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Original Article

Evaluation of cucumber cultivars for resistance to *Tetranychus urticae* Koch. and *Tetranychus turkestani* Ugarov & Nikolski (Acari: Tetranychidae)

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Abstract

Forty-three cucumber‶*Cucumis sativus* L.″ cultivars were tested against two spotted spider mite ‶*Tetranychus urticae* Koch″ and strawberry spider mite‶*Tetranychus turkestani* Ugarov & Nikolskii″. The experiment was implemented in a randomized complete block design with 43 treatments (cucumber cultivars) and check treatments in greenhouse conditions between 2015 and 2016. The results showed that the highest numbers of eggs laid per female per day by the *T. urticae* and *T. turkestani* were 8.98 and 9.23, respectively, which were recorded on the Taha cultivar. The lowest numbers of eggs laid per female per day were 2.23 and 2.21 by the *T. urticae* and *T. turkestani*, on the Samer star and Danitu cultivars, respectively. The highest visual damage ratings were recorded on the Taha cultivar (5.75 for *T. urticae* and 5.78 for *T. turkestani*) and the lowest damage ratings of 2.49 and 4.25 were recorded on the Samer star and Storm cultivars for *T. urticae* and *T. turkestani*, respectively. Based on our findings, Taha cultivar was found to be a susceptible cultivar and Samer star cultivar proved to be a resistant one. These two cultivars could be used as differential hosts in further studies.

Keywords: cucumber, damage rating, resistance, spider mites

1. Introduction

Greenhouse cultivations are rising in recent years and at the same time problems from pests are increasing. Cucumber (*Cucumis sativus*) is one of the most important and widely grown crops in greenhouse conditions (Rich, Webb, Paret, & Momol, 2013). Greenhouse crops are affected by many pests that cause extensive yield losses (Zhang, 2003). Growers use chemical pesticides to control the pests but this threatens the health of consumers, particularly because these crops are consumed as raw and fresh (Hoy, 2011). Among the wide range of pests which attack greenhouse crops, the two-spotted spider mite, *Tetranychus urticae*, and the strawberry spider mite, *T. turkestani*, are economically important pests (Martinez-Ferrer, Jacas, Piolles-Moles, & Aucejo-Romero, 2006). The mite population increases enormously in the constantly warm and moist conditions of greenhouses (Zhang, 2003). The pest further infests the underside of leaves, where it produces plenty of web and feeds in a piercing-sucking manner that damages plant cells and tissue. The feeding results in the appearance of yellow chlorotic spots on leaves (Martinez-Ferrer *et al.*, 2006). Hot and dry weather conditions, coupled with suppressed population of natural enemies due to indiscriminate use of pesticides could lead to a population explosion of the pest, which demands further use of chemicals (Yang, Buschman, Zhu, & Margolies, 2002). This chain reaction could ultimately lead to development of resistance in the pest to the pesticides. Management of the *T. urticae* has become difficult, primarily due to miticide resistance (Bynum, Archer, & Plapp, 1990; Perring, Archer, Bynum, & Hollingsworth, 1981).

A number of natural enemies (lady beetles; *Stethorus* spp. and predatory mites) exist and if given protection, can efficiently control the spider mites. Biological control alone

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and in combination with the host plant resistance can play a vital role in the management of the pest (Dionysios, Eleftheria, & Georgios, 2008). In addition to the use of natural enemies, one of the strategies for sustainable management of the spider mites is the use of resistant host plants. Initial work on the resistance in cucumber to the *T. urticae* was conducted by Dacosta and Jones (1971), De Ponti (1979) and Hussey and Parr (1963). Bitter gene (cucurbitacin-C) in the host plant (*C. sativus*) was considered to be responsible for resistance to both the *T. urticae* and *T. turkestani*. However, De Ponti (19 79) denied the absolute role of bitterness against both the *T. urticae* and *T. turkestani*. The role of cucurbitacin was later confirmed by Balkema-Boomstra *et al.* (2003) and Bouwmeerster, Verstappen, Posthumus, and Dicke (1999).

