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Magnitude of Resistance and Metabolism of Acaricides in Two-spotted Spider Mite, *Tetranychus urticae* Koch on Vegetables in Southern Districts of Tamil Nadu

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ABSTRACT

Background: Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a destructive, polyphagous and cosmopolitan pest infesting a wide range of vegetables. Farmers frequently use acaricides in the vegetable ecosystem for mite management leading to increased probability for the development of resistance against acaricides in mite species. Literature about resistance in *T. urticae* to novel acaricides is scanty from this region. Hence, this study was aimed to evaluate the magnitude of acaricide resistance in *T. urticae* populations on vegetable crops from Southern districts of Tamil Nadu against seven acaricides. The role of detoxification enzymes in conferring bio-chemical resistance in the mite species was also studied.

Methods: The laboratory experiments were conducted at completely randomized design (CRD) during 2019-2021 in central instrumentation laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. The level of resistance in *T. urticae* populations to fenazaquin, propargite, spiromesifen, buprofezin, fenpropathrin, diafenthiuron and chlorfenapyr was evaluated following the leaf dip bioassay technique. The activity of the detoxifying enzymes *viz.,* Glutathione S Transferase (GST), Mixed Function Oxidase (MFO) and Carboxylesterase (CarE) were estimated.

Result: Fenazaquin was highly toxic to *T. urticae*, which recorded a low level of resistance (2.00 to 8.62-fold). *T. urticae* exhibited a low to moderate level of resistance to fenpropathrin (1.86 to 37.28-fold), moderate to a high level of resistance to diafenthiuron (15.81 to 50.53-fold), high level of resistance to propargite (45.16 to 65.10-fold) and chlorfenapyr (54.67 to 100.14-fold) and extremely high level of resistance to spiromesifen (193.04 to 452.61-fold) and buprofezin (377.97 to 514.44-fold). Among all the test populations, the highly resistant DAmRg population exhibited the highest specific activity of the detoxifying enzymes, *viz.,* GST (26.98 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), MFO (5.07 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and CarE (827.90 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$). The CarE activity was found to be positively correlated with highly resistant acaricide, propargite (r = 0.995) and MFO activity with buprofezin having an extremely high level of resistance (r = 0.997). This preliminary knowledge would be instrumental in framing the acaricide resistant management (ARM) strategies.

Key words: Acaricides, Detoxification enzymes, LC_{50}, RR, *Tetranychus urticae*, Vegetables.

INTRODUCTION

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an economically important phytophagous pest of field cum greenhouse vegetable crops like tomato, brinjal, bhendi, lablab, cluster bean, amaranthus and cucurbits (Kumari *et al.*, 2015). It is cosmopolitan in distribution.

The mites primarily colonize the abaxial leaf surface and they shift to the adaxial leaf surface upon severe infestation (CABI, 2019). They cause damage by piercing the host plant using chelicerae, suck up cellular contents using the rostrum which creates white specks on the adaxial leaf surface (Brust and Gotoh, 2018). The mite has the capability of destroying 1-2 dozen cells/ minute (IRAC, 2009). It arrests photosynthetic activity and results in leaf abscission (Gorman *et al.,* 2002). The severely infested leaves can be recognized by bronzing and webbing which leads to ballooning. They can be carried easily through the wind. Higher temperature (30 to 32°C), lower humidity (20 to 40%) and better aeration favour for the multiplication and

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development of the mite pest. It can cause 10 to 50% yield loss in tomato and 15.29 to 81.10% fruit loss in brinjal (CABI, 2019).

