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Status of acaricide resistance in field collected two-spotted spider mite, *Tetranychus urticae* Koch from vegetable growing areas of Punjab, India

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Abstract
Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acarina: Tetranychidae), has gained importance in view of their widespread occurrence as a pest on vegetables in Punjab. So far, Scanty information is available on the status of acaricide resistance in *T. urticae* populations in North India. So the study was conducted in 2015-16 to monitor the magnitude of resistance in *T. urticae* on brinjal against different acaricides *viz*., propargite, spiromesifen, fenpyroximate and fenazaquin collected from different vegetable growing areas (Amritsar, Hoshiarpur, Malerkotla and Patiala) of Punjab. Patiala population was least susceptible to fenazaquin (RR 24.65) among all tested acaricides while Amritsar population was found to be highly susceptible (RR 6.67) Among all tested acaricides, fenpyroximate (0.00205 – 0.00954 per cent) was found to be the most toxic against all the tested populations except Malerkotla population. Low to moderate levels of resistance (3.19-24.65 fold) was reported in *T. urticae* to tested acaricides in Punjab.

Keywords: *Tetranychus urticae*, acaricides resistance, susceptible, monitor

1. Introduction
Brinjal, *Solanum melongena* L. is one of the important vegetable crop which is cultivated in all seasons throughout India. Brinjal is intensively grown in Punjab with production of 82800 metric tonnes [1]. Insects, diseases and mites are the main bottlenecks in brinjal productivity. Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) is the most economically important plant-feeding pest mite on the brinjal. This mite has a great potential to produce a high population which depends particularly on temperature, humidity and host plant and these in turn make it one of the most important pests of greenhouses, farms and orchards in different regions of the world [2, 3]. Plant damage results in chlorophyll degradation as well as woven webs with dust accumulation which interferes with photosynthesis in the leaves [4]. There was a reduction in yield of brinjal due to *T. urticae* incidence [5-8].

More than 550 species of insects and mites have developed resistance to at least one class of insecticides/acaricides [9]. It has been found that *T. urticae* has the potential to quickly develop resistance to almost all kinds of acaricides because of their high prolific rate, short life-cycle, high reproductive potential, arrhenotokous reproduction, polyphagous feeding habit, coupled with their extremely dispersal behavior [10-12]. The persistent exposure of *T. urticae* to diverse pesticides in order to contain it below economic threshold has resulted in resistant populations found in more than 40 countries in both greenhouses and field conditions [13] and led to resistance to at least 92 different compounds [14]. Resistance and control failures against *T. urticae* have been reported for pesticides such as organophosphates [15], organotins [16], hexythiazox [17], propargite [18-19], fenpyroximate [11, 20], Spiromesifen [21-22], fenazaquin [23-24, 6], and bifenazate [25]. *T. urticae* can become fully resistant to new acaricides within two to four years, and as a result control of multi-acaricide resistant *T. urticae* has become increasingly difficult [26]. As a consequence, *T. urticae* has attained the dubious reputation to be "the most resistant species" in terms of the total number of pesticides to which it has become resistant [12]. Keeping these in view, monitoring the level of resistance in *T. urticae* populations collected from diverse vegetable (brinjal) growing areas of Punjab towards recommended and new acaricides were planned as scanty information is available on the status of acaricides resistance.

2. Material and methods

Collection of the test insects

The populations comprising of *T. urticae* adults were collected from brinjal from vegetable growing areas i.e Malerkotla, Patiala, Amritsar and Hoshiarpur, where extensive pesticides were being sprayed on all vegetables. Leaves which were infested with *T. urticae* were collected from each location and brought to the laboratory. They were transferred with a fine brush to brinjal plants and were maintained separately in screenhouse. F_1 generation of each population was tested in Acarology laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana.

Raising of mite culture

Brinjal plants, *Solanum melongena*, were planted regularly once a week in small earthern pots in the screen house at the Entomological Research Farm, Punjab Agricultural University Ludhiana. Plants were kept in a screenhouse at 25 ± 2 °C temperature and they were transferred to a growth chamber to be infested by mites. Field collected populations of *Tetranychus urticae* Koch were released on the plants and mite culture for each population was maintained separately for further experiments.

