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Imidacloprid in processed tea and tea liquor*

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Abstract: Imidacloprid is a newly introduced broad-spectrum chloronicotinyl insecticide and will find its way in agricultural production, particularly in Asia. However, information on the fate of imidacloprid in crop plants is lacking. The degradation of imidacloprid in processed CTC tea and tea liquor was investigated in the present study in which imidacloprid was applied at recommended application rate (30.0 g a.i./ha) and twice the recommended application rate (60.0 g a.i./ha) for three consecutive seasons. Imidacloprid was rapidly dissipated in processed tea following first order reaction kinetics at all application rates and had half-lives of 0.91~1.16 d with the residue in tea liquor found to be below detectable limit on 3rd day samples. The study revealed that imidacloprid is safe for human consumption and will not pose any residual toxicity problem.

Key words: Imidacloprid, Processed tea, Tea liquor, Residue, Dissipation
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INTRODUCTION

Tea (*Camellia sinensis*) is known as queen of beverages. India is the biggest producer of tea in the world and is being cultivated mainly in northeast and south India where it is the most important cash crop due to its export potentialities. Tea production levels in India were about 1000 kg/ha during the 1950's and increased up to about 1800 kg/ha in the mid 1980's due to the introduction of green revolution technologies such as external chemical inputs (Senapati *et al*., 1994). In northeast India tea is the main commercial crop receiving wide range of pesticides to boost production. Pests, pathogens and weeds are important factors limiting the productivity and quality of tea in almost all the tea growing countries of the world. During the last few decades the use of synthetic chemicals achieved control of pests, diseases and weeds in tea fields. Though broad-spectrum chemicals offer powerful incentives in the form of good control, increased yield and high economic returns, they have serious drawbacks such as resistance to pesticides, pest resurgence, outbreak of secondary pests, harmful effects on human health and environment and presence of undesirable residues.

Imidacloprid, 1-[(6-chloro-3-pyridyl)-methyl]-2-nitroimidazolidin (Fig.1), represents a modern potent insecticide group, chloronicotinyl that interacts like nicotine, epibatidine and nereis toxin analogues with the postsynaptic nicotinic acetylcholine receptor (Bai *et al*., 1991; Tomizawa and Yamamoto, 1993). It is effective for controlling aphids, plant bugs, leafhoppers, plant hoppers, thrips, scales, whiteflies and various other harmful pest species including resistant strains (Ishii *et al*., 1994). Imidacloprid was found most effective against tea mealy bug (Ignacimuthu and Jayaraj, 2003) and also has excellent systemic properties, which makes it suitable for seed, soil and foliar treatment (Wamhoff and Schneider, 1999).

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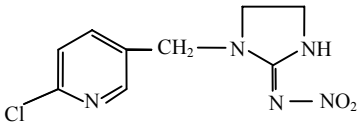

Fig.1 Chemical structure of imidacloprid

Imidacloprid undergoes rapid photochemical degradation with 1-(6-chloro-3-piridinyl)-methyl-2-imidazolidinone being identified as the major photo metabolite (Wamhoff and Schneider, 1999). Roy *et al*.(2005) reported the photodegradation pathway of a commercial formulation of imidacloprid in irrigated water. The photo metabolites obtained were 1-[(6-chloro-3-pyridinyl)-methyl] imidazolidin-2-one, 1-[(6-chloro-3-pyridyl)-methyl]-N-nitro-4-imidazolidene-2-ylideneamine, 6-hydroxy nicotinic acid and 6-chloronicotinic acid. The photolytic process affected the imidazolidine moiety while the 6-chloropyridine moieties remained unchanged. The proposed phototransformation pathway involved hydrolysis, oxidation and dehydration.

In the Indian market, the active ingredient (imidacloprid) is embodied in the trade products Gaucho for seed treatment and Confidor for leaf and soil treatment. Its use as a replacement for other insecticides is increasing. Based on the above information, a three-season field study of imidacloprid was conducted in northeast India for determination of the dissipation pattern and the residue level in processed tea and tea liquor.

