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Persistence of acetamiprid in tea and its transfer from made tea to infusion

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ABSTRACT

Acetamiprid, a new-generation, highly active neonicotinoid insecticide has been used to control mites and insect pests. In the present study, the disappearance trend of acetamiprid residue in tea under field conditions was studied at two dosages for two seasons (dry and wet), and transfer of residues from made tea to infusion was also determined. Acetamiprid dissipation rate was found to be faster in the wet season. Half-life of acetamprid was found to be 1.82–2.33 days in green tea shoots and 1.84–2.25 days in made tea for both dry and wet seasons. The percent transfer of acetamiprid residues from made tea to infusion was 36.84–50.00%; however, 31.11–44.40% of the residues remained stuck to the spent leaves during both the dry and wet seasons. On the basis of transfer of residues from made tea to infusion, a waiting period of 15 days for tea plucking after pesticide application at recommended dose may be suggested.

1. Introduction

Neonicotinoid insecticides represent the fastest growing class of insecticides introduced to the market since the launch of pyrethroids. The current market share of this class of chemical is well above €600 million per year. Acetamiprid ((*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine), (Fig. 1), is a new-generation, highly active neonicotinoid insecticide, which has been used to control insects belonging to the orders Hemiptera, Thysanoptera, Coleoptera and Lepidoptera on a wide range of crops, especially vegetables, fruits and tea (Mateu-Sanchez, Moreno, Arrebola, & Martinez Vidal, 2003; Roberts & Hutson, 1999; Tomlin, 2000). Acetamiprid was registered in Japan, New Zealand, China and other European countries, in the 1990s, for use in tea, with a minimal risk level provisionally established by the Japanese Union at 50.0 mg/kg and by China at 2.0 mg/kg. Field dissipation studies conducted in the US (Washington, Florida, New York, California and New Jersey) yielded half-life values ranging from 2.8 to 14.1 days (Pest Management Regulatory Agency, 2002–2005). In recent years, a number of field trials of acetamiprid were done on various crops. Half-lives were reported to be 1.02–1.59 days in mustard plant and 1–2 days in field soils (Pramanik, Bhattacharyya, Dutta, Dey, & Bhattacharyya, 2006; Tokieda, Ozawa, Kobayashi, Gomyo, & Takeda, 1999).

Tea is the most popular beverage throughout the world. Among the factors limiting the quality and quantity of tea production, the role of insect pests is important. Management of pests in tea plantations has largely depended on the use of conventional, neurotoxic, broad-spectrum synthetic chemical pesticides, viz., organophosphates, carbamates, synthetic pyrethroids and a number of new chemical classes, such as neonicotinoids. As tea makes an important contribution to the human diet, data on the fate of acetamiprid residues in tea after application are essential, for the establishment of MRL's in various agroclimatic conditions.

In recent years, a number of research works have dealt with the behaviour of quinalfos (Jaggi, Sood, Kumar, Ravindranath, & Shanker, 2000), dimethoate, dicofol, deltamethrin (Sood, Jaggi, Kumar, Ravindranath, & Shanker, 2004), hexaconazole (Kumar, Ravindranath, & Shanker, 2004) fenvalerate (Sharma, Gupta, & Shanker, 2008) and imidacloprid (Gupta, Sharma, & Shanker, 2008) pesticides in tea, the influence of various manufacturing processes on their residues in the made tea, and their fate from made tea to infusion. As tea is subjected to infusion prior to consumption, residue levels of many pesticides in made tea and in its infusion have been reported (Bhattacharya, Chowdhury, Somchowdhury, Pallarl, & Roy, 1995; Chen, Wan, Wang, Xue, & Xia, 1987; Jaggi, Sood, Kumar, Ravindranath, & Shanker, 2001; Kumar et al., 2004). To the best of our knowledge, no work has been published on the dissipation of acetamiprid residue in tea, its transfer from made tea to infusion and its retention in spent leaves. Therefore, the present work was carried out with the following objectives: (1) to evaluate the loss of acetamiprid in green tea shoots, made tea, infusion and spent leaves at different time intervals during dry (April–June) and wet (July–September) seasons, and (2) and its transfer potential from made tea to hot water infusion. The study would be helpful in establishing adequate monitoring of the residue of acetamiprid and its judicious incorporation in pest management strategies in the tea plantation.

