PLANT PROTECTION BULLETIN

Bitki Koruma Bülteni

Volume 64 Number 1 January-March 2024 ISSN 0406-3597 E-ISSN 1308-8122

Published by Plant Protection Central Research Institute Ankara, Turkey

TAGEM JOURNALS

PLANT PROTECTION BULLETIN / BİTKİ KORUMA BÜLTENİ

Volume 64	No 1	January-March 2024

Aims and Scope

Plant Protection Bulletin has been published by Plant Protection Central Research Institute since 1952. The journal is published four times a year with original research articles in English or Turkish languages on plant protection and health. It includes research on biological, ecological, physiological, epidemiological, taxonomic studies and control methods of disease, pest, weed, and natural enemies that cause damage in plants and plant products. In addition, studies on residue, toxicology and formulations of plant protection products and plant protection machinery are also included. Article evaluation process is based on double blind referee system and published as open access. Annual biological studies, short communication, first report and compilations do not publish in the journal.

Abstracted/indexed by EBSCOhost, CAB Abstracts, Clarivate Analytics-Zoological Record, TR-Dizin.

Plant Protection Bulletin is quarterly publication of the Directorate of Plant Protection Central Research Institute on behalf of General Directorate of Agricultural Research and Policies.

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http://dergipark.gov.tr/bitkkorb

Grafik Tasarım : Filiz ERYILMAZ

Printing: Tarım ve Orman Bakanlığı - Eğitim ve Yayım Dairesi Başkanlığı İvedik Caddesi Bankacılar Sokak No: 10 Yenimahalle, Ankara Türkiye

Tel: (0312) 315 65 55 - Fax: 0312 344 81 40

E-Posta : yayin@tarim.gov.tr

PLANT PROTECTION BULLETIN / BİTKİ KORUMA BÜLTENİ

Volume 64 | No 1 | January-March 2024

Contents / İçindekiler

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
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 10th and 20th 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 dis 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^2 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, F=18.85, df=5). Moreover, 10 days after the release, the leaves with 10 or more mites were completely destroyed.

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
p Value	0.000
F Value	18.85
df Value	5

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

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 10th 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^2 leaf under noninfested and *T. urticae* infested (26.91 mites/4 cm^2 leaf) conditions, respectively. On the other hand, those of the resistant groups were 167.71±11.08 and 145.21±11.41 nmol/cm^2 leaf under noninfested and infested (8.16 mites/4 cm^2 leaf) conditions, respectively.

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)					*p* Value	*F* Value	*df* Value
	5th day	10th day	15th day	20th day	25th 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
p Value	0.286	0.001	0.000	0.000	0.000			
F Value	1.26	4.55	12.08	36.35	76.39			
df 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).

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
T. urticae	Leaf disc	-0.846***	-----
	Potted plant	-0.392***	-0.719***
P. latus	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^2 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^2. 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 infected 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 an 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 ± SE) (SPAD)				
	5^{th} day	10^{th} day	*p* Value	*T* Value	*df* Value
0 (Control)	44.85 ± 1.34 Aa	42.28 ± 1.76 Aa	0.374	0.98	5
5	42.53 ± 1.73 Aa	34.31 ± 1.29 Bb	0.014	3.72	5
10	44.11 ± 0.99 Aa	33.21 ± 0.87 Bb	0.001	6.54	5
15	41.81 ± 0.79 Aa	33.69 ± 1.52 Bb	0.009	4.09	5
20	44.19 ± 2.18 Aa	33.55 ± 1.60 Bb	0.005	4.68	5
25	39.97 ± 1.35 Aa	29.67 ± 1.05 Bb	0.006	4.63	5
p Value	0.202	0.000			
F Value	1.56	7.21			
df 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 ± SE) (SPAD)					*p* Value	*F* Value	*df* Value
	5th day	10th day	15th day	20th day	25th day			
0 (Control)	46.31 ± 0.44 Aa	42.24 ± 0.94 Ba	41.52 ± 0.93 Ba	40.42 ± 0.97 Ba	39.75 ± 1.25 Ba	0.000	6.22	4
5	44.16 ± 0.69 Aa	40.16 ± 0.69 Ba	38.68 ± 0.82 Bab	31.55 ± 1.26 Cb	23.93 ± 0.86 Db	0.000	81.63	4
10	44.54 ± 0.42 Aa	40.23 ± 0.51 Ba	38.82 ± 0.64 Bab	28.63 ± 1.24 Cbc	22.33 ± 0.59 Dbc	0.000	153.11	4
15	45.76 ± 0.69 Aa	41.30 ± 0.62 Ba	38.67 ± 0.66 Bab	27.98 ± 0.81 Cbc	20.86 ± 0.88 Dbc	0.000	195.07	4
20	45.95 ± 0.84 Aa	42.12 ± 0.75 Ba	37.35 ± 0.71 Cb	29.61 ± 0.89 Dbc	21.34 ± 0.59 Ebc	0.000	144.08	4
25	45.23 ± 0.64 Aa	42.52 ± 0.49 Aa	37.68 ± 0.82 Bb	26.97 ± 1.11 Cc	19.60 ± 0.87 Dc	0.000	185.86	4
p Value	0.159	0.052	0.003	0.000	0.000			
F Value	1.62	2.27	3.88	21.47	74.22			
df 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 5th and 10th 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 (20th 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 2nd International Congress on

Engineering and Life Science (11-14 April 2019, Kastamonu, Türkiye) and 8th 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 döllenmiş 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

Atıf 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

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Evaluation of propylene oxide fumigation against *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae) in dried figs and hazelnuts

Kuru incir ve fındıkta *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae)'ya karşı propilen oksit fümigasyonunun değerlendirilmesi

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ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1339441
Received : 08-08-2023
Accepted : 03-11-2023

Keywords:
fig moth, dried figs, hazelnuts, fumigant, propylene oxide

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ABSTRACT

This study aims to investigate the potential use of propylene oxide (PPO) for rapid control of the fig moth, *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae). To this end, the biological efficacy of PPO (10 µl/l) against all biological stages of the fig moth was evaluated for a short exposure period (4 hours) under three different conditions: PPO alone (normal pressure), PPO+vacuum (100 mm Hg low pressure), and PPO+CO_2 (92% CO_2 concentration) in the absence and presence of dried figs and hazelnuts. In the absence of commodities, the biological tests showed 100% mortality rates in all biological stages of *E. cautella*, except for the pupa stage, when using the PPO+vacuum treatment. However, the PPO alone and PPO+CO_2 treatments did not achieve a 100% mortality rate for the biological stages of *E. cautella*. Conversely, in the presence of dried figs, the mortality rates for all biological stages of *E. cautella* ranged from 19.7% to 100% in the PPO+vacuum and PPO+CO_2 treatments. As for the shelled hazelnuts, all PPO treatments resulted in low mortality rates ranging from 0.7% to 10.6% with PPO+vacuum and PPO+CO_2 treatments. In conclusion, the study suggests that the PPO+vacuum treatment can have a viable potential for rapid insect control, particularly in dried figs, making it suitable for quarantine applications.

INTRODUCTION

Infestation of storage pests during drying and storage of dried figs and hazelnuts can cause significant problems in the dried fig and hazelnut industry (Yıldız 2013). Addressing the problem of storage pests is crucial to ensure the sustainable growth and development of the dried fig and hazelnut sector in Türkiye and to enable the country to maintain its status as a leading producer and exporter of dried figs and hazelnuts worldwide. The following pests can be mentioned as significant threats to stored dried figs and hazelnuts: Fig moth (*Ephestia cautella* Walk), Indian meal moth (*Plodia*

interpunctella Hübner; Pyralidae: Lepidoptera), dried fruit beetle (*Carpophilus* spp.; *Nitidulidae*: Coleoptera), sawtoothed grain beetle (*Oryzaephilus surinamensis* L.; Silvanidae: Col.) and dried fruit mite (*Carpoglyphus lactis* L.; *Carpoglyphidae*: Acari) (Turanlı 2003). In particular, the fig moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae), which is among the stored dried fruit pests and nuts, rapidly infests the products and causes significant damage by forming high populations (Tripathi 2018). Its larvae feed on the fruit, leading to a reduction in the fruit's quality (Küçüktopçu 2023). In addition, the presence of the pest's body residues, excreta, and secreted web-like substances further contributes to a significant reduction in the quality and commercial value of the product (Bilgili 2015, Celik et al. 2008, Ferizli and Emekci 2013). Effective measures to control these storage pests are essential to preserve the integrity of dried figs and hazelnuts during storage, transport, and export, thus safeguarding the reputation of the industry and its economic importance for the country.

