Research Article *Journal of Plantation Crops, 2012, 40 (1): 23-27*

Assessment of resistance level in tea red spider mite, *Oligonychus coffeae* against certain acaricides

A. Roobak Kumar[1], A. Babu[2]*, M. Sankara Rama Subramaniam[1] and P. Kumar[1]

[1]*UPASI Tea Research Foundation, Tea Research Institute, Nirar Dam B.P.O., Valparai 642 127, Coimbatore District, Tamil Nadu.*

[2]*Tea Research Association, Tocklai Experimental Station Jorhat - 785 008, Assam*

(Manuscript Received: 14-10-11, Revised: 03-01-12, Accepted: 08-02-12)

Abstract

The level of resistance in red spider mite populations infesting tea to acaricides like, fenpropathrin, ethion and dicofol was assessed. Field collected red spider mites from three different tea growing regions of southern India were subjected to dose mortality studies to obtain the median lethal concentration of the acaricides and the resistance ratio of susceptible and resistant populations. The resistance ratio ranged from 1.96 to 2.13, 1.08 to 1.11 and 1.11 to 1.26 fold for fenpropathrin, ethion and dicofol, respectively. The general esterase activity with α- naphthyl acetate as substrate also showed increased activity (1.78 to 2.53 fold) in field collected populations than the laboratory reared mites (without exposing to any acaricides) for more than 20 generations. Esterase activity in Poly Acrylamide Gel Electrophoresis (PAGE) profile also indicated a higher level of resistance in mites collected from the Nilgiris region compared to that of Central Travancore and the Anamallais region.

Keywords: Esterase, *Oligonychus coffeae*, pesticides, red spider mite, resistance, tea

Introduction

The red spider mite (RSM), *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) is a serious pest of tea (*Camellia sinensis* (L.) O. Kuntze) in south India and it caused considerable crop loss (Muraleedharan *et al*., 2005). Infestation by *O. coffeae* starts along midrib and veins initially and gradually spreads to the entire upper surface of leaves. As a result of feeding, the maintenance foliage turns ruddy bronze, making red spider mite infested fields distinct even from distance and severe infestation leads to defoliation. The impact of red spider mite on tea, has led the tea growers to use acaricides extensively in order to keep their field unblemished. The high reproductive potential and the shorter lifecycle of mite with frequent acaricide applications, facilitate resistance build-up. Several studies have been carried out in other mite species for acaricide resistance in the recent years (Kim *et al*., 2004; Ay and Gurkan, 2005; Van Leeuwen *et al*., 2006; Van Leeuwen and Tirry, 2007). Hitherto, there is no such report on the development of resistance to the acaricides in red spider mite infesting tea in southern India. However, of late, this pest could not be managed by the application of such acaricides. Hence, the present study has been focused on assessing the level of resistance developed in RSM populations due to the continuous use of different classes of acaricides like fenpropathrin, ethion and dicofol which requires a demand for new molecules with novel modes of action for better management.

Materials and Methods

Acaricides

The acaricides used were commercial formulations of ethion (Fosmite 50 % EC, PI Industrial Ltd., Udaipur), dicofol (Colonel-S 18.5%

*Corresponding Author: azariah.babu@gmail.com

EC, Indofil Chemicals Company, Mumbai) and fenpropathrin (Meothrin 30% EC, Sumitomo Chemicals India Pvt. Ltd. Hyderabad). The fenpropathrin and dicofol are sodium channel modulator and electron transport inhibitor respectively, while ethion is acytylcholinesterase inhibitor.

Spider mite populations

Red spider mites were collected from unsprayed tea field in the Nilgiris (Coonoor) (organic tea garden) during 2007 and a reference laboratory susceptible population (SP) was maintained by transferring onto one year old potted tea plants kept in a green house at 25 ± 1°C and 75± 5% RH which served as a stock culture. From this stock, RSM adults were transferred onto fresh tea leaf (6 cm^2) placed on moistened cotton pads (ca. 1.5 cm thick) in plastic trays (42 x 30 x 6.5 cm) for various laboratory bioassays. Rearing trays were kept under controlled conditions where temperature was maintained at 25 ± 1°C, 75 ± 5% RH and 16L : 8D photoperiod. Withered and drying leaves were regularly replaced. Similarly, mites were also collected from conventional tea gardens of Nilgiris, Anamallais and Central Travancore and were cultured separately in the laboratory. Synchronized cultures of *O. coffeae* were produced from each of the three field collected populations as well as the laboratory reared susceptible population (control) and were used for all laboratory bioassays.

