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SHORT COMMUNICATION

First report on heat shock protein expression in red spider mites (*Oligonychus coffeae*) in response to pesticide exposure

S Roy[1], AK Prasad[2], G Handique[2], A Barua[2], S Roy[2]

[1]Natural Product Chemistry Group, CSIR-North East Institute of Science & Technology, Jorhat, Assam 785006, India
[2]Entomology Department, Tocklai Tea Research Institute, Tea Research Association Jorhat, Assam 785008, India

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Abstract

Red spider mites (RSM) is one of the major pest of tea and reported from all tea producing regions around the world. Chemical acaricides are the primary control method against this pest which induced biochemical changes in the RSM. In this study early expression of heat shock proteins have been observed in RSM exposed to commonly used acaricides viz., ethion, dicofol and fenpropathrin. In case of pesticide exposed RSM, hsp90 showed more prominent bands than the hsp70 in immunoblotted membrane, indicating that the intensity of expression of hsp90 in RSM against pesticides is higher than hsp70. Hsp70 expression is higher in ethion and fenpropathrin induced RSM than that of dicofol. Ethion and fenpropathrin treated RSM were not showing any difference when compared based on exposure time, whereas dicofol treated once showed more expression in short time. The expression of hsp90 is significantly higher in the dicofol treated samples followed by fenpropathrin, however ethion treated sample showed marginally higher hsp90 expression than control.

Key Words: red spider mites; *Oligonychus coffeae;* heat shock protein (hsp); ethion; fenpropathrin; dicofol

Introduction

Tea is currently produced by 30 countries including China, India, Sri Lanka, Kenya, Turkey and Indonesia which contributes major part of the production (Anonymous, 2013). Tea is perennial plantations crop, provides a stable microclimate to thousands of pests which led to 5 - 55 % annual crop loss (Hazarika *et al*., 2009). Red spider mite, *Oligonychus coffeae* Nietner (Acari: Tetranychidae) is one of the major pest of tea and reported from most of the tea-producing countries. It is widely distributed, starting from Afro-tropical region to Australian, Nearctic, Neotropical, Oriental and Palearctic regions in 48 countries and feed on around 133 crops of these regions (Roy *et al*., 2014). In last few decades, it caused a huge damage to tea plantations of North East India (Roy *et al*., 2014). Nymphs and adults of *O. coffeae* lacerate cells, producing minute characteristic reddish brown marks on the upper surface of mature leaves, which turn red in severe cases of infestation, resulting in crop loss. The pest is present on tea all the year round, although numbers vary depending on season and reported to cause 17 - 46 % of crop loss per year (Roy *et al*., 2014). Chemical control is the primary mode of management of *O. coffeae* and a wide range of acaricides belonging to different chemical groups is currently used worldwide to control this pest. The difference in relative toxicity of different commonly used acaricides to *O. coffeae* was observed in plantations of the tea-growing region of India (Roobakkumar *et al*., 2012; Roy *et al*., 2012) as well as of Japan (Gotoh *et al*., 2001). In North East India, resistance in *O. coffeae* populations has been reported to a range of organochlorine (dicofol) and organophosphate (ethion) acaricides (Roy *et al*., 2012).

Insects/mites are known to evolve resistance in about a decade after the introduction of a new pesticide by virtue of their genetic plasticity. The introduced toxicant present in the environment exerts a selection pressure on an insect population and only those individuals that can survive the toxic effect are able to reproduce. Endemic population of a pest usually comprises a variety of

Corresponding author:
Somnath Roy
Entomology Department
Tocklai Tea Research Institute
Tea Research Association
Jorhat, Assam 785008, India
E-mail: somnathento@gmail.com

biotypes with minute differences from one another. Pesticide selection may favour the fittest in the toxic environment. Selection operates at biochemical, physiological, and behavioural level as well. Zhao and Jones (2012) reported that in insects Heat Shock Protein (HSP) expressed in response of various stress factors. Abiotic stress (including temperature, ultraviolet radiation, drought and anhydrobiosis, chemicals and metals), biotic stress (including parasites, pathogens) and developmental regulators and mutants can induce the hsp gene expression. HSP 70 and HSP 90 are also involved in various essential activities such as protein folding, localization and dehydration (King and MacRae, 2015). *Hsp90* genes are also involved in tolerance and resistance not only to temperature but pesticides too. Sun et al. (2014) studied the effect of pesticides on *Apolygus lucorum* (Meyer-Dür) and subsequent expression of *hsp90* gene.

