

# Bitki Koruma Bülteni / Plant Protection Bulletin

<http://dergipark.gov.tr/bitkorb>

Original article

## Effect of *Tetranychus urticae* and *Polyphagotarsonemus latus* (Acari: Tetranychidae, Tarsonemidae) at different infestation levels and feeding durations on chlorophyll content of bean plants

*Tetranychus urticae* ve *Polyphagotarsonemus latus*'un (Acari: Tetranychidae, Tarsonemidae) farklı yoğunluk düzeyleri ve beslenme sürelerinde fasulye bitkisinin klorofil içeriğine etkisi

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### ARTICLE INFO

Article history:

DOI: [10.16955/bitkorb.1302239](https://doi.org/10.16955/bitkorb.1302239)

Received : 25-05-2023

Accepted : 17-11-2023

Keywords:

feeding time, mite density, correlation, injury assessment, two-spotted spider mite, yellow tea mite

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### ABSTRACT

The study investigated the effects of different initial infestation levels and feeding durations of *Tetranychus urticae* Koch and *Polyphagotarsonemus latus* (Banks) (Acari: Tetranychidae, Tarsonemidae) on the chlorophyll content of the pinto bean plants [*Phaseolus vulgaris* L. (Fabaceae)]. The experiment was carried out on 3 cm diameter leaf discs and potted plants. To determine the effects of mite feeding on the chlorophyll content of leaf disc, 0 (control), 5, 10, 15, 20, and 25 mated adult females (24-48 hours old) were separately placed on each disc. Chlorophyll measurements were made 5 and 10 days after the initial infestation. For the potted plant bioassay, young plants were infested separately with different densities of *P. latus* or *T. urticae* (5, 10, 15, 20, and 25 females per plant) while noninfested plants acted as the control. Data were obtained at intervals of 5 days for a total of 5 times. The chlorophyll contents at infestation levels of 15, 20, and 25 *T. urticae* per disc were statistically lower than the control discs after exposure for 5 days. The heavily infested discs with 10 or more *T. urticae* were destroyed until the second measurement. On the other hand, there was no significant difference between the chlorophyll contents of *P. latus*-infested and noninfested discs 5 days after infestation. However, the content was significantly lower in infested discs at all infestation levels than in noninfested discs 10 days after infestation. According to the potted plant experiment, the chlorophyll contents of *T. urticae* and *P. latus*-infested plants were significantly lower than the noninfested plants at all infestation levels from the 10<sup>th</sup> and 20<sup>th</sup> days of the infestation, respectively. A highly significant negative correlation was recorded between chlorophyll content and mite density, as well as exposure time indicating that the leaf chlorophyll content of infested bean plants decreased with increasing mite density and time. It was also determined that *P. latus* required a longer feeding time than *T. urticae* to affect the chlorophyll content of the bean plants.

## INTRODUCTION

*Tetranychus urticae* Koch (Two-spotted spider mite) (Acari: Tetranychidae) is a cosmopolitan pest mite species that feeds on a wide variety of plants (Liburd et al. 2020, Nyoike and Liburd 2013, Park and Lee 2002). *Polyphagotarsonemus latus* (Banks) (Broad or yellow tea mite) (Acari: Tarsonemidae) is also considered a polyphagous pest of diverse crops (Devi et al. 2019, Pena et al. 2000). These mites feed by piercing leaf tissue with their stylets and sucking the host plant sap. It is noted that probing mesophyll cells with stylets harms them and leads to the deterioration of their chloroplasts, ultimately resulting in a reduction of chlorophyll content (Bueno et al. 2009, Campbell et al. 1990, Ziaee and Nikpay 2016). Chlorophyll plays a direct role in photosynthesis, which is closely related to the crop's ability to develop, and yield (Ghimire et al. 2015). It has been reported that there is a notable association between the chlorophyll content and the grain yield and some yield-attributing traits (Bahar 2015, Ghimire et al. 2015, Wang et al. 1999), the nitrogen content of bean leaf and yield (Güler and Özçelik 2007), the water-use and transpiration efficiency as well as specific leaf area and specific leaf nitrogen in peanuts (Lal et al. 2006, Nageswara Rao et al. 2001, Sheshshayee et al. 2006), the leaf anatomy and plant morphology in Triticale (Kabanova and Chaika 2001). Consequently, it is obvious that the reduction in chlorophyll will damage the plant.

