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Thiacloprid Residues and Its Safety Evaluation in Darjeeling Tea

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Abstract In order to examine the persistence behavior, safety evaluation and utilization of residue data for fixation of thiacloprid MRL, a supervised field trial in tea was conducted at Darjeeling. The HPLC analysis of thiacloprid in green tea leaves indicates that the initial deposits of 2.14 and 3.95 mg kg^{-1}, which declined gradually and persisted until day 14 to the tune of 0.23 and 0.45 mg kg^{-1} respectively. The residues in processed tea samples prepared from green tea leaves of 7 and 14th day were 3.0–3.8 times less. Thiacloprid did not infuse to tea liquor from processed tea. The half-life value in green tea leaves ranged from 4.29 to 4.31 days. Considering the EU MRL value of 10 mg kg^{-1} and risk assessment calculation, thiacloprid at 30 g a.i. ha^{-1} appears to be safe in plant protection schedules and first round of plucking of green tea leaves on day 7 is recommended.

Keywords Thiacloprid · Persistence · Green tea · Processed tea · Tea liquor · Safety evaluation

The state West Bengal in India has diversified agro -climatic zones and earned reputation not only in technology led cultivation but also in exporting agricultural commodities like tea. Darjeeling tea (*Camellia sinensis*) is famous for its exquisite flavor as well as health-promoting properties and India earns huge foreign exchange from it. It is well known that tea plants are susceptible to pest and diseases that are responsible for a significant loss in tea production in terms of both quality and quantity. Neonicotinoid insecticides represent the fastest growing class of insecticides introduced to the market since the launch of pyrethroids. Thiacloprid, {(Z)-N-[3-(6-chloro-pyridin-3-ylmethyl)-thiazolidin-2-ylidene]-cyanamide} belonging to neonicotinoids family encompasses high insecticidal activity with a favorable eco-biological profile and is useful in modern crop protection systems. It acts agonistically on the insect nervous system for fast knockdown of pests through contact and ingestion with systemic and translaminar properties. It is found to be active against tea mosquito bug and fits well in resistance management strategies. With this view, M/s Bayer Crop Science has come forward for the registration of thiacloprid as an alternative insecticide management in tea. However, the application of thiacloprid may do leave residues in tea matrices. Pesticide residues in/on food commodities are a matter of concern and are regulated by national and international laws and the residue data are the prime requirement by the registration authorities for the fixation of MRL. Little information is available on thiacloprid analysis in tea matrices and persistence behavior in Darjeeling tea under West Bengal agro-climatic condition. With this background, the present study was aimed to examine the residual fate of thiacloprid in green tea leaves, processed tea and its transfer potential from processed tea to tea infusion in hot water and safety evaluation in order to protect the consumer and utilization of residue data in MRL fixation for our country.

Materials and Methods

The experiment was carried out in a randomized block design replicated thrice at Kamalpur Tea Estate, Dist. Darjeeling (27°03′N, 88°18′E), W.B. in the year 2006

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using mixed clone variety (TV-1 and TV-19). A plot of 100 m^2 was taken for individual treatment leaving two rows of bushes as guard rows between the control and the different treatment plots. Thiacloprid (Calypso 240 SC) was applied to tea bushes thrice at an interval of 10 days in the recommended dose at 30 g a.i. ha^{-1} (T_1) and double the recommended doses at 60 g a.i. ha^{-1} (T_2) along with untreated control (T_3). The volume of water was used 400 L ha^{-1}.

Green tea leaves consisting of two leaves and a bud (1 kg) were plucked randomly from each replicate of the treated and control plots at different time interval [0 (2 h after third spray), 3, 5, 7, 10 and 14 days] after the last application of the chemical. The fresh green tea leaf samples (1 kg) of 7 and 14 days were then processed in the factory of the tea garden following standard manufacturing method to get processed tea (CTC, 250 g) since tea leaves are plucked and processed after 7 and 14 days of application. The processed tea (3.0 g) of 7 and 14 days was boiled in 100 mL distilled water on a heater for 2 min and filtered through a stainless steel filter to get the tea liquor.