Cucumber has bitter foliage and non-bitter fruits, but the fruits may become bitter under stress. The gene *Bi* confers bitterness to the entire plant which is determined in the cotyledons (Balkema-Boomstra *et al.,* 2003). In a preliminary study with dihaploid (completely homozygous) progenies from the F1 generation of a cross between the non-bitter, susceptible inbred line G6 and the resistant bitter accession 9140, Balkema-Boomstra *et al.* (2003) noticed an absolute link between spider mite resistance and bitterness in cucumber judged by testing the young cotyledons. All non-bitter-tasting dihaploid lines were highly susceptible while the bitter-tasting dihaploid lines were shown to be resistant. Perring *et al.* (1981) reported that the *Bi* gene confers bitterness to the entire plant while the plants with bi (the recessive allele) are bitter-free.

The objective of this study was to evaluate the bitter-taste cucumber cultivars for any possible variation in terms of resistance among them. We hypothesized that resistant in cucumber to the *T. urticae* and strawberry spider mite was dependent on the level of cucurbitacin.

2. Materials and Methods

2.1 Plants cultivars

Forty-three cucumber (*C. sativus*) cultivars were purchased in the open market in Bandarabbas, Hormozgan Province, Iran. Twenty-seven were Dutch cultivars (Khasib, Nasim, Danitu, Negeen, Tunca, Fadia, Caspian, Taha, Nahid, Donya, Borhan, Sucrates, Iver, Amiral, Padideh, Mehr, Tania, Yalda, Samer star, Bit alfa, Diva, Nageen, Datis, Sina, Storm, Shahin, and Storia). Six were American cultivars (Super star, Royal 24189, Maxim, Maxwell, Davos, and Super n3). Five were Turkish cultivars (F1 asker, F1 82-8183, F1 karim, Nefes, and Y.green). Three were Japanese cultivars (F1 saso, Salvador, and Jasmin), and two were Italian cultivars (Super dominus and Sultan).

2.2 Plant cultivation

Two to three seeds of each cultivar were sown in a plastic cup (7.5 cm diameter by 7.5 cm deep) in potting materials containing sterilized soil with vermiculite (minimum size) in a 3:2 ratio. After planting, the cups were watered and placed in an incubator at 25±2 ºC with 16:8 hours (lightness:darkness) photoperiods. The plants were watered twice a week and no fertilizers or chemicals were added throughout the experiment.

2.3 Rearing of the mites

The adults of *T. urticae* were originally collected from infested green bean fields and the initial populations of *T. turkestani* were collected from infested cucumber greenhouses in the Roodan region, Hormozgan Province, Iran in May 2015. The collected mites were separately reared on common bean (*Phaseolus vulgaris* L.) plants. Rearing of the mites was done in a growth chamber at 25±2 °C and 60±5% RH with a photoperiod of 16:8 hours (lightness:darkness).

2.4 Preparation for experiments

All experiments were performed at the some above mentioned conditions in a greenhouse. At the two-leaf stage, one set of all the test cucumber plants were inoculated with spider mites and individually caged.

These plants provided us mature females for egg production on the required leaf disc of the test cultivars. A second set of test plants was maintained in isolated incubator at 25±2 ºC with 16:8 hours (lightness:darkness) photoperiod for the regular supply of leaves for leaf discs. Circular leaf discs of 2 cm diameter were cut with the help of a punch from leaves of common bean and from each cucumber cultivar and placed upside down on wet cotton (8 cm diameter) in a petri dish (8.5 cm diameter). Leaf discs were arranged in a circular fashion. The position of each disc was marked for proper identification. Additional water was added to each petri dish to arrest the movement of the female mites from one disc to another. Each petri dish was covered with a lid (9 cm diameter) having 50–60 tiny holes to allow the escape of excessive moisture. A single young female of *T. urticae* was placed with a help of a fine camel hairbrush. At three day intervals, the numbers of eggs were counted and the females were transferred to a fresh leaf disc. The numbers of eggs were counted for 9 days and the number of eggs laid/female per day was calculated for each cultivar. In a separate experiment, two cucumber plants per cultivar were grown in plastic pots (15 cm diameter and 15 cm deep) with the same material as stated above. The common bean was also tested for comparison for damage rating. Plants were raised on string as suggested by De Ponti (1977). The experiment was run at 25±1 ºC and 60±5%RH with a photoperiod of 16:8 hours (lightness:darkness). At the four-leaf stage, each plant was inoculated with 20 adult female mites. After six weeks of infestation, plant damage was scored using the 1–5 damage rating scheme of De Ponti (1977) where 1=slight damage (1–20%) and 5=heavy damage (80–100%). The experiments for the two mite species were carried out separately to avoid cross infestation and each experiment was replicated three times.

