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Creative Commons-BY-NC-ND which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.
Acaricide susceptibility of *Oligonychus coffeae* Nietner (Acari: Tetranychidae) with corresponding changes in detoxifying enzyme levels from tea plantations of sub-Himalayan Terai, India

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**Abstract** — *Oligonychus coffeae* Nietner is cosmopolitan in its distribution and is an important pest of a number of economically important tropical and sub-tropical crops including tea. It is the most damaging acarine pest of tea crops in the sub-Himalayan Terai region of India which is mostly controlled chemically in the conventionally managed tea plantations of the region. Objectives of the present study were to i) investigate the tolerance level of *O. coffeae* collected from bio-organically managed plantations (BMP) (with no synthetic acaricide application) and conventionally managed plantations (CMP) (with periodic application of synthetic acaricide) to the acaricides, 'ethion' and 'fenpropathrin', ii) Quantify the detoxifying enzymes, general esterases (GE) of phase I and glutathione S-transferases (GST) of phase II, in *O. coffeae* as these are deemed important in acquiring pesticide tolerance, iii) Establish the relation of GE and GST activity levels with acaricide tolerance levels in populations of *O. coffeae*. The study revealed that i) BMP populations of *O. coffeae* were susceptible to both of the acaricides whereas CMP populations were tolerant. CMP populations of the pest showed low to medium tolerance to the organophosphate acaricide ‘ethion’ whereas tolerance to the synthetic pyrethroid, ‘fenpropathrin’ was high; ii) Corresponding GE and GST levels were significantly higher in CMP populations compared to that of BMP population. Electrophoretic analysis of GE isozymes of CMP and BMP populations further corroborated the quantitative study; iii) activity of the detoxifying enzymes, GE and GST were positively correlated with the tolerance level of *O. coffeae* populations indicating involvement of these enzymes in the development of acaricide tolerance.

**Keywords** — *Oligonychus coffeae*; acaricides; bioassay; tolerance level; detoxifying enzymes

**Introduction**

*Oligonychus coffeae* Nietner (Acari: Tetranychidae), commonly known as red spider mite (RSM) is distributed widely over the world. It is found in more than 40 countries spread through Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palaeartic regions of the world (Migeon and Dorkeld, 2006-2015). Being polyphagous, RSM is recorded on at least 130 host plants (Migeon and Dorkeld, 2006-2015; EPPO, 2014). It is considered a serious pest of many agricultural, horticultural and plantation crops besides tea, such as, Jute, mango, grapes, avocado, guava, citrus, strawberry, mulberry, cashew nut, rubber, coffee, cotton etc (Jefferson et al., 1975; Meyer, 1987; Gotoh and Nagata, 2001; CABI and EPPO, 2013). Mites as a group are the most serious and persistent pests of tea in almost all tea producing countries (Cranham, 1966;
Among twelve species of mites recorded on tea, the RSM, *O. coffeae* is the major one (Banerjee, 1988; 1993) in north east India. RSM normally infests the upper surface of mature tea leaves imparting them a reddish bronze colour and impairing their photosynthetic capacity leading to their nutritional deficiency and shedding. If the tea bush is under draught stress, tender leaves may also be attacked. Injury caused by RSM may be frequently followed by pathogenic infections (Light, 1927).

Acaricides play a major role in the management of phytophagous mites. In spite of application of different types of synthetic pesticides, such as, organochlorides, organophosphates and synthetic pyrethroids, the mite pest is becoming increasingly difficult to control. This failure of pest suppression is attributed to the development of pesticide tolerance resulting from repeated and extensive use of acaricides in the tea plantations of sub-Himalayan Terai-Dooars under conventional (chemical) management practices (Das, 1959; Sahoo et al., 2004; Roy et al., 2008b). The high reproductive potential and extremely short life cycle combined with frequent acaricide applications facilitate tolerance/resistance build-up in the mites (Van Leeuwen et al., 2005). A very high level of resistance to several compounds can develop within one to four years of continuous use and can often induce a high degree of cross-resistance (Crannham and Helle, 1985). Roy et al. (2010, 2014) reported and reviewed the tolerance build up against commonly used acaricides in the conventionally managed tea plantations (CMP) of sub-Himalayan Terai and the Dooars regions. Majority of the tea plantations of this region are under conventional management practices which rely on periodic application of different pesticides including synthetic insecticides and acaricides to keep the pest population under control. Bio-organically managed plantations (BMP) are only a few in this region which depends on botanical and microbial formulations instead of synthetic agrochemicals and different cultural practices for managing the pest populations. Ethion (0.0, 0’0’, – Tetraethyl – S.S’ – Methyene Bisphosphorodithioate), an organophosphate, was recommended and used as an acaricide in the tea plantations of sub-Himalayan Terai and the Dooars region of West Bengal even a few years back (Gurusubramanian et al., 2008) while fenpropatrin (*α*-cyano-3-phenoxypybenzyl 2,2,3,3-tetramethyl cyclopropane carboxylate), a synthetic pyrethroid with repellent and contact activities is still recommended (Plant Protection Code (PPC), Tea Board of India, 2014) and widely used to control RSM in the region. Most common types of resistance found in insects and mites are due to increased enzymatic detoxification and target site insensitivity (Oppenoorth, 1984; Scott, 1999; Ay and Yorulmaz, 2010). Objectives of the present study were to i) investigate the acaricide tolerance level of RSM (*O. coffeae*) collected from BMP (with no synthetic acaricide application) and CMP (with periodic synthetic acaricide application) to ethion and fenpropatrin using bioassay method, ii) Quantify general esterases (GE) and glutathione S-transferases (GST) as these are important as phase I and phase II detoxifying enzymes for development of acaricide tolerance in mites, iii) Find the relation of GE and GST activity and acaricide tolerance level of the mite pest.

