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Dissipation Pattern and Residue of Fenvalerate in Tea of Bangladesh

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Abstract

Fenvalerate, a non-systematic insecticide is extensively used for protection of tea leaves in Bangladesh. Excessive use of insecticides with improper pre-harvest intervals may cause the tea unsuitable for consumption and trade. The study was designed to determine the safe per-harvest interval after the application of fenvalerate on tea trees at two different doses. Fenvalerate was applied on tea plants in experimental plots at the full and half of the recommended doses (0.1 kg a.i /ha and 0.05 kg a.i /ha, respectively). Tea leaves were collected at 0 (2 h after application), 1, 3, 5, 7, 10 and 14 days after application of the insecticide and made into black tea which was infused with hot water. Both brew and brew free residue were extracted, cleaned up and analyzed by GC-ECD. The residue levels in the brew were 0.189, 0.033 and 0.007 mg/kg at zero, 7 and 10 days, respectively, when it was applied at half of the recommended dose. In case of the recommended dose, residue levels were 0.644 and 0.010 mg/kg at 0 and 10 day, respectively. Residues were below the maximum residue level (MRL: 0.1 mg/kg) on 5 day at half of the recommended dose and on 7 day at recommended dose. Dissipation of fenvalerate followed first order kinetics at both doses with half lives of 2.6 days in brew part and 4.6 days in brew free part. Recoveries were 6.56±0.003% and 90.6±0.033% in brew part and brew free residue part, respectively, giving a total recovery of 96.6±0.036%. LOD and LOQ were 0.002 and 0.006 mg/kg, respectively.

Key words: Bangladesh, dissipation, GC-MS, fenvalerate, pesticide, tea,

I. Introduction

Tea is one of the most popular beverages all over the world including Bangladesh. Bangladesh exported nearly 3.15 million kg tea in 2009[1]. However, tea production in Bangladesh is greatly affected by various pest like chewing and sucking insects, mites etc[2]. For protection of tea leaves from pest attack several pesticides including fenvalerate are used all over the world[3]. Fenvalerate which is a non-systemic pyrethroid insecticide is one of the common pesticides used in tea plantations in Bangladesh. It acts against a wide variety of insects by interference with sodium ion channel permeability in stimulated nerve membranes[4]. However, indiscriminate use of pesticides may render the tea unsuitable for consumption and trade[5]. Therefore, determination of a suitable pre-harvest non-spraying interval is necessary to produce safe tea for export as well as for local consumption. Pesticide residue in the processed tea depends on the dose of application, climatic condition of each country and also on seasons like winter and summer. The present study was made to find out how fenvalerate dissipates from tea after applying the recommended and half of the recommend dose under agro climate condition of Bangladesh.

II. Materials and Methods

Field experiment and sampling

Tea was grown in an experimental field (open field) of Bangladesh Tea Research Institute (BTRI), Srimongal, Moulvibazar. Three treated and one control plots were chosen for the studies. Commercial grade of fenvalerate (active ingredient 20EC i.e 20% emulsifiable concentrate) were applied at full and half of recommended doses (0.1 kg a.i /ha and 0.05 kg a.i /ha, respectively) and tea leaves were collected at 2 h and 1, 3, 5, 7, 10 and 14 days after application during June 2009. Both of the batches of collected tea sample were made into black tea at the tea production plant of the BTRI. Sample were packed, labeled and transferred to the laboratory and stored at -20 ºC until analysis was carried out.

Reagents and materials

Florisil was purchased from Sigma-Aldrich, USA. Anhydrous sodium sulfate and extra pure analytical grade ethyl acetate (EtOAc), dichloromethane (DCM), n-hexane and acetone were purchased from E. Merck, Germany. Anhydrous sodium sulfate was purified by heating at 300^0C for 10 h in a furnace and alumina & florisil were activated by heating for 12 h at 130 ºC in an oven and were allowed to cool at room temperature before use. The analytical standard fenvalerate (purity 98.5%) was purchased from Dr. Ehrenstrofer GmbH, Augsberg-Germany. Standards were stored at -20 ºC in a freezer and away from the experimental samples.

Instruments

Quantifications were performed using a Shimadzu-2010 gas chromatograph (GC) equipped with an AOC-20i auto sampler and an electron capture detector (ECD). Injector and detector temperatures were set at 280 ºC and 290 ºC, respectively. The injection (1 µL) was made in the split-less mode opening after 2 min. Separations were performed on a HP-5 capillary column (30 m x 0.25 *i.d*. and film thickness, 0.25 µm) where nitrogen was used as carrier and make-up gas, and flow rate was 1.78 mL/min. Oven temperature was programmed at initial temperature 120 ºC (2 min hold) and raised at 10 ºC/min up to 280 ºC (hold 10 min).