2.5 Data analysis

The experiment was implemented in the randomized complete block design with 43 treatments (cucumber cultivars) and check treatments (common bean) in greenhouse conditions. The experiment was replicated 25 to 30 times depending on the availability of young females. For easy handling, a set of ten replicates was handled at a time. The same procedure was adopted for *T. turkestani*. Data were analyzed using SAS packages. Means were compared with Duncan's multiple range ($P<0.01$).

3. Results

The number of eggs laid per female and the damage ratings of the various cucumber cultivars from the *T. urticae* and *T. turkestani* are shown in Tables 1 and 2. The results showed that the numbers of eggs that were laid per female and the damage ratings of the various cucumber cultivars were significantly different in both species of mites.

In the case of the *T. urticae*, the highest number of eggs/female/day was 13.21 which was recorded on the common bean followed by 8.98 eggs/female/day on the Taha cultivar. On the other hand, the lowest number of eggs/female/day was 2.23 which was recorded on the Samer star cultivar. Also, the highest and lowest means of the damage ratings were recorded on the common bean (5.90±0.06) and Samer star cultivar (2.49±0.12), respectively (Table 1).

In the case of *T. turkestai*, the highest number of eggs/female/day was also recorded on the common bean (13. 97 eggs/female/day). On the Taha cultivar the number of eggs laid was 9.23/female/day and the lowest number of eggs/female/day was recorded on the Danitu cultivar (2.21). Also, the highest and lowest means of the damage ratings were recorded on the common bean (5.80±0.13) and Iver cultivar (2.70±0.61), respectively (Table 2).

The Dutch cultivars showed a wide range of response. In the case of *T. urticae*, 8.98 and 8.60 eggs/female/day were recorded on the Taha and Nasim cultivars, respectively, while 5.95 and 2.23 eggs were recorded on the Iver and Sumer star cultivars, respectively (Table 1). In the case of *T. turkestai*, the highest numbers of 9.23, 8.85, and 8.83 eggs/female/day were recorded on the Taha, Nahid, and Nasim cultivars, respectively. The lowest numbers of 4.95, 3.58, and 2.21 eggs/female/day were recorded on the Caspian, Fadia, and Danitu, respectively (Table 2). Somewhat similar responses were exhibited by the other cucumber cultivars.

4. Discussion

Our results demonstrated a significant variation in the cucumber cultivars response to the two mite species, *T. urticae* and *T. turkestai*. The numbers of eggs/female/day on some cucumber cultivars were high but the damage ratings were low (Tables 1 and 2). For example in Table 1, the number of eggs/female/day and the damage rating on the Taha cultivar were 8.98 and 5.75±0.11 and on the Y.green cultivar the results were 7.63 and 3.55±0.17, respectively. Also in Table 2, the number of eggs/female/day and the damage rating on the Taha cultivar were 9.23 and 5.78±0.09 and on the Mehr cultivar the results were 7.67 and 3.55±0.11, respectively. Our findings established a relative susceptible response for the Taha and Nasim cultivars which can be classified as preferred hosts. The Samer star and Iver cultivars were tolerant and can be classified as non-preferred host for *T. urticae* and *T. turkestai* eggs (Tables 1 and 2). It could be concluded from these findings that the Taha cultivar is susceptible for both mites and the Samer stara and Danitu cultivars are somewhat resistant for *T. urticae* and *T. turkestai*, respectively. There seems some discrepancy in the numbers of eggs/female/day between the two mite species but this is quite natural. The number of eggs laid by a female on a particular cucumber accession could be an index of acceptance of the host for oviposition and the amount of nutrients or secondary metabolites provided by the host. The results of this research are in line with the results presented by other researchers.