**MATERIALS AND METHODS**

**Acaricides and Chemicals**

The acaricides used were commercial formulations of the organophosphate, ethion (Ethion® 50% EC, Ankur Industries Private Limited, Kolkata) and a 4th generation synthetic pyrethroid ester, fenpropatrin (Meothrin® 30% EC, Sumitomo Chemicals India Pvt. Ltd., Hyderabad). Bovine serum albumin (BSA), *α*-naphthyl acetate (*α*-NA), *α*-naphthol, 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), Fast Blue BB salt, acrylamide, bis-acrylamide was procured from Sisco Research Laboratory (SRL), Mumbai, India. Solutions of *α*-NA (30 mM) and CDNB (50 mM) were prepared fresh just before use.

**Spider mite populations**

Tea twigs containing RSM were collected from tea plantations of sub-Himalayan Terai region managed bio-organically and conventionally. The
bio-organically managed plantation in the present study, BMP 1 (26°49’N and 88°16’E) is maintained without use of any synthetic acaricides/pesticides at the foothill whereas, the conventionally managed plantations selected for the present investigation, CMP 1 (26°47’N and 88°18’E), CMP 2 (26°52’N and 88°57’E) and CMP 3 (26°54’N and 88°54’E) are maintained using synthetic pesticides periodically is at the plain of Darjeeling district. Tea twigs containing mites were kept immersed at their base in conical flasks containing water to avoid fall in turgidity. The flasks were then kept inside plastic containers, with their mouths covered with fine cloths. Both toxicity bio-assays and detoxifying enzyme analysis were done using female mites. For enzyme analysis adult female RSM were collected in 1.5 ml centrifuge tube and preserved in -20°C for further analysis. Bio-assays were performed with female mites from field-collected stocks after preconditioning them for two days in the laboratory. Collection of the mite samples and bioassays were performed during summer seasons (May – July) of 2013-2014.

**Toxicity bioassay**

The selected acaricides for this study were tested simultaneously against the populations of RSM collected from bio-organically and conventionally managed tea plantations of Terai region. For laboratory bioassay the standard method recommended by the Insecticide Resistance Action Committee (IRAC method no. 4) was used (Reghupathy et al., 2007). Mature tea leaves were collected from the experimental tea garden maintained organically by the Department of Zoology, University of North Bengal. These were washed thoroughly with distilled water and air dried. Then leaf discs of 2 cm diameter were cut from whole leaves. These leaf discs were dipped into different concentrations of acaricides and air dried. After that the treated leaf discs were placed on moist cotton towelling in a petridish to keep them fresh. Ten adult female RSM were transferred from the colony with a fine brush onto each leaf disc. Mites on leaf discs dipped in distilled water (instead of acaricide solutions) served as untreated control. All experiments were conducted with five replicates for each concentration of acaricide. Six such concentrations were tried. Based on percent mortality data lethal concentrations LC$_{50}$ and LC$_{95}$ were calculated using the statistical package for social sciences (SPSS) version 10.0 SPSS Inc., USA, based on probit analysis method (Finney, 1973).

To assess the tolerance/resistance of a given population of RSM, the resistance coefficient (RC) was calculated after Wegorek et al. (2009) as follows: $\text{RC} = \frac{\text{LC}_{95}}{\text{recommended field dose}}$.

The following values of ratios were considered for assessment of resistance level: RC $\leq 1$, lack of resistance; RC $1.1 – 2$, low resistance; RC $2.1 – 5$, medium resistance; RC $5.1 – 10$, high resistance and RC > 10, very high resistance.

