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Response of Seven Populations of the Two-Spotted Spider Mite *Tetranychus Urticae* Koch) for Amitraz Acaricide on) Cucumber (*Cucumis Sativus* L.) Under Plastic Houses in Jordan

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Tawfiq M. AL-Antary, Mohammad Raed "Kamel Al-Lala" and Marwan I. Abdel-Wali; Response of Seven Populations of the Two-Spotted Spider Mite Tetranychus Urticae Koch) for Amitraz Acaricide on) Cucumber (Cucumis Sativus L.) Under Plastic Houses in Jordan

ABSTRACT

Laboratory bioassays were conducted during the period from June 2009 till August 2010 to evaluate the toxicitiy of amitraz against adult females of seven populations of the two-spotted spider mite (TSSM) (*Tetranychus. urticae* Koch). Six of the seven tested populations were collected from Al-Ramtha, Baq'a, Zyzya, Krimeh, Deir-Alla and Karamah covering the main regions devoted to cucumber cultivation in Jordan. The seventh population was brought from Syria and was considered as a susceptible strain (SSS). All the seven populations except the SSS strain were relatively resistant to amitraz action. The krimeh and Karamah populations were moderate to amitraz, RF = 4.33 and 3.6 respectively, while Baqa population was the least resistant one (RF =2.06). However, Resistance build up for amitraz was obvious for all the tested populations. The results indicated obviously that most of the local TSSM populations displayed different levels, of resistance to amitraz.

Key words: Acaricides, toxicity, Amitraz, two spotted spider-mite cucumber, plastic houses, Jordan.

Introduction

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae). It has recently become a serious problem because of the extensive use of acaricides, resulting in resistance among the mite populations [7,2]. The development of resistance is also known to be accelerated under confined environmental conditions such as plastic-houses. Since the mite has a very short and prolific life cycle, its resistance to acaricides has more readily emerged than the rate of the resistance in other pests. In addition, the mites resistance to certain acaricides has been shown to have cross resistance to other acaricides. Thus, most commercial acaricides have been often proved to be ineffective to control the field mite populations [4].

From field surveillance and screening of various acaricides, it seems that TSSM has developed resistance to the most conventional acaricides, but toxicological data are scarce and poorly documented. It is quite possible that TSSM susceptibility to acaricides would differ from one location to another of cucumber cultivation in Jordan. Therefore, it was important to monitor the acaricide susceptibilities of *T. urticae* populations that were collected from cucumber cultivation in Jordan and to evaluate the efficacy of testing acaricides. This study showed the results of laboratory-based tests that determined the response of six field populations (Al-Ramtha, Baq'a, Zyzya, Krimeh, Deir-Alla, and Karamah) and one susceptible strain of *T. urticae.* To amitraz.

The Jordanian farmers rely heavily on acaricides to control the two-spotted spider mite. Therefore, they have increased the rate of application, applied a mixture of acaricides and applied acaricides more frequently than they should. They have complained about unsatisfactory results in controlling *T. urticae*. The total quantity of acaricides imported to Jordan in 2009 was 120,000 liters and / or kilograms. The imported acaricides belong to 18 active ingredients, from the most important imported active ingredients was amitraz (9000 liters). In addition to imported acaricides, there are more than 15 local agrochemical factories that produce different formulations of active ingredients. The majority of these factories production is for export and the quantity of their product that goes to the local market is unknown.

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Although TSSM represents a real threat to cucumber plantation under plastic-houses in Jordan, few toxicological studies on this pest have been conducted. Therefore, this study aimed to achieve the following objectives to evaluate the susceptibilities of TSSM collected from cucumber cultivation in Jordan to the studied amitraz acaricides and to find the resistance factors to the tested amitraz among the two spotted spider mite populations.

Materials And Methods

Populations of T. urticae:

Six *T. urticae* populations were collected from cucumber plants grown under plastic houses conditions in different regions of Jordan. These geographical regions include Al-Ramtha (100 Km north west of Amman), Baq'a (20 Km north west of Amman), Zyzya (30 Km South of Amman), Krimeh (South of Jordan Valley), Deir-Alla (Central of Jordan Valley) and Karamah (South of Jordan Valley). These regions are considered the main area for cucumber production in the country. A susceptible strain of *T. urticae* was obtained from Lattakia Center for Rearing and Production of Biological Agents (LCRPBA) in Syria. This strain was reared in (LCRPBA) for 5 years without the application of acaricides.

Production of bean Phaseolus vulgaris L. plants:

Bean (*Phaseolus vulgaris* L. cv. Bronco) was chosen for rearing and for the toxicological tests of the (TSSM) because it is one of the mite's favorite host, and for its ease of producing transplants. Seeds of bean (Bronco, Asgrow, USA) were directly sowed inside 10 cm. pots. Potting media used was Peat-moss and Perlite with 3:1 ratio. Plants were infested with SSS *T. urticae* when they reached the true leaf stage. These plants were irrigated and replaced as needed. No pesticides were applied on the plants except for acaricide tests. These plants were grown under greenhouse conditions at a temperature of 25-35 °C , relative humidity of 45% to 60% and a photoperiod of L16:D8 regime.