Türkiye is known worldwide as the largest producer and exporter of dried figs (Özer 2020). With a significant share of 58% and a production volume of 85.500 tons in 2020-2021, it is at the forefront of global dried fig production (INC 2021). Such a significant production volume underlines dominant position of the country in the dried fig sector. Similarly, Türkiye, which accounts for about 80% of the world's hazelnut production, is the world's leading country in hazelnut production and export, producing 684 thousand tons of hazelnuts on 7.4 million acres of land in 2021 (Uzundumlu et al. 2022). In 2021, the hazelnut production area in Türkiye was 7.4 million da and the total hazelnut production was 684 thousand tons (Bayyurt and Kocakoç 2023). Hazelnuts are used as a basic ingredient in various food products such as chocolate, biscuits, confectionery desserts, cakes, ice cream, meals, and salads. This shows that hazelnut is a versatile material and plays an important role in the food industry. As a result, ensuring the sustainability and growth of the dried fig and hazelnut sectors has become a critical priority in supporting the agricultural and economic progress of Türkiye.

Methyl Bromide (MeBr) has been widely used in controlling stored product pests due to its broad-spectrum activity, low cost, and fast insect-killing ability (Fields and White 2002). However, its application has been restricted due to its ozone-depleting effect on the ozone layer (Schneider et al. 2003, Tütüncü and Emekci 2014). Under the Montreal Protocol, MeBr has been banned in developed countries since 2005 and in Türkiye since 2007, except for specific quarantine and pre-shipment applications. Given the restrictions on the use of MeBr and its impact on the environment, research into possible methods of replacing MeBr is becoming increasingly crucial. The hard-shell fruit and nut industry relies mainly on phosphine as a chemical fumigant to prevent insect infestation after harvest. However, due to the carcinogenic effect of phosphine (Alavanja et al. 1990, Garry et al. 1990), its flammability (Ohtani et al. 1989), insect resistance (Benhalima et al. 2004, Daglish et al. 2014, Gautam et al. 2016, Sağlam et al. 2015) and the requirement for a longer exposure time (4 to 6 days or more), the use of phosphine is not suitable for quarantine applications (Isikber et al. 2006). The lack of readily available potentials that can quickly control insect infestations has led to significant adverse effects on the dried fig and hazelnut industry due to the loss of MeBr. Therefore, there is a critical need to develop new fumigants that can achieve rapid insect mortality (exposure periods of less than one day), especially for quarantine treatments.

Propylene oxide (PPO), a clear, colorless, volatile liquid fumigant, with an ether-like odor and 35 °C boiling point, is a significant organic chemical raw material (Weast et al. 1986). In the study by Meylan et al. (1986), the impact of PPO as a fumigant on human health and the environment was investigated. They reported that PPO poses significantly lower environmental risks compared to MeBr. They also observed that PPO has no ozone-depleting properties and rapidly converts to non-toxic propylene glycol in the soil and human stomach. The main disadvantage of PPO is its flammability in air, which ranges from 3% to 37%. Therefore, precautions need to be taken to reduce the fire risk to ensure the safe use of PPO. One such measure is using PPO gas under low pressure or in an atmosphere enriched with carbon dioxide (CO_2). Recent studies have shown that PPO has an increased fumigation potential when used at low pressure (100 mm Hg) for a short exposure period (Isikber et al. 2006, Isikber et al. 2012, Navarro et al. 2004). Although previous laboratory studies have demonstrated that PPO can be effective against stored product pests at low pressure and enriched-CO_2 atmosphere for short-term exposure, there is limited research available in the literature regarding the efficacy of PPO with vacuum and CO_2 against stored product Lepidopteran insects in the presence of the commodity.

This study aimed to investigate the potential use of PPO for the rapid control of the fig moth as a potential fumigant to MeBr. For this reason, the biological efficacy of PPO alone at a concentration of 10 µl/l, 10 µl/l PPO+vacuum (100 mm Hg low pressure), and 10 µl/l PPO+CO_2 (92% CO_2 concentration) was investigated against all developmental stages of *E. cautella* (eggs, larvae, pupae and adults) in the absence and presence of dried figs and hazelnuts.

MATERIALS AND METHODS

Products used in biological tests

In the biological tests, sun-dried Sarılop (Calimyrna) (*Ficus carica* L.) (Moraceae) dried fig variety with 21% ± 1 moisture content and shelled Çakıldak hazelnut variety, which are hybrids of *Corylus avellana* L. and *Corylus maxima* Mill. (Betulaceae), containing 10-12% moisture content was utilized.

The source and rearing of insect culture used in biological tests

In biological tests, all biological stages of *Ephestia cautella* were used. The main material of the *E. cautella* culture used in biological tests was obtained from Namık Kemal University, Faculty of Agriculture, Plant Protection Department Laboratory.

For rearing of *E. cautella* culture, a food mixture was prepared to consist of 350 g cornmeal for every 2 kg of wheat bran, along with 350-400 ml of glycerin, 450-500 ml of glucose syrup, and 1 teaspoon of inactive yeast. The wheat bran and cornmeal were kept in a deep freezer at -20 °C for 3-4 days to prevent insect infestation. The ingredients were thoroughly mixed by hand and then processed in a mixer. To obtain the eggs of adult *E. cautella*, the adults were transferred from 3-L culture jars using the laboratory type of vacuum pump (KNF, Germany) to a 3-L culture glass jar, and the culture jar's mouth was covered with a mesh. After keeping the culture jar with mixed-sex adults in an air-conditioning cabinet for one day, the jar was inverted to allow the eggs to fall onto a piece of paper. The collected eggs ranging from 400 to 500 were added to 3-L jars containing 350-400 g of food and covered with a mesh that allowed air to pass through. The culture jars were placed in completely dark conditions within an incubator at a constant temperature of 30±1 ºC and 65±5% humidity. Throughout the study, the insect culture was monitored daily, and these procedures were repeated to ensure continuity.

Fumigant

The fumigant PPO was obtained from SERVA Electrophoresis GmbH company (Heidelberg, Germany) with >%99 purity (CAS no. 75569, Cat. no. 33715). PPO was transferred into a 100 ml glass bottle and securely sealed with a septum. During the treatment phase, a predetermined amount of PPO was drawn from the glass bottle using a gas-tight micro syringe (Hamilton, Switzerland).

Carbon dioxide (CO_2) gas

Carbon dioxide (CO_2) used in biological tests has been supplied by Linde Gas (Ankara, Türkiye) Company in a pressurized steel cylinder with a purity of 99%.

Fumigation chamber

The fumigation chamber consists of 3-L glass jars, each equipped with metal lids, a metal tube housing an inlet and an outlet hole. Two silicone flexible hoses, each with a length of 5 cm and a diameter of 0.62 cm, have been placed over the inlet and outlet metal tubes. These silicone hoses were securely attached using metal clamps to create a gas-tight environment. To ensure no gas leakage, silicone is carefully applied around the edges of the metal lids before closing them. This gas-tight system allows to use of vacuum and PPO safely without any leakage.

Biological tests conducted in a commodity-free atmosphere

In biological experiments, 20 adults, pupae (1 to 2 days old), late-stage larvae (28 to 32 days old), and 50 eggs (1 to 2 days old) of *E. cautella* were used. Each developmental stage was carefully placed inside separate 50 ml glass vials. Food medium was added to the vials to meet the larvae's dietary needs, filling approximately 1/3 of their volume (equivalent to 10 g for 50 ml vials). To allow the PPO gas to enter the vials and prevent the insects from escaping, the mouths of the vials were covered with a fine muslin mesh and securely fastened with rubber bands. Afterwards, each insect vial was placed in a 3-L glass jar with a metal lid (fumigation chamber). Thus, the tested insects and PPO gas were kept in a gas-tight atmosphere in the fumigation chamber. To apply PPO under a low-pressure (vacuum) atmosphere, the vacuum pump (KNF, Germany) evacuated the air from the 3-L fumigation chamber, effectively reducing the pressure to 100 mm Hg. To ensure accurate monitoring of the low-pressure level inside the fumigation chamber, the low-pressure level was measured using a Celesco model SE-2000 vacuum gauge. After achieving the desired low-pressure level, PPO at 10 µl/l was injected into the fumigation chamber using a 50 µl gas-tight micro syringe (Hamilton Company, Bonaduz, Switzerland).

For applying PPO under a CO_2 atmosphere, we first established a low pressure of 60.8 mm Hg in the fumigation chamber. Subsequently, CO_2 gas was circulated within the fumigation chamber until the pressure returned to a normal atmospheric level. The CO_2 gas concentration inside the fumigation chamber was measured throughout this process using a precise CO_2/O_2 measurement device (CheckPoint, PBI-Dansensor, Denmark). Once the desired 92% CO_2level was achieved, PPO at a 10 µl/l c was injected into the fumigation chamber using a 50 µl micro syringe. In applying PPO alone (under normal pressure), 10 µl/l concentration of PPO was directly injected into the fumigation chamber. In the biological tests, all stages of *E. cautella* were exposed to a combination of 10 µl/l PPO alone, 10 µl/l PPO with 92%

CO_2, and a vacuum of 100 mm Hg for 4 hours. In addition, all developmental stages of *E. cautella* were exposed to separate treatments of vacuum (100 mm Hg low pressure) and CO_2 gas (92% CO_2 atmosphere) for 4 hours without PPO treatment. After completing the biological tests, the lids of the fumigation chamber were quickly closed and were kept in a completely dark climate chamber with a temperature of 26±1 °C and a relative humidity of 65±5% for 4 hours. To ensure the reliability and robustness of the results, each treatment of PPO was performed with 4 replicates and 4 control groups were included for each treatment.

