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FULL LENGTH ARTICLE

Comparative toxicities of novel and conventional acaricides against different stages of *Tetranychus urticae* Koch (Acarina: Tetranychidae)

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Abstract Adulticidal, ovicidal and nymphicidal effects of different newer acaricides along with some conventional one were evaluated on the life stages of a susceptible laboratory strain of two-spotted spider mite (TSMM), *Tetranychus urticae* Koch (Acarina: Tetranychidae) using a spray method. Abamectin was found to be the most toxic to the adults (LC_{50} = 0.39 ppm) followed by fenpyroximate (5.67 ppm), spiromesifen (12.53 ppm), chlorfenapyr (32.24 ppm), propargite (77.05 ppm) and dicofol (146.65 ppm). Hexythiazox was least toxic. There was no egg hatching when eggs were sprayed with one third of the recommended concentration of spiromesifen. This was statistically significantly different from all other treatments (fenpyroximate, chlorfenapyr, propargite, dicofol and hexythiazox) which were, however, at par with each other. Based on the 10^{th} day observations, the ovicidal activity of spiromesifen (100%) was followed by dicofol (7.78% egg mortality) and hexythiazox (6.67%). Almost no effect on hatching was observed in both abamectin and chlorfenapyr treatment (0.54%). In case of propargite, all the treated eggs hatched. Abamectin resulted in highest nymphal mortality (96.05%) followed by dicofol (94.51%), hexythiazox (90.24) propargite (90.00), chlorfenapyr (89.33) and fenpyroximate (86.84%) and all the treatments were at par with each other and statistically different from the control. The present study revealed that abamectin, spiromesifen, hexythiazox, fenpyroximate and chlorfenapyr acaricides can alternatively be used for effective management of the mites.

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1. Introduction

Phytophagous mites are among the most common plant pests, responsible for significant yield losses in many economically important crops, such as fruits, cotton, vegetables and ornamentals. One of the most important species of mite is the two-spotted spider mite (TSMM), *Tetranychus urticae* Koch

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(Acari: Tetranychidae) which is an economically important pest of many field and greenhouse crops of the world. It is probably the most important species in the family Tetranychidae associated with 900 plant species (Jeppson et al., 1975; Meyer, 1996). It affects crops by direct feeding, reducing the area of photosynthetic activity and may result in leaf abscission in severe infestation (Gorman et al., 2002). It is reported to cause significant economic losses in tomatoes, peppers, roses, and beans. Many insecticides and acaricides have been used for their control (Kim et al., 2006). Control of plant-feeding mites is a continual struggle. However, their control is hindered by rapid evolution of resistance to many chemical classes of pesticides (Stumpf and Nauen, 2001; Sato et al., 2005; Whalon et al., 2008; Nicastro et al., 2010, 2013; Tirello et al., 2012). The resistance development is favored by its high reproductive potential and extremely short life cycle (Ismail et al., 2007).

The degree of resistance to many established acaricides has resulted in a demand to develop and introduce compounds with novel modes of action (Devine et al., 2001). Therefore, considerable research efforts have been done in finding alternative strategies for the suppression of mites especially in the search for bio-active substances. It resulted in an increasing interest for natural pesticides derived from plants and micro-organisms (Tedeschi et al., 2001; Attia et al., 2013) or development of other risk reduced pesticides as they are generally perceived to be safer. In recent years, a number of acaricides with novel or under-exploited modes of action have been introduced (Kumari et al., 2012) for controlling mites as a result of acaricide research which is mainly focussed on the identification of suitable and novel target sites. To overcome the resistance problems, exploitation of new chemistries that operate on novel sites of miticidal action and the judicious use of acaricides from different modes of action are currently the best approaches (Dekeyser, 2005).