Table 1. Mean number of eggs laid per female/day* by *T. urticae* and damage rating response of various cucumber cultivars in comparison with common bean.

No.	Cultivars	Eggs/ female	Damage rating (mean±SD[1])	No.	Cultivars	Eggs/ female	Damage rating (mean±SD[1])
1	Common bean	13.21	5.90±0.06a[2]	23	Super star	7.47	3.75±0.19jkl
2	Taha	8.98	5.75±0.11a	24	Nefes	7.46	3.35±0.18l
3	Nasim	8.60	5.75±0.83ab	25	F1 82-8183	7.32	5.55±0.14abcd
4	Donya	8.14	5.40±0.40ab	26	Maxim	7.23	5.20±0.20abcdefg
5	Bit alfa	8.11	5.75±0.11ab	27	Maxwell	7.12	5.30±0.17abcdef
6	Nahid	8.10	4.90±0.23ab	28	Storia	7.09	4.40±0.16ghijk
7	Sucrates	8.08	5.70±0.15ab	29	Salvador	6.99	5.05±0.13abcdefgh
8	F1 saso	8.04	5.67±0.13abc	30	Tunca	6.90	4.80±0.15bcdefgh
9	Fadia	8.02	5.50±0.15abcd	31	Storm	6.86	4.85±0.18bcdefgh
10	Super dominus	7.98	5.40±0.12abcde	32	Yalda	6.79	4.30±0.13ghijkl
11	Datis	7.94	5.50±0.14abcd	33	Diva	6.76	3.70±0.17jkl
12	Caspian	7.90	5.20±0.13abcdefg	34	Padideh	6.68	3.55±0.13kl
13	F1 karim	7.90	5.10±0.23abcdefgh	35	Tania	6.68	3.65±0.15kl
14	Royal 24189	7.89	4.60±0.16defghij	36	Borhan	6.61	3.80±0.13ijkl
15	F1 asker	7.83	4.70±0.21defghi	37	Negeen	6.58	3.55±0.14kl
16	Danitu	7.80	5.50±0.17abcd	38	Amiral	6.58	4.15±0.15hijkl
17	Nageen	7.73	5.70±0.13ab	39	Shahin	6.54	3.80±0.13ijkl
18	Jasmin	7.72	5.40±0.22abcde	40	Super n3	6.43	3.80±0.15ijkl
19	Sultan	7.71	4.90±0.23bcdefg	41	Mehr	6.17	3.35±0.11l
20	Sina	7.68	5.00±0.21abcdefgh	42	Davos	6.17	2.72±0.52lm
21	Y.green	7.63	3.55±0.17kl	43	Iver	5.95	2.50±0.05m
22	Khasib	7.59	4.50±0.16efghij	44	Samer star	2.23	2.49±0.12m

*: During a 9-day period of reproduction
1: Damage rating is based on a 1–5 scale (1 being no or low damage and 5 highly damaged)
2: Means in a column followed by the same letter are not significantly different (P>0.05)

Table 2. Mean number of eggs laid per female/day* by *T. turkestani* and damage rating response of various cucumber cultivars in comparison with the common bean.