Sarker and Mukhopadhyay (2006), Roobakkumar *et al*. (2012) and Mukhopadhyay *et al*. (2016) reported enhancement of general esterase and glutathione-*S*-transferase as two defense enzymes responsible for development of resistance (biochemical resistance) in *O. coffeae* against acaricides in India. Beside the xenobiotic detoxifying enzymes, synthesis of heat shock proteins could be another phenomenon by which insects respond to any abiotic stress (Zhao and Jones, 2012; *Z*hang *et al*., 2015). In this paper, we compared the expression of hsps in *O. coffeae* exposed to commonly used different pesticides (acaricides) to assess the *O. coffeae* response against the effect of pesticides.

Material and Methods

Chemicals

The primary antibody anti-heat shock protein 70 and anti-heat shock protein 90 (monoclonal antibody, raised in mouse), ammonium persulfate, Coomassie brilliant blue, phenylmethylsulfonly fluoride (PMSF), and Nonidet were procured from Sigma Chemical Co. (St. Louis, MO). The molecular weight marker for polyacrylamide gel electrophoresis was purchased from Genetix Biotech Asia Pvt Ltd. Rabbit anti-mouse IgG linked to alkaline phosphatase was procured from Santacruz Biotechnology, Inc. All other chemicals used were of analytical grade, purchased from Sisco Research Laboratories (Mumbai, India) and E. Merck (Mumbai, India). Acaricides used for induction of hsp are ethion 50 emulsifiable concentrates (EC) (aliphatic organothiophosphate: Tafethion, Rallis India Ltd., Mumbai, India), dicofol 18.5 EC (diphenyl aliphatics organochlorine: Klin XL, Krishi Rasayan India Ltd., Delhi, India) and fenpropathrin 30 EC (fourth generation pyrethroid ester: Meothrin, Sumitomo Chemicals India Pvt. Ltd., Mumbai, India).

Maintenance of red spider mite

A culture of red spider mite was maintained in the laboratory at 25 ± 2 °C and 70 - 80 % RH on a susceptible tea clone, TV1 by following the detached leaf culture method of Roy *et al*. (2010).

Estimation of heat shock protein of 70 and 90 kDa
Preparation of sample

Groups of two hundred adult mites each were taken in mature tea leaves and sprayed with selected acaricides viz., ethion (2500 ppm), dicofol (2500ppm) and fenpropathrin (500ppm) at their recommended dose. The mortality of RSM after 1 h of exposure to dicofol, ethion and fenpropathrin was 0%, 7.5 %, 7.5 % and after 6 h, mortality was recorded as 42.5 %, 65 %, 67.5 % respectively for three acaricides.

The mites treated with water were taken as control. After 1 h and 6 h interval of spraying the treated mites were collected in a centrifuge tube. A 20 % homogenate of the whole body of control and treated red spider mite was prepared in 50mM Tris buffer (pH 7.6) containing 0.1mM PMSF and 1 % Nonidet and centrifuged at 10,000*g* for 10 min. The cytosolic supernatant was collected very carefully and protein content of the sample was measured following the method of Lowry *et al*. (1951).

Methods for western blotting

A 10 µl aliquot of cytosol with a concentration of 5 µg/µl protein, prepared from control and treated mite samples containing total 50 µg protein, was run through a 10 % SDS-PAGE at constant voltage (100V) for 2 h following the method of Laemmli (1970) and blotted on a polyvinylidene fluoride membrane following the method of Sambrook *et al*. (1989) as modified by Roy and Bhattacharya (2006). Western blots were performed according to the method of Sambrook *et al.* (1989) and transfer was carried out for 60 min at 50 V constant. Anti-hsp70 monoclonal antibody raised in mouse (diluted 1:1,000) and Anti-hsp90 monoclonal antibody raised in mouse (diluted 1:1,000) was used as primary antibody and rabbit anti-mouse IgG linked to alkaline phosphatase (diluted to 1:500) was used as secondary antibody. The membrane was washed thoroughly and then incubated in the presence of the substrate (SIGMAFAST™ BCIP/NBT)

