the control of leafhoppers, mealy bugs and sharpshooter (Pfeiffer et al. 2007). The chemical has colourless crystal with weak characteristic odour, mp 144°C, vp 4×10^{-7} mPa (20°C) and KOW log $P = 0.57$ (21°C) and stable to hydrolysis at pH 5–11. It is commonly used on rice, cereals, maize, potatoes, vegetable, sugar beat, fruit, cotton, hops and turfs and is especially systemic when used as seed or soil treatment. The chemical works by interfering with the transmission of stimuli in the insect nervous system. Specially, it causes a blockage in a type of neuronal path ways (nicotinergic) that is more abundant in insects than warm blooded animals. This blockage leads to accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventually death. It is effective on contact and via stomach action the half-life of imidacloprid in soil is 48–190 days depending upon the amount of ground cover, manure in soil and of course the sunlight. Imidacloprid is the greatest step vise to the primary metabolites 6-chloro nicotinic acid, which eventually breaks down to carbon dioxide. The chemical is moderately stable and has moderate binding affinity to organic materials in soil. A number of coworkers have reported residues of imidacloprid in different fruit substrate (Anonymous 2005a). As considerable concern is being expressed by various agencies over the magnitude of pest control chemicals left in foodstuffs following their use while raising the crops, therefore, it is important to ensure that the levels of harvest time residues of imidacloprid in grape berries do not pose any hazard to consumers and are admissible in domestic as well as international trade.

Materials and Methods

Field experiments were conducted during 2005–2006 on vines of grape cultivar Perlette at P.A.U. Regional Research Station, Abohar, Punjab, India, and the crop was raised according to recommended agronomic practices (Anonymous 2005b).

Three replications were selected for each treatment i.e. control, recommended dose and double the recommended dose. A total of four applications of imidacloprid (Confidor 200SL) at 400 and 800 g ha^{-1} 1,000 L^{-1} were made as foliar spray. First application was given 15 days after pruning on 24.02.2006 and 2nd application after a fortnight of the 1st application, 3rd application was made at full bloom stage on 23.03.2006 and the 4th application at berry setting stage on 04.04.2006. Control vines were treated with water only.

About 200 g of grape leaves were collected randomly from control and treated vines at 0 (2 h), 1, 3, 5, 7, 10 and 15 days of the last application of the insecticide. Grapes and soil samples (1 kg) were collected at harvest time. The interval between last application and harvest was found to be 86 days.

Various researchers used different methods for extraction and determination of imidacloprid residues depending upon the infrastructure of instrumentation and facilities available (Obana et al. 2002, 2003; Gonzalez 2004; Blasco et al. 2002, 2005; Zywitz et al. 2003). The analytical methodology used for grape leaves and grape barriers is detailed below.

A representative 50 g sample of grape leaves/grape berries was blended with 50 mL acetonitrile in a blender and filtered through Whatman filter paper No.1. The acetonitrile extract was evaporated to near dryness (5 mL) using rotary vacuum evaporator and diluted with 50 mL of saturated sodium chloride solution and partitioned thrice into hexane (3 × 50 mL). Discarded the hexane layers and again partitioned the lower aqueous phase with hexane: ethyl acetate (98:2, v/v). Again discarded the organic layers. Lower aqueous phase was again partitioned thrice into dichloromethane using 50 mL each time. The pooled dichloromethane extracts were passed through anhydrous sodium sulfate, treated with 500 mg activated charcoal powder for 2 h. Filtered the clear extract through Whatman filter paper No. 1 along with rinsing of dichloromethane and evaporated to near dryness using rotary vacuum evaporator. The residues were dissolved in 5 mL of acetonitrile (HPLC grade) and estimated by HPLC.

A representative 50 g soil was mixed with 50 mL mixture of acetonitrile:water (7:3, v/v) in 250 mL conical flask and shaken for 3 h on an electrical shaker at 150 rpm. Soil suspension was filtered through Whatman filter paper No.1, washed twice with 50 mL acetonitrile and water mixture (7:3 v/v) and finally with 30 mL acetonitrile only. Filtrate was concentrated to about 50 mL on rotary vacuum evaporator and partitioned thrice into dichloromethane using 50 mL each time. Dichloromethane fractions were collected and dried over anhydrous sodium sulfate. The extract was finally concentrated to near dryness under rotary vacuum evaporator and the residues were dissolved in 5 mL acetonitrile (HPLC grade) and estimated imidacloprid residues by HPLC.

Table 1 Per cent recovery of imidacloprid from spiked samples of grape leaves, grape berries and soil

Substrate	Level of fortification (mg kg^{-1})	Recovery, %[a] (Mean ± SD)
Grape leaves	0.1	89.67 ± 5.87
	0.2	90.07 ± 1.62
Grape berries	0.1	91.8 ± 0.04
	0.2	97.9 ± 0.95
Soil	0.1	87.0 ± 1.32
	0.2	90.33 ± 1.52

[a] Each value in the mean ± standard deviation of the three replicate determinations

The residues of imidacloprid were estimated using Shimadzu HPLC equipped with UV detector, model SP-10A, dual pump, LC-10AT, fraction collector FRC-10A and communication bus module model CBM-10A and C_{18} column. The mobile phase used was acetonitrile at 1 mL min^{-1} and retention time of imidacloprid under these conditions was observed to be 3.10 min. The residues of imidacloprid were estimated in different substrates by comparison of peak height of the sample with that of standard imidacloprid run under identical conditions. The syringe used injected 20 µL volume of the sample manually. A concentration of 5 ng of imidacloprid give fairly good response and limit of determination of imidacloprid worked out to be 0.05 mg kg^{-1}.