MATERIALS AND METHODS

A three seasons (1st season, March 2003; 2nd season, September 2003; 3rd season, March 2004), field experiment on tea (variety TV1) was conducted at Kamalpur Tea Estate, Darjeeling, West Bengal, India. Confidor 200SL was applied thrice on tea bushes at an interval of 7 d as high volume spray (400 L/ha) by a knapsack sprayer. It was applied at 30.0 g a.i./ha (recommended application rate, i.e., T_1) and 60.0 g a.i./ha (twice the recommended application rate, i.e., T_2). Untreated control (T_3) was simultaneously maintained. Each treatment including control was replicated thrice in a randomized block design (RBD). The number of bushes per treatment was 100 and spacing between the bushes was regular double hedge type. Green tea leaves (two leaves and a bud, 1 kg) were plucked randomly from each treatment replication at different time intervals [0 (2 h after last spraying), 1, 2, 3 and 5 d] and the green leafs were into processed CTC tea (100 g) at Kamalpur Tea Estate factory following standard manufacturing methods.

Imidacloprid was quantified using high performance liquid chromatography (JASCO UV 1575 UV-Vis detector equipped with 3392A integrator). For recovery studies, processed tea samples (1:2, w/v) were fortified with acetonitrile solution of imidacloprid to obtain concentrations corresponding to different doses. The samples were immediately extracted three times with 100 ml of acetonitrile on an electric shaker (1 h), each time followed by ultrasonic vibration for 5 min. After centrifugation at 3000 r/min for 10 min, the extracts were combined and imidacloprid was partitioned into $CHCl_3$ (100 ml+50 ml+50 ml). The combined organic extract was put into a 250 ml round bottomed flask and evaporated to dryness with rotary vacuum evaporator with the water bath temperature adjusted to 40 °C. The concentrated extract was then subjected to adsorption chromatography over florisil (60~120 mesh) with 10 cm layer of anhydrous sodium sulphate on the top. The column was eluted with 200 ml of acetonitrile:methanol (95:5, v/v). The organic fraction was evaporated to dryness, rinsed with HPLC grade methanol and filtered (0.2 μm) for direct HPLC analysis. Imidacloprid was separated on an Intersil 150 mm×4.6 mm ODS 2, 5 μm (RP C_{18}) using a mobile phase of methanol and water (60:40, v/v) at a flow rate of 1 ml/min and column temperature at 40 °C. Quantification was performed against imidacloprid standard at a wavelength of 270 nm. Under this condition the retention time of imidacloprid was 3.9 min, the limit of detection was 0.01 mg and the sensitivity of the method was 0.005 mg/kg. The average imidacloprid recovery was 91.2%. Determination of imidacloprid residues in the treated samples was carried out as per the recovery study.

The liquor was prepared by adding 10 g of processed CTC tea in 200 ml of boiling water and was stirred for 5 min with spoon and then filtered. The same procedure was followed for the tea liquor after extraction of the compound by liquid-liquid partition with dichloromethane.

RESULTS AND DISCUSSION

Initial deposits, dissipation percent, half-life values and regression equation of imidacloprid in processed CTC tea and residues remaining in tea liquor are presented in Tables 1~3. The results showed that the residues of imidacloprid in processed tea decreased progressively with time irrespective of application rates and seasons. The initial deposits of imidacloprid on day 0 (2 h after last spraying) were found to be 0.2163×10^{-6}~0.2314×10^{-6} and 0.4190×10^{-6}~0.4520×10^{-6} for the treatments T_1 and T_2 respectively among the seasons. The dissipation rate was found to be rapid and the initial deposit dissipated upto 26.16%~33.10% after the 1st day followed by 78.18%~85.40% on the 3rd day irrespective of

Table 1 Persistence of imidacloprid in processed tea at recommended application rate (T_1)

