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Acaricide resistance in *Tetranychus urticae* on cucumber (*Cucumis sativus*) under protected cultivation

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ABSTRACT

The present study was conducted to determine the effects of four acaricides on four different populations of two-spotted spider mite, *Tetranychus urticae*, collected from cucumber (*Cucumis sativus* L.) under protected cultivation in 2016 and 2017. The statistical differences were found in susceptibility against treated acaricides and between populations collected from different regions of Punjab. The population with a resistance ratio of more than 60-folds with respect to an acaricide was considered as resistant population. Among all tested acaricides, the highest resistance ratio was reported to fenazaquin (62.52 to 212.55 folds) in population collected from Hoshiarpur, Patiala and Bathinda. Low to moderate level of resistance was reported against spiromesifen (3.76 to 32.10 folds). Propargite (14.64 to 22.17 folds) and fenpyroximate (17.10 to 32.10-folds) showed moderate level of resistance in *T. urticae*. The collected populations of *T. urticae* on cucumber under protected cultivation showed moderate to very high level of resistance to spiromesifen, propargite, fenpyroximate and fenazaquin, respectively in Punjab.

Key words: Acaricides, Cucumber, Monitoring, Resistance, *Tetranychus urticae*

The two-spotted spider mite, *Tetranychus urticae* is the most economically important arthropod pest among phytophagous mite species in India. This mite has the potential to build its population in favourable temperature and relative humidity. These two factors make it one of the most important key pest of vegetables grown under protected cultivation (Zhang 2003). Due to continued or repeated use of various acaricides at shorter intervals against *T. urticae* has disrupted natural biological control system and led to resistance development. Increased levels of resistance to the most widely used acaricides have caused multiple treatments and excessive doses, raised serious environmental and human health concerns. Based on field surveillance and screening of various acaricides, it was speculated that *T. urticae* has developed resistance to most of the conventional acaricides (Hoy 2011). There are reports of resistance development in more than 550 species of insects and mites to one class of insecticides and acaricides (Van Leeuwan 2012). This wide spread acaricide resistance has been a major obstacle in the cost-effective integrated mite management programme in India. In addition, factors such as increased costs of labour and pesticide application and safety issues have made cultivation of crops difficult for farming community. The continuous exposure of two-spotted spider mite to different acaricides has resulted in resistance development both in greenhouse and field conditions. The higher levels of resistance in two-spotted spider mite to different acaricides were detected for dicofol (Kaur and Bhullar 2011), propargite and spiromesifen (Kaur and Bhullar 2016), organophosphates (Van *et al.* 2009), organotin (Moghadam *et al.* 2012), hexythiazox (Vassiliou and Kitsis 2013), fenpyroximate (Sato *et al.* 2004), fenazaquin (Sharma and Bhullar 2018, Kaur and Bhullar 2016) and bifenazate (Ay *et al.* 2005). Due to the fast evolution of resistance development, two-spotted spider mite has now attained status of most resistant species in the world (Van *et al.* 2010). In India, little work has been done on resistance patterns in two-spotted spider mite to currently used acaricides on vegetables grown under protected cultivation. It is quite possible that their susceptibility to acaricides would differ between localities under ployhouse. Therefore, it is important to study acaricide susceptibilities of *T. urticae* collected from cucumber grown under protected cultivation in Punjab. Our paper reports the results of laboratory-based tests that determine the response of the four collected populations and susceptible strain of *T. urticae* to four commonly used acaricides (propargite, spiromesifen, fenpyroximate and fenazaquin) by the farmers.

MATERIALS AND METHODS

Chemicals: Commercial formulations of propargite 57% EC (Inhibitors of mitochondrial ATP synthase Energy metabolism), fenazaquin 10% EC, spiromesifen 22.9 SC and

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fenpyroximate 5 % EC (Mitochondrial complex I electron transport inhibitors) were used for the present study.

Collection of T. urticae from cucumber under protected cultivation: Survey was done for the collection of two-spotted spider mite on cucumber under protected cultivation from different districts, i.e. Bathinda, Hoshiarpur, Patiala and Sangrur (Malerkotla) of Punjab, India in 2016 and 2017. The mite infested cucumber foliage were collected**,** placed in polyethylene bags and brought to the laboratory. Each sample was checked for the presence of adults. Each collected population was tested with different commonly used acaricides at different concentrations.

Maintenance of susceptible population: Susceptible population was maintained in polyhouse at Entomological Research Farm, Punjab Agricultural University (PAU), Ludhiana on potted plants of French bean without exposing to any acaricides. Then this population was bioassayed with different acaricides at different concentrations in Acarology Laboratory, Department of Entomology, PAU, Ludhiana.