**Estimation of detoxifying enzyme activities**

**Enzyme preparation**

Defense enzyme (=detoxification enzyme) (General Esterases, abbreviated as GE and Glutathione S-transferases, abbreviated as GST) activity was measured using adult female mites. Adult female mites were homogenized in ice cold 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 12,000 g for 15 min at 4°C in a high speed refrigerated centrifuge (Sigma 3K30). The resultant post-mitochondrial supernatant was used as the enzyme source for assay of GE and GST activity and to estimate the amount of total protein.

**General Esterase (GE) activity**

General Esterase activity was measured using $\alpha$-naphthyl acetate ($\alpha$-NA) as substrate according to the method of Van Asperen (1962) with minor modifications. Twenty microlitre (µl) of supernatant was taken in each well of the microplate reader (Opsys MR, DYNEX Technologies, Chantilly, VA, USA) in triplicate. Two hundred µl of 30 millimole (mM) $\alpha$-NA was added to each well for the reaction to occur. The reaction was stopped after 10 minutes by adding 50 µl of staining solution containing 0.1% Fast Blue BB salt and 5% SDS (2:3). The plate was left for 5 min for equilibration and absorbance was recorded at 450 nm (Zamani, et al., 2014). The change in absorbance was converted to
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end product (α-naphthol) using the standard curve of α-naphthol. Blanks were set at the same time using a reaction mixture without enzyme extracts.

Glutathione S-transferase (GST) activity

GST activity was estimated using the method of Habig et al. (1974) with minor modifications. To prepare a reaction mixture, fifty µl of 50 mM CDNB and 150 µl of 50 mM GSH were added to 2.70 ml of sodium phosphate buffer (100 mM, pH 6.5). One hundred µl of enzyme extract was then added as the source of enzyme. The contents were shaken gently, incubated 3 mins at 25°C and then transferred to a quartz cuvette in the sample cuvette slot of UV-Visual Spectrophotometer (Rayleigh UV-2601, China). The reaction was carried out in duplicate. The reaction mixture (3 ml) without enzyme was placed in the reference slot for zeroing. Absorbance at 340 nm was recorded for 10 minutes employing kinetics (time scan) menu. The GST activity was calculated using the formula

\[ \text{CDNB-GSH conjugate (µM mg protein}^{-1} \text{min}^{-1}) = \frac{(\text{Absorbance increase in 5 min} \times 3 \times 1000) \times 9.6 \times 5 \times 1000}{9.6 \text{ mM/cm is the extinction coefficient for CDNB-GSH conjugate at 340 nm).} \]

Protein quantification

Enzyme activities were corrected for protein concentration. The total protein content of the homogenate was determined by Folin – Lowry method (Lowry et al. 1951) using BSA as standard.

Gel electrophoresis and densitometric analysis of Esterase isozymes

Polyacrylamide gel electrophoresis (Native PAGE) with 7.5% resolving and 4% stacking gel was carried out at fixed voltage in a Genei Vertical Gel Electrophoresis apparatus at 4 °C. The volume of supernatant was taken in such a way that each well was loaded with an equal amount of protein. Tris-glycine (pH 8.8) was used as an electrode buffer. Fast blue BB dye staining with α-naphthyl acetate as a substrate was used for visualization of protein bands with esterase activity (Georghiou and Pas-teur, 1978). Depending on resolution and based on mobility esterase isozyme bands were divided into zones which were designated as Z-1, Z-2 etc. Densitometric analysis of the stained esterase bands in the gel was performed using Image Aide Gel Analysis Software (Spectronics Corp., Lincoln, NE, USA). In densitometric analysis certain peaks were obtained which corresponded to the pixel density of the isozyme bands. Staining intensity of the bands was reflected in the amplitude of the peaks re-

Table 1: Toxicity of ethion and fenpropathrin to red spider mite (RSM) in tea plantations of sub-Himalayan Terai-Dooars under different management practices.