For use in the toxicological tests, polystyrene trays of 84 cells were filled by Peat-moss and Perlite (3:1 ratio). Then, these trays were sown by 1-2 bean seeds for each cell, after complete germination the cotyledon leaves were used in the toxicological tests.

Rearing of the Syrian susceptible strain (SSS):

Rearing of the Syrian susceptible strain was done inside special insectaria in University of Jordan at Al Jubaiha area. The Syrian TSSM was reared and maintained on *Phaseolus vulgaris* plants under greenhouse conditions; at temperature 27±5°C and relative humidity 57±8% and a photoperiod of L16:D8 regime. *P. vulgaris* Plants were irrigated and replaced as needed.

Plant materials:

P. vulgaris seeds were sowed in 84 polystyrene trays filled with Peat-moss and Perlite at ratio of 3:1. Each cell was sowed with one or two seeds. Prior to germination, cotyledon leaves of seedling were used for all experimental sets.

Tested acaricide:

Amitraz 20% W/V, EC (Mitac®). Produced by Bayer Crop Science in 1975, with higher recommended rate of 500 mg/L H2O. Its Molecular formula is $C_{19}H_{23}N_3$ (Tomlin 205).

Preparing of stock solutions:

For each test, fresh stock solution was prepared by dissolving a calculated quantity of the acaricide enough to run the whole concentrations needed. The acaricides was used as its commercial formulations, Mitac 20%EC

Bioassays of acaricides toxicity:

Toxicological bioassays were conducted according to the procedures described by IRAC [8]. Cotyledon leaves from untreated bean plants were placed, lower side up, in Petri-dishes lined with water-saturated cotton wool. 25 adult females of *T. urticae* were introduced into each cotyledon leaf by using a binocular microscope and a fine paintbrush. 24 hours after mite release, each Petri-dish was sprayed with a constant amount of the acaricide solutions for 2 seconds using a hand sprayer. The sprayed Petri-dishes were left to dry for 30 minutes, then they were placed under room temperature. Mites condition was assessed by gentle probing with a fine paintbrush. Mites were classed as dead when they didn't move or displayed some movement not exceeding their body length. Mites which were able to move were considered alive. This assessment of mites conditions were recorded 48 hours after acaricide application. The LC_{50}, and LC_{90} values and their 95% confidence limits were calculated from probit regressions using the SPSS13 program.

The application of the acaricide was done with four replicates per each concentration, and with seven concentrations for the acaricide. These concentrations were chosen based on preliminary studies and they were different for each *T. urticae* population. Tap water was sprayed as control. These experiments were carried out in the Pesticide laboratory at room temperature between 25±5°C and 57±8% relative humidity.

Statistical analysis:

Adv. Environ. Biol., 6(3): 951-954, 2012

Data were subject to probit analysis [5] which incorporated Abbott's correction for natural mortality (Abbott, 1925). The SPSS (version 13 USA) computer program was used for data analysis to estimate LC50 and LC90 values, regression coefficient (slope) and its standard error, intercept and its standard error, Pearson goodness of fit chi-square, expected mortality, and 95% confidence limits (95%CL) for effective level of concentrations. This programme used normal equivalent deviate (NED) instead of probit numbers. However, NED numbers can be readily adjusted to probit by adding 5 to each NED number [5]. Y value for each line estimated by probit regression was equal to 0.0 and 1.28 when LC50 and LC90 (X) value was converted to log base 10, respectively.

LC50 and LC90 values were considered significant when (95% CL) did not overlap. To determine the resistance factor (RF) for each population, the LC50 of each acaricide of the field population was divided by the corresponding LC50 for the susceptible strain. The resistance factors were categorized according to Fukami [6] as follows: low. RF<10, moderate 10<RF≤ 40, high 40-60 and very high resistance >60. LC90 values in ppm divided by the higher recommended field rate in ppm were calculated and tabulated for each TSSM strain (Ratio value).

Goodness of line fitting was checked by Chi-square test X2. According to Finney [5], the value of X2 at 0.05 level of probability equals to 14.1 at 5 degree of freedom (df). Results obtained in this study revealed that (X2) Goodness of fit chi-square were less than that tabulated for each regression line indicating goodness of fit at 0.05 level of probability.