Bioassay: Response of populations of two-spotted spider mite collected from different areas was studied by bioassay conducted on female adult mites in 2016 and 2017. Leaf-dip method was followed for bioassay studies. Leaf discs were prepared by punching leaves of cucumber plants and were dipped in respective test acaricidal solutions for 10 sec. However, control leaf discs were treated with water. After shadow drying, they were placed on wet cotton pads in Petri dishes (9 cm diameter and 2 cm in height) and maintained in laboratory conditions at 26±1°C, RH 60–70%. Twenty adult females were transferred to each leaf disc. Each acaricide concentration and control treatment was replicated thrice to get more accuracy in result. Mortality of mites was determined after 24 h of treatment, and mites were considered dead is appendages did not move when prodded with camel hair brush. Treated adult mites were kept at 25–27°C temperature.

Statistical analysis: Lethal concentration values (LC_{50}) were calculated using the Probit analysis with chi-square and the slope associated with dose-response relationship (Finney 1971).The level of resistance (RR) acquired by two-spotted spider mite was calculated by dividing LC_{50} of resistant population with LC_{50} of susceptible population. The RR values of <10, 10-40, 40-60 and >60 indicate low, moderate, high and very high resistance levels, respectively (Fukami *et al.* 1983).

RESULTS AND DISCUSSION

LC_{50}s of propargite, fenazaquin, spiromesifen and fenpyroximate were 0.002, 0.0004, 0.0005 and 0.0001%, respectively for susceptible population. The susceptible population was compared with the populations collected from cucumber grown under protected cultivation of different locations, viz. Malerkotla (Sangrur), Hoshiarpur, Patiala and Bathinda for finding out the level of resistance.

Response of T. urticae to propargite: The highest LC_{50} value of propargite was obtained in population collected from Patiala (44.34%) followed by Malerkotla (35.45%) and Bathinda (29.29%). The population collected from Hoshiarpur was found to be susceptible resulting in death of all the individuals (Table 1). The level of resistance to propargite was found to be moderate in population collected from Patiala (RR = 22.17 folds), Malerkotla (RR = 17.72 folds) and Bathinda (RR = 14.64 folds). The population of *T. urticae* collected from Hoshiarpur showed high susceptibility to propargite (Table 3). Moderate level of resistance was reported to propargite (9.03 to 14.33-folds) in two-spotted spider mite on brinjal (Sharma and Bhullar 2018). Ay *et al.* (2005) also reported moderate level of resistance against propargite (39-135-folds) in two-spotted spider mite populations. Low level of resistance was reported against propargite (6-15-folds) in two-spotted spider mite population collected from apple orchards and hop yards in Korea and Czech Republic (Koh *et al.* 2009, Vostre *et al.* 2010). In India, there are reports of low to moderate level of resistance in Punjab (2.85 - 38.57-folds) in two-spotted spider mite on brinjal against propargite (Kaur and Bhullar 2011). The results corroborate with the earlier studies conducted at Gandevi and Gadat areas of Navsari against propargite in two-spotted spider mite on the brinjal exhibiting moderate level of resistance 32.08 and 28.43 folds (Anonymous 2015). Kaur and Bhullar (2016) reported high level of resistance to propargite in field collected two-spotted spider mite population which is in contrast to our study.

Response of T. urticae to fenazaquin: The results obtained from the study revealed that the LC_{50} value of fenazaquin was observed highest in Bathinda population (85.02 %) followed by Patiala (35.57%), Hoshiarpur (25.01%) and Malerkotla (8.15%) (Table 1). In comparison

Table 1 LC_{50}s of propargite and fenazaquin against *Tetranychus urticae* on cucumber under protected cultivation in Punjab

Acaricides	Locations	LC_{50}s	Fiducial Range	Chi-square	Heterogeneity	Slope
Propargite	Malerkotla	35.45	15.503-59.694	2.13	0.71	1.778±0.525
	Hoshiarpur	death of all the individuals				
	Patiala	44.34	9.347-73.363	0.89	0.29	0.761±0.456
	Bathinda	29.29	9.347-73.363	1.85	0.62	0.688±0.469
Fenazaquin	Malerkotla	8.15	0.602-19.590	1.68	0.56	1.424±0.621
	Hoshiarpur	25.01	10.802-41.291	1.38	0.46	2.090±0.631
	Patiala	35.57	13.090-81.601	0.98	0.23	1.131±0.479
	Bathinda	85.02	59.924-601.196	1.80	0.60	2.883±0.491

Table 2 LC_{50}s of spiromesifen and fenpyroximate against *Tetranychus urticae* on cucumber under protected cultivation in Punjab