| Acracide          | Management practices of TE | Slope ± SE | LC_{95} (ppm) | RF^c | x^c | LC_{95} Field dose | RC^c resistance classification |
|-------------------|----------------------------|------------|---------------|------|-----|---------------------|--------------------------------|
| Ethion 50% EC     | BMP^f 1                    | 2.51 ± 0.0004 | 2.36 | 2.05 – 2.72 | 1    | 0.658 | 10.79 | 0.0043 |
|                   | CMP^f 1                    | 1.15 ± 0.0010 | 186.18 | 136.69 – 253.38 | 78.89 | 0.497 | 50.90 | 2.02  |
|                   | CMP 2                      | 1.36 ± 0.0018 | 229.94 | 177.32 – 298.17 | 97.43 | 2.35  | 381.49 | 1.52  |
|                   | CMP 3                      | 1.22 ± 0.0027 | 256.15 | 163.46 – 341.17 | 100.06 | 3.42  | 537.21 | 2.15  |
| Fenpropathrin 30% EC | BMP 1                     | 0.97 ± 0.0025 | 14.712 | 10.383 – 21.015 | 1    | 1.24  | 70.41 | None |
|                   | CMP 1                      | 1.75 ± 0.0008 | 1383.8 | 1136.8 – 1684.5 | 93.93 | 1.6   | 12190 | None |
|                   | CMP 2                      | 2.05 ± 0.0006 | 1075.84 | 899.11 – 1287.3 | 73.03 | 6.37  | 684.34 | 5.51  |
|                   | CMP 3                      | 2.45 ± 0.0007 | 1587.77 | 1316.7 – 1914.7 | 107.79 | 2.77  | 7506.1 | None |

Notes: ^f TE, tea estate; ^T, Fiducial limit; ^c, Resistance Factor: LC_{95} of each population/ LC_{95} of susceptible population; ^RC, Resistance Coefficient: LC_{95} of a population/recommended field dose; BMP, bio-organically managed plantations; CMP, conventionally managed plantations.
results of the detoxifying enzyme activity of red spider mite (RSM) populations in differently managed sub-Himalayan tea plantations of Terai-Dooars, India.

### Table 2: Detoxifying enzyme activity of red spider mite (RSM) populations in differently managed sub-Himalayan tea plantations of Terai-Dooars, India.

| Management practices at multilocational TE | GE (µM/min/mg protein) | GST (µM/min/mg protein) |
|------------------------------------------|------------------------|-------------------------|
| BMP 1                                    | 0.308 ± 0.035<sup>a</sup> | 98.35±8.06<sup>a</sup> |
| CMP 1                                    | 0.704 ± 0.088<sup>b</sup> | 205.62±14.60<sup>b</sup> |
| CMP 2                                    | 0.759 ± 0.076<sup>b</sup> | 202.43±15.41<sup>b</sup> |
| CMP 3                                    | 0.964 ± 0.084<sup>b</sup> | 198.21±15.69<sup>b</sup> |

Notes: <sup>a,b</sup> Mean values with different letters within a column were significantly different (P < 0.05; Tukey’s multiple comparison test); <sup>c</sup>GE, general esterases; <sup>d</sup>GST, glutathione S-transferases; <sup>e</sup>TE, tea estate; <sup>f</sup>BMP, bio-organically managed plantations; <sup>g</sup>CMP, conventionally managed plantations.

In general a strong correlation existed between the toxicity levels of both ethion and fenpropathrin and the activities of GE and GST.

### Isozyme profile of GE

Isozyme profiles of GE of RSM populations collected from CMP and BMP appeared different, which were evident on the native PAGE (Figure 1).

Three activity zones of isozymes were exhibited that differed in staining intensity of the bands between the populations from CMP and BMP. All the three zones (Z-1, Z-2 and Z-3) of GE of the mites from CMP were with high staining intensity of bands. This was reflected as high amplitudes (profile height) in densitometric analysis, implying presence of higher quantity and activity of the enzyme as compared to that of BMP.
Fig. 1: A – Esterase isozyme profile of Oligonychus coffeae collected from different tea plantations. Lane 1 and 2, from conventionally managed plantations (CMP); Lane 3 – 5, from bio-organically managed plantations (BMP); B – Densitometric analysis of the isozyme pattern of the O. coffeae population from different plantations with graphical representation of pixel.

Discussion

Populations of red spider mite (RSM) of different tea plantations of sub-Himalayan Terai region varied considerably in susceptibility to commonly used acaricides as well as in their detoxifying enzyme activity. The development of resistance/tolerance may be related to the history of the commonly sprayed insecticides or other pesticides of similar groups (Zhu et al., 2011). Acaricide application pattern may possibly have a bearing on the observed variability in susceptibility of RSM populations in the present study. Synthetic acaricides are not applied for managing RSM populations in BMP; whereas RSM populations from CMP are under constant pressure of synthetic acaricides (Sannigrahi and Talukdar, 2003; Roy et al., 2008a). Such differential selection pressure of acaricides may be a major cause of high tolerance of the RSM populations occurring in CMP as compared to the populations from BMP that are 70 – 100 times less tolerant (susceptible).

