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Establishment of base line values for susceptibility of *Tetranychus urticae* Koch to major acaricides over successive generations

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Abstract
The susceptible culture of two spotted spider mite, *T. urticae* Koch was successfully maintained on mulberry leaves under laboratory condition without exposing to any acaricides for two years designated as the susceptible reference population. The susceptibility of the spider mite to selected acaricides *viz.*, fenazaquin, propargite, chlorfenapyr, dicofol, diafenthiuron, spiromesifen and abamectin was determined after every five generations starting from 118th to 128th generation. The LC_{50} values of different acaricides in 128th generation were 0.18 ppm for fenazaquin, 0.20 ppm for propargite, 0.29 ppm for spiromesifen, 0.32 ppm for abamectin, 0.42 ppm for chlorfenapyr and 0.30 ppm for diafenthiuron and dicofol. This laboratory population was the reference population and the dose-mortality values estimated for laboratory population during 128th generation was considered as base-line susceptibility of spider mite for corresponding acaricide and was further used to estimate the level of resistance in different field population.

Keywords: Base-line values, acaricides, susceptible, dose-mortality

Introduction
Two spotted spider mites belong to Tetranychidae family are most notorious ones during later stage of the crop growth. However, during recent years mites have become notably serious pests and are gaining tremendous importance owing to their devastating nature and severe damage potential. The total number of mite species reported from India was 2670; among these 700 species are phytophagous mites (Mondal *et al.*, 2020) [1]. They causes significant yield losses in many horticultural, ornamental and agronomic crops globally. *T. urticae* can develop resistant to new acaricides within two to four years, and as a result management of *T. urticae* has become increasingly difficult (Grbic *et al.*, 2011) [2]. As a result, it is "most resistant species" in terms of the total number of pesticides to which populations have become resistant (Van Leeuwen *et al.*, 2010) [3].
Ranjeethkumar (2008) [4] estimated the baseline value susceptibility of spider mite, *T. urticae* infesting tomato crop from Kolar and Bangalore districts of Karnataka to major acaricides *viz.*, abamectin, dicofol, diafenthiuron, fenazaquin, propargite and wettable sulphur after successfully rearing under laboratory condition up to 38th generation. Najeer-E-Noor and Srinivasa (2018) [5] collected *T. urticae* in tomato crop from Vadgur village of Kolar district of Karnataka was reared in laboratory condition for more than 60th generations and its susceptibility to selected acaricides was determined. The German susceptible strain of spider mite was reared under laboratory condition without exposing to any acaricides since 1965 and it was used as susceptible population to estimate the level of resistance in different field populations (Nauen *et al*. (2001); Stumpf and Nauen (2001); Van Pottelberge *et al*. (2009); Ay and Kara (2011) [6, 7, 8, 9]. The process of evolution of resistance is driven by the basic genetics of organism. Moreover, a base line data estimated with regard to the toxicity of popular acaricides would greatly help in understanding the potentiality of the mite pest species to develop resistance to acaricides (Sharma, 2017) [10].

Materials and methods
Development of susceptible culture in the laboratory
The susceptible culture of 106th generation of two spotted spider mite, *T. urticae* was brought from All India Network Project (AINP) on Agriculture Acarology, UAS, GKVK, and Bengaluru and culture was maintained on mulberry leaves under laboratory condition.

This culture served as susceptible laboratory population without exposing it to any acaricide and used for determining base-line values for susceptibility to acaricides, namely fenazaquin, propargite, chlorfenapyr, dicofol, diafenthiuron, spiromesifen and abamectin.

Leaf dip bioassay: Fresh tomato leaflets were dipped in desired concentration of acaricides for 5-10 seconds and air dried under a ceiling fan for 3-5 minutes. Such treated leaflets were kept on wet cotton wads in a Petri plate and on these leaflets twenty five active adult females were released as one replication, and three such replications were maintained along with a water treated control. Observations on the spider mites mortality was recorded at 24, 48 and 72 hrs after treatment. The mite mortality recorded was corrected by the using of Abbott's method (1925) [11] based on the mite mortality found in control. Then the corrected mortalities were subjected to Probit analysis (Finney, 1971) [12] to determine the concentration-mortality responses and Median Lethal Concentration (LC_{50}) values. The susceptibility may be defined as the shift in the dose/per cent mortality (d/pm) response of the mite.

Results

The susceptibility of *T. urticae* to different acaricides over successive generations:

The results found over succeeding generations, the susceptibility of *T. urticae* to major acaricides was increased from 118th generation to 128th generations. During 118th generation of the susceptible laboratory population of *T. urticae* recorded the LC_{50} value of 0.20 ppm for fenazaquin, 0.25 ppm for propargite, 0.42 ppm for dicofol, 0.43 ppm for diafenthiuron, 0.68 ppm for spiromesifen, 0.69 ppm for chlorfenapyr and 0.72 ppm for abamectin. Acaricides fenazaquin, propargite and dicofol were most toxic, with a value of 3.60, 2.88 and 1.71 folds of potency ratio, followed by diafenthiuron, spiromesifen, chlorfenapyr and abamectin with potency ratio of 1.67, 1.05, 1.04 and 1 folds, respectively (Table 1).

During 123rd generation of susceptible laboratory population of *T. urticae* recorded the LC_{50} values of 0.19 ppm for fenazaquin, 0.22 ppm for propargite, 0.32 ppm for diafenthiuron, 0.36 ppm for dicofol, 0.42 ppm for spiromesifen, 0.46 ppm for abamectin and 0.55 ppm for chlorfenapyr. Acaricides fenazaquin, propargite and diafenthiuron were found to be most toxic, with a value of 2.89, 2.50 and 1.71 folds of potency ratio, followed by dicofol, spiromesifen, abamectin and chlorfenapyr with potency ratio of 1.52, 1.30, 1.19 and 1 folds, respectively (Table 1).

Similarly, the LC_{50} values were also observed for 128th generation of susceptible laboratory population of *T. urticae*. The LC_{50} values were 0.18 ppm for fenazaquin, 0.20 ppm for propargite, 0.29 ppm spiromesifen, and 0.30 ppm for diafenthiuron, 0.30 ppm for dicofol, 0.32 ppm for abamectin and 0.42 ppm for chlorfenapyr. Acaricides like fenazaquin, propargite and spiromesifen were found to be most toxic, with a value of 2.33, 2.10 and 1.44 folds of potency ratio, followed by diafenthiuron, dicofol, abamectin and chlorfenapyr with potency ratio of 1.40, 1.40, 1.31 and 1 fold (Table 1).

Discussion

The susceptibility of *T. urticae* to major acaricides was increased over successive generations for laboratory reared population of spider mite without any acaricidal exposure. Baseline susceptibility is the character of a population which is more stable having majority of homozygous individuals compared to the heterozygous individuals in the field population having the past history of selection pressure by different groups of compounds.

Najeer-E-Noor and Srinivasa, 2018 [5] reported the LC_{50} value of 0.23 ppm for fenazaquin during 60th generation of the susceptible laboratory population of *T. urticae.* Najeer-E-Noor and Srinivasa, 2020 [13] reported the LC_{50} value of 0.22 ppm for fenazaquin in 91st generation. Cho *et al*. (1995) [14] estimated the baseline susceptibility of fenpyroximate (a METI acaricide like fenazaquin) as 0.53 ppm. Van Pottelberge *et al*. (2009) [8] determined the susceptibility of German susceptible strain of *T. urticae* to fenazaquin as 40 ppm, which was much higher than baseline susceptibility of 0.18 ppm at 128th generation of *T. urticae* in the present study compared to the values from the results of Cho *et al*. (1995) [14] and Ranjeeth Kumar (2008) [4].

Mohammadzahad *et al*. (2014) [15] reported the efficacy of propargite for susceptible population of *T. urticae* and recorded the LC_{50} value of 0.0720 ppm from Karaj population in Iran. The LC_{50} value obtained during our study are too high when compared to the findings of Kaur and Bhullar, 2019 [16] recorded the LC_{50} value of 0.002 ppm for susceptible population of *T. urticae* to propargite from Punjab.

Sridhar and Jhansi Rani (2002)[17] reported LC_{50} value of 0.0404% for dicofol against *T. urticae*. Ranjeethkumar (2008) [4] recorded LC_{50} value of 0.1 ppm for dicofol and diafenthiuron during 38th generation.

Najeer-E-Noor and Srinivasa, 2018 [5] reported the LC_{50} value of 0.29 ppm for spiromesifen during 60th generation. Najeer-E-Noor and Srinivasa, 2020 [13] recorded the LC_{50} value of 0.92 ppm for spiromesifen during 91st generation of the susceptible laboratory population of *T. urticae*. The LC_{50} value of 0.29 ppm was much lower in our present study when compared to Najeer-E-Noor and Srinivasa, (0.92 ppm). Mohammadzahad *et al*. (2014) [15] studied the efficacy of abamectin for susceptible population of *T. urticae* and reported the LC_{50} value of 0.0273 ppm.

Beers *et al*. (1998) [18] concluded that the important parameters underlying the acaricide resistance management is the availability of effective baseline susceptibility data of the target mite to the acaricides. The establishment of baseline values as reference against the acaricide before its widespread use may helpful for effective monitoring and understanding the changes in its susceptibility over a period of time and can provide opportunity to monitor the resistance before the instances of field failures.

Baseline toxicity estimation is more necessary to plan and execute resistance monitoring surveys. But unfortunately, the most of the baseline susceptibility ratios were designated in relation to the most susceptible population from among the field population. The field population with least LC_{50} value is often used as the baseline susceptibility value while calculating the level of resistance in other field population.

Baseline values estimated in the present study was the highest susceptibility level of *T. urticae*, when completely deprived of acaricide selection pressure. The baseline value calculated through generation study with wide range of test dose values at different generations indicates apparent genetic diversity of the mite. The most susceptible population identified from the field population represents the field level of toxicity which was not evident with the continuously reared laboratory population used for determining the baseline susceptible values.

Table 1: Establishment of base-line values/susceptibility data to different acaricides against *Tetranychus urticae*

Sl. No.	Acaricides	IRAC MoA Group	Generations	LC_{50} (ppm)	Fiducial limits (95%)		χ^2 value (DF)	Regression equation	Potency ratio
					Lower	Upper			
1	Fenazaquin	Mitochondrial complex I electron transport inhibitors (21, 21A)	118	0.20	0.20 - 0.21		4.48 (5)	Y= - 14.93 + 14.57X	3.60
			123	0.19	0.18 - 0.20		5.12 (5)	Y= - 14.91 + 14.00X	2.89
			128	0.18	0.17 - 0.20		6.62 (5)	Y= - 9.84 + 6.66X	2.33
2	Propargite	Inhibitors of mitochondrial ATP synthase (12, 12C)	118	0.25	0.24 - 0.27		6.81 (5)	Y= - 10.47 + 9.20X	2.88
			123	0.22	0.22 - 0.23		6.49 (5)	Y= - 15.94 + 16.90X	2.50
			128	0.20	0.19 - 0.21		7.20 (5)	Y= - 15.68 + 15.46X	2.1
3	Spiromesifen	Inhibitors of acetyl CoA carboxylase (23)	118	0.68	0.50 - 0.98		10.12 (5)	Y= - 5.31 + 1.80X	1.05
			123	0.42	0.34 - 0.52		8.73 (5)	Y= - 5.99 + 2.63X	1.30
			128	0.29	0.21 - 0.39		11.33 (5)	Y= - 5.97 + 1.81X	1.44
4	Diafenthiuron	Inhibitors of mitochondrial ATP synthase (12, 12A)	118	0.43	0.32 - 0.89		12.47 (5)	Y= - 6.16 + 3.16X	1.67
			123	0.32	0.30 - 0.35		9.74 (5)	Y= - 7.03 + 4.15X	1.71
			128	0.30	0.27 - 0.34		10.02 (5)	Y= - 7.49 + 4.75X	1.40
5	Dicofol	Compounds of unknown or uncertain MoA (UN)	118	0.42	0.38 - 0.46		25.23 (5)	Y= - 6.26 + 3.35X	1.71
			123	0.36	0.29 - 0.53		16.39 (5)	Y= - 6.60 + 3.62X	1.52
			128	0.30	0.29 - 0.32		15.41 (5)	Y= - 7.51 + 4.86X	1.40
6	Abamectin	Glutamate-gated chloride channel allosteric modulators (6)	118	0.72	0.47 - 1.34		17.37 (5)	Y= - 5.27 + 1.88X	1
			123	0.46	0.37 -0.58		13.90 (5)	Y= - 5.93 + 2.66X	1.19
			128	0.32	0.28 -0.36		11.40 (5)	Y= - 7.08 + 4.22X	1.31
7	Chlorfenapyr	Uncouplers of oxidative phosphorylation via disruption of the proton gradient (13)	118	0.69	0.45 - 1.28		14.38 (5)	Y= - 5.30 + 1.84X	1.04
			123	0.55	0.47 -0.65		10.06 (5)	Y= - 5.88 + 3.49X	1
			128	0.42	0.28 - 0.63		12.00 (5)	Y= - 6.05 + 2.76X	1

Potency ratio = LC_{50} of the least toxic chemical/ LC_{50} of the most toxic chemical (Generation wise)

Conclusion

Based on our present investigations it was concluded that for effective management of *T. urticae* under field conditions only those acaricides which possess the low stability and higher reversion rate in terms of response by the mites need to be used. From the above results, it is clear that the LC_{50} values decreased from 118th generations to 128th generations as there was increase in the susceptibility of laboratory culture during successive generations for all the major acaricides tested for their susceptibility. The baseline data derived from laboratory reared population, which would be more helpful for acaricide resistance related studies.

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