

Resistance to *Tetranychus urticae* Koch (Acari: Tetranychidae) in *Phaseolus vulgaris* L.

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Abstract: This research was conducted to investigate the bean resistance against *Tetranychus urticae* in Khomein region, Iran, in two parts. At first, the study was initiated by screening 458 chiti bean *Phaseolus vulgaris* germplasm for their resistance to *T. urticae* in field experiments among several planting seasons, during 2005-2008. In these experiments, foliar damage was measured according to CIAT 1-9 damage, intervals 8 and 15 days after plot infestation. The second, 15 genotypes were found to be resistant to the infestation by *T. urticae*. From these resistant genotypes, the four most resistant bean genotypes, KS21247, KS21181, KS21212 and KS21189, were chosen for investigation of their underlying resistance mechanism in greenhouse tests, during 2008-2010. To antixenosis mechanism of resistance, 200 adult mites were released in the center of five potted bean plants of four different resistant genotypes and one susceptible control genotype (KS21258). Mites were counted 24 and 48 h after releasing. The genotype KS21189 could be defined as preference, while KS21247 was not preferred by the mites. Antibiosis was studied on excised leaf discs (4cm²) of the same five bean genotypes mentioned above in 80 replicates for each genotype. Adult mites were reared on these discs and life parameters were determined. Based on the life-table comparisons, both KS21247 and KS21181 were found to be less favorable for the mite development.

Key words: *Tetranychus urticae* % *Phaseolus vulgaris* % Antixenosis % Antibiosis % Khomein region

INTRODUCTION

Bean *Phaseolus vulgaris* L. was domesticated from a wild-growing vine distributed in the highlands of tropical America [1]. It has now become one of the most important and widely grown crops in the world and commercially produced in Markazi, Lorestan, Fars and Zanjan provinces of Iran. Based on reports by the Iranian Ministry of Agriculture in 2005, overall, this crop is grown on more than 105000 ha annually in Iran [2]. Various pests have negative effects on bean production in Iran which among them the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, has been considered as a major pest in many bean-growing areas of Iran [3-7]. TSSM infests the underside of leaves, where profuse webbing may be present. The rapid developmental rate, short generation time and high net reproductive rate of *T. urticae* allows them to achieve damaging population

levels very quickly when growth conditions are good, resulting in an equally rapid decline of host plant quality. The population growth parameters of *T. urticae* such as developmental rate, survival, reproduction and longevity may vary in response to changes in temperature, host plant species, host plant nutrition, cultivar kind, phenological stage, exposure to pesticides, relative humidity, etc. [8-13].

Crop plants may use different ways to protect themselves from the damage of insect infestation. Painter [14] described three types or mechanisms of plant response, or host plant resistance (HPR): No preference (later replaced by Antixenosis), Antibiosis and Tolerance. Antixenosis refers to the plant properties that reduce colonization by pests seeking food or oviposition sites. This can be due to morphological characteristics or lacking of attractants [15].

Antibiosis is a resistance influencing biological processes of insects, like survival, growth, generation time, fecundity and longevity [15]. As in antixenosis, antibiosis involves both insect and plant factors. The quantity and quality of primary as well as secondary plant metabolites are frequently associated with antibiosis [16]. Therefore, knowledge of cultivar susceptibility or resistance might be a fundamental component of an integrated pest management program (IPM) for any crop. Such information can be used in developing an insect-resistant cultivar [17] or designing and good assays for breeding new varieties [18]. The life table parameters, including net reproductive rate (R_0), mean generation time (T), doubling time (DT), finite rate of increase (λ) and intrinsic rate of natural increase (r_m) have been used to evaluate the susceptibility or resistance of several host plants in relation to various pests [19-21]. Among these parameters, the intrinsic rate of natural increase represents one measure commonly used to evaluate the level of plant resistance to insects [22-23]. Unlike antixenosis and antibiosis, tolerance involves plant responses only. If a crop cultivar is tolerant to insect infestation, it will show a reduced response to damage compared with other non-tolerant genotypes [16]. When different resistance mechanisms are combined, high levels of overall resistance can be achieved [24].

In this research, resistance in beans to two-spotted spider mite was studied under field and greenhouse conditions at Bean Research Institute of Khomein, Iran. The study was initiated by screening 458 chiti bean germplasm for their resistance to *T. urticae* in field experiments over several planting seasons and so, according to this scoring system, resistance genotypes were chosen for investigation of their underlying resistance mechanisms in greenhouse tests.