Metabolic detoxification of pesticides is an important mechanism in arthropods leading to development of tolerance. GE is categorised as phase I (primary) detoxifying enzyme which metabolizes pesticides mainly by hydrolysis of ester bonds. GST is phase II detoxifying enzyme which conjugates polar products with various endogenous compounds such as sugars, sulphate, phosphate, amino acids or glutathione (Yu, 2008). Esterases have great versatility and are generally involved in the metabolism of organophosphorus and pyrethroid pesticides (Campbell, 2001; Karunaratne and Hemingway, 2001; Limoe, 2007). GST activity also is reported to increase with development of pesticide resistance (Wu et al., 2004; Nehare et al., 2010). Increased GST activity is found to be particularly associated with organophosphate and pyrethroid resistance (Cheng et al., 1983; Yu and Nguyen, 1992), indicating its role in metabolic detoxification. An increase in GE and GST activity in the more tolerant forms of RSM in the present study to both the acaricides.
cides in question indicates that these two enzymes play a significant role in acaricide metabolism. Likewise, increased levels of GE, GST and cytochrome P450 monoxygenase activity have been found to be involved in the development of pesticide (acaricide) resistance in different arachnid species including mites (Matsumara and Voss, 1964; Jamroz et al., 2000; Wang and Yu, 2007; Pasay et al., 2009; Yorulmaz and Ay, 2009). The activity of GE and GST vis-à-vis levels of acaricide tolerance in female RSM showed a high positive correlation ($r > 0.9$) both for ethion and fenpropathrin in the present investigation. A strong correlation between pesticide tolerance and detoxifying enzyme activity has also been reported in Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) (Chen et al., 2005) and Helopeltis theivora Waterhouse (Saha et al., 2012). Further studies in insects by Perera et al. (2008), Sarker et al. (2009) and Zhu et al. (2011) have shown that field populations of insects exposed to pesticide application, record an enhanced activity of the detoxifying enzymes.

Resistance/tolerance developed in an arthropod to a particular pesticide may not be a fixed one and can be closely related to the number of applications of pesticides sharing same mechanism of action (Campos et al., 1995). High tolerance to ethion was reported earlier, while fenpropathrin was found to be very effective against RSM population of Terai-Dooars plantations some five years back (Roy et al., 2008b; 2010). Repeated use of fenpropathrin on RSM population of these plantations in the recent past is possibly responsible for its increasing tolerance to this acaricide. On the other hand, a comparative decrease in tolerance to ethion in RSM in last five years implies that its higher tolerance level recorded in the past (Roy et al.; 2010) was possibly temporarily acquired. The earlier status has reversed in the present mite populations which show more susceptibility to ethion, possibly due to lack of exposure to the organophosphate acaricide in recent past (from personal communication with the planters). Thus periodic monitoring of tolerance status of RSM to different acaricides is required in the Terai-Dooars tea plantations to design proper and more effective management strategy. In fact, more information can play an important role in circumventing problems associated with acaricide resistance and assist in choice of acaricides and their rotations (Ghadamyari et al., 2008).

Qualitative changes of esterases can give rise to pesticide resistance (Devonshire, 1977; Devonshire and Field, 1991; Wu et al., 2011). Resistance due to qualitative changes in esterases has been recorded in *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Devonshire, 1977) and *Musca domestica* L. (Diptera: Muscidae) (Van Asperen and Oppenooorth, 1959). The differential expressions of esterase isozymes in different RSM populations as recorded in the present study could also be related with acaricide exposure. Higher expression level of esterase isozymes in tolerant RSM populations from CMP (pesticide exposed) than that from susceptible BMP-populations (pesticide unexposed) indicated the involvement of the isozymes of GE in determining the tolerance level in RSM for the said acaricides. The development of resistance in insects and mites is mainly induced by frequent application of pesticides as has been found in case of the mite *T. urticae* Koch (Yorulmaz and Ay, 2009). Higher tolerance/resistance of RSM (*O. coffeae*) to the acaricides in the field populations of south Indian tea plantations (with pesticide exposure) in comparison to laboratory cultured population (without pesticide exposure) has also been observed (Roobakkumar et al., 2012). In pest management programmes, pesticide treatment decisions are based on economic thresholds or aesthetic injury levels. Pesticide resistance, however, reduces the efficacy of insecticide treatments (Kawai, 1997), and therefore influence the decision process by curtailing the number of viable treatment options. Variation in the activity of the defense enzymes, GE and GST in red spider mite (*O. coffeae*) from multi location tea plantations may be used as an index to monitor the level of tolerance or resistance of the mite pest populations against the said acaricides.

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REFERENCES

Ay, R. and Yorulmaz S. 2010 — Inheritance and detoxification enzyme levels in *Tetranychus urticae* Koch (Acari: Tetranychidae) strain selected with chlorpyriphos — J. Pest Sci., 83: 85–93. doi:10.1007/s10340-009-0274-9

Banerjee, B. 1988 — An Introduction to Agricultural Acarology — Associated Publishing Co., New Delhi. pp. 118.