Toxicity bioassay

The selected acaricides were simultaneously tested against the susceptible population of red spider mite and field collected red spider mites. For laboratory bioassay, mature tea leaves were collected from the field and leaf discs of 2 cm diameter were cut from whole leaves and placed on moist cotton in a Petri dish (9 cm dia.). Laboratory bioassays were carried out on adult females of RSM. Ten adults (less than 48 h old) were transferred directly from the colony with a fine brush onto each leaf disc. Leaf discs with RSM were sprayed with a constant quantity of spray solution for 5 s with the respective acaricides using a glass atomizer (50 ml, Vensil). Leaf discs sprayed with distilled water served as untreated control. The leaf discs were left to dry for 30 minutes and then placed in a climatic chamber at 25 ± 1°C, 75 ± 5% RH and a 16L: 8D photoperiod. Mortality of adults was observed after 24 h using binocular stereomicroscope (Olympus No.1220) with 10x magnification. Death of individual mites was confirmed by touching each mite with a fine brush and mites that were unable to walk at least a distance equivalent to their body length were considered as dead. Mortality tests were done before each experiment to determine a range of concentration that would produce 10-95% mortality. All experiments were conducted with five replicates of a five concentration design with distilled water as control. Pooled data were subjected to probit analysis (SPSS 10) and LC_{50}, LC_{60} and LC_{90} at 95% CL were estimated. The LC_{50} of the field collected populations was compared with that of laboratory reared population.

Estimation of esterase activity

Esterase assays were performed according to the method developed by Stumpf and Nauen (2002). Hundred adult females were homogenized in 500 µl ice-cold 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1% (w/v) Triton X-100, was diluted 10-fold and used as the enzyme source. Twenty five micro liter aliquots (0.5 mite equivalents) were added to the centrifuge tube, containing 25 µl of 0.2 M sodium phosphate buffer, pH 6.0. Wells with buffer alone served as a control for the non-enzymatic reaction. The assay was started by adding 200 µl of substrate solution to each well, giving a final volume of 250 µl. Substrate solution consisted of 15 mg of fast Blue BB salt dissolved in 25 ml of sodium phosphate buffer, pH 6.0, and 250 µl of 100 mM α-naphthyl acetate in acetone. Esterase activity was measured continuously at 450 nm and 25º C in a UV- visible spectrophotometer (LABINDIA UV 3000^+) in kinetic mode for 10 minutes. The amount of protein in the enzyme source was determined according to the original procedure of Bradford (1976). The formation of the 1-naphthol-Fast Blue RR dye complex was measured at 500 nm and converted to specific activity using a standard curve of 1-naphthol and Fast Blue BB (Van Leeuwen *et al*. 2006).

Gel electrophoresis

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was done according to the procedures of Walker (1994) and Goka & Takafuji (1992). The gel system was 1mm thick, 106 mm long and 101 mm wide (Hofer mini VE, Amersham Biosciences, USA). The concentrations of acrylamide monomer were 7.5% in separating gel and 3.5% in stacking gel. Electrophoresis was carried out at a constant current of 70V for 3 h at 4°C. Undefined esterases were stained by placing the gels for 1 hour on 0.4% (w/v) Fast Blue BB salt after incubation for 30 minutes in 0.02% (w/v) of α-napthylacetate in 0.2 M phosphate buffer (pH 6.5) containing 1% acetone. All the stained gels were quenched in 7.5% glacial acetic acid. Five mites from each population were used for the analysis of esterase enzyme pattern.