Biological tests conducted in the presence of commodity

The biological tests conducted in the presence of products (figs and hazelnuts) were carried out following the same experimental procedures as those conducted without commodities. The only difference was using fumigation chambers formed within 3-liter glass jars with metal lids, where 1.3 kg of dried figs and shelled hazelnuts were placed.

Data processing and analysis

Following each treatment, the larvae, pupae, and adult insects were transferred to 200-milliliter jars containing standard diets. These containers were maintained at a temperature of 26 ± 1°C and a relative humidity of 70 ± 5% until they were inspected for mortality. The eggs, placed on Perspex slides, were also subjected to the same environmental conditions until the sites where they were laid were examined to determine egg hatch rates. Mortality counts for adults were made 4-5 d after exposure; for larvae, they were based on those insects that had failed to pupate 9 d after exposure; pupal mortality was based on those pupae that failed to produce adults 9 d after exposure; and egg hatch was counted 7 d after treatment.

Mortality data were corrected using Abbott's formula (Abbott 1925). All mortality data for each biological stage in PPO treatments were normalized using arcsine transformation. Subsequently, a two-way ANOVA was conducted using the GLM Procedure of SAS/STAT® 12.1 (SAS 2012), with PPO treatment and biological stage as the main factors. Mean mortality percentages for each biological stage and PPO treatment were separated using Tukey's HSD (Honestly Significant Difference) test.

RESULTS

Mortality of life stages of Ephestia cautella exposed to Propylene oxide treatments in a commodity-free atmosphere

The results showed that only PPO+vacuum achieved complete mortality (100%) of all life stages of *E. cautella* except its pupa stage, while PPO alone and PPO+CO_2 did not result in 100% mortality of life stages (Table 1). In contrast, PPO+vacuum exhibited significantly higher efficacy against all life stages than PPO alone and PPO+CO_2 (except the adult stage for PPO+CO_2). Similarly, PPO+CO_2 achieved significantly higher efficacy against all life stages than PPO alone. PPO+CO_2 and PPO alone generally produced very low larva and pupa stage mortalities, ranging from 8 to 38%. These results indicated that the larva and pupa were the most tolerant stages for PPO treatments, whereas the adult and egg were the most susceptible.

It was observed that there was no statistically significant difference in mortality rates of all life stages of *E. cautella* exposed to only 92% concentration of CO_2, 100 mm Hg vacuum and control treatments (Table 2). Only vacuum (100 mm Hg low pressure) and CO_2 gas (92% CO_2 concentration) treatment without PPO for 4 hours caused very low mortality levels of all life stages, ranging from 1.3% to 18.8%.

Table 1. Corrected percentage mortality (%) of life stages of *Ephestia cautella* exposed to PPO alone, PPO+vacuum, and PPO+CO_2 for 4 hours in a commodity-free atmosphere

PPO treatments	Corrected Percentage Mortality (%) ± Standard Error				F and P value
	Adult	Larva	Egg	Pupa	
PPO	74.7±3.4 Ba*	8.0±1.3 Cb	77.7±2.4 Ca	18.5±2.9 Cb	$F_{3,12}$=146.2 P<0.0001
PPO+vacuum	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	87.7±4.4 Ab	$F_{3,12}$=29.5 P<0.0001
PPO+CO_2	97.3±1.5 Aa	14.7±0.0 Bd	89.0±0.5 Bb	38.5±2.5 Bc	$F_{3,12}$=183 P<0.0001
Control	6.3±2.4	8.8±1.3	13.0±1.3	18.8±2.4	For PPO treatment: $F_{2,36}$=544.31, P<0.0001 For Biological stage: $F_{3,36}$=275.06, P<0.0001 For PPO treatment*Biological stage: $F_{6,36}$=52.08, P<0.0001
F and P value	$F_{2,9}$=37.1 P<0.0001	$F_{2,9}$=2054.3 P<0.0001	$F_{2,9}$=204.4 P<0.0001	$F_{2,9}$=80.3 P<0.0001	

*One-way ANOVA was applied to the mortality data for PPO treatments in each column and biological stages in each row. This means that a row with the same lower-case letter and a column with the same upper-case letter did not differ significantly (Tukey's HSD test at 5% level).

Table 2. Percentage mortalities (%) of all life stages of *Ephestia cautella* exposed to 92% CO_2, 100 mm Hg vacuum alone and control treatment for 4 hours

PPO treatments	Percentage Mortality (%) ± Standard Error			
	Adult	Larva	Egg	Pupa
Control	3.8±1.3 A*	0.0±0.0 A	4.0±0.0 A	16.3±1.3 A
100 mm Hg vacuum	5.0±2.0 A	1.3±1.3 A	6.5±0.5 A	18.8±1.3 A
92% CO_2	3.8±1.3 A	1.3±1.3 A	5.5±0.5 A	17.5±1.4 A
F and P value	$F_{2,9}$=0.05 P=0.9491	$F_{2,9}$=0.50 P=0.6224	$F_{2,9}$=1.42 P=0.055	$F_{2,9}$=0.90 P=0.4402

*One-way ANOVA was applied to the mortality data for the treatments. This means that a column with the same upper-case letter did not differ significantly (Tukey's HSD test at 5% level).

Mortality of all life stages of Ephestia cautella exposed to PPO treatments in the presence of dried figs

Only PPO+vacuum treatment resulted in 100% mortality of *E. cautella* adults in the presence of dried figs (Table 3). PPO+vacuum achieved higher mortality rates of all life stages of *E. cautella* than PPO alone and PPO+CO_2 (except the egg for PPO+CO_2). Similarly, PPO+CO_2 caused higher mortality rates in all life stages of *E. cautella* than PPO alone. PPO+CO_2 and PPO alone generally produced very low mortalities of larva and pupa stage, ranging from 1 to 40%, while they resulted in relatively high mortality rates of *E. cautella* adults and eggs, ranging from 64 to 94%. 10 ul/l PPO+vacuum was insufficient to kill 100% of *E. cautella* larvae and pupae, even though it caused 100% or close to 100% mortality rates of *E. cautella* adults and eggs (Table 3).

Mortality of all life stages of Ephestia cautella exposed to PPO treatments in the presence of shelled hazelnut

PPO alone, PPO+vacuum, and PPO+CO_2 treatments for 4 hours of exposure in the presence of shelled hazelnuts caused very low mortality rates of all life stages of *E. cautella*, ranging from 0 to 10.6% (Table 4). None of the PPO treatments in the presence of shelled hazelnuts had fumigant toxicity to all life stages of *E. cautella*.

Table 3. Corrected percentage mortality (%) of life stages of *Ephestia cautella* exposed to PPO alone, PPO+vacuum, and PPO+CO_2 for 4 hours in a commodity-free atmosphere

PPO treatments	Corrected Percentage Mortality (%) ± Standard Error				F and P value
	Adult	Larva	Egg	Pupa	
PPO	64.0±2.6 Cb*	1.3±1.3 Cd	82.9±0.9 Ba	13.6±2.9 Cc	$F_{3,12}$=168.33 P<0.0001
PPO+vacuum	100.0±0.0 Aa	36.8±2.1 Ad	93.6±1.5 Ab	65.2±2.9 Ac	$F_{3,12}$=247.06 P<0.0001
PPO+CO_2	86.6±1.5 Bb	19.7±1.3 Bd	94.1±1.0 Aa	40.9±2.9 Bc	$F_{3,12}$=315.20 P<0.0001
Control	6.3±2.4	5.0±2.0	6.5±1.5	17.5±3.2	For PPO treatment: $F_{2,36}$=277.40, P<0.0001 For Life stage: $F_{3,36}$=622.39, P<0.0001 For PPO treatment*Life stage: $F_{6,36}$=14.76, P<0.0001
F and P value	$F_{2,9}$=256.31 P<0.0001	$F_{2,9}$=66.62 P<0.0001	$F_{2,9}$=17.62 P=0.0008	$F_{2,9}$=68.70 P<0.0001	

*One-way ANOVA was applied to the mortality data for PPO treatments in each column and biological stages in each row. This means that a row with the same lower-case letter and a column with the same upper-case letter did not differ significantly (Tukey's HSD test at 5% level).