Results:

The efficacy of amitraz towards *T. urticae* populations collected from different locations is shown in Table 1. The lowest LC50 value was recorded for Syrian population (147 ppm) followed by Baq'a (303 ppm), Al-Ramtha (346 ppm), Deir-Alla (376 ppm), Karamah (503 ppm), Zyzya (558 ppm) and Krimeh (639 ppm). However, there were no significant differences among LC50 values for the mite populations from Al-Ramtha, Baq'a, and Deir-Alla. As well as LC50 for the populations from Zyzya and Karamah didn’t differ significantly from each other. In contrast, LC50 value for Krimeh population was significantly higher than those for other populations.

The LC90 of amitraz against different populations showed that Deir-Alla exhibited the greatest susceptibility with 763 ppm LC90, followed by Baq'a (820.82), Karamah (903.86), Krimeh (976.8), Al-Ramtha (1238.34) and Zyzya (1349.7) populations. However, the LC90 values for Al-Ramtha, Zyzya and Krimeh were not significantly different.

Table 1: Susceptibilities of field-collected populations of *T. urticae* adult females to amitraz (Higher recommended field rate =500 mg/L).

Chi Square Calculated	RF5 LC50	Ratio4 LC90	Slope ± S.E3.	L.E.P.R2 Y=a + b(x)	LC90(mg/l) 95%CL	LC50(mg/l) 95%CL1	Population name
7.3	2.35	2.48	2.31±0.19	Y=-5.88+2.31(x)	1238.34 a6 1049.95-1531.02	345.95 c6 302.10-389.36	Ar-Ramtha
6.4	2.06	1.64	2.96±0.27	Y=-7.36+2.96(x)	820.82 cb 711.42-996.73	303.33 c 293.27-331.92	Baq'a
5.9	3.79	2.69	3.34±0.8	Y=-9.19+3.34(x)	1349.7 a 1202.14-1570.54	558.48 b 504.02-608.43	Zyzya
4.4	4.33	1.95	6.96±0.55	Y=-19.51+6.96(x)	976.80 ab 920.96-1053.83	639.05 a 612.38-664.43	Krimeh
5.8	2.55	1.53	4.18±0.34	Y=10.76+4.18(x)	763.03 c 695.69-859.95	376.58 c 348.98-402.27	Deir-Alla
6.8	3.60	1.81	5.54±0.45	Y=-15.11+5.54(x)	903.94 bc 838.98-996.40	530.86 b 503.15-557.15	Karamah
5.7	_	0.8	2.96±0.24	Y=-6.41+2.96(x)	400.07 d 352.87-470.40	147.5 d 131.80-162.25	SSS

1. 95% Confidence limits for LC50 or LC90 in ppm.
2. L.E.P.R. = Line Estimated by Probit Regression.
3. S.E. = Standard error.
4. Ratio LC90 = LC90 / higher recommended field rate.
5. R.F. Resistance Factor= LC50 of field population / LC50 of susceptible population (SSS).
6. LC50 or LC90 values having different letters are significantly different (95% CL did not overlap).

Discussion:

Monitoring of local populations for susceptibility towards acaricides is the first step in resistance management of *T. urticae*. It is essential to carry out regularly acaricide resistance tests to avoid resistance development in target mites. In addition, control tactics must depend on the use of different acaricides to avoid or delay resistance.

The toxicity of amitraz on one foreign susceptible mite strain obtained from Syria and six local populations collected from different locations revealed that all the local populations of *T. urticae* were moderately resistance to this acaricide whereas foreign population was susceptible. Resistance to amitraz could be resulted of the frequent use of amitraz by Jordanian farmers in a large scale, And presumably due to its moderate price compared to other acaricides in Jordanian markets. Since Krimeh and Al Karamah regions are mainly cultivated with eggplant throughout the year which is a favorite host for *T. urticae*, farmers use acaricides including amitraz in a large scale. Therefore, mite populations originated from this location exhibited the highest resistance to amitraz.

Outcomes of this study indicated that *T. urticae* had developed a moderate resistance to amitraz. The maximum resistance factor to amitraz was 4.33 for Krimeh population and the lowest one was 2.06 for Baq'a population. In contrast to these results, Nazer (1982) obtained that amitraz was the most effective acaricide against Baq'a population of *T. urticae* with resistance index of 0.98. Resistance factors ranged from 1.2 - 2.1 to amitraz among *T. urticae* had been reported by Ay and Gurkan, (2005) in Turkey.

In conclusions , all the tested field populations were moderately resistant to amitraz. The resistance factor ranged from 2.06 to 4.33. At its high recommended field rate amitraz was ineffective in controlling *T. urticae.*

However, in order to have safe and high quality and quantity of cucumber product and to manage resistance development by *T .urticae* to acaricides, the following are recommended; Regular monitoring should be carried out to detect the extent of resistance to the pesticides used, particularly amitraz and restricting the use of acaricides to which the magnitude of resistance is moderate like amitraz. And applying acaricides that have different active ingredients and different mode of action.

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