Acaricides	Locations	LC_{50}s	Fiducial range	Chi-square	Heterogeneity	Slope
Spiromesifen	Malerkotla	5.70	0.181-5.877	0.93	0.62	1.208±0.517
	Hoshiarpur	1.88	0.535-6.112	2.01	0.68	0.979±0.532
	Patiala	2.02	0.895-9.037	1.28	0.42	0.781±0.511
	Bathinda	16.05	4.237-35.706	2.35	0.78	1.6±0.4795
Fenpyroximate	Malerkotla	2.21	0.180-5.877	0.75	0.25	1.094±0.582
	Hoshiarpur	2.32	0.930-5.204	0.75	0.25	2.520±1.372
	Patiala	1.59	1.463-3.927	0.59	0.19	1.648±0.643
	Bathinda	1.71	0.1806-5.877	1.62	0.54	1.151±0.594

to susceptible population of *T. urticae*, the resistance ratio was found to be very high to fenazaquinin population collected from Bathinda (RR = 212.55 folds), Patiala (RR = 88.92 folds) and Hoshiarpur (RR = 62.52 folds) (Table 3). However, moderate level of resistance was recorded in Malerkotla population to fenazaquin (RR = 20.37 folds). Similar results of high level of resistance were reported on tomato (310 folds) and roses (189 folds) to fenazaquin in two-spotted spider mite in Cyprus (Vassiliou and Kitsis 2013).The high level of resistance was also reported from Vadugar (168-249 folds) of Kolar district in 2007 to 2009 from Tomato (Anonymous 2009). In Punjab, high level of resistance to fenazaquin in two-spotted spider mite on brinjal was recorded (Kaur and Bhullar 2016). The studies conducted in Iran, revealed that resistance ratios of Isfahan, Yazd and Rasht population of two-spotted spider mite were 3109, 439.5 and 10.53 folds, respectively, in comparison to susceptible population (Moghadam *et al.* 2012).

Response of T. urticae to spiromesifen: Spiromesifen was found least effective against Bathinda population as compared to other populations. The LC_{50} value of three populations collected from Malerkotla, Patiala and Hoshiarpur was found to be 5.70%, 2.02% and 1.88%, respectively (Table 2). Moderate level of resistance against spiromesifen was reported in population collected from Malerkotla (11.40 folds) and Bathinda (32.10 folds) and low level of resistance in population collected from Hoshiarpur (3.76 folds) and Patiala (4.04 folds) (Table 3). In Jordan, studies were conducted on acaricide resistance development and observed moderate level of resistance (17.96 folds) in *T. urticae* population collected from cucumber (Mohammad *et al.* 2012). Sharma and Bhullar (2018) reported moderate level of resistance (11.14 to 21.40 folds) to spiromesifen in *T. urticae*. The results of studies conducted on resistance monitoring at Kangra are in accordance with our study where *T. urticae* showed resistance ratio of 32.13 folds to spiromesifen (Kumari *et al.* 2017).

Table 3 Resistance ratios of various acaricides against *Tetranychus urticae* on cucumber under protected cultivation in Punjab

Acaricides	Resistance ratios			
	Propargite	Fenazaquin	Spiromesifen	Fenpyroximate
Malerkotla	17.72	20.37	11.40	22.10
Hoshiarpur	0.0	62.52	3.76	23.20
Patiala	22.17	88.92	4.04	32.10
Bathinda	14.64	212.55	32.10	17.10

Response of T. urticae to fenpyroximate: The data revealed that population of two-spotted spider mite collected from four districts were found to show low LC_{50} value for fenpyroximate as compared to other tested acaricides. The range of LC_{50} value was found to be in range of 1.59 to 2.32% for fenpyroximate in all four tested acaricides (Table 2). The population of *T. urticae* collected from four districts exhibited moderate level of resistance to fenpyroximate and was in range of 17.10 to 32.10 folds (Table 3).There are reports of resistance development in *T. urticae* to fenpyroximate in Korea. The resistant strain of *T.urticae* from apple in Korea after selection pressure for 20 generations with fenpyroximate exhibited 252 folds resistance to fenpyroximate (Kim *et al.* 2004). The studies conducted in Korea revealed that resistance to fenpyroximate ranged from 6.07 to 977.39 folds in *T. urticae* population collected from commercial green house crops and from 1.43 to 933.26 folds from 10 apple orchards, respectively (Suh *et al.* 2006). There are reports of cross resistance in pyridaben resistant strain (373 folds) and dicofol resistant strain (67.7 folds) of *T. urticae* to fenpyroximate (Kim *et al.* 2006, Kim *et al.* 2007).The population of *T. urticae* collected from rose showed 74.1 and 25.9 folds resistance to fenpyroximate in SAN and PSE strains, respectively (Tirello *et al.* 2012). In India, the studies conducted on acaricidal resistance in *T. urticae* population collected from Palampur revealed moderate level of resistance (14.54 folds) to fenpyroximate (Kumari *et al.* 2017). Sharma and Bhullar (2018) studied that status of acaricide resistance and worked out the resistance ratios in range of 2.8 to 10.15 in *T. urticae* to fenpyroximate.

The development of resistance is expected to be increased in situations where spider mite control only relies on acaricides. There was statistical difference in level of resistance depending on the acaricide treated and the locality from where the population was collected. The population of *T. urticae* showed very high level of resistance to fenazaquin and moderate level to spiromesifen, propargite, fenpyroximate in Punjab.

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