The analytical standard of thiacloprid (99.6 % purity) and Calypso 240 SC formulation were supplied by M/S Bayer Crop Science, Mumbai, India. Stock solution (1,000 mg L^{-1}) was prepared in acetonitrile and the solutions required for preparing a calibration curve (5, 1, 0.5, 0.1, 0.05 and 0.01 mg L^{-1}) were prepared from the stock solution by serial dilutions. All other solvents and chemicals used were of analytical grade.

The green tea leaves, processed tea and tea liquor samples were analyzed for thiacloprid residues by HPLC using a method for thiacloprid determination in plant material (Placke and Schöning 2001) with minor modifications.

A representative sample (25 g) each of green tea leaves and processed tea was extracted with 150 mL acetone:water (3:1), filtered through celite 545 (5 g), concentrated to an aqueous remainder of 20 mL by a rotary vacuum evaporator (RVE) at 40°C. The aqueous remainder loaded on a Chem Elut column and was eluted with 60 mL cyclohexane: ethyl acetate (1:1) and evaporated to dryness using a RVE at 40°C. The residue was dissolved in 2 mL of ethyl acetate.

Tea liquor (100 mL) was taken in a separating funnel and 10 mL of saturated sodium chloride solution was added to it followed by partition with (3 × 60 mL) cyclohexane: ethyl acetate (1:1 by volume). The combined upper organic layer was collected over anhydrous sodium sulphate and concentrated using RVE. The residues were dissolved in 2 mL of ethyl acetate.

The concentrated ethyl acetate fraction of green tea leaves, processed tea and tea liquor were separately subjected to column chromatography using 10 g florisil (deactivated with 5 % water). The column was washed with 80 mL ethyl acetate and eluate was discarded. The thiacloprid residues were eluted with 100 mL acetonitrile, concentrated and the residues were dissolved in 5 mL of acetonitrile:water (4:6 by volume) and subjected to HPLC analysis.

Agilent 1200 HPLC equipped with a UV–VIS detector was employed for analysis using a mobile phase consisting of acetonitrile: water (4:6 by volume). Chromatographic separation was performed on a BDS, Hypersil RP-C18 column (25 cm long, 4 mm i.d, particle size: 5 µm) at constant flow rate of 0.5 mL/min at λ_{max} 242 nm with an injection volume of 100 µL. Data analyses were performed using Chemstation software (Agilent Technologies™).

Results and Discussion

The retention time (RT) of thiacloprid was recorded to be 10.762 ± 0.5 min and confirmation in samples was assessed by comparing with the RT. The quantification was accomplished using a 6 point calibration graph with an excellent linear correlation coefficient (R^2) >0.99. The stated extraction and cleanup of green tea leaves, processed tea and infusion was acceptable as no interfering peak was detected at the RT of thiacloprid (Fig. 1). The method efficiency was tested by fortifying thiacloprid in tea matrices in the range of 0.005–0.50 mg kg^{-1}. The overall mean recovery and relative standard deviation (RSD) of all the matrices under study ranged from 86 to 89 % and 1.16–7.87 % respectively. Considering recovery percentage, RSD and S/N ratio >10, indicated that the method was efficient, repeatable with the limit of quantification of 0.05 mg kg^{-1} in green tea leaves and processed tea and 0.005 mg L^{-1} for tea liquor. The data relating to the thiacloprid residues in tea from the field experiments carried out in November 2006 (winter season) is presented in Table 1. No residues were detected in any analyzed control tea sample. The initial deposit of thiacloprid residues in green tea leaves was found to be 2.14 and 3.95 mg kg^{-1} in T_1 and T_2 respectively, which declined to 0.70, and 1.13 mg kg^{-1} on day 7 with a corresponding loss of 67.29 and 71.39 %.

