The Effects of Three Acaricides on Egg Hatchability of Three Populations of the Two–Spotted Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in all of the irrigated and arid regions either in Jordan and most countries in the world is considered to be a destructive pest all the year around. However, laboratory bioassays were conducted to evaluate the effects of chemical compounds on egg hatchability of the two-spotted spider mite, after treatment of egg stage of Syria, Baq'a and Deir-Alla populations in three successive generations against three acaricides selected from different groups. Mite eggs laid on bean leaves were separately treated with LC_{50} acaricides' concentrations. The percentages of the eggs that survived after hexythiazox treatments of the females were increased from the first generation up to third generation for Deir-Alla and Baq'a populations. The two field populations treated with hexythiazox had a significant effect on the egg hatching for the three generations. Etoxazole and clofentezine acaricides have a significant decrease of three different populations. Etoxazole had high toxicity to the spider mite eggs since it has a sterilizing effect on the adult female.

Keywords: *Tetranychus urticae*, Acaricides, Two-Spotted Spider Mite, Egg Hatchability, Generations.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari:Tetranychidae) is one of the most important arthropods pest worldwide. It is the most polyphagous species attacking fruits, vegetables, field crops and ornamentals (Zhang, 2003; Mark, 2005). The two-spotted spider mite feed on many species of plants. It is a major pest of vegetables, ornamentals, fruit trees, hops, cotton and strawberries (Van Den Boom *et al.,* 2003). At present, it is safe to assume that most of the major spider mite problems in greenhouses are involved the two-spotted spider mite. This mite has been reported infesting over 200 species of plants (Van Den Boom *et al.,* 2003). This mite generally feeds underneath the leaves and causes graying of the leaves due to mesophyll collapse and yellowing. Neccroting spots occur in the advanced stages of leaves damage (Van Den Boom *et al.,* 2003; Zhang, 2003; Mark, 2005). Al-Mommany and Al-Antary (2008) reported that the two - spotted spider mite in Jordan causes great loss in different crop yields whether in open fields including eggplants, tomato, cotton, cabbages, cucurbits, legumes, fruit trees and ornamental, or on those planted under plastic houses. However, in 2009, the total number of plastic houses in Jordan is being affected by this pest, which were planted with vegetables were about 66,000 (Ministry of Agriculture, 2009).

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The life cycle of the tetranychids consists of an egg, larva, protonymph, deutonymph and adult stages. The mite takes less than a week at optimal temperatures (30-32°C) (Zhang, 2003) for complete its development.

Chemical control is the primary method for the control of spider mite on vegetables. The ability of the mite to develop resistance is a major cause of control failure (Stumpf *et al.*, 2001; Van Leeuman *et al.*, 2008). Reliance on chemicals has caused mite resistance and public concerns on their high residues in products (Dagli and Tunc, 2001).

The use of insect growth regulators is an interesting approach. Benzoylphenyl urea inhibits chitin synthesis in a wide range of insect and mite groups, resulting in abortive molting. They act mainly as larvicides and ovicides (Saenz-de-Cobezon and Frank, 2006). Effects on adult's fertility have also been reported (Saenz-de-Cabezon *et al.*, 2002). Other pesticides used for controlling spider mites are available, and act as ovicides, but also little information about their effects are known. Nazer (1985) studied the toxicity of different acaricides against *T. urticae* population collected from Baq'a region in Jordan. He found that cyhexatin and fenpropathrin acaricides were the most effective ones, followed by dicofol. However, Ako *et al.* (2006) reported there is a need to determine the effectiveness of acaricides on different mite stages starting with egg stage for three successive generations. So, the aim of this study is to determine the efficacy of three recent acaricides from different groups on egg hatchability rate of three different populations of the two-spotted spider mite collected from different locations.

MATERIALS AND METHODS

Bean Plants

Seeds of bean, *Phaseolus vulgaris* L cv Bronco were continuously sown in seed trays until reaching 5-6 true leaves. Hereafter, the seedlings were transplanted in plastic pots containing peat moss and kept in a glass house in University of Jordan for mite rearing. The temperature in the glass house ranged between 27-30°C during the period of rearing. Plants were irrigated whenever needed. Plants were kept pesticide free during the plant rearing period.