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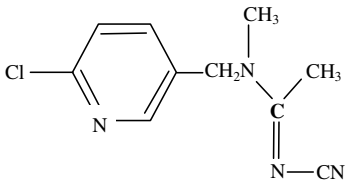

Fig. 1. Structure of acetamiprid.

2. Materials and methods

2.1. Field trials

Field trials were carried out at IHBT tea experimental farm at Banuri, Palampur (32°N × 76°E), Himachal Pradesh, India. A plot size of 100 bushes (10 × 10) was selected for the control and each treatment of the pesticide under study, leaving two rows of bushes as guard rows between the control and the different treatment plots. Acetamiprid was sprayed at two doses, 125 ml/ha (recommended) and 250 ml/ha (double the recommended), in three replications, with a hand-operated knapsack sprayer, using a recommended formulation volume of 400 l/ha.

2.2. Sampling

For studying the dissipation of acetamiprid in tea, samples (two leaves and a bud) were collected at time 0 days (2 h post application, when the spraying mixture had dried) and 1, 3, 5, 7, 9, 11, 15 and 21 days after the application. About 0.5 kg of the green tea shoots (two leaves and a bud) was harvested from each replicate of both the treatment and control plots and brought to the laboratory each time.

2.3. Tea leaves processing and infusion preparation

The untreated control and treated green tea shoots from the field were processed in the laboratory's mini manufacturing unit, using a conventional orthodox tea manufacturing process. The manufacturing process, in brief, involved withering of shoots (50–55% water loss) at ambient temperature for 15–20 h; rolling (twisting and rupturing the tissue to express the juice) using a piezy roller for about 30 min with pressure, followed by fermentation (oxidation) for 1–2 h at 25–30 °C and 95% RH; finally drying the shoots in a tea dryer, using hot air at 100 ± 5 °C, to a final moisture content of 2–3%. Made tea (5 g) was infused in 150 ml of boiled water. After 2 min of brewing, the water extract was filtered through a stainless steel filter, cooled and examined for residue transfer from the made tea. The matrices used for residue determination were the green tea shoots, made tea, the infusion prepared and the spent leaves left in the stainless steel filter.

2.4. Chemicals

An analytical standard of acetamiprid (CAS-No-135410-20-7) was supplied by Krishi Rasayan Exports Pvt. Ltd., Baddi, Solan, Himachal Pradesh, India. Standard solution (1000 mg/l) was prepared in acetonitrile and the solutions required for preparing a standard curve (0.2, 0.4, 0.6, 0.8 and 1.0 mg/l) were prepared from the stock solution by serial dilutions. All the solvents and chemicals used were of analytical grade from Merck (Darmstadt, Germany).

2.5. Analytical procedures

All green tea shoots, made tea, infusion and spent leaves samples were analysed for acetamiprid residues by HPLC with diode array detection, according to a method for imidacloprid determination in tobacco (Liu et al., 2005) with modifications in the extraction, partitioning and cleanup step, in order to remove the interfering coextractives in the tea samples. Extraction, partitioning and cleanup steps are briefly described below.