Statistical analysis

The percent mortality was calculated using the "Abbot's correction analysis" (Abbott, 1925). Pooled data were subjected to probit analysis and LC_{50} with 95% CL were estimated using SPSS software. Resistance ratio (RR) was calculated by substituting the LC_{50} value of the resistant strain and LC_{50} value of the susceptible strain in the following formula. Resistance factor (RF) = LC_{50} or LC_{90} of field collected population / LC_{50} or LC_{90} of susceptible population (Ay and Yorulmaz, 2009). Results of esterase activity were subjected to analysis of variance (ANOVA) and means were separated by Turkey's test

Results and Discussion

Results on the level of resistance of field collected and laboratory cultured (without exposing to acaricides) red spider mites to acaricides are presented in Table 1. The resistance levels ranged between 1.96 to 2.13, 1.08 to 1.11 and 1.11 to 1.26 fold for fenpropathrin, dicofol and ethion, respectively. Among the different acaricides tested, the mites showed more resistance towards fenpropathrin compared to other two acaricides which may be attributed to the extensive use and repeated application of the same over a long period of time that resulted in the rapid development of resistance. The development of resistance in insects and mites to insecticides was mainly induced by the frequency of insecticide application (Ay and Yorulmaz, 2009).

Data on the variation of general esterase activity obtained with α - naphthyl acetate as substrate in susceptible population (SP) and field collected population of red spider mites from different tea growing regions are given in Table 2. Esterase activity was significantly higher in RSM population collected from the Nilgiris region followed by Central Travancore, the Anamallais and laboratory reared mites. The present study indicated that higher

Table 1. Concentration probit- analysis of acaricides on the susceptible population (SP) and field collected populations of *Oligonychus coffeae*

Acaricide	Population	Slope ± SE	LC_{50} (ml/L) (95% CI)[a]	RR[b]	LC_{90} (ml/L) (95% CI)	RR
Fenpropathrin	Control	3.86 ± 0.63	0.23 (0.10-0.29)	-	0.45 (0.39-0.57)	-
	The Anamallais	3.88 ± 0.40	0.45 (0.42-0.48)	1.96	0.78 (0.71-0.87)	1.73
	The Nilgiris	3.49 ± 0.40	0.48 (0.45-0.52)	2.09	0.85 (0.77-0.97)	1.89
	Central Travancore	3.30 ± 0.39	0.49 (0.44-0.56)	2.13	0.92 (0.78-1.21)	2.04
Dicofol	Control	3.04 ± 0.65	2.04 (1.87-2.11)		2.21 (2.14-2.46)	
	The Anamallais	5.06 ± 0.44	2.20 (2.07-2.32)	1.08	2.44 (2.31-2.98)	1.10
	The Nilgiris	3.62 ± 0.40	2.24 (2.15-2.37)	1.10	2.57 (2.42-3.14)	1.16
	Central Travancore	3.26 ± 0.41	2.26 (2.23-2.29)	1.11	2.60 (2.52-2.72)	1.18
Ethion	Control	6.58 ± 0.65	1.46 (1.43-1.48)		1.64 (1.62-1.68)	
	The Anamallais	4.80 ± 0.43	1.62 (1.44-1.76)	1.11	1.87 (1.74-2.62)	1.14
	The Nilgiris	3.53 ± 0.39	1.74 (1.71-1.78)	1.19	2.09 (2.01-2.21)	1.27
	Central Travancore	3.39 ± 0.39	1.84 (1.79-1.89)	1.26	2.22 (2.11-2.39)	1.35

[a] - Confidence interval, [b] - Resistance ratio = LC_{50} of field collected mites/ LC_{50} of control mites

Table 2. Esterase activity of susceptible and field collected populations of red spider mites

Population	Enzyme activity[a]*	R/S[#]
Control	0.061 ± 0.005a	
Anamallais	0.109 ± 0.007b	1.78
The Nilgiris	0.154 ± 0.006d	2.53
Central Travancore	0.142 ± 0.004c	2.32

[a]- Enzyme activity was expressed as nmol 1-naphthol $min^{-1}mg^{-1}$ protein (± SEM). *- Means followed by the same letter are not significantly different at 0.05 levels as determined by Turkey's test. # - enzyme activity of field collected mites / enzyme activity of control mite

esterase activity in RSM populations which might have involved in detoxification of acaricides. Ay *et al*. (2005) also highlighted the variations in esterase enzyme in two spotted spider mite, *Tetranychus urticae* as a result of prolonged application of insecticide and acaricide for the management**.** Sarkar and Mukhopadhyay (2006) reported an enhanced esterase activity in tea mosquito bug, *Helopeltis theivora* (Hemiptera: Miridae) collected from the conventionally managed tea fields.