Table 4. Corrected percentage mortality (%) of all life stages of *Ephestia cautella* exposed to PPO alone, PPO+vacuum, and PPO+CO_2 for 4 hours in the presence of 1.3 kg of shelled hazelnut

PPO treatments	Corrected Percentage Mortality (%) ± Standard Error				F and P value
	Adult	Larva	Egg	Pupa	
PPO	0.0±0.0 Ac*	1.3±0.7 Abc	2.1±0.5 Aba	6.1±1.7 Aa	$F_{3,12}$=10.67 P=0.0011
PPO+vacuum	1.4±0.8 Ab	1.9±0.6 Ab	4.8±0.6 Aba	10.6±2.9 Aa	$F_{3,12}$=6.55 P=0.0072
PPO+CO_2	0.7±0.7 Ab	1.3±0.7 Ab	5.9±0.9 Aa	7.6±1.5 Aa	$F_{3,12}$=10.12 P=0.0013
Control	6.3±2.4	5.0±2.0	6.5±1.5	17.5±3.2	For PPO treatment: $F_{2,36}$=3.68, P<0.0001 For Life stage: $F_{3,36}$=25.35, P<0.0001 For PPO treatment*Life stage: $F_{6,36}$=0.39, P<0.0001
F and P value	$F_{2,9}$=1.29 P=0.3227	$F_{2,9}$=0.28 P=0.7642	$F_{2,9}$=9.42 P=0.0625	$F_{2,9}$=0.90 P=0.4409	

*One-way ANOVA was applied to the mortality data for PPO treatments in each column and biological stages in each row. This means that a row with the same lower-case letter and a column with the same upper-case letter did not differ significantly (Tukey's HSD test at 5% level).

DISCUSSION AND CONCLUSION

PPO has shown promising results even in a short exposure period in the studies on its use in controlling some stored product pests (Isikber et al. 2002, Isikber et al. 2006, Navarro et al. 2004). However, it is known that various factors during the fumigation process, such as environmental conditions (temperature, relative humidity) during fumigation, the type of commodity used, and the performance of the equipment, can affect the efficacy of commercial fumigation. Understanding the insecticidal effectiveness of PPO and its penetration ability within the commodity is of great importance for using PPO gas as a commercial fumigant in the food industry.

In the present study, PPO applied at 10 µl/l concentration under 100 mm Hg low pressure was enough to obtain 100% mortality in the adult and egg stages of *E. cautella*, whereas it was not enough to reach complete mortality in its pupa stage. In parallel to these results, Isikber et al. (2004) reported that the LC99 toxicities of PPO+vacuum for the adult, egg, larvae, and pupa of *E. cautella* were 5.7, 6.1, 13.0, and 14.4 µl/l, respectively. When PPO was applied under a vacuum or at a high CO_2 concentration, they had greater fumigant toxicity against all life stages of *E. cautella* than PPO alone. Similarly, Navarro et al. (2004) reported that when PPO was used in combination with 100 mm Hg vacuum and 92% concentration of CO_2, the mortality rates of all life stages of *T. castaneum* except the egg stage were significantly higher compared to those of PPO applied alone. The results from our bioassay experiments, where we subjected all life stages to either low pressure or CO_2 alone for 4 hours, demonstrated minimal mortality rates akin to those observed in the control group. Consequently, our findings assert that the combination of low pressure and CO_2 substantially heightened the potency of PPO against *E. cautella*. Notably, the utilization of low pressure or CO_2 in isolation exhibited negligible impact on the insects' well-being. Navarro et al. (2004) also reported that a 100 mm Hg vacuum and 92% concentration of CO_2 have a synergistic effect on the toxicity of PPO against insect pests. Previous studies conducted on several fumigants, particularly MeBr, and phosphine, have also demonstrated that insecticidal effectiveness could be enhanced by employing vacuum fumigation or blending with CO_2 (Calderon and Leesch 1983, Donahaye and Navarro 1989, Monro et al. 1966).

Based on our results from a single concentration test, the larva and pupa were the most tolerant stages when exposed to PPO alone for 4 hours. Similarly, Isikber et al. (2017) reported that the eggs and adults of *E. cautella* were the most sensitive life stage to PPO with an LC_{99} value of 16.52 and 18.91 µl/l for 4h, whereas pupae and larvae were the most tolerant with an LC_{99} value of 134.06 and 48.72 µl/l, respectively. The eggs and pupae of stored-product insects are commonly recognized to exhibit greater tolerance compared to larvae and adults when exposed to MeBr (Athanassiou et al. 2015), phosphine (Aulicky et al. 2015), carbonyl sulfide (Plarre and Reichmuth 1996) and sulfuryl fluoride (Athanassiou et al. 2012). Consequently, achieving effective control over the eggs of stored-product insects proves challenging using the majority of commonly employed fumigants and contact insecticides. Typically, significantly extended exposure periods are necessary to adequately manage the eggs. In contrast to phosphine, MeBr, and sulfuryl fluoride, PPO is easy to kill *E. cautella* eggs during short exposure periods, which is particularly

important in providing a potential for fumigants that have a weak effect or a long exposure time on eggs.

There were significant differences in the toxicities of PPO alone, PPO+vacuum, and PPO+CO_2 treatments against all life stages of *E. cautella* in the presence of dried figs. PPO+vacuum achieved higher mortality rates of all life stages of *E. cautella* than PPO alone and PPO+CO_2 (except the egg for PPO+CO_2). Even though 10 µl/l PPO+vacuum caused 100% or close to 100% mortality rates of *E. cautella* adults and eggs, it was not enough to kill 100% of *E. cautella* larvae and pupae. It shows that there is a need to increase PPO concentration to achieve the complete mortality of all life stages of *E. cautella* in the presence of dried figs. Generally, mortality rates of all life stages of *E. cautella* decreased when PPO alone, PPO+vacuum, and PPO+CO_2 were applied in the presence of dried figs. This decline could likely be attributed to the pronounced gas adsorption by the commodity, leading to a reduction in the accessible concentration of active gas. This phenomenon was also noted in tests of various fumigants on *Tribolium castaneum* (Herbst) (Punj 1969); the LC_{50} values indicated a notable increase, ranging from 2.7 to 7.5 times, during fumigation exercises when paddy and groundnut kernels were present in comparison to treatments without these commodities.

On the other hand, in the presence of shelled hazelnuts, PPO alone, PPO+vacuum, and PPO+CO_2, and 100 mm Hg vacuum treatments gave very low mortality rates of all life stages of *E. cautella* ranging from 0 to 10.6%. There were dramatic decreases in mortality rates of all life stages of E. cautella when PPO alone, PPO+vacuum, and PPO+CO_2 were applied in the presence of hazelnuts. The reduction in mortality rates of all life stages was much higher in the presence of hazelnuts than in the presence of dried figs. This situation may be due to the higher PPO absorption rate of hazelnuts compared to those of dried figs. Isikber et al. (2006) documented significant sorption of PPO by oily products like peanuts, almonds, and walnuts following a 5-hour exposure period. The level of sorption was notably substantial ranging between 87% and 91% of the initial concentration. Zettler et al. (2003) also found that PPO sorption in almonds, pecans, and walnuts reached 97.3%, 99.2%, and 98.6% f, respectively, within 48 hours of the start of the fumigation process. In contrast to the nuts, Isikber et al. (2012) noted that PPO was absorbed to a lesser extent in dried figs (50% of the initial PPO concentration). These findings indicate that the toxicity of PPO to insects of stored products varies depending on the type of fumigated commodity, as the type of commodity can strongly influence the absorption of PPO. The results of this study show that the combination of PPO with 100 mm Hg low pressure and 92% CO_2 can be used as a potential alternative fumigant for rapid control of insect contamination, especially in dried figs. Nonetheless, additional investigations are imperative to acquire comprehensive insights into PPO's ability to permeate commodity masses, its potential phytotoxicity, and its ramifications for commodity quality. To realize the practical application of PPO on a commercial scale, precise treatment protocols need to be established to effectively control insect pests that infest stored dried figs.

ACKNOWLEDGEMENTS

This study contains some data from the master's thesis conducted at Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection.

