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ACARICIDE RESISTANCE IN FIELD-COLLECTED TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH

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ABSTRACT

Two spotted spider mite *Tetranychus urticae* Koch is an economically serious pest posing threat to major vegetable crops. Roving survey in and around Coimbatore region revealed that farmers do not target mites with acaricides instead they use higher dose of insecticides at frequent intervals which results in development of resistance. The bioassay results revealed that fenpropathrin (2.07 to 6.86-folds) and fenazaquin (2.74 to 7.13-folds) exhibit higher susceptibility, whereas diafenthiuron (5.35 to 12.25-folds) revealed a low to moderate level of resistance. The propargite (43.80 to 60.63-folds) and chlorfenapyr (61.01 to 75.10-folds) exhibited high resistance, followed by spiromesifen (222.28 to 300.26-folds) and buprofezin (382.60 to 417.87-folds), with extremely high level of resistance. The higher specific activity of GST (4.54-folds), MFO (10.06-folds) and CarE (15.06-folds) in Puthupalayam population suggested the role of biochemical resistance. A significant positive correlation was observed between diafenthiuron and CarE activity (r = 0.981*), fenpropathrin and MFO activity (r = 0.964*).

Key words: Fenazaquin, propargite, spiromesifen, buprofezin, fenpropathrin, diafenthiuron, chlorfenapyr, LC_{50}, RR, GST, MFO, CarE, resistance, vegetables.

Two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a world-wide pest, mesophyll feeder, and major vegetable crop pest in field and greenhouse conditions (Titiksha and Sood, 2019). It is responsible for 10 to 50% yield loss on tomato and 15.29 to 81.10% fruit loss of brinjal. On depletion of nutrient content, they form ballooning and gets migrated to another plant through the wind (Shukla et al., 2017). Modern agricultural practices viz., dumping of pesticides when the population is below thresholds and monocropping system lead to resistance development. The biological characteristics of *T. urticae* accelerate the development of resistance (Van Leuween et al., 2009); and to date, *T. urticae* has developed resistance to 96 chemicals, and 551 resistance cases have been reported worldwide (Mota-Sanchez and Wise, 2021). The resistance development leads to reduced efficacy and increased costs. Survey in major vegetable growing areas of Coimbatore and Tiruppur districts revealed a insecticide usage pattern requiring evaluation. Since, *T. urticae* was resistant, farmers targeted mites with varied insecticides, and at higher doses at frequent intervals, The farmers were not aware of the acaricides. Hence, the present study with selected insecticides along with standard checks to ascertain the level of resistance and detoxification enzymes associated with them.

MATERIALS AND METHODS

A roving survey was conducted in vegetable growing areas of Coimbatore and Tiruppur regions during August 2019- April 2021, and the populations of *T. urticae* were collected from four locations viz., Puthupalayam (10.9965° N; 76.8542° E), Pichanur (10.8623° N; 76.8727° E), Muthur (11.0449° N; 77.7352° E) and Nallur (11.1014° N; 77.3927° E) covering two districts. In order to obtain uniform aged mites, the collected adults were released on potted bhendi plants (variety: Arka Anamika) in polyhouse at the Department of Horticulture, AC & RI, Madurai, separately and allowed to multiply. The F_1 mites were utilized as a source for bioassay and enzyme assay studies. The initial susceptible culture of *T. urticae* was obtained from All India Network Project (AINP) on Agricultural Acarology, TNAU, Coimbatore and reared under laboratory condition (26± 1°C; 70± 10%RH) in mulberry leaves at the Mass Culture Laboratory, Department of Agricultural Entomology, AC & RI, Madurai till 25th generation to calculate base-line

LC_{50} values. The acaricides selected for assessing the resistance level were fenazaquin 10%EC, propargite 57%EC, spiromesifen 22.9%SC, buprofezin 25%SC, fenpropathrin 10%EC, diafenthiuron 50%W/W and chlorfenapyr 10%SC. The chemicals required for detoxification enzyme assays were purchased from Sigma Aldrich Pvt. Ltd. The IRAC (2009) recommended leaf dip bioassay method (Method No. 004) was used. The fresh mulberry leaf discs (5x 5 cm) were dipped in the test solutions for 30 sec and allowed them to air dry. An untreated control was maintained by dipping leaf discs in distilled water. Twenty F_1 adult female mites were transferred to the treated leaves. The mortality of mites was determined by their inability to walk at least a distance equivalent to their body length when prodded with a brush after 48 hours of acaricide exposure.

