pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2025, 45 (1), 63-74

Research Article

Resistance of promising genotypes of Chitti bean to two-spotted spider mite *Tetranychus urticae* Koch in greenhouse conditions

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Abstract. *Tetranychus urticae* Koch (Acari: Tetranychidae), two-spotted spider mite (TSSM), is one of the most significant pests infesting beans and a large amount of insecticides are used annually against it. The present research was conducted to identify resistance levels of chitti bean genotypes to ward *T. urticae*. For the experiments, Type 1 bean genotypes (standing) (KS-21216, KS-21181, KS-21538, KS-21565, KS-21563, KS-21602, KS-21500, KS-21601, KS-21600, and KS-21607), Type 2 genotypes (half standing) (KS-21573, KS-21597, and KS-21606), and Type 3 genotypes (prostrate) (KS-21255 and KS-21574), along with the susceptible variety (Sadri), were cultivated in the field at the Khomein National Bean Research Station in a randomized complete block design with three replications during two successive years (2022 and 2023). To examine the population (egg, nymph, larval and adult stages), ten plants were randomly selected from each plot, and from each plant, two heaves from the top, two from the middle, and two from the bottom of the canopy, were selected. The sampling program was conducted weekly from the third leaf stage until the end of the season. The resistance mechanism (not from the biochemical aspects) was determined (tolerance, antixenosis and antibiosis) under greenhouse conditions on the same varieties. The results of population changes revealed the highest number of eggs, nymphs, larvae, and adults of *T. urticae* were observed in the Sadri variety and genotype KS-21607. In contrast, the lowest was ascertained in genotypes KS-21216, and KS-21184. According to greenhouse results Sadri variety and genotype KS-21607 were classified as susceptible to TSSM with damage scales of 5.90±0.10 and 4.80±0.12 respectively. Genotypes KS-21538, KS-21216, KS-21184, and KS-21255 exhibited desirable antibiosis-type resistance mechanisms. But, the Sadri variety and genotypes KS-21607, KS-21573 and KS-21597, supported a high reproductive and growth fitness of *T. urticae*. The highest resistance indices were observed in genotypes KS-21538 (36.73) and KS-21216 (21.26). Hence, genotypes KS-21538 and KS-21216 are suggested for cultivation in the region because of their higher resistance index than other genotypes. If they also demonstrate superior performance and lower damage scale in future field studies, they would be ideal choices.

Keywords: Pest management, Plant resistance, Resistance mechanism, Tolerance, Antixenosis, Antibiosis

Article history
Received: 13 June 2024
Accepted: 22 October 2024
Published: 01 January 2025

Subject Editor: Azadeh Farazmand

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DOI: https://doi.org/10.61186/jesi.45.1.5

Introduction

In Iran, a significant portion of the provinces, including Fars, Lorestan, Markazi, Chaharmahal and Bakhtiari, Zanjan, and East Azerbaijan, cultivate beans every year. According to statistics published by the Ministry of Agriculture, the area under bean cultivation in Iran was 91,798. In Markazi province, the area under bean cultivation is 13,401 hectares, with an average yield of 2,786 kg per ha, making it one of the major areas for bean cultivation in the country (Ahmadi *et al*., 2022).

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests of beans that large amount of acaricides are used to control it every year. The feeding of two-spotted spider mite, *T. urticae* Koch on leaf sap reduces photosynthesis and, increases transpiration in damaged leaves, ultimately leading to a reduction in the number of viable seedlings, the size of the bean pods, and a decline in the quality and nutritional value of the product (Saeidi & Arbabi, 2014). Symptoms of this mite on bean leaves consist of the formation of yellow spots on the leaf surface (Dorri *et al*., 2015). Chemical pesticides, aside from the numerous problems they pose for human health and the environment, do not effectively solve this problem (Abolfathi *et al*., 2018). The use of resistant varieties is one of the most effective and cost-effective methods of pest control in integrated pest management programs (Pedigo, 2002). Saeidi (2020) examined the tolerance of 55 pinto bean lines to *T. urticae* infestation. Their results showed that lines 'D521', 'J633', 'D524' and 'D532' were highly susceptible and susceptible, while, lines 'L31', 'L329', 'L321', 'L16', 'B417', 'B425', 'L328', 'J29', 'J67', 'L19', 'D3', 'L25' and 'L1' were resistant to two-spotted spider mite. In a study conducted at the International Center for Tropical Agriculture (CIAT) on the *Tetranychus desertorum* Banks mite, 1500 genotypes were screened for resistance to the mite, and three resistant genotypes (BAT417, BAT82, and BAT93) were identified under field conditions. The resistance mechanism was identified as being of the type "antibiosis" in one of these genotypes, BAT 93. The antibiosis mechanism manifests as a reduction in the number of laid eggs, a decline in the size of adult mites, and a shorter life cycle (Flexner *et al*., 1995). Examination of the population density of the two-spotted spider mite on four different bean varieties (including Talash, Sadaf, Goli, and Parastou) in Tehran revealed that the highest and lowest population densities were observed on the Talash and Parastou varieties, respectively. This study indicated that the host type can influence the biological parameters of the two-spotted spider mite (Ahmadi *et al*., 2006).

Plants with an antibiosis mechanism may cause a reduction in the survival, size, weight, lifespan, and reproduction of adult pests and indirectly prolong the exposure period of the pest to natural enemies (Dent, 2000; Sarfraz *et al*., 2006). Resistant cultivars not only reduce pest damage but also ensure production stability (Saeidi & Mallik, 2006). Every year, a relatively large amount of acaricide is used against this pest, but currently, the result of using these pesticides is not very satisfactory despite the high consumption and significant costs. In other words, apart from the many problems they cause in the field of human health and the environment, chemical acaricides cannot solve this problem, For this reason, the investigation of various factors affecting the pest population can reduce the consumption of these compounds and prevent farmers from spending enormous costs (Abolfathi *et al*., 2011). The use of resistant cultivars is one of the most effective and cost-effective methods of pest control in the integrated pest management program (Pedigo, 2002). Tolerant cultivars or genotypes are those that exhibit (more performance and lower damage scale and population, higher resistance index) in field and greenhouse conditions. With this background, in the present study, we aimed to identify resistant genotypes of beans introduced in the collection of the Khomein Bean Research Station against the two-spotted spider mite through the examination of resistance mechanisms and the determination of plant resistance indices, ultimately aiming to reduce the adverse effects of pesticide use and to produce resistant varieties and thus produce beans in sustainable agriculture.

Materials and methods

1-Experiment under field conditions

The bean genotypes of Type 1 (standing) (KS-21216, KS-21181, KS-21538, KS-21565, KS-21563, KS-21602, KS-21500, KS-21601, KS-21600, and KS-21607), type 2 genotypes (half standing) (KS-21573, KS-21597, and KS-21606), and type 3 genotypes (prostrate) (KS-21255 and KS-21574) along with the susceptible treatment (Sadri) were planted in a field located at the Khomein national bean research station (This station is located at altitude of 1930 meters above sea level, with a longitude of 49 degrees and 57 minutes and latitude of 33 degrees and 39 minutes) using a complete randomized block design with three replications during two successive years (2022 and 2023). Each plot was composed of four rows of three meters. The planting was done using irrigation and ridges, with a distance of 50 cm between the ridges and a distance of 10 cm between the seeds on the ridges. For sampling TSSM populations, ten plants were randomly selected from each plot, and from each of them, two leaves from the top, two from the middle, and two from the bottom of the canopy, were selected and then the number of eggs, nymph, larva, and adult, were counted and recorded. Sampling was conducted weekly from the beginning of infestation until harvest. Sampling was carried out uniformly. The samples were individually labeled in nylon bags and promptly transported to the laboratory on ice. Sampling was performed 11 times during the season. To immobilize the active stages of the mite and ensure accurate counting, the collected leaves were kept in a refrigerator at 4°C for 1 hour. Then the number of different stages of the mite were counted within a 2×2 cm leaf area (A 2 ×2 cm cardboard frame was placed on the back and middle of the leaf in the place where the mites were concentrated).

2-Experiment under greenhouse conditions

Mite colony

The experiment was conducted in a research greenhouse under the following conditions: a temperature of 25±5 °C, relative humidity of 65%±15, and a photoperiod of L16: D8 hours. To establish a colony of the two-spotted spider mite, the bean-susceptible (Sadri) (Beyzaei & Dorri, 2011) was used. The colony was inspected almost daily, and every two weeks, new plants were cultivated to feed the mites, while the dried plants were removed from the greenhouse. The colonies obtained from three generations were used for the experiments. To synchronize the eggs, female mites were selected from the colony and placed on healthy plants (Plants were covered by insect-proof nets to avoid any contamination). After 24 hours, the female mites were removed and only the laid eggs remained for the experiment.

Tolerance mechanism

The pots with a diameter of 30 cm, filled with a mixture of regular soil, sandy soil, and leaf mold, were initially used for the experiments. The desired genotypes, along with the sensitive susceptible genotypes (as control) were used, separately cultivated in these pots, (five pots for each genotype and each pot containing one plant). The completely randomized experimental design was used with five replicates. Plants were covered by insect-proof nets to avoid any infestation. Infestation of the genotypes was performed when the plants were at the two-leaf stage by introducing 10 eve-aged adult females (3 days old) from the colony from the stock colony. A leaf damage scale was used to assess the susceptibility of the bean plants to the Two-spotted spider mite (Saeidi, 2020). After two weeks, six leaves of each plant were selected randomly and scored based on the intensity of damage (from 0, without damage to 6, feeding patches more than 80% of leaf area). The assessment scale was based on the level of chlorotic spots on the leaf surface and, includes: 1- No damage, 2- Less than 5% damage, 3- Damage between 5-25%, 4- Damage between 26-45%, 5- Damage between 46-65%, and 6- More than 65% damage (Smith, 2005; Yousefi & Dorri, 2012). The genotypes that had damage levels higher than 4.6 to 6 were categorized as susceptible, between 3.7 and less than 4.6 as semi-susceptible, between 2.5 and less than 3.7 as semi-tolerant, and less than 2.5 as tolerant (Saeidi, 2020).

Antixenosis mechanism

This experiment was conducted in a completely randomized design with five replications. To perform this experiment, circular culture trays covered with mesh were used. In this experiment, plants from different genotypes were placed in a circular pattern around the trays. Each circular tray was considered as one replication. In the center of each tray, facing each genotype separately, a leaf containing 8 adult female mites (3 days in age) was placed. Sampling was carried out twice with 7-day interval after infestation. The number of mites on each plant was counted and applied as a criterion for determining the resistance (Franca *et al.*, 2018).

Antibiosis mechanism

The method of cultivation and the experimental design (with five replications) were similar to the tolerance mechanism. Each pot was enclosed with cylindrical plastic cages (12 cm in diameter and 50 cm in height). Each plant, at the two-leaf stage, was infested with 10 eggs of two-spotted spider mites. Sampling was carried out 14,21 and 28 days after infestation, 3 leaves of each plant (one leaf from the top, one from the middle, and one from the bottom of the canopy) were selected randomly and the number of adult mites (the whole leaf area) was counted (Franca *et al.*, 2018).

Plant resistance index

The plant resistance index was calculated using the method developed by Webster *et al.* (1993). This index allows for the simultaneous evaluation of three resistance mechanisms (antixenosis, antibiosis, and tolerance) and provides a unified value. This approach enables quicker access to a summary of the results to aid in the selection of resistant plants. The following formula was used for this purpose.

$$\text{plant resistance index (PRI)} = 1/(az+0.5)\ (ab+0.5)\ (tol+0.5)$$

Where az is the antixenosis index, ab is the antibiosis index, and (tol) shows the tolerance index. Since some of these indices may be zero for certain genotypes, the PRI value can become infinite. Thus, to address this issue, before calculating the resistance index, 0.5 units should be added to all mechanisms for all genotypes. To standardize the data, for each trait, the largest data point was identified separately, and then all data points were divided by it, ensuring that all data points become less than or equal to one.

Statistical analysis

After collecting all the data and inputting them into Excel software (2016), analysis of variance was performed using SAS (version 9.1) and SPSS (version 21) software, and the mean comparisons were done using Tukey's test at the 1% and 5% levels. In field condition (complete randomized block design), SPSS (version 21), a repeated measure of Anova for two years (2022 and 2023), and in greenhouse condition (completely randomized experimental design), SAS (version 9.1) Proc ANOVA, was conducted. Kolmogorov-Smirnov test was used to determine the normality of the data. To normalize the data obtained from the experiments, they were converted to the Log_{10} (x+1).

Results

Changes in the population of different stages of the two-spotted spider mite in the years 2022 and 2023

The results of the analysis of variance (complete randomized block design, repeated measure of ANOVA for two years (2022 and 2023) revealed no significant difference between the two experimental year (egg ($F_{(1, 160)}$ =0.11, P=0.73)), nymph ($F_{(1, 160)}$ =0.26, P=0.61)), larvae ($F_{(1, 160)}$ =0.19, P=0.66)) and adult ($F_{(1, 160)}$ =2.77, P=0.09)). But the interaction effect between year and treatment was found to be statistically significant in adult stage at 0.05 level ($F_{(15, 160)}$ =2.060, P=0.014)), (egg ($F_{(15, 160)}$ =0.72, P=1.00)), nymph ($F_{(15, 160)}$ =0.13, P=1.00)) and larvae ($F_{(15, 160)}$ =0.11, P=1.00)). Additionally, the treatment effect was also significant at the 1% level ($F_{(15, 160)}$ =5.359, P<0.001)) (Table 1).

In the first year (2022), two distinct peaks in population were observed on 18 August and 1 September, which were almost similar for each developmental stage. A slight peak for nymph, larvae, and egg was also observed on 4 August. In the second year (2023), a distinct peak on 9 August was observed for all stages. A second distinct peak was also found for the nymph, larvae, and egg on 30 August (Fig 1 and 2). In the first and second years, the highest numbers of egg, nymph, and adult stages were observed in the genotype KS-21607 and variety Sadri, and the lowest number in genotypes KS-21216 and KS-21184 (Tables 1 and 2).

Tolerance mechanism

The results of the analysis of variance showed a significant difference among treatments at the 1% level (Table 3). The genotypes with damage levels higher than 4.6 to 6 were classified as susceptible, those with damage levels between 3.7 and less than 4.6 were classified as semi-susceptible, those with damage levels from 2.5 to less than 3.7 were categorized as semi-tolerant, and those with damage levels less than 2.5 were classified as tolerant. Therefore, the variety Sadri and the genotype KS-21607 were classified as susceptible to damage from *T. urticae*. The genotypes KS-21573, KS-21597, KS-21574, KS-21600, KS-21601, KS-21500, and KS-21565 were considered semi-susceptible, whereas the genotypes KS-21216, KS-21184, KS-21538, KS-21563, KS-21602, KS-21255, and KS- 21606 were classified as semi-tolerant (Table 5).

Antixenosis mechanism

The results of the analysis of variance demonstrated a significant difference in means among treatments on 7 and 14 days after infestation at the 1% level (Table 3). The comparison of means revealed that the genotypes KS-21538, KS-21216, and KS-21184 exhibited desirable antixenosis properties, with 3.40, 5.00, and 5.10 adult mites respectively. This resulted in a lower attraction of mites to these genotypes (Table 5).

Antibiosis mechanism

The results of the analysis of variance at the 1% level indicated a significant difference among treatments (Table 4). The mean comparisons resulting from this mechanism showed that genotypes KS-21538, KS-21216, KS-21184, and KS-21255 exhibited desirable antibiosis-type resistance mechanisms, each with mean (14,21 and 28 days after infestation) number of 11.20±0.13, 14.93±0.19, 16.33±0.23, and 17.07±0.19 adult mites, respectively. But, the Sadri variety and genotypes KS-21607, KS-21573, and KS-21597 with a mean (14,21 and 28 days after infestation) numbers of 62.73±0.29, 43.53±0.23, 41.60±0.12 and 40.27±0.12 adult mite, respectively, were suitable for the growth of this pest. The high number of mites in these genotypes was considered as a negative trait (Table 6).

Plant resistance index

The results of the resistance index calculation revealed that the KS-21538 and KS-21216 showed high resistance indices of 36.73 and 21.26 respectively, and were classified as resistant genotypes (or less susceptible) to damage and population of *T. urticae*.

Table 1. Repeated measures of one-way ANOVA in *Tetranychus urticae* for two years (2022 and 2023)

Source of variation	df	Mean square	F	P value
Year	1	57.284	2.767	0.098
Year × Treatment	15	42.636	2.060	0.014
Error (Year)	60	20.701		
Treatment	15	681.120	5.359	P<0.001
Error	60	127.108		

Table 2. Mean comparison of Compound analysis of different stages of *Tetranychus urticae* on the studied genotypes in years (2022 and 2023)

Row	(Genotypes and cultivar)	(Eggs)	(Nymphs)	(Larvae)	(Adults)
1	KS-21216	23.77^{f}±8.44	6.04^{g}±2.85	3.36^{h}±1.56	1.68^{f}±0.66
2	KS-21184	27.00^{fg}±9.10	6.95^{g}±3.08	3.77^{h}±1.61	2.18^{f}±0.74
3	KS-21538	39.27^{ef}±12.49	12.77^{efg}±4.91	6.82^{fgh}±2.59	4.04^{f}±1.38
4	KS-21565	44.91^{ef}±14.89	17.50^{defg}±6.47	9.27^{efgh}±3.36	4.41^{f}±1.54
5	KS-21563	35.86^{ef}±11.14	11.64^{efg}±4.46	6.09^{fgh}±2.29	4.04^{f}±1.35
6	KS-21602	31.64^{ef}±10.10	9.14^{fg}±3.47	5.01^{gh}±1.98	3.23^{f}±1.11
7	KS-21500	55.50^{de}±17.18	23.68^{cde}±7.96	12.64^{cdef}±4.16	6.54^{def}±2.04
8	KS-21601	51.59^{de}±16.52	21.68^{cdef}±7.61	11.32^{defg}±3.90	5.50^{ef}±1.76
9	KS-21600	43.27^{ef}±14.77	17.77^{defg}±6.65	9.23^{efgh}±3.32	4.36^{f}±1.39
10	KS-21607	107.04^{ab}±29.46	45.45^{ab}±14.89	22.91^{b}±7.40	15.50^{b}±4.42
11	KS-21573	88.00^{bc}±25.95	35.14^{bc}±12.22	18.41^{bc}±6.17	12.18^{bc}±3.80
12	KS-21597	82.09^{c}±24.55	32.5^{bc}±10.03	16.77^{bcd}±5.05	10.45^{cd}±3.21
13	KS-21255	29.68^{ef}±9.62	8.00^{fg}±3.43	4.27^{gh}±1.75	2.54^{f}±0.88
14	KS-21574	74.68^{cd}±22.53	29.59^{cd}±10.59	15.50^{cde}±5.39	9.41^{cde}±2.92
15	KS-21606	31.95^{ef}±9.84	9.45^{efg}±3.82	5.09^{gh}±1.98	3.41^{f}±1.15
16	Sadri	129.82^{a}±35.42	52.14^{a}±15.16	27.18^{a}±7.78	21.68^{a}±5.8

* The number obtained for each column is equal mean. means in columns followed by different letters are significantly different.

Table 3. Analysis of variance of resistance mechanisms (tolerance and antixenosis) of different bean genotypes to *Tetranychus urticae* Koch., in greenhouse conditions in the year 2023

		Mean of squares			
S.O.V	df	Tolerance (14 days)	Antixenosis (7 days)	Antixenosis (14 days)	Mean
Treatment	15	2.19**	**10.90	**60.13	26.89**
Error	64	0.08	0.23	0.37	0.16
Total	79	-	-	-	-
CV	-	7.40	8.64	8.18	6.1

ns : Non-significant; * and **: Significant at 5% and 1% probability levels, respectively

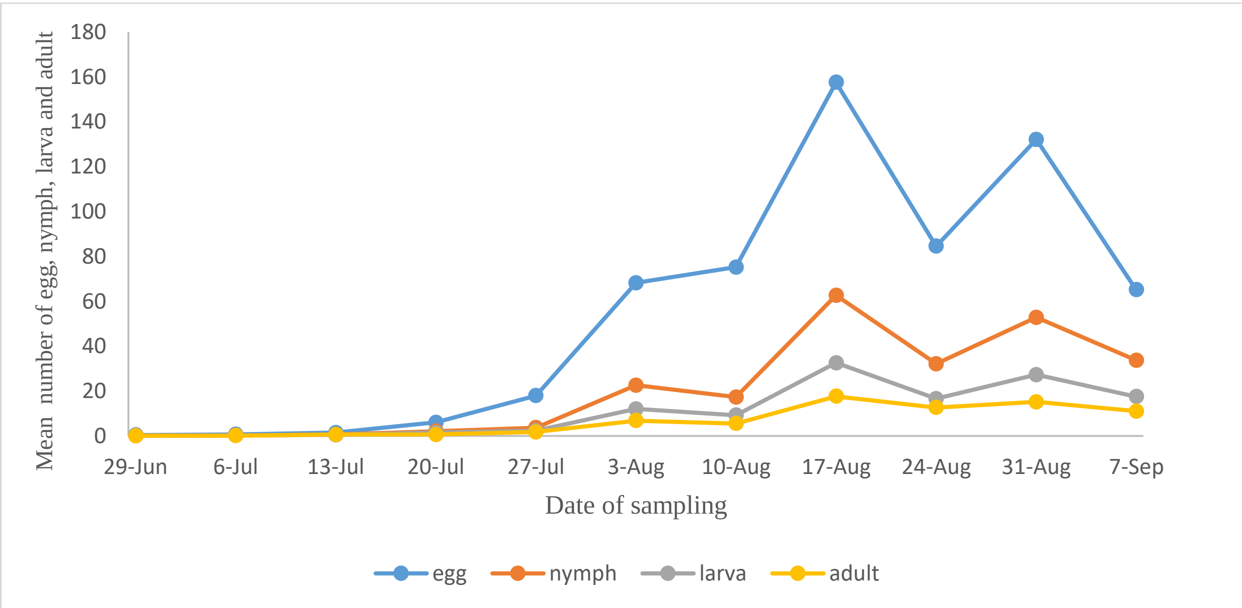

Fig. 1. Population dynamics of different stages of *Tetranychus urticae* in the year 2022

Table 4. Analysis of variance of resistance mechanisms (antibiosis) of different bean genotypes to *Tetranychus urticae* Koch., in greenhouse conditions in the year 2023

		Mean of squares			
S.O.V	df	Antibiosis (14 days)	Antibiosis (21 days)	Antibiosis (28 days)	Mean
Treatment	15	**10.799	**714.71	4127.94**	978.58**
Error	64	0.44	0.69	0.70	0.22
Total	79	-	-	-	-
CV	-	6.58	3.06	1.88	1.73

Table 5. Comparison of mean resistance mechanisms (tolerance and antixenosis) of different bean genotypes to *Tetranychus urticae* in greenhouse conditions in the year 2023

Row	Genotype and cultivar	Tolerance (14 days)	Antixenosis (7 days)	Antixenosis (14 days)	Mean of Antixenosis
1	KS-21216	$3.30^{g}\pm0.12$	$5.80^{b}\pm0.20$	$4.20^{h}\pm0.20$	$5.00^{i}\pm0.16$
2	KS-21184	$3.40^{fg}\pm0.10$	$5.60^{bcd}\pm0.24$	$4.60^{fg}\pm0.24$	$5.10^{hi}\pm0.10$
3	KS-21538	$3.70^{efg}\pm0.12$	$3.60^{f}\pm0.24$	$3.20^{h}\pm0.20$	$3.40^{j}\pm0.19$
4	KS-21565	$3.80^{defg}\pm0.20$	$4.40^{ef}\pm0.23$	$7.40^{d}\pm0.25$	$5.90^{efgh}\pm0.10$
5	KS-21563	$3.66^{efg}\pm0.14$	$5.64^{bcd}\pm0.38$	$6.00^{e}\pm0.10$	$5.82^{efghi}\pm0.19$
6	KS-21602	$3.56^{efg}\pm0.11$	$6.02^{b}\pm0.02$	$5.00^{efg}\pm0.10$	$5.51^{fghi}\pm0.01$
7	KS-21500	$4.00^{cdef}\pm0.13$	$4.64^{cdef}\pm0.20$	$8.00^{d}\pm0.32$	$6.32^{def}\pm0.23$
8	KS-21601	$3.90^{defg}\pm0.10$	$4.60^{def}\pm0.24$	$7.80^{d}\pm0.37$	$6.20^{defg}\pm0.25$
9	KS-21600	$3.74^{defg}\pm0.11$	$4.44^{ef}\pm0.23$	$7.60^{d}\pm0.24$	$6.02^{defg}\pm0.14$
10	KS-21607	$4.80^{b}\pm0.12$	$5.86^{b}\pm0.17$	$10.00^{b}\pm0.45$	$7.93^{b}\pm0.27$
11	KS-21573	$4.60^{c}\pm0.19$	$5.70^{c}\pm0.18$	$9.60^{bc}\pm0.24$	$7.65^{bc}\pm0.16$
12	KS-21597	$4.40^{bcd}\pm0.11$	$5.20^{bcde}\pm0.20$	$8.60^{cd}\pm0.25$	$6.90^{cd}\pm0.19$
13	KS-21255	$3.50^{fg}\pm0.16$	$6.00^{b}\pm0.10$	$4.80^{efg}\pm0.20$	$5.40^{ghi}\pm0.10$
14	KS-21574	$4.20^{bcde}\pm0.12$	$5.00^{bcde}\pm0.10$	$8.40^{cd}\pm0.24$	$6.70^{de}\pm0.12$
15	KS-21606	$3.60^{efg}\pm0.19$	$5.80^{b}\pm0.20$	$5.60^{ef}\pm0.23$	$5.70^{fghi}\pm0.13$
16	Sadri	$5.90^{a}\pm0.10$	$10.4^{a}\pm0.23$	$18.00^{a}\pm0.46$	$14.20^{a}\pm0.25$

* The number obtained for each column is equal mean. means in columns followed by different letters are significantly different.

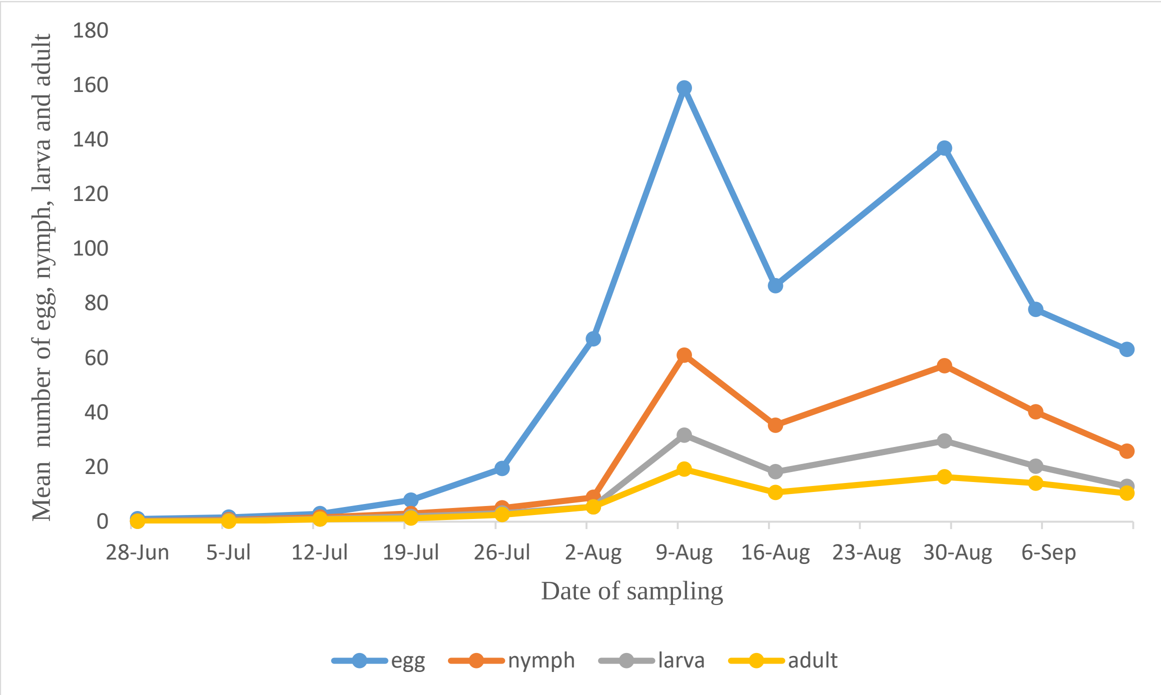

Fig. 2. Population dynamics of different stages of *Tetranychus urticae* in the year 2023

The other genotypes were placed in the group of susceptible genotypes to the pest. The resistance index was the final basis for the evaluation of the studied genotypes (Table 7).

Discussion

In the study of the population changes of this pest over two times, the population density had an initial ascending trend, which then decreased in September. During the two-time study, the peak population of various stages of the two-spotted spider mite was observed in July-August. According to the results, the highest numbers of egg, nymph, and adult stages of *Tetranychus urticae* were observed in the Sadri cultivar and the KS-21607 genotype, while the lowest was observed in the KS-21216 and KS-21184 genotypes. The population density had an upward trend in the beginning, which decreased after September. Probably, factors such as yellowing, reduction in quality and fall of leaves due to aging, as well as the decrease in ambient temperature at the end of the season, played an effective role in reducing the reproduction and population of the two-spotted spider mite (Mohiseni *et al*., 2016). Differences in the population density of the two-spotted spider mites in the studied genotypes and varieties may be related to morphological characteristics, chemical substances, nutritional quality, and inhibitory substances (Roozbahani *et al*., 2016). A study on the population density of the two-spotted spider mite on four different varieties of beans (Talash, Sadaf, Goli, and Parastoo) demonstrated that the highest and lowest population densities were observed on the Talash and Parastoo varieties, respectively. This study revealed that the host type can influence the biological parameters of the two-spotted spider mite (Ahmadi *et al*., 2006). In another study by Ashtari *et al*. (2020) showed that in the population dynamic of *T. urticae*, in the first year, two distinct population peaks were observed on 30 Jul 2017 and 13 Aug 2017, which was almost similar for different stages. In the second year, on 14 Aug 2018 and 28 Aug 2018, a clear and slight peak was observed in all age stages of *T. urticae*. In the first and second years, the highest numbers of eggs, nymphs, larvae, and adults were observed in the Sadri variety, and the lowest in the KS-21517 genotype (Ashtari *et al*., 2020).

Regarding the trend of population changes, the results of this study are somewhat similar to the present study. The results obtained regarding Sadri variety are similar to the present study. The results of population changes under greenhouse conditions revealed that the highest number of eggs, nymphs, and adult mites were observed in Kara Casehir, while the lowest number of eggs was observed in D81083 and G-11867. The genotypes examined in this study do not have similarities with the current research. Plant resistance to pests is based on inherited traits and is generally divided into three distinct categories: antixenosis, antibiosis, and tolerance (Kant *et al*., 2015; Smith, 2005). The low quality of leaves and the absence of sufficient desirable stimuli on the leaves can lead to antixenosis resistance (Seki, 2016). The results demonstrated that the genotypes KS-31286 and KS-21492 as well as the Dadfar variety were selected as tolerant genotypes to the damage of the two-spotted spider mite, with damage scales of 3.6, 4, and 3.8, respectively. The results of this research are not consistent with the results of the current study due to the difference in genotypes. Saeidi & Salehi (2005) investigated the resistance of seven genotypes of native kidney beans to the two-spotted spider mite. They concluded that genotype number one was resistant to the two-spotted spider mite, and its resistance mechanism was of the antibiosis type. Additionally, this genotype was introduced for mass production and identification of resistant genes, and for transfer to high-yielding and high-quality varieties. The results of this project do not have similarities with the current study due to differences in the genotypes under study. In a study conducted at the International Center for Tropical Agriculture (CIAT) on the *Tetranychus desertorum* mite, 1500 genotypes were screened for mite resistance, where three resistant genotypes were identified under field conditions. The genotypes BAT417, BAT82, and BAT93 showed resistance to the mite. The resistance mechanism was identified as antibiosis in one of these genotypes, BAT93. The genotypes examined in this study do not have similarities with the present study. By examining the resistance of several genotypes and varieties of beans to the two-spotted spider mite, the red bean variety Dadfar and the white bean genotype KS-41247 exhibited higher tolerance with resistance indices of 12.5 and 14.28, respectively, compared to other varieties and genotypes (Ashtari *et al*., 2020).

The genotypes examined in this study do not have similarities with the present study. Mohammadi *et al*. (2012) conducted a research project on the mechanisms of resistance of different bean genotypes to the two-spotted spider mite under greenhouse conditions. The varieties Sadaf, Dehghan, and genotype D10 exhibited antixenosis resistance. In this study, the Dehghan variety and genotype D81083 have antixenosis mechanisms. Yousefi & Dorri (2012) examined the response of several bean genotypes to the two-spotted spider mite. In this study, the genotypes KS-21364, KS-21484, KS-21452, and KS-21461 were selected as tolerant genotypes to the damage of the two-spotted spider mite. The genotypes KS-21342, KS-21485, KS-21364, and KS-21191 had antibiosis mechanisms. The genotypes KS-21461, KS-21375, KS- 21484, KS-21193, and KS-21191 had antixenosis mechanisms. Genotype KS-21191 was selected as the most resistant genotype due to its high plant resistance index of 75.6. Considering the differences in the studied genotypes between this and the two recent studies, the results obtained do not concur with each other. In a research project, Ashtari *et al.* (2020) investigated the resistance mechanism of several genotypes to the two-spotted spider mite. The Dadfar variety and the KS-31286 genotype exhibited antibiosis resistance mechanisms. The genotype KS-31286 and the Dadfar variety exhibited antixenosis mechanisms. The results of the plant resistance index calculation conducted in the greenhouse revealed that the Dadfar variety has high resistance index of 14.28 (Ashtari *et al*., 2020). Considering the differences in the studied genotypes between this and the recent study, the obtained results do not accord with each other. In this research, the Sadri cultivar was selected as the control due to its susceptibility to the two-spotted spider mite in cultivar introduction projects (Salehi, 2015; Beyzaei & Dorri, 2011). Furthermore, in a study conducted by Ashtari *et al.* (2020) to assess the tolerance of bean genotypes, Sadri cultivar showed the highest population and damage scale.

Table 6. Comparison of mean resistance mechanisms (antibiosis) to *Tetranychus urticae* in greenhouse conditions in the year 2023

Row	Genotype and cultivar	Antibiosis (14 days)	Antibiosis (21 days)	Antibiosis (28 days)	Mean of Antibiosis
1	KS-21216	8.20^{fg}±0.20	15.80^{h}±0.37	20.80^{k}±0.37	14.93^{n}±0.19
2	KS-21184	8.60^{efg}±0.24	18.40^{g}±0.51	22.00^{k}±0.32	16.33^{m}±0.23
3	KS-21538	8.00^{g}±0.32	10.20^{i}±0.37	15.40^{l}±0.40	11.20^{o}±0.13
4	KS-21565	9.70^{cde}±0.20	23.80^{f}±0.36	31.60^{i}±0.40	21.70^{i}±0.18
5	KS-21563	9.50^{cdef}±0.22	20.20^{g}±0.20	27.60^{j}±0.41	19.10j±0.10
6	KS-21602	9.30^{defg}±0.20	19.60^{g}±0.51	24.60^{jk}±0.40	17.83^{kl}±0.17
7	KS-21500	10.40^{bcd}±0.40	29.40^{e}±0.24	41.40^{f}±0.25	27.07^{f}±0.27
8	KS-21601	10.10^{cd}±0.23	25.00^{f}±0.32	36.00^{g}±0.45	23.70^{g}±0.18
9	KS-21600	9.90^{cde}±0.10	24.20^{f}±0.37	33.62^{h}±0.40	22.57^{h}±0.18
10	KS-21607	11.80^{b}±0.21	38.80^{b}±0.37	80.00^{b}±0.32	43.53^{b}±0.23
11	KS-21573	11.60^{b}±0.24	37.80^{bc}±0.37	75.41^{c}±0.25	41.60^{c}±0.12
12	KS-21597	10.80bc±0.37	36.80^{cd}±0.35	73.20^{d}±0.31	40.27^{d}±0.12
13	KS-21255	9.20^{defg}±0.36	19.20^{g}±0.20	22.80^{jk}±0.37	17.07^{lm}±0.19
14	KS-21574	10.60^{bcd}±0.24	35.40^{d}±0.40	66.00^{e}±0.45	37.33^{e}±0.30
15	KS-21606	9.40^{cdefg}±0.51	19.80^{g}±0.20	25.40^{k}±0.40	18.20^{jk}±0.31
16	Sadri	13.80^{a}±0.37	58.60^{a}±0.51	115.80^{a}±0.37	62.73^{a}±0.29

Table 7. Normalized indexes of resistance to *Tetranychus urticae* in the studied genotypes in the year 2023

Row	Genotype and cultivar	Tolerance	Antixenosis	Antibiosis	Resistance index
1	KS-21216	0.56	0.35	0.24	21.26
2	KS-21184	0.58	0.36	0.26	18.42
3	KS-21538	0.63	0.24	0.18	36.73
4	KS-21565	0.64	0.41	0.34	11.21
5	KS-21563	0.62	0.41	0.30	13.11
6	KS-21602	0.60	0.39	0.28	15.26
7	KS-21500	0.68	0.44	0.43	7.77
8	KS-21601	0.66	0.44	0.38	9.06
9	KS-21600	0.63	0.42	0.36	10.50
10	KS-21607	0.81	0.56	0.69	3.19
11	KS-21573	0.78	0.54	0.66	3.60
12	KS-21597	0.74	0.48	0.64	4.40
13	KS-21255	0.59	0.38	0.27	16.52
14	KS-21574	0.71	0.47	0.59	5.08
15	KS-21606	0.61	0.4	0.29	14.13
16	Sadri	1	1	1	1

The two genotypes introduced in the current study displayed a higher resistance index compared to other genotypes and also had a relatively lower population across different stages of the two-spotted spider mite. By studying the mechanisms of resistance of common bean genotypes to *T. urticae* under greenhouse conditions, it was concluded that in the antixenosis experiment, the Naz cultivar had the lowest number of mites, while the genotype 405-071-65 had the highest. The Naz cultivar showed a desirable antibiosis mechanism compared to genotype 405-071-65, which had the highest number of mites. In the study of resistance index, the Naz cultivar indicated the highest resistance index, while genotypes 107-062-65 and 400-071-65 had the lowest resistance index (Mohammadi *et al*., 2020). Both antixenosis and antibiosis mechanisms were observed in the resistance of common bean cultivars to *T. urticae* (Shoorooei *et al*., 2018). In this project, both mechanisms were also observed in some of the examined genotypes (56, 63 and 238). The results of these two studies are not similar to the present research due to the difference in the studied genotypes.

In a recent study, KS-21538 and KS-21216 genotypes exhibited antibiosis resistance mechanisms with mean numbers of 11.20±0.13 and 14.93±0.19 adult mites, antixenosis mechanisms with mean numbers 3.40±0.19 and 5.00±0.16 adult mite, respectively and high resistance indices of 36.73 and 21.26, respectively. The resistance indices found in the current study are higher compared to those in this study. Considering the differences in the studied genotypes between this and the two recent studies, the obtained results do not accord with each other. The efficacy of chemical acaricides in controlling the two-spotted spider mites has been limited by the numerous health and environmental issues they pose. Therefore, examining various influential factors on the population of this pest, such as utilizing resistant cultivars, can decrease the usage of these compounds and prevent farmers from facing exorbitant costs. Incorporating resistant cultivars is among the most effective and economical strategies in integrated pest management programs. On the other hand, resistance mechanism investigation experiments under greenhouse conditions showed that the highest resistance index was found in the KS-21538 and KS-21216 genotypes. Therefore, genotypes KS-21538 and KS-21216, due to having a higher resistance index compared to other genotypes, if they also show higher performance and lower damage scale in future studies under field conditions, are recommended for cultivation in the region. Breeders should undertake more research projects to develop genotypes and cultivars with tolerance to the two-spotted spider mite. Additionally, researchers need to conduct further studies in field conditions to enhance the applicability of the results. It is also essential that if cultivars are selected from these genotypes, tests should be repeated on the cultivars.

Author's Contributions

Sedighe Ashtari: conceptualization, methodology, formal analysis, investigation, draft preparation, final review and edit, visualization, supervision, project administration and funding acquisition; **Behrouz Asadi**: investigation, final review and edit, **Hamid Reza Dorri**: final review and edit

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Funding

This study was supported by Markazi Agricultural and Natural Resources Research and Education Center 3-61-1603-022-010119.

Data Availability Statement

All data supporting the findings of this study are available within the paper.

Acknowledgments

This research was financially supported by Markazi Agricultural and Natural Resources Research and Education Center, which is greately appreciated. The authors would like to express many thanks to the anonymous referees and the editor for their corrections and comments on this manuscript.

Ethics Approval

Mites and plant were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Citation: Ashtari, S., Asadi, B. & Dorri, H. R. (2025) Resistance of Chitti bean genotypes of promising to two-spotted spider mite *Tetranychus urticae* Koch in greenhouse conditions. *J. Entomol. Soc. Iran*, 45 (1), 63–74.

DOI: https://doi.org/10.61186/jesi.45.1.5

URL: https://jesi.areeo.ac.ir/article_131275.html pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2025, 45 (1), 63-74

مقاومت ژنوتیپ‌های امید بخش لوبیا چیتی به کنه تارتن دولکه‌ای *Tetranychus urticae* Koch در شرایط گلخانه

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چکیده: کنه تارتن دو لکه ای *Tetranychus urticae* Koch (Acari: Tetranychidae) یکی از مهم ترین آفات لوبیا بوده و سالانه مقدار زیادی حشره کش برای کنترل آن استفاده می شود. پژوهش حاضر به منظور شناسایی سطوح مقاومت ژنوتیپ های لوبیا چیتی به *T. urticae* انجام شد. برای انجام آزمایش‌ها، ژنوتیپ‌های لوبیا نوع ۱ (ایستاده)KS-21216 ، KS-21181، KS-21538، KS-21565، KS-21563، KS-21602، KS-21500، KS-21601، KS-21600 وKS-21607 ، ژنوتیپ‌های نوع ۲ (نیمه رونده) KS-21573، KS-21597 و KS-21606 و ژنوتیپ‌های نوع ۳ (رونده) KS-21255 و KS-21574 همراه با رقم حساس (صدری) در مزرعه ایستگاه تحقیقات لوبیا خمین در قالب طرح بلوک های کامل تصادفی با سه تکرار طی دو سال متوالی ۱۴۰۱ و ۱۴۰۲کشت شدند. برای بررسی جمعیت (مراحل تخم، نمف، لارو و بالغ)، از هر کرت ده بوته به طور تصادفی انتخاب و از هر بوته، دو برگ از بالا، دو برگ از وسط و دو برگ از پایین گیاه انتخاب شدند. نمونه‌برداری به صورت هفتگی از مرحله‌ی سه برگی تا پایان فصل انجام شد. مکانیسم مقاومت (نه از جنبه‌های بیوشیمیایی) (تحمل، آنتی زنوز و آنتی‌بیوز) در شرایط گلخانه‌ای روی ژنوتیپ‌ها تعیین شد. نتایج تغییرات جمعیت نشان داد که بیشترین تعداد تخم، نمف، لارو و بالغ کنه تارتن دو لکه‌ای در رقم صدری و ژنوتیپ KS-21607 مشاهده شد. در مقابل، کمترین جمعیت در ژنوتیپ‌های KS-21216 و KS-21184 مشاهده شد. بر اساس نتایج گلخانه ای، رقم صدری و ژنوتیپ KS-21607 به ترتیب با مقیاس خسارت ۵/۹۰±۱۰ و ۴/۸۰±۰/۱۲ به عنوان حساس به کنه تارتن دو لکه‌ای طبقه بندی شدند. ژنوتیپ‌های KS-21538، KS-21216، KS-21184 و KS-21255 مکانیسم‌های مقاومت مطلوبی را از نوع آنتی‌بیوز نشان دادند. اما رقم صدری و ژنوتیپ‌هایKS-21607، KS-21573 و KS-21597 برای رشد و تولید مثل این آفت مطلوب بودند. بیشترین شاخص های مقاومت در ژنوتیپ های KS-21538 (۳۶/۷۳) و KS-21216 (۲۱/۲٦) مشاهده شد. از این رو ژنوتیپ های KS-21538 و KS-21216 به دلیل شاخص مقاومت بالاتر نسبت به سایر ژنوتیپ ها برای کشت در منطقه توصیه می شوند. اگر آنها عملکرد برتر و مقیاس خسارت کمتری را در مطالعات مزرعه‌ای آینده نیز نشان دهند، انتخاب های ایده‌آلی خواهند بود.

کلمات کلیدی: مدیریت آفت، مقاومت گیاه، مکانیسم مقاومت، تحمل، آنتی زنوز، آنتی بیوز

تاریخچه مقاله
دریافت: ۱٤۰۲/۰۳/۲٤
پذیرش: ۱٤۰۳/۰۸/۰۱
انتشار: ۱۴۰۳/۱۰/۱۲

دبیر تخصصی: آزاده فرازمند

نویسنده مسئول: صدیقه اشتری

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DOI: https://doi.org/10.61186/jesi.45.1.5