Most of the modern acaricides exert their effects through disruption of respiratory processes or effects on growth and development (Dekeyser, 2005; Krämer and Schirmer, 2007). Abamectin is a risk reduced neuroactive insecto-acaricide (chloride channel activator). It is a mixture of natural products avermectin B1a (> 80%) and avermectin B1b (< 20%) isolated from the fermentation of a soil bacterium *Streptomyces avermitilis*. Abamectin is considered safe to beneficial arthropods under field conditions due to their short environmental persistence, rapid uptake into treated plants and fast degradation of surface residues (Krämer and Schirmer, 2007). Spiromesifen is a novel insecticidal/acaricidal compound derived from spirocyclic tetronic acids and is a lipid biosynthesis inhibitor. It is recommended for control of spider mites, white fly and psyllids that acts effectively via inhibition of acetyl-CoA-carboxylase, a lipid metabolism enzyme (Kontsedalov et al., 2009). Hexythiazox is a growth inhibitor. Fenpyroximate (pyrazole), whose mode of action is inhibition of mitochondrial electron transport (MET) at complex I is reported to show high efficacy with quick knockdown effect against both tetranychid and eriophyid mites. Chlorfenapyr, a pyrrole compound, at biochemical level acts as uncoupler of oxidative phosphorylation via disruption of the proton gradient. It is included in the Environmental Protection Agency (EPA) list as an alternative to organophosphorus compounds (Marčić et al., 2011).

One of the most important factors governing the management of acaricide use is the availability of sound baseline data on the susceptibility of target mite species to the acaricides. Therefore, the relative toxicity of some acaricides with novel mode of action was evaluated against TSSM under the laboratory conditions and compared with the conventional ones. For chemical control, apart from measuring acute mortality, it is better to evaluate the impact of an acaricide on eggs and nymphs to measure the tolerance to pesticides. Therefore, in the present study acaricides namely abamectin, spiromesifen, chlorfenapyr, hexythiazox, fenpyroximate, propargite and dicofol were evaluated for toxicity studies (lethal concentrations, ovicidal and nymphicidal activities) against *T. urticae* under laboratory conditions.

2. Materials and methods

2.1. Rearing of two-spotted spider mite

The mites, *T. urticae* used in the study were obtained from our laboratory where it was reared for more than 40 generations on potted bean plant (*Phaseolus vulgaris*) at 25 ± 2 °C and 60 ± 10% RH and 14:10 h photoperiod (Light:Dark). Fresh plants were supplemented at regular intervals to maintain the culture for experimentation.

2.2. Acaricidal formulations

The commercial formulations of abamectin, spiromesifen, hexythiazox, fenpyroximate, bifenazate, propargite and dicofol were purchased from the local market. The details of these pesticides are given in Table 1.

2.3. Toxicity evaluation against adults

Toxicity of these acaricides was evaluated by spray method using Potter's Spray Tower (Burkard, UK). Briefly, 15 adult mites were placed on a bean leaf disc (4 cm diameter) which was kept on a cotton pad saturated with water and placed in a Petri dish (9 cm diameter). Based on the preliminary evaluations, 5–8 concentrations of test acaricides were prepared in distilled water containing 0.05% Triton (LR Spreader). This acaricide suspension (2 ml) was sprayed onto the leaf disc containing mites using Potter's Spray Tower at one bar pressure. There were three replicates of each concentration. Distilled water having 0.05% Triton only served as a control. After spraying, the Petri plates were kept at 25 ± 2 °C and 65 ± 10% RH with a 14:10 h (L:D) photoperiod. Observations on mortality were recorded after 48 h. Individual mite survival was determined by touching each mite with a fine hair camel brush. Mites were considered dead which were unable to walk at least a distance equivalent to their body length. Each experiment was repeated thrice.

2.4. Ovicidal and nymphicidal activities

Ovicidal activities of the formulated products were studied using spray method. Briefly, bean leaf discs (4 cm) were cut and kept on distilled water saturated cotton pad kept in a 90 mm Petri plate. Then 10 adult mites were gently transferred onto the leaf discs and allowed to lay eggs. After egg laying for ~18 h, eggs were counted under the stereo microscope and

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Table 1 Details of test acaricides.

Acaricides	Recommended dose (a.i./ha)	Trade name	Manufacturer/suppliers
Abamectin	7.2 g in 500 L	Dynamite 1.9% EC	Crystal Phosphate Ltd., Delhi
Fenpyroximate	15 g in 400 L	Pyromite 5% EC	Excel Crop Care Ltd., Gujarat
Spiromesifen	96 g in 500 L	Oberon 22.9% SC	Bayer Crop Science Ltd., Mumbai
Chlorfenapyr	75 g in 750 L	Lepido 10% SC	PI Industries Ltd., Udaipur, Rajasthan
Propargite	430 g in 400 L	Omite 57% EC	Dhanuka, Agritech Ltd., Jammu and Kashmir
Dicofol	500 g in 500 L	Colonel-S 18.5% EC	Indofil Industries Limited, Mumbai
Hexythiazox	15 g in 400 L	Maiden 5.45% EC	Biostadt India Ltd., Mumbai

g-gram; L-liter; EC: emulsifiable concentrate; SC: suspension concentrate.