Results

Profile of the heat shock protein 70 and 90

The gel obtained from the SDS-PAGE was examined by immunoblotting with anti-hsp 70 monoclonal antibody. Clear bands developed at regions of 70 kDa and 90 kDa respectively against hsp70 and hsp90 antibody. No other additional bands or smear are present on the immunoblot. The immunoblotted membrane of the hsp90 showed more prominent bands than the hsp70, indicating that the intensity of expression of hsp90 in red spider mite against pesticide namely ethion, fenpropathrin and dicofol is higher than hsp70 which is distinctly represented in the graph showing integrated density values (IDV; calculated with the help of Alpha easer software) (Fig. 1). It is also clear that ethion and fenpropathrin induce hsp70 expression remarkably higher than dicofol. The exposure period-wise profile did not show any

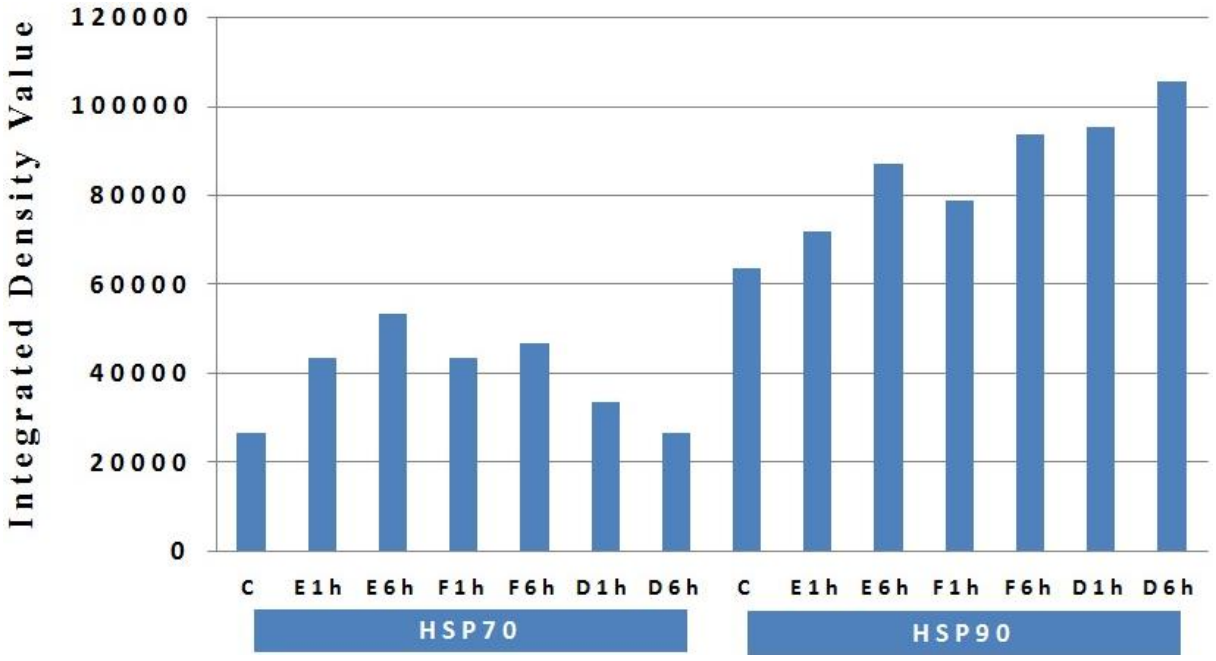

Fig. 1 Graph showing Integrated Density Values of each band of HSP70 and HSP90 developed in PVDF membrane (C: Control, E1 h: after 1 h exposure to ethion, E6 h: after 6 h exposure to ethion, F1h: after 1 h exposure to fenpropathrin, F6h: after 6 h exposure to fenpropathrin, D1h: after 1 h exposure to dicofol, D6h: after 6 h exposure to dicofol).

difference in ethion and fenpropathrin treated samples where as dicofol samples showed more expression in shorter time period. The expression of hsp90 is significantly higher in the dicofol treated samples followed by fenpropathrin (Fig. 2). The level of hsp90 is marginally higher than control in ethion treated samples.