Grape leaves, berries and soil samples were spiked with imidacloprid at 0.10 and 0.20 mg kg^{-1} and analysed as per the methodology described above. Per cent recovery for imidacloprid for grape leaves, grape berries and soil were found to be consistent and more than 87% (Table 1).

Results and Discussion

Following application of imidacloprid (Confidor 200SL) at 400 and 800 g ha^{-1} 1,000 L^{-1} of water (0.008% and 0.016% a.i.) resulted in average initial deposits of 10.01 and 16.97 mg kg^{-1} of imidacloprid on grape leaves at single and double dosages, respectively. These residue levels of imidacloprid dissipated to the extent of more than 85% during one week. Fifteen days after application, residues of imidacloprid on grape leaves were observed to be 0.12 and 0.51 mg kg^{-1} at recommended and double the recommended dosages, respectively, thereby showing dissipation to the extent of 98.8% and 97.0%, respectively (Table 2). Half-life ($t_{1/2}$) values of imidacloprid on grape leaves at single and double dosages were observed to be 2.35 and 2.97 days, respectively. The interval between last application and the harvest of the crop was found to be 86 days. Average levels of imidacloprid residues on grape berries at harvest time were observed to be below limit of determination (BDL) calculated to be 0.05 and 0.06 mg kg^{-1}, respectively, at single and double dosages. Soil samples collected at harvest were also found to contain residues of imidacloprid at 0.12 and 0.22 mg kg^{-1} at recommended and double the recommended dosages, respectively (Table 2).

These residues levels seems to be quite safe in view of the fact that maximum residue limit (MRL) of imidacloprid on grapes is fixed at 1.00 mg kg^{-1} (Anonymous 2004).

The use of pesticides on food crops lead to unwanted residues, which may constitute barriers to exporters and domestic consumptions when they exceed MRLs. Gonzalez (2004) studied dissipation of abamectin, spinosad, imidacloprid and thiacloprid on horticultural crops following FAO guidelines and local good agricultural practices and reported negligible residues in all instances. Further it was also revealed that non-detection residue level reached within about 30 days after the last spray.

Table 2 Residues of imidacloprid (mg kg^{-1}) on grape leaves, grape berries and soil

Days after treatment	400 mL ha^{-1} 1,000L^{-1} water (0.008% a.i.)			800 mL ha^{-1} 1,000L^{-1} water (0.016% a.i.)		
	Replication	Mean ± SD	Dissipation (%)	Replication	Mean ± SD	Dissipation (%)
Before application	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
Leaves (0 days)	9.44	10.01 ± 0.67	–	18.49	16.97 ± 1.89	–
	9.84			14.85		
	10.75			17.56		
1	5.56	5.29 ± 0.24	47.16	10.59	9.54 ± 0.98	43.79
	5.07			9.41		
	5.23			8.63		
3	4.00	3.97 ± 0.32	60.34	5.95	5.56 ± 0.60	67.24
	4.28			5.87		
	3.64			4.87		
5	2.43	2.45 ± 0.25	75.53	3.66	3.44 ± 0.19	79.73
	2.71			3.39		
	2.20			3.28		
7	1.30	1.45 ± 0.17	85.52	2.03	2.23 ± 0.18	86.86
	1.40			2.40		
	1.64			2.26		
10	0.60	0.50 ± 0.09	95.01	1.36	1.38 ± 0.14	91.87
	0.48			1.25		
	0.42			1.53		
15	0.12	0.12 ± 0.02	98.81	0.65	0.51 ± 0.12	97.00
	0.14			0.41		
	0.10			0.48		
Grape berries (86 days)	BDL	BDL	–	0.05	0.06 ± 0.01	–
	BDL			0.07		
	BDL			0.06		
Soil (86 days)	0.10	0.12 ± 0.01	–	0.20	0.22 ± 0.05	–
	0.13			0.28		
	0.13			0.18		
$t_{1/2}$	2.35 days			2.97 days		

BDL-Below Determination Limit of 0.05 mg kg^{-1}

Neonicotinoid residues were detected in 33 samples out of 382 analysed in German and 15 of these samples exceeded the German maximum residues limit (MRLs). The residues were most frequently found in sweet pepper (Zywitz et al. 2003).

The applicability of the method to detect and quantify imidacloprid and hexythiazox was demonstrated successfully in 159 bunch and nectarine samples obtained from an agricultural cooperative. The calculations of the acceptable daily intake were much lower than the acceptable daily intake proposed by international agencies (Blasco et al. 2002).

The application of the imidacloprid (Confidor 200 SL) at 400 and 800 g ha^{-1} 1,000 L^{-1} water leaves residues of imidacloprid in grape berries below its determination limit of 0.05 mg kg^{-1} which is quite low as compared to its MRL of 1.00 mg kg^{-1}. Moreover, acceptable daily intake of imidacloprid is 0.06 mg kg^{-1} body weight day^{-1} which means that an adult of 60 kg and a child of 10 kg can safely tolerate intake of 3,600 and 600 µg of imidacloprid without any appreciable risk to their life. Assuming a consumption of 200 g grapes contaminated at 0.06 mg kg^{-1} as found in the present study at application rate of 800 g ha^{-1}, it will lead to intake of 12 µg imidacloprid which is quite safe for a child as well as an adult and constitutes only 0.02% and 0.003% of ADI value.

Acknowledgements Authors thanks the Director, PAU Regional Research Station Abohar and the Head, Department of Entomology for providing the necessary research facilities during the tenure of the study.

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