2.5.1. Green tea shoots, made tea, infusion and spent leaves extraction and clean-up procedure

Green tea shoots (25 g) and made tea (10 g) were extracted with an appropriate amount of acetonitrile (150 ml and 100 ml, respectively) by mechanical shaking for 2 h. Extracts were filtered through Whatman No. 1 filter paper containing 2 g of sodium sulfate. The filtrate was evaporated to 50 ml and transferred into a 500 ml separating funnel, to which 100 ml of petroleum ether and 100 ml of 5% NaCl was added. After thoroughly mixing the two phases the aqueous layer was separated and the organic layer was partitioned twice with 5% NaCl. Aqueous phases were combined and partitioned with dichloromethane (75 ml × 4). The extract was dried completely and reconstituted in eluent (5 ml) and then transferred to a silica-carbon column (30 cm × 1.1 cm i.d.; 6 g of Merck brand activated silica (60–100 mesh) thoroughly mixed with 0.1 g carbon), prewashed with 50 ml acetone + *n*-hexane (4:6 v/v). The column was eluted with 150 ml of acetone + *n*-hexane (5:5 v/v). The eluent was concentrated to dryness. The residue was reconstituted in 2 ml of mobile phase (acetonitrile/water, 30:70 v/v) for final analysis.

From the infusion prepared (described above), a 50 ml aliquot (cooled to room temperature) was transferred to a separating funnel (500 ml). The pesticide was extracted with dichloromethane (75 ml × 4). The organic layer was separated, combined and concentrated to 5 ml. The spent leaves were dried between the folds of filter paper and residues were extracted by shaking them with 100 ml acetonitrile for 2 h. The extract was filtered through Whatman No. 1 filter paper and transferred to a separating funnel. The clean-up procedure was similar to that used for the green tea shoots.

2.5.2. HPLC determination

A high-performance liquid chromatograph, (LA-Chrom; Merck), equipped with a LiChrospher®100 reverse phase (RP)-18 end capped (30 cm long, 5 μm dia) column (Merck) and diode array detector was used. The mobile phase was acetonitrile/water (30:70 v/v) with 0.6 ml/min flow rate. Detection was at 270 nm and injection volume was 20 μl.

3. Results

3.1. Efficiency of acetamiprid determination method

The described method of analysis of acetamiprid residues in green tea shoots, made tea, infusion and spent leaves samples by HPLC is fast and relatively simple. Quantification was accomplished by using a standard curve, prepared by diluting the stock solution in acetonitrile/water (30:70 v/v). Good linearity was achieved with a correlation coefficient of 0.9995. The limit of detection (LOD) was determined, based on the lowest concentration level of standard detected, and was found to be 0.05 mg/kg. No control samples showed any evidence of chromatographic interference.

Confirmation of acetamiprid in samples was performed by measuring its retention time. In Fig. 2 only chromatograms of acetamiprid in made tea are reported because those of green tea shoots, infusion and spent leaves were similar. The efficiency of the method has been evaluated by spiking green tea shoots, made tea, infusion and spent leaves samples with acetamiprid working solutions at various levels (1–20 mg/l for green tea shoots, made tea, infusion and spent leaves). Recovery values for green tea shoots, made