Discussion

Hsps are expressed in numerous tissues from several animal species, and their presence is often associated with a response to a harmful stress situation or to adverse life conditions. The highly conserved nature of hsp 70 and hsp 90 allows the use of a monoclonal antibody raised in mouse to detect the induced hsps in red spider mite. This is the only report of findings from pesticide-induced red spider mite which resemble those described in many other animals, including invertebrate species (Zhao and Jones, 2012).

It is surmised that induction of hsps is an early response against prescribed doses of selected pesticide viz., ethion, dicofol and fenpropathrin. The hsp induction profiles of pesticide treated red spider mite clearly indicate the early response which can be a significant survival strategy of the test species. Information about the induction of hsp in response to repeated or continuous exposures to pesticide is limited. Pesticides, including organophosphate, organochlorine, and pyrethroid compounds, are widely used in agricultural including tea crop. In general, organophosphates are known to inhibit acetylcholine esterase (key enzyme of the cholinergic system, regulating the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine) mostly leading to death of the insect (Stenersen, 2004). In the other hand, organochlorines are GABA-gated chloride channel antagonists and pyrethoid acts as Sodium channel modulators (Stenersen, 2004). All these pesticide classes affect the insect physiology in different way, ultimately causing stress to insect body. Further, Pesticides induce up regulation of detoxifying enzymes which in turn involves in glycolysis, the TCA cycle and oxidative phosphorylation and this will lead to increase energy metabolism to support detoxification and the stress response (Despres *et al.,* 2007) as well as production of ROS (reactive oxygen species) (Du Rand *et al.,* 2015). The ROS will then induce oxidative and heat shock stress responses (Du Rand *et al.,* 2015). Therefore the response of red spider mite to organochlorine (dicofol), organophosphate (ethion) and pyrethroid (fenpropathrin) with respect to hsps is highly significant. It is reported that the induced response of noctuidae *Spodoptera litura* hsp90 to zinc was more sensitive than that of hsp70 (Zhao and Jones, 2012). The observation in this study also demonstrate that the intensity of expression of hsp90 in red spider mite against pesticide namely ethion, fenpropathrin and dicofol is higher than hsp70. Joshi and Tiwari (2000) suggested that a common set of gene loci encoding heat shock proteins is responsive to diverse environmental

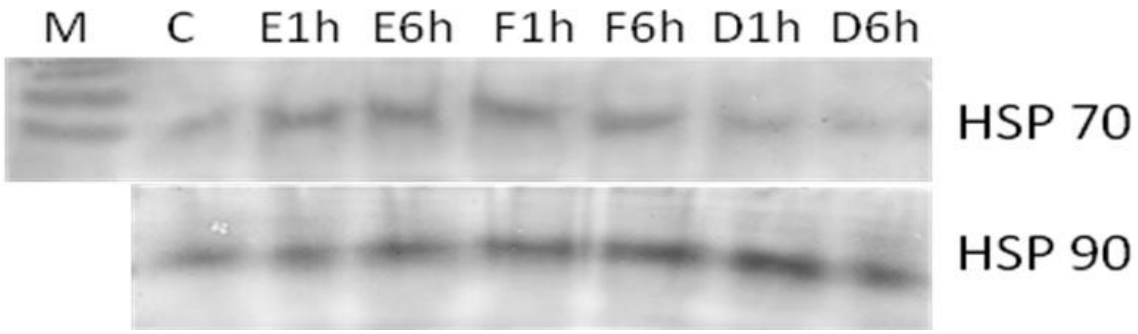

Fig. 4 Expression of HSP bands developed in PVDF membrane

stresses in blowfly *Lucilia cuprina*.

Hsp genes are induced and modulated in insects in response to environmental factors including abiotic and biotic stresses. It may be likely that via *hsp* activity, many pest and beneficial species will be able to adapt to global warming more than previously thought (Zhao and Jones, 2012). The function of *hsp*s and other genes has been recently studied using dsRNA interference (RNAi) knock down techniques (Papaconstantinou *et al*., 2010).

This is the first report of hsp from red spider mites and can be of great importance while contemplating alternate methods of their control where any compound which can suppress gene expression of these hsp proteins can be employed as a new pesticide.

From the present investigation it may be opined that the expression of hsp has a protective role in red spider mite. In future the pesticide-induced stress response can appear as an evident biomarker as indicated in our present study which may provide a great boost to biological control and reducing pesticide use.

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