their number was adjusted to 20 eggs/leaf disc. 2 ml of each acaricide was sprayed at one third of the recommended concentration (Table 1) on the leaf discs containing eggs using Potter's Spray Tower at one bar pressure. After spraying, the Petri plates were kept under the controlled laboratory conditions. There were three replicates per treatment and the experiment was repeated thrice. Water spray served as control. The nymphs that hatched from the eggs were allowed to develop on the same treated leaf discs. Observations on egg hatching and nymphal mortality were recorded regularly up to 10 DAT.

2.5. Statistical analysis

Data were pooled and mortality was converted to percent mortality. Any mortality in the control was corrected using the Abbott's formula (1925). Data were analyzed using EPA Probit Analysis Program; Version 1.5 for calculating $LC_{50/90}$ values. General linear model procedures were used to perform the analysis of variance (ANOVA) using the computer program SPSS 7.5 for windows. Means were separated with the Duncan Multiple Range Test (DMRT).

3. Results

3.1. Toxicity to the adults

Based on the adulticidal activity, abamectin was found to be the most toxic (LC_{50} = 0.39 ppm) to the adults followed by fenpyroximate, spiromesifen, chlorfenapyr, propargite and dicofol (Table 2). Hexythiazox was the least toxic (LC_{50} = 277.47 ppm). Taking the LC_{50} of abamectin as unity, the relative resistance to different pesticides is calculated. The lethal concentrations ($LC_{50/90}$) along with other parameters are presented in Table 2.

3.2. Ovicidal activities

Egg hatching started on 5th day after treatment (DAT) in the control as well as in the treatments. Observations were recorded up to 10 days post-spray. The test acaricides exhibited different levels of ovicidal activities (Table 3). Spiromesifen exhibited the best ovicidal activity as there was no egg hatching and was significantly different from all the treatments i.e. fenpyroximate, chlorfenapyr, propargite, dicofol and hexythiazox. However, all other treatments were statistically at par with each other. Based on the 10th day observations, ovicidal activity of spiromesifen (100%) was followed by dicofol (7.78%), hexythiazox (6.67%), fenpyroximate (4.44%), chlorfenapyr (0.56%) and abamectin (0.56%). Propargite exhibited no effects on the hatching of eggs, as there was a 100% egg hatching.

3.3. Nymphicidal activities

There were differential mortalities of the nymphs that hatched from eggs which were sprayed with different acaricides (Table 4). Based on the results of 10 DAT, all the treatments were at par with each other and statistically different from the control. Results were not recorded for the spiromesifen as there was no egg hatching. Abamectin resulted in highest nymphal mortality (96.05%) followed by dicofol (94.51), hexythiazox, propargite, chlorfenapyr and fenpyroximate.

4. Discussion

Abamectin was the most toxic to adult mites (Table 2) followed by fenpyroximate (relative resistance i.e. RR = 15), spiromesifen (RR = 32), chlorfenapyr (RR = 83), propargite

Table 2 Toxicities of different acaricides against *T. urticae*.

Acaricides	LC_{50} (ppm)	LC_{50} (fiducial limits, ppm)		LC_{90} (ppm)	LC_{90} (fiducial limits, ppm)		Chi square	Intercept	Relative resistance
		Lower	Upper		Lower	Upper			
Abamectin	0.39	0.33	0.48	1.81	1.29	2.96	4.83	0.78	1.00
Fenpyroximate	5.67	4.53	6.90	26.12	20.14	37.02	3.54	−1.46	14.54
Spiromesifen	12.53	10.56	14.72	39.01	31.10	53.22	3.31	−2.85	32.13
Chlorfenapyr	32.24	25.16	40.60	72.79	53.86	144.12	7.66	−5.46	82.67
Propargite	77.05	66.68	87.25	178.78	152.04	224.22	2.99	−6.61	197.56
Dicofol	146.65	97.57	210.91	517.56	330.46	1265.40	13.38	−5.07	376.03
Hexythiazox	277.47	265.85	288.79	395.26	371.49	430.28	3.15	−20.37	711.46

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Table 3 Ovicidal activity of different acaricides against *T. urticae*.

