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Acaricides resistance and its consequences on biology of *Tetranychus urticae* Koch (TSSM) on gerbera under protected cultivation in Tamil Nadu

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Abstract
Spider mites are important crop pests that rapidly develop resistance to Acaricides. *Tetranychus urticae* Koch collected from Hosur, Tamil Nadu during 2022 was reared in the laboratory for more than 10 generations and its susceptibility to selected three Acaricides was determined after every 2 generations. The lowest LC_{50} values of selected Acaricides in the 10 generations *viz*., propargite - 1.81ppm; fenazaquin - 2.20ppm, and spiromesifen - 1.30ppm were used as the baseline data for determining the level of resistance in the field populations of *T. urticae*. Mites samples collected from gerbera crop were subjected to bioassay with three major Acaricides *viz*., Propargite, fenazaquin, and spiromesifen. The level of resistance to spiromesifen (274.98 folds) was high for all three Acaricides tested. The consequence of acaricide resistance on the development of male mites was apparent as the mean duration of development of males in the resistant Hosur population was significantly more (12.2 days *versus* 10.4 days) than in the laboratory susceptible population.

Keywords: Acaricides, LC_{50}, *Tetranychus urticae* and susceptibility

Introduction
Two-spotted spider mite (TSSM), *Tetranychus urticae*, is a very common pest of greenhouse crops and is found throughout the world. Gerbera are grown successfully under protected condition and causes 25 to 30 per cent yield loss (Anon., 2009). This pest has often been referred to as a red-spider. The TSSM is soft-bodied, oval-shaped, and has two dark spots one on either side of the top. This mite is more problematic, particularly on vegetables, fruits, flower crops, and ornamental plants (Guanilo *et al*., 2008) [3]. Loss in brinjal crop due to TSSM infestation in Ludhiana region of Punjab was estimated to be 18 percent (Khadri & Srivivasa, 2018) [4] but according to Jayasinghe & 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 percent loss of yield in Kolar area of Karnataka. Being extremely polyphagous, this pest has an extraordinary ability to develop resistance to pesticides. Since 1990, worldwide populations of TSSM across many crops have developed resistance to several newer Acaricides (Arthropod Pesticide Resistance Database, http:// www.pesticide resistance.org/).

Eggs are laid singly on the surface of leaves. The eggs are spherical and found on the under surface of the leaf often where mite feeding is noticeable. They cause damage by piercing the host plant using chelicerae, and sucking up cellular contents using the rostrum which creates white specks on the adaxial leaf surface (Brust & Gotoh, 2018) [1]. The mite has the capability of destroying 18 to 22 cells/ minute (IRAC, 2009) [6]. The severely infected leaves can be recognized by bronzing and webbing which leads to ballooning. They can be carried easily through the wind. The development rate is significantly determined by temperature. Under greenhouse conditions, the average development time from egg to adult is 14 to 18 days. However, mites developed quickly under hot, dry conditions and may mature in as few as 7 days during this period. Higher temperature (30 to 32 °C), lower humidity (20 to 40%), and better aeration favour the multiplication and the rapid evolution of resistance in mite pests are favored by extensive and frequent use of Acaricides cum insecticides ((Van Leeuwen *et al*., 2011) [12]; Tang *et al*., 2014). The first case of resistance in *T. urticae* was reported to Ammonium potassium selenosulfide (Selecide) by Compton & Kearns (1937) and Saito *et al*., (1983) [9].

The *T. urticae* ranks first in terms of the number of chemicals against which, it had developed resistance (FAO, 2012) [2] and was designated as the 'most resistant species' (IRAC, 2009) [6]. 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 behavior and arrhenotokous reproduction.

Materials and Methods

Rearing of susceptible population

The test insect collected from the infested gerbera crops in Hosur during 2022 were reared on mulberry leaves placed on wet absorbant cotton in Petri plate in the laboratory under pesticide-free conditions for "n" generation to have a highly susceptible source to calibrate the discriminating dose for different Acaricides.

Field Population

Mites collected from Hosur on gerbera crops were maintained separately on mulberry leaves in laboratory conditions at room temperature. Depending on the availability, mites from the field sample or the F1 generation were used for acaricide bioassay studies.

Bioassay

Acaricide bioassays were carried out following the method of the Insecticide Resistance Action Committee (IRAC, 2009) [6] with minor modifications. At least five concentrations of each acaricide (after preliminary assay with 2-3 extreme doses) were used for bioassay with 5 cm^2 mulberry leaf discs following the procedure of Leaf Dip technique (leaf discs were dipped in desired acaricide concentrations for 2 to 3 minutes). 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 predatory mites, insect pests, and dirt before using the bioassay experiment. Air-dried treated leaf discs were placed on a wet cotton wad in a Petri plate and 20 adult female mites were gently transferred onto the leaf disc (using a needle) under a microscope. Three such replications were maintained for each of the test concentrations and observations on mortality were recorded at 24 hours and 48 hrs after the mite release.