Two-spotted spider mite

Three different mite populations of the two-spotted spider mite collected from different geographic locations were used in the experiments. One population was obtained from Latakia Center for Rearing and Production of Biological Agents in Syria. This Syrian population was reared in a special compartment in the glass house in a muslin cage placed on a tray on a bench with water in its basement. The other two mite populations were collected from cucumber plants from Deir-Alla (Latitude: 32° 13' 0" altitude: -223 meters) in Central Jordan Valley, and Baq'a (latitude: 32°24´N, 36.03 E at an altitude of 860 m) eight km northwest of Amman. Mites of all populations were separately reared and continuously on potted bean plants in muslin cages with water in its basement were kept inside different locations in the glass house inside sterilized bench under conditions of the glass houses, and were used in the experimental work during 1 to 2 days after collecting from fields. The plants were gradually renewed and whenever necessary.

Acaricides

Among many acaricides used in Jordan, three were selected for the experimental work. The acaricides belong to different groups and relatively recent compounds in Jordan to control the two-spotted spider mites. A fresh stock solution of each was prepared in a tap water on each test daily for finding LC_{50s} in a

separated experiment (Salim and Al-Antary, 2012) to use them in the present study. All further dilutions were prepared from the stock solution. Tap water was used as a control. The acaricides used in this study as commercial formulations are shown in Table (1).

Table (1): Trade name, common name, formulation, manufacture and application rate for the three used acaricides in the experiments.

Trade name	Common name	Acaricide family	Formulation % a.i	Manufacture	Application rate
Baroque	Etoxazole	diphenyl oxazoline	10% Sc	Valena Yashima Chemical Industry, Sumicomo Chemical – Japan	0.5 gm/L of water
Nissorum	Hexythiazox	carboxamide	10% WP	Nippon Soda Co.LTD-Japan	0.5 gm/L of water
Appollo	Clofentezine	Tetrazine	50% Sc	Irvita Plant Protection – Canada	0.5 gm/L of water

Effect of

Acaricides on Hatchability for Three Generations

To obtain eggs for experimental work, bean leaves were placed into Petri-dishes lined with water saturated cotton wool, in which 10-20 female mites were introduced on the lower surfaces of the leaves before spraying and allowed to lay eggs for 24 hr, then they were removed. Four replicates were used (20-50 eggs/leaf) for each acaricide from each population. Immediately after removing the females, the leaves were sprayed with LC_{5o} acaricides' concentrations. The LC_{5o} for each acaricide from each population was separately and previously determined (Salim and Al-Antary, 2012). The SPSS computer program revision 13 (S. Hardware) (SAS, 2002) was used for data analyses to estimate LC_{5o} values, for more details see Salim and Al-Antary (2012). The Petri-dishes were dried for two min. and then placed under laboratory room conditions, under natural room light during the day and under fluorescent light during night. Average temperature was 25.2°C and average relative humidity of 57.8%. All Petri-dishes were daily examined for seven successive days. Hatchability percent was recorded after six to eight days by counting the number of hatched eggs on the leaf. For getting control treatment, tap water was used instead of acaricides. All treatments were separately examined for three generations of survived mites to determine acaricidal activity on the egg hatchability of the *T. urticae* for the three populations (Table 2).

Table (2): LC_{50} concentrations (PPM) of the two-spotted spider mite. (Salim and Al-Antary, 2011)

Population	LC_{50} (PPM) for		
	Hexythiazox	Etoxazole	Celfentezine
Syria	0.1 a	0.08a	16.56a
Baq'a	33b	0.43c	29.70c
Deir–Alla	370c	0.15 b	27.40 bc

LC_{50} values in the same column having different letters are significantly different at 5% probability level using LSD test.

Statistical analysis

The layout of experiments was complete randomized design (CRD). Obtained data were subjected to analysis, using SAS program (SAS, 2002). Means were separated using LSD at 5% level.

RESULTS

Results of hatchability were analyzed by paired t-test. The comparison between the effects of different acaricides on the egg hatchability of different acaricides on the egg hatchability of different population is shown in Tables 3, 4 and 5. After 8 days of the eggs exposure to LC_{50} concentrations of the tested acaricides, the percentage of the eggs that survived after hexythiazox treatment was 60.50% for the first generation (G1) and 74% for third generation (G3), 58.3% for generation one and 67.75% for generation three, for field population and 42.35% (G1) and 29.37% (G3) for Syrian population (Table 3).

The percentage of the egg hatching after 8 days of etoxazole was 42.62% (G1) and 29.62% for (G3) for Deir-Alla field population. The percentage of the egg hatching after 8 days of etoxazole was 54.4%, 45.68% for (G1) and 42.4%, 35.62 for (G2) and 25% for Baq'a and Syrian populations, respectively (Table 4). The percentage of Syrian population egg hatching with clofentezine egg treatment was 42.55% for (G1) and 26.66% for (G3). The results for Deir-Alla field population were 54.94% for (G1) and 42.42% (G3) and 48.75% for (G1) and 36.49% for (G3) for Baq'a field population (Table 5).