The protein content was estimated using a standard, bovine serum albumin (BSA) and the values were expressed as mg g^{-1} (Lowry et al., 1951). The glutathione S transferase (GST) activity was quantified using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate and the enzyme extract of 600 adult female mites (15 mg) was prepared with ice-cold Tris-HCl buffer (0.1 M, pH 8.0) containing 10 mM reduced glutathione. To the 100 µl of enzyme extract, 3.824 ml Tris-HCl buffer (0.1 M, pH 8.0) was added and allowed for pre-incubation of 10 min at 25°C. Then, 76 µl of 0.1 M CDNB prepared in acetone was added. The change in absorbance was recorded for 5 mins with every 1 min interval in UV-Vis spectrophotometer at 340 nm and the specific activity of enzyme was expressed as nmoles of CDNB conjugated ml^{-1} min^{-1} mg^{-1} protein (Bose, 2019). The *p*-nitroanisole was used as a substrate to estimate mixed function oxidase (MFO) activity. The enzyme extract of adult mites was prepared with 50 mM ice-cold Tris-HCl buffer containing 1.15 % KCl and 1.0 mM ethylenediamine tetraacetic acid (EDTA) (pH 7.7). The assay mixture containing 1.7 ml Tris-HCl buffer, 1 ml 50 Mm *p*-nitroanisole (in ethanol) and 100 µl enzyme extract was incubated at 34°C for 3 min. Then 200 µl of 10.0 mM nicotinamide adenine dinucleotide phosphate (NADPH) in 0.1 M phosphate buffer at pH 7.8 was added and the reaction mixture was again incubated at 34°C for 30 min. The activity was immediately measured at 405 nm for every 15 sec interval till 10 min against the blank at 34°C and the enzyme activity was expressed as nmoles of *p*-nitrophenol formed ml^{-1} min^{-1} mg^{-1} protein (Sharma, 2017). The carboxyl esterase (CarE) activity was estimated by preparing enzyme source with ice-cold phosphate buffer (0.04 M, pH 7.0). The reaction mixture contained 100 µl of enzyme source, 450 µl of 0.04 M phosphate buffer and 1.80 ml of 0.3 Mm α-naphthyl acetate was taken in a test tube where α-naphthyl acetate was used as a substrate. Then, the reaction was stopped by adding 0.9 ml of mixture containing two parts of 1% fast blue BB salt and five parts of 5% sodium dodecyl sulfate (SDS) and the reaction mixture was incubated at 30°C for 20 min under natural light conditions. The color was allowed to develop at room temperature for 15 min. The absorbance was measured at 600 nm using UV-Vis spectrophotometer and the specific activity was expressed as nmoles of α-naphthol produced ml^{-1} min^{-1} mg^{-1} protein (He, 2003).