There are studies on the effect of feeding *T. urticae* and *P. latus* on different host plant chlorophyll content. Among them, Reddall et al. (2007) detected a reduction in the chlorophyll content of the basal region of cotton leaves infested with *T. urticae*. Sivritepe et al. (2009) found that the chlorophyll content of *T. urticae*-infested grapevines was lower than that of noninfested grapevines. Tehri et al. (2014) and Park and Lee (2002) also stated that the chlorophyll content in the cucumber plants was reduced with *T. urticae* infestation. Rajashekharappa et al. (2018) indicated that the chlorophyll content was higher in predator-released rose plants than in pesticide-sprayed ones to control *T. urticae*. Shaabani et al. (2021) determined that *T. urticae* feeding also reduced chlorophyll content in 104 common bean genotypes by 14% - 20% in 45 - 75 days after sowing under open field conditions in Iran. Additionally, there was a significant negative correlation between the chlorophyll content in chili leaves and *P. latus* densities (Gulati 2015, Latha and Hunumanthraya 2018). It was also noted that the relative chlorophyll content of cucumber leaves increased under light and moderate *P. latus* invasion but decreased under heavy invasion (Jiang et al. 2019).

Despite some studies conducted in the past on the feeding effect of *T. urticae* and *P. latus* on the chlorophyll content of some plants, no research has been done to explore the effect on pinto bean plants at different initial densities and feeding duration of these two mites. It is well known *T. urticae* is a worldwide crop pest with a strong preference for bean plants (Al-Shammery and Al-Khalaf 2022). *Polyphagotarsonemus latus* is also a significant crop pest that affects various crops including beans (Androcioli et al. 2021). It is therefore of great importance to conduct a study that will consider the effects of *T. urticae* and *P. latus* feeding on pinto bean plants at different initial densities and exposure times. Moreover, the amount of damage caused by a pest species is a major factor in determining its economic injury level. In general, it is difficult to assess the amount of injury caused by a pest with sucking-type mouthparts such as *T. urticae* and *P. latus* as the symptoms of damage are often hidden and hard to quantify, especially at low densities and during the early infestation period. However, physiological damage or the reduction of chlorophyll content in leaves may already be occurring even at low levels of mite-days. Such a study would provide valuable insight into the possibilities of these species to influence the chlorophyll content of pinto bean plants, which could enhance the understanding of their roles in the ecosystem and aid in early pest intervention or control. So, this study aimed to investigate how different initial infestation densities (0, 5, 10, 15, 20, 25 females per plant or leaf disc) and exposure times of *T. urticae* and *P. latus* affect the chlorophyll content of pinto bean plants under controlled conditions.

## MATERIALS AND METHODS

### *Rearing of plants and mites*

In the study, pinto bean plants [*Phaseolus vulgaris* L. (Fabaceae)] were used as the test substrate and host for *Tetranychus urticae* Koch and *Polyphagotarsonemus latus* (Banks) (Acari: Tetranychidae, Tarsonemidae). Plastic pots (26 x 14 cm) were utilized to rear plants in a mixture of vermiculite and soil. Seeds were planted at two-day intervals to provide plants with 2- to 3-day-old primary leaves for leaf disc.

*Tetranychus urticae* was obtained from a laboratory stock colony. The initial population of *T. urticae* was collected from infested bean plants in Ordu in 2010, while the *P. latus* population was initially obtained from tea plants in Rize in 2012. Adult females of *P. latus* and *T. urticae* were collected and individually placed on 2 cm leaf discs, which were left

upside down on water-saturated cotton pads. After a 5–6-day period of oviposition, the females were removed from the discs and placed in lactophenol, a clearing medium. Subsequently, the cleared specimens were mounted in Hoyer's medium and dried at 50 °C. Mites were identified at the species level according to Pritchard and Baker (1955), Cho et al. (1993), Jeppson et al. (1975), Lindquist (1986) and Zhang (2003). To confirm the identifications, Edward A. Ueckermann (North–West University, South Africa) was consulted, an expert in the field who was able to provide a clear and definite verification of the classifications. This ensured that the identifications were correct and provided a reliable basis for further study.

