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Metabolism of Fenazaquin, an Acaricide in Tea Plant

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India is one of the major tea producing countries and earns much foreign exchange by exporting this commodity. In recent years, it has been observed that the income of foreign currency through tea export is declining and this may be probably due to loss of tea production to a great extent. Red spider mite i.e. *Oligonychus coffea* is becoming one of the major pest problems in tea plantation especially in Terai and Dooars region of West Bengal. The wide spread use of pesticides has now become an established practice in the field of agriculture all over the world in the control of pests attacking cultivated plants. At the same time they leave toxic residues of the parent molecule along with several toxic and non-toxic metabolites in soil, water, crop and grains. Various new molecules are extensively used around the world in the control of plant feeding mites. Recent studies has established that fenazaquin (I) [4-t-butylphenethyl quinazoline-4-yl-ether], a new acaricide with an unique chemical configuration consisting of a quinazoline moity (Fig. 1), is highly effective against red spider mite. It has excellent contact activity against *tetranychid* and *eriophyid* mites (*Eutetranychus*, *Panonychus*, *Tetranychus* spp) on cotton, stone and pome fruits, citrus, grapes and ornamental (Laffia and Raboni 1995). It is being used as an ideal mite management tool. It inhibits mitochondrial electron transport at site I of the mitochondrial respiratory chain (Hollingworth *et al.* 1994). To date, there is no information regarding the metabolism of fenazaquin in tea leaves. The purpose of the present study was to investigate the residues and dissipation pattern of fenazaquin in green tea leaves and also to characterize the metabolites formed in tea leaves.

MATERIALS AND METHODS

The field experiment was laid out at Kamalpur Tea Estate (Terai Region), Darjeeling, West Bengal in April-2000 using TV-23 variety and the laboratory study was conducted at Pesticide Residue Laboratory, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India. Fenazaquin (10% EC) was applied to tea bushes, at the recommended doses 125 g.a.i/ha (T_1) and double the recommended doses 250 g.a.i./ha (T_2) along with an untreated control. For each treatment 50 no. tea bushes were selected with three replication and 500 g sample was taken from each group and formed a single sample for analysis. Tea leaves were plucked at different time intervals [0,3,7 and 14 days] after application of the chemical.

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Green tea leaves were first sequentially washed with hexane : chloroform (1:1 v/v, wash-1) and methanol (wash-2) and the washed tea samples were finally extracted with acetonitrile : water (9:1, v/v) in a Remi Automix blender. The acetonitrile : water extract was purified by partitioning into dichloromethane (100 + 50 + 50 mL) after adding 5% sodium bicarbonate solution followed by concentration to a minimum volume by rotary vacuum evaporation at 40°C. All three fractions after extraction were subjected to column chromatography over a mixture of florisil and charcoal (19:1). The column was prepared using hexane and eluted successively with the following solvents i) dichloromethane (100 mL) (fraction-A); ii) dichloromethane : ethylacetate (200 mL) (fraction-B) and iii) dichloromethane : ethylacetate (1:1) (200 ml) (fraction-C). All the eluates were evaporated to dryness by rotary vacuum evaporation at 40°C and the final volume was made with acetonitrile for analysis. The analysis of fenazaquin residue in tea leaves was by HPLC using a Hewlett Packard Model 1050 equipped with a UV/VIS variable detector, set at λ_{max} 214 nm, coupled to a HP 3392A integrator. The column used was reversed phase Hypersil (ODS) of Shandon HPLC, UK (μ Bondapak C_{18}; 250 × 4.6 mm i.d.) and the mobile phase was acetonitrile (100%) at a flow rate of 0.6 ml/min. The retention time (RT) of fenazaquin was 5.50 min.

Isolation and identification of different metabolites of fenazaquin formed in tea leaves were achieved by gas chromatographic analysis of tea samples followed by GC-MS analysis. For gas chromatographic analysis, the glass column DB-5 (30 cm length with 0.33 i.d) was used equipped with flame ionization detector coupled to a HP 3392A integrator. The initial temperature was 150°C and the final temperature 240°C. Flow rate of nitrogen, hydrogen and air were 30 mL/min, 150 mL/min and 150 mL/min respectively. GC-MS spectra were obtained on Shimadzu QP 2000 (70 eV) and the GC conditions were as follows : an ULBON HQ-1 equivalent to OV-1; fused silica capillary (0.24 mm x 12.5 m) with film thickness 0.25 μm, temperature programme - 100°C for 6 min increased at 10°C/min to 250°C.