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

Bu çalışmanın temel amacı, propilen oksit (PPO)'in *Ephestia cautella*'nın hızlı kontrolü için potansiyel kullanımını araştırmaktır. Bu amaçla, PPO'nun (10 µl/l) incir güvesinin tüm biyolojik aşamalarına (yumurta, larva, pupa ve ergin) karşı biyolojik etkinliği, ürünlü (kuru incir ve fındık) ve ürünsüz ortamlarda üç farklı koşul altında [10 µl/l PPO tek başına (normal basınç), 10 µl/l PPO+vakum (100 mm Hg düşük basınç) ve 10 µl/l PPO+CO_2 (%92 CO_2 konsantrasyonu)] kısa maruz kalma süresince (4 saat) değerlendirilmiştir. Ürünsüz ortamda gerçekleştirilen biyolojik testlerde, PPO+vakum uygulamasında zararlının pupa dönemi hariç diğer tüm biyolojik dönemlerinde (ergin, yumurta ve larva) %100 ölüm oranı tespit edilirken, tek başına PPO ve PPO+CO_2 uygulamalarında *E. cautella*'nın biyolojik dönemlerinde hiçbir zaman %100 ölüm oranına ulaşamamıştır. Elde edilen bulgular sonucunda, *E. cautella*'ya karşı PPO+vakum uygulamasının tek başına PPO ve PPO+CO_2 uygulamalarına kıyasla daha yüksek insektisidal etkinlik gösterdiği tespit edilmiştir. Kuru incir kullanılan ortamda yürütülen biyolojik testlerde, PPO+vakum ve PPO+CO_2 uygulamalarında *E. cautella*'nın tüm biyolojik dönemlerinde meydana gelen ölüm oranları %19.7 ile %100 arasında değişiklik göstermiştir. Diğer yandan kabuklu fındık kullanılan ortamda yürütülen biyolojik testlerde, tüm PPO uygulamalarında %0.7 ile %10.6 arasında değişen düşük ölüm oranları gözlemlenmiştir. Bu bakımdan yapılan çalışma sonucunda, kuru incir ve kabuklu fındık bulunan ortamlarda yürütülen tüm PPO uygulamalarının *E. cautella*'ya karşı toksisitelerinde önemli farklılıklar tespit edilmiştir. Genel olarak yapılan bu çalışma, özellikle kuru incirlerde böcek kontaminasyonunu hızla kontrol etmek için umut vaat eden bir alternatif fümigant olarak PPO'nun potansiyelini ortaya koymuştur. Ancak, bu tür uygulamaların pratikte kullanılabilirliğini belirlemek için daha büyük ölçekli ticari deneylere ihtiyaç duyulmaktadır.

Anahtar kelimeler: incir güvesi, kuru incir, fındık, fumigant, propilen oksit

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Cite this article: Küçüktopcu, Y., & Işıkber, A. A. (2024). Evaluation of propylene oxide fumigation against *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae) in dried figs and hazelnuts. Plant Protection Bulletin, 64-1. DOI: 10.16955/bitkorb.1339441

Atıf için: Küçüktopcu, Y., & Işıkber, A. A. (2024). Kuru incir ve fındıkta *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae)'ya karşı propilen oksit fümigasyonunun değerlendirilmesi. Bitki Koruma Bülteni, 64-1. DOI: 10.16955/bitkorb.1339441

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Investigation of consciousness levels of Çanakkale farmers on environmental and toxicological risks of pesticides

Çanakkale çiftçilerinin pestisitlerin çevresel ve toksikolojik riskleri konusundaki bilinç düzeylerinin araştırılması

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ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1338505
Received : 06-08-2023
Accepted : 14-11-2023

Keywords:
farmers' awareness, Cronbach Alpha coefficient, environment, Likert scale, questionnaire, pesticide

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ABSTRACT

Pesticides are increasingly being used against pests in agricultural fields. However, unconscious use of pesticides results in health risks for humans and the environment. Efforts should therefore be spent to reduce the negative impacts of pesticides. The objective of this study was to measure farmers' awareness of pesticide use in the agricultural fields of Çanakkale. The sample size was calculated using the "Simple Random Sampling Based on Means" method. The present survey was conducted with 270 farmers. Of the farmers who participated, 25.55% had 31-40 years of farming experience, 47.78% were primary school graduates, and 35.56% were 55-65 years old. Farmers' knowledge of pesticide use was assessed through Likert scale. Chi-Square test was used to investigate the relationship of farmers' knowledge level with education, age, farming experience, land size and farming type. Only education was significant. As the education level increased, the knowledge level increased. In terms of farmers' attitudes, 78.89% of the farmers indicated that they changed their clothes after spraying, 46.67% cared about the information on pesticide labels, 45.18% used protective equipment during spraying, 69.63% cared about PHI (pre-harvest intervals), and 15.92% had knowledge about MRL (Maximum Residue Limit). 41.85% disposed of remaining pesticide solutions to the edge of agricultural fields, 40.74% to garbage and 6.3% to environment. It was concluded based on the present findings that farmers need serious training on pesticide use and the potential effects of pesticides on human health and the environment.

INTRODUCTION

Pesticides constitute an essential component of agricultural production. They are highly effective in protecting agricultural products against pests and diseases. Annually about $5x10^6$ tons of pesticides are used worldwide. Such a huge quantity may end up with significant damage to non-target organisms, food chains and biodiversity. Unsafe or misuse of pesticides pose serious risks to human health and the environment (Verger and Boobis 2013). Knowledge, attitude, practices, and

behaviours on pesticide usage play a vital role in the prevention of the negative effects of pesticides. For sustainable agriculture, the environment and human health, and food safety should be considered together (WCED 1987). According to 2022 data, the total pesticide consumption of Türkiye was 55.374 tons (TSI 2023). Çanakkale ranks 8th among the provinces in Turkey in terms of pesticide use, with 2.014,7 tons of consumption in 2022 (Anonymous 2022). Most of these quantities are used in irrigated agriculture. Possible residues as a result of unconscious use of pesticides affect our foreign trade. EU-RASFF (European Union-Rapid Alert System for Food and Feed) portal lists the number of warnings issued to each country about pesticide residues on agricultural commodities marketed within the EU boundaries. In this sense, Türkiye was issued 354 warnings for pesticide residues on fresh vegetables and fruits in 2021, 292 warnings in 2022 and 109 warnings in 2023 (the first 7 months) (RASFF 2023).

It is important to reveal the awareness levels of producers to reduce the negative effects of pesticide use. A Likert scale was used in previous survey studies to assess the responses of participants who were asked questions about their pesticide use awareness (Akar and Tiryaki 2018, Erdil and Tiryaki 2020, Likert 1932). Scale reliability is generally checked through Cronbach's alpha coefficient (Anonymous 2023, Cronbach 1951, Kılıç 2016). Compatibility and consistency of the questions asked are highly significant issues (Kaygısız Ertuğ and Göksel 2019). In similar survey studies, Chi-Square independence test is commonly used to assess the correlations of pesticide use awareness levels with different variables such as age, education, and farming experience. The degree of relationship is calculated by Coefficient of Contingency, CC (Düzgüneş et al. 1983).

Fan et al. (2015) conducted a survey study with 307 farmers in the Wei River basin of northern China and investigated farmers' knowledge of pesticide use in agriculture. It was reported that farmers dealing with vegetable and fruit production had a higher knowledge of pesticide use than farmers dealing with cereal farming. However, they were using greater quantities of pesticides to ensure reliable yield levels. It was also observed that there was mistrust among farmers, retailers and government bodies.

Aldosari et al. (2018) conducted a survey study with 195 farmers in Central Punjab-Pakistan to assess sustainable use of pesticides. The majority of the respondents did not receive any training on sustainable use of pesticides and about 66.7% farmers did not receive any training on alternative pest control methods. A positive correlation was encountered between educational level and the other parameters of the farmers.

Quinteiro et al. (2013) conducted a survey study with pesticide applicators in Spain's Galician greenhouses to investigate the effects of education level on safe pesticide application. It was reported that there was no relationship between education level and safe pesticide application. Jallow et al. (2017) conducted a survey study with 250 farmers of Kuwait to investigate farmers' knowledge and behaviour of safe pesticide use. About 71% of participant farmers indicated pesticides as harmful to health, 65% harmful to the environment, 70% did not care about label information and 58% did not use any protective equipment.

Erdil and Tiryaki (2020) conducted a survey study with 384 farmers to assess farmers' awareness of pesticide use in agriculture in Manisa provinces of Türkiye. Farmers' knowledge of pesticide use was high in 63.8% of participants and moderate in 25.3% and low in 10.9%. These values in another study, carried out in Antalya province were 58.2%, 28.3% and 13.5%, respectively (Akar and Tiryaki 2018). Chi-Square independence test revealed that there was a significant correlation knowledge and education of Manisa farmers and between knowledge and farming experience of Antalya farmers. It was also observed that 12.7% of Antalya farmers and 15.4% of Manisa farmers did not care about PHI (preharvest interval) of the pesticides.