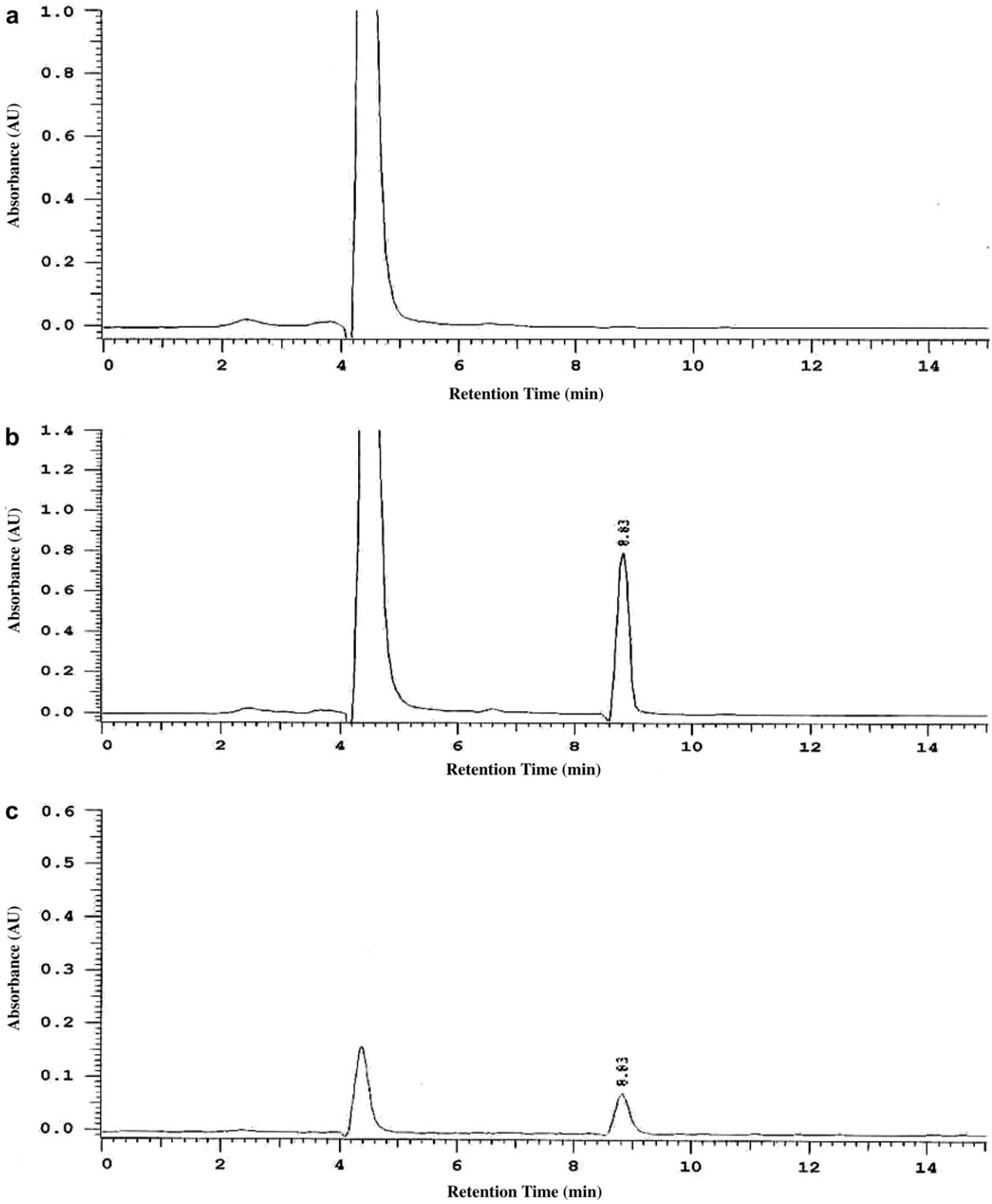

Fig. 2. Chromatograms of acetamiprid (t_R = 8.83 min): (a) control (untreated sample), (b) made tea (0 day sample with 22.59 mg/kg of the insecticide), (c) standard (solution in acetonitrile: water at 10 mg/l).

tea and spent leaves ranged from 95% to 108% and for infusion from 92% to 98%. All of these values of recovery indicated good method accuracy and repeatability, as they are within the accepted range for residue determinations (Commission of the European Union, 2003; Greve, 1984). The experimental data were subjected to statistical analysis using Microsoft Excel (Windows 2000). The half-life of acetamiprid in different matrices was calculated using the first order rate equation:

$$C_t = C_0 e^{-kt}$$

where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration and k is the rate

constant per day. The half lives ($t_{1/2}$) were determined from the k value for each experiment

$$t_{1/2} = In2/k.$$

3.2. Dissipation of residues

3.2.1. Green tea shoots and made tea

The data relating to the residues in tea from the field experiments carried out in June 2006 (dry season) and August 2006 (wet season) are reported in Tables 1 and 2. No residues of acetamiprid were detected in any analysed control tea sample. The initial deposits of the acetamiprid residues in green shoots at the two dosages were 4.43 and 9.53 mg/kg in dry, and 3.96 and 8.25 mg/kg in wet season, respectively. In the dry season the residues declined sharply to 0.50 and 1.12 mg/kg in 5th day samples, as compared to 3rd day samples, i.e., 2.42 and 6.10 mg/kg at recommended and double the recommended dosage, respectively. In the case of the made tea initial deposits of residue observed were 13.4 and 26.4 mg/ kg in dry and 11.2 and 22.9 mg/kg in