Rearing of Susceptible population of *T. urticae*

The susceptible population of *T. urticae* was maintained on brinjal without exposure to any acaricide in the screen house and isolated from possible contaminants (i.e. pesticides and other arthropods for 25 generations.

Test Acaricides: Commercially available acaricides propargite (57% EC), spiromesifen (240 SC), fenazaquin (10% EC) and fenpyroximate (5% EC) were used for bioassay of test insect

Bioassay: The toxicity of four acaricides viz propargite, spiromesifen, fenazaquin and fenpyroximate on two spotted spider mite was assayed using leaf dip method of bioassay. Five different doses of acaricides were prepared that produced 0-100% mortality. Leaf discs (2cm diameter) was punched from three week old brinjal leaves. Cut discs were examined for predatory thrips or predatory mites), to confirm an absence of contamination. Leaf discs were immersed in the respective test chemical solutions for ten seconds. Control leaf discs were treated with water. After shadow drying in room temperature each treated discs were placed on wet cotton in a Petridish of 8 cm diameter and height of 1.5 cm with a lid. The Petri dishes were moistened with distilled water to maintain leaf disc turgidity. Twenty *T. urticae* adult females (F_1 generation) were transferred to each leaf disc. Each concentration and the control treatment had three replicates.

The number of live and dead mites in each replication was counted under stereo microscope. Petri dishes were covered with a ventilated lid and incubated (27 ± 2^0C) for 24 h. The resistance ratios (RR) were categorized as follows: low, RR ≤ 10; moderate, 10 < RR ≤ 40; high, 40 < RR ≤ 160; very high, 160 < RR.

Observations

The criterion for mortality was an inability on the part of mites to move appendages when lightly prodded with a camel's hair brush. Mortality was recorded after 24 hours of acaricide exposure.

Statistical analysis

The log concentration-mortality regression was estimated by probit analysis using the POLO programme [27]. The degree of resistance acquired by *T. urticae* (Resistance ratio) was calculated by dividing the higher LC_{50} value of a population with a lower LC_{50} value of another population for each acaricide and the relative degree of resistance acquired was calculated. The toxicity ratios were worked out by dividing the LC_{50} value of the least toxic acaricides with the LC_{50} value of the insecticide in question.

3. Result and Discussion

Susceptibility of *T. urticae* to propargite

The lowest LC_{50} value (0.0605%) of propargite was obtained against the population collected from Amritsar. However, the highest LC_{50} value (0.123%) was found against the population collected from Hoshiarpur, which was followed by Patiala and Malerkotla populations with LC_{50} value of 0.113 and 0.096 per cent, respectively (Table 1). The levels of resistance from Amritsar, Hoshiarpur, Patiala and Malerkotla populations were 9.03, 18.36, 16.86 and 14.33 times to propargite when compared with the susceptible population value (0.0067%). Similar results were obtained while monitoring acaricides resistance from Gandevi and Gadat areas of Navsari against propargite in TSSM on the brinjal exhibiting moderate level of resistance 32.08 and 28.43 fold [6]. Low to moderate level of resistance was reported in *T. urticae* on brinjal against propargite 1.0 - 8.97 fold in 2008 [28], 2.85 - 38.57 fold in 2010 [5], 5 - 9 fold in 2012 [29] and 1- 4 fold in 2014 [6] which are in conformity with our results. Moderate level of resistance against propargite (39-135 fold) in *T. urticae* populations [30-31]. In India low to moderate level of resistance in *T. urticae* to propargite was reported in Bangalore district (4-12 fold) and Kolar district (6-28 fold) in 2007 to 2009 from the tomato crop [28] while in Korea and Czech Republic low level of resistance against propargite (6-15 fold) was exhibited by *T. urticae* populations collected from apple orchards [32] and hop yards [33].

The fenpyroximate resistant strain of *T. urticae* in Turkey exhibited 6.43 fold resistance against propargite [34]. On comparing relative toxicities of various acaricides against laboratory reared *T. urticae* population, high level of resisatance i.e 197.56 fold resistance against propargite was reported which is in contrast to the present findings [19].