RESULTS AND DISCUSSION

The residues of fenazaquin in tea samples at different days interval are presented in Table 1. The majority of fenazaquin residue associated with green tea leaves was removed by washing with methanol (Wash-2). It is evident from the table that the initial deposits of fenazaquin was found to be 2.56 ppm for T_1 and 4.75 ppm for T_2. Afterwards, there was sharp decline in the fenazaquin concentration for both the doses. In the 3rd day after application, the residue of 1.05 ppm for T_1 and 2.86 ppm for T_2 were obtained, thus representing a loss of 59% and 40% respectively. At 7 days after application 94% fenazaquin had dissipated for T_1 and for T_2, the dissipation was 89%. No fenazaquin was detected at 14 days after application. Thus, the molecule was found to persist only upto 7 days after application in tea leaves. The dissipation rate followed first order kinetics irrespective of any treatment and the calculated half-life ($T_{1/2}$) from regression equation in tea leaves were found to be 1.70 days for T_2 and 2.16 days for T_2.

Hexane chloroform (1:1) washed samples and also acetonitrile : water (9:1) extract of tea leaves did not give any fenazaquin residue irrespective of any doses.

The gas-chromatographic analysis of tea samples with flame ionization detector revealed that there were some diagnostic peaks with significant area and different RT values observed in hexane : chloroform (1:1) washed samples and in acetonitrile : water (9:1) extracted samples at 7 days after application and thus indicated the formation of some metabolites, as these peaks were absent in untreated tea samples. The above samples individually were chromatographed over silica gel and it resulted number of fractions which does not give rise any pure compound. The individual fractions were subjected to GC-MS analysis and from a careful study of GC-MS analytical data (Table 2), three metabolites (F_1, F_2 & F_3) of fenazaquin were characterized in tea leaves.

The metabolite F_1 was identified from the fraction of benzene : ethyl acetate (7:3) of acetonitrile : water (9:1) extract samples. The mass spectrum analysis of F_1 (Table 2) gave the molecular ion peak (M^+) at m/z 146 along with other structurally informative peaks at m/z 145 (M^+-$H^\cdot$), 129 (M^+-$OH^\cdot$) and m/z 91 (for tropilium ion formed). Hence, the structure of this compound could be characterized as 4-hydroxy quinazoline (II) (Fig.1). The metabolite F_2 was obtained from the fraction of hexane : benzene (1:1) of hexane : chloroform (1:1) wash samples. The mass spectrum of this compound (Table 2) showed a molecular ion peak (M^+) at m/z 178 from which several other peaks originated viz. m/z 163 (M^+-$CH_3^\cdot$), 147 (m/z 163-$OH^\cdot$ + $H^\cdot$), 105 [m/z 147-$(CH_3)_2C^\cdot$] and 91 (m/z 105-$CH_3^\cdot$ + $H^\cdot$). Thus, the structure of this metabolite was elucidated as β-phenyl (*p-tert*-butyl) ethyl alcohol(III) (Fig. 1). The metabolite F_3 was isolated from the fraction of hexane : benzene (1:1) of hexane : chloroform (1:1) wash samples. The mass spectrum analysis of F_3 (Table-2) exhibited a molecular ion peak at m/z 160 (M^+) along with other significant peaks at m/z 145 (M^+-$CH_3^\cdot$), 131 (m/z 145-$CH_3^\cdot$ + $H^\cdot$), 117 (m/z 131-$CH_3^\cdot$ + $H^\cdot$) and 91 (m/z 117-$C_2H_3^\cdot$ + $H^\cdot$). Hence the structure of this compound could be constituted as *p-tert*-butyl vinyl benzene (IV) (Fig. 1).

It is an interesting point that all these three metabolites were also identified from sunlight and UV irradiated aqueous methanolic and aqueous isopropanolic solution of fenazaquin (Bhattacharyya *et al.* 2003). The major degradation products are formed as a result of breakage of the ether bridge linking the quinazoline and phenyl ring system of the molecule and this leads to the formation of two metabolites F_1 and F_2. Metabolite F_2 on dehydration leads to the formation of the other metabolite F_3. Therefore, from the present study we may concude that fenazaquin rapidly undergoes metabolism in green tea leaves and metabolism was influenced by photolight.