No.	Cultivars	Eggs/ female	Damage rating (mean±SD[1])	No.	Cultivars	Eggs/ female	Damage rating (mean±SD[1])
1	Common bean	13.97	5.80±0.13a[2]	23	Royal 24189	8.13	4.80±0.32abcdef
2	Taha	9.23	5.78±0.09a	24	Maxim	8.05	4.63±0.22abcdef
3	Nahid	8.85	5.51±0.08ab	25	F1 asker	7.97	4.60±0.08abcdef
4	Nasim	8.83	5.31±0.15abcd	26	Storia	7.96	4.50±0.14abcdefg
5	Negeen	8.82	5.10±0.06abcde	27	Diva	7.96	4.55±0.32abcdefg
6	Tunca	8.73	5.25±0.18abcd	28	Samer star	7.84	4.53±0.90abcdefg
7	Borhan	8.68	5.14±0.26abcde	29	Yalda	7.80	4.40±0.89abcdefg
8	Bit alfa	8.65	5.01±0.11abcdef	30	Tania	7.72	4.39±0.31abcdefg
9	Super star	8.62	5.00±0.12abcdef	31	Mehr	7.67	3.55±0.11efgh
10	F1 saso	8.58	5.20±0.13abcd	32	Padideh	7.43	3.70±0.21defgh
11	F1 82-8183	8.57	5.35±0.17abc	33	Amiral	7.41	4.00±0.11bcdefgh
12	Nefes	8.54	5.52±0.08ab	34	Super n3	7.14	3.40±0.13fgh
13	Shahin	8.54	4.95±0.23abcdef	35	Y.green	6.27	3.80±0.22cdefgh
14	Sina	8.47	5.00±0.21abcdef	36	Davos	6.31	2.95±0.08gh
15	Jasmin	8.45	4.97±0.19abcdef	37	Iver	6.28	2.70±0.61h
16	Salvador	8.41	4.85±0.07abcdef	38	Sucrates	5.85	5.35±0.90abc
17	Maxwell	8.38	4.82±0.18abcdef	39	Donya	5.68	5.30±0.09abcd
18	Datis	8.34	4.80±0.15abcdef	40	Caspian	4.95	5.15±0.29abcde
19	Sultan	8.33	4.75±0.21abcdef	41	Fadia	3.58	5.00±0.20abcdef
20	F1 karim	8.30	4.73±0.30abcdef	42	Danitu	2.21	4.80±0.21abcdef
21	Super dominus	8.24	4.86±0.21abcdef	43	Khasib	---[3]	4.70±0.31abcdefg
22	Nageen	8.14	4.70±0.12abcdef	44	Storm	---	4.25±0.12abcdefgh

*: During the 9-day period of reproduction
1: Damage rating is based on a 1–5 scale (1 being no or low damage and 5 highly damaged)
2: Means in a column followed by the same letter are not significantly different (P>0.05)
3: Eggs were not collected

Various genotypes of a plant, due to experiencing environmental stress or their genetic make-up, possess physiological and biochemical variations, which can affect their nutritional values (primary metabolites) for herbivores (Goncalves-Alvim, Collevatti, & Fernandes, 2004; Zakir, Sarwar, Allen, Khan, & Butt, 2006). In the present study, some life-history traits of the *T. urticae* and *T. turketani*, the number of deposited eggs and damaging rates on various cucumber cultivars were evaluated. The results showed significant differences among the cultivars, interaction of the cultivars, and mite species in terms of the numbers of deposited eggs/ female/day and the damaging rates. In both mite species the highest number of eggs/female/day was on the Taha and Nasim cultivars and the lowest was on the Iver and Samer star cultivars. Therefore, it can be reasonably argued that the Taha and Nasim cultivars are the preferred/susceptible and the Iver and Samer star cultivars are the relatively non-preferred host. Variation in the life history traits of *T. urticae* and *T. turketani* on different genotypes of cucumber were documented by different researchers in multiple-choice tests in greenhouse studies. For example, the highest number of *T. urticae* eggs/ female/day (5.98) was recorded on the cucumber genotype Blackish Green while the lowest eggs/female/day (2.95) was recorded on Winter Long Green (Ullah, Lee, & Farhatullh, 2006).

The highest total fecundity of *T. turketani* was observed on the Hedieh cultivar (29.784 eggs/female) and the lowest on the Puia cultivar (15.773 eggs/female). The highest gross and net fecundity rates of this pest were seen on the Hedieh cultivar (91.721 and 41.171 eggs/female) and the lowest on the Puia cultivar (35.765 and 9.154 eggs/female, respectively). Therefore, *T. turketani* had the highest gross and net fertility rates on the Hedieh cultivar (44.913 and 26. 720 eggs/female, respectively). Hence, it seems that the Hedieh and Milad Jadid cultivars were the more susceptible and resistant cultivars to the *T. turkestani* than the other tested cultivars, respectively (Mohammadi, Seraj, & Rajabpour, 20 15). This research of two diverging categories of cucumber cultivars, previously defined as resistant and susceptible (Mohammadi *et al.,* 2015; Ullah *et al.,* 2006), showed differential eggs laid per female/day and damage ratings in response to natural mite infestations in the greenhouse trials.