Leaf discs containing the female specimens identified as *P. latus* or *T. urticae* were placed on clean bean plants for mass rearing. The *T. urticae* colony was maintained by regularly replanting pinto bean plants. For this purpose, cut foliage containing *T. urticae* was laid on the top of clean plants at the 3- to 6-leaf stage. This cycle was repeated to sustain the colony of *T. urticae*. The cycle of planting pinto bean seeds was also used to maintain a colony of *P. latus*.

Experiments were conducted using 1-2 day-old mated adult females of each species. Ten *T. urticae* and 20 *P. latus* female mites were taken from the stock culture and placed on each leaf disc to acquire individuals of the same age. Mites were given 24 hours to lay eggs and then removed from the disc. Once the eggs had hatched, the discs were kept until the adult females emerged. Two males were then introduced to each disc containing adult females and left for a 24-hour period to mate. These females were then used for the experiments.

The plant and mite rearing were conducted in a climate-controlled room to maintain consistent conditions [25±2 °C, 70-80% humidity, 16:8 h L:D photoperiod (daylight, 1200 lux)].

#### *Experimental design for bean leaf disc assays*

Bean leaf discs with a diameter of 3 cm were used in experiments. The discs were placed upside down on wet cotton in a 15x11 cm plastic tray to prevent escape and maintain freshness. Adult females of *P. latus* and *T. urticae* were placed on the leaf discs at densities of 0 (control), 5, 10, 15, 20 and 25 mites per disc to determine the effect on chlorophyll content. Six replicates were used for each treatment.

Chlorophyll contents were measured five and ten days after the infestation with *P. latus*. However, just 5 days after infestation, the chlorophyll content of infested discs with *T. urticae* could be measured, as the heavily infested discs were destroyed by the second measurement. The chlorophyll

content of each disc was measured at four different points using a calibrated portable leaf chlorophyll meter (SPAD-502, Konica Minolta, Inc., Japan). The chlorophyll meter has a measurement area of 0.06 cm<sup>2</sup> and weighs 225 g. It can calculate an index in SPAD units based on transmittance at 650-940 nm (Markwell et al. 1995).

#### *Experimental design for potted bean plant assays*

An experiment was conducted on potted pinto bean plants [*Phaseolus vulgaris* L., (Fabaceae)]. In the experiment, there were 6 treatments for each mite species: 0 mites/plant (control), 5 mites/plant, 10 mites/plant, 15 mites/plant, 20 mites/plant and 25 mites/plant. Five replicates were used per treatment. Each replicate contained four plants giving a total of 20 plants for each treatment. Seven days after planting bean plants, mites were released on the plants that were about 15 cm high and in a 2-leaf stage. The plants were infested with either *P. latus* or *T. urticae* at a density of either 5, 10, 15, 20 or 25 adult females/plant. For this purpose, mites were carefully transported to small leaf discs (2 cm in diameter) with a brush from the colony. These leaf discs were then placed on each plant. Each disc was supplied with either 5, 10, 15, 20 or 25 mites. One noninfested group served as a control.

Four points on a single leaf of each plant were measured for chlorophyll levels. Data was collected in 5-day intervals for a total of 5 times.

The experiments were conducted in a climate-controlled room, where the temperature was held at 25 ± 1 °C and the humidity was measured at 60% ± 5. Furthermore, the photoperiod was 16 hours of light and 8 hours of darkness with a light intensity of 1200 lux (daylight). The measurements were all kept within these parameters to ensure the accuracy of the experiment.

#### *Statistical analysis*

Analysis of variance was carried out for the continuous data for leaf-disc and potted-bean plant experiments. Before ANOVA, the assumptions, data normality and homogeneity of variance, were checked by Kolmogorov-Smirnov test and Levene's test, respectively. Then, the variables between groups (mite densities) were analyzed by one-way ANOVA. Secondly, the variables within groups (exposure times) were analyzed by repeated measurement ANOVA using the General Linear Model (GLM) for potted-bean plant experiments. The mean results of ANOVAs were then compared in letters by Tukey's post-hoc test for both analyses. Additionally, the differences between the chlorophyll data of the two exposure times of *P. latus*-leaf-disc experiment were determined with the Paired-Sample *T*-test. Differences

among means were considered statistically significant when  $p < 0.05$ .