Three Acaricides representing different activity groups or modes of action such as 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.

Biology of Resistance and susceptible population of TSSM

To know the consequence of Acaricides resistance on the development of mite, a study with a susceptible laboratory population and resistance field population from kotagiri was carried out. This experiment was done by using a 1.5cm^2 mulberry leaf disc, on which a single adult female mite was released. The leaf disc kept on wet absorbent cotton in Petri plates was placed in a BOD at 28±1 °C and 60-70% RH. Five leaf discs of male and female susceptible laboratory population and resistance field population were maintained separately. Observation from egg to adult emergence were recorded at 4-5 hrs intervals under the microscope and the duration of each development stage of mite was recorded.

Statistical analysis

LC_{50} and LC_{95} values of susceptible and resistance population was calculated by using Finneys (1971) method of Probit analysis. Corresponding Resistance ratio (RR) was calculated by using LC_{50} values of susceptible and LC_{50} values of resistance population. In the present study LC_{50} value of the 30th generation was used for determining the RR values. 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) [5].

$$RR = \frac{\text{LC50 of resistant poulation}}{\text{LC50 of susceptible population}}$$

Male and female mites were examined independently using Tukey's HSD test and compared at a 5% level of significance for data relevant to developmental biology of susceptible and resistant populations.

Results and Discussion

As per the survey report, farmers utilized the same Acaricides to suppress mite incidence, and they generally used mono-cropping in polyhouses. To control red spider mites, carnation growers used to spray Acaricides every week (Table 1).

Base line susceptibility of *T. urticae* to several Acaricides

A fundamental need for creating a real-time sensitive population is the continuous multiplication or culturing of individuals under ideal rearing conditions of temperature and humidity and without any acaricide exposure for numerous generations. Individuals from these communities are tested to determine whether they are susceptible to an acaricide that is of interest at the time (generation). A progressive increase in susceptibility to the corresponding acaricide is shown by a progressive decrease in the LC_{50} values of an acaricide over subsequent generations, and this susceptibility is anticipated to maintain over time or generations. At mite's 10th generation, *T. urticae* baseline vulnerability to the Acaricides used in this study became apparent. Baseline susceptibility of *T. urticae* to Acaricides used in the present study was apparent at the 10th generation of the mite. Therefore, resistance-related toxicological studies with *T. urticae* may use the LC50 value of the relevant acaricide at the 30th generation. The three selective Acaricides with the lowest LC_{50} values at the 30th generation are Propargite (1.81 ppm), Fenazaquin (2.20 ppm), and Spiromesifen (1.30 ppm).

Resistance to Acaricides in *T. urticae* species in the field

Relative toxicity data of Acaricides, such as propargite, fenazaquin and spiromesifen to different field populations (Hosur) are presented in Table 2 to table 4.

Resistance to spiromesifen: Spiromesifen showed an extremely high level of resistance (274.98 folds) followed by propargite (241.36 folds) and fenazaquin (156.46 folds). Thus the intensity of resistance to spiromesifen in *T. urticae* from gerbera crops at Hosur was high. This might be due to the frequently used of spiromesifen at weekly intervals. High level of resistance to spiromesifen in *T. urticae* population from Kolar district, Bangalore (KHADRI & Srinivasa, 2018) [4]. (Sato *et al*., 2016) [10] reported a high frequency of resistant individuals in *the T. urticae* population infesting open cultivated rose and chrysanthemum crops in Brazil. The variability of spiromesifen resistance may be the cause of the

fluctuation in spiromesifen resistance levels between crops or geographical areas.

Resistance to propargite: Propargite showed a very high level of resistance (241.46 folds) followed by fenazaquin (156.46 folds).High level of resistance to propargite with *T. urticae* Koch on vegetable crops in southern districts of Tamil Nadu (Naveena *et al*., 2022) [7].

Resistance to fenazaquin: Fenazaquin also showed a high level of resistance (156.46 folds). Low to moderate level of resistance to fenazaquin with the *T. urticae* population reported from the brinjal crop in different districts of Punjab (Sharma *et al*., 2018) [11] and fenzaquin also showed a low level of resistance in grapes of Northern Karnataka (Patil *et al*., 2020) [8].