Table 1
Acetamiprid residue in different stages of tea (green tea shoots, made tea, infusion and spent leaves) at different time intervals during the dry season

Time interval (days)	Acetamiprid residue in mg/kg ± standard deviation							
	Green tea shoots		Made tea		Infusion		Spent leaves	
	T1[a]	T2[b]	T1	T2	T1	T2	T1	T2
0	4.43 ± 0.29 (0.00)[c]	9.53 ± 0.31 (0.00)	13.4 ± 0.44 (0.00)	26.4 ± 0.61 (0.00)	6.37 ± 0.13 (47.5)[d]	12.6 ± 0.33 (47.8)	5.95 ± 0.19 (44.4)	10.2 ± 0.39 (38.8)
1	3.80 ± 0.33 (14.2)	7.77 ± 0.44 (18.5)	10.8 ± 0.78 (19.5)	21.7 ± 0.19 (17.6)	5.33 ± 0.06 (49.4)	9.85 ± 0.51 (45.3)	4.34 ± 0.05 (40.2)	8.62 ± 0.26 (39.7)
3	2.42 ± 0.07 (45.4)	6.10 ± 0.06 (36.0)	7.16 ± 0.28 (46.6)	17.3 ± 0.83 (34.6)	3.06 ± 0.06 (42.7)	6.76 ± 0.07 (39.2)	2.35 ± 0.11 (32.8)	6.63 ± 0.30 (38.4)
5	0.50 ± 0.03 (88.7)	1.12 ± 0.11 (88.3)	1.44 ± 0.05 (89.3)	3.16 ± 0.04 (88.0)	0.58 ± 0.06 (40.3)	1.32 ± 0.03 (41.8)	0.46 ± 0.02 (31.9)	1.06 ± 0.07 (33.5)
7	0.24 ± 0.00 (94.6)	0.51 ± 0.02 (94.7)	0.68 ± 0.03 (94.9)	1.47 ± 0.03 (94.4)	0.34 ± 0.01 (50.0)	0.61 ± 0.06 (41.5)	0.30 ± 0.06 (44.1)	0.51 ± 0.01 (34.7)
9	0.15 ± 0.01 (96.6)	0.42 ± 0.01 (95.6)	0.45 ± 0.01 (96.6)	1.21 ± 0.03 (95.4)	0.18 ± 0.02 (40.0)	0.54 ± 0.02 (44.6)	0.14 ± 0.01 (31.1)	0.45 ± 0.02 (37.2)
11	0.07 ± 0.02 (98.4)	0.23 ± 0.01 (97.6)	0.21 ± 0.00 (98.4)	0.62 ± 0.01 (97.7)	0.08 ± 0.01 (38.1)	0.31 ± 0.00 (50.0)	0.07 ± 0.00 (33.3)	0.23 ± 0.05 (37.1)
15	0.03 ± 0.00 (99.3)	0.11 ± 0.00 (98.9)	0.08 ± 0.01 (99.4)	0.26 ± 0.01 (99.0)	ND[e]	0.13 ± 0.01 (50.0)	ND	0.09 ± 0.00 (34.6)
21	ND	ND	ND	ND	ND	ND	ND	ND
$t_{1/2}$[f]	2.08	2.33	2.03	2.25				

[a] Treatment 1 (125 ml/ha).
[b] Treatment 2 (250 ml/ha).
[c] % Degradation after spraying.
[d] % Transfer of residues from made tea.
[e] Not detected.
[f] Half-life in days.