Gas Chromatography-Mass Spectrometry (GC-MS) analyses were carried out using an Agilent 6890 series GC coupled with a 5973 mass selective detector (MSD) (Agilent Technologies, Wilmington, DE) having auto-injector and auto-sampler. Separations were accomplished using a capillary column HP-5MS (30 m x 0.25 *i.d*. and film

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thickness, 0.25 µm) connected with a Quadrupole Mass analyzer with Electron Ionization at 70 eV in scan mode. Helium was used as the carrier gas with average linear velocity of 36 cm/s. The injector was maintained constant at 250 ºC and the oven temperature was programmed at initially 120 ºC up to 1 min, 10 ºC/min till 200 ºC and hold for 5 min, 5 ºC/min till 280 ºC and hold for 5 min.

Extraction

Extraction of tea by hot water and clean up

Tea sample (5 g) was infused with 100 mL hot water at 80 ºC for 5 min in a thermostated water bath. Brew was filtered by vacuum filtration through a filter paper into a conical flask and transferred into a separatory funnel (250 mL). Then NaCl (5 g) was added and the mixture was partitioned twice with ethyl acetate (100 & 50 mL). The organic layer was separated, dried completely by evaporation and reconstitued in 1 mL DCM.

The samples were cleaned up using florisil–alumina column, the column first packed with florisil (12 g) followed by neutral alumina (5 g). DCM (60 mL) was used for packing and equilibration the column. The extract (1 mL) was applied to the column and eluted with DCM (100 mL) and was collected in a round bottomed flask, dried completely by rotary evaporator and reconstituted in 1.0 mL n-hexane.

Extraction of brew free tea by ethyl acetate and clean up[3]

After brewing, the residue was extracted twice with ethyl acetate (100 and 50 mL) and filtered through anhydrous sodium sulfate on porcelain Buchner funnel, dried completely and re-dissolved in 1 mL DCM. Sample extracts were cleaned up using same procedure described in brew part.

Standard calibration curves

The calibration curve of fenvalerate was made by injecting solutions at concentration 0.025, 0.05, 0.25, 0.5, 1.0 and 2.0 mg/kg of the standard fenvalerate into the GC-ECD, plotted integrated areas of the peaks against the standard concentration using MS Excel software. Correlation coefficient (r^2) was 0.997. LOD and LOQ were found to be 0.002 (Signal to noise ration 3:1) and 0.006 mg/kg (S/N:: 10:1), respectively.

Recovery

Control tea sample (5.0 g) was spiked with 2.5 mg/kg of standard fenvalerate solution and were extracted and cleaned up following the same procedure as described above for brew and brew free tea. The recoveries were found to be 6.56±0.003% and 90.04±0.033% in brew part and brew free residue part, respectively. The total recovery was 96.6%.

III. Results and Discussion

The residual level of fenvalerate was analyzed by GC-ECD in brew and brew free residue part for all the doses. Fenvalerate showed two sharp and symmetrical peaks. These two peaks were confirmed by analysis of the standard fenvalerate by GC-MSD. The amount of fenvalerate was calculated by combining area of two peaks of the stereo isomers.

The efficacy of the extraction and clean up procedure was evaluated by performing triplicate recovery experiments at spiking level 0.5 mg/kg and was found to be 6.56±0.003% and 90.6±0.033% in brew part and brew free residue part, respectively. The total recovery was 96.6%. LOD and LOQ of fenvalerate were calculated as 0.002 (Signal to noise ration, 3:1) and 0.006 (S/N, 10:1) mg/kg, respectively.

When half of the recommended dose was applied, the residue in the brew was 0.189 mg/kg at day 0, 0.033 mg/kg at day 7 and gradually declining to 0.007 mg/kg on day 10 and below quantification limit (BQL) at day 14 (Table 1). At full recommended dose the brew contained 0.644 mg/kg on day 0, gradually declining to 0.010 mg/kg on day 10 and BQL at day 14. The brew free part contained 2.722 & 0.367 mg/kg and 28.670 & 0.468 mg/kg at day 0 and 14, respectively after application of half and full recommended doses, respectively (Table 1).

Table 1. Residual fenvalerate in brew part and brew-free part of black tea

Fenvalerate Residues in ppm (Mean± SD)				
	Brew Part		Brew-free Part	
Days	T_1	T_2	T_1	T_2
0	0.189 ±0.013	0.644 ± 0.103	2.722± 0.533	28.670± 0.934
1	0.157 ±0.001	0.356 ± 0.012	2.333 ± 0.162	24.255 ± 0.737
3	0.133 ± 0.177	0.192 ± 0.004	1.538± 0.071	20.409 ± 0.662
5	0.097 ±0.001	0.111 ±0.020	1.250±0.094	15.298±0.220
7	0.033 ±0.001	0.049 ±0.002	0.787±0.027	5.616±0.0.626
10	0.007 ± 0.001	0.010 ± 0.001	0.598± 0.021	2.125± 0.064
14	BQL*	BQL	0.367±0.015	0.468±0.627

