Bull Environ Contam Toxicol (2012) 89:340–343
DOI 10.1007/s00128-012-0671-2

Degradation Dynamics of the Insecticide: Clothianidin (Dantop 50 % WDG) in a Tea Field Ecosystem

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Received: 30 January 2012 / Accepted: 3 May 2012 / Published online: 16 May 2012

Abstract The fate of clothianidin [(*E*)-1-(2-chloro-1, 3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] applied to tea plant was studied at two location in West Bengal, India. The insecticide was applied in Tea field at two doses @30 and 60 g.a.i./ha during June–July 2009. Solid-phase extraction and liquid–liquid extraction was employed for the determination of this insecticide in tea samples. Clothianidin residues were analyzed and estimated quantitatively by HPLC at λ_{max} 250 nm. The observed half life values of made tea and green tea leaf ranges from 3.71 to 4.07 days and 4.07 to 4.49 days respectively.

Keywords Persistence · Residue · Clothianidin · Tea · HPLC

Clothianidin is a novel neonicotinoid insecticide. Neonicotinoid insecticides exhibit excellent insecticidal activity with a high level of safety for vertebrates. Laboratory studies have demonstrated that that the clothianidin is highly active against not only hemipterous insects but also coleopterous, thysanopterous, dipterous and some lepidopterous pests (Uneme et al. 2006). Neonicotinoid acts agonistically at the postsynaptic nicotinic acetylcholine receptor (nAChR) (Yamamoto et al. 1995) in central nervous system of insect. Because of its broad spectrum of insecticidal activity, good systemic properties and low mammalian toxicity, clothianidin is a compound that is considered to be compatible with integrated pest management strategies (Ohkawara et al. 2002).

However, the contamination of pesticide residues in tea is a potential threat to the health of tea drinkers. In this respect, our objective of the present study to understand the dissipation and the fate of Clothianidin residue in/on tea and soil at different location and to develop an efficient residue analytical method (Table 1).

Materials and Methods

A field study in two locations was conducted at Kamalpur Tea Estate, Darjeeling, West Bengal, India and Hantapara Tea Estate, Hantapara, Birpara, Dooars, Jalpiguri India. The insecticide was applied in two doses @30 g.a.i./ha (recommended dose-T_1) and 60 g.a.i./ha (double dose-T_2). Randomised block design with plot size 100 m^2 with three replications each were maintained.

Green tea leaves of 500 g at different days intervals (0, 1, 3, 5, 7, 10 days) were collected each treatment replicationwise. A composite sample was also collected from the untreated control. Green tea leaves were processed in the tea factory for manufacturing of CTC tea. These CTC tea samples were collected for residue analysis. Simultaneously one portion of green tea leaf samples were kept as such for residue analysis.

Soil sample 1 kg was collected at different days interval (0, 1, 3, 5,7,10 days) from each treatment replicationwise. Five soil cores were randomly taken from each plot from 0 to 15 cm depth using a soil auger. The cores were bulked together from each plot, air dried,

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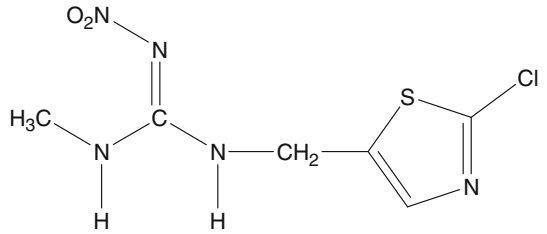
Table 1 Physico-chemical properties of clothianidin

1.	IUPAC name	(*E*)-1-(2-chloro-1, 3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
2.	Empirical formula	$C_6H_8ClN_5O_2S$
3.	Molecular weight	249.7 g/mol
4.	Melting point	176.8°C (99.7 %)
5.	Water solubility	0.327 g/L at 20°C
6.	Vapor pressure	3.8×10^{-11} Pa at 20°C
7.	Chemical structure	

powdered and passed through a 3 mm sieve to achieve uniform mixing. Samples from the controlled plots were collected similarly. In each case 100 g representative sample of soil has been prepared by quartering in the laboratory and taken for final analysis.

Pesticide analytical standard (99.7 %) and clothianidin (50 % WDG Dantop) formulation were procured from M/S Nagarjuna Industries Ltd., Hyderabad, India. Solvents like methanol, ethyl acetate were obtained from Qualigens. Celite, Hexane, Chloroform, Silica, anhydrous sodium sulfate and sodium chloride were purchased from Merck. Solid phase extractor used in this study were Alumina-Neutral (AL-N) SEP Pak cartridge of Accubond brand, SEP-PAK Si cartridge by Orochem. CHEM-ELUT cartridge of Varian was used. Spectrochem's acetonitrile of HPLC grade was selected as mobile phase.