Susceptibility of *T. urticae* to Spiromesifen

The data presented in Table 1 revealed that the LC_{50} values and the related parameters of spiromesifen for *T. urticae* population collected from vegetable growing areas of Punjab ranged between 0.0047 and 0.0092 per cent. The lowest LC_{50} value (0.0047) was obtained from Hoshiarpur populations while the highest LC_{50} value (0.0092) was found in population from Malerkotla which was followed by Patiala (0.0084) and Amritsar (0.0068) populations, respectively.

On comparing the results obtained with LC_{50} value of 0.00043 of susceptible population, the resistance ratio (RR) calculated under the present study was found to be 11.14, 15.81, 19.53 and 21.40 fold for *T. urticae* population collected from Hoshiarpur, Amritsar, Patiala and Malerkotla, respectively. These results are in accordance with the studies where laboratory reared *T. urticae* population exhibited 32.13 fold resistance against spiromesifen in Kangra [19].

Compared to the laboratory selected hexythiazox populations, moderate levels of cross-resistance to spirodiclofen (8.12 fold), etoxazole (14.41 fold), spiromesifen (17.96 fold), propargite (17.48 fold), clofentezine (12.67 fold) and milbectin (11.22 fold) respectively, was observed fro cucumber in Jordan [21].

Susceptibility of *T. urticae* to fenpyroximate

Fenpyroximate was found to be the most toxic among the tested acaricides against *T. urticae* populations collected from different locations of Punjab with LC_{50} values ranging from 0.00205 to 0.00954 in the present studies (Table 1). The highest LC_{50} value (0.00954) was recorded from Malerkotla population followed by Patiala (0.0073), Amritsar (0.0030), and Hoshiarpur populations (0.00205). Considering the LC_{50} value of the laboratory susceptible strain as the baseline 0.00094, the resistance ratio worked out in the present study was 10.15 fold for Malerkotla, 7.77, 3.19 and 2.18 for Patiala, Amritsar and Hoshiarpur populations, respectively. Development of resistance to fenpyroximate in laboratory reared *T. urticae* was 14.54 fold from Palampur [19]. A study from Korea revealed that *T. urticae* had developed 182-fold resistance to fenpyroximate when compared with susceptible strain which is in contrast to the present studies [35]. The present studies are in accordance with observation that fenpyroximate resistant strain of *T. urticae* from apple in Korea, when selected for 20 generations with fenpyroximate with selection pressure of 30-50 per cent mortality, exhibited 252 fold resistance to fenpyroximate [36]. Similarily, pyridaben resistance strain of *T. urticae* showed high level of cross resistance to fenpyroximate (373 fold) [18]. Results on monitoring the development of the resistance in *T. urticae* to fenpyroximate throughout Korea revealed that resistance to fenpyroximate ranged from 6.07 to 977.39 fold in 16 commercial green house crops and from 1.43 to 933.26 fold from 10 apple orchards, respectively [37]. Similarily, dicofol resistant strain (after selection for 20 generations with dicofol) of *T. urticae* collected from apple in Korea showed 67.7, 63.3 and 373 fold resistance against fenpyroximate, pyridaben and acrinathrin, respectively [38]. Monitoring the acaricides resistance in *T. urticae* strains collected from rose (SAN and PSE) showed 48.4 and 163.6 fold resistance against tebufenpyrad and 74.1 and 25.9 fold resistance against fenpyroximate in SAN and PSE strains, respectively when compared to susceptible BOSA strain [39].

