

## Abamectin in Tea and Tea Liquor Under Northeastern Indian Climatic Conditions

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Received: 5 July 2005/Accepted: 12 October 2005

Tea (*Camellia sinensis*) is the second most consumed beverage after water and about one and a half billion cups of tea is consumed daily all over the world. India is the highest producer of tea in the world. Tea is being cultivated mainly in northeast and south India and is the most important cash crop for its export potentialities. The tea crop is attacked by a variety of pests, which multiply rapidly, and cultivators apply a combination of several types of pesticides over larger areas to combat these pests. Red spider mite is one of the major pests in tea cultivation especially in northeast region of India. There are a number of miticides available, out of which ethion, dicofol etc. are causing residual problem in made tea. Abamectin belongs to the family of avermectins, which are macrocyclic lactones produced by the actinomycetes *Streptomyces avermitilis* (Diserens and Henzelin 1999) and have been used in recent years as an insecticide and acaricide with contact and stomach action (Cayrol et al. 1993; Tomlin 1997). Abamectin acts by stimulating the release of  $\gamma$ -aminobutyric acid, an inhibitory neurotransmitter causing paralysis. It has limited plant systemic activity but exhibits translaminar movement. It controls the motile stages of mites, leaf miners, suckers, etc. on various crops and is notably used on apples, oranges, pears, vegetables (cucumbers, tomatoes, etc.) and cotton (Moye et al. 1990; Iskander 1993; Laffi and Raboni 1995; Salas et al. 1997; Diserens and Henzelin 1999; Gonzalez and Barria 1999; Salas et al. 2002; Walunz et al. 2002). Its use is increasing as a replacement for other acaricides, like amitraz, dicofol etc. Based on the above information, a three season field study of abamectin was conducted in northeast India for determination of the dissipation pattern as well as the residue level of abamectin present in made tea and tea liquor.

### MATERIALS AND METHODS

A three seasons [1<sup>st</sup> season, April 2002; 2<sup>nd</sup> season, March 2003; 3<sup>rd</sup> season, September 2003], field experiment on tea (variety TV1) was conducted at Kamalpur Tea Estate, Darjeeling, West Bengal, India. Abamectin (Vertimec 1.9 EC) was applied on tea bushes thrice at an interval of 7 days as high volume spray (400L/ha) by a knapsack sprayer. It was applied @ 5.0 g a.i./ha (recommended dose i.e. T<sub>1</sub>) and 10.0 g a.i./ha (double the recommended dose i.e. T<sub>2</sub>) and untreated control (T<sub>3</sub>) were simultaneously maintained. Each treatment including

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control was replicated thrice in a randomized block designed (RBD). The number of bushes per treatment was 100 and spacing between the bushes was regular double hedge type. Green tea leaves (Two leaf and a bud, 1 kg) were plucked randomly from each treatment replication wise at different time intervals [0 (2 hr after last spraying), 1, 2 and 3 days] and the green tea leaf samples processed for made tea (CTC, 100 g) at Kamalpur Tea Estate factory following standard manufacturing methods.

Made tea (10 g) was homogenized and blended into a 200 mL extraction tube with 100 mL of acetone/water (1/1, v/v). Isooctane (15 mL) was added and contents of the extraction tube were mixed as before. The tube was centrifuged for 10 min at 2000 rpm. After centrifugation the isooctane layer was transferred to a reservoir connected to an alumina SPE cartridge. The isooctane extract was allowed to pass through the cartridge while the sample was re-extracted twice more with isooctane; 3 x 15 mL volumes of isooctane were used in total and passed through the alumina cartridge. The SPE cartridge was washed with 10 mL hexane/ethyl acetate (70/30, v/v) and the analytes were eluted with 8 mL of methanol/ethyl acetate (70/30, v/v). The eluate was collected in a siliconized test tube and evaporated to dryness by rotary vacuum evaporator at 40°C.

The liquor was prepared by adding 10 g of made 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 isooctane.

For derivatisation, 1 mL of methylimidazole/acetonitrile (1/1, v/v) were added to the test tube, which was stoppered and vortexed for 2 min. Trifluoroacetic anhydride/acetonitrile (1/2, v/v) mixture (1.5 mL) were added and the tube was stoppered and vortexed for 1 min.

Final analysis of abamectin residues were done by High Performance Liquid Chromatograph (JASCO) with JASCO FP 1520 fluorescence detector equipped with Chemito 5000 Data Processor. Shandon Hypersil 250 x 4.6 mm ODS 5 $\mu$ m (RPC<sub>18</sub>) column was used for the chromatographic separation of abamectin. The mobile phase consisted of methanol/acetonitrile/water (containing 1% triethylamine and phosphoric acid) 61/30/9 (v/v/v) and the flow-rate was 1 mL/min. The entire system was allowed to stabilise for 15 to 20 min. The fluorescence detector was operated at 365 nm for excitation and 470 nm for emission. Aliquots of 20  $\mu$ L of standard or test portion extract were injected. The retention time, sensitivity and limit of detection were 8.1 min, 0.01  $\mu$ g/g and 0.01  $\mu$ g/g respectively. The average recovery of abamectin in made tea and tea liquor spiked at 0.05 – 1.0 ppm were 92.0 – 93.0% respectively.