Made Tea sample (20 g) was blended with 100 mL of acetone in a waring blender and it was then filtered on Celite bed with Buchner flask and the extract was then concentrated using rotary vacuum evaporator below 50°C. The mixture thus obtained was concentrated and transferred to CHEM-ELUT column. The column was eluted with 100 mL hexane and discarded. The residue was then eluted with 200 mL ethyl acetate. The ethyl acetate fraction was concentrated in rotary vacuum evaporator at 40°C and subjected to clean up in AL-N Sep-Pak cartridge. Clothianidin was eluted with 40 mL methanol and concentrated in rotary vacuum evaporator at 40°C. The concentrated extract was again subjected to silica Sep-Pak cartridge column clean up. The cartridge was eluted with 20 mL ethyl acetate and concentrated in rotary vacuum evaporator at 40°C and the final volume was made up with acetonitrile for HPLC analysis.

Green tea leaf sample 20 g was taken in conical flask and extracted by acetone using similar procedure as mentioned in made tea.

Soil sample (20 g) was taken in 250 mL conical flask and 100 mL acetone was added to it. It was kept overnight and then shaken in mechanical shaker for about 1 h. It was then filtered on Celite bed with Buchner flask. The filtrate was then concentrated using rotary vacuum evaporator below 50°C. The concentrated solution was diluted with 150 mL of water saturated with NaCl and was partioned thrice with 200 mL (100 + 50 + 50) ethyl acetate. The ethyl acetate fraction was then concentrated and was subjected to column clean up using silica gel (10 g) column. First it was applied on the column and kept for about 1 h. The column was then eluted subsequently with 100 mL hexane and 50 mL $CHCl_3$: ethyl acetate (9:1) and discarded. The residue was finally eluted with 200 mL ethyl acetate and collected. The ethyl acetate fraction was evaporated to dryness in rotary vacuum evaporator below 50°C and finally reconstituted with acetonitrile for HPLC analysis.

Shimadzu High Performance Liquid Chromatograph with SPD-10A Detector connected to CBM-101 module and CR LC 10 software was used for residue analysis. A Thermo Hypersil ODS- C 18, 5 μm, 4.6 × 250 mm was employed as analytical column. Mobile phase was used acetonitrile and water with the ratio of 3:7. The pump flow was set at 0.8 mL min^{-1}. The wavelenghth (λ_{max}) was maintained at 250 nm during the HPLC analysis. Residues of clothianidin were quantified by comparison of peak height/peak area of standards with that of unknown or spiked samples run under identical conditions. The retention time of this compound was 8.60 ± 0.20 min. Standard calibration curve of clothianidin was constructed by plotting concentration against peak area. Good linearity was achieved, limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1 respectively. LOD and LOQ were determined as 0.01 and 0.05 mg/kg respectively.

Table 2 Recovery study of clothianidin in different substrates

Substrate	Amount fortified (mg/kg)	Amount recovered (mg/kg)	Recovery (%)	Average recovery (%)
Made tea	0.05	0.0495	89.00	87.33
	1.00	0.825	85.00	
	5.00	4.40	88.00	
Green tea leaf	0.05	0.0485	87.00	86.00
	1.00	0.850	85.00	
	5.00	4.30	86.00	
Field soil	0.05	0.044	88.00	86.50
	1.00	0.845	84.50	
	5.00	4.35	87.00	

Results and Discussion

Percent recoveries generated during the validation of the method are presented in Table 2. The average recovery for soil, made tea and green tea leaf was in the ranges 86 %–87.33 % over a fortification range from 0.05 to 5 mg/kg. as the recovery results are found satisfactory so, this method was followed for the residue analysis of clothianidin in soil, made tea and green tea leaf.

The results of the field study of persistence of clothianidin in green tea leaf,made tea and soil samples of two different locations,viz., Hantapara and Kamalpur tea estate has been summarized in Tables 3 and 4. The residue gradually decreased with time following first order kinetics in all cases. In Hantapara green tea leaf deposits of clothianidin immediately after application (2 h) were found to be 0.42 and 1.02 mg/kg for single and double dose, respectively. The half life of the molecule was calculated to be 4.07 and 4.63 days for the single dose and double dose respectively. Residues in green tea leaf at Kamalpur location were found to be 0.51 and 1.25 mg/kg for single (T_1) and double (T_2) dose, respectively. The calculated half life value was nearly same to another location 4.36 days and 4.49 for T_1 and T_2. Interestingly the initial concentration of clothianidin in made tea was found much higher than green tea leaf sample irrespective of dose and location. The initial deposit (after 2 h spraying) of clothianidin in made tea at found higher at Kalampur compare ti Hantapara location. For Kamalpur it was 2.69 mg/kg (T_1), 4.54 (T_2) mg/kg and for Hantapara it was 1.97 mg/kg (T_1), 2.83 (T_2) mg/kg. The observed