Banerjee, B. 1993 — Tea production and processing — Oxford and IBH Publishing, New Delhi.

CABI, EPPO. 2013 — *Oligonychus coffeae*. Database on quarantine pests [Internet] — Prepared by CABI and EPPO for the EU. Available from http://www.cabi.org/cpc (Accessed on May 2013).

Campbell, B.E. 2001 — The role of esterases in pyrethroid resistance in Australian populations of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) [Ph.D. Thesis] — Australian National University, Canberra.

Campos, F., Dybas, R.A. and Kruba, D.A. 1995 — Susceptibility of two-spotted spider mite (Acari:Tetranychidae) populations in California to abamectin — J. Econ. Entomol. 88: 225–231. doi:10.1093/jee/88.2.225

Chen, S., Yang, Y. and Wu, Y. 2005 — Correlation between fenvalerate resistance and cytochrome P450 mediated O-demethylation activity in *Helicoverpa armigera* (Lepidoptera: Noctuidae) — J. Econ. Entomol. 98: 943–946. doi:10.1603/0022-0493-98.3.943

Cheng, E.Y., Kao, C.H., Lin, D.F. and Trai, T.C. 1983 — Insecticide resistance study in *Plutella xylostella* The specificity of oxidative detoxification mechanism in larval stage — J. Agric. Res. China 35:375–386.

Cranham, J.E. 1966 — Tea pests and their control — Annu. Rev. Entomol. 11: 491–514. doi:10.1146/annurev.en.11.010166.002423

Cranham, J.E. and Helle, W. 1985 — Pesticide resistance in Tetranychidae — In: Helle, W., Sabelis, M. W. (Eds.), Spider mites: Their biology, natural enemies and control Vol. 1B Elsevier, Amsterdam, The Netherlands. p. 405–421.

Das, G.M. 1959 — Bionomics of the red spider mite, *Oligonychus coffeae* (Nietner) — Bull. Entomol. Res. 50: 265–275. doi:10.1017/S0007485300054572

Devonshire, A.L. 1977 — The properties of a carboxylesterase from the peach potato aphid, *Myzus persicae* (Sulz.), and its role in conferring insecticide resistance — Biochem. J. 167: 675–683. doi:10.1042/bj1670675

Devonshire, A.L. and Field, L.M. 1991 — Gene amplification and insecticide resistance. Annu. Rev. Entomol. — 36: 1–23. doi:10.1146/annurev.en.36.010191.000245

EPPO, 2014 — DP 03: Morphological identification of spider mites (Tetranychidae) affecting imported fruits [Internet] — EPPO Expert Group, The Secretariat of the North American Plant Protection Organization, Canada. Available from www.nappo.org (Accessed on 4.08.2016)

Finney, D.J. 1973 — Probit Analysis — Cambridge University Press, Cambridge. pp.333.

Georghiou, G.P. and Pasteur, N. 1978 — Electrophoretic esterase pattern in insecticide resistant and susceptible mosquitoes — J. Econ. Entomol. 71: 201–205. doi:10.1093/jee/71.2.201

Ghadamyari M., Talebi K., Mizuno H., Kono, Y. 2008 — Oxydemeton-methyl resistance, mechanisms, and associated fitness cost in green peach aphids (Hemiptera: Aphididae) — J. Econ. Entomol. 101: 1432-1438. doi:10.1093/jee/101.4.1432

Gotob T, Nagata T. 2001 — Development and reproduction of *Oligonychus coffeae* (Acari:Tetranychidae) on tea — Int. J. Acarol. 27: 293-298. doi:10.1080/01647900108684269

Gurusubramanian, G., Rahman, A., Sarmah, M., Ray, S. and Bora, S. 2008 — Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures — J. Environ. Biol. 29(6): 813-26.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. 1974 — Glutathione S-transferases: The first enzymatic step in mercapturic acid formation — J. Biol. Chem. 249: 7130-7139.

Hazarika, L.K., Bhuyan, M. and Hazarika, B.N. 2009 — Insect pests of tea and their management — Annu. Rev. Entomol. 54: 267–284. doi:10.1146/annurev.env.53.101006.004446

Jamroz, R.C., Guerrero, F.D., Pruett, J.H., Oehler, D.D. and Miller, R.J. 2000 — Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican strains of the southern cattle tick, *Boophilus microplus* — J. Insect Physiol. 46: 685-695. doi:10.1016/S0022-1910(99)00157-2

Jeppson L, Keifer H, Baker E. 1975 — Mites Injurious to Economic Plants — University of California Press, Berkeley, USA. pp.614

Karunarathne, S.H.P. and Hemingway, J. 2001 — Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of...
mosquito vectors of disease in Sri Lanka — Bulletin WHO 79: 1060-1064.

