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Persistence Behaviour of Milbemectin in/on Tea Under North-East Indian Climatic Condition

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Abstract A multi-location field trial was conducted under North-East Indian climatic condition viz. Siliguri and Dooars, West Bengal, India during Monsoon 2005 to evaluate the dissipation pattern of Milbemectin formulation (Milbeknock 1% EC) in/on tea field at two application rates (5 and 10 g a.i. ha^{-1}). The quantitative analysis was performed using High Performance Liquid Chromatography (HPLC) with fluorescence detection at 460 nm. Following the first order kinetics the acaricide dissipates with half-life ($T_{1/2}$) value ranges between 4.93–5.28 days and 6.84–10.76 days in made tea samples of Siliguri and Dooars field, respectively.

Keywords Persistence · Dissipation · Milbemectin · Residue

Tea is a popular beverage throughout the world and is valued for its specific aroma and flavor as well as for its health-promoting properties (Yang and Landau 2000). India is one of the major tea-producing countries in the world. Among the factors limiting the quality and quantity of tea production, the role of insect pests is important (Gandhi and Patel 1997). Management of pests in tea plantations largely depends on the use of broad-spectrum synthetic chemical pesticides. Mite is one of the most important insect pest of tea garden causes significant yield losses under North-East Indian climatic condition (FAOSTAT 2004).

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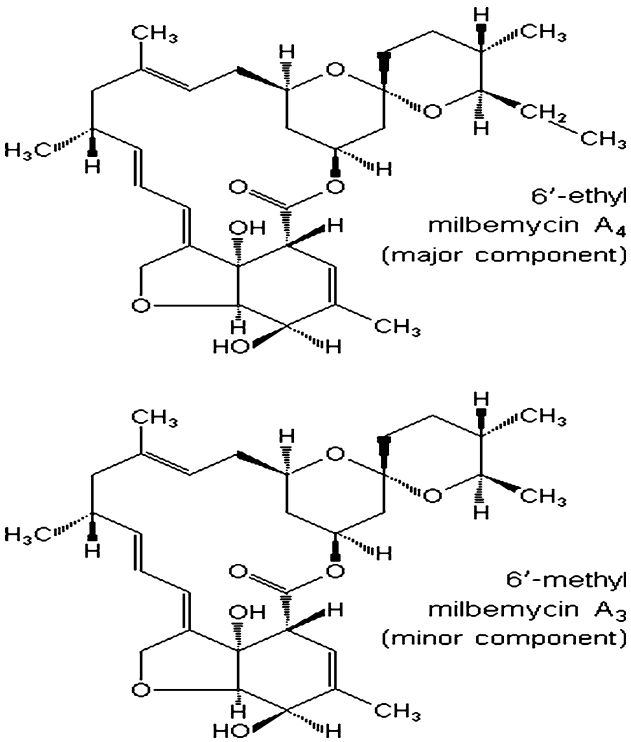
Milbemectin is a member of the Mectin class of insecticide/acaricide. Very recently Milbemectin is being introduced in India as an acaricide. It controls red spider mite (*Oligonychus caffeaeae*), yellow mite, red slugs etc. (Aoki et al. 1994). The target of Milbemectin is r-amino butyric acid (GABA) receptor in the eripheral nervous system. The compound stimulates the release of GABA from nerve endings and enhances the binding of GABA to receptor sites on the post-synaptic membrane of inhibitory motor neurons of mites and other arthropods. Milbemectin consists of two active ingredients of MA_3 [Milbemycin MA_3 (methyl)] and MA_4 [Milbemycin MA_4 (ethyl)] in the ratio of 3–7. The metabolism of Milbemectin in apples, oranges and strawberries has been studied earlier. It was found that the parent molecules (Milbemycin A_3 and Milbemycin A_4) are the only metabolites found at significant levels in plant metabolism studies or in field residue studies under conditions of use, and are the only expected metabolites of toxicological concern in plants. The photolytic metabolites of Milbemectin (8,9Z-M.A_3 and 8,9Z-M.A_4) were not found at toxicologically significant levels in field residue studies (source: The pesticide manual). The objective of the present work was to study the dissipation and fate of Milbemectin in/on made tea grown under North-East Indian climatic condition (Table 1).