Finally, the residues reached to 0.23 and 0.45 mg kg^{-1} on 14th day with corresponding reduction of 89.25 and 88.61 %. The dissipation of thiacloprid occurred quite rapid during the first seven days (67.29–71.39 %) compared to the disappearance rate of 17.22–21.96 % for the next seven days. Moreover, the residues in green tea leaves reached well below the EU MRL of 10.0 mg kg^{-1} (htpp://ec.Europa.eu/sanco_pesticides/public/index.cfm?event=substance.selection) within 2 h after spraying and therefore, 1st round of plucking schedule in green tea leaves may be

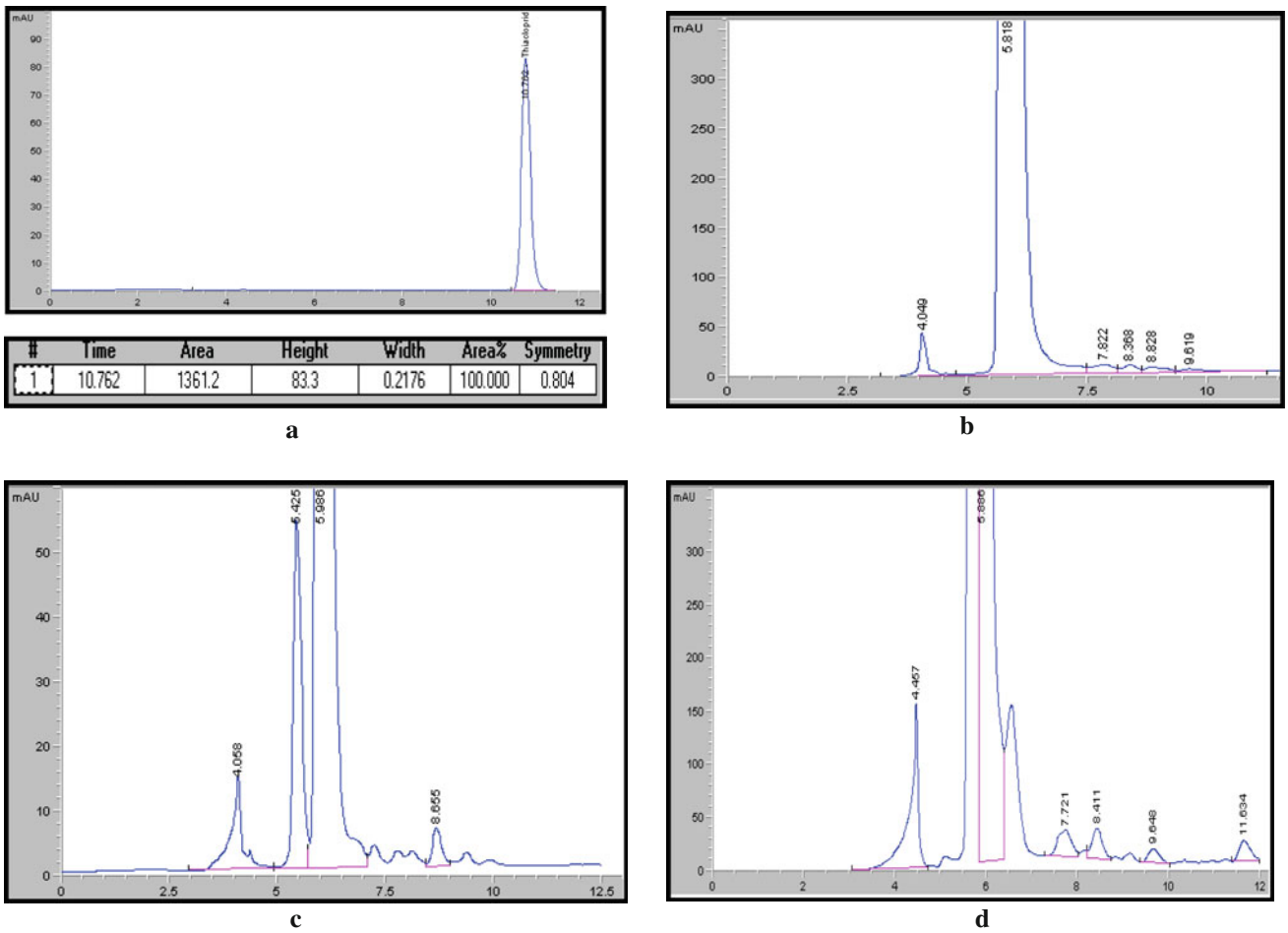

Fig. 1 Chromatograms of thiacloprid standard and tea matrices. **a** Thiacloprid standard 1.0 mg kg^{-1}. **b** Control green tea leaves. **c** Control processed tea. **d** Control tea liquor

recommended. The significant correlation co-efficient ($R^2 \approx 0.98$) indicated statistical conformity of the dissipation data to pseudo-first order kinetics. The calculated half-life value in green tea leaves was found to be 4.29 (T_1) and 4.31 (T_2) days. Dubey et al. (2008) also reported similar observations.