Susceptibility of *T. urticae* to fenazaquin

The data presented in Table 1 revealed that the LC_{50} values and the related parameters of fenazaquin for *T. urticae* populations collected from four districts of Punjab ranged from 0.018 to 0.004 per cent. The lowest LC_{50} value (0.004) was obtained against the population of *T. urticae* from Amritsar, while the highest LC_{50} value (0.018) was found against population collected from Patiala. Considering the LC_{50} value of laboratory susceptible strain as baseline 0.00073, the resistance ratio worked out in the present study was 24.65 fold for population collected from Patiala. This population exhibited maximum resistance among all the tested acaricides on *T. urticae* populations collected from different locations. While populations collected from Malerkotla, Hoshiarpur and Amritsar exhibited 17.12, 15.18 and 6.67 fold, repectively. The results are in agreement with the results exhibiting 310 fold resistance against fenazaquin in *T. urticae* population collected from tomato grown in fields in Cyprus and 189 fold resistance against *T. urticae* population collected from rose grown in greenhouse from Cyprus [24]. These studies are in accordance with some of the studies carried out in India where low to moderate level of resistance in *T. urticae* to fenazaquin was reported in Bangalore district (5 -32 fold) and Kolar district (8-25 fold) and contradictory to the report where high level of resistance was reported from Vadugar (168-249 folds) of Kolar district in 2007 to 2009 from Tomato crop [28]. In earlier studies carried out in Punjab, on resistance monitoring low to moderate level of resistance was reported in *T. urticae* on brinjal against fenazaquin 1.0-7.5 fold, 22.22-55.55 fold, 36-30 fold and 1.43-7.14 fold from different districts of Punjab, when compared with the susceptible strain [5,6,28,29]. A Belgian field strain (MR-VP) of *T. urticae* exhibited 35 fold resistance against fenazaquin which is in accordance with the present studies [20]. Surveying of Iranian populations of the two-spotted spider mite, *T. urticae*to to determine resistance mechanisms against fenazaquin was done. Leaf disc dip method of bioassay was carried out on four populations, collected from Isfahan, Yazd and Rasht. The results revealed that resistance ratios of Isfahan, Yazd and Rasht populations were 3109, 439.5 and 10.53 fold, respectively, compared with the susceptible population [23].

Table 1: Toxicity and resistance ratio of various acaricides against *T urticae* in major brinjal growing areas of Punjab

Sr. No	Acaricides	Locations	LC_{50} (%)	FL at 95% CL	Slope	Heterogeneity	Resistance ratio
1	Propargite	Amritsar	0.0605	0.0292-0.0929	1.962±0.460	0.52	9.03
		Hoshiarpur	0.123	0.057-0.190	2.020±0.487	0.68	18.36
		Patiala	0.113	0.0603-0.165	1.991±0.442	0.71	16.86
		Malerkotla	0.096	0.048-0.144	1.956±0.446	0.28	14.33
2	Spiromesifen	Amritsar	0.0068	0.0027-0.0095	1.785±0.428	0.56	15.81
		Hoshiarpur	0.00479	0.00278-0.00692	2.141±0.458	0.47	11.14
		Patiala	0.0084	0.004-0.0127	2.121±0.506	0.49	19.53
		Malerkotla	0.0092	0.0048-0.0139	1.806±0.414	0.86	21.40
3	Fenpyroximate	Amritsar	0.0030	0.00152-0.00450	1.954±0.446	0.28	3.19
		Hoshiarpur	0.00205	0.0011-0.0029	2.107±0.447	0.37	2.18
		Patiala	0.0073	0.0038-0.0107	1.928±0.429	0.44	7.77
		Malerkotla	0.00954	0.0044-0.0145	2.157±0.529	0.57	10.15
4	Fenazaquin	Amritsar	0.00487	0.00247-0.00719	2.011±0.456	0.22	6.67
		Hoshiarpur	0.01108	0.0052-0.0168	2.038±0.481	0.26	15.18
		Patiala	0.018	0.0104-0.0257	2.203±0.477	0.59	24.65
		Malerkotla	0.0125	0.0063-0.0189	1.825±0.421	0.71	17.12

Relative toxicity of different acaricides to *T. urticae* populations collected from different locations

The relative toxicity of different acaricides against *T. urticae* collected from four vegetables growing areas of Punjab was calculated and presented in Table 2.Fenpyroximate with LC_{50} value of 0.003 per cent was found to be 20.17 times more toxic than the propargite against Amritsar population, while fenazaquin and spiromesifen showed 12.42 and 8.90 times more toxicity than propargite, respectively (Table 2). In Hoshiarpur populations, the toxicity of fenpyroximate,

spiromesifen and fenazaquin was found to be 60, 25.68 and 11.10 times more toxic than propargite, respectively. The *T. urticae* populations collected from Patiala when treated with different acaricides by leaf dip technique showed the LC_{50} values to be 0.0073, 0.0084, 0.018 and 0.113 for fenpyroximate, spiromesifen, fenazaquin and propargite, respectively, thereby showing 15.48, 13.45 and 6.28 times toxicity than propargite. In the *T. urticae* populations collected from Malerkotla, when treated with different insecticides by leaf dip technique, the LC_{50} values were observed to be 0.0092, 0.0095, 0.0125 and 0.096 for spiromesifen, fenpyroximate, fenazaquin and propargite, respectively, thereby showing 10.43, 10.06 and 7.68 times toxicity than propargite.