There are several other detailed studies on farmers' practices of pesticide use in different provinces of Türkiye such as in Isparta province (Demircan and Yılmaz 2005), Adana province (Akbaba 2010), Tokat province (Kızılaslan and Kızılaslan 2005), Bingöl province (Çelik and Karakaya 2017), Samsun province (Eryılmaz et al. 2018), Manisa province (Özyörük et al. 2019), and Gaziantep province (Atakan et al. 2020). Although there is no detailed study on pesticide applications in Çanakkale province, a few local studies have been conducted. In a study conducted in a village of Çanakkale-Evreşe-Yülüce (Cevizci et al. 2012), the use of pesticides was associated with cancer diseases. Researchers recommended safe use of pesticides and storage conditions, they also recommended farmers' training on safe use of pesticides. In another study, farmers living in Çanakkale province were asked about the safe use of pesticides and disposal of containers. Environmental impacts of pesticides were also assessed. The information obtained from the participant farmers and observations made in the villages revealed that their knowledge levels were insufficient. It was concluded that there was a need for training (Cevizci and Bakar 2012). Present study focused on farmers' knowledge, attitudes, practices and awareness of pesticide use on agricultural fields of Çanakkale province. Farmers' attitudes on environmental and toxicological risks of pesticides were assessed proportionally. Reliability of the Likert scale was checked with Cronbach's alpha test and the relationships between the level of knowledge and the other factors (such as age and education) were assessed through Chi-Square test.

MATERIALS AND METHODS

Study area and data collection

This study was conducted in the province of Çanakkale in Türkiye. The province is located between 25-35 and 27-45 east (°E) longitudes and 39-30 and 40-42 north (°N) latitudes. It has an average altitude of 2 m (Figure 1). Face-to-face interviews were made with the participant farmers to gather data through a structured questionnaire between May 2022 and January 2023. The structured questionnaire contained questions on socio-demographic and economic characteristics of farmers, pest control methods, measures to be taken in case of poisoning, storage and disposal of pesticides, personal protective equipment, attitudes towards the hazardous effect of pesticides, farmers' practices in applying pesticides and health problems.

Figure 1.

Determination of sample size

In survey studies, the sample size, i.e. the number of farmers to be interviewed, should be able to represent the study area. Statistical methods compatible with the nature of the data should be used for this purpose. In this study, the method of "Simple Random Sampling Based on Means" was used to determine the sample size (Collins 1986, Erdil and Tiryaki 2020, Miran 2003). Following equation was used to calculate sample size (Eq.1):

$$n = \frac{(Z\alpha/2)^2 \times p \times (1-p)}{d^2} \quad 1$$

where;

n=sample size (number of farmers)

$Z_{\alpha/2}$= The tabulated value (Zα/2) corresponding to the desired confidence level (90%, $Z_{\alpha/2}$=1.645)

p= Estimated proportion of the population that presents the characteristic (p=0.5)

d= Tolerated margin of error (0.05)

$$n = \frac{(1.645)^2 \times 0.5 \times (1-0.5)}{0.05^2} = 270.61$$

The number of farmers to be surveyed was calculated as 270 with a tolerated error of 0.05 and a 90% confidence interval. If the P value is unknown, 0.5 is an accepted value for high sample size (Collins 1986, Eryılmaz et al. 2018, Niyaz and Inan 2016).

With this approach, a survey was conducted among 270 farmers in 164 villages in 12 districts of Çanakkale province. The number of farmers to be surveyed in each district was calculated by the proportional distribution of villages in each district according to the total population (149.893). The number of farmers to be interviewed in the villages was calculated using the same method. Table 1 shows the number and distribution of the number of farmers surveyed in Çanakkale province by districts.

Data analyses

The data obtained from the questionnaires were assessed through Likert Scale and Chi-Square Independence Test.

Likert scale

The data were also evaluated proportionally with tables and graphs. Farmers' awareness of pesticide use was evaluated with the scores given to the answers to questions. The evaluation was based on the positive answers given by the farmers to the survey questions. The answers received were grouped using a four-point Likert Scale (Likert 1932). In accordance with this rating, a farmer can get a maximum of 96 points. Accordingly, the score ranges that determine the pesticide use consciousness level are as follows:
Max score: 96 points
Scale ranges: 0-96 points
Low: 0-40 points
Medium: 41-60 points
High: 61-80 points
Very high: 81-96 points

Chi-square (x^2) test of independence

The significance of the relationship between farmers' consciousness of pesticide uses and other parameters was assessed with the Chi-Square (x^2) Independence Test (Eq.2) and P values were also found (Düzgüneş et al. 1983).

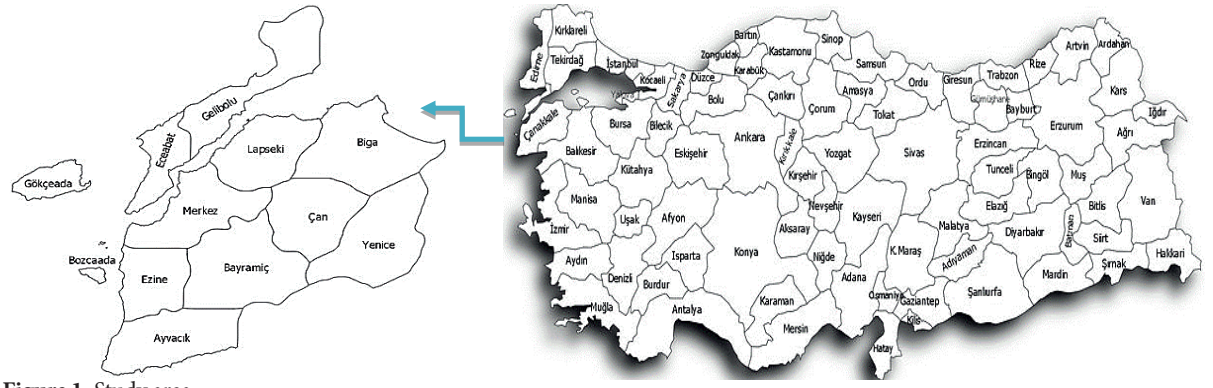

Figure 1. Study area

$$x^2 = \sum \frac{(Oi-Ei)^2}{Ei} \quad 2$$

x^2: Chi-Square test

O_i: Observed value of *i*

E_i: Expected value of *i*

If the critical x^2 value [0.05 Significance level (α) and determined Degrees of Freedom (df)] is less than the calculated one, there is a significant relationship between the two variables. The parameters investigated in relation to the level of pesticide use awareness are as follows: Education, age, farming experience, land size and type of farming (irrigated or dry farming)

Coefficient of Contingency (CC), indicating the degree of significance of the relationship between the variables, was also calculated with the use of Eq. 3 (Düzgüneş 1983).

$$CC = \sqrt{\frac{x2}{N+x^2}} \quad 3$$

CC:Coefficient of Contingency

x^2: Khi-Kare

N: Number of farmers surveyed

Cronbach's alpha co-efficient

Cronbach's alpha coefficient was calculated with the use of Eq. 4.

$$\alpha = \frac{K}{K-1} * \left(\frac{1-\sum_{i=1}^{K} \sigma^2_{yi}}{\sigma^2_{yi}}\right) \quad 4$$

where;

Yi = Observed values of question *i*

$X = Y_1 + Y_2 + \ldots + Y_K$ = Sum of observed values

σ^2_{yi} = Variance of question *i*

K = Number of questions

$\sum_{i=1}^{K} \sigma^2_{yi}$ = Sum of variance of questions

σ^2_X= Variance of total score.

Coefficients range between 0 - 1. The reliability of the scale is accepted as good if the coefficient is ≥0.70. The closer the Cronbach's alpha coefficient is to 1.0, the greater the internal consistency of the scale items (Forst 2023, Gliem and Gliem 2003). George and Mallery (2003) indicated the reliability of Cronbach's alpha coefficients as ">9 – Excellent, > 8 – Good, > 7 – Acceptable, > 6 – Questionable, > 5 – Poor, and < .5 – Unacceptable."

RESULTS AND DISCUSSION

Cronbach's reliability test

The overall Cronbach's alpha coefficient was calculated as 0.7563. Since the reliability of the scale is accepted as good (George and Mallery 2003, Özsayın and Everest 2019), no changes were made in the survey questions, and the evaluations were made based on the answers given to these questions.

Likert scale and assessment of pesticide use consciousness

Farmers' awareness of pesticide use was calculated by giving points to the responses received, and assessments were made over a four-point Likert scale. Farmers' awareness was assessed as low for 1.85%, moderate for 25.18%, high for 66.29%, and very high for 6.67% of participant farmers (Table 2). Kızılaslan and Kızılaslan (2005) reported farmers' awareness of pesticide use as low at 27.45%, moderate at 49.02% and high at 23.53%, Akar and Tiryaki (2018) reported the ratios as 13.5%, 28.3%, and 58.2%, respectively and Erdil and Tiryaki (2020) reported as 10.9%, 25.3%, and 63.8%, respectively.

Five parameters (education, age, farming experience, land size, and type of farming), which contributed to the level of pesticide use awareness, were also assessed with Chi-Square Independence Test (Eq. 2). Pesticide use awareness level of the farmers based on their education level is given in Table 2. Based on Chi-Square test, the relationship between these 2 parameters was found to be significant. As the education level increases, the awareness level of the farmers also increases. The Coefficient of Contingency (CC) was calculated as 0.467 (Eq.3). The highest level of awareness (76.19%) was among university graduates.