Materials and Methods

A multi-location field trial was conducted under North-East Indian climatic condition viz. Siliguri and Dooars during Monsoon 2005. Milbemectin formulation (Milbeknock 1% EC) was applied on tea bushes at 5 g a.i.ha^{-1} (T_1) and 10 g a.i.ha^{-1} (T_2) along with untreated control. In dissipation study, green tea leaf samples were plucked at 0, 1, 5,

Table 1 Physico-chemical properties of Milbemectin

1.	IUPAC name	Mixture of 70% (2a*E*,4*E*,8*E*)-(5′*S*,6*R*,6′*R*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-ethyl-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17-one and 30% (2a*E*,4*E*,8*E*)-(5′*S*,6*R*,6′*R*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydro-20,20b-dihydroxy-5′,6,6′,8,19-pentamethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17-one
2.	Chemical formula	MA_3: $C_{31}H_{44}O_7$; MA_4: $C_{32}H_{46}O_7$
3.	Molecular weight	MA_3: 528.7; MA_4: 542.7
4.	Melting point	213°C
5.	Water solubility (20°C + 1°C)	0.88 mg/L
6.	Vapour pressure at 25°C (mPa)	1.30×10^{-5}
7.	Chemical structure	

Milbemectin consists of two active ingredients of MA_3 [Milbemycin MA_3 (methyl)] and MA_4 [Milbemycin MA_4 (ethyl)] in the ratio of 3–7
Source: The pesticide manual (Tomlin 1997)

8, 15 days after application and processed and manufactured to made tea at tea garden factory. Tea samples (20 g) were collected on each day treatment-wise along with untreated control samples for both the locations.

Made tea sample was extracted with 100 mL mixture of methanol and water (7:3, v/v) followed by filtration through Celite bed and again washed with 50 mL mixture of methanol and water (7:3, v/v). The filtrate was concentrated at rotary vacuum evaporator at 40°C and the extract was chromatographed over silica gel followed by elution with methanol.

The eluate from above procedure was evaporated at ~45°C under reduced pressure and then 1 mL of 0.5 M triethylamine in benzene solution (Previously prepared) and 0.1 mL of trifluoroacetic anhydride were added to it. The mixture was shaken under tightly sealed glass vessel at 40°C for few minutes. After the derivatization little amount of 0.5 M triethylamine (5 µL) was added to each reaction mixture, the reaction mixture was shaken further for few minutes without heating and then concentrated with rotary vacuum evaporator at ~45°C and reconstituted with methanol. Finally the residue of Milbemectin was analysed by HPLC coupled with fluorescence detector. The similar analytical procedure was followed by Kimihiko et al. 2004 and Nagata et al. 2003.

The quantitative analysis was performed using High Performance Liquid Chromatography (Jasco – Japan Model PU 1580) with fluorescence detection at 460 nm.

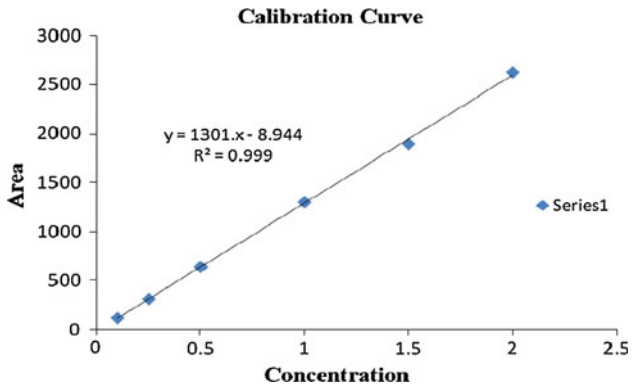


Fig. 1 Calibration curve of analytical standard of Milbemectin ($MA_3 + MA_4$)

Equipped with Thermo Hypersil ODS column (250 × 4.6 m) using Methanol: Water (98:2, v/v) as mobile phase. The limit of detection (LOD) and limit of quantification (LOQ) were found 0.10 and 0.25 μg/g, respectively. A calibration curve (Fig. 1) was prepared by plotting different concentration (0.10–2.0 μg/g) of the standards injected versus peak area observed and the curve was found linear up to the lowest concentration range of 0.10 μg/g.

The efficiency of extraction and clean-up method was checked by recovery study, by fortifying the made tea samples with different levels of analytical standard solution of Milbemectin The recovery percentage of Milbemectin from made tea samples of Siliguri and Dooars were 86.33% and 86.67%, respectively.

Table 2 Dissipation of Milbemectin ($MA_3 + MA_4$) in made tea of Siliguri, West Bengal

Days after application	Treatment	Residues in μg/g				Dissipation (%)
		R_1	R_2	R_3	Mean ± SD	
0	T_1	1.65	1.47	1.39	1.50 ± 0.11	–
1		1.26	1.22	1.18	1.22 ± 0.03	18.67
5		1.12	1.10	1.07	1.09 ± 0.02	27.33
8		0.40	0.37	0.45	0.41 ± 0.03	72.67
15		BDL	BDL	BDL	–	–
0	T_2	6.38	6.61	5.28	6.09 ± 0.58	–
1		4.03	3.88	3.29	3.73 ± 0.32	38.75
5		3.01	2.61	2.35	2.65 ± 0.27	56.48
8		2.04	1.79	1.72	1.85 ± 0.13	69.62
15		BDL	BDL	BDL	–	–
T_1: $Y = -0.061x + 3.194$				T_2: $Y = -0.057x + 3.711$		
$T_{1/2} = 4.93$ days				$T_{1/2} = 5.28$ days		