MATERIALS AND METHODS

Plant Materials: 1- Screening genotype for Resistance in Field: Screening bean germplasm for resistance to *T. urticae* was initiated in field during 2005-2008. A total of 458 bean genotypes have been screened for resistance. Bean genotypes were chosen from the Bean Research Institute of Khomein bean germplasm bank, one of the biggest Phaseolus collections in Iran. These include 103 germplasm accessions obtained from CIAT's germplasm bank (CIAT: Centro Intemacional de Agricultura Tropical, Colombia), 98 commercial bean genotypes representing the main seed types grown in provinces of Iran, 257 elite

bred lines from CIAT. Genotypes were selected to represent all major seed types and colors as well as all known growth habits within the different bean races as described by Singh *et al.* [25]. These genotypes were screened in naturally infested field plots. During the experiments, all plants were irrigated at the same time and no fertilizers or pesticides were used. Experiments were conducted at Bean Research Institute of Khomein, Iran. All screening trials were planted in randomized complete blocks, with two or four replications. Seed was sown in 5m long and the seeds planted every 5cm, single-row plots with 60cm between rows. Plots were hand-planted and thinned to 100 plants per plot.

Plots were estimated for reaction to *T. urticae* using a 9-point scale of visual damage: 1: no visible damage; 2: initial damage on the upper surface of the leaves in the middle portion of the plant that show slight mottling with white-colored spots; 3: damage extended on the upper surface of all leaves; 4: obvious damage extended to all leaves; 5: the mottling, covering approximately one-third of the leaf area; 6: the mottling extended to all of the intermediate and terminal leaves; 7: a white-dotted mottling covers approximately two-third of the leaf area; 8: a white-dotted mottling extended to all of the leaves; 9: severe damage, all leaves show mottling that covers almost all of the leaves, severe leaf yellowing and necrosis combined with defoliation and weblike structures occur. Damage scores were recorded at 50 and 60 days after planting. Genotypes were classified as resistant, intermediate, or susceptible on the basis of mean score as follows: 1-5, resistant; 5.1-7, intermediate; \$7, susceptible [26].

At maturity, genotypes were rated using a 1-9 reproductive adaptation (RA) scale, a visual estimate of pod and seed set under high mite pressure: 1: no pod setting; 2: very few, deformed and empty pods have been set, 80% or more of the pod setting potential seems to have been lost; 3: many pods are small, many are empty and deformed, the plant seems to have lost 70% of its pod setting potential; 4: some pods are small, many are deformed and empty, the plant seems to have lost 60% of its pod setting potential; 5: some pods are small, a few are deformed and empty, the plant seems to have lost 50% of its pod setting potential; 6: a few pods are deformed and empty, half or more of the pods look normal; 7: most pods look normal, very few are deformed and empty; 8: most pods look normal, good seed setting; 9: all pods look normal, excellent pod and seed setting. Genotypes were classified on the basis of mean score as follows: 1-3, susceptible; 3.1-5, intermediate; \$5, resistant [27].

Selection of Bean Genotypes for Mechanisms of Resistance in Greenhouse:

The bean genotypes used in this study (Mechanisms of Resistance: Antixenosis, Antibiosis and Tolerance) were selected on the basis of field evaluation for resistance to *T. urticae* in greenhouse conditions during 2008-2010. They included four moderately resistant genotypes: KS21247, KS21181, KS21212 and KS21189. Bean plants of each genotype used for antixenosis tests were grown individually in plastic pots (20 cm diameter × 25 cm height) filled with fertilized field soil and used at the age of 21 days, with at least two trifoliolate leaves developed. For antibiosis tests, bean plants were grown in the same way and leaf disks (4cm²) were cut when the young leaves had reached the necessary size.

Mite Colony: For greenhouse trials, adults of two-spotted spider mite were originally collected from common bean fields of the Khomein region, Iran in May 2008. This mites were reared on bean plants that cultivated on plastic pots (20cm diameter × 25 cm height) in a growth chamber (27±2°C, 70±5% RH and a photoperiod of 16 L:8 D h.) for at least two months (several generations) before conducting the experiments. All experiments were performed at the some above mentioned conditions in growth chambers.

Antixenosis Tests: Greenhouse and field choice tests were conducted to evaluate the preference of *T. urticae* for different bean genotypes. In the multiple-choice test, five bean plants (four different resistant genotypes: KS21247, KS21181, KS21212 and KS21189 and one susceptible control genotype: KS21258) representing the five genotypes selected for this study were arranged in a circle around a platform (30 cm diameter, with a distance of about 20 cm between plants) within an insect proof cage (100 × 70 × 60 cm). One trifoliolate leaf from each plant was placed on the platform, without touching the others. Two hundred adult female mites (collected 18 days after oviposition by the parental females) were released in the center of the platform. Individual mites moved and colonized different plants by their own free choice. The choice of plant was scored by carefully counting the number of mites on each entire plant. Care was taken not to disturb the mites. Two independent multiple-choice tests were carried out with 20 replicates each. The number of mites on plants was counted at eight hours after the release of adult mites. Ovipositional preference was recorded in the test 56 hours after release. Eggs were counted on all leaves under a stereomicroscope with transmitting light.