Table 2
Acetamiprid residue in different stages of tea (green tea shoots, made tea, infusion and spent leaves) at different time interval during the wet season

Time interval (days)	Acetamiprid residue in mg/kg ± standard deviation							
	Green tea shoots		Made tea		Infusion		Spent leaves	
	T1[a]	T2[b]	T1	T2	T1	T2	T1	T2
0	3.96 ± 0.38 (0.00)[c]	8.25 ± 0.10 (0.00)	11.2 ± 0.15 (0.00)	22.9 ± 0.45 (0.00)	5.40 ± 0.04 (48.1)[d]	11.3 ± 0.11 (49.1)	4.60 ± 0.04 (41.0)	9.27 ± 0.13 (40.4)
1	1.91 ± 0.08 (51.8)	4.48 ± 0.23 (45.7)	5.60 ± 0.08 (50.1)	12.2 ± 0.23 (46.8)	2.69 ± 0.03 (48.0)	5.73 ± 0.02 (47.0)	2.30 ± 0.02 (41.1)	4.60 ± 0.01 (37.7)
3	0.70 ± 0.01 (82.3)	1.40 ± 0.03 (83.0)	1.98 ± 0.02 (82.4)	4.17 ± 0.09 (81.8)	0.89 ± 0.01 (45.0)	2.04 ± 0.07 (48.9)	0.80 ± 0.05 (40.4)	1.70 ± 0.02 (40.8)
5	0.37 ± 0.08 (90.7)	0.85 ± 0.03 (89.7)	1.07 ± 0.02 (90.5)	2.24 ± 0.08 (90.2)	0.49 ± 0.01 (45.8)	0.96 ± 0.04 (42.9)	0.41 ± 0.01 (38.3)	0.84 ± 0.01 (37.5)
7	0.20 ± 0.02 (95.0)	0.38 ± 0.00 (95.4)	0.54 ± 0.03 (95.2)	1.10 ± 0.02 (95.2)	0.24 ± 0.02 (44.4)	0.44 ± 0.01 (40.0)	0.20 ± 0.02 (37.0)	0.43 ± 0.00 (39.1)
9	0.12 ± 0.00 (97.0)	0.26 ± 0.00 (96.9)	0.34 ± 0.01 (97.0)	0.67 ± 0.01 (97.1)	0.13 ± 0.00 (38.2)	0.27 ± 0.00 (40.3)	ND	0.23 ± 0.01 (34.3)
11	0.06 ± 0.00 (98.5)	0.15 ± 0.01 (98.2)	0.18 ± 0.00 (98.4)	0.38 ± 0.02 (98.3)	ND[e]	0.14 ± 0.00 (36.8)	ND	ND
15	ND	ND	ND	ND	ND	ND	ND	ND
21	ND	ND	ND	ND	ND	ND	ND	ND
$t_{1/2}$[f]	1.82	1.90	1.84	1.86				

[a] Treatment 1 (125 ml/ha).
[b] Treatment 2 (250 ml/ha).
[c] % Degradation after spraying.
[d] % Transfer of residues from made tea.
[e] Not detected.
[f] Half-life in days.