Based on LC_{50} values obtained, the order of toxicity of newer/recommended acaricides was found to be fenpyroximate > fenazaquin > spiromesifen > propargite for the Amritsar populations. However, for *T. urticae* populations from Hoshiarpur, the trend was fenpyroximate >spiromesifen > fenazaquin > propargite, while for Patiala population the order of toxicity was fenpyroximate >spiromesifen > fenazaquin > propargite wherein the trend of toxicity for Malerkotla populations was spiromesifen > fenpyroximate > fenazaquin > propargite.

Fenazaquin was 1.5, 1.63 and 3.75 times more toxic to population of Malerkotla, Hoshiarpur and Amritsar in comparison to Patiala population. According to LC_{50} value, propargite was found to be 1.08, 1.28 and 2.05 times more toxic in Patiala, Malerkotla and Amritsar than Hoshiarpur populations, respectively. Similarily fenpyroximate was 1.3, 3.17 and 4.75 times more toxic to population of Patiala, Amritsar and Hoshiarpur in comparison to Malerkotla populations, respectively, whereas spiromesifen showed 1.09, 1.35 and 1.95 times more toxicity in Patiala, Amritsar and Hoshiarpur than Malerkotla population, respectively. Fenpyroximate was found to be the most toxic to *T. urticae* among all other acaricides tested i.e fenazaquin, spiromesifen and propargite. Propargite, spiromesifen, and fenpyroximate showed less toxicity to Malerkotla population as compared to Patiala, Amritsar and Hoshiarpur populations.

The present findings are also similar to the studies conducted on the relative toxicity of common acaricides to *T. urticae* on tomato. Results revealed milbemectin (1.05ppm) and abamectin (1.44ppm) were more toxic to adults. Next in the order of toxicity to adults, was fenpyroximate (20.82ppm) and fenazaquin (32.75ppm). The order of toxicity of different acaricides to adult females was, milbemectin> abamectin> fenpyroximate> fenazaquin> buprofezin> dicofol> diafenthiuron> bifenthrin> flufenzin> clofentezine> propargite [40]. Results on toxicity of acaricides reported that fenpyroximate resulted in the maximum reduction (96-98%) of *T. urticae* population on brinjal in Bangalore with significant increase in fruit yield (>64q/acre vs 31q/acre in control) [41]. Results suggests that fenpyroximate is highly effective against TSSM and this acaricides can prove to be a promising substitute for the management of resistance. Other acaricides are also effective but if they are used judiciously and alternatively to prevent development of resistance.

Table 2: Comparative toxicity of different acaricides against *T. urticae* populations

Locations	LC_{50} (%) Acaricides			
	Propargite	**Spiromesifen**	**Fenpyroximate**	**Fenazaquin**
Amritsar	0.0605 (1.00)	0.0068 (8.90)	0.0030 (20.17)	0.00487 (12.42)
Hoshiarpur	0.123 (1.00)	0.00479 (25.68)	0.00205 (60.00)	0.01108 (11.10)
Patiala	0.113 (1.00)	0.0084 (13.45)	0.0073 (15.48)	0.018 (6.28)
Malerkotla	0.096 (1.00)	0.0092 (10.43)	0.00954 (10.06)	0.0125 (7.68)

Figures in parentheses are the toxicity ratios

As fenpyroximate has not been recommended against any crop in Punjab low level of resistance build up in *T. urticae* has been reported in my present studies. Although farmers are using acaricide and is giving efficient control against mites but there is possibility of resistance The present study, thus, presents a comprehensive resistance profile of *T. urticae* populations collected from vegetable growing areas of Punjab, suggesting that low to moderate level of resistance has been observed against recommended and new acaricides. To manage the development of resistance, a resistance management strategy of decreased selection pressure could be achieved by alternating these acaicides.

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