Antibiosis Tests: To evaluate possible antibiotic effects, the age-specific life table of *T. urticae* reared on bean plants of each of the five genotypes (KS21247, KS21181, KS21212, KS21189, KS21258) was constructed to compare performance of the mites in climatic chambers (27±2°C, 70±5% RH and a photoperiod of 16 L:8 D h.). Several life-history traits (development time and survivorship of immature stages, adult body length, longevity of adults, daily oviposition rate) and demographic parameters (net reproductive rate (R_0), generation time (T), intrinsic rate of natural increase (r_m), finite rate of increase (λ) and doubling time (D_7)) were determined for the cohort reared on each bean genotype. To perform the experiments, the leaf disc method was used [28-29]. Each leaf disc was 4cm² of area center of leaves that this unit separated by plastic padding 2cm×2cm. Each leaf disc was placed on plastic Petri dishes (8cm diameter×1.5cm height with a hole in its center). Thereafter, one fully expanded young leaf (third leaf below the apical meristem of one month-old plants) was randomly collected and used for the leaf disc preparation. The leaves of different common bean genotypes were selected from all replications and cut into a leaf disc (2 × 2cm) and then placed on water-saturated cotton in the Petri dish with the underside facing upward. During the experiments, all the common bean genotypes were periodically planted in the greenhouse (every 10 days) and to reduce the effects of plant age on mite development and fecundity, the new leaf discs were prepared from their leaves and the mites transferred on them. Newly hatched larvae were transferred into a separate dish using a small soft brush. The larval cohort on each bean genotype consisted of 78 to 96 individuals. The duration and mortality of immature stages, i.e. egg, larva, nymph (nymph I + II combined) and adult, were recorded. The longevity of resulting adults and the number of eggs laid per female per day were measured. To determine the oviposition rate and longevity of adult mites on the different bean genotypes, the individuals that developed on each genotype were separated at the stage of egg and transferred singly into individual petri dishes. Upon emergence of adults, unmated females were observed separately throughout their lifetime. An adult cohort of 48 to 74 individuals was started for each bean genotype. Every second day, living and dead individuals were recorded. Each live mite was transferred into a new petri dish, while the number of eggs on the old leaf disk was counted under a stereomicroscope with transmitting light.

Statistical Analyses:

Screening Genotype for Resistance in Field: All data were analyzed using the Statistix package [30]. Descriptive statistics were calculated for each response variable. Simple and rank correlation coefficients between response variables were calculated. Data were analyzed using the general linear model (GLM) procedure. Means were separated by least significant difference (LSD) ($P=0.05$) using the LSD test only when the overall F test was significant. Insect counts were transformed to $\log(x)$; percentages were transformed to arcsine square root of proportion. Untransformed means and standard errors are presented. Scheffe's F method of significance testing for arbitrary simultaneous linear contrasts was used to test for differences between susceptible and resistant groups of genotypes.

Antixenosis Tests in Greenhouse: In the greenhouse choice tests for antixenosis, only those *T. urticae* that were present on the plants were taken into account. Therefore, we calculated the percentage of mites recorded from each plant within a test cage and the data analysis was based on the percentage of total encountered insects rather than on the absolute number of mites on a plant. All percentage data were subjected to arcsine-square-root transformation prior to analysis. A general linear model with repeated measures (times of counting) was applied to the analysis of data from these choice tests, using the program SPSS 10 [31].

Antibiosis Tests in Greenhouse: The life table parameters were included: net reproductive rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase (λ), mean generation time (T) and doubling time (DT). Data on immature developmental period and adult longevity of TSSM were analyzed with one-way analyses of variance (ANOVA). When the variation among cultivars was significant, means comparison were done based on Duncan's multiple range test ($P<0.05$). The statistical differences of life table parameters among various bean cultivars were detected using the jackknife procedure [32-33]. In this procedure, jackknife pseudo values of each life table parameter were calculated for n females by following equation:

$$A_{(j)} = n \times A_{(all)} - (n - 1) \times A_{(i)}$$

Where $A_{(j)}$ is the jackknife pseudo value, n is the number of females, $A_{(all)}$ is the calculated life table parameters for all females and $A_{(i)}$ is the calculated parameters for ($n-1$)

females. Various life table parameters including: r_m , R_0 , T , λ and DT were inserted in this equation instead of (A). Consequently, n calculated jackknife pseudo values were subjected to one-way ANOVA and if significant differences were detected a Duncan's multiple range test were run ($P<0.05$). The obtained sex ratio of offspring were compared to expected ratio of 1:1 by a chi-square test (χ^2 , $P<0.05$). A t -test was run for comparison of total immature developmental times of males and females on the same cultivar. All statistical analyses were carried out using the Minitab statistical software [34] and SPSS statistical packages [31].