The laboratory experiments were conducted at completely randomized design (CRD) with three replications during 2019-2021 in Central Instrumentation Laboratory, AC & RI, Madurai. The detoxification enzyme assay was replicated thrice and a control without enzyme extract was maintained for each replication. The median lethal concentration (LC_{50}) was determined by Finney's Probit analysis (Regupathy and Dhamu, 2001). The resistance ratio (RR) was computed by dividing the LC_{50} of field population with that of susceptible population. The level of resistance was categorized based on the RR values as follows, <10 as low resistance, 10-40 as moderate resistance, 40-160 as high resistance and >160 as extremely high resistance (Kim et al., 2004). The specific activity (SA) of detoxification enzymes was calculated by dividing the mean of OD difference (nm) and total volume of reaction mixture (ml) with extinction coefficient, volume of substrate (ml), incubation time (min) and protein (mg). The final value was multiplied with 1000 to obtain results in nmoles ml^{-1} min^{-1} mg of $protein^{-1}$ where, extinction coefficient of CDNB is 0.0096 $\mu M^{-1}cm^{-1}$; extinction coefficient of *p*-nitroanisole is 0.00332 $\mu M^{-1}cm^{-1}$ and extinction coefficient of α-naphthol is 0.00222 μM^{-1} cm^{-1}.

RESULTS AND DISCUSSION

The laboratory population was observed to be highly susceptible to fenazaquin (LC_{50} of 0.11 ppm) followed by fenpropathrin (0.12 ppm), chlorfenapyr (0.15 ppm), diafenthiuron (0.22 ppm), propargite (0.91 ppm), spiromesifen (2.00 ppm) and buprofezin (5.17 ppm), respectively. The highly toxic acaricides with lowest LC_{50} were fenpropathrin (0.26 ppm) and fenazaquin (0.30 ppm) to Muthur and Puthupalayam populations, respectively showing low level of resistance. All the four field populations tested were highly resistant to

Table 1. Toxicity of acaricides against field populations of *Tetranychus urticae*