Season	Period (d)	Mean residue±*SD* ($\times10^{-6}$)	Dissipation (%)	Regression equation [half-life (d)]
1st	0	0.2208±0.0078		*y*=−0.3296*x*+1.8137
	1	0.1525±0.0054	30.95	
	2	0.1085±0.0082	50.84	[0.91]
	3	0.0400±0.0004	81.86	
	5	BDL		
2nd	0	0.2163±0.0003		*y*=−0.2096*x*+1.5780
	1	0.1447±0.0043	33.10	
	2	0.1116±0.0029	48.41	[1.14]
	3	0.0472±0.0037	78.18	
	5	BDL		
3rd	0	0.2314±0.0002		*y*=−0.2709*x*+1.7060
	1	0.1633±0.0016	29.43	
	2	0.1020±0.0073	55.92	[1.11]
	3	0.0338±0.0048	85.40	
	5	BDL		

BDL: Below detectable limit

Table 2 Persistence of imidacloprid in processed tea at twice the recommended application rate (T_2)

Season	Period (d)	Mean residue±*SD* ($\times10^{-6}$)	Dissipation (%)	Regression equation [half-life (d)]
1st	0	0.4520±0.0005		*y*=−0.2633*x*+1.9994
	1	0.3159±0.0027	30.10	
	2	0.2287±0.0019	49.40	[1.14]
	3	0.0776±0.0002	82.82	
	5	0.0440±0.0034	90.26	
	7	BDL		
2nd	0	0.4190±0.0053		*y*=−0.2586*x*+1.9634
	1	0.2853±0.0007	31.91	
	2	0.2316±0.0083	44.73	[1.16]
	3	0.0758±0.0061	81.91	
	5	0.0414±0.0003	90.12	
	7	BDL		
3rd	0	0.4430±0.0069		*y*=−0.2610*x*+1.9932
	1	0.3271±0.0018	26.16	
	2	0.2150±0.0008	51.47	[1.15]
	3	0.0831±0.0001	81.24	
	5	0.0436±0.0040	90.16	
	7	BDL		

BDL: Below detectable limit

Table 3 Residue of imidacloprid in tea liquor at recommended and twice the recommended application rate

Season	Period (d)	Mean residue±*SD* ($\times10^{-6}$)	
		T_1	T_2
1st	0	0.0160±0.0013	0.0230±0.0021
	1	0.0083±0.0006	0.0121±0.0017
	2	BDL	0.0076±0.0011
	3		BDL
2nd	0	0.0139±0.0003	0.0228±0.0053
	1	0.0071±0.0029	0.0137±0.0077
	2	BDL	0.0081±0.0043
	3		BDL
3rd	0	0.0145±0.0020	0.0251±0.009
	1	0.0086±0.0027	0.0128±0.0037
	2	BDL	0.0079±0.0004
	3		BDL

BDL: Below detectable limit

application rates and seasons. The residue level decreased to below detectable limit on the 5th day for T_1 and 7th day for T_2. In the untreated control (T_3), no imidacloprid residues were detected. The half-life values calculated from the best-fit lines of the logarithm of residual concentrations versus incubation period, suggested first order reaction kinetics in the dissipation of imidacloprid residue. The study revealed that the dissipation rate was independent of initial deposit and the that half-life ($t_{½}$) values of imidacloprid in processed tea ranged from 0.91 to 1.16 d according to the seasons and application rate.

Imidacloprid residues in tea liquor from zero (0) day samples were found to be 0.0139×10^{-6}~0.0160×10^{-6} and 0.0228×10^{-6}~0.0251×10^{-6} irrespective of the seasons for the treatments T_1 and T_2 respectively. Those from the 1st day sample were found to be 0.0071×10^{-6}~0.0086×10^{-6} and 0.0121×10^{-6}~0.0137×10^{-6}. The residue decreased to below detectable limit on the 2nd day for T_1 and 3rd day for T_2. In the untreated control (T_3), no imidacloprid residues were detected irrespective of the seasons.

The MRL (maximum residue limit) value of imidacloprid has not yet been established in tea. There is no recommended MRL value of imidacloprid in tea by WHO/FAO. But MRL value of imidacloprid in potato, tomato, apple, mango etc., has been fixed to 0.2×10^{-6}~0.5×10^{-6} by FAO (FAOSTAT, 2005). As no residue was detected in the 3rd day sample it might be stated that imidacloprid may not pose any residual toxicity problem in processed tea, which also fits the plucking schedule of India's Tea Estates of the northeastern region of the country.

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