T_1= Half of recommended dose; T_2= Recommended dose; BQL = Below Quantification Limit

In the present study, recovery of fenvalerate in brew part was 6.56% only, which is below acceptable limit, whereas ethyl acetate extract of brew free residue part gave 90.04% recovery. Thus, the total recovery was 96.60±0.036% which is reasonable and acceptable for an analytical method. Solubility of fenvalerate in water is low (0.002 mg/L) and most of the pesticide residue remained in the brew free residue part[6]. Normally tea is subjected to infusion prior to consumption, but in this subcontinent i.e. India, Pakistan, Sri-Lanka and Bangladesh, tea leaves are boiled for hours and served with milk and sugar to get concentrated liquor. So, if the tea is boiled for hours, more residues might come into the liquor and that should be avoided. The FAO recommended maximum residue limit (MRL) of fenvalerate on tea is 0.1 ppm. When fenvalerate was applied in tea at half of the recommended dose, residue in brew was found to be below MRL value on 5 days was 0.097±0.001 ppm (mean & standard deviation) and at recommended dose it was found to be below MRL on 7 days was 0.049±0.002 ppm.

Fenvalerate gradually dissipated from tea leaves following first order kinetics at recommended and half of the recommended doses. This result is also in good agreement of the dissipation of acetamiprid in tea[7]. In brew part, 96.2% dissipation was found when sprayed at half of the recommended dose and it was dissipated up to 84.4% when sprayed at recommended dose at day 10. On the other hand, 86.5% was dissipated at day 14 in brew free part when sprayed at half of the recommended dose and that was 98.3% at the same day sprayed at recommended dose (Figure 1 & 2). The half lives were found to be 2.6 days in brew part and 4.6 days in brew free part which were in agreement with the reported photo degradation fenvalerate on soil surface with half life being 2-18 days[6]. It was also found that fenvalerate, a common pesticide used in tea garden of Bangladesh for protection of tea leaves was degraded quickly and went down to below MRL value (0.1 ppm) within 5 and 7 days after application at half of the recommended and recommended doses, respectively.

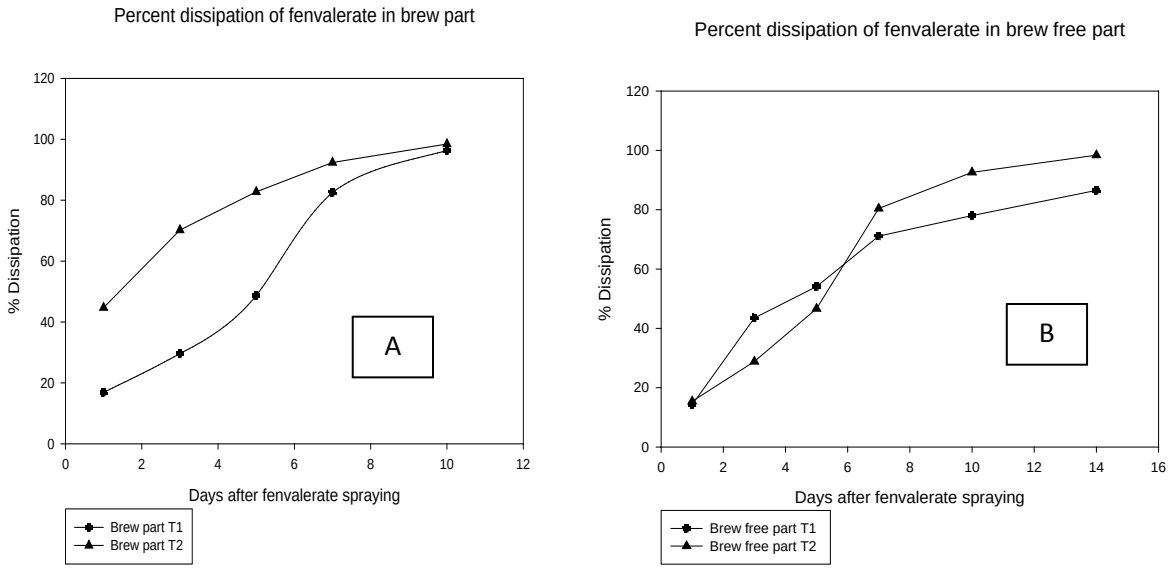

Fig. 1. Percent dissipation of fenvalerate in brew part (A) and in brew-free part (B)

IV. Conclusion

From the present study, it can be concluded that at or after the 7th day of harvest, there was no detectable residue transfer to infusion at the half of the recommended and the recommended dose. Thus, infusion consumption is safe in samples harvested 7 days after fenvalerate treatment.

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