Pesticide use awareness of farmers based on their age is given in Table 3. There was no significant relationship between these parameters. However, the highest level of awareness (91.68%) was found in 25-34 years age group.

Similarly, there was no significant relationship between farmers' awareness of pesticide use with farming experience (Table 4) and type of farming (Table 5). The highest level of awareness was seen in farmers with 21-30 years of farming experience (75.75%) and farmers dealing with mixed farming (67.09%).

Although the relationship was found to be significant between the land size and awareness level, $x^2_{critical}$ (21.026) and $x^2_{calculated}$ (21.024) values were very close to each other (Table 6). Indeed, Kızılaslan and Kızılaslan (2005) could not find a relationship between the level of awareness and land size.

Table 2. The relationship between the pesticide use awareness level and education

Education	Pesticide use awareness level										
	Low		Moderate		High		Very high		Sum, Σ		
	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Survey basis*
illiterate	0 (0.00)	0.02	1(100.00)	0.25	0	0.67	0	0.07	1 (100)	1	0.37
literate	0 (0.00)	0.04	1 (50.00)	0.50	1 (50.00)	1.32	0	0.13	2 (100)	2	0.74
primary school	2 (1.55)	2.40	45 (34.88)	32.49	76 (58.91)	85.52	6 (4.65)	8,60	129 (100)	129	47.78
secondary school	1 (2.00)	0.92	11 (22.00)	12.59	36 (72.00)	33.15	2 (4.00)	3.33	50 (100)	50	18.52
high school	2 (2.98)	1.24	10 (14.92)	16.87	50 (74.62)	44.42	5 (7.46)	4.47	67 (100)	67	24.82
university	0 (0.00)	0.39	0 (0.00)	5.29	16 (76.19)	13.92	5 (23.81)	1.4	21 (100)	21	7.78
Master or Ph D	0 (0.00)	0	0 (0.00)	0	0 (0.00)	0	0 (0.00)	0	0 (0.00)	0	0
Sum, Σ	5 (1.85)	5	68 (25.18)	68	179 (66.29)	179	18 (6.67)	18	270 (100)	270	100.00
Null hypothesis (H_0): No relation between two variables $x^2_{critical}$=28.87 with the df=18 and α =0.05 $x^2_{calculated}$=76.39											
$x^2_{calculated} > x^2_{critical}$ H_0:reject											
p=0.009 CC=0.467											

*1x100/270=0.37

Table 3. The relationship between the pesticide use awareness level and age

Age, year	Pesticide use awareness level										
	Low		Moderate		High		Very high		Sum, Σ		
	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Survey basis*
25-34	0 (0.00)	0.44	1 (4.17*)	6.04	22(91.68)	15.911	1 (4.16)	1.60	24 (100)	24	8.89**
35-44	1 (2.22)	0.83	17 (37.77)	11.33	25(55.55)	29.833	2 (4.44)	3.00	45 (100)	45	16.67
45-54	1 (1.47)	1.26	18 (26.47)	17.12	46(67.64)	45.081	3 (4.41)	4.53	68(100)	68	25.18
55-65	2 (2.08)	1.78	21 (21.87)	24.18	66(65.75)	63.644	7 (7.29)	6.40	96 (100)	96	35.56
66≥	1 (2.70)	0.68	11 (29.72)	9.32	20(54.05)	24.529	5 (13.51)	2.47	37 (100)	37	13.70
Sum, Σ	5 (1.85)	5.00	68 (25.18)	68.00	179 (66.29)	179.000	18 (6.67)	18.00	270 (100)	270	100.00
Null hypothesis (H_0): No relation between two variables $x^2_{critical}$ =21.026 with the df=12 and α =0.05											
$x^2_{calculated}$ =18.95 $x^2_{calculated} < x^2$critical H_0:accept											
p=0.177 CC=0.256											

*100x1/24=4.17
**24x100/270=8.89

Table 4. The relationship between the pesticide use awareness level and farming experience

Farming experience, year	Pesticide use awareness level										
	Low		Moderate		High		Very high		Sum, Σ		
	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Survey basis*
1.0-10	0 (0.00)	0.65	7 (20.00*)	8.81	26 (74.28)	23.20	2 (5.72)	2.33	35 (100)	35	12.96**
11-20	2 (3.38)	1.09	14 (23.72)	14.86	38 (64.40)	39.11	5 (8.47)	3.93	59 (100)	59	21.85
21-30	1 (1.52)	1.22	14 (21.21)	16.62	50 (75.75)	43.75	1 (1.52)	4.40	66 (100)	66	24.44
31-40	0 (0.00)	1.28	21 (30.43)	17.38	42 (60.86)	45.74	6 (8.69)	4.60	69 (100)	69	25.55
41≥	2 (4.87)	0.76	12 (29.26)	10.32	23 (56.10)	27.18	4 (9.75)	2.73	41 (100)	41	13.70
Sum, Σ	5 (1.85)	5.00	68 (25.18)	68.00	179 (66.29)	179.000	18 (6.67)	18.00	270 (100)	270	100.00
Null hypothesis (H_0): No relation between two variables $x^2_{critical}$ =21.026 with the df=12 and α =0.05											
$x^2_{calculated}$ =14.30 $x^2_{calculated} < x^2$critical H_0:accept											
p=383 CC=0.224											

*100x1/24=4.17
*35x100/270=12.96

Table 5. The relationship between the pesticide use awareness level and the type of farming

Type of farming	Pesticide use awareness level										
	Low		Moderate		High		Very high		Sum, Σ		
	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Survey basis*
Dry	0 (0.00)	1.20	16 (24.61*)	16.37	42 (64.61)	43.09	7 (10.77)	4.33	65 (100)	65	24.07**
Irrigated	1 (2.00)	0.92	14 (28.00)	12.59	33 (66.00)	33.15	2 (4.00)	3.33	50 (100)	50	18.52
Mixed	4 (2.58)	2.87	38 (24.51)	39.04	104 (67.09)	102.76	9 (5.80)	10.33	155 (100)	155	57.41
Sum, Σ	5 (1.85)	5.00	68 (25.18)	68.00	179 (66.29)	179.000	18 (6.67)	18.00	270 (100)	270	100.00
Null hypothesis (H_0): No relation between two variables $x^2_{critical}$ =12.59 with the df=6 and α =0.05 $x^2_{calculated}$ =5.41 $x^2_{calculated} < x^2$critical H_0:accept p=0.644 CC=0.140											

*100*16/65=24.61

**65x100/270=24.07

Table 6. The relationship between the pesticides use awareness level and land size

Land size, da	Pesticide use awareness level										
	Low		Moderate		High		Very high		Sum, Σ		
	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Observed (%)	Expected	Survey basis*
1-50	2 (1.88*)	1.96	35 (33.02)	26.70	63 (59.43)	70.274	6 (5.66)	7.066	106	106	39.26**
51-150	1 (1.01)	1.83	23 (23.23)	24.93	70 (70.70)	65.633	5 (5.05)	6.600	99	99	36.67
151-350	2 (4.76)	0.78	7 (16.66)	10.58	28 (66.66)	27.844	5 (11.90)	2.800	42	42	15.55
351-500	0 (0.00)	0.26	3 (21.42)	3.52	9 (64.28)	9.281	2 (14.28)	0.933	14	14	5.18
501≥	0 (0.00)	0.17	0 (0.00)	2.27	9 (100.00)	5.966	0 (0.00)	0.600	9	9	3.33
Sum, Σ	5 (1.85)	5	68 (25.18)	68.00	179 (66.29)	179	18 (6.67)	18.00	270 (100)	270	100.00
Null hypothesis (H_0): No relation between two variables $x^2_{critical}$ =21.026 with the df=12 and α =0.05 $x^2_{calculated}$ =21.04 $x^2_{calculated} < x^2$critical H_0:reject p=0.205 CC=0.268											

*100*2/106=1.88

**106x100/270=39.26

Akar and Tiryaki (2018) reported significant correlations only between pesticide use awareness and farming experience. In another study, relationship between the knowledge level and education was found to be significant and coefficient contingency was 0.344 (Erdil and Tiryaki 2020). Kızılaslan and Kızılaslan (2005) reported significant correlations of farmers' awareness with age and education.

Proportional assessment

Demographic characteristics of the participant farmers were calculated proportionally and evaluated in tables. Considering the parameters with the maximum % value, 47.78% of the participant farmers were primary school graduates (Table 2), 35.56% were in the 55-65 years age range (Table 3), 25.55% had 31-40 years of farming experience (Table 4), 57.41% applied mixed farming (Table 5) and 39.26% owned 1-50 da land area (Table 6). Information about the farmers' farming experience and land size is given in Table 7. The average farming experience of the farmers was determined as 13 years and the average land size was 140 da. In addition, the smallest farming experience is 2 years and the largest is 55 years, and the standard deviation is calculated as 13 years. As to land size, the smallest land amount is 3 da and the largest land is in 2.000 da, and its standard deviation is 217 da.

