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ORIGINAL RESEARCH ARTICLE

Susceptibility of ten tomato cultivars to attack by *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) under laboratory conditions

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Abstract
We evaluated, in a laboratory experiment, the susceptibility of 10 tomato cultivars to the attack by the tomato red spider mite *Tetranychus evansi*, an invasive pest of this crop in Benin. Among the 10 cultivars tested, six were imported (i.e. Roma VF, Tima, Rio Grande, Buffalo, Petomech, and TLCV15) and four local (i.e. Akikon, Tounvi, TomL4, and Kèkèfo). Leaf disks (2.5 cm diameter) excised from leaves of tested cultivars, and placed upper side down on top of water-soaked cotton wool laying in 9.5 cm diameter Petri dishes (6 leaf disks per Petri dish), served both as experimental units and feeding source for the mites. Mites were individually placed on leaf disks of each cultivar and several biological parameters – development, reproductive parameters and longevity – were assessed. The experiments were performed at 27 ± 1 °C, 65–70% RH, and 12:12 h (L: D) photoperiod. Our results revealed significant effects of tomato cultivars on several biological parameters of *T. evansi*, especially the developmental time of the egg stage ($P < 0.0001$), and the larval stage ($P = 0.0113$), as well as female oviposition period ($P = 0.0002$). Likewise, female longevity ($P = 0.0095$) and fecundity (i.e. egg/♀, $P = 0.0055$) were significantly affected. The sex-ratio of the progeny was generally similar for all the cultivars except for Buffalo and Tounvi on which most juveniles did not develop beyond the larval stage. These two cultivars appeared as potential tomato cultivars for implementation of IPM strategies for the control of *T. evansi*.

Keywords Spider mite · Biological parameters · Varietal control · IPM

Introduction

Among vegetable crops, tomato, *Lycopersicon esculentum* Mill. (Solanales: Solanaceae), appears as the most consumed since it is regularly used as condiment in the daily food ration of households worldwide (FAOSTAT 2012). Unfortunately, this crop is very attractive to many arthropod pests and diseases, particularly during the dry season (Kennedy 2003). Whiteflies, thrips, bedbugs, aphids, moths, leafminer and mites are the major tomato pests in greenhouse as well as in open field (Escudero and Ferragut 1999). The fruit fly, *Dacus punctatifrons* (Karsch) was also recognized in open field as pest in tomatoes (Tindo and Tamo 1999). One of the key mite pest attacking tomato in Africa and also in the world is *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), commonly referred to as 'Tomato Red Spider Mite' (Saunyama and Knapp 2003; Duverney and Gueye-Ndiaye 2005; Martin et al. 2010; Migeon et al. 2014; Azandémè-Hounmalon et al. 2015). Probably native to South America (Migeon et al. 2009; Boubou et al. 2011), and reported for the first time in Benin in 2008 (Martin, Pers communication), *T. evansi* causes severe damage to many tropical vegetable crops and constitutes the most serious constraint to the growth and development of tomato plants (Saunyama and Knapp 2003; Duverney and Gueye-Ndiaye 2005; Martin et al. 2010; Migeon et al. 2014; Azandémè-Hounmalon et al. 2015). This invasive species feeds on its host-plants by piercing the underside leaf surface and sucking all the cellular

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contents with its cheliceral stylets (André and Remacle 1984; Tomczyk and Kropczynska 1985). The feeding activity of the mite leads to a real handicap to the photosynthetic activity of leaves, thereby inducing enormous yield losses and even death of the plants (Azandémè-Hounmalon et al. 2015). Since 2008, *T. evansi* has become the pest of first importance in Benin, not only of tomato, but also of other solanaceous plants (Azandémè-Hounmalon et al. 2014, 2015). This pest has a very high reproductive capacity and can quickly reach very high population densities on tomato plants.