The thiacloprid residues in processed tea on day 7 were 0.19 and 0.29 mg kg^{-1} in T_1 and T_2 respectively, which is comparable with the results, reported by Dubey et al. (2008) and dissipated below detectable level of 0.05 mg kg^{-1} on 14th day at T_1 and was 0.15 mg kg^{-1} at T_2. In general, it is expected that residual content of made tea should increase by a concentration factor of 3–4 times than green tea leaves (Nagayama 1996) but it was 3.00–3.80 times lower in the present study. It appears that during manufacturing of processed tea from green tea leaves, thiacloprid residues either degraded or were lost in the environment due to physico-chemical factors of processing unit. The loss of many pesticides during processing has been reported by Chen and Wan (1988), Jaggi et al. (2000) and Dubey et al. (2008).

Tea liquor prepared from processed tea of 7 and 14th day for both the doses were analyzed and it was found that thiacloprid did not infuse to tea liquor. The water solubility and partition coefficient of a pesticide determines the transfer rate of pesticide residues from processed tea to the tea liquor (Nagayama 1996; Wan et al. 1991; Jaggi et al. 2001; Tsumura-Hasegawa et al. 1992). Lower the water solubility and higher the octanol–water partition coefficient, lower is the residues transfer from processed tea to tea liquor. In the present study no transfer of thiacloprid occurred because of low water solubility (0.185 g L^{-1} at 20°C and pH 7) and high octanol/water partition coefficient (Pow = 18). Furthermore, it might bind to suspended organic matter in the infusion (proteins, carbohydrates, pigments, polyphenols, etc.) and remain in the spent leaves. Similar infusion rate was recorded by Dubey et al. (2008).

Based on the residue data of thiacloprid in processed tea and average daily consumption of tea to the extent of 1.92 g, the theoretical maximum residue contribution

Table 1 Thiacloprid residues in different stages of tea and reduction percentage in processed tea

DAT	Thiacloprid residues (mg kg^{-1}) ± standard deviation					
	Green tea leaves		Processed tea		Tea infusion	
	T_1	T_2	T_1	T_2	T_1	T_2
0	2.14 ± 0.09	3.95 ± 0.07				
	(-)	(-)				
3	1.66 ± 0.09	2.79 ± 0.05				
	(22.43)	(29.37)				
5	1.16 ± 0.07	2.16 ± 0.04				
	(45.79)	(45.32)				
7	0.70 ± 0.01	1.13 ± 0.05	0.19 ± 0.01	0.29 ± 0.04	BDL	BDL
	(67.29)	(71.39)	[72.85]	[74.36]		
10	0.52 ± 0.02	0.78 ± 0.01				
	(77.10)	(80.25)				
14	0.23 ± 0.02	0.45 ± 0.02	BDL	0.15 ± 0.01	BDL	BDL
	(89.25)	(88.61)		[66.67]		
Regression equation	y = 3.377−0.07x	y = 3.612−0.069x				
R^2	0.98	0.98				
Half-life (days)	4.29	4.31				

BDL Below detectable limit, Values in parentheses are the dissipation %; and brackets reduction % from green tea leaves to processed tea

(TMRC) is calculated according to Banerjee et al. (2010) and compared with maximum permissible intake (MPI) for safety evaluation. It was found that TMRC of thiacloprid in processed tea were lower than MPI, indicating residues are safe and application of Calypso 240 SC at 30 g a.i. ha^{-1} can be recommended for use in tea. Apparently, the risk posed by insecticide residues to the consumers' health is negligible.

The overall residue scenario of green tea leaves, processed tea and tea liquor coupled with safety risk assessment indicated that thiacloprid (Calypso 240 SC) could be safely used in plant protection schedule of tea and the data might be helpful for the fixation of MRL from the viewpoint of national context.

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