RESULTS

Screening Genotype for Resistance in Field: The results of screening genotype for resistance to *T. urticae* in field trials showed that most of the entries tested were susceptible to *T. urticae*, with damage scores ≤ 7 and reproductive adaptation (RA) scores of < 3 . Fifteen of the 458 genotypes tested (3.2%) were rated as resistant to *T. urticae*. These resistant entries included five germplasm accessions, three commercial variety and seven elite breeding lines (Fig. 1). From these resistant genotypes, the four most resistant bean genotypes were elite breeding lines. These genotypes included: KS21247, KS21181, KS21212 and KS21189 that were chosen for investigation of their underlying resistance mechanism in greenhouse tests. Also, the most susceptible genotype was KS21258 from elite breeding lines. Reaction to mites was not associated with maturity, growth habit, pubescence and seed color or seed size. However, all determinate, large-seeded genotypes tested were susceptible. The result of tests revealed significant differences between resistant and susceptible genotypes. The elite breeding lines KS21247 and KS21181 repeatedly showed less damage and higher reproductive adaptation (RA) scores than the susceptible check KS21258. Although damage and RA ratings change with various levels of infestation, genotype responses and resistance ratings were consistent. Correlations between visual damage scores and RA scores were high ($r=-0.914$; $P<0.001$; $n=457$), meaning that selection for damage is useful in the selection of genotypes that may have tolerance in terms of yield response as a mechanism of resistance. Overall, resistance levels in beans can be considered as moderate, because none of the genotypes tested received damage scores of less than 3 and none was ever rated as highly resistant in terms of reproductive adaptation scores. When these materials were tested

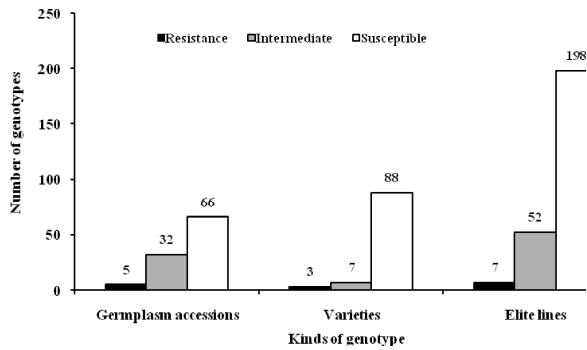


Fig. 1: Frequency distribution of response to *T. urticae* attack in 458 genotypes tested for resistant.

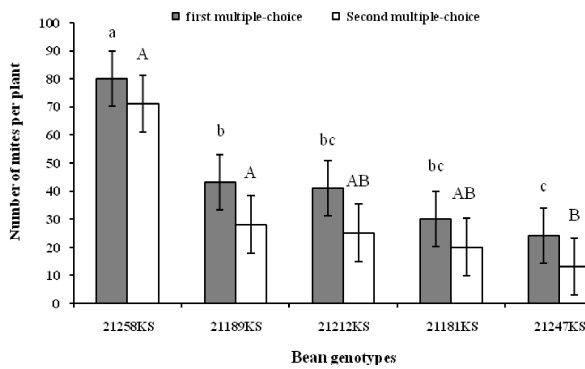


Fig. 2: Number of *T. urticae* per bean genotypes in first and second multiple-choice tests for antixenosis. Each test was analyzed separately, block with different letters are significantly different ($P < 0.05$) within a test.

again using 3 replications per genotype, mean damage scores ranged from 3 to 9, with some materials showing relatively high levels of resistance. Again, there was a significant correlation between damage and RA scores ($r = -0.901$; $P < 0.001$; $n = 457$).