The control of *T. evansi* in tomato is done mainly with applications of synthetic pesticides. Despite its relative efficiency, chemical control has several negative impacts such as the selection of resistant individuals due to the continuous use of certain active ingredients, the reduction or elimination of beneficial species, the high toxicity of the products to applicators, and the presence of pesticide residues in food (Maniania et al. 2008). This has called for the need to search for environmentally safe and effective alternative methods for controlling *T. evansi* on tomato plants. In the search for such methods, natural enemies are being evaluated as biological control agents of *T. evansi* (Wekesa et al. 2005; Furtado et al. 2007), but none of the natural enemies identified until now, has proven to be efficient against the pest.

Another control strategy being investigated against that pest is the use of pest-resistant varieties, considered as a basic component of IPM. Indeed, several studies suggested that the use of resistant varieties could offer viable alternative approach for the control of this pest on tomato plants (Maluf et al. 2001; Resende et al. 2002; Gonçalves et al. 2006; Resende et al. 2008; Murungi et al. 2009, 2010; Onyambus et al. 2011; Alba et al. 2015).

In Benin, no such studies have yet been carried-out. The present study aims therefore, at assessing the susceptibility of some tomato cultivars commonly produced in Benin to the attack of *T. evansi*.

Materials and methods

The study was conducted in the laboratory at the International Institute of Tropical Agriculture (IITA), Benin-Station located at 12 km NW Cotonou (6°25'N; 2°19′E; 15 m asl), from June to October 2016. The 10 tomato cultivars used in this study included four local (i.e. Akikon, Tounvi, TomL4, and Kèkèfo) and six imported (i.e. Roma VF, Tima, Rio Grande, Buffalo, Petomech, and TLCV15), that are commonly grown in Southern Benin. Seeds of the local cultivars (Akikon, Tounvi, TomL4, and Kèkèfo) and of TLCV15 were provided by the Institut National des Recherches Agricoles du Bénin (INRAB), while the five other imported ones were purchased at the "Benin Seed", a seed company based at Cotonou, the Economic capital of Benin.

Tomato seeds were sown in plastic trays (dimensions: 62 cm × 31 cm), each tray containing 100 holes (1 cm diam.; 2.5 cm depth), filled with topsoil collected from a field fallowed for more than 4 years, enriched with poultry manure and maintained in a greenhouse at IITA-Benin. Seedlings were transplanted into plastic pots (diam.: 29 cm; height: 25 cm) 2 weeks after sowing, and water was supplied on a daily basis. No chemical fertilizers were applied to the plants.

The population of *T. evansi* used in this study was originally collected from a vegetable farm at Sèmè-Kpodji (6°22'N; 2°37E) in March 2016, and maintained in a greenhouse for 12 weeks on nightshade (*Solanum macrocarpon* L.) and tomato plants, at 25 ± 2 °C, 60–70% RH and 12:12 h (L:D) photoperiod prior to the commencement of the experiments.

The experimental units consisted of 2.5 cm -diameter leaf discs excised from leaves of each tomato cultivar and placed on top of water-soaked cotton laying in a 9.5 cm diameter plastic Petri dish. The cotton wool was watered on a daily basis for the triple purposes of keeping the leaf discs turgid, preventing mobile stages of *T. evansi* to escape and to prevent ants from reaching the leaf discs and feeding on the mites. Leaf discs were renewed every 96 h to avoid colonization by saprophytic fungi.

Effects of tomato cultivars on the duration of the developmental stages of *T. evansi*

About 10 gravid adult females *T. evansi* taken from the rearing colonies were transferred on leaflets of each tomato cultivar. Six eggs laid within a 24 h-period were transferred individually onto one experimental unit, thereby totaling six leaf discs per tomato cultivar. The six leaf discs from a given cultivar were kept in the same Petri dish. Experimental units were observed daily to record the developmental time of *T. evansi* on each tomato cultivar. The experiment was replicated six times.

Fecundity, longevity and sex-ratio of *T. evansi* on the different tomato cultivars

For this purpose, 60 deutonymph females of *T. evansi* (i.e. six deutonymphs/tomato cultivar), were picked from the mother colony reared on leaflets of each tomato cultivar, and allowed to mate by adding two males to each of them until the first egg was laid; thereafter the males were removed while the gravid females were transferred individually and delicately on six leaf discs cut from the same tomato cultivar. The experimental units were observed daily until death of females to record the fecundity, the pre-oviposition, oviposition and post-oviposition periods and the longevity of females. Eggs laid on each cultivar were reared until adulthood to determine the sex ratio of the progeny (i.e. the percentage of females in the progeny).