## RESULTS AND DISCUSSION

Data regarding the initial deposits, dissipation percentage, half-life values and the regression equation of abamectin residues in made tea and tea liquor following the

application @ 5.0 g a.i./ha ( $T_1$ ) and 10.0g a.i./ha ( $T_2$ ) have been presented in the tables (1-4). Interestingly, the result showed that the residues of abamectin in made tea declined progressively with time irrespective of any dose and season. The initial deposits of abamectin after two hour of spraying were found to be 0.0400 - 0.0430 and 0.0800 - 0.0850 mg/kg irrespective of the seasons for the treatments  $T_1$  and  $T_2$  respectively. It was observed from this study that the dissipation rate was very fast which after 1<sup>st</sup> day dissipated upto 81.19 – 84.63 % irrespective of the dosage applied. The residue level goes to below detectable limit on 2<sup>nd</sup> day for  $T_1$  and 3<sup>rd</sup> day for  $T_2$ . For the Untreated control ( $T_3$ ), no residues of abamectin were detected irrespective of the seasons. The dissipation of abamectin residue followed first order reaction kinetics in all the doses. From this study it appears that the rate of dissipation is independent of initial deposit and the half-life ( $T_{1/2}$ ) values of abamectin in made tea ranged between 0.40 – 0.42 days irrespective of the seasons and application rate. The half-life found in the present study corroborates well with the previous report by Wislocki et al. (1989).

**Table 1.** Persistence of abamectin in made tea for consecutive three seasons at recommended dose.

Season	Days after application	Residue in ppm (M*±S.D)	Dissipation (%)	Regression equation [Half life (days)]
1 <sup>st</sup>	0	0.410±0.0110	-	Y= 1.61-0.76X (0.40)
	1	0.0070±0.0060	82.23	
	2	N.D	-	
2 <sup>nd</sup>	0	0.0430±0.0030	-	Y= 1.63-0.73X (0.41)
	1	0.0080±0.0020	81.19	
	2	N.D	-	
3 <sup>rd</sup>	0	0.0400±0.0080	-	Y= 1.60-0.75X (0.40)
	1	0.0070±0.0020	82.50	
	2	N.D	-	

The residue of abamectin in tea liquor in the zero (0) day sample were ranged between 0.0080 - 0.0100 and 0.0168 - 0.0175 ppm irrespective of the seasons for

the treatments T<sub>1</sub> and T<sub>2</sub> respectively which for the 1<sup>st</sup> day sample were in the range of 0.0085 – 0.0091 ppm for the double recommended dosage applied. The residue goes to below detectable limit on 1<sup>st</sup> day for T<sub>1</sub> and 2<sup>nd</sup> day for T<sub>2</sub>. For the untreated control (T<sub>3</sub>), no residues of abamectin were detected irrespective of the seasons. The MRL value of abamectin has not yet been established in tea. There is no recommended MRL value of abamectin in tea by WHO/FAO. But MRL value of abamectin in different substrates has been fixed in the range of 0.01 - 0.05 ppm by WHO/FAO (2000). As no residue was detected in the 3<sup>rd</sup> day sample it might be stated that abamectin may not pose any residual toxicity problem in tea, which is also befitting with the plucking schedule of Tea Estates of northeastern region of our country.

**Table 2.** Persistence of abamectin in made tea for consecutive three seasons at double the recommended dose.

Season	Days after application	Residue in ppm (M*±S.D)	Dissipation (%)	Regression equation [Half life (days)]
1 <sup>st</sup>	0	0.0820±0.0100	-	Y= 1.8917-0.755X (0.40)
	1	0.0126±0.0000	84.63	
	2	0.0025±0.0003	96.59	
	3	N.D	-	
2 <sup>nd</sup>	0	0.0800±0.0270	-	Y= 1.8733-0.710X (0.42)
	1	0.0129±0.0070	83.86	
	2	0.0030±0.0010	96.17	
	3	N.D	-	
3 <sup>rd</sup>	0	0.0850±0.0070	-	Y= 1.9017-0.725X (0.41)
	1	0.0132±0.0001	84.48	
	2	0.0030±0.0005	95.89	
	3	N.D	-	

**Table 3.** Residue of abamectin in tea liquor for consecutive three seasons at recommended dose.

Season	Days after application	Residue in ppm (M*±S.D)
1 <sup>st</sup>	0	0.0090±0.0007
	1	N.D
2 <sup>nd</sup>	0	0.0080±0.0003
	1	N.D
3 <sup>rd</sup>	0	0.0100±0.0020
	1	N.D

**Table 4.** Residue of abamectin in tea liquor for consecutive three seasons at double the recommended dose.

Season	Days after application	Residue in ppm (M*±S.D)
1 <sup>st</sup>	0	0.0175±0.0004
	1	0.0085±0.0005
	2	N.D
2 <sup>nd</sup>	0	0.0168±0.0007
	1	0.0091±0.0010
	2	N.D
3 <sup>rd</sup>	0	0.0171±0.0003
	1	0.0087±0.0006
	2	N.D

M\* = Mean of three replications

ND = Not detectable (<0.01 ppm)

*Acknowledgments.* We are very grateful to M/s Syngenta, Mumbai for sponsoring this research programme and Manager, Kamalpur Tea Estate, Darjeeling, West Bengal for conducting the field experiment.

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