Antixenosis Tests in Greenhouse: The first multiple-choice test demonstrated a significant effect of the bean genotype on the number of *T. urticae* colonizing a plant. The number of mites on a given bean genotype did not vary over different periods of time after mite release. Ovipositional preference was not influenced since the bean genotype did not have a significant effect on the number of eggs laid per female. Concurrently, the second multiple-choice test showed that the number of mites on bean plants of different genotypes was significantly different ($P < 0.001$) and that the number of mites on bean plants of the same genotype did not change significantly during the experimental period. The results of percentage

of mites on bean plants showed that KS21189 was the largest at all counting times and that on KS21247 was always the least. The percentage of mites tended to increase on KS21189 from 24 to 48 hours after mite release and to decrease on KS21247 over this time period. This might underlay the significance of interaction between mites numbers on bean plants counted at different times and on different bean genotypes (Test 1: $P < 0.05$ and Test 2: $P < 0.05$). The number of mites on bean plants reflected preferential choices of *T. urticae* for different genotypes after 24 and 48 h in the first and second multiple-choice tests, respectively. When the pooled mean percentages of mites recorded on the five bean genotypes were compared (Fig. 2), the ranking order of preference for different genotypes by *T. urticae* was consistent in both tests. After KS21258 genotype (check genotype), KS21189 was the most preferred, having 43 ± 1.4 and 28 ± 1.8 of *T. urticae* counted in Test 1 and Test 2, respectively. KS21247 genotype was the least preferred, with the number of *T. urticae* on the bean plants accounting for 24 ± 1.1 in Test 1 and 13 ± 1.7 in Test 2. The preference of mites for other genotypes was intermediate. All these tests in both greenhouse and field have shown that KS21247 genotype exhibits a strong antixenotic effect, as a significantly lower number of *T. urticae* was always found on KS21247 genotype in comparison with other genotypes. This may explain the phenomenon noticed in previous field resistance trials that *T. urticae* populations were consistently low on KS21247 genotype.

Antibiosis Tests in Greenhouse: The development times of various stages (males and females) of TSSM on five bean genotypes are given in Table 1. No significant variations among five host plants were observed for egg incubation period. While the development periods of larvae, protonymphs and deutonymphs ($P < 0.05$) showed significant differences among various host plants. Total immature developments of both males and females ($P < 0.05$) were significantly different among five bean genotypes and ranged from 15.96 ± 0.20 to 18.71 ± 0.24 days for females and from 15.89 ± 0.24 to 19.37 ± 0.31 days for males on KS21258 and KS21247, respectively (Table 1). Overall, no significant differences were observed between development times of males and females on the same bean genotypes ($P = 0.05$).

The pre-oviposition, oviposition, post-oviposition periods and females longevities of TSSM on five bean genotypes are shown in Table 2. The post-oviposition periods and oviposition periods ($P < 0.05$) of TSSM are

Table 1: Development times (in days) of immature stages of *T. urticae* on five bean genotypes (Mean± SE)

Stage		Bean genotype				
		Chiti Ks21258	Chiti Ks21189	Chiti Ks21212	Chiti Ks21181	Chiti Ks21247
Egg	&	2.00±0.04 a	2.00±0.04 a	2.00±0.03 a	2.00±0.03 a	2.00±0.03 a
	%	2.01±0.09 a	2.10±0.08 a	2.00±0.09 a	2.00±0.07 a	2.05±0.12 a
Larva	&	4.21±0.06 e	4.45±0.02 d	4.75±0.06 c	5.08±0.02 b	5.29±0.11 a
	%	4.45±0.04 d	4.80±0.05 c	4.99±0.03 c	5.20±0.08 b	5.52±0.09 a
Protonymph	&	4.85±0.02 d	4.98±0.08 d	5.15±0.05 c	5.31±0.06 b	5.72±0.06 a
	%	4.45±0.06 d	4.81±0.05 c	5.22±0.09 b	5.75±0.02 a	5.89±0.08 a
Deutonymph	&	4.90±0.08 d	5.10±0.09 c	5.28±0.03 c	5.49±0.05 b	5.70±0.04 a
	%	4.98±0.05 e	5.15±0.06 d	5.32±0.04 c	5.70±0.03 b	5.91±0.02 a
Total development time	&	15.96±0.20 e	16.53±0.23 d	17.18±0.17 c	17.88±0.16 b	18.71±0.24 a
	%	15.89±0.24 e	16.86±0.24 d	17.53±0.25 c	18.65±0.20 b	19.37±0.31 a

Means followed by the same letters within rows are not significantly different (one-way ANOVA, " $\alpha=0.05$).

Table 2: Pre-oviposition, Oviposition, Post-oviposition periods and Reproduction rate of *T. urticae* on five bean genotypes (Mean± SE)

	Bean genotypes				
	Chiti Ks21258	Chiti Ks21189	Chiti Ks21212	Chiti Ks21181	Chiti Ks21247
Ovipositional parameters					
Pre-oviposition	1.00±0.04 a	1.37±0.07 ab	1.28±0.04 a	1.50±0.08 ab	2.10±0.23 b
Oviposition	8.95±0.80 a	8.58±1.02 a	8.63±0.95 a	8.36±1.10 a	6.98±1.22 b
Post-oviposition	1.40±0.40 a	1.00±0.59 a	1.20±0.51 a	1.00±0.71 a	1.00±0.93 a
Total	11.35±1.24 b	10.95±1.68 ab	11.11±1.50 b	10.86±1.89 ab	10.08±2.38 a
Reproduction rate					
Daily Fecundity	16.16±1.25 a	12.09±1.69 bc	13.33±1.47 b	10.98±1.89 c	12.63±2.12 bc
Total Fecundity	142.05±6.58 a	99.55±8.23 bc	116.54±6.98 b	90.52±8.54 c	82.45±8.89 c

Means followed by the same letters within rows are not significantly different (one-way ANOVA, " $\alpha=0.05$).

Table 3: Life table parameters of *T. urticae* reared on five bean genotypes (Mean± SE)

Parameter	Bean genotype				
	Chiti Ks21258	Chiti Ks21189	Chiti Ks21212	Chiti Ks21181	Chiti Ks21247
r_m (&/&/day)	0.269±0.031 a	0.187±0.055 b	0.203±0.063 b	0.152±0.024 c	0.129±0.048 d
R_0 (&/&/generation)	62.38±1.65 a	51.84±1.55 b	60.39±1.48 a	38.51±1.36 c	26.11±1.40 d
T (day)	15.24±1.12 d	21.09±1.10 c	20.19±1.18 c	24.04±1.21 b	25.55±1.09 a
δ (&/&/day)	1.30±1.02 a	1.20±1.08 b	1.22±1.01 b	1.16±1.01 c	1.13±1.10 d
DT (day)	2.54±1.14 f	3.70±1.20 d	3.40±1.22 e	4.54±1.18 c	5.33±1.25 a

Means followed by similar letters in rows are not significantly different (one-way ANOVA, " $\alpha=0.05$).

significantly influenced by the bean genotypes. No significant host plant effects were observed on the pre-oviposition period of TSSM. Host plant genotypes significantly affected on the total females longevities ($P<0.05$). The shortest and longest females longevities were observed on KS21247 (10.08±2.38 days) and KS21258 (11.35±1.24 days), respectively. The mean number of daily eggs laid by each female and total fecundity data of TSSM are given in Table 3. The mean daily eggs laid exhibited significant differences among five bean genotypes ($P<0.05$). Two spotted spider mite,

laid the highest daily number of eggs on KS21258 (16.16±1.25), which was significantly more than on the other genotypes. This was followed by Ks21189 and KS21247. Also, total fecundity of TSSM ($P<0.05$) was significantly different among the tested bean genotypes.

Life table parameters of the TSSM on the five bean genotypes are presented in Table 3. The analysis of the all life table parameters (R_0 , r_m , δ , T and DT) indicated significant differences among five host plants ($P<0.05$). The net reproductive rate (R_0) was the highest on KS21258 (62.38±1.65 females/ females/ generation) and

lowest on KS21247 (26.11 ± 1.40 females/ females/ generation). The intrinsic rate of natural increase (r_m) and the finite rate of increase (λ) showed a pattern similar to R_0 in which it was highest on KS21258 and lowest on KS21247 genotypes. The mean generation time (T), is required time for population of TSSM to multiply as R_0 , and varied from 15.24 ± 1.12 to 25.55 ± 1.04 days KS21258 and KS21247 genotypes, respectively. The lowest and greatest values of doubling times (DT) were estimated to be 2.54 ± 1.14 and 5.33 ± 1.25 days on KS21258 and KS21247 genotypes, respectively.

In summary, both antixenosis and antibiosis have shown to be categories of resistance in common beans to *T. urticae*. Antixenotic effects are strongly exhibited in the genotype KS21247. A certain degree of antibiotic effects is shown in some genotypes, particularly in the genotype KS21247, which causes a high mortality of immatures and a low intrinsic rate of natural growth in *T. urticae*. It must be pointed out, however, that the two other moderately resistant genotypes (KS21181 and KS21212) also show low damage and good reproductive adaptation in the field. These findings suggest that the resistance of common beans to *T. urticae* might even be the combined functions of antixenosis and antibiosis.

DISCUSSION

In the present study, resistance mechanisms to *T. urticae* investigated on the bean (*Phaseolus vulgaris* L.) genotypes. Infestation by adult *T. urticae* on bean plant can cause significant economic injury on common beans in the field. This infestation level was reached and surpassed on all bean genotypes at 8 and 15 days after plot in the field trials. Differences in the mite abundance were observed between some genotypes; for example, the density of mites was high on Chiti KS21258 and very low on Chiti KS21247. This result is consistent with findings from previous field screening studies [35-36] and from multiple-choice tests with different plant genotypes in the greenhouse [27]. The low level of mites populations on the susceptible genotypes towards the end of the experimental period in the field trials was most likely due to the leaf desiccation caused by this mite, rendering these genotypes unsuitable for the mite's survival and reproduction. Thus, it is indispensable to compare the infestation level on a given bean genotype with the resulting damage and reproductive adaptation scores, as well as with the yield losses in order to evaluate the tolerance type of resistance [37]. The two diverging categories of bean genotypes, previously defined as

resistant and susceptible [35], showed differential damage and reproductive adaptation scores in response to natural mites infestation in the field trials. For example, the resistant genotypes Chiti KS21258 and KS21224 had particularly low damage and high RA in spite of high infestation, indicating a high degree of tolerance. These results verify the ratings of the genotypes into the resistant/susceptible categories [35]. Regarding pod, seed and yield production, all genotypes showed significant differences in both protected (with acaricide treatment) and non-protected trial subplots. In general, genotypes categorized as 'resistant' suffered less pod and seed losses and produced higher yield, as compared to the genotypes categorized as 'susceptible'. These two genotypes, originally characterized as resistant [35], have shown some degree of antixenosis and/or antibiosis [27].

To estimating resistance mechanisms to *T. urticae* on the bean genotypes, the population growth parameters were recognized. These parameters indicate insect population growth rates in the current and next generations [38] and understanding them is essential to develop an integrated pest management (IPM) strategy. The results showed that the population growth parameters of *T. urticae* may vary in response to changes in bean genotypes. Total developments of both males and females were significantly different among five bean genotypes. Chiti KS21258 with 15.96 ± 0.20 and 15.89 ± 0.24 days and Chiti KS21247 with 18.71 ± 0.24 and 19.37 ± 0.31 days for females and males were the shortest and longest, respectively (Table 1). This results are conformance with Mondal and Ara [39] on fresh bean (*Lablab purpureus* L.) and Deciyanto *et al.* [40] on six cultivars of *Mentha piperita* L. and *M. arvensis* L. results. Puttaswamy [41] on cucurbit, Adango *et al.* [42] on *Amaranthus cruentus* L. and *Solanum macrocarpon* L., Moros and Aponte [43] on *P. vulgaris* and da Silva [44] on cotton, recorded mean duration of developmental stages of the same spider mite species were shorter than our findings on bean cultivars. So, other external factors (temperature and relative humidity) have effect upon the population growth parameters of *T. urticae*. This research was studied at constant laboratory conditions ($27 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH). Shih [45] stated the optimum temperature for development of spider mite was between $23-30^\circ\text{C}$. He suggested that the mean longevity of generation time of *T. urticae* declined with increasing temperature. This period 6.5 days at 30°C [10], 17.7, 14.3 and 11.6 days at 22.7 , 26.6 and 30.5°C [46] and 7.8 and 6.3 days at 31 and 36°C , respectively [47].

Our findings indicated that the development times of immature stages of TSSM females were similar to males on each bean genotype in greenhouse conditions. This result is close to the results of Laing [48], who found similar development times for males and females (16.1 and 16.9 days, respectively) but the results of other researchers [49] are different from what was reported here (Table 2). van de Vrie *et al.* [49] emphasized the occurrence of the differences between males and females as to development rate. Shih *et al.* [50] reported lower values for longevity of females (19.1 days) and males (14.6 days), but according to van de Vrie *et al.* [49] specimens of different stages can be vary considerably in relation to their exposure to environmental conditions. The increase in the longevity of females may be an important adaptation for the pest to maintain its generation when food quality is low, because only a limited number of females are able to remain [51-52]. According to this study, the number of dead TSSM immature at the end of experiment was very low on the bean cultivars (Table 1). Low mortality of immature stages suggests that the bean cultivars were not harmful to this mite. So, these results conform Shih and Wang [53], except the development times of deutonymphs. They also demonstrated that these variations could be ascribed to differences in host plant quality. The host plant affected on fecundity and longevity of development times of mite immature stages.

Egg incubation period in this study calculated from 2.00 ± 0.03 to 2.10 ± 0.08 days and no significant variations were observed among five bean genotypes (Table 1). Moros and Aponte [43] and da Silva [44] found that the egg incubation period is the longest of all other life stages of *T. ludeni*. Egg hatchability, development time and survival to adult stage were similar among bean cultivars. Razmjou *et al.* [54] reported total immature stages of *T. urticae* on three legumes including soybean, cowpea and bean (9.23, 9.38 and 9.12 days, respectively) that were shorter than our results. Chahine and Michelak [55] pointed out that no difference was found in longevity when eggplant, tomato and bean were used as hosts, but fecundity was indeed affected by the host plants. These results indicates that the developmental cycle of *T. urticae* is influenced by several factors.