BDL below detection limit

Table 3 Dissipation of Milbemectin ($MA_3 + MA_4$) in made tea of Dooars, West Bengal

Days after application	Treatment	Residues in μg/g				Dissipation (%)
		R_1	R_2	R_3	Mean ± SD	
0	T_1	3.34	3.44	3.46	3.41 ± 0.05	–
1		2.46	2.57	2.73	2.58 ± 0.11	24.34
5		2.08	2.20	2.39	2.22 ± 0.13	34.89
8		0.93	1.39	1.68	1.33 ± 0.30	60.99
15		BDL	BDL	BDL	–	–
0	T_2	7.14	6.08	6.61	6.61 ± 0.43	–
1		5.13	5.88	5.50	5.50 ± 0.31	16.79
5		4.28	4.48	4.27	4.34 ± 0.09	34.34
8		3.61	3.78	3.84	3.74 ± 0.09	43.42
15		BDL	BDL	BDL	–	–
T_1: $Y = -0.044x + 3.509$				T_2: $Y = -0.028x + 3.794$		
$T_{1/2} = 6.84$ days				$T_{1/2} = 10.76$ days		

BDL below detection limit

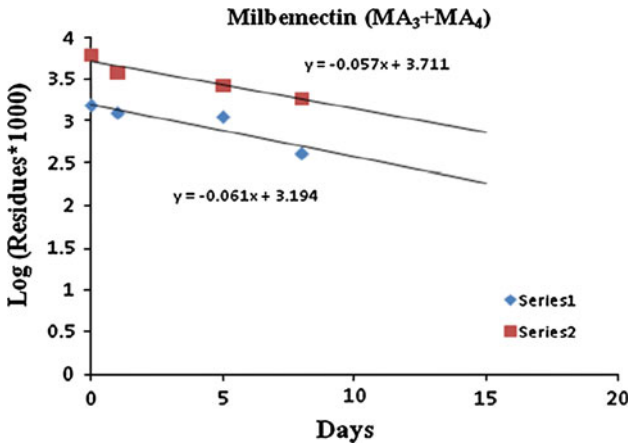


Fig. 2 Linear plot of dissipation of Milbemectin in made tea of Siliguri Garden

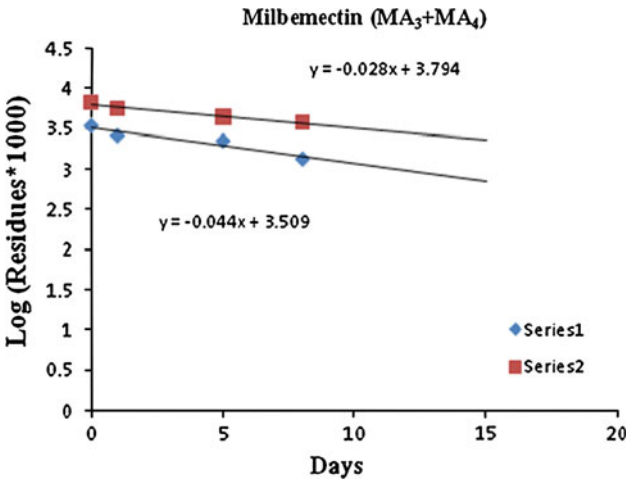


Fig. 3 Linear plot of dissipation of Milbemectin in made tea of Dooars Garden

Results and Discussion

The results of field study of persistence of Milbemectin in made tea samples has been summarized in Table 2 (For Siliguri) and Table 3 (For Dooars). It was found that, in both the cases the residues gradually decreased with time following 1st order kinetics. The initial deposits of Milbemectin ($MA_3 + MA_4$) in made tea of Siliguri garden were found 1.50 µg/g (T_1) and 6.09 µg/g (T_2) and the half-life values ($T_{1/2}$) were found to be 4.93 days (T_1) and 5.28 days (T_2). In case of Dooars garden the initial deposits of Milbemectin ($MA_3 + MA_4$) in made tea sample were found 3.41 µg/g (T_1) and 6.61 µg/g (T_2) and the half-life values ($T_{1/2}$) were found to be 6.84 days (T_1) and 10.76 days (T_2). The dissipation patterns as well as half-life values derived in the present study are more or less similar with the earlier results reported by Omata (1997). More than 60% of the initial deposit of Milbemectin was dissipated within 8 days irrespective of any doses and locations (Figs. 2, 3).

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