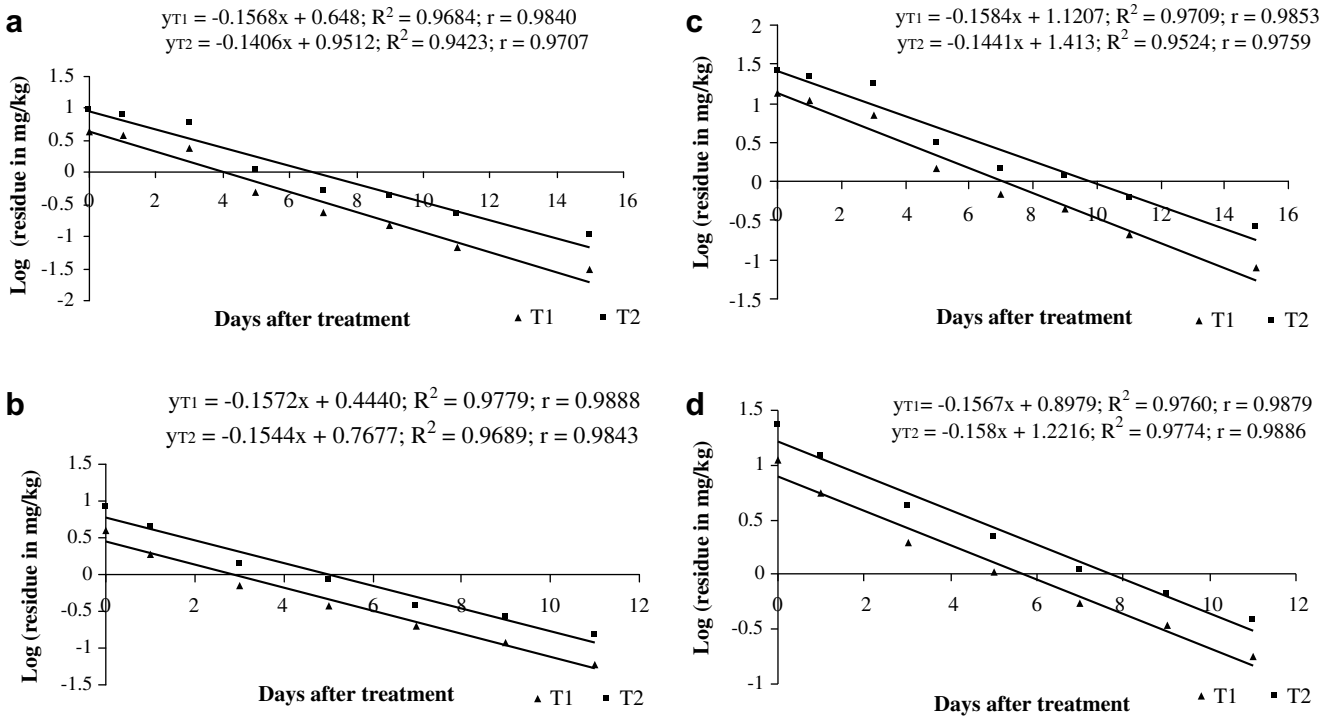

Fig. 3. Persistence of acetamiprid in (a, b) green tea shoots and (c, d) made tea during the dry and wet seasons, respectively for both treatments.

wet season at the two dosages which showed a residue 2.45–2.68 times higher than that in the corresponding sample from green shoots. The persistence pattern is presented in Fig. 3. The dissipation trend of acetamiprid in both seasons followed first order kinetics. The values of the correlation coefficient, *r*, were nearer to 1, which indicated a linear correlation. The half life values (Tables 1 and 2) of acetamiprid were in the range 2.08–2.33 and 1.82–1.90 days, and 2.03–2.25 and 1.84–1.86 days in made tea from dry and wet seasons, respectively, which again indicated faster residue dissipation in the wet season than in the dry season.

3.2.2. Infusion and spent leaves

The percent transfer of residue during the brewing process was 38.1–50.0 in the dry season and 36.8–49.1 in the wet season at both dosages. The percent residue that remained on spent leaves were 31.1–44.4 in the dry season and 34.3–41.1 in the wet season at both dosages, respectively.

4. Discussion

4.1. Dissipation of residues

4.1.1. Green tea shoots and made tea

The level of acetamiprid in tea during the dry season showed a sharp decline in the residue from the 3rd to the 5th day; this may be due to the rainfall, which was recorded at 9.9, 27.0 and 100.3 mm on the 4th, 5th and 6th day after the treatment, respectively (Fig. 4). In the tea field, besides the effects of rainfall, physical and chemical factors, like light, heat, pH and moisture (Agnihothrudu & Muraleedharan, 1990; Chen et al., 1987; Cosby, Moilanon, Nakagawa, & Wong, 1972; Miller & Donaldson, 1994; Miller & Zepp, 1983) on the degradation of insecticides, growth dilution factor (Agnihothrudu & Muraleedharan, 1990; Bisen & Ghosh Hajara, 2000; Chen & Wan, 1988) might have played a significant role in the degradation of acetamiprid residue. It is evident from the data