Locations	N	Slope± SE	χ^2	LC_{50} (ppm) (50% FL)	LC_{95} (ppm) (95% FL)	RR	Class
				Respiration targets			
				Fenazaquin			
Puthupalayam	360	4.91± 0.04	0.69	0.30 (0.24-0.36)	0.65 (0.53-0.79)	2.74	Low
Pichanur	360	9.45± 0.02	0.60	0.51 (0.46-0.57)	0.77 (0.69-0.86)	4.70	Low
Muthur	360	7.29± 0.02	0.09	0.78 (0.69-0.88)	1.32 (1.17-1.49)	7.13	Low
Nallur	360	4.05± 0.04	0.00	0.59 (0.48-0.72)	1.51 (1.23-1.85)	5.40	Low
Susceptible	360	3.56± 0.05	0.67	0.11 (0.08-0.14)	0.32 (0.25-0.42)	-	-
				Propargite			
Puthupalayam	360	24.04± 0.00	0.96	55.23 (53.31-57.22)	64.68 (62.43-67.01)	60.63	High
Pichanur	360	36.93± 0.00	0.90	54.62 (53.31-55.97)	60.54 (59.08-62.03)	59.96	High
Muthur	360	27.82± 0.00	0.97	43.05 (41.68-44.46)	49.24 (47.68-50.85)	47.25	High
Nallur	360	17.54± 0.01	0.96	39.90 (38.02-41.88)	49.53 (47.19-51.99)	43.80	High
Susceptible	360	2.21± 0.08	0.99	0.91 (0.61-1.34)	5.03 (3.41-7.42)	-	-
				Diafenthiuron			
Puthupalayam	360	2.19± 0.09	0.39	2.70 (1.79-4.08)	16.93 (11.22-25.55)	12.25	Moderate
Pichanur	360	3.25± 0.06	0.39	1.18 (0.90-1.55)	3.95 (3.00-5.20)	5.35	Low
Muthur	360	4.30± 0.04	0.65	1.64 (1.34-2.02)	4.04 (3.29-4.97)	7.44	Low
Nallur	360	3.17± 0.06	0.82	2.25 (1.70-2.99)	7.63 (5.75-10.12)	10.21	Low
Susceptible	360	2.87± 0.06	0.83	0.22 (0.16-0.30)	0.85 (0.63-1.16)	-	-
				Chlorfenapyr			
Puthupalayam	360	14.51± 0.01	0.85	10.50 (9.87-11.18)	13.65 (12.83-14.53)	67.78	High
Pichanur	360	18.29± 0.01	0.78	11.64 (11.06-12.24)	14.35 (13.64-15.10)	75.10	High
Muthur	360	13.95± 0.01	0.80	9.45 (8.85-10.10)	12.45 (11.66-13.30)	61.01	High
Nallur	360	13.90± 0.01	0.62	10.40 (9.75-11.09)	13.70 (12.84-14.62)	67.10	High
Susceptible	360	3.23± 0.06	0.38	0.15 (0.11-0.20)	0.52 (0.38-0.70)	-	-
				Mite growth regulators			
				Spiromesifen			
Puthupalayam	360	10.64± 0.01	0.99	504.69 (465.35-547.37)	720.95 (664.75-781.91)	251.84	Extremely high
Pichanur	360	12.31± 0.01	0.95	601.72 (561.19-645.18)	819.23 (764.05-878.39)	300.26	Extremely high
Muthur	360	9.52± 0.02	0.99	445.45 (406.87-487.69)	663.47 (606.00-726.39)	222.28	Extremely high
Nallur	360	27.50± 0.00	0.86	452.02 (437.74-466.76)	519.58 (503.16-536.52)	225.56	Extremely high
Susceptible	360	2.85± 0.06	0.62	2.00 (1.46-2.73)	7.75 (5.67-10.58)	-	-
				Buprofezin			
Puthupalayam	360	22.37± 0.00	0.99	1985.81 (1910.14-2064.47)	2352.51 (2262.87-2445.71)	383.65	Extremely high
Pichanur	360	32.58± 0.00	0.99	2162.91 (2102.39-2225.16)	2429.55 (2361.57-2499.48)	417.87	Extremely high
Muthur	360	66.52± 0.00	0.97	1980.35 (1952.10-2009.02)	2097.11 (2067.19-2127.46)	382.60	Extremely high
Nallur	360	27.47± 0.00	0.99	2189.06 (2119.61-2260.78)	2512.78 (2433.06-2595.11)	422.92	Extremely high
Susceptible	360	3.73± 0.05	0.16	5.17 (4.04-6.61)	15.63 (12.22-19.99)	-	-
				Sodium channel modulator			
				Fenpropathrin			
Puthupalayam	360	6.75± 0.03	0.76	0.85 (0.74-0.97)	1.50 (1.30-1.72)	6.64	Low
Pichanur	360	5.76± 0.03	0.91	0.87 (0.75-1.02)	1.71 (1.46-1.99)	6.86	Low
Muthur	360	2.59± 0.07	0.80	0.26 (0.19-0.36)	1.15 (0.83-1.61)	2.07	Low
Nallur	360	2.44± 0.07	0.62	0.49 (0.35-0.70)	2.41 (1.70-3.41)	3.89	Low
Susceptible	360	3.33± 0.05	0.76	0.12 (0.09-0.16)	0.41 (0.31-0.54)	-	-

N - Number of mites tested, SE - Standard Error,
LC_{50} - Median lethal concentration, FL - Fiducial limit, RR - Resistance Ratio

Table 2. Estimation of detoxification enzymes in populations of *Tetranychus urticae*

Locations	Protein content (mg/ g)	*SA of Glutathione S Transferase (GST)	Ratio	*SA of Mixed Function Oxidase (MFO)	Ratio	*SA of Carboxylesterase (CarE)	Ratio
Puthupalayam	123.97	20.63	4.54	0.75	10.06	673.65	15.06
Pichanur	127.96	9.71	2.14	0.63	8.49	267.02	5.97
Muthur	112.87	6.63	1.46	0.24	3.21	375.87	8.40
Nallur	124.48	6.45	1.42	0.36	4.85	481.99	10.77
Susceptible	80.54	4.53	-	0.07	-	44.71	-