Kawai, A. 1997 — Prospect for integrated pest management in tea cultivation in Japan — Jpn. Agric. Res. Q. 31: 213-217.

Light, S.S. 1927 — Mites as pests of the tea plant — Trop. Agric. — 68(4): 229-238.

Limooe M., Enayati A.A., Ladonni H., Vatandoost H., Baseri, H., Oshaghi M.A. 2007 — Various mechanisms responsible for permethrin metabolic resistance in seven field-collected strains of the German cockroach from Iran, *Blatella germanica* (L.) (Dictyoptera: Blattellidae) — Pestic. Biochem. Physiol. 87: 138-146. doi:10.1016/j.pestbp.2006.07.003

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951 — Protein measurement with the folin phenol reagent — J. Biol. Chem. 193: 265-275.

Matsumara F., Voss G. 1964 — Mechanism of malathion resistance, parathion resistance in the twospotted spider mite, *Tetranychus urticae* — J. Econ. Entomol. 57: 911-917. doi:10.1093/jee/57.6.911

Meyer M.K.P. 1987 — African Tetranychidae (Acari: Prostigmata) with reference to the world genera. Department of Agriculture and water supply, Republic of South Africa — Entomology memoir 69: 1-175.

Migeon A., Dorkeld F. 2006-2015 — Spider Mites Web: a comprehensive database for the Tetranychidae [Internet] — Montpellier: INRA/CBGP; Available from: http://www1.montpellier.inra.fr/CBGP/spmweb/, [Accessed on 8 August 2016].

Nehare S., Moharil M.P., Ghodki B.S., Lande G.K., Bisane M.D., Thakre A.S., Barkhade U.P. 2010 — Biochemical analysis and synergistic suppression of indoxacarb resistance in *Platella xylostella* L. — J. Asia Pacific Entomol. 13: 91-95. doi:10.1017/S1742758412000161

Oppenorth F.J. 1984 — Biochemistry of insecticide resistance — Pestic. Biochem. Physiol. 22: 187-193. doi:10.1016/0048-3575(84)90088-9

Pasay C., Arlian J., Morgan M., Gunning R., Rossiter L., Holt D., Walton S., Beckham S., MacCarthy J. 2009 — The Effect of Insecticide Synergists on the Response of Scabies Mites to Pyrethroid Acaricides — PLoS Negl. Trop. Dis. 3(1): e354. doi:10.1371/journal.pntd.0000354

Perera M.D.B., Hemingway J., Karunaratne S.H.P.P. 2008 — Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka — Malar. J. 7: 168. doi:10.1186/1475-2875-7-168

Plant Protection code, 2014, Ver. 2.0. — Tea Board of India, Ministry of Commerce and industry, Government of India [Internet] — Available from: (http://www.teaboard.gov.in/pdf/notice/plant_protection_code.pdf), [Accessed on 15 June 2015]

Reghupathy A., Radhakrishnan B., Ayyasamy R. 2007 — Need for acaricide resistance monitoring in mites affecting tea in South India — Resistant Pest Management Newsletter 17(1): 4-8.

Roobakkumar A., Babu A., Rahman V.K.J., Subramaniam M.S.R.S., Kumar D.V. 2012 — Comparative susceptibility and detoxifying enzyme activities to fenpropathrin in field-collected and laboratory-reared *Oligonychus coffeae* infesting tea — Two and a Bud 59: 55-59.

Roy S., Mukhopadhyay A., Gurusubramanian G. 2008a — Use pattern of insecticides in tea estates of the Dooars in North Bengal, India — NBU J. Anim. Sci. 2: 35-40.

Roy S., Mukhopadhyay A., Gurusubramanian G. 2008b — Preliminary toxicological study of commonly used acaricides of tea red spider mite (*Oligonychus coffeee* Nietner) of North Bengal, India. — Resistant Pest Management Newsletter 18: 10-15.

Roy S., Mukhopadhyay A., Gurusubramanian G. 2010 — Baseline susceptibility of *Oligonychus coffeae* to acaricides in North Bengal tea plantations, India — Int. J. Acarol. 36(5): 357-362. doi:10.1080/16749591003733731

Roy S., Muraleedharan N., Mukhopadhyay, A. 2014 — The red spider mite, *Oligonychus coffeae* (Acari: Tetranychidae): its status, biology, ecology and management in tea plantations — Exp. Appl. Acarol. 63: 431-463. doi:10.1007/s10493-014-9800-4

Saha D., Roy S., Mukhopadhyay A. 2012 — Insecticide susceptibility and activity of major detoxifying enzymes in female *Helopeltis theivora* (Heteroptera: Miridae) from sub-Himalayan tea plantations of North Bengal, India — Int. J. Trop. Insect Sci. 32(2): 85-93. doi:10.1174/1071472758412000161

Sahoo B., Sahoo S.K., Somchaudhury, A.K. 2004 — Studies in the toxicity of newer molecules against Tea red spider mite — In: Proceedings of the National Symposium on Frontier Areas of Entomological Research 5–7 November, 2003, Division of Entomology, IARI, New Delhi. p. 301–302.