Table (3): Effect of hexythiazox on % of egg hatchability of the different populations of the two-spotted spider mite after the treatment with its LC_{50}, for three generations under laboratory conditions.

% Egg hatchability ± SE						
Populations	**First generation**		**Second generation**		**Third generation**	
	Control	**Hexythiazox**	**Control**	**Hexythiazox**	**Control**	**Hexythiazox**
Syrian	96.81±3.045a	42.35± 2.39b	97.29± 1.402a	35.625± 1.173b	95.11± 2.12a	26.37± 2.196b
Baq'a	96.88±2.041a	58.3± 4.26b	97± 0.123a	63.5±1.19b	95.98± 1.089a	67.75± 1.49b
Deir-Alla	95.50±1.32a	60.500± 1.32b	98.25± 1.18a	71.75±1.75b	96.34± 0.045a	74.00± 1.19b

Means within the same row for the same generation that do not share the same letter are significantly different at 5% level according to paired t-test

Table (4): Effect of etoxazole on % of egg hatchability of the different population of the two-spotted spider mite after the treatment with its LC_{50}, for three generations under laboratory conditions.

% Egg hatchability ± SE						
Populations	**First generation**		**Second generation**		**Third generation**	
	Control	**Etoxazole**	**Control**	**Etoxazole**	**Control**	**Etoxazole**
Syrian	96.50± 0.523a	42.687±1.23b	97.29± 1.402a	35.625± 1.173b	96.25±1.52a	22.500± 2.0.2b
Baq'a	96.27± 1.045a	54.37±1.19b	97.55± 1.20a	42.35±0.39b	95.62±0.197a	25.0± 1.023b
Deir-Alla	96.47± 0.033a	42.62±2.57b	96.24± 1.423a	35.62±2.37b	96.34±0.045a	29.62± 1.19b

Means within the same row for the same generation that do not share the same letter are significantly different at 5% level according to paired t-test

Table (5): Effect of clofentezine on % of egg hatchability of the different population of the two-spotted spider mite after the treatment with its LC_{50}, for three generations under laboratory conditions.

% Egg hatchability ± SE						
Populations	**First generation**		**Second generation**		**Third generation**	
	Control	**Clofentezine**	**Control**	**Clofentezine**	**Control**	**Clofentezine**
Syrian	96.15± 1.38a	42.55± 1.616b	96.25±2.42a	30.0±2.347b	96.0 ±1.625a	26.66± 1.175b
Baq'a	97.25± 1.22a	48.75±0.72b	96.20±1.32a	42.42±2.45b	96.05±1.62a	36.49± 0.748b
Deir-Alla	96.43± 2.73a	54.94±0.33b	96.43±2.73a	48.88±2.091b	95.83±1.59a	42.42± 2.46b

Means within the same row for the same generation that do not share the same letter are significantly different at 5% level according to paired t-test.

DISCUSSION

Generally, hexythiazox was the least toxic against egg stage of *T. urticae* than the other pesticide used in this study. Etoxazole was the highest toxic against egg stage of the two field populations (Baq'a and Dier-Alla field populations) of *T. urticae* followed by clofentezine. Ochiai et al. (2007) studied the toxicity of etoxazole and other pesticides against adults, larval and eggs' stages of *T. urticae*. Etoxazole showed no activity against adults of *T. urticae*. However, etoxazole was highly effective in controlling the larval and egg stages of *T. urticae* mite. The present results indicated that clofentezine was considerably toxic on the egg stage of *T. urticae*. The eggs treated with 29.7 PPM and 27.4 PPM in Baq'a and Dier-Alla field populations, respectively, both field populations had low resistance factor (Salim and Al-Antary, 2012), which means that the clofentezine was still highly toxic against the two-spotted spider mite. Similar effect was recorded by Dingxu *et al.* (2006) for clofentezine and other pesticides against the *T. urticae*. They found that clofentezine was highly toxic to different mite stages. Also, clofentezine acaricide used in this study had effect on the hatchability of the three populations of *T. urticae*. Historically, mites have developed resistance very quickly to ovicidal acaricides (Luis *et al.*, 2008).