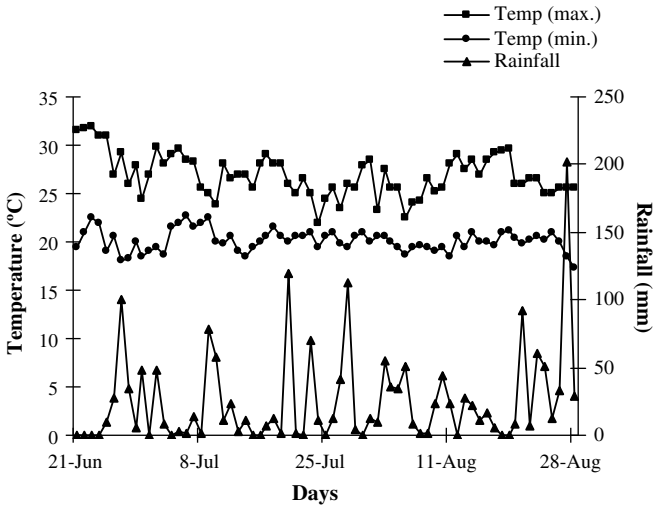

Fig. 4. Weather parameters.

that acetamiprid degraded more rapidly during the wet season than in the dry season. Over 98% of the residue had dissipated on the 11th day in the wet and on the 15th day in the dry season in green shoots. During processing, leaves undergo dehydration, which causes a concentration factor of 3–4 (Nagayama, 1996). Therefore, theoretically the residue in made tea should increase by a similar factor, if it is not lost during manufacturing. However, results showed that the residue deposit was smaller (~75%) than the concentration effect. This indicates that during the processing of green tea shoots the residue decreased by approximately 25% but was still higher than that of the corresponding samples of unprocessed green tea shoots. The present finding supports the studies reporting loss of many pesticides during processing (Chen & Wan, 1988; Jaggi et al., 2000). Over 98% of the residue had

dissipated on the 11th day in the wet and on the 15th day in the dry season in made tea at both dosages. In mustard plant 98% of acetamiprid residue had dissipated by the 7th day after the treatment, this may be due to differences in the matrix and the agroclimatic conditions.

4.1.2. Infusion and spent leaves

No significant relationship was observed between the transfer of acetamiprid residue and rate of application. The transfer rate of the pesticide residue to the infusion depends on its water solubility (Nagayama, 1996; Wan, Xia, & Chen, 1991), partition coefficient (Jaggi et al., 2001; Tsumura-Hasegawa, Tonogai, Nakamura, & Ito, 1992) and low vapour pressure (Chen & Wan, 1988). This insecticide has high water solubility, i.e., 4.2 g/l at 25 °C but low organic matter adsorption capability, with organic adsorption coefficient values (K_{oc}) in the range of 132–267 ml/g at 20 °C. Furthermore, it binds to suspended organic matter in the infusion (proteins, carbohydrates, pigments, etc.) and has a high octanol/water partition coefficient (K_{ow} = 6.27 at 25 °C) (U.S. Environmental Protection Agency, 2002). These may be the reasons for the reasonably low transfer of acetamiprid from made tea to infusion, as it remains stuck to the spent leaves.

5. Conclusions

Acetamiprid showed a relatively slow dissipation rate in the tea plantation, as compared to mustard. The transfer of acetamiprid residue from made tea to infusion was observed to depend on the amount of acetamiprid present in the made tea. On the basis of the above findings it can be concluded that at or after the 15th day of harvest, there was no detectable residue transfer to the infusion at the recommended dose (125 ml/ha) in both wet and dry seasons. Thus, infusion consumption is safe in samples harvested 15 days after acetamiprid treatment. Half-life of acetamiprid in made tea ranged from 1.84–2.25 days during both dry and wet seasons. A waiting period of 15 days can be recommended for safe harvesting of the crop. The described method of analysis of acetamiprid residues is suitable for determination of residue in tea and the method can be suitably applied to other members of the neonicotinoid group.

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