Pearson correlation analyses were also conducted to explore the relationships between chlorophyll content and mite density or exposure duration.

The statistical analysis was conducted using the software Minitab® version 17.1.0.

## RESULTS AND DISCUSSION

### *Effects of different densities and exposure times of Tetranychus urticae on the chlorophyll content of bean plants*

The mean chlorophyll content of bean discs was significantly impacted by feeding in accordance with the duration of exposure (Table 1). The results of the analysis indicated a marked decrease in chlorophyll content as the density of mites increased and the duration of exposure lengthened. After exposure for 5 days, the mean chlorophyll contents of bean discs were 50.73, 48.17, 46.86, 45.10, 39.68 and 39.72 SPAD at densities of 0 (control), 5, 10, 15, 20 and 25 mites per disc, respectively. Additionally, the mean chlorophyll contents at infestation levels of 15, 20 and 25 mites per disc were statistically lower than the control discs ( $p = 0.000$ ,

**Table 1.** Effects of varying densities of *Tetranychus urticae* on the chlorophyll content in bean leaf discs after 5 days of feeding

Density (mite/ disc)	Chlorophyll content (Mean ± SE) (SPAD)
0 (Control)	50.73 ± 1.64 a
5	48.17 ± 0.80 ab
10	46.86 ± 1.42 ab
15	45.10 ± 1.74 b
20	39.68 ± 0.42 c
25	39.72 ± 0.49 c
<i>p</i> Value	0.000
<i>F</i> Value	18.85
<i>df</i> Value	5

The values with different lower-case letters in the same column are significantly different according to the Tukey test ( $p < 0.05$ ).

**Table 2.** Effects of varying densities of *Tetranychus urticae* on the chlorophyll content of bean plants grown in pots

Density (mite/plant)	Chlorophyll content (Mean ± SE) (SPAD)					<i>p</i> Value	<i>F</i> Value	<i>df</i> Value
	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day	20 <sup>th</sup> day	25 <sup>th</sup> day			
0 (Control)	43.83 ± 0.46 Aa	42.10 ± 0.47 ABa	41.60 ± 0.61 ABCa	40.52 ± 0.50 BCa	39.40 ± 0.81 Ca	0.000	8.34	4
5	44.02 ± 0.77 Aa	39.61 ± 0.45 Bb	38.42 ± 0.53 Bb	37.22 ± 1.00 Bb	32.54 ± 0.79 Cb	0.000	30.83	4
10	43.46 ± 0.48 Aa	39.68 ± 0.37 Bb	38.16 ± 0.41 BCb	36.77 ± 0.80 Cb	31.77 ± 1.01 Db	0.000	39.72	4
15	43.67 ± 0.69 Aa	38.93 ± 0.71 Bb	35.53 ± 0.77 Cbc	31.31 ± 0.78 Dc	22.87 ± 0.78 Ec	0.000	112.15	4
20	42.14 ± 0.56 Aa	39.40 ± 0.65 ABb	36.52 ± 0.91 Bbc	30.71 ± 0.75 Cc	21.67 ± 0.94 Dcd	0.000	109.84	4
25	43.47 ± 0.57 Aa	39.33 ± 0.45 Bb	35.22 ± 0.59 Cc	28.52 ± 0.62 Dc	19.11 ± 1.03 Ed	0.000	196.22	4
<i>p</i> Value	0.286	0.001	0.000	0.000	0.000			
<i>F</i> Value	1.26	4.55	12.08	36.35	76.39			
<i>df</i> Value	5	5	5	5	5			

Means with different lower-case letters in the same column are significantly different according to the Tukey test ( $p < 0.05$ ). Means with different upper-case letters in the same row are significantly different according to the Tukey test ( $p < 0.05$ ).

$F = 18.85$ ,  $df = 5$ ). Moreover, 10 days after the release, the leaves with 10 or more mites were completely destroyed.

The chlorophyll contents of the pinto bean leaves of the potted plants are given in Table 2. The results showed that the chlorophyll content of the leaves also decreased with an increase in the density of mites and time of exposure as in the leaf discs. The chlorophyll levels of the noninfested plants were significantly higher than those infested with 5 or more mites per plant on the 10<sup>th</sup> day of the infestation ( $p = 0.001$ ,  $F = 4.55$ ,  $df = 5$ ). This was also demonstrated for all infestation levels during the rest of the experiment. At the end of the experiment, the mean chlorophyll content of noninfested bean leaves was 39.40 SPAD, while those of infested ones ranged from 19.11 to 32.54 SPAD.