Sannigrahi S., Talukdar T. 2003 — Pesticide use patterns in Dooars tea industry — Two and a Bud 50: 35-38.

Sarker M., Bhattacharyya I.K., Borkotoki A., Goswami D., Rabha B., Baruah I., Srivastava R.B. 2009 — Insecticide resistance and detoxifying enzyme activity in principal bankroftian filariasis vector, *Culex quinquefasciatus*, in northeastern India — Med. Vet. Entomol. 23: 122–131. doi:10.1111/j.1365-2915.2009.00805.x

Scott J.G. 1999 — Cytochrome P450 and insecticide resistance — Insect Biochem. Molec. Biol. 29: 757-777. doi:10.1016/S0965-1748(99)00338-7
Das et al.

Tsagkarakou A., Pasteur N., Cuany A., Chevillon C., Navajas M. 2002 — Mechanisms of resistance to organophosphates in *Tetranychus urticae* (Acari: Tetranychidae) from Greece — Insect Biochem. Molec. Biol. 32: 417–424. doi:10.1016/S0965-1748(01)00118-7

Van Asperen K. 1962 — A study of housefly esterases by means of a sensitive colorimetric method — J. Insect Physiol. 8: 401–416. doi:10.1016/0022-1910(62)90074-4

Van Asperen K., Oppenoorth F.J. 1959 — Organophosphate resistance and esterase activity in houseflies — Entomol. Exp. Appl. 2: 48-57. doi:10.1111/j.1570-7458.1959.tb02096.x

Van Leeuwen T., Van Pottelberge S., Tirry L. 2005 — Comparative acaricide susceptibility and detoxifying enzyme activities in field-collected resistant and susceptible strains of *Tetranychus urticae* — Pest Manag. Sci. 61: 499–507. doi:10.1002/ps.1001

Wang L., Wu Y. 2007 — Cross-resistance and biochemical mechanisms of abamectin resistance in the B-type *Bemisia tabaci* — J. Appl. Entomol. 131: 98-103. doi:10.1111/j.1439-0418.2006.01140.x

Wegorek P., Mrówecki M. Zamojska J. 2009 — Resistance of pollen beetle (*Meligethes aeneus*) to selected active substances of insecticides in Poland — J. Plant Prot. Res. 49(1): 119 – 127. doi:10.2478/v10045-009-0016-2

Wu G., Jiang S., Miyata T. 2004 — Seasonal changes of methamidophos susceptibility and biochemical properties in *Plutella xylostella* (Lepidoptera: Yponomeutidae) and its parasitoid, *Cotesia plutellae* (Hymenoptera: Braconidae) — J. Econ. Entomol. 97: 1689–1698. doi:10.1603/0022-0493-97.5.1689

Wu S., Yang Y., Guorui Y., Campbell P.M., Teese M.G., Russell R.J., Oakeshott, J.G., Wu Y. 2011 — Overexpressed esterases in a fenvalerate resistant strain of the cotton bollworm *Helicoverpa armigera*. Insect Biochem. Molec. Biol. 41: 14–21. doi:10.1016/j.ibmb.2010.09.007

Yorulmaz S., Ay, R. 2009 — Multiple resistance, detoxifying enzyme activity, and inheritance of abamectin resistance in *Tetranychus urticae* Koch (Acarina: Tetranychidae) — Turk. J. Agric. For. 33, 393–402.

Yu S.J., Nguyen S.N. 1992 — Detection and biochemical characterization of resistance in diamondback moth. Pestic. Biochem. Physiol. 44: 74–81. doi:10.1016/0048-3575(92)90011-N

Yu S.J. 2008 — The toxicology and Biochemistry of Insecticides — CRC Press, Taylor and Francis Group, Boca Raton, London, New York. pp. 296.

Zamani P., Sajedi R. H., Ghadamyari M., Memarizadeh N. 2014 — Resistance mechanisms to chlorpyrifos in Iranian populations of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) — J. Agr. Sci. Tech. 16:277-289.

Zhu Y.C., West S., Snodgrass G., Luttrell R. 2011 — Variability in resistance-related enzyme activities in field populations of the tarnished plant bug, *Lygus lineolaris* — Pestic. Biochem. Physiol. 99: 265–273. doi:10.1016/j.pestbp.2011.01.005

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