Statistical analyses

A single-factor analysis of variance (PROC GLM; SAS, 2009) was used to test the effects of tomato cultivar on the biological parameters of *T. evansi* (duration of development, pre-oviposition, oviposition and post-oviposition periods, female fecundity and sex-ratio of the progeny), and means separated using the Student-Newman-Keuls multiple range test (SNK). To correct for homogeneity of the variances, data on mite counts were transformed using $\log_{10}(x + 1)$, whereas data on proportions were transformed using arcsine square root (*arsin (sqrt Proportion)*) before their use in the statistical analyses.

Results

Effects of tomato cultivars on the duration of the developmental stages of *T. evansi*

Developmental times of *T. evansi* on the 10 tomato cultivars tested are presented in Table 1. The egg-adult developmental times varied between 11.40 (on cultivar Kèkèfo and Roma VF) and 13.75 days (cultivar Petomech). However, no larvae developed to the nymphal stage on cultivar Buffalo whereas only one specimen reached the adult stage on cultivar Tounvi (Table 1).

The results of the analysis of variance, show that durations of egg and larval stages differed significantly among the 10 tomato cultivars tested ($P < 0.05$); whereas, in contrast, the durations of the nymphal stages as well as the total egg-to-adult developmental times did not differ significantly among cultivars ($P > 0.05$; Table 1).

Effects of tomato cultivars on the oviposition periods, and on longevity of female *T. evansi*

Tomato cultivar did not affect the pre-oviposition ($P = 0.1560$) and post-oviposition ($P = 0.5466$) periods, while it did affect oviposition period ($P = 0.0002$) and female longevity ($P = 0.0095$) of *T. evansi* (Table 2). Survival of adult female *T. evansi* decreased faster on Tounvi than on the other cultivars, and longevity did not go beyond 18 days (Roma VF and Rio Grande) on any of the tested cultivars (Fig. 1). On average, females lived longer on Kèkèfo and Akikon (10.7 and 9.8 days, respectively) and shorter on Buffalo (4.2 days) (Table 2) on which none adult female *T. evansi* reached the post-oviposition stage.

Effects of tomato cultivars on the fecundity, egg viability and sex-ratio of *T. evansi*

There was significant effect of tomato cultivar on the fecundity of *T. evansi*. Egg production decreased faster on Buffalo just after 2 days and was the longest on Kèkèfo and Rio Grande (Fig. 2). Total fecundity varied significantly among cultivars ($P = 0.0055$), being the highest on Kèkèfo 29.3/female) and the lowest on Buffalo (2.7/female) (Table 3). The egg hatching rate ranged between 44.4% (Buffalo) and 84.0% (Tima), and did not differ significantly among the 10 tomato cultivars tested ($P = 0.3541$). Meanwhile, the number of hatched larvae ranged between 1.2 (Buffalo) and 24.7

Table 1 Mean development duration (days; ± SE) of *T. evansi* reared on 10 different tomato cultivars at 27 ± 1 °C, 65–70% RH and 12:12 h (L:D) photoperiod