Among the researchers working on this subject, Sivritepe et al. (2009) reported that the chlorophyll contents of Muskule and Sultana grapevine cultivars infested with 500 mites per plant at the 5-leaf stage were 27.62 and 26.10 SPAD at the end of a 6-day exposure period. Jayasinghe and Mallik (2010) detected that mite-infested tomato plants (400 mites per plant initially released) had significantly low total chlorophyll content ranging from 1.016 to 1.177 mg/g leaf at the 14th week after planting. Tehri et al. (2014) found that total chlorophyll contents at 0, 5, 10, 15 and 20 mites per initially infested leaf were 2.02, 1.13, 1.09, 1.06 and 1.04 mg/g, respectively after 60 days of *T. urticae* feeding on cucumber leaves. Shaabani et al. (2021) indicated that the chlorophyll contents of susceptible bean cultivars were 139.20 ± 13.38 and 113.61 ± 16.13 nmol/cm<sup>2</sup> leaf under noninfested and *T. urticae* infested (26.91 mites/4 cm<sup>2</sup> leaf) conditions, respectively. On the other hand, those of the resistant groups were 167.71 ± 11.08 and 145.21 ± 11.41 nmol/cm<sup>2</sup> leaf under noninfested and infested (8.16 mites/4 cm<sup>2</sup> leaf) conditions, respectively.

**Table 3.** Correlation of the chlorophyll content of bean leaf disc and potted plants with initial mite density and exposure time.

Mite Species	Chlorophyll Content	Correlation (r)	
		Mite Density	Exposure Time
<i>T. urticae</i>	Leaf disc	-0.846***	-----
	Potted plant	-0.392***	-0.719***
<i>P. latus</i>	Leaf disc	-0.348***	-0.698***
	Potted plant	-0.212***	-0.811***

----- Chlorophyll content of discs infested with *T. urticae* could be measured only once after infestation, as heavily infested discs were destroyed before the second measurement.

\*\*\*  $p < 0.001$

In the current study, the correlation analysis showed a negative relationship between chlorophyll content and *T. urticae* density as well as exposure time (Table 3). Similarly, Iatrou et al. (1995) conducted a study to determine the effects of *T. urticae* density (ranging from 1 to 32 mites per 1.5 cm<sup>2</sup> leaf) and feeding period (from 1 to 5 days) on the chlorophyll content of bean plant leaves. Their research showed a clear correlation between decreasing leaf chlorophyll content and increasing feeding duration and mite density. Park and Lee (2002) found that the total chlorophyll content of cucumber leaves was notably reduced by approximately 55% and 80% by feeding immatures and adults of *T. urticae*, respectively, at 1000 mite-days per 6 cm<sup>2</sup>. Landeros et al. (2004) noticed a significant decrease in the amount of chlorophyll content in roses affected by *T. urticae*. Sivritepe et al. (2009) further documented the observation that vines infested with *T. urticae* had lower chlorophyll content than noninfested vines. Tehri et al. (2014) discovered a strong negative correlation between the population of *T. urticae* and the total chlorophyll, chlorophyll a, and chlorophyll b content in cucumber leaves. Their data showed that the chlorophyll content decreased to 40-47.27% at the infestation density of 20 mites per grown leaf in a field setting compared to a noninfested leaf. However, a contrasting result was found by Bounfour et al. (2002). They indicated that the infestation of *T.*

*urticae* did not affect the chlorophyll content of raspberry leaves following 2 weeks of feeding. Bueno et al. (2009) also reported that the feeding of *T. urticae* did not affect the chlorophyll content of soybean leaves from 5 to 10 days and 12 days after infestation under field and greenhouse conditions, respectively.