Table 3 Dissipation of clothianidin in different substrate of tea sample in Hantapara Tea Estate

Substrate	Days	Residue in mg/kg [M* ± SD]	
		T_1 (30 ga.i.ha^{-1})	T_2 (60 ga.i.ha^{-1})
Made tea	0	1.97 ± 0.37	2.83 ± 0.56
	1	1.25 ± 0.04	2.71 ± 0.09
	3	1.04 ± 0.18	2.49 ± 0.36
	5	0.70 ± 0.01	2.43 ± 0.23
	7	BDL	BDL
	10	BDL	BDL
	Regression equation and half life	$y = 3.246 - 0.081x$ $T_{1/2} = 3.72$ days	$y = 3.544 - 0.079x$ $T_{1/2} = 3.81$ days
Green tea	0	0.42 ± 0.14	1.02 ± 0.31
	1	0.28 ± 0.10	0.71 ± 0.11
	3	0.21 ± 0.09	0.54 ± 0.12
	5	0.17 ± 0.06	0.46 ± 0.05
	7	BDL	BDL
	10	BDL	BDL
	Regression equation and half life	$y = 2.571 - 0.074x$ $T_{1/2} = 4.07$ days	$y = 2.960 - 0.065x$ $T_{1/2} = 4.63$ days
Field soil	0	0.25 ± 0.06	0.45 ± 0.09
	1	BDL	0.23 ± 0.08
	3	BDL	BDL
	5	BDL	BDL
	7	BDL	BDL
	10	BDL	BDL

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

Table 4 Dissipation of clothianidin in different substrate of tea sample in Kamalpur Tea Estate

Substrate	DAYS	Residue in mg/kg [M* ± SD]	
		T_1 (30 ga.i.ha^{-1})	T_2(60 ga.i.ha^{-1})
Made tea	0	2.69 ± 0.65	4.54 ± 0.45
	1	1.85 ± 0.25	3.19 ± 0.44
	3	1.52 ± 0.24	2.59 ± 0.62
	5	1.52 ± 0.22	1.81 ± 0.62
	7	BDL	BDL
	10	BDL	BDL
	Regression equation and half life	y = 3.394 − 0.075x	y = 3.624 − 0.074x
		$T_{1/2}$ = 4.01 days	$T_{1/2}$ = 4.07 days
Green tea	0	0.51 ± 0.13	1.25 ± 0.15
	1	0.36 ± 0.13	0.86 ± 0.17
	3	0.27 ± 0.08	0.65 ± 0.12
	5	0.22 ± 0.08	0.52 ± 0.12
	7	BDL	BDL
	10	BDL	BDL
	Regression equation and half life	y = 2.665 − 0.069x	y = 3047 − 0.067x
		$T_{1/2}$ = 4.36 days	$T_{1/2}$ = 4.49 days
Field soil	0	0.25 ± 0.06	0.37 ± 0.09
	1	BDL	BDL
	3	BDL	BDL
	5	BDL	BDL
	7	BDL	BDL
	10	BDL	BDL

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

half life value ranges from 3.72 to 3.81 days for Hantapara location and 4.01–4.07 days for Kamalpur location. No residue was found after 5 days of application in tea leaf and made tea irrespective of location for recommended dose (T_1). The clothianidin residues in soil were detected only at 0 day in all the cases except from Hantapara tea estate where residues were detected on the 1st day for double dose.

The result almost consistent with that of the dissipation pattern of imidacloprid and acetamiprid – neonicotinoid insecticides on tea was applied at a almost similar rate which went below detectable limit after 5th day of application (Gupta and Shanker 2009)

References

Gupta M, Shanker A (2009) Fate of imidacloprid and acetamiprid residues during black tea manufacture and transfer into tea infusion. Food Addit Contam Part A 26(2):157–163

Ohkawara Y, Akayama A, Matsuda K, Andersch W (2002) Clothianidin: a novel broad spectrum neonicotinoid insecticide. The BCPC conference, Brighton, pp 51–58

Uneme H, Konobe M, Akayama A (2006). Discovery and development of a novel insecticide "Clothianidin".Sumitomo Chemical Co. Ltd. Available via http://www.sumitomochem.co.jp/english/rd/report/theses/docs/20060202_h6t.pdf. Accessed 3 Jan 2012

Yamamoto A, Yabuta G, Tomizawa M, Saito T, Miyamoto T, Kagabu S (1995) Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. J Pesticide Sci 20:33–40