The net reproductive rate (R_0) and the intrinsic rate of natural increase (r_m) are important indicators of tetranychid population dynamics [56-57]. Comparisons of R_0 and r_m often provide considerable insight beyond that available from the independent analysis of individual life-history parameters [58]. In the present study, bean cultivars greatly affected TSSM fecundity and life-table

parameters (Table 3). The r_m values ranged from 0.269 ± 0.031 to 0.129 ± 0.048 females/female/day. Hence, the population development of two-spotted spider mite was the shortest on Chiti KS21258. This was mainly due to short development time, an early peak in reproduction, high daily egg production and high total fecundity. Our findings revealed that Chiti KS21247 and KS21181 were less suitable cultivars for two-spotted spider mite. These values are close to those estimated for the spider mites reared on other host plants [56, 59-61]. Razmjou *et al.* [62] reported Sayyad cultivar was the most favorable host for two-spotted spider mites with $r_m = 0.295$ and Talash cultivar with $r_m = 0.214$ was unfavorable host. Sabelis [56,63] has reported r_m values of *T. urticae* from 0.219 to 0.336 and Ahmadi *et al.* [64] has estimated r_m values from 0.038 to 0.142 females/female/day on common bean.

The net reproductive rate (R_0) found on bean cultivars are similar to those reported by Silva *et al.* [65] for *T. urticae* on cotton. Ahmadi *et al.* [64] has reported R_0 values of *T. urticae* from 2.043 to 8.822 (females/female/generation) that were shorter than those reported in this study. The same situation may have influenced the mean generation time (T), where Silva *et al.* [65] found values between 22.2 and 24.9 days on cotton and beans, respectively, which was similar to values of this study. The higher values of r_m and R_0 indicate the susceptibility of a bean cultivar to TSSM, while the lower ones indicate that the bean cultivar is resistant to TSSM. Therefore, among examined cultivars, Chiti KS21258 and Chiti KS21247 are the most susceptible and resistant cultivars for TSSM, respectively.

Daily fecundity of TSSM, was estimated from 10.98 ± 1.89 to 16.16 ± 1.25 eggs/female/day and total fecundity was between 142.05 ± 6.58 and 82.45 ± 8.89 eggs/female. These parameters on cucumber (*Cucumis sativus* L.) has been reported as 5.98 and 104.85, respectively [66]. The total number of eggs laid per female in her lifetime was averaged as 108.3 ± 3.23 in the laboratory condition on fresh bean (*Lablab purpureus* L.) [39]. The mean number of eggs laid and the lifetime of *T. urticae* was 34.50 eggs/female and 14.10 days respectively on bean that lower than results in this study [54]. The life table parameters of two-spotted spider mite on 14 soybean genotypes were evaluated and the highest r_m was recorded on L17 (0.392 females/female/day) and the lowest values of this parameter was obtained on Tms (0.233 females/female/day). So, R_0 and 8 of the TSSM had the highest value on L17 as 45.521 females/ female/ generation and 1.475 females/female/day, respectively.

The lowest values of these parameters were recorded on Tms as 12.149 and 1.258, respectively. DT varied significantly on different genotypes and the shortest and longest values of this period were obtained on L17 and 032 genotypes, respectively [67-68]. According to Gallo *et al.* [69], the two spotted spider mite feeds on a large number of plant species such as cotton, strawberry, rose, tomato, bean, soybean, peach and among others, evidence of a potential pest for Brazil. On suitable host plants, spider mites had r_m values between 0.220 and 0.340 [64]. The reported r_m values of *T. cinnabarinus* on bean ranged between 0.197 and 0.440, while the same values of *T. urticae* were 0.282 and 0.143 on cucumber (susceptible line), strawberry, bean and cucumber (resistant line), respectively, at 25°C [48, 56, 70-71].

These results complemented previous studies, demonstrating variation in mite performance on different cultivars of the same crop. Examples include *T. truncatus* Ehara on corn [72], *Amphitetranychus viennensis* Zacher and *T. urticae* on apple [13, 69], *T. urticae* on strawberry [73] and on cucumber [74]. The use of crop cultivars that support only low pest population growth or even resistant varieties is an important part of integrated pest management (IPM) [62]. An important goal of future research will be to compare the varieties susceptibility to two-spotted spider mite with other economically relevant traits.

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