On the other hand, it should be noted that the chlorophyll content in a plant can react sensitively to a variety of external factors. For example, Reddall et al. (2007) found that the chlorophyll content of cotton leaves infested with *T. urticae* was not reduced in the distal region, unlike the basal region. Atar et al. (2020) detected that the chlorophyll content of the plant can change according to its growth period and the species of plant, even without being exposed to mites. Furthermore, Shaabani et al. (2021) observed chlorophyll levels of bean plants that had been infested with *T. urticae* every ten days from 45 to 75 days after the planting. Their findings suggested that mite feeding caused a decrease in the chlorophyll content of the plants, from 14% in the resistant plants to 20% in the susceptible plants in comparison to the noninfested plants. Therefore, the chlorophyll content in plants can be easily affected by external factors, and it is important to be aware of this to ensure the health and growth of the plant.

#### *Effects of different densities and exposure times of Polyphagotarsonemus latus on the chlorophyll content of bean plants*

The chlorophyll contents of pinto bean leaf discs infested with *P. latus* are presented in Table 4. After 5 days of being exposed to the pest, no significant differences were detected in the mean chlorophyll content of the infested and noninfested discs. However, the leaf discs infested at any level had a noticeably lower chlorophyll content compared to the noninfested discs after 10 days of exposure to the pest ( $p=0.000$ ,  $F=7.21$ ,  $df=5$ ).

**Table 4.** Effects of varying densities of *Polyphagotarsonemus latus* on the chlorophyll content in bean leaf discs after five and ten days of feeding.

Density (mite/ disc)	Chlorophyll content (Mean $\pm$ SE) (SPAD)		<i>p</i> Value	<i>T</i> Value	<i>df</i> Value
	5 <sup>th</sup> day	10 <sup>th</sup> day			
0 (Control)	44.85 $\pm$ 1.34 Aa	42.28 $\pm$ 1.76 Aa	0.374	0.98	5
5	42.53 $\pm$ 1.73 Aa	34.31 $\pm$ 1.29 Bb	0.014	3.72	5
10	44.11 $\pm$ 0.99 Aa	33.21 $\pm$ 0.87 Bb	0.001	6.54	5
15	41.81 $\pm$ 0.79 Aa	33.69 $\pm$ 1.52 Bb	0.009	4.09	5
20	44.19 $\pm$ 2.18 Aa	33.55 $\pm$ 1.60 Bb	0.005	4.68	5
25	39.97 $\pm$ 1.35 Aa	29.67 $\pm$ 1.05 Bb	0.006	4.63	5
<i>p</i> Value	0.202	0.000			
<i>F</i> Value	1.56	7.21			
<i>df</i> Value	5	5			

Means within the same column are significantly different if lower-case letters are different according to the Tukey test ( $p < 0.05$ ). Means with different upper-case letters in the same row are significantly different ( $p < 0.05$ ).

**Table 5.** Effects of varying densities of *Polyphagotarsonemus latus* on the chlorophyll content of bean plants grown in pots

Density (mite/plant)	Chlorophyll content (Mean $\pm$ SE) (SPAD)					<i>p</i> Value	<i>F</i> Value	<i>df</i> Value
	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day	20 <sup>th</sup> day	25 <sup>th</sup> day			
0 (Control)	46.31 $\pm$ 0.44 Aa	42.24 $\pm$ 0.94 Ba	41.52 $\pm$ 0.93 Ba	40.42 $\pm$ 0.97 Ba	39.75 $\pm$ 1.25 Ba	0.000	6.22	4
5	44.16 $\pm$ 0.69 Aa	40.16 $\pm$ 0.69 Ba	38.68 $\pm$ 0.82 Bab	31.55 $\pm$ 1.26 Cb	23.93 $\pm$ 0.86 Db	0.000	81.63	4
10	44.54 $\pm$ 0.42 Aa	40.23 $\pm$ 0.51 Ba	38.82 $\pm$ 0.64 Bab	28.63 $\pm$ 1.24 Cbc	22.33 $\pm$ 0.59 Dbc	0.000	153.11	4
15	45.76 $\pm$ 0.69 Aa	41.30 $\pm$ 0.62 Ba	38.67 $\pm$ 0.66 Bab	27.98 $\pm$ 0.81 Cbc	20.86 $\pm$ 0.88 Dbc	0.000	195.07	4
20	45.95 $\pm$ 0.84 Aa	42.12 $\pm$ 0.75 Ba	37.35 $\pm$ 0.71 Cb	29.61 $\pm$ 0.89 Dbc	21.34 $\pm$ 0.59 Ebc	0.000	144.08	4
25	45.23 $\pm$ 0.64 Aa	42.52 $\pm$ 0.49 Aa	37.68 $\pm$ 0.82 Bb	26.97 $\pm$ 1.11 Cc	19.60 $\pm$ 0.87 Dc	0.000	185.86	4
<i>p</i> Value	0.159	0.052	0.003	0.000	0.000			
<i>F</i> Value	1.62	2.27	3.88	21.47	74.22			
<i>df</i> Value	5	5	5	5	5			