Acaricides	% Egg hatching			
	7 DAT	8 DAT	9 DAT	10 DAT
Abamectin	81.67 ± 7.26 c	95.56 ± 3.47 c	97.78 ± 0.96 b	99.44 ± 0.96 b
Hexythiazox	63.33 ± 6.01 b	72.22 ± 11.82 b	90.00 ± 10.00 b	93.33 ± 6.01 b
Chlorfenapyr	93.89 ± 2.55 c	96.11 ± 4.19 c	98.33 ± 1.67 b	99.44 ± 0.96 b
Fenpyroximate	85.00 ± 11.55 c	91.11 ± 5.85 c	93.33 ± 5.77 b	95.56 ± 7.70 b
Dicofol	81.11 ± 15.40 c	86.67 ± 10.14 c	92.22 ± 7.52 b	92.22 ± 7.52 b
Propargite	96.11 ± 1.92 c	97.22 ± 2.55 c	99.44 ± 0.96 b	100.00 ± 0.00 b
Spiromesifen	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Control	89.44 ± 9.48 c	95.56 ± 7.70 c	99.44 ± 0.96 b	100.00 ± 0.00 b

Means within a column followed by the same letter do not differ significantly ($P > 0.05$; DMRT).

Table 4 Nymphicidal activity of different acaricides against *T. urticae*.

Acaricides	% Nymphal mortality			
	7 DAT	8 DAT	9 DAT	10 DAT
Abamectin	20.73 ± 5.94 b	59.27 ± 8.92 bc	94.90 ± 6.10 c	96.05 ± 5.45 b
Hexythiazox	30.49 ± 5.75 bc	82.86 ± 5.63 d	85.11 ± 9.61 c	90.24 ± 8.26 b
Chlorfenapyr	56.95 ± 9.12 d	83.69 ± 7.22 d	85.79 ± 9.45 c	89.33 ± 9.35 b
Fenpyroximate	25.67 ± 6.65 bc	47.29 ± 9.60 b	63.17 ± 8.83 b	86.84 ± 6.97 b
Dicofol	47.89 ± 5.57 d	87.15 ± 0.43 d	90.88 ± 8.15 c	94.51 ± 5.01 b
Propargite	34.67 ± 9.68 c	74.06 ± 19.85 cd	84.94 ± 8.61 c	90.00 ± 8.82 b
Control	0.00 ± 0.00 a	1.67 ± 2.89 a	2.23 ± 2.54 a	8.89 ± 2.55 a

Means within a column followed by the same letter do not differ significantly ($P > 0.05$; DMRT).

(RR = 198) and dicofol (RR = 376). Toxicities of these acaricides to mite have been reported by various researchers. Ismail et al., 2007 calculated LC_{50} of abamectin as 0.34 ppm which was higher than the LC_{50} (0.0135 ppm) reported by Salman (2007). LC_{50} values of chlorfenapyr and abamectin against *T. urticae* were calculated as 59.34 and 1.50 ppm, respectively (Vásquez and Ceballos, 2009). Sato et al. (2005) reported LC_{50} of abamectin as 0.17 and 58.10 ppm against susceptible and resistant strains of *T. urticae*, respectively. It was observed that resistance ratio at LC_{50} reached 342-fold values. The resistance was reported to be unstable in the absence of selection pressure and there was no cross-resistance to fenpyroximate and propargite. However, Sato et al. (2005) reported cross-resistance to milbemectin. Due to reports of chlorfenapyr resistance against TSSM in Brazil, the use of acaricides *viz*. spiromesifen, milbemectin, fenpyroximate and diafenthiuron is recommended for an acaricide rotation program so that efficacy of chlorfenapyr against TSSM can be maintained (Nicastro et al., 2013).

In the present study, hexythiazox was the least toxic (LC_{50} = 277.47 ppm) i.e. 711 times less effective than abamectin. Alzoubi and Cobanoglu (2008) calculated LC_{50} of hexythiazox 537.45 and 175.75 ppm after 24 and 72 h, respectively, against *T. urticae*. High level of resistance to hexythiazox has been detected in *Panonychus ulmi* (>2500-fold) (Edge et al., 1987) and *T. urticae* (>1000-fold) (Gough, 1990) in Australia, and in *Panonychus citri* (>24,000-fold) in Japan (Yamamoto et al., 1995). In Cyprus, widespread use of abamectin by farmers for the management of TSSM resulted in the development resistance to this acaricide (Vassiliou and Kitsis, 2013). In a study conducted against chlorfenapyr-resistant and susceptible strains of *T. urticae*, chlorfenapyr resistance was found to be stable in the absence of selection pressure. A possible positive cross-resistance between chlorfenapyr and abamectin, propargite and etoxazole, and a possible negatively correlated cross-resistance between chlorfenapyr and spiromesifen was observed (Nicastro et al., 2013). Therefore it is realized that the acaricides for which resistance has been developed should be avoided in order to reduce further increase of resistant strains in nature (Ullah and Gotoh, 2013).

Based on the 10th day observations, ovicidal activity was in the following order: spiromesifen > dicofol > hexythiazox > fenpyroximate > chlorfenapyr > abamectin > propargite. None of the acaricides except spiromesifen exhibited significant ovicidal activity. In case of propargite, there was 100% egg hatching. In the present study, spiromesifen was found to be a potent ovicidal agent as there was no egg hatching even when eggs were sprayed with one third of the recommended concentrations. Spiromesifen (a tetronic acid derivative) has been commercialized as acaricides with a novel mode of action (inhibition of acetyl-CoA-carboxylase) against spider mites and whiteflies. Present findings gain support from the reports that spiromesifen acts against eggs and nymphal stages of whiteflies besides reducing fecundity and fertility of adult females when applied at concentration levels well below field recommended rates (Nauen et al., 2005; Prabhaker et al., 2008; Kontsedalov et al., 2009). Further, it is reported that the spiromesifen at concentrations ranging from 0.064 to 40 ppm reduced the fecundity of TSSM females and exhibited

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pronounced residual effect under practical conditions (Nauen et al., 2005). Spiromesifen is reported to be effective against the egg and juvenile stages of spider mites and whiteflies (Dekeyser, 2005). Eggs less than 72 h old were reported to be more sensitive than the other development stages to the spiromesifen and the oviposition rate was significantly affected (Sato et al., 2011). Eggs of spider mites, *Tetranychus macfarlanei* and *Tetranychus truncatus* were reported to be highly susceptible to the acaricides viz. spiromesifen, hexythiazox, and chlorfenapyr at the LC_{50} level (Ullah and Gotoh, 2013).

In the present study, hexythiazox resulted in 96.33% egg hatching. However, Alzoubi and Cobanoglu, 2008 reported 11.1% egg hatching after spraying with one third of the recommended dose of hexythiazox. In the present study, abamectin did not exhibited ovicidal activity and resulted in 99.44% egg hatching. There are different reports on the ovicidal action of abamectin. Kumar and Singh (2004) and Ismail et al. (2007) reported that abamectin did not affect eggs of TSSM at a range of concentrations. Whereas, Salman (2007) reported that abamectin did not affect mite fertility, but that it was highly toxic for eggs at all ages.

A varying degree of toxic effects on newly hatched nymphs were observed in all the treatments. The nymphs hatched from the sprayed eggs kept on the leaf discs started dying (Table 4). In case of spiromesifen, there was no egg hatching, therefore, observations on the nymphal mortality were not taken and shown in Table 4. Abamectin was the most toxic to the nymphs followed by dicofol, hexythiazox, propargite, chlorfenapyr and fenpyroximate. Abamectin is reported to significantly reduce the female fecundity and killed offspring when applied directly on the eggs (Ismail et al., 2007). They further reported that all the larvae which hatched from abamectin treated eggs died on day 5 after laying. The mortality can be attributed to the direct contact of the newly emerged nymphs to the acaricides or there might be some adverse effects on the development of the eggs. Fenpyroximate is reported to show higher efficacy against larvae than against other life stages of mites (Hamaguchi et al., 1995). Study revealed that abamectin, spiromesifen, hexythiazox, fenpyroximate and chlorfenapyr can be used alternatively for the control of mites.

5. Conclusions

In the present study, spiromesifen was the best ovicidal whereas the abamectin the most toxic to the adults as well as nymphs. Hexythiazox, chlorfenapyr and fenpyroximate were more toxic to the nymphal stages. The present study revealed that these acaricides (abamectin, spiromesifen, hexythiazox, fenpyroximate and chlorfenapyr) can alternatively be used for effective and sustainable management of the mites.

Conflict of interest

There is no conflict of interest.

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