Tomato cultivar	Egg	Larva	Protonymph	Deutonymph	Egg-adult
Akikon	5.33 ± 0.49ab(117)	3.33 ± 0.21ab (61)	2.33 ± 0.21a (34)	2.00 ± 0.32a (29)	13.20 ± 0.73a
Buffalo	4.50 ± 0.22b (16)	3.00 ± 0.45ab (7)	–	–	–
Kèkèfo	4.67 ± 0.21b (176)	2.33 ± 0.21b (122)	2.60 ± 0.24a (62)	1.80 ± 0.20a (48)	11.40 ± 0.24a
Petomech	6.00 ± 0.37a (80)	2.33 ± 0.33b (48)	3.25 ± 0.48a (33)	1.75 ± 0.25a (32)	13.75 ± 0.48a
Rio Grande	4.67 ± 0.33b (141)	2.60 ± 0.40ab(107)	2.50 ± 0,29a (66)	2.00 ± 0.00a (43)	11.75 ± 0.63a
Roma VF	4.67 ± 0.21b (132)	2.33 ± 0.33b (82)	2.40 ± 0.24a (43)	1.80 ± 0.20a (20)	11.40 ± 0.24a
Tima	4.33 ± 0.21b (115)	4.00 ± 0.52a (113)	2.83 ± 0.31a (95)	1.60 ± 0.24a (58)	12.20 ± 0.49a
TLCV15	4.50 ± 0.22b (107)	3.00 ± 0.41ab(102)	2.33 ± 0.33a (69)	1.67 ± 0.33a (38)	11.67 ± 0.88a
TomL4	4.17 ± 0.17b (99)	3.17 ± 0.31ab (70)	3.00 ± 0.00a (48)	1.40 ± 0.24a (31)	12.00 ± 0.32a
Tounvi	6.33 ± 0.33a (67)	2.17 ± 0.31b (37)	2.00 ± 0.82a (4)	1.75 ± 0.00a (1)	12.00 ± 1.58a
F	**6.18**	**2.76**	**1.12**	**0.42**	**1.43**
P	**<0.0001**	**0.0113**	**0.3782**	**0.8972**	**0.2236**

Means followed by the same letters within columns are not significantly different ($P > 0.05$; Student-Newman-Keuls, multi-range test) Numbers in brackets represent the population size

Table 2 Pre-oviposition, oviposition, and post-oviposition periods and longevity (days ±SE) of female *T. evansi* reared on 10 tomato cultivars at 27 ± 1 °C, 65–70% RH and 12:12 h (L:D) photoperiod

Tomato cultivars	Pre-oviposition	Oviposition	Post-oviposition	Longevity
Akikon	3.33 ± 0.42a	5.00 ± 0.89ab	1.80 ± 0.37a	9.83 ± 1.01a
Buffalo	2.67 ± 0.61a	1.50 ± 0.45c	–	4.17 ± 0.60b
Kèkèfo	2.50 ± 0.34a	7.33 ± 1.09a	1.67 ± 0.67a	10.67 ± 1.82a
Petomech	2.50 ± 0.34a	4.00 ± 0.77bc	2.17 ± 0.54a	8.67 ± 1.12a
Rio Grande	1.83 ± 0.17a	4.33 ± 0.61bc	2.67 ± 0.49a	8.83 ± 0.75ab
Roma VF	2.00 ± 0.45a	5.33 ± 0.42ab	2.00 ± 0.71a	8.67 ± 0.95ab
Tima	2.50 ± 0.50a	4.00 ± 0.37bc	1.83 ± 0.40a	8.33 ± 0.49ab
TLCV15	1.67 ± 0.21a	4.17 ± 0.87bc	3.20 ± 0.97a	8.50 ± 1.31ab
TomL4	2.00 ± 0.45a	4.50 ± 0.57b	1.80 ± 0.49a	8.00 ± 1.10ab
Tounvi	2.83 ± 0.40a	3.17 ± 0.48bc	1.33 ± 0.33a	6.67 ± 0.80ab
F	**1.55**	**4.70**	**0.88**	**2.81**
P	**0.1560**	**0.0002**	**0.5466**	**0.0095**

Means followed by the same letters within columns are not significantly different ($P > 0.05$; Student-Newman-Keuls, multi-range test)

(Kèkèfo), and differed significantly among cultivars ($P = 0.0004$). As for the number of juveniles *T. evansi* that reached the adult stage, it ranged between 0.0 (Buffalo) and 6.7 (Tima), and was significantly affected by the cultivars ($P = 0.0132$) (Table 3). The sex-ratio (expressed as percentage of females within the adult progeny), ranged between 75.8% (TLCV15) and 100% (Tounvi), and did not differ among tomato cultivars ($P = 0.8884$). On cultivar Tounvi, however, the only one individual that reached the adult stage was a female thereby explaining the 100% female recorded for the sex-ratio on this cultivar (Table 3).

Discussion

This is the first study conducted in Benin to evaluate the susceptibility of some tomato cultivars to attack by the Tomato Red Spider Mite, *T. evansi*. Our results suggest that the use of resistant/tolerant tomato cultivars may contribute to reducing the population of this mite pest. Among the 10 cultivars tested, two (i.e. Buffalo and Tounvi) were found to show some resistance to *T. evansi* with regard to the developmental time of its juvenile stages as well as other biological parameters such as oviposition, egg hatchability and adult female longevity. Indeed, the fecundity was significantly lower on Buffalo (and to some extent on Tounvi) than on the other cultivars. In addition, no juveniles developed beyond the larval stage on Buffalo, while only 1.5% of individuals (from a starting population of 60 eggs) reached the adult stage on Tounvi. Female longevity was also significantly lower on Buffalo followed by Tounvi compared with the eight other cultivars. The shorter longevity of *T. evansi* was so obvious on Buffalo that females could not even reach the post-oviposition period before dying. These among-cultivars differences are certainly linked with the deleterious effects of certain chemical compounds contained in Buffalo and Tounvi on the embryonic development of *T. evansi,* as has been reported by Adkisson and Dyck (1980) in some tomato cultivars for other arthropod pests. It is also well known that plants of some tomato cultivars are capable of secreting chemical substances that could be repellent or toxic to some mite species (Chatzivasileiadis and Sabelis 1997; Kennedy 2003; Murungi et al. 2013).

The negative effects of cultivars Buffalo and Tounvi on the biological parameters of *T. evansi* let us suspect the presence in these cultivars of some chemical compounds that repel or prevent the mite from feeding efficiently on it, thereby interfering with its development and survival. It also well-known that the abaxial surface of some tomato leaves has glandular trichomes or hairs that generally disturb and hinder the development of arthropods by trapping them with the sticky substances that they exude, as they prevent their movement on the leaves, thus limiting their access to the food (Valverde et al. 2001; Kennedy 2003; Simmons et al. 2003; Handley et al. 2005). Indeed, it has been shown in some previous studies that the type VI and V trichomes present on tomato leaves are involved in the resistance of *Lycopersicon hirsutum* var. *hirsutum* Dunal (Solanales: Solanaceae) to *T. urticae*, and of *L. pennelli* Corr to *T. evansi* (Luckwill 1943; Snyder and Carter 1985; Weston et al. 1989; Maluf et al. 2001; Resende et al. 2002, 2008; Simmons and Gurr 2005; Gonçalves et al. 2006).

Since *T. evansi* had acquired the status of a devastating tomato pest worldwide, it can clearly be deduced that this mite species had succeeded in breaking those chemical and/or

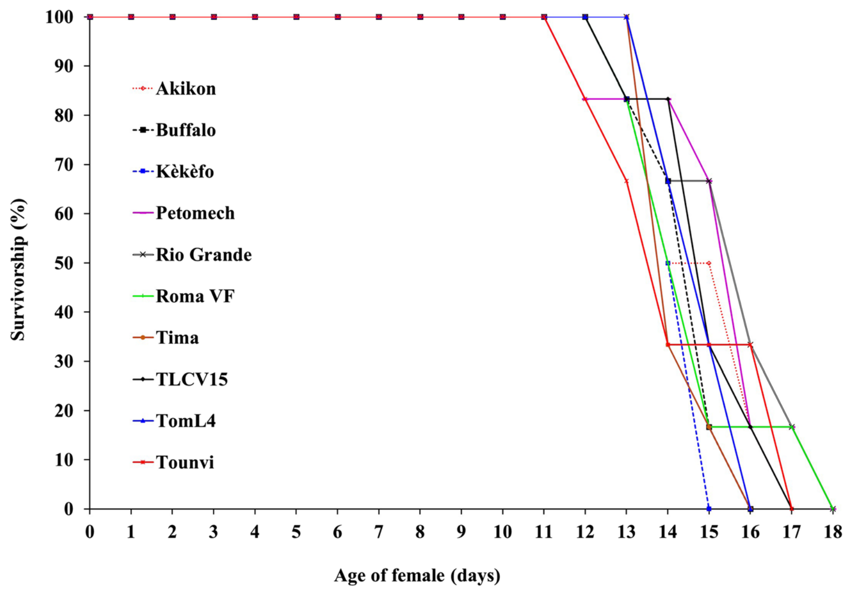

Fig. 1 Age-specific survival of female adults *T. evansi* on 10 tomato cultivars. Day 0 is the day on which the females became adults

physical barriers to feed and to develop on tomato plants. However, and interestingly, our results suggest that some of the cultivars tested were able to defend themselves against this mite pest, certainly by relying on the presence of such glandular trichomes on their leaves or stems, or on other biochemical properties that are specific to them. Whereas our study did not aim at identifying the types and characteristics of the trichomes on the 10 tomato cultivars tested, their presence on the leaves may be played a role in preventing the mite from feeding and establishing colonies on some of them.

Contrasting with the other parameters measured, the sex-ratio of the offspring was higher than 50% (in favor of females) for almost all the cultivars except for Buffalo on which this parameter could not be calculated since no *T. evansi* larvae reached the nymphal stage. The 100% female observed on Tounvi was due to the fact that the only one individual that reached the deutonymphal stage on this cultivar was a female. In that respect, the lack of significant among-cultivar differences for this parameter should be taken with caution. However, the sex-ratio obtained in our study for the 10 cultivars pooled together was very close to the 75% females vs. 25% males that generally characterizes mite species of the family Tetranychidae (Helle and Sabelis 1985). As our results were obtained in laboratory conditions, they could not, therefore, be extrapolated directly into natural habitats, since several biotic (eg. predators, entomopathogens), as well as abiotic factors (temperature, humidity and food quality) that occur in the fields could well affect the sex-ratio of the herbivore

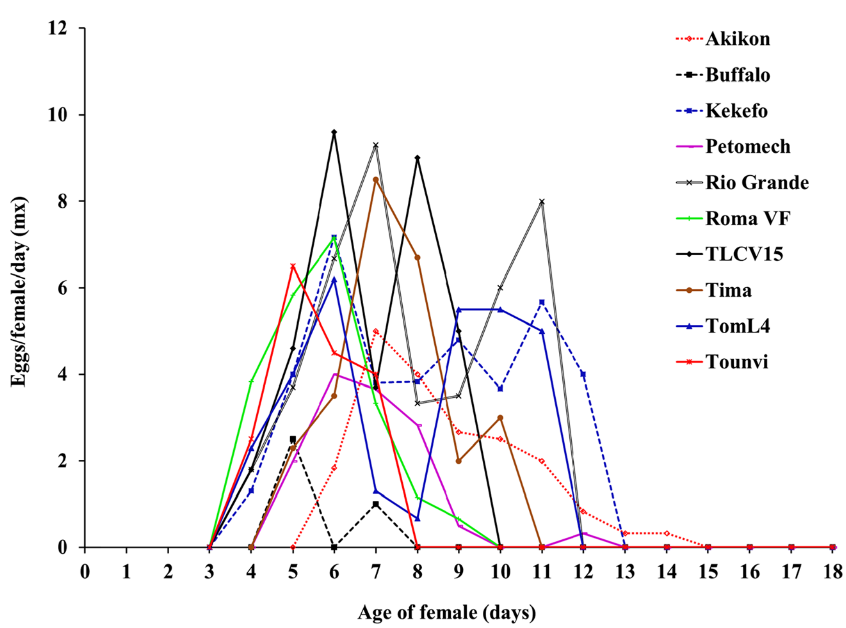

Fig. 2 Fecundity of *T. evansi* on 10 tomato cultivars. Day 0 is the day on which the females became adult

Table 3 Number of eggs/female/day, #eggs/female, hatching rate, population size per developmental stage, and sex ratio of *T. evansi* reared on leaf disks of 10 tomato cultivars at 27 ± 1 °C 65–70% RH, and 12:12 h (L:D) photoperiod