Means within the same column are significantly different if lower-case letters are different according to the Tukey test ( $p < 0.05$ ). Means with different upper-case letters in the same row are significantly different ( $p < 0.05$ ).

The chlorophyll contents of pinto bean leaves of potted plants are given in Table 5. The chlorophyll levels of infested plants were not significantly different from those of noninfested plants on 5<sup>th</sup> and 10<sup>th</sup> days of the infestation ( $p=0.159$ ,  $F=1.62$ ,  $df=5$ ;  $p=0.052$ ,  $F=2.27$ ,  $df=5$ , respectively). Noninfested plants had a much higher chlorophyll content than infested plants from the fourth measurement (20<sup>th</sup> day) at all infestation levels ( $p=0.000$ ,  $F=21.47$ ,  $df=5$ ). The chlorophyll value of infested plants was quite low, ranging between 19.60 SPAD and 23.93 SPAD while the mean chlorophyll content of noninfested plants was 39.75 SPAD at the end of the experiment (after 25 days). Among the researchers working on this subject Latha and Hunumanthraya (2018) reported that the chlorophyll contents of chili plants at *P. latus* density that ranged from 0.54 to 1.14 mites per leaf were between 19.20 - 84.40 SPAD values.

According to statistical analysis, there was a significant negative correlation between the chlorophyll content and *P. latus* densities and exposure time (Table 3). This indicates that the chlorophyll content of the bean plant decreased as the initial density of *P. latus* and the exposure time of the leaves increased in accordance with the report of Latha and Hunumanthraya (2018) on chili plants. Jiang et al. (2019) discovered that the relative chlorophyll content of cucumber leaves decreased significantly when it was severely infested with *P. latus*, however, this only happened under greenhouse conditions. Evaristo et al. (2013) also conducted a study and found that the *P. latus* infestation did not influence the total chlorophyll concentration of *Jatropha curcas* L. (Euphorbiaceae) plants ten days after the infestation. Similarly, Girish et al. (2019) found no relationship between the *P. latus* population level (including egg and active stages between 15 and 75 days from planting) and the chlorophyll content in chili leaves. The results of the last two studies may be because the plants were not exposed long enough to the mites to cause a reduction in the chlorophyll content.

In summary, this study showed that the leaf discs infested with *P. latus* at all levels had lower chlorophyll content than the noninfested discs after 10 days of feeding. Conversely, due to the drying of all discs infested with 10 or more *T. urticae* within 10 days of infestation, there were no leaf discs left to measure the amount of chlorophyll during the same period. Furthermore, *T. urticae* feeding influenced the chlorophyll content of the potted plants at all infestation levels 10 days after release. However, only from the fourth measurement (20 days after release), infested plants with *P. latus* had statistically lower chlorophyll content than noninfested plants.

It can be asserted that the smaller size of *P. latus* compared to that of *T. urticae* suggests that this mite feeds on a much smaller quantity of cellular content. Differences between life table parameters of two mite species may also cause different feeding capacities, which can affect chlorophyll content. These could partially explain why the reduction in the chlorophyll level of infested plants with *P. latus* was lower and occurred after a longer period than that of *T. urticae*.

Moreover, we believe that the level of chlorophyll content impacted by mite feeding may vary significantly depending on a multitude of factors such as mite species, population density, feeding duration, host plant susceptibility, and plant part mites fed on. These are among the essential elements to consider when attempting to ascertain the effect of mite feeding on chlorophyll content.