SA - Specific activity, *Enzyme activity in nmoles ml^{-1} min^{-1} mg of $protein^{-1}$

propargite (43.80 to 60.63-folds) and chlorfenapyr (61.01 to 75.10-folds) when compared with laboratory susceptible population. The Pichanur, Muthur and Nallur populations exhibited low resistance to diafenthiuron (5.35 to 10.21-folds), while Puthupalayam population was moderately resistant (12.25-folds). The mite growth regulators, spiromesifen (222.28 to 300.26-folds) and buprofezin (382.60 to 417.87-folds) had shown extremely high resistance to all the field populations. Among the field populations, Puthupalayam one had developed high resistance to propargite (60.63-folds) and diafenthiuron (12.25-folds), Pichanur population to chlorfenapyr (75.10-folds), spiromesifen (300.26-folds) and fenpropathrin (6.86-folds), Muthur population to fenazaquin (7.13-folds) and Nallur population to buprofezin (422.92-folds). The toxicity of acaricides in descending order is as follows, fenpropathrin > fenazaquin > diafenthiuron > chlorfenapyr > propargite > spiromesifen > buprofezin (Table 1).

Sharma (2017) and Titiksha (2019) reported low fenazaquin resistance in *T. urticae* (6.67-folds, 3.62-folds) from brinjal and capsicum, respectively which is in confirmation with our present findings. In *T. urticae*, resistance to propargite was moderate (9.03 to 18.36-folds) in brinjal at Bangalore (Sharma, 2017) and extremely high (3,725-folds) in Okra at Punjab (Hany et al., 2020). The magnitude of resistance reported by Mohin (2020) in tomato viz., propargite (149.0 to 164.0-folds), diafenthiuron (41.73 to 55.93-folds), chlorfenapyr (58.21 to 68.59-folds) and spiromesifen (592.31 to 625.86-folds) were more or less correlated. Similarly, low diafenthiuron resistance (10-folds) was reported in *T. truncatus* collected from okra at Kerala (Anushree et al., 2019). In *T. urticae*, Xu et al. (2018) and Lu et al. (2016) reported low to extremely high (2.38 to 952.22-folds) and high (44.64 ppm) chlorfenapyr resistance in vegetables and rose at China, respectively. Similarly, extremely high spiromesifen resistance (431.26 to 969.10-folds) was observed by Syed et al. (2018) in tomato. The extremely high buprofezin resistance was found by Wu et al. (2018) to *Nilparvata lugens* in China. The *O. coffeae* infesting tea was examined low fenpropathrin resistance (1.23 to 2.04-folds) (Roy et al., 2018, Amsalingam et al., 2016). Pan et al. (2020) observed low to moderate level of fenpropathrin resistance to *Panonychus citri* from Southwestern China.

The variation in results of resistance level in field populations depend on the extent of acaricides usage pattern by the farmers in a particular area. The enhanced resistance in the Puthupalayam and Pichanur populations possibly may result from a long history of continuous exposure to acaricides since these areas has been highlighted as major vegetable growing areas following mono-cropping patterns in Coimbatore. The acaricides which exhibited low level of resistance viz., fenpropathrin (pyrethroid) and fenazaquin (MET-inhibitor) can be recommended to control *T. urticae* in Coimbatore region of Tamil Nadu. The Puthupalayam population recorded higher specific activity of GST (20.63 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) which was 4.54-folds higher than that of susceptible population. Similarly, the MFO (0.75 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and CarE activity (673.65 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) were 10.06 and 15.06-folds higher than the susceptible population (Table 2). A pairwise correlation coefficient analysis between resistance ratio of diafenthiuron and CarE activity (r = 0.981*), fenpropathrin and MFO activity (r = 0.964*) were positively significant at p = 0.05. Similarly, Riaz et al. (2014) found elevated level of CarE activity in diafenthiuron treated *Brevicoryne brassicae* (313.33 µmol/ min/ mg) at LC_{50} after 24 hours when compared to control (250 µmol/ min/ mg). Xin-Ju and Hui-Min (2011) reported 17.386- folds increased MFO activity in fenpropathrin resistant *T. urticae* (247.35-folds).

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