Because etoxazole is a relatively new acaricide, studies on the etoxazole toxicity and its effect on the life

biology of the two-spotted spider mite could not be found. In the present toxicity test, it was found that etaxozole had high toxicity to the spider mite eggs. Its activity on egg hatchability was very high after direct treatment on eggs. It is significantly reduced egg hatchability in this study. Etoxazole is effective against eggs, larvae and nymphs' stages of mites but lacks any efficacy against adults (Salim and Al-Antary, 2012). However, its sterilizing effect on adult female by exhibiting significant transovarial ovicidal activity was reported by several authors (Nauen and Smagghe, 2006). Therefore, when applications of the etoxazole are made at recommended field rate, newly laid eggs from treated females do not hatch. Nauen and Smagghe (2006) observed that ovicidal activity against egg stage of *Orius insidious* seven days after treatment, *O. insidious* eggs hatching rate varied significantly among the acaricides.

The chemical unrelated ovicide hexythiazox was released in 1987 in Australia and is also known to be very active on the eggs of *Panonychus ulmi* in the laboratory (Welty *et al*.,1988) and in field (Bower, 1990). It was expected that clofentezine and hexythiazox would provide a period of excellent control of *P. ulmi* during which time new acaricides would be developed. The present work has investigated the effects of the hexythiazox acaricide on hatchability of the two-spotted spider mite eggs. The results indicated that females that survived treatment with hexythiazox as egg stages were found to have lower vitality with reduced hatchability significantly in Syrian population, and also in the 2nd and 3rd generations compared with the first generation in all populations. In the two field's population hexythiazox has increased significantly effect on the egg hatchability during its application, for the three generations, but the egg hatching deceased significantly for each generation compared with the control. Results indicated that etoxazole and clofentezine compounds had reduced significantly the effect on the egg hatching for three generations of three different populations compared with the control. However, recommended the field rate hexythiazox had decreased longevity and net fecundity rate and causing over 90% mortality of the two-spotted mite (Sekulic, 1999).

In conclusion, there were several fitness factors (increase fecundity, short egg stage, short development times for males and reduced adult longevity) associated with the susceptible population which may have contributed to develop resistance. The same result was observed in the several fitness factors; decrease longevity and increase fecundity in the two field populations. Females that survived treatment with hexythiazox as egg stage had lower vitality with reduced hatchability in Syrian population and had reduced the effect on the adult longevity for second and third generations of all populations compared with first generation. In the two field populations treated with hexythiazox had a significant the effect on the egg hatching for the three generations. Etoxazole and clofentezine acaricides had a significant decrease effect on the egg hatching for three generations for the three different populations. Etoxazole had no effect on adult longevity and adult fecundity, compared to the other two acaricides.

It is recommended to use clofentezine and etoxazole as selective ovicidal products against spider mite eggs, since both ovicides are selective and aid in the conservation of populations of beneficial arthropods as indicated by the parent company. For managing hexythiazox resistance and keeping successful control at the species in the field, rotated program is needed. When choosing an acaricide to treat the two-spotted spider mite infestation. It is important to know how it will affect different life stages .It would be beneficial to have one acaricide that is effective against all life stages.

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تأثير ثلاثة مبيدات حلم على بيض ثلاثة مجتمعات من الحلم الاحمر
Tetranychus urticae Koch (Acari :Tetranychidae)

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ملخـص

يعد الحلم الاحمر ذو بقعتين في كل المناطق المروية والجافة في الاردن ومعظم دول الشرق الاوسط آفة وبائية على كثير من النباتات طوال العام. لقد تم القيام بالتقيم الحيوي المخبري لمعرفة فعالية ثلاثة مبيدات حلم (اختيرت من مجاميع مختلفة) على قابلية فقس بيض الحلم لثلاثة مجتمعات جمعت من سورية ، والبقعة ودير علا من الاردن ، لثلاثة اجيال متتابعة. عملت بيض الحلم الموضوعة على أوراق الفاصولياء بطريقة منفصلة بتراكيز الجرعة النصف مميتة لمبيدات الحلم . بيوض الإناث التي عاشت (لم تمت) بعد المعاملة بمبيد هكسيثيازوكس وكانت اقل حيوية مع انخفاض القابلية للفقس . وكان المجتمعان الحقليان من الحلم المعاملان بمبيد هيسثيازوكس معنويا أقل تأثيرا على قدرة البيض على الفقس لثلاثة أجيال من الحلم. وكان تأثير مبيدي اتوكسازول وكلوفنتازين معنويا أقل للثلاثة أجيال . وكان مبيد اتوكسازول أعلى سمية على بيض الحلم لأن له تأثيرا تعقيميا على الاناث البالغة.

الكلمات الدالة: *Tetranychus urticae* ، مبيدات حلم, الحلم الأحمر ذو البقعتين ,أجيال ، قدرة البيض على الفقس.

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