#### ACKNOWLEDGEMENTS

We are grateful to Eddie A. Ueckermann (North-West University, South Africa) for confirming the identification of *P. latus*. We also thank the anonymous reviewers for their deep, thorough reviews and constructive comments that helped us to improve the manuscript. Part of this research was presented as a poster at the 2<sup>nd</sup> International Congress on

Engineering and Life Science (11-14 April 2019, Kastamonu, Türkiye) and 8<sup>th</sup> Plant Protection Congress with International Participation (August 24-28, 2021, Online), and was published as an abstract in the abstract book.

#### Author's Contributions

Authors declare the contribution of the authors is equal.

#### Statement of Conflict of Interest

The authors have declared no conflict of interest.

#### ÖZET

Çalışmada, *Tetranychus urticae* Koch ve *Polyphagotarsonemus latus* (Banks) (Acari: Tetranychidae, Tarsonemidae)'un farklı başlangıç yoğunluk seviyeleri ve beslenme sürelerinin barbunya fasulyesi [*Phaseolus vulgaris* L. (Fabaceae)]'nin klorofil içeriğine etkisi araştırılmıştır. Denemeler, 3 cm çapındaki yaprak diskleri ve saksılı bitkiler üzerinde yürütülmüştür. Yaprak disk denemelerinde, her diske 0 (kontrol), 5, 10, 15, 20 ve 25 dölleniş ergin dişi *P. latus* veya *T. urticae* (24-48 saat yaşlı) yerleştirilmiştir. Bulaştırmadan beş ve on gün sonra klorofil ölçümleri yapılmıştır. Saksılı bitki denemesi için ise, fasulye bitkileri, farklı yoğunluklarda *P. latus* veya *T. urticae* (5, 10, 15, 20 ve 25 ergin dişi/bitki) ile bulaştırılmışken, temiz bitkiler kontrol grubunu oluşturmuştur. Klorofil ölçümleri 5 gün arayla toplam 5 kez gerçekleştirilmiştir. Salımdan 5 gün sonra, disk başına 15, 20 ve 25 *T. urticae* bulunduran disklerin klorofil içeriğinin, kontrol disklerinden daha düşük olduğu belirlenmiştir. Disk başına 10 ve üzeri miktarda *T. urticae* ile bulaşık diskler ise ikinci ölçüme kadar kurumuşlardır. Diğer yandan, bulaştırmadan 5 gün sonra, *P. latus* ile bulaşık ve temiz yaprak disklerinin klorofil içerikleri arasında herhangi bir farklılık belirlenmemiştir. Salımdan ancak 10 gün sonra, klorofil içeriği, tüm yoğunluk seviyelerinde, kontrol grubuna göre istatistiki olarak düşük çıkmıştır. Saksılı bitki denemelerinde ise, bulaşık bitkilerin klorofil içeriklerinin, temiz bitkilerinkinden *T. urticae* için salımın 10, *P. latus* için ise denemenin 20. gününden itibaren tüm bulaşıklık seviyelerinde önemli ölçüde düşük olduğu belirlenmiştir. Sonuçlar, fasulye bitkisinin klorofil içeriği ile akar yoğunluğu ve beslenme süresi arasında negatif bir korelasyon olduğunu ve klorofil içeriğinin artan akar yoğunluğu ve zamanla orantılı olarak azaldığını göstermiştir. Ayrıca *P. latus*'un, fasulye bitkisinin klorofil içeriğini etkileyebilmesi için, *T. urticae* ye göre daha uzun süre beslenmesi gerektiği de tespit edilmiştir.

Anahtar kelimeler: beslenme süresi, akar yoğunluğu, korelasyon, zarar değerlendirmesi, iki noktalı kırmızı örümcek, sarı çay akarı

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**Cite this article:** Akyazı, R., & Soysal, M. (2024). Effect of *Tetranychus urticae* and *Polyphagotarsonemus latus* (Acari: Tetranychidae, Tarsonemidae) at different infestation levels and feeding durations on chlorophyll content of bean plants. *Plant Protection Bulletin*, 64-1. DOI: 10.16955/bitkorb.1302239

**Atf için:** Akyazı, R., & Soysal, M. (2024). *Tetranychus urticae* ve *Polyphagotarsonemus latus*'un (Acari: Tetranychidae, Tarsonemidae) farklı yoğunluk düzeyleri ve beslenme sürelerinde fasulye bitkisinin klorofil içeriğine etkisi. *Bitki Koruma Bülteni*, 64-1. DOI: 10.16955/bitkorb.1302239