Table 7. Descriptive statistics of farmers

Statement	Lowest	Highest	Mean	Standard deviation
Farming experience (year)	2	55	29	13
Land size (da)	3	2000	140	217

Farmers' opinions on the environmental impacts of pesticides are given in Table 8-A. Of the participant farmers, 72.23% indicated that pesticides polluted lakes/streams, 73.70% indicated that pesticides can be harmful to beneficial insects or bees and 72.23% indicated that pesticides could be harmful to birds. Farmers' opinions on effects of pesticides

on human health are provided in Table 8-B. Of the participant farmers, 7.03% were strongly disagree with the carcinogenic effects of pesticides, 8.15% were undecided (no opinion), 31.12% were mostly agree, 53.70% were strongly agree. When asked "Pesticides can cause diseases we do not know about", 10.74% disagreed, 26.30% were undecided, 32.22% mostly agreed and 30.74% totally agreed.

Farmers' opinions on empty pesticide containers were shown in Table 9-A. Of the producers, 48.89% stated that they burned empty pesticide containers, 40.74% left them into garbage bin, 6.30% threw them into the environment, and 4.07% buried them in the ground. Of the farmers, 41.85% stated that they disposed of leftover pesticide solutions at the edge of agricultural fields, 27.04% sprayed them on

Table 8. Farmers' opinions on impacts of pesticides on environment and human health

Statement	Disagree		Indecisive /no opinion		Mostly agree		Totally agree	
	Number	%	Number	%	Number	%	Number	%
A) Farmers' opinions about environmental effects of pesticides								
Creates pollution in lake and rivers	18	6.66	13	4.81	44	16.30	195	72.23
Harmful to beneficial insects or bees	15	5.56	13	4.81	43	15.93	199	73.70
Harmful to birds	18	6.66	14	5.18	43	15.93	195	72.23
Harmful to reptiles	18	6.66	14	5.18	43	15.93	195	72.23
Harmful to mammals	18	6.66	14	5.18	43	15.93	195	72.23
B) Farmers' opinions about the effects of pesticides on human health								
Causes short-term toxicity	19	7.02	24	8.88	82	30.40	145	53.70
It has a carcinogenic effect	19	7.03	22	8.15	84	31.12	145	53.70
Irritate to the skin	16	5.92	26	9.63	82	30.37	146	54.08
Causes some unknown diseases	29	10.74	71	26.30	87	32.22	83	30.74

Table 9. Farmers' opinions and behaviours about pesticide residues and remaining pesticide solutions, and their disposal methods

Statement	Number of responder	%
A) Methods of disposal of empty pesticide containers		
Destroying by burning	132	48.89
Leave into garbage box	110	40.74
Throw out to the environment	17	6.30
to bury in the ground	11	4.07
Sum	270	100.0
B) Farmers' behaviours on remaining pesticide solutions		
to dispose on the edge of agricultural fields	113	41.85
to spray on a uncultured field	73	27.04
to pour into the canal	8	2.96
Discharge into an irrigation canal or river	50	18.52
Others	26	9.63
Sum	270	100.0
C) Farmers' opinions on pesticide residues in agricultural products		
Some pesticides have residue on the product	10	3.70
Pesticide residues can be eliminated by washing process	10	3.70
There are no pesticide residues in the products	192	71.11
I have no idea about the pesticide residues	58	21.49
Sum	270	100.0

uncultivated fields, 2.96% poured into the canal, and 18.52% discharged them into irrigation canal or river (Table 9-B). Of the producers, 3.70% stated that pesticide residues can be eliminated by washing process, 71.11% were no pesticide residues in the products, and 21.49% had no idea about the pesticide residues (Table 9-C).

Farmers' opinions about pesticide application are given in Table 10-A. Of the participant farmers, 18.89% stated that they sprayed pesticides when there was no pest, 46.67% indicated that they have knowledge about special signs and warnings on pesticide labels, 69.63% indicated that they cared about PHI, 78.89% stated that they changed their clothes after spraying, 45.18% indicated that they used protective equipment during pesticide application, 7.78% stated that they eat or drink while spraying. Farmers' opinions about pesticides and environmental behaviours are provided in Table 10-B. Of the participant farmers, 3.33% stated that they used empty pesticide packages for other purposes, and 15.92% stated that they had knowledge about MRLs.

Table 10. Farmers' opinions about pesticide applications and environmental behaviours

Question	Yes		Mostly/*not remember**		Sometime/*No idea**		No	
	Number	%	Number	%	Number	%	Number	%
A) Farmers' opinions on the pesticide applications								
Do you apply pesticides when there are no pests?	51	18.89	32	11.85	26	9.63	161	59.63
Do you read the label (special signs, warnings, instructions, expiration date, dosage, registration) before spraying?	126	46.67	69	25.55	33	12.22	42	15.56
Do you pay attention to the PHI?	188	69.63	39	14.44	20	7.41	23	8.52
Do you record the time of use and the amount of pesticide used?	52	19.26	16	5.92	16	5.92	186	68.90
Do you take protective measures while spraying and cleaning the materials?	122	45.18	40	14.81	45	16.68	63	23.33
Have you ever sprayed the pesticides with your hand?	68	25.18	43	15.93	64	23.70	95	35.19
Do you change your clothes after the spraying?	213	78.89	25	9.26	8	2.96	24	8.89
Do you ventilate where you store pesticides?	120	44.44	71	26.30	28	10.37	51	18.89
Do you spray when you are tired or sweaty?	46	17.04	45	16.65	67	24.81	112	41.50
Do you eat or drink (cigarettes, etc.) while spraying?	21	7.78	21	7.78	46	17.04	182	67.40
Do you take a bath after pesticide application?	242	89.63	24	8.89	2	0.74	2	0.74
Do you take break frequently while spraying?	28	10.37	36	13.33	85	31.50	121	44.80
Do you spray in windy weather?	0	0	2	0.74	30	11.11	238	88.15
Do you have someone with you during the application?	72	26.67	53	19.63	54	20	91	33.70
B) Farmers' opinions about pesticides and environmental behaviours								
Do you act carefully to avoid harmful effects on the environment at the time of spraying?	222	82.22	31	11.48	6	2.22	11	4.08
Do you believe that pesticides harm the environment?	219	81.11	25	9.26	9	3.33	17	6.30
Do you know that excessive pesticide consumption has a negative impact on the country's economy?	221	81.85	13	4.81	11	4.07	25	9.25
Do you check the presence of animals in the environment before spraying?	154	57.05	22	8.16	29	10.7	65	24.09
Do you use empty pesticide packages for other purposes (water transport)?	9	3.33	0	0	11	4.07	250	92.60
Did you hear the term of "maximum residue limit"?	43	15.93	19	7.03	34	12.59	174	64.45

* "not remember" and "no idea" alternatives are related to "Farmers' opinions on pesticide residues and environmental behaviours" section of table.

Survey studies are used to reveal the behaviour of producers during the pesticide use process. Although the present findings showed that farmers' awareness of pesticide use was not significantly related to age and farming experience, awareness of pesticide use was high among young farmers (25-34 years old). Likewise, the awareness levels of farmers with 21-30 years of farming experience were high. The relationship between education and pesticide awareness level was found to be significant. As the education level increases, the awareness level of the farmers also increases. With this result, the importance of education, as in most disciplines, has once again become clear. The awareness level of well-educated farmers with many years of farming experience was quite high. Although 66.29% of farmers have a high knowledge level, only 53.70% of them agreed that pesticides had a carcinogenic effect, 46.67% cared about the information on pesticide labels, 45.18% used protective equipment during spraying, 69.63% cared about PHI, 15.92% had knowledge about MRLs and 41.85% disposed remaining pesticide solutions to the edge of agricultural fields. The present results suggest that farmers need to be seriously educated about the use of pesticides and the potential effect of pesticides on human health and the environment.

ACKNOWLEDGEMENTS

This article is a part of the master thesis of Umut TAŞKIR (ÇOMÜ Institute of Graduate Studies - Department of Plant Protection) and was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit under the project number FYL-2022-4135. The authors are thankful to Elif Betül Serbes for her help in conducting the study. Thanks are also extended to Prof. Dr. Zeki Gokalp (Certified English Translator) for his critical reading and syntactic corrections of the manuscript.

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

Tarım alanlarında karşılaşılan zararlı organizmalara karşı pestisitler giderek daha fazla kullanılmaktadır. Ancak bilinçsiz pestisit kullanımı insan ve çevre sağlığını tehdit etmektedir. Bu nedenle pestisitlerin olumsuz etkilerