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Determining baseline susceptibility of *Tetranychus urticae* Koch (Acari: Tetranychidae) to acaricides by generation method

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Abstract
Culture of *Tetranychus urticae* Koch successfully maintained under laboratory conditions unexposed to acaricidal stress for more than two years was designated as the susceptible reference population. Susceptibility of the mite to four major acaricides *i.e.*, dicofol, fenazaquin, propargite and spiromesifen was ascertained after every 10 generations starting from 20th to 91st generation. The susceptibility of *T. urticae* by 91st generation was found increased to 282, 89, 31 and 221 folds for dicofol, fenazaquin, propargite and spiromesifen, respectively as compared to the susceptibility of the initial population at the 20th generation. This baseline susceptibility data could be helpful in monitoring as well as in the management of acaricide resistance of *T. urticae*. It is inferred that propargite can be included in the control program of *T. urticae* more conveniently, due to its more consistent toxicity to the mite with a novel mode of action of inhibiting mitochondrial ATPase and its faster reversion rate.

Keywords: Baseline, susceptible, acaricide, dose-mortality, generations

Introduction
Tetranychus urticae Koch is the most polyphagous spider mite species known today globally and is a major pest in many cropping systems, which severely infests vegetable as well as other crops like eggplant, okra, rose, cotton, apple *etc*. (Onkarappa *et al*., 2007; Singh and Raghuraman, 2011) [1, 2]. It has been reported to show resistance to commonly used acaricides. Since 1990s, worldwide populations of Two Spotted Spider Mite have shown resistance to several newer acaricides also (Arthropod Pesticide Resistance Database, http:// www.pesticideresistance.org). Frequent exposure of *T. urticae* to variety of pesticides on diverse crops has resulted in the development of resistance to at least 92 compounds both in green house and open field conditions in more than 40 countries (Ranjeethkumar, 2008) [3] and according to Zhu *et al*. (2016) [4] in recent years, resistance is found increased to 94 compounds.

Ranjeethkumar (2008) [3] determined the baseline susceptibility of *T. urticae* infesting tomato crop in Kolar district of South Karnataka to important acaricides *viz*., wettable sulphur, dicofol, abamectin, diafenthiuron, fenazaquin and propargite after continuous rearing for 38 generations in the laboratory without any acaricidal exposure. The potentiality of the organisms to show or acquire resistance to acaricide stress is often understood and studied by developing acaricide resistant populations in the laboratory. The German susceptible strain of *T. urticae* (GSS) was maintained in the laboratory without acaricide treatment since 1965 (Dennehy *et al*., 1993) [5] and it was used as susceptible population to determine the resistance level in different populations (Nauen *et al*. (2001); Stumpf and Nauen (2001) & (2002); Van Pottelberge *et al*. (2009b); Yorulmaz and Ay (2009); Ay and Kara (2011)) [6,7,8,9,10,11].

Phenomenon of evolution of resistance is influenced by the basic genetics of the organism. Fundamentally, resistance is influenced by number of genes, mode of inheritance, dominance of resistance *etc*. Genetics of acaricide resistance build-up in *T. urticae* has been studied by Zilbermints *et al*. (1969) [12], Overmeer and Van Zon (1973) [13], Cranham and Helle (1985) [14], Croft and Van de Baan (1988) [15], Uesugi *et al*. (2002) [16], Kwon *et al*. (2015) [17] and Xu *et al*. (2018) [18]. Generally, it is stated that the development of resistance is monogenic with dominant or semi-dominant inheritance. Hitherto the baseline values have been determined for only few acaricides without considering allele frequency and inheritance of the resistant gene and they are less realistic. Wherein field collected base population which was more

heterozygous has been designated as a susceptible population. It is important to keep in mind the genetics of inheritance of the resistance allele, while determining reliable data from the baseline population based on its true genetic background.

Knowledge about the stability of pesticide resistance has practical implications in developing effective resistance management strategies (Abbas *et al*., 2015; Afzal *et al*., 2015) [19, 20]. The development of pesticide resistance may be delayed through good management strategies. Thus, the acaricide resistance needs to be monitored continuously as a means of integral part of chemical control because it enables the early detection of resistance to initiate necessary measures of resistance management. Moreover, a base line data determined with regard to the toxicity of all available or popular acaricides would greatly help in understanding the potentiality of the mite pest species to show or develop resistance to acaricides (Sharma, 2017) [21].

The mite population from the field would have been exposed to acaricide pressure and is bound to have individuals having both susceptible gene and resistant gene and the frequency of these genes would vary depending on the extent or level of resistance in the population. Certainly, such populations have their susceptibility level altered and which in turn would have acquired resistance of varying intensities. For more precise and accurate estimation of resistant levels, it is often suggested to have a baseline susceptibility values necessarily established or derived from a base population Ranjeeth Kumar (2008) [3]. Base population is expected to have high frequency of susceptible genes and low frequency of resistant genes which can be achieved by continuous laboratory rearing, unexposed to acaricide pressure or stress. This population needs to be used as reference population for obtaining baseline toxicity values for different acaricides.

By making use of the field population having lowest median lethal dose value as reference value will only provide the information on the relative level of resistance among the populations sampled. But for understanding the true potential resistance to acaricide, the base population with the baseline susceptibility values need to be the reference values for all comparisons and assessment of intensities of resistance. When median lethal values of different field populations are within a narrow range, it is clear that resistance levels are more or less similar. On the other hand it can also be an erroneous inference of similar levels of resistance across different populations (Najeer-E-Noor, 2018) [22]. When once the baseline values are used, all these ambiguities are resolved and the true picture of acaricide resistance will be more evident. For this reason the establishment of baseline population and determination of baseline susceptibility values for different acaricides to *T. urticae* by generations' method is largely justified in the present study.

In the present investigation, baseline values of acaricides such as dicofol, fenazaquin, propargite and spiromesifen with respect to the susceptibility of *T. urticae* have been determined, which is indispensable for ascertaining the intensity of acaricide resistance and to undertake other resistance related studies. Baseline value determined in the present study is the highest susceptibility level of *T. urticae* to a target acaricide when it is completely deprived of acaricide selection pressure and is suggested to use it for determining the level or extent of resistance to an acaricide in question.

Materials and Methods

The base population of *T. urticae* was initially collected from tomato crop in Vadagur village of Kolar District in southern Karnataka during II week of April 2016 and further maintained in the laboratory at room temperature conditions (24-26 °C) by rearing on excised mulberry leaves placed on wet polyethylene foam kept in plastic trays. The lab population was designated as *Tu*SSL (*Tetranychus urticae* susceptible laboratory population). After every ten generations of rearing and upto 90 generations lab bioassay was carried out to determine median lethal concentration (LC_{50}) values of acaricides by leaf dip bioassay technique and to ascertain the progress of susceptibility to selected acaricides such as dicofol, fenazaquin, propargite and spiromesifen.

Leaf dip bioassay: Mulberry leaf discs of 5 cm^2 were dipped in desired acaricide concentration (prepared by serial dilutions) for 10-15 seconds and then gently air-dried under a ceiling fan for 10-15 minutes. Later the leaf discs were placed on wet cotton wad kept in Petri plate. One hundred *T. urticae* adult females were transferred on to three leaf discs. Mortality was recorded at 24 h interval up to 72 h. Mites which were not able to move at least the distance of their body length were considered as moribund or dead. The mortality data were then subjected to probit analysis using SPSS® version 23 and dose-mortality response curves were prepared in Sigma Plot version 14 and used for illustrations.

When once the acaricide exposure stress is withdrawn the mite susceptibility level to different acaricides after every 10 generations would reflect the mite mortality pattern in terms of susceptibility. The susceptibility or resistance may be defined as the shift in the dose/per cent mortality (d/pm) response of the mite. The acaricide resistance within the population may be attributed to heterogeneous or homogeneous conditions of exposure of the individuals of the population to the acaricidal stress as:

i) **Indication of Heterogeneity in the Population:** A low level of resistance (high level of susceptibility) may be described as the response of *T. urticae* population (d/pm) having relatively wide range of doses (or slightly higher range of doses) and have one or more plateaus
ii) **Indication of Homogeneity in the Population:** A high level of resistance (low level of susceptibility) may be described as the response of *T. urticae* population (d/pm) having a narrow range of doses (slopes from low values) with few or no plateaus.

Results

Progressive Susceptibility of *T. Urticae* to Different Acaricides over Generations:

It was found that over successive generations' susceptibility of *T. urticae* to dicofol increased from initial LC_{50} value of 129.67 ppm to 0.54 ppm and 0.46 ppm from 20th (*Tu*SSL 20) to 60th (*Tu*SSL 60) and 91st (*Tu*SSL 91) generation, respectively. The slope values of the probit lines ranged from 0.98 to 1.42 showing increase in the slope values after every ten generations of laboratory rearing to become relatively more homogenous with respect to its susceptibility to dicofol (Table 1). From 20th generation to 40th generation, the laboratory reared population was found more heterogeneous in its response (mortality) to incremental concentrations of dicofol (1.5 to 300 ppm) showing more distinct plateaus. The homogeneity was evident from 50th generation onwards, upto 91st generation for a narrow range of test dose values (*i.e*., from 0.1 to 10 ppm) with fewer indistinct plateaus (Figure 1). The corresponding LC_{50} values were found reduced by 282 times from 20th to 91st generation (Table 1). The rate of decrease of resistance in *T. urticae* to dicofol was 0.0195

(Table 2).

For fenazaquin, LC_{50} values ranged from 17.78 ppm to 0.23 and 0.22 ppm from 20th to 60th and 91st generation, respectively. The slope values of probit lines ranged from 2.7 to 1.53 and the slope values decreased after every ten generations of laboratory rearing. Corresponding LC_{50} values decreased by 90 times (88.90 folds) from 20th generation to 91st generation of lab rearing. Initially the mite population was relatively more heterogeneous with regard to fenazaquin susceptibility at the 20th generation, with a wider range of test dose values (4 to 75 ppm) and with more distinct plateaus. Slope values remained almost similar from 30th generation to 40th generation *i.e*., 0.74 and 0.73, respectively with a comparatively narrow range of dosage values (0.5 to 30 ppm) and with one or two plateaus being slightly heterogeneous (Figure 2). The homogeneity with respect to susceptibility to fenazaquin was more apparent from 50th generation up to 91st generation with test dose values (0.1 to 2 ppm) and with few indistinct plateaus (Figure 2). The rate of decrease being of narrow range in LC_{50} values of fenazaquin was 0.0065 (Table 2).

For propargite, LC_{50} values ranged from 9.31 ppm to 0.32 ppm and 0.29 ppm from 20th to 60th and 91st generation, respectively. The slopes of the probit lines ranged from 1.84 to 1.80 and the slope values of the laboratory reared *T. urticae* population remained more or less same over generations. The LC_{50} values decreased by 32.10 times from 20th to 91st generation. The lab population was found with respect to its susceptibility to propargite from 20th to 50th generation, exposed to wide range of test dose concentrations of 0.1 to 20 ppm with more distinct plateaus (Figure 3). The lab population was comparatively more homogeneous at 60th and 91st generation with more narrow range of test dose concentrations (0.1 to 2 ppm) and the response curve has fewer indistinct plateaus (Figure 3). The rate of decrease in LC_{50} values of propargite was 0.0047 (Table 2).

For spiromesifen LC_{50} values ranged from 202.93 ppm to 1.00 ppm and 0.92 ppm for 20th to 60th and 91st generation, respectively. The slope values ranged from 1.52 to 1.37. The LC_{50} values decreased by 220.58 times from 20th generation to 91st generation (Table 1). The behaviour response to spiromesifen was heterogeneous from 20th to 50th generation, getting exposed to a wide range of test doses 1 to 400 ppm showing more distinct plateaus. The homogeneity was apparent at 60th generation and 91st generation, for a narrow range of test concentration values (0.1 to 2 ppm) and response curve showed less number of indistinct plateaus (Figure 4). The rate of decrease in LC_{50} values of spiromesifen was 0.0250.

Discussion

The susceptibility of *T. urticae* to dicofol increased over generations of lab rearing as the mite population was without any acaricidal exposure. Ranjeeth Kumar (2008) [3] reported increase in susceptibility from 5th to 38th generation of *T. urticae*, as LC_{50} values for dicofol decreased from 183 ppm to 0.1 ppm a.i. Cho *et al*. (1995) [23] collected *T. urticae* population from fields of Chibaken of Japan and used as susceptible strain after rearing on kidney bean (*Phaseolus vulgaris* var *humilis* Alefeld) and used to determine its baseline susceptibility (LC_{50}) to dicofol as 21.25 ppm. The baseline susceptibility value in the present study is 0.46 ppm and is 46.20 times less, attributed to the age of the laboratory population *i.e.,* 91 generations, less than the susceptibility recorded by the Ranjeeth Kumar (2008) [3] at 38th generation of lab rearing. The allele frequency for dicofol resistance probably got stabilised after the 40th generation of lab rearing and it be opined that the withdrawal of dicofol application for almost one year (as acaricide holiday) may lead to restoration of mite's susceptibility to dicofol.

The susceptibility to fenazaquin was also found to increase over generations of laboratory rearing without any acaricidal exposure. Ranjeeth Kumar (2008) [3] reported an increase in susceptibility of *T. urticae* to fenazaquin from 5th to 38th generation, as the LC_{50} values decreased from 5.1 to 0.1 ppm a.i. Cho *et al*. (1995) [23] determined the baseline susceptibility of (LC_{50}) fenpyroximate (a METI acaricide like fenazaquin) as 0.53 ppm. Van Pottelberge *et al*. (2009a) [24] also ascertained the susceptibility of German susceptible strain of *T. urticae* to fenazaquin as 40 ppm, which is much higher than baseline susceptibility of 0.22 ppm at 91st generation of *T. urticae* in the present study compared to the values from the studies of Cho *et al*. (1995) [23] and Ranjeeth Kumar (2008) [3].

Ranjeeth Kumar (2008) [3] reported a decrease in LC_{50} values of propargite 3 ppm to 0.3 ppm a.i., from 15th to 38th generation of lab rearing. In Japan Cho *et al*. (1995) [23] determined baseline susceptibility (LC_{50}) to propargite as 50.21 ppm, which is much higher (173.14 times) than the LC_{50} value of 0.29 ppm at the 91st generation of lab rearing in the present study. The present study data indicated that acaricide resistance in the field collected samples was diluted further by continuous laboratory rearing without any selection pressure from propargite (0.0047) and was more stable with respect to rate of decrease in LC_{50} values as compared to other three acaricides, fenazaquin (0.0065); dicofol (0.0195), and spiromesifen (0.0250) (Table 2). The reversion rate of acaricide in *T. urticae* when reared free of acaricidal stress was highest for spiromesifen and was lowest for propargite under controlled rearing conditions.

One of the most important parameters underlying the acaricide resistance management is the availability of reliable baseline susceptibility data of the target mite to the acaricide(s) in vogue. The establishment of baseline values (either LC_{50} or LC_{90}) as reference against an acaricide before its widespread use may allow better monitoring and understanding the changes in its susceptibility over time and can provide opportunity to detect resistance before the instances of field failures (Beers *et al*., 1998) [25].

Baseline toxicity determination is also helpful 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 populations. The field population with lowest LC_{50} value is often used as the baseline susceptibility value while determining the level of resistance in other field populations. This method may not be accurate because the field population experiences a) intensive acaricide pressure (where different types of compounds are used) b) environmental stress and c) host plant resistance. There may be possibility of presence of mite colonies surviving and their potential activity cannot be determined easily. As the field population is the mixture of different phenotypes and genotypes which one can categorize into homogenous and heterozygous populations. Baseline susceptibility is a character of a population which is more stable having more or 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.

Baseline value(s) determined in the present study is the highest susceptibility level of *T. urticae*, when completely deprived of acaricide selection pressure. The baseline value determined through generation study with wide range of test dose values at different generations indicates apparent genetic diversity of the mite which gets stabilized from 60th generation onwards. Genetically, laboratory reared dicofol resistant population is monogenic with a recessive nature (Zilbermints *et al*., 1969; Overmeer and Van Zon, 1973) [12, 13]. Similarly, *Panonychus citri* field resistant population was monogenic with a recessive trait (Inoue, 1979) [26]. Van Pottelberge *et al*. (2009a) [24] stated that without the selection pressure the resistance (toxicity) was unstable indicating intermediate and polygenic mode of inheritance. In the present investigation, acaricide toxicity to the laboratory population was found initially more variable which rapidly declined in further generations in the absence of acaricidal stress. Thus, the response of such homozygous individuals (as LC_{50} values) could be the highest susceptibility or the lowest toxicity of the corresponding acaricide.

According to Devine *et al*. (2001) [27] and Stumpf & Nauen (2001) [7], majority of field populations was moderately resistant to fenazaquin and propargite; resistant phenotypes were incompletely dominant and inherited both paternally and maternally. Similarly, when the homozygous diploid females were reciprocally crossed with haploid males showed that METI-resistance was inherited both paternally and maternally. However, heterozygous females resulting from crosses between METI-resistant males (R) and susceptible females (SS) exhibited lower levels of resistance to pyridaben and fenpyroximate (similar to fenazaquin) than those heterozygotes resulting from RR females and S males. Therefore, only the maternal trait of METI resistant inheritance was fully dominant. This slight maternal effect might be caused by a resistance gene located in the mitochondrial DNA (Stumpf and Nauen, 2001) [7]. METI compounds are known to target the mitochondrial respiratory pathway and hence the resistance to METI compounds is more related to mitochondrial DNA, inherited from the female gamete. But in haplodiploidy condition, where the resistance inherited paternally excludes the possibility of resistance encoded by the mitochondrial genes.

Additionally, fitness cost may be associated with resistance in *T. urticae* to dicofol, fenazaquin and spiromesifen, but not with propargite resistance (Najeer-E-Noor, 2018) [22]. It has been found that relative fitness differences, initial gene(s) frequencies and dominance relation of susceptible & resistant alleles of the phenotype which are from original field population are important factors that influenced on the reversion rate of insecticide resistance in the laboratory (Roush and Croft, 1986) [28], but this phenomenon in mites necessitates further investigation. Resistance is a temporal phenomenon and there are many examples of pesticide resistant insects and mites that revert to susceptible nature when reared without any insecticide exposure under laboratory conditions (Abedi and Brown, 1960; Flexner *et al*., 1989; Kristensen *et al*., 2000) [29, 30, 31]. The dominance of resistance mechanisms and stability of METI acaricide resistance in the laboratory without selection indicated a high risk for increasing widespread METI resistance in *T. urticae* (Stumpf and Nauen, 2001) [7]. Under such circumstances, better resistance management strategies with different class of acaricides, while use of METI-compound in a season needs to be restricted.

The most susceptible population identified from the field populations represents the field level toxicity which is not evident with the continuously laboratory reared population used for determining the baseline susceptible values. The population interactions between different phenotypic or genotypic individuals & the immigrant individuals and emigrations between the treated & untreated fields may alter the real time susceptibility of individuals.

The base line susceptibility of *T. urticae* to dicofol, fenazaquin, propargite and spiromesifen was determined by using a laboratory population of *T. urticae* free of any acaricides exposure continuously for 91 generations. Initial bioassay showed LC_{50} values of 129.67, 17.78, 9.31 and 202.93 ppm for dicofol, fenazaquin, propargite and spiromesifen, respectively. Further the susceptibility increased with successive generations on rearing in the laboratory in the absence of any acaricide selection. The susceptibility of *T. urticae* at 91st generation increased to 282, 89, 31 and 221 folds for dicofol, fenazaquin, propargite and spiromesifen, respectively as compared to corresponding values with previous generations for *e.g.*, 20th generation. These baseline values may be used for monitoring acaricide resistance in *T. urticae* in India as well as in other countries.

Based on our findings it can be concluded that for effective management of *T. urticae* under field conditions only those acaricides with low stability and higher reversion rate in terms of response by the mites need to be used. Resistance to such acaricides can be easily managed by rotation of acaricides. Resistant population will have more number of heterozygous individuals. When the noticeable symptoms of resistance appear, the frequency of resistant gene(s) will have already increased substantially. Unless there is a very heavy fitness cost, the resistant gene(s) may gradually accumulate in the pest population. The development of resistance is a dynamic process and is continuously evolving; as a result resistance management practices should be flexible to suit the resistance evolving strategy. Also, resistance or susceptibility monitoring attempts to measure changes in the degree or frequency of resistance in time & space. The acaricides which possess high stability and lower reversion rate may lead to more serious problems and would result in control failures. Propargite can be a good candidate acaricide in *T. urticae* control program in view of its low stability and higher reversion rate in addition to its novel mode of action of inhibiting mites' mitochondrial ATPase. Baseline date on the pest organism's susceptibility to the pesticide need to be collected ideally before the introduction of the product in a given area. Irrespective of the resistance verification method used, the outcome of the tests is always compared to the baseline population. For insecticides, laboratory strains are often used to establish baseline susceptibility values, useful in providing information as the highest susceptibility imminent with the test organism. If the range of baseline values is large, this indicates there is considerable genetic diversity within the target organism population and resistance may develop more rapidly than if the range of baseline values is quite small. In our study, baseline susceptibility was determined by using the laboratory population by generations' method and the rate of increase in the susceptibility over generations was evident which got stabilized from 60th generation onwards. The baseline data derived from a more stabilized (lab reared) population, would be more helpful for acaricide resistance related studies besides resistance intensity studies.

Table 1: Acaricide dosage-mortality response in laboratory susceptible population of *T. urticae* over generations

Acaricides	Generations	*n*	Slope ± SEM	χ^2 (df)	P value (Sig ≤ 0.05)	LC_{50} in ppm (95% CL)
Dicofol	20	270	0.98 ± 0.04	1.13 (2)	0.569	129.67 (84.17-199.01)
	30	450	0.84 ± 0.06	2.22 (2)	0.330	104.98 (51.84-169.88)
	40	360	1.52 ± 0.22	4.57 (2)	0.102	35.66 (4.55-76.87)
	50	450	1.17 ± 0.19	1.82 (2)	0.402	0.75 (0.05-19.2)
	60	450	1.94 ± 0.06	5.98 (2)	0.050	0.54 (0.35-0.73)
	91	450	1.42 ± 0.45	1.58 (2)	0.454	0.46 (0.11-0.89)
Fenazaquin	20	270	2.7 ± 0.23	2.44 (2)	0.295	17.78 (4.74-28.33)
	30	450	0.74 ± 0.06	1.02 (2)	0.601	9.79 (2.29-15.79)
	40	450	0.73 ± 0.10	4.98 (2)	0.083	8.82 (0.04-17.90)
	50	375	1.84 ± 0.13	0.14 (2)	0.711	0.42 (0.11-0.69)
	60	450	1.71 ± 0.09	19.17 (3)	<0.001	0.23 (0.00-0.61)
	91	450	1.53 ± 0.08	6.59 (3)	0.086	0.22 (0.15-0.29)
Propargite	20	270	1.84 ± 0.06	1.25 (2)	0.535	9.31 (6.47-11.34)
	30	360	1.09 ± 0.06	2.66 (2)	0.265	5.23 (1.77-7.96)
	40	360	0.86 ± 0.52	4.61 (2)	0.100	0.61 (0.00-6.10)
	50	450	1.26 ± 0.23	3.13 (2)	0.209	0.50 (0.03-1.32)
	60	450	1.65 ± 0.08	13.70 (3)	<0.001	0.32 (0.03-0.71)
	91	450	1.80 ± 0.08	14.99 (3)	<0.001	0.29 (0.02-0.66)
Spiromesifen	20	270	1.52 ± 0.06	0.02 (2)	0.989	202.93 (119.16-292.89)
	30	525	0.63 ± 0.05	4.64 (3)	0.200	302.38 (188.01-478.74)
	40	270	1.10 ± 0.05	1.05 (2)	0.592	185.05 (132.33-253.15)
	50	450	1.89 ± 0.02	3.03 (2)	0.219	18.36 (16.93-19.66)
	60	450	1.23 ± 0.06	4.09 (2)	0.130	1.00 (0.58-1.27)
	91	450	1.37 ± 0.06	5.26 (2)	0.072	0.92 (0.56-1.17)

Table 2: Stability of acaricide resistance in *Tetranychus urticae* (at 91st generation)

Acaricide	Initial LC_{50} (log)	Final LC_{50} (log)	Rate of decrease in LC_{50} (R)
Dicofol	129.67 (2.1128)	0.46 (-0.3372)	0.0195
Fenazaquin	17.78 (1.2499)	0.22 (-0.6576)	0.0065
Propargite	9.31 (0.9689)	0.29 (-0.5376)	0.0047
Spiromesifen	202.93 (2.3073)	0.92 (-0.0362)	0.0250

R = rate of decrease in LC_{50} [log (final LC_{50}-initial LC_{50})/N] where, N is number of generations population reared without acaricide exposure

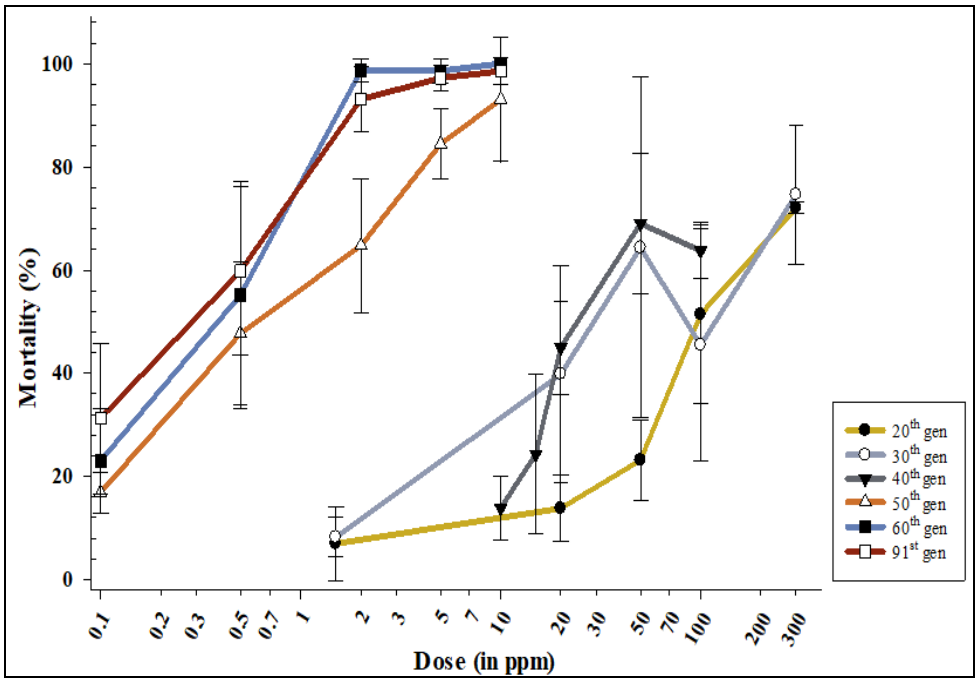

Fig 1: Progress of susceptibility in laboratory population of *T. urticae* (*Tu*SSL) to dicofol over different generations (with *SE* values)

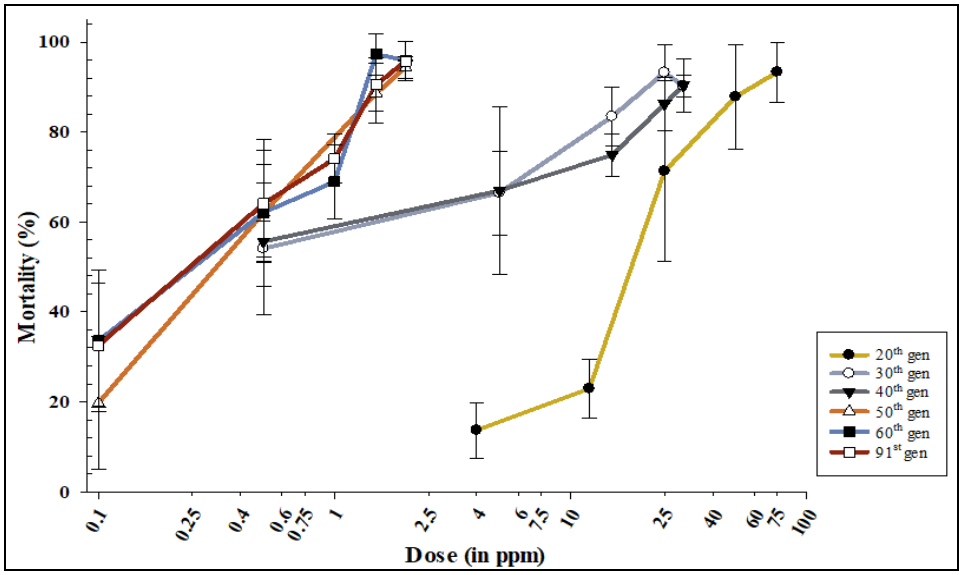

Fig 2: Progress of susceptibility in laboratory population of *T. urticae* (*Tu*SSL) to fenazaquin over different generations (with *SE* values)

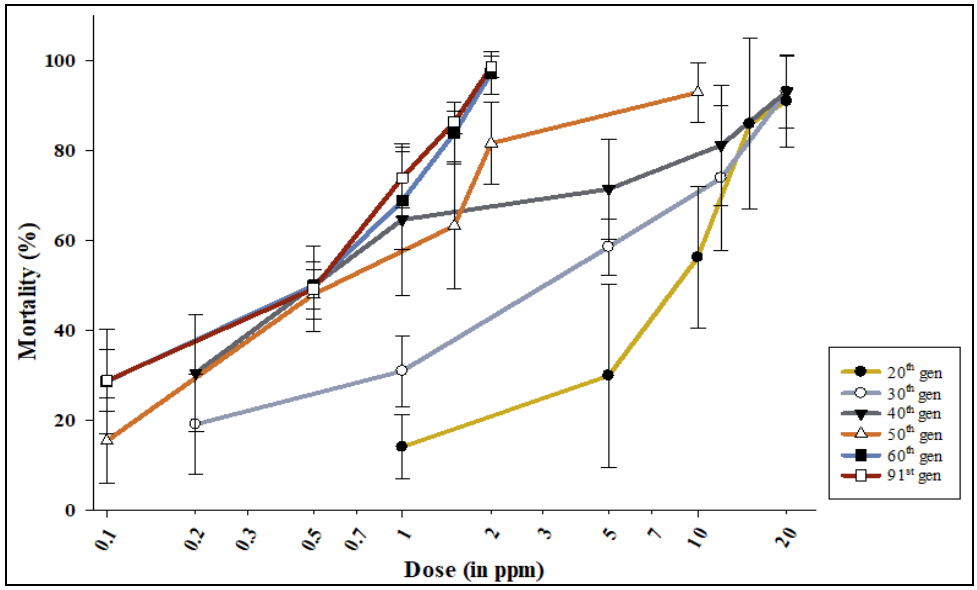

Fig 3: Progress of susceptibility in laboratory population of *T. urticae* (*Tu*SSL) to propargite over different generations (with *SE* values)

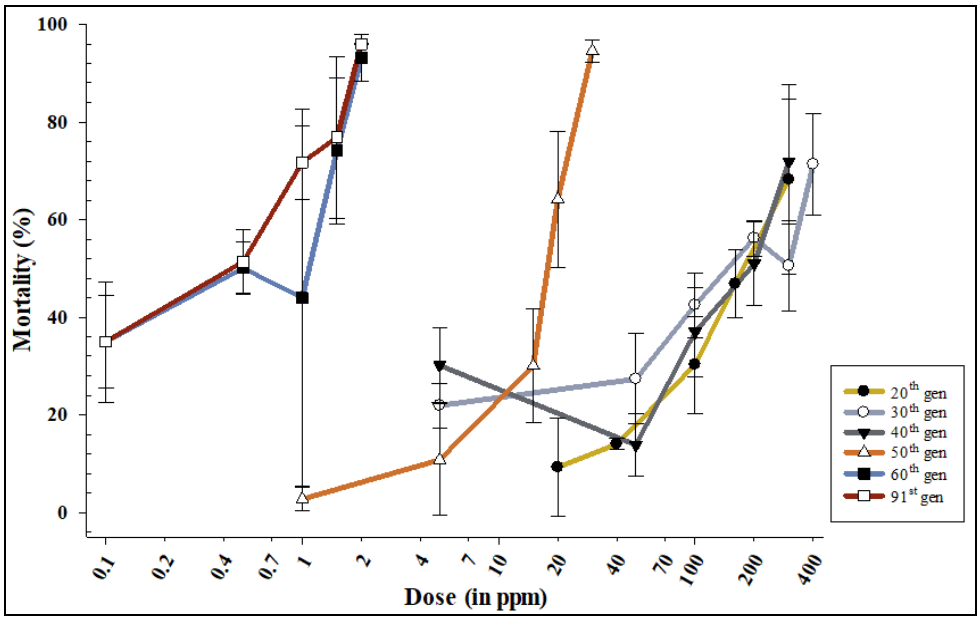

Fig 4: Progress of susceptibility in laboratory population of *T. urticae* (*Tu*SSL) to spiromesifen over different generations (with *SE* values)

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