Our results are similarly supported by the findings of De Ponti (1985) who reported 21 eggs/female/3days and 14 eggs/female/9days of *T. urticae* on a susceptible cucumber line (Bitter cucumber line which was equivalent to 14 eggs/ female/9 days).

Plants exploit various ways to minimize damage of insect infestation (Egenborde & Trumble, 1994). Painter (19 51) described three types of defense mechanisms in plants or host plant resistance including antixenosis, antibiosis, and tolerance in which antixenosis proved to be an effective defensive mechanism against insect pests in many crops and vegetables (Egenborde & Trumble, 1994; Rodriguez, Dabrowski, Stoltz, Chaplin, & Smith, 1971).

Antixenosis includes some morphological properties and the lack of plant attractants that reduce the available diet and the ability of residential colonization on the plant (Van Emden, 1997). In total, resistance mechanisms like this usually induce the morphological and biochemical features of a plant (Harrison & Karban, 1986; Stadler, 1992) to impair the normal feeding or oviposition of various pest insects or induce other mortality factors that collectively create phonetic resistance (Harrison & Karban, 1986; Underwood, 1999).

The results of the current study revealed a high variation in damaging rates of the various cucumber cultivars from the *T. urticae* and *T. turkestai*. A positive correlation was also disclosed between the number of eggs laid/female/day and the damaging rate. The existence of highly diverse damaging rates in the cucumber cultivars possibly stemmed from a variety of physio-morphic characteristics in plants which can function as inhabitant-preventing factors (Ernest, 1989). These prevention factors may include some characteristics related to the plant shape and size (Prokopy & Owens, 1983), plant color and surface texture, presence of trichomes and wax crystals on the surface, and the thickness and toughness of the tissue (Ernest, 1989).

The observed variations among the cucumber cultivars from the damaging rates and the numbers of deposited eggs/female/day were possibly also due to variable levels of secondary plant metabolites, especially, cucurbitacin-C (Balkema-Boomstra *et al.,* 2003). Highly variable levels of cucurbitacin-C in bitter dihaploid cucumber lines were demonstrated by Balkema-Boomstra *et al.* (2003). They showed a negative relevance between the survival rate in *T. urticae* and the amount of cucurbitacin-C in bitter dihaploid cucumber lines. They also demonstrated a higher survival rate of *T. urticae* on cucumber lines with lower cucurbitacin-C level and vice versa. It was shown that formation of cucurbitacin in the cucumber can be induced by mite feeding which can subsequently decrease the mite population up to 40% (Agrawal, Gorski, & Tallamy, 1999).

Two different strategies have been ascertained regarding mite feeding on resistant cucumber cultivars. First, the mites avoid feeding and remain slim and weak. Second, they keep on feeding but cannot digest the food material which results in swelling and become more or less bloated and turn black (Rodriguez *et al.,* 1971). This phenomenon usually happens more in *T. urticae* compared with *T. turkestai* and leads to an evident change in the *T. urticae* in only a few days after feeding on the resistant cultivars (Rodriguez *et al.,* 1971). It might be one of the reasons for different responses of the two mite species on the same cucumber cultivars. This hypothesis is supported by the findings of Wehner, Liu, and Staub (1998) who reported a second recessive gene (bi-2) which segregates independently of the first gene (bi). The role of the dominant gene, Bi-2 has yet to be investigated (Balkema-Boomstra *et al.,* 2003).

In the present study, a diverse array of responses, high variation in the number of deposited eggs/female and damaging rates, were seen rather than only resistance or susceptibility. It could be speculated that two or more genes may be involved in the resistance of cucumber to the mites. De Ponti (1979) hypothesized there are polygenic resistance in cucumber cultivars to the *T. urticae*. Balkema-Boomstra *et al.* (2003) showed that resistance genes in the cucumber were expressed independently and their effects are cumulative in nature. In this study, we documented the variation in susceptibility of the most common commercial cucumber varieties of which we introduced two varieties, Iver and Samer star, as the most tolerant to mite injuries in greenhouse cultures. Further investigations are needed to determine the level of cucurbitacin in these cucumber cultivars and the impact on life table parameters of the mite species.

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