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Resistance of Two Spotted Spider Mite, *Tetranychus urticae* Koch to Major Acaricides and its Consequences on Biological Characteristics of Mites

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ABSTRACT

Two Spotted Spider Mite, *Tetranychus urticae* Koch collected from Vadagur of Kolar district in April 2016 was reared in the laboratory for more than 60 generations and its susceptibility to major acaricides was determined after every 10 generations. The lowest LC_{50} values of different acaricides in the 60th generation *viz.*, dicofol - 0.29 ppm; fenazaquin - 0.23 ppm; propargite - 0.32 ppm and spiromesifen - 0.29 ppm were used as the baseline data for determining the level of resistance in the field populations of *T. urticae* from different locations. Mites sampled from tomato crop in Hassan (*Tu*HSN), Kolar (*Tu*KLR), Chickballapur (*Tu*CKB) and Chitradurga (*Tu*CTD) districts were subjected to bioassay with four major acaricides namely, dicofol, fenazaquin, propargite and spiromesifen to determine the level of acaricide resistance. The level of resistance to dicofol was high at all the four locations (143 to 1038 folds) and resistance to fenazaquin was found to be high in *T. urticae* population from Hassan (*Tu*HSN). Resistance to propargite was moderate (15.65 to 32.83 folds), while resistance to spiromesifen was high in all the four districts. The consequence of acaricide resistance on the development of male mites was apparent as the mean duration of development of males in the resistant Kolar population (*Tu*KLR) was significantly more (10.4 days *versus* 10.0 days) than the laboratory susceptible population. Male biased sex ratio was also evident in the acaricide resistant population (♂:♀ ratio of 1.22:1.00 *versus* 0.98:1.00)

Keywords : Acaricides, two spotted spider mite, biological characteristics

THE two-spotted spider mite, *Tetranychus urticae* Koch (Acari : Tetranychidae) has been an important mite pest of cultivated crops worldwide (Vacante, 2015). It can develop on over 1100 host plant species of more than 70 plant genera. This mite is more problematic particularly on vegetables, fruits and ornamental plants (Moraes and Flechtmann, 2008). Loss in brinjal crop due to TSSM infestation in Ludhiana region of Punjab was estimated to be 18 per cent (Anon., 2011), but according to Jayasinghe and Mallik (2010), 9-10 weeks of tomato crop most crucial for TSSM infestation as mite feeding caused severe damage to the leaves by reducing the chlorophyll content and resulted in more than 50 per cent loss of yield in Kolar area of Karnataka. Being extremely polyphagous, this pest has an extraordinary ability to develop resistance to pesticides. Since 1990, world wide populations of TSSM across many crops have developed resistance to several newer acaricides (Arthropod Pesticide Resistance Database, http:// www.pesticide resistance.org/). The continuous exposure of *T. urticae* to diverse pesticides has resulted in the occurrence of resistance to at least 92 compounds both in green house and open field conditions in more than 40 countries (Ranjeet Kumar, 2008). There are reports of resistance development in *T. urticae* to pesticides in general and to acaricides in particular, such as bifenazate (Van Leeuwen *et al.*, 2006), fenazaquin (Vassiliou and Kitsis, 2013), propargite (Kumari *et al.*, 2015) and Spiromesifen (Sato *et al.*, 2016).

Instability in the resistance of mites to avermectin compounds like abamectin and milbemectin was associated with the reproductive disadvantage of 21 per cent decrease in its oviposition rate compared to the susceptible strain (Nicastro *et al.*, 2011). According to Nicastro *et al.* (2011 & 2013), instability of acaricide resistance was probably due to lack of fitness characteristics with the resistant strain. The present study was carried out to assess the level of resistance to major acaricides in the populations of *T. urticae* infesting tomato crop in different districts of Karnataka where pesticides are being used extensively. Also the consequence of acaricide

resistance was studied by comparing the development and reproduction of mites in resistant Kolar population (*Tu*KLR) and susceptible laboratory population (*Tu*SSL).

Material and Methods

Establishment of susceptible population in the laboratory

Mites collected from infested tomato crop in Vadagur near Kolar during April 2016 were reared on mulberry leaves placed on wet foam in plastic trays and maintained as susceptible population in the laboratory (*Tu*SSL). *Tu*SSL was used in all comparative studies with the field populations collected from different locations.

Field Population: Mites collected from Hassan (*Tu*HSN), Kolar (*Tu*KLR), Chickballapur (*Tu*CKB) and Chitradurga (*Tu*CTD) on tomato crop were maintained separately on mulberry leaves in the laboratory at room temperature. Depending on the availability, mites from the field sample or from the F1 generation were used for acaricide bioassay studies.

Bioassay: Acaricide bioassays were carried out following the method of Insecticide Resistance Action Committee (IRAC, 2009) with minor modifications. At least five concentrations of each acaricide (after preliminary assay with 2-3 extreme doses) were used for bioassay with 5cm^2 mulberry leaf discs following the procedure of Leaf Dip technique (leaf discs were dipped in desired acaricide concentrations for 15 – 20 seconds). Air dried treated leaf discs were placed on wet cotton wad in Petri plate and 25-30 adult females gently transferred on to leaf disc (using a fine Camel Hair brush) served as one replication. Three such replications were maintained for each of the test concentrations and observations on mortality were recorded at 24 hours interval for 3 days.

Four acaricides representing different activity group or mode of action such as conventional acaricide dicofol (Sumfol 18.5EC); a METI acaricide, fenazaquin (Magister 10EC); a sulfite ester compound, propargite (Omite 57EC); tetronic acid derivative, Spiromesifen (Oberon 240 SC) were used for bioassay and resistance related investigations in the present study.

Comparative biology of susceptible (TuSSL) and resistant (TuKLR) populations: To understand the consequence of acaricide resistance on the development of the mite, a comparative study with susceptible laboratory population (*Tu*SSL) and resistant population from Kolar (*Tu*KLR) was carried out. Development of mite was studied using 1.5 cm^2 mulberry leaf disc, on which a single egg was retained (after releasing a single female mite from the respective stock culture for 4- 6 hours). Leaf discs kept on wet cotton wad in Petri plates were placed in a BOD incubator at 25±1^{0}C temperature; 50-60 per cent RH and 14:10 Light-Dark hours. Approximately100 such leaf discs (each with one egg) were maintained for *Tu*SSL and *Tu*KLR populations separately. Observations on development from egg to adult emergence were recorded periodically (at 3-6 hours interval) and duration of each developmental stage was computed.

Statistical analysis: Acaricide bioassay data were analyzed using the method of probit analysis in SPSS software package version 23 and LC_{50} (Median Lethal Concentration) values were determined. Corresponding Resistance Ratio (RR) was calculated using LC_{50} of the field population (as numerator) and LC_{50} of susceptible population (as denominator). In the present study LC_{50} value of the 60th generation was used for determining the RR values. Intensity of resistance was categorized as low (RR values ≤10), moderate (RR values >10 ≤ 40) and high (RR values >40) (Young *et al*., 2004)

Data pertaining to developmental biology of susceptible (*Tu*SSL) and resistant (*Tu*KLR) populations were analyzed for male and female mites separately using Tukey's HSD test and compared at 5 per cent level of significance.

Results and Discussion

Baseline Susceptibility of *T. urticae* to major acaricides

Continuous multiplication or culturing of individuals under optimum rearing conditions of temperature & humidity and without any acaricide selection pressure for several generations is a basic requirement for establishing a real time susceptible

population. Individuals from such populations are assayed to ascertain their susceptibility to an acaricide of interest at that point of time (generation). Progressive reduction in the LC_{50} values of an acaricide over successive generations indicates progressive increase in the susceptibility to the corresponding acaricide and this susceptibility is expected to get stabilized over time or generations. Data pertaining to the establishment of susceptible population of *T. urticae* in the laboratory and progressive increase in its susceptibility to four different acaricides determined at an interval of 10 generations from 20th to 60th generation are presented in Table I. Baseline susceptibility of *T. urticae* to acaricides used in the present study was apparent at the 60th generation of the mite. Thus the LC_{50} value of the corresponding acaricide at the 60th generation may be used for resistance related toxicological studies with *T. urticae*. The lowest LC_{50} value at the 60th generation for different acaricides (Baseline susceptibility) is as follows: Dicofol - 0.29 ppm; Fenazaquin - 0.23 ppm; Propargite - 0.32 ppm and Spiromesifen - 0.29 ppm.

Resistance to different acaricides across locations

Relative toxicity data of acaricides (such as dicofol, fenazaquin, propargite and spiromesifen) to different field populations (Hassan, Kolar, Chickballapur and Chitradurga) are presented in Table II.

TABLE I

Establishment of TSSM susceptible population in the laboratory indicating its generation-wise baseline susceptibility to major acaricides

Acaricides	Generation	LC_{50} (ppm)	Fiducial limits (ppm)		Chi Square (χ^2) value	Regression equation
			Lower	Upper		
Dicofol	20th	129.67	84.18	199.01	1.126	$\hat{Y}$ = -2.07+0.98X
	30th	104.97	51.84	169.87	2.217	$\hat{Y}$ = -1.59+0.84X
	40th	35.66	4.56	76.87	4.57	$\hat{Y}$ = -2.43+1.52X
	50th	0.75	0.50	1.92	1.82	$\hat{Y}$ = 0.21+1.17X
	60th	0.29	0.12	0.55	8.172	$\hat{Y}$ = 1.11+1.92X
Fenazaquin	20th	17.48	4.74	28.33	2.441	$\hat{Y}$ = -3.45+2.7X
	30th	9.79	2.30	15.79	1.017	$\hat{Y}$ = 0.20+0.74X
	40th	8.82	0.37	17.90	4.982	$\hat{Y}$ = -0.01+0.73X
	50th	0.42	0.11	0.69	0.138	$\hat{Y}$ = 0.94+1.84X
	60th	0.23	0.001	0.61	19.182	$\hat{Y}$ = 1.11+1.71X
Propargite	20th	9.31	6.46	11.34	1.25	$\hat{Y}$ = -1.35+1.84X
	30th	5.23	1.77	7.96	2.659	$\hat{Y}$ = -0.32+1.09X
	40th	0.61	0.001	6.10	4.609	$\hat{Y}$ = 0.15+0.86X
	50th	0.50	0.03	1.32	3.134	$\hat{Y}$ = 0.29+1.26X
	60th	0.32	0.29	0.71	13.719	$\hat{Y}$ = 0.85+1.65X
Spiromesifen	20th	202.93	119.16	292.89	0.022	$\hat{Y}$ = -3.43+1.52X
	30th	302.38	188.01	478.74	4.644	$\hat{Y}$ = -1.38+0.63X
	40th	185.05	132.33	253.15	1.049	$\hat{Y}$ = -2.47+1.10X
	50th	18.36	16.93	19.66	3.034	$\hat{Y}$ = -1.99+1.89X
	60th	0.29	0.001	0.71	11.347	$\hat{Y}$ = 0.67+1.21X

Resistance to Dicofol – The highest LC_{50} value (303.30 ppm) was with *Tu*CTD followed by *Tu*CKB (196.46 ppm), *Tu*KLR (133.49 ppm) and *Tu*HSN (41.9). *Tu*CTD showed high level of resistance (1039 folds) followed by *Tu*CKB (673 folds), *Tu*KLR (457 folds) and *Tu*HSN (143 folds). Thus the intensity of resistance to dicofol in *T. urticae* population from tomato crop at all the locations was high and the extent of resistance was relatively less in Hassan population. This might be due to the persistance of dicofol beyond 3 years under field conditions. In 2008, Ranjeet Kumar also reported very high level of resistance to dicofol with *T. urticae* population from Bangalore (767 to 3690 folds) and Kolar districts (500 – 6491 folds). Mable and Pree (1992) proposed that dicofol resistance in European Red Mite in Southern Ontario, Canada subsequent to the report of Ranjeet Kumar in 2008 from the same district of Kolar.

Resistance to Fenazaquin: Levels of resistance to fenazaquin remained moderate to high at different locations (Table II). It was highest with *Tu*HSN (75 folds), while moderate level of resistance observed at other locations was almost of similar intensity (12 to 16 folds). Anonymous (2009) reported low to moderate level of resistance to fenazaquin (5-32 folds) with *T. urticae* populations from tomato crop in Bangalore and Kolar districts. *T. urticae* population from brinjal crop in different districts of Punjab also showed low to moderate level of resistance (1 to 36 folds). The results are in accordance with the findings of Herron and Rophail (1998). According to them dicofol selected strains of *T. urticae* (with RR of 465 folds) showed cross resistance to METI acaricides, where high level of resistant to dicofol - low to moderate level of resistance to fenazaquin (a METI acaricide) is evident with *T. urticae* populations from all the locations.

TABLE II

Relative toxicity of major acaricides to different field populations of TSSM, Tetranychus urticae *infesting tomato crop and intensity of acaricide resistance*

Acaricide	Location	LC_{50} (ppm)	Fiducial limits (ppm)		Chi Square (χ^2) value	Regression equation	Resistance Ratio*(RR)
			Lower	Upper			
Dicofol	*Tu*HSN	41.90	5.04	182.04	5.98	$\hat{Y}$ = -1.6+0.97X	143.49
	*Tu*KLR	133.50	69.48	240.59	11.42	$\hat{Y}$ = -3.01+1.44X	457.18
	*Tu*CKB	196.46	156.71	247.84	5.07	$\hat{Y}$ = -3.28+1.44X	672.81
	*Tu*CTD	303.30	156.06	893.89	12.08	$\hat{Y}$ = -2.88+1.15X	1038.70
Fenazaquin	*Tu*HSN	17.40	8.58	25.86	0.48	$\hat{Y}$ = -1.54+1.48X	75.00
	*Tu*KLR	3.39	2.54	4.36	6.51	$\hat{Y}$ = -0.72+1.31X	14.62
	*Tu*CKB	2.79	1.86	3.86	0.93	$\hat{Y}$ = -0.45+1.02X	12.02
	*Tu*CTD	3.73	2.39	5.35	4.21	$\hat{Y}$ = -0.52+0.91X	16.06
Propargite	*Tu*HSN	4.99	3.89	6.16	4.95	$\hat{Y}$ = -1.38+2.03X	15.65
	*Tu*KLR	6.68	5.41	8.42	4.67	$\hat{Y}$ = -1.5+1.84X	20.95
	*Tu*CKB	10.47	5.26	32.89	6.77	$\hat{Y}$ = -1.31+1.31X	32.83
	*Tu*CTD	6.23	4.90	8.07	0.25	$\hat{Y}$ = -1.23+1.54X	19.53
Spiromesifen	*Tu*HSN	124.64	76.05	285.60	3.27	$\hat{Y}$ =-2.17+1.05X	431.26
	*Tu*KLR	146.57	87.05	255.53	14.51	$\hat{Y}$ = -3.04+1.41X	507.16
	*Tu*CKB	280.07	172.70	558.10	12.12	$\hat{Y}$ = -3.37+1.38X	969.10
	*Tu*CTD	206.84	164.94	264.78	6.29	$\hat{Y}$ = -3.3+1.43X	715.72

**LC_{50} value of 60th generation used; Tu*HSN*:* Hassan*; Tu*KLR*:* Kolar*; Tu*CKB*:* Chikkaballapur*; Tu*CTD*:* Chitradurga

Resistance to propargite : Though the higher LC_{50} value of propargite was found associated with *T. urticae* population from *Tu*CKB, the overall level of resistance across four different locations was only moderate *i.e.*, 16 to 33 folds (Table II). Earlier studies with *T. urticae* population from brinjal crop (Anony., 2009 & 2015) in Navsari of Gujarat and from tomato crop in Kolar and Bangalore of Karnataka (Anon., 2009) also showed low to moderate level of resistance to propargite, *i.e.*, 28 -32 folds and 4-28 folds, respectively. Thus *T. urticae* populations irrespective of the host crop showed low to moderate level of resistance to propargite. Similarly in Punjab Rakesh and Manmeet (2018) also observed 9 to 14 folds resistance to propargite in brinjal populations and the intensity of resistance was between low and moderate.

Resistance to spiromesifen: *T. urticae* populations occurring on tomato crop irrespective of the location showed extremely high level of resistance only next conventional acaricide dicofol. RR values ranged from 431 (with *Tu*HSN) to 969 (with *Tu*CKB) and the corresponding LC_{50} values ranged from 125ppm – 280ppm. Sato *et al.* (2016) reported high frequency of resistant individuals in *T. urticae* population infesting open cultivated rose and chrysanthemum crops in Brazil. Rakesh and Manmeet (2018) noticed low to moderate resistance (11 to 21 folds) in *T. urticae* population from brinjal crop in Punjab. This variation in the level of resistance to spiromesifen across crops or geographical locations may be attributed to instability in spiromesifen resistance. Similarly results were observed by Sato *et al*. (2005).

Developmental biology and reproduction in acaricide resistant population

In susceptible population *Tu*SSL successful egg hatching was maximum (94.5%) compared to the resistant population *Tu*KLR (82.1%). Data pertaining to duration of different developmental stages in susceptible and resistant population are presented in Table III. Total developmental time from egg to adult for male in resistant population was 10.40 days, which is the significantly different from 10.00 days duration for male in the susceptible population. There was no significant difference in the total duration of development for female between susceptible and resistant populations (10.635 days and 10.640 days, respectively) (Table IV).

TABLE III

Comparative development of lab susceptible population (TuSSL) and resistant Kolar population (TuKLR) of TSSM, Tetranychus urticae under laboratory conditions (25±1ºC; 60-70% RH; 14h:10h Light & Dark)

Developmental stage	Susceptible (TuSSL) Male (n= 53)	Resistant (TuKLR) Male (n= 50)	Susceptible (TuSSL) Female (n=54)	Resistant (TuKLR) Female (n= 42)
Egg	109.9 ± 0.99	114.2 ± 0.77	110.1 ± 1.00	110.9 ± 0.88
Larva	24.7 ± 0.62	26.1 ± 0.85	30.3 ± 1.00	27.9 ± 0.57
Nymphochrysalis	22.5 ± 0.45	22.8 ± 0.44	22.6 ± 0.41	22.9 ± 0.65
Protonymph	17.9 ± 0.54	18.5 ± 0.54	20.9 ± 0.44	19.5 ± 0.61
Deutochrysalis	18.9 ± 0.60	21.1 ± 0.40	21.9 ± 0.54	22.0 ± 0.50
Deutonymph	21.0 ± 0.67	19.6 ± 0.72	23.6 ± 0.79	23.7 ± 0.82
Teleiochrysalis	25.1 ± 0.49	27.4 ± 0.63	25.5 ± 0.56	28.3 ± 0.83
Total development (Egg to adult)	240.0 ± 0.49 10.01 days	246.4 ± 0.47 10.41 days	254.6 ± 0.70 10.64 days	252.9 ± 0.34 10.64 days

TABLE IV

Comparative development of susceptible and resistant populations of TSSM, T. urticae

Population	Development from egg to adult (in days)	
	Male	Female
Susceptible (*Tu*SSL)	10.006 [a]	10.635 [a]
Resistant (*Tu*KLR)	10.408 [b]	10.640 [a]

Values with alphabetical superscript within the column are significantly different according to Tukey's HSD test ($P<0.05$)

Data with respect to the number of male and female adults emerged out of 100 randomly selected eggs and used in separate developmental studies for susceptible and resistant populations showed that relatively equal number of males and females were found emerged. However, the proportion of male and female adults computed as male to female ratio for susceptible (*Tu*SSL) and resistance population (*Tu*KLR) was 0.98:1 and 1.22:1, respectively. Further detailed demographic studies would throw light on the exact number of male and female offsprings per female in the resulting progeny as a consequence of acaricide resistance.

It is evident that the level of resistance to different acaricides in *T. urticae* populations varied across locations. The order of level of resistance to different acaricides in *T. urticae* population from Hassan (*Tu*HSN) was; spiromesifen > dicofol > fenazaquin > propargite, the order for both Kolar (*Tu*KLR) and Chickballapur (*Tu*CKB) populations was; spiromesifen > dicofol > propargite > fenazaquin, while the order for Chitradurga population (*Tu*CTD) was; dicofol > spiromesifen > propargite > fenazaquin. The effect of acaricide resistance on biological characteristics of mite population from Kolar (*Tu*KLR) showed reduction in the egg hatchability as well as male biased sex ratio in the resulting progeny. The probable impact of acaricide resistance on reproduction parameters of the mite needs further investigation.

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