Tsagkarakou *et al.* (2002) documented the resistance mechanism to the acaricides on the basis of separation of bands using electrophoresis method. The esterase patterns of field collected and laboratory cultured populations of *O. coffeae,* visualized using α -napthylacetate as a substrate also revealed that the number of esterase bands and density which found to vary from the susceptible population (SP). Similarly, Goka and Takafuji (1992) reported the presence of genetic variation in phosphoglucoisomerase (PGI) and malatedehydrogenase (MDH) of red pigmented Japanese spider mites.

In the present study, the analysis of zymograms also revealed the distribution and type of esterase bands showing distinct variation in susceptible and field collected populations, in which the former showed more homogenous esterase band pattern than the later ones. Interestingly, E1 band was detected in all the populations including laboratory reared mites. Similar results were reported in the populations of *T. urticae* also (Ay *et al.*, (2005). On the other hand, esterase band pattern obtained from pesticide exposed populations were more heterogeneous in nature. The esterase bands of field collected populations were much thicker and showed higher staining intensity than the laboratory reared population. For instance, mite collected from Anamallais, Nilgiris and Central Travancore regions showed dark E1 band and an additional E2 band. These bands were completely absent in pesticide unexposed control mites (Fig.1). The enhanced quantity of esterase as well as an additional band in *O. coffeae* from the pesticide exposed population is possibly involved in the detoxification acaricides. Higher esterase activity has been reported in *T. urticae* which are continuously exposed to pesticides (Goka and Takafuji, 1992, 1998). The results of the present study indicated that the RSM population exposed to acaricides over a long period of time might have developed the mechanism of detoxification of acaricides when compared to population of pesticide unexposed mites. Our investigation on general esterase banding pattern brings to our knowledge the level of resistance/ tolerance that has developed in RSM population exposed to similar pesticide applications over a long period of time. Studies on detoxifying enzymes and LC_{50} values of *O. coffeae* have provided base-line

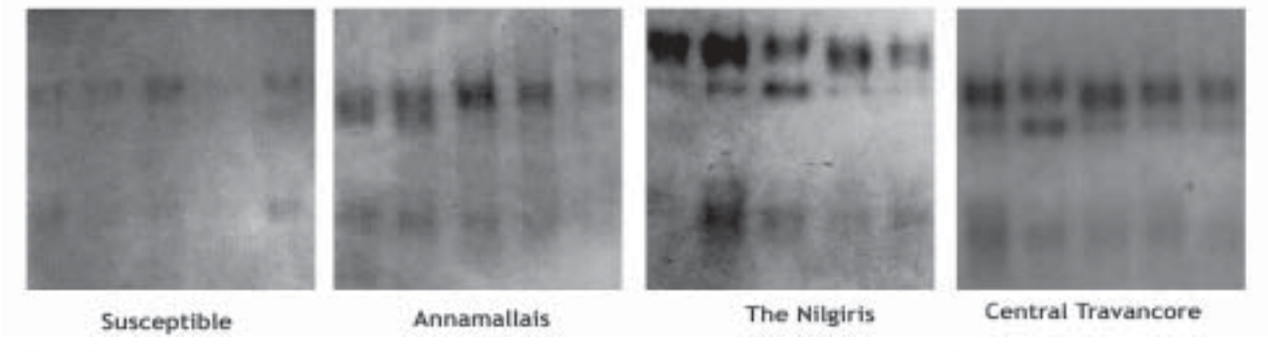

Fig. 1. Different general esterase-zones in the laboratory susceptible population and field collected strains of *Oligonychus coffeae*

information on the level of tolerance/ resistance developed in red spider mites. Esterase enzyme activity plays a role in pyrethroid resistance (Ay and Gurkan, 2005). The results indicated the possibility of resistance development to pyrethroid insecticides, in the mites collected from the Nilgiris and Central Travancore zones, that require newer molecules with different modes of action for effective management.

Acknowledgements

This study was supported by Tea Board, Ministry of Commerce, Govt. of India as XI plan project and authors are also indebted to Dr. P. Mohan Kumar, Director of UPASI Tea Research Institute for providing the facilities and support. Special gratitude is due to Dr. Recep Ay, Suleyman Demirel University, Turkey for critically reviewing the manuscript and the necessary corrections.

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