Table 1. Persistence/Dissipation of Fenazaquin in Green tea leaves at different intervals.

Washing solvent	Days after application	Treatment	Residue in ppm* (% dissipation)	Regression equation	$T_{1/2}$ (days)
Methanol	0	125g.a.i/ha T_1	2.56	Y=3.455 -0.177 X	1.70
	3		1.05(59)		
	7		0.15(94.2)		
	14		N.D.		
	0	250g.a.i/ha T_2	4.75	Y=3.742 -0.139 X	2.16
	3		2.86(39.8)		
	7		0.52(89.1)		
	14		N.D.		

* Average of three replicates

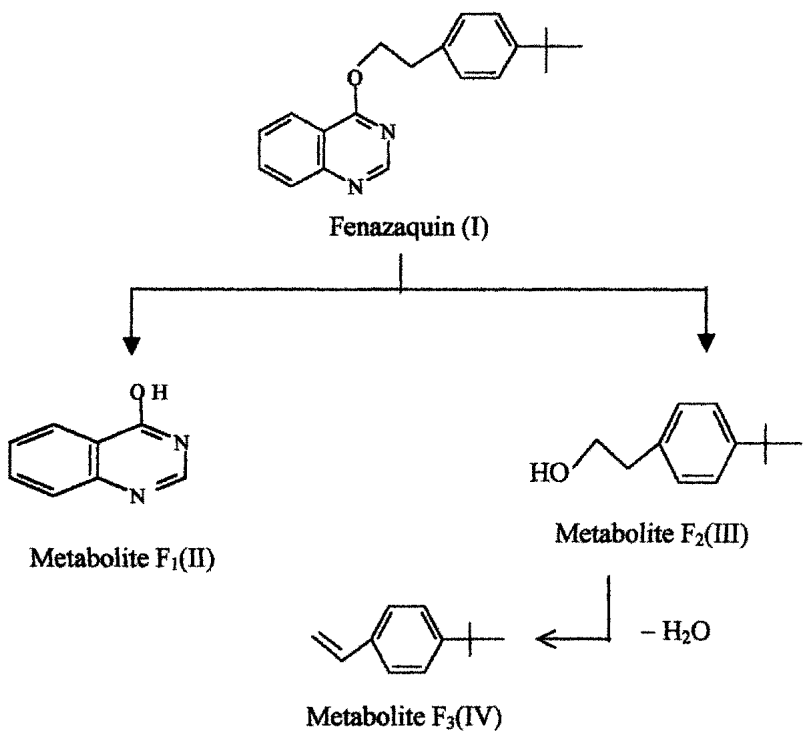

Figure 1. Structure of Fenazaquin and metabolites identified in tea leaves.

Table 2. Mass spectral data of metabolites of fenazaquin in tea leaves.

Metabolite	Mass found	Structure
F_1	146	M^+
	145	$M^+ - H^{\cdot}$
	129	$M^+ - OH^{\cdot}$
	91	tropilium ion
F_2	178	M^+
	163	$M^+ - CH_3^{\cdot}$
	147	$M^+ - CH_3^{\cdot} - OH^{\cdot} + H^{\cdot}$
	105	$M^+ - CH_3^{\cdot} - OH^{\cdot} + H^{\cdot} - (CH_3)_2C^{\cdot}$
	91	$M^+ - CH_3^{\cdot} - OH^{\cdot} + H^{\cdot} - (CH_3)_2C^{\cdot} - CH_3^{\cdot} + H^{\cdot}$
F_3	160	M^+
	145	$M^+ - CH_3^{\cdot}$
	131	$M^+ - CH_3^{\cdot} - CH_3^{\cdot} + H^{\cdot}$
	117	$M^+ - CH_3^{\cdot} - CH_3^{\cdot} + H^{\cdot} - CH_3^{\cdot} H^{\cdot}$
	91	$M^+ - CH_3^{\cdot} - CH_3^{\cdot} + H^{\cdot} - CH_3^{\cdot} + H^{\cdot} - C_2 H_3^{\cdot} + H^{\cdot}$

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