Developmental biology and reproduction in acaricide resistant population: In susceptible population successful egg hatching was maximum compared to the resistant population. Data pertaining to duration of different developmental stages in susceptible and resistant population are presented in Table. 5. Total developmental time from egg to adult for male in resistant population was 12.2 days, which is the significantly different from 10.2 days duration for male in the susceptible population and female in resistance population was 12.0 days which is not significantly different from 11.6 days duration for male of susceptible population. (Table 6). In 2018, Najeer *et al.* reported that 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 1: Pesticides usage pattern in Gerbera under protected cultivation

Locations	Host plant	Cropping pattern	Assayed generation	Coordinates	Acaricides /Insecticides used	Doses	Number of spraying during this season
Hosur	Gerbera	Mono-cropping	F_1	12.6942°N 77.862128°E	Omite Magister Oberon Fenpyroximate Reagent	2ml/l 1.5ml/l 2ml/l 1.5ml/l 0.5ml/l	1 2 3 1 2

Table 2: LC_{50} values propargite 57 EC to *Tetranychus urticae* on gerbera at Hosur

Location	Population	LC50 (ppm)	50% fiducial limit		Regression equation	LC95(ppm)	95% fiducial limit		χ^2	Resistance ratio(RR)
			UL	LL			UL	LL		
Hosur	Field	437.36	396.87	481.98	Y= 3.65x-4.65	1232.13	955.99	1588.04	6.22	241.36
	Susceptible	1.81	1.29	2.55	Y=0.96x+4.74	90.58	2.94	2782.57	0.27	

Table 3: LC_{50} values of fenazaquin 10 EC to *Tetranychus urticae* on gerbera at Hosur

Location	Population	LC50(ppm)	50% fiducial limit		Regression equation	LC95 (ppm)	95% fiducial limit		χ^2	Resistance Ratio
			UL	LL			UL	LL		
Hosur	Field	344.23	302.98	391.09	Y = 2.67x-1.78	1419.41	978.08	2059.82	4.88	156.46
	Susceptible	2.20	1.49	3.25	Y = 0.92x+4.68	134.48	2.51	7196.46	0.17	

Table 4: LC_{50} values of spiromesifen 240 SC to *Tetranychus urticae* on gerbera at Hosur

Location	Population	LC50`(ppm)	50% fiducial limit		Regression equation	LC50`(ppm)	95% fiducial limit		χ^2	Resistance ratio
			UL	LL			UL	LL		
Hosur	Field	357.48	316.36	403.93	Y = 2.71x-1.93	1442.63	995.30	2091.003	6.27	274.98
	Susceptible	1.30	1.03	1.64	Y= 1.95x+4.77	9.04	4.33	18.90	1.60	

Table 5: Comparative development of lab susceptible population and resistant population of TSSM, *Tetranychus urticae* under laboratory conditions.

(25±1 ºC; 60-70% RH; 14h: 10h Light & Dark)

Development stage (Days)	Development duration(Days)			
	Susceptible male (Mean±standard error)	Susceptible female (Mean±standard error)	Resistance male (Mean±standard error)	Resistance female (Mean±standard error
Egg	3.2 ± 0.374166	3.6± 0.509901	3.4± 509902	4± 0.316227
Larvae	2.8± 0.374165	3± 0.316227	3.2± 0.374165	3± 0.447213
Protonymph	2.4± 0.244989	2.6± 0.4	2.6± 0.244948	2.8± 0.374165
Deutonymph	1.6± 0.244989	1.8± 0.374165	2± 0.316227	2± 0.316227
Total development (Egg to adult)	10.2± 0.812403	11.6± 0.509901	12.2± 0.663324	12± 0.7071067

Table 6: Comparative development of lab susceptible population and resistant population

Populations	Mean difference	HSD values	Significant
Male(S)- Male(R)	2.0	1.98	Yes
Male(S)-Female(S)	1.4	1.98	No
Female(S)-Female(R)	0.4	1.98	No
Female(S)-Male(R)	0.6	1.98	No

S-susceptible; R-resistance

If mean difference more than HSD values its indicate significant

Conclusion

Among the tested Acaricides, the level of resistance in the field population of *T.urticae* to Acaricides in ascending order is, fenazaquin < propargite < spiromesifen. Due to the action of detoxifying enzymes, mono-cropping of gerbera flowers, and recurrent use of the same Acaricides to manage the mite infestation, the Hosur population displays a high level of resistance ratio to the three Acaricides examined.Female resistance population takes more times for development. Farmers' past use of insecticides has an impact on the beginning and upkeep of the establishment of resistance in mite populations in a given area.

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