Tomato cultivars	Egg /♀/day	Egg/female	Hatching rate(%)	Larva	Protonymph	Deutonymph	Adult	Sex-ratio (% female)
Akikon	4.06 ± 0.60a	19.50 ± 4.89a	56.30 ± 6.23a (61)	10.17 ± 1,99ab	5.67 ± 2.01ab	4.83 ± 1.45abc	4.00 ± 1.44ab	77.00 ± 9.04a (19)
Buffalo	0.94 ± 0.06b	2.67 ± 0.42b	44.44 ± 20.48a (7)	1.17 ± 0.54c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b	–
Kèkèfo	4.88 ± 0.78a	29.33 ± 4.20a	76.82 ± 4.13a (122)	24.67 ± 4.17a	10.33 ± 4.93a	8.00 ± 3.12a	4.50 ± 2.73ab	83.34 ± 10.54a (35)
Petomech	3.35 ± 0.99a	13.33 ± 4.29ab	59.91 ± 3.24a (48)	8.00 ± 2.52ab	5.50 ± 2.38ab	5.17 ± 2.18abc	4.00 ± 1.71ab	81.25 ± 6.57a (25)
Rio Grande	4.72 ± 1.30a	23.50 ± 8.10a	67.39 ± 16.57a (107)	17.83 ± 7.28ab	11.00 ± 4.23a	7.17 ± 2.36ab	4.50 ± 1.61ab	93.65 ± 3.72a (33)
Roma VF	4.72 ± 1.14a	22.00 ± 4.33a	63.37 ± 9.18a (82)	13.67 ± 3.74ab	7.16 ± 2.35a	3.33 ± 1.12abc	2.50 ± 0.89ab	86.29 ± 8.59a (15)
Tima	4.68 ± 0.62a	19.17 ± 3.06a	84.02 ± 6.32a (113)	18.83 ± 3.50ab	15.83 ± 2.54a	9.67 ± 3.69a	6.67 ± 2.36a	80.83 ± 6.40a (50)
TLCV15	4.40 ± 0.97a	17.83 ± 5.42a	76.37 ± 9.08a (102)	17.00 ± 5.59ab	11.50 ± 3.69a	6.33 ± 2.81abc	1.17 ± 0.65ab	75.78 ± 9.51a (26)
TomL4	3.38 ± 0.56a	16.50 ± 4.79a	73.19 ± 8.97a (70)	11.67 ± 3.35ab	8.00 ± 3.44ab	5.17 ± 2.75abc	3.50 ± 2.39ab	89.29 ± 10.72a (26)
Tounvi	2.55 ± 0.66a	11.17 ± 3.80ab	54.25 ± 15.24a (37)	6.17 ± 2.95bc	0.67 ± 0.49bc	0.17 ± 0.17bc	0.17 ± 0.17b	100.00 ± 0.00a (1)
F	**3.39**	**3.05**	**1.14**	**4.21**	**4.78**	**3.30**	**2.66**	**0.44**
P	**0.0025**	**0.0055**	**0.3541**	**0.0004**	**0.0001**	**0.0031**	**0.0132**	**0.8884**

Means followed by the same letters within columns are not significantly different ($P > 0.05$; Student-Newman-Keuls, multi-range test)

Numbers in brackets represent the population size

populations. It appears from the present study that among the 10 tomato cultivars tested, the most susceptible to *T. evansi* were Kékèfo, Tima and Akikon; the moderately susceptible ones were Rio Grande, Petomech, Roma, TomL4 and TLCV15; whereas the less susceptible ones were Buffalo and Tounvi. These last two cultivars could, therefore, be considered tolerant/resistant to *T. evansi* attacks. However, complementary/additional studies on the population dynamics of *T. evansi* on the 10 tomato cultivars in greenhouse and fields, as well as the establishment of life table characteristics of the mite on those cultivars, followed by the determination of the chemical compound contained in their leaves are required for a stronger conclusion.

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Compliance with ethical standards

Conflict of interest The author declare that they have no conflict of interest.

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