The rapid evolution of resistance in mite pests is favoured by extensive and frequent use of acaricides cum insecticides (Van Leeuwen *et al.,* 2009; Tang *et al.,* 2014). The first case of resistance in *T. urticae* was reported to Ammonium potassium selenosulfide (Selecide) by Compton and Kearns in the year 1937 (Saito *et al.,* 1983). The *T. urticae* ranks first in terms of the number of chemicals against which, it had developed resistance (FAO, 2012) and was designated as the 'most resistant species' (IRAC, 2009). The resistance development is accelerated by its shorter developmental period (15 to 20 days), microscopic size (Male: 0.3 mm; Female: 0.5 mm), higher fecundity (100 eggs/ 10 days), inbreeding, cross-fertilization, high mutation rates, dispersal behaviour and arrhenotokous reproduction of *T. urticae*.

In *T. urticae*, 551 resistance cases were reported to 96 chemicals worldwide which include fenazaquin (3 cases were reported in Cyprus and UK), propargite (9 cases in Brazil, China, USA and New Zealand), spiromesifen (6 cases in Brazil and Belgium), fenpropathrin (3 cases in Greece and Korea), diafenthiuron (2 cases in Brazil) and chlorfenapyr (12 cases in Brazil, China, Belgium and Australia). The distribution of documented cases of acaricide resistance based on their mode of action include AChE inhibitors (31%), MET inhibitors (25%), others (24%), abamectin (12%) and pyrethroids (7%) (Mota-Sanchez and Wise, 2021).

The detoxification process enhances the elimination of xenobiotics, hence reducing the dose that reaches the target site (Adesanya *et al.,* 2021). The over-expression of mixed function oxidase is responsible for the detoxification of neonicotinoids, organophosphates and growth regulator acaricides (Fahnbulleh, 2007).

An extensive survey on pesticide usage patterns in Tamil Nadu during 2016 revealed that only very few farmers used the recommended dose of insecticides (20.83%) and considered the pesticide label (5.83%), but the majority of them did not follow the waiting period of 1 day after spraying (65.00%) and 10-14 days interval between sprayings (52.33%). The farmers have scanty knowledge on the recommended pesticide, dosage, safe harvest interval, label claim, *etc.* (Meenambigai *et al.,* 2017). It shows that the farmers in Tamil Nadu largely rely upon chemical control for instant recovery from mite infestation on vegetables. Hence, monitoring the magnitude of acaricide resistance in *T. urticae* on major vegetable crops grown in Tamil Nadu and elucidating the role of detoxification enzymes are essential to formulate resistance management strategies. In this background, the novel acaricides predominantly used by farmers in the region were selected for this study.

MATERIALS AND METHODS

Collection and maintenance of two-spotted spider mite

A survey was conducted in vegetable-growing fields in Southern districts of Tamil Nadu namely Madurai, Dindigul and Theni between December 2019 and April 2021. The details on the GPS coordinates, name of the vegetable crops from where the mites were collected, insecticide usage history, number of sprayings and cropping pattern in the locality were collected during the survey (Table 1). Mites associated with the vegetable crops were collected and maintained on potted bhendi plants (Hybrid: Arka Anamika) (to simulate the field condition until bioassay) in Polyhouse, Department of Horticulture, Agricultural College and Research Institute, TNAU, Madurai as four field populations separately and designated as MACBh, MAlBh, DAmRg and TPaBr.

Development of susceptible laboratory culture

The susceptible culture of *T. urticae* was obtained from All India Network Project (AINP) on Agricultural Acarology, TNAU, Coimbatore and maintained on mulberry leaves under laboratory condition in the Mass culture laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai at 26±1°C temperature and 70±10% RH,. The culture was reared till the 25th generation without exposing them to acaricides to calculate baseline LC_{50} values.

Toxicity of acaricides

The toxicity of seven commercial formulations of acaricides namely fenazaquin 10% EC (Magister®, DuPont India Pvt. Ltd.), propargite 57% EC (Simbaa®, PI Industries Ltd.), spiromesifen 22.9% SC (Oberon®, Bayer Crop Science India Pvt. Ltd.), buprofezin 25% SC (Applaud®, Rallis India Ltd.), fenpropathrin 10% EC (Danitol®, Sumitomo Chemical India Ltd.), diafenthiuron 50% W/W (Pegasus®, Syngenta India Pvt. Ltd.) and chlorfenapyr 10% SC (Lepido®, PI Industries Ltd.) was assessed in the laboratory.

Bioassay

The level of resistance of different mite populations to acaricides was assessed using the leaf dip bioassay technique (IRAC method No. 004) recommended by insecticide resistance action committee (IRAC, 2009) with slight modifications. The primary stock solutions *viz.,* 1000 ppm, 2000 ppm and 3000 ppm were prepared from seven selected commercial formulations of acaricides. Preliminary range finding assay was done by diluting primary stock solution to reveal five concentrations causing 15-20 to 75-80% mortality. The control was maintained simultaneously by dipping leaf discs in distilled water. The fresh untreated mulberry leaves were taken and washed with tap water to remove dirt, predatory mites and other pests before using for the experiment. The test solutions were agitated well and the mulberry leaf discs (5×5 cm) were dipped in the test solutions for 30 sec. The leaf discs were air-dried to drain surface liquid completely for 20 mins on tissue paper. The treated leaves were placed upside down on tissue paper saturated with tap water at the base of the petri dish (90×17 mm). Twenty F_1 adult female mites collected from the polyhouse

were transferred to the treated surface using a fine sable hairbrush. A thread was stuck on the outer border of the leaves to arrest the movement of mites to wet tissue paper and to confine them to the treated surface. The experiment was carried out under laboratory conditions (Temp: 26±1°C, RH: 70±10%) with three replications and mortality was recorded after 48 hours of exposure using magnascope. Mite survival was determined by touching each mite with a brush. Mites were considered dead when unable to walk at least a distance equivalent to their body length.

Activity of detoxifying enzymes

Protein estimation

The protein content was estimated on F_1 mites as per Lowry's method (Lowry *et al.*, 1951) using Bovine Serum Albumin (BSA) as a standard and expressed as mg g^{-1}.

Glutathione S Transferase (GST)

Preparation of enzyme extract

The 600 F_1 female adult mites (15 mg) were taken in a sterile 1.5 ml Eppendorf® tube and macerated with 1 ml of Tris-HCl buffer (0.1 M, pH 8.0) containing 10 mM reduced glutathione using a micro pestle under ice-cold condition. The homogenate was centrifuged at 10,000 rpm for 15 mins at 4°C in a refrigerated centrifuge and the supernatant was transferred into a new sterile Eppendorf® tube without disturbing the pellet. The clear supernatant was used as the enzyme source.

Enzyme assay

GST activity was quantified using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Enzyme extract of (100 µl) was added to test tubes containing 3.824 ml Tris-HCl buffer (0.1 M, pH 8.0). After pre-incubation of 10 mins at 25°C, the 76 µl of 0.1 M CDNB prepared in acetone was added. The changes in the density of enzyme were recorded for 5 mins with every 1 min interval in UV-Vis spectrophoto meter at 340 nm.

Mixed Function Oxidase (MFO)

Preparation of enzyme extract

The enzyme extract was prepared by homogenizing 600 F_1 female adult mites (15 mg) in 500 µl of 50 mM Tris-HCl buffer on ice (containing 1.15% KCl and 1.0 mM Ethylenediaminetetraacetic acid (EDTA), pH 7.7). The homogenate was centrifuged at 14,000 rpm for 20 mins at 4°C. The clear supernatant collected was used as an enzyme source.

Enzyme assay

MFO activity was quantified using *p*-nitro anisole as a substrate. The assay mixture containing 1.7 ml Tris-HCl buffer, 1 ml 50 Mm *p*-nitro anisole (in ethanol) and 100 µl enzyme extract was incubated at 34°C for 3 mins. 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 incubated at 34°C for 30 mins. The activity of *p*-nitro anisole O-demethylation (PNOD) forming O-demethylated product *p*-nitrophenol was immediately measured at 405 nm for every 15 secs interval till 10 mins against the blank at 34°C.

Carboxylesterase (CarE)

Preparation of enzyme extract

The 600 F_1 female adult mites (15 mg) were collected and homogenized in 500 µl of ice-cold phosphate buffer (0.04 M, pH 7.0). The homogenate was centrifuged at 10,000 rpm for 20 mins at 4°C and the clear supernatant was used as an enzyme source.

Enzyme assay

CarE activity was quantified using *á*-naphthyl acetate as a

Table 1: Details on sampling sites of *Tetranychus urticae* field populations and insecticide usage history in Southern Tamil Nadu on vegetables.

Code	Locations	Host plants	Cropping pattern	Collection date	Assayed generation	Coordinates	Insecticide usage history in the standing crop	Number of sprays during this season
MACBh	Madurai, AC and RI	Bhendi	Crop rotation	21.12.2019	F_1	9.9584°N, 78.1877°E	Chlorpyrifos 16% + Alphacypermethrin 1% EC	1
MAlBh	Madurai, Alagapuri	Bhendi	Sequential cropping	20.04.2021	F_1	10.0611°N, 78.0623°E	Cyantraniliprole 10.26% OD, Emamectin benzoate 5% SG, Propargite 57% EC	2
DAmRg	Dindigul, Ambilikai	Ridge gourd	Mono-cropping	15.12.2019	F_1	10.5475°N, 77.7257°E	Acephate 75% SPFenpropathrin 10% EC Diafenthiuron 50% WP Fipronil 7% + Hexythiazox 2% SC	4
TPaBr	Theni, Palarpatty	Brinjal	Crop rotation	29.12.2020	F_1	10.0106°N, 77. 3497°E	Spiromesifen 22.9% SC Chlorpyriphos 20% EC Thiamethoxam 25% WG	3

substrate. The assay 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. The reaction was stopped by adding 0.9 ml of a mixture containing two parts of 1% Fast Blue BB salt and five parts of 5% Sodium Dodecyl Sulfate (SDS). Then, the reaction mixture was incubated at 30°C for 20 mins under natural light conditions. The color was allowed to develop at room temperature. The absorbance was measured after 15 mins at 600 nm using a UV-Vis spectrophotometer. The specific activity (SA) of detoxification enzymes was calculated by the following formula:

$$SA = \frac{\text{Mean of OD difference (nm)} \times \text{Total volume of reaction mixture (ml)}}{\text{Extinction co-efficient} \times \text{Volume of substance (ml)} \times \text{Incubation time (min)} \times \text{Protein (mg)}} \times 1000$$

Where,

The extinction coefficient of CDNB is 0.0096 $\mu M^{-1}cm^{-1}$ and expressed as nmoles of CDNB conjugated ml^{-1} min^{-1} mg^{-1} protein (Bose, 2019) and the extinction coefficient of *p*-nitro anisole is 0.00332 μM^{-1} cm^{-1} and expressed as nmoles of *p*-nitrophenol formed ml^{-1} min^{-1} mg^{-1} protein (Sharma, 2017). The extinction coefficient of α-naphthol is 0.00222 μM^{-1} cm^{-1} and expressed as nmoles of α-naphthol produced ml^{-1} min^{-1} mg^{-1} protein (He, 2003).

Statistical analysis

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} value of a field population by the LC_{50} value of the susceptible population. Acaricide resistance levels were 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 detoxification enzyme assay was replicated thrice and control without enzyme extract was maintained.

RESULTS AND DISCUSSION

Base-line susceptibility of *T. urticae* to acaricides

The laboratory population of *T. urticae* was highly susceptible to fenazaquin with an LC_{50} value of 0.11 ppm and was least susceptible to buprofezin (5.17 ppm). The population recorded LC_{50} values of 0.91, 2.00, 0.12, 0.22 and 0.15 ppm for propargite, spiromesifen, fenpropathrin, diafenthiuron and chlorfenapyr, respectively (Table 2-4).

Acaricide resistance in field populations of *T. urticae*

Among the acaricides evaluated, fenazaquin and fenpropathrin were found to be highly toxic to the field-collected mite populations with lower LC_{50} values (0.22 to 0.94 ppm and 0.23 to 4.77 ppm, respectively), whereas spiromesifen and buprofezin were the least toxic acaricides with the higher LC_{50} values (386.85 to 907.04 ppm and 1956.38 to 2662.77 ppm, respectively) for all the four field populations. Based on the RR values, field populations were categorized as low resistant mites towards fenazaquin (2.00 to 8.62-fold), low to moderately resistant to fenpropathrin (1.86 to 37.28-fold), moderate to highly resistant to diafenthiuron (15.81 to 50.53-fold), highly resistant to propargite (45.16 to 65.10-fold) and chlorfenapyr (54.67 to 100.14-fold) and extremely high resistance to spiromesifen (193.04 to 452.61-fold) and buprofezin (377.97 to 514.44-fold) (Table 2-4).

Among the different field populations tested, all the populations were responded similarly towards all the test acaricides, except fenpropathrin and diafenthiuron. The MACBh population exhibited the lowest LC_{50} values, whereas the DAmRg population exhibited the highest LC_{50} values towards all the acaricides evaluated. All the field populations exhibited low resistance to fenazaquin (0.22 to 0.94 ppm), while towards fenpropathrin the MACBh (0.23 ppm) and TPaBr (0.55 ppm) populations exhibited less resistance, MAlBh and DAmRg populations showed moderate resistance (3.77, 4.77 ppm, respectively). Concerning diafenthiuron, the MACBh, MAlBh and TPaBr populations exhibited moderate resistance with LC_{50} values of 3.49, 5.59, 6.73 ppm, respectively while the DAmRg population recorded high resistance with an LC_{50} value of 11.16 ppm. All the field populations were categorized as highly resistant towards propargite (41.14 to 59.30 ppm) and chlorfenapyr (8.47 to 15.52 ppm). All the four populations were extremely high resistant towards both spiromesifen (386.85 to 907.04 ppm) and buprofezin (1956.38 to 2662.77 ppm) (Table 2-4).

Various scientists reported the extent of resistance exhibited by mites collected from different locations towards acaricides. A low level of resistance to fenazaquin was recorded in Cyprus populations of *T. urticae,* collected from field beans, greenhouse cucumber, field tomato (3.1, 3.7, 6.8-fold, respectively) (Vassiliou and Kitsis, 2013) and Amritsar population collected from brinjal (6.67-fold) (Sharma, 2017). Likewise, the *Oligonychus coffeae* of Assam collected from tea also recorded a low level of resistance to fenazaquin (1.77-fold) (Roy *et al.*, 2018). On contrary, Bangalore *T. urticae* populations collected from tomato were found moderately resistant to fenazaquin (12.02 to 75-fold) (Najeer *et al.,* 2018). Sumathi *et al.* (2020) found that Kurkuthi (217.86-fold) and Kapati (312.17-fold) populations of *T. urticae* collected from greenhouse carnation were highly resistant to fenazaquin in Tamil Nadu.

The *T. urticae* populations of Himachal Pradesh registered 197.56-fold resistance to propargite (Kumari *et al.*, 2015). According to Sharma (2017), *T. urticae* populations of Punjab showed low to moderate level of resistance (9.03 to 18.36-fold) to propargite on brinjal. Assam population of *O. coffeae* on tea registered low resistance (11.94-fold) (Roy *et al.,* 2018) while *T. urticae* on tomato collected from

Table 2: Toxicity of acaricides targeting respiration to field populations of *Tetranychus urticae*.

Locations	N	Slope±SE	χ^2	LC_{50} (ppm) (50% FL)	LC_{95} (ppm) (95% FL)	RR	Class
				Fenazaquin			
MACBh	360	3.46±0.05	0.60	0.22 (0.16-0.28)	0.68 (0.52-0.89)	2.00	Low
MAlBh	360	4.18±0.04	0.90	0.74 (0.61-0.91)	1.86 (1.52-2.26)	6.80	Low
DAmRg	360	12.33±0.01	0.80	0.94 (0.88-1.02)	1.30 (1.21-1.40)	8.62	Low
TPaBr	360	7.12±0.02	0.92	0.75 (0.66-0.85)	1.28 (1.14-1.45)	6.87	Low
Susceptible	360	3.56±0.05	0.67	0.11 (0.08-0.14)	0.32 (0.25-0.42)	-	-
				Propargite			
MACBh	360	25.53±0.00	0.99	41.14 (39.74-42.60)	47.73 (46.10-49.42)	45.16	High
MAlBh	360	27.09±0.00	0.99	55.07 (53.35-56.85)	63.34 (61.36-65.39)	60.45	High
DAmRg	360	30.71±0.00	0.97	59.30 (57.67-60.99)	67.10 (65.25-69.00)	65.10	High
TPaBr	360	21.05±0.00	1.00	52.67 (50.51-54.91)	63.05 (60.47-65.73)	57.81	High
Susceptible	360	2.21±0.08	0.99	0.91 (0.61-1.34)	5.03 (3.41-7.42)	-	-
				Diafenthiuron			
MACBh	360	2.78±0.07	0.16	3.49 (2.50-4.87)	14.99 (10.74-20.91)	15.81	Moderate
MAlBh	360	4.56±0.04	0.70	5.59 (4.56-6.86)	13.12 (10.70-16.09)	25.33	Moderate
DAmRg	360	3.63±0.05	0.55	11.16 (8.65-14.41)	32.88 (25.48-42.42)	50.53	High
TPaBr	360	9.26±0.02	0.79	6.73 (6.11-7.42)	10.19 (9.25-11.23)	30.47	Moderate
Susceptible	360	2.87±0.06	0.83	0.22 (0.16-0.30)	0.85 (0.63-1.16)	-	-
				Chlorfenapyr			
MACBh	360	12.37±0.01	0.76	8.47 (7.87-9.12)	11.57 (10.75-12.46)	54.67	High
MAlBh	360	18.20±0.01	0.82	11.56 (10.98-12.17)	14.32 (13.61-15.08)	74.60	High
DAmRg	360	21.55±0.00	0.74	15.52 (14.88-16.19)	18.57 (17.80-19.37)	100.14	High
TPaBr	360	16.69±0.01	0.49	11.47 (10.86-12.12)	14.56 (13.78-15.38)	74.04	High
Susceptible	360	3.23±0.06	0.38	0.15 (0.11-0.20)	0.52 (0.38-0.70)	-	-

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

Table 3: Toxicity of acaricides targeting growth and development to field populations of *Tetranychus urticae*.

Locations	N	Slope±SE	χ^2	LC_{50} (ppm) (50% FL)	LC_{95} (ppm) (95% FL)	RR	Class
				Spiromesifen			
MACBh	360	9.37±0.02	0.96	386.85 (352.22-424.89)	580.78 (528.78-637.89)	193.04	Extremely high
MAlBh	360	7.77±0.02	0.95	765.04 (685.33-854.03)	1248.70 (1118.60-1393.95)	381.76	Extremely high
DAmRg	360	18.17±0.01	0.90	907.04 (865.27-950.83)	1119.35 (1067.80-1173.38)	452.61	Extremely high
TPaBr	360	12.43±0.01	0.86	462.35 (429.93-497.20)	630.65 (586.43-678.19)	230.71	Extremely high
Susceptible	360	2.85±0.06	0.62	2.00 (1.46-2.73)	7.75 (5.67-10.58)	-	-
				Buprofezin			
MACBh	360	23.37±0.00	1.00	1956.38 (1884.10-2031.44)	2300.46 (2215.47-2388.72)	377.97	Extremely high
MAlBh	360	29.71±0.00	0.91	2064.13 (2001.59-2128.63)	2349.86 (2278.66-2423.29)	398.79	Extremely high
DAmRg	360	34.16±0.00	0.86	2662.77 (2594.15-2733.21)	2980.85 (2904.02-3059.70)	514.44	Extremely high
TPaBr	360	26.70±0.00	0.92	1965.02 (1899.79-2032.50)	2265.75 (2190.53-2343.55)	379.64	Extremely high
Susceptible	360	3.73±0.05	0.16	5.17 (4.04-6.61)	15.63 (12.22-19.99)	-	-

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

Table 4: Toxicity of nerve and muscle target acaricides to field populations of *Tetranychus urticae*.

Locations	N	Slope±SE	χ^2	LC_{50} (ppm) (50% FL)	LC_{95} (ppm) (95% FL)	RR	Class
				Fenpropathrin			
MACBh	360	3.63±0.05	0.43	0.23 (0.18-0.30)	0.71 (0.55-0.92)	1.86	Low
MAlBh	360	12.41±0.01	0.99	3.77 (3.50-4.07)	5.13 (4.75-5.53)	29.51	Moderate
DAmRg	360	6.00±0.03	0.74	4.77 (4.11-5.53)	9.08 (7.83-10.53)	37.28	Moderate
TPaBr	360	1.58±0.12	0.25	0.55 (0.31-0.96)	6.89 (3.95-12.01)	4.32	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 5: Detoxification enzymes in different field populations of *Tetranychus urticae*.

Location	Protein content (mg/g)	*SA of glutathione S transferase (GST)	Ratio	*SA of mixed function oxidase (MFO)	Ratio	*SA of carboxylesterase (CarE)	Ratio
MACBh	117.22	8.73	1.92	0.38	5.15	111.00	2.48
MAlBh	119.56	16.35	3.60	0.75	10.10	723.02	16.17
DAmRg	124.63	26.98	5.94	5.07	67.85	827.90	18.51
TPaBr	130.83	9.21	2.03	0.43	5.84	604.53	13.52
Susceptible	80.54	4.53	-	0.07	-	44.71	-

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

Table 6: Pairwise correlation coefficient analysis between resistance ratio of acaricides and detoxification enzymes in *Tetranychus urticae*.

Acaricides	GST	MFO	CarE
Fenazaquin	0.722 NS	0.630 NS	0.985*
Propargite	0.785 NS	0.662 NS	0.995**
Diafenthiuron	0.868 NS	0.916 NS	0.797 NS
Chlorfenapyr	0.907 NS	0.887 NS	0.886 NS
Spiromesifen	0.951*	0.794 NS	0.843 NS
Buprofezin	0.962*	0.997**	0.628 NS
Fenpropathrin	0.937 NS	0.761 NS	0.806 NS

*Significant at p = 0.05; **Significant at p = 0.01; NS = Not significant.

different locations of Bangalore recorded moderate resistance (15.65 to 32.83-fold) (Najeer *et al.,* 2018) and on greenhouse carnation at Tamil Nadu recorded extremely high resistance (272.50, 233.31-fold) to propargite (Sumathi *et al.,* 2020).

Anushree *et al.* (2019) reported 10-fold resistance to diafenthiuron in *Tetranychus truncatus* Ehara collected from Okra in Kerala. The present studies are in confirmation with the findings of Mohin (2020), who reported the field evolved resistance in various populations of *T. urticae viz., Tu*CKM (a) *Tu*CKM (b) *Tu*SMG (a) *Tu*SMG (b) in tomato at Karnataka where the RR of mites towards propargite (149.0 to 164.0-fold), diafenthiuron (41.73 to 55.93-fold), chlorfenapyr (58.21 to 68.59-fold) and spiromesifen (592.31 to 625.86-fold) were recorded.

The *T. urticae* population collected from field beans was highly resistant (2.67-fold) to chlorfenapyr (Kumari *et al.*, 2015). Najeer *et al.* (2018) reported an extremely high level of resistance in *T urticae* to spiromesifen on tomato (431.26 to 969.10-fold), while a moderate level of resistance was reported in the populations on brinjal (11.14 to 21.40-fold) (Sharma, 2017) and field bean (32.13-fold) (Kumari *et al.,* 2015).

The *O. coffeae* populations collected from tea showed low resistance to fenpropathrin *viz.,* 1.23-fold (Roy *et al.,* 2018) and 1.31 to 2.04-fold (Amsalingam *et al.,* 2016). The *Panonychus citri* populations sampled from Liangping, Wanzhou, Daying and Anyue in Southwestern China observed low to moderate levels of resistance to fenpropathrin (Pan *et al.*, 2019).

Activity of detoxification enzymes in different populations of *T. urticae*

The highly resistant, DAmRg population recorded the higher specific activity of GST (26.98 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), which was 5.94-fold higher than that of the susceptible population (4.53 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$). Similarly, MFO (5.07 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and CarE activity (827.90 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) were 67.85 and 18.51-fold higher than the susceptible population (Table 5). A highly significant positive correlation was observed between the resistance ratio of mites to propargite and CarE activity (r = 0.995), buprofezin and MFO activity (r = 0.997) at p = 0.01 which indicates the role of detoxification enzymes in the bio-chemical resistance of mites. In addition, there was a significant positive relationship between fenazaquin and CarE (r = 0.985) as well as spiromesifen and buprofezin with GST (r = 0.951, 0.962 respectively). The increased activity of detoxification enzymes was observed, as the level of resistance to acaricides got increased (Table 6).

Several researchers reported the relationship between detoxification enzymes and acaricides. Sharma (2017) reported 3.21-fold higher MFO activity, 1.40-fold higher GST activity and 1.13-fold higher esterase activity in *T. urticae* population with high resistance to fenazaquin when compared with the susceptible population in Punjab. Amsalingam *et al.* (2016) confirmed that the presence of detoxifying enzymes, *i.e.,* CarE (1.43 to 2.53-fold), GST (1.11 to 1.86-fold) and overexpression of the AChE gene (1.4 to 2.7-fold) could be the reason for the fenpropathrin resistance in *O. coffeae* infesting tea. Ay and Yorulmaz (2010) estimated detoxification enzyme activities in three populations of *T. urticae* namely GSS (Susceptible strain), SAK (Original Turkish strain collected from greenhouse bean) and CHLO 12 (91.45-fold chlorpyrifos resistant strain). The increased esterase activity was observed in CHLO 12 strain (3.24-fold) than the SAK strain (1.86-fold) when compared with the susceptible strain suggesting the role of the biochemical mechanism of resistance in spider mites. The 0.86-fold and 2.62-fold higher GST and MFO activity was also recorded

in CHLO 12 strain. GST, MFO and CarE are the important detoxification enzymes and consequent to their increased activity there is a possibility for the development of resistance in different insects or mites to organophosphate, carbamate and pyrethroid insecticides. CarE efficiently catalyze the hydrolysis of esters, detoxify the toxins and play role in the metabolism of lipids (Ran *et al.,* 2009), GST act *via* de-ethylation of insecticides/ acaricides and MFO by oxidative reaction (Cumming *et al.,* 2008).

CONCLUSION

Among the tested acaricides, the level of resistance in field populations to acaricides in descending order is, buprofezin > spiromesifen > propargite > chlorfenapyr > diafenthiuron > fenpropathrin > fenazaquin. The DAmRg population had shown a relatively high level of resistance ratio to all the acaricides tested, which might be due to the activity of detoxification enzymes, mono-cropping of vegetable crops and repeated use of the same acaricides/ insecticides for the control of mites and other pests. The insecticide usage history of farmers decides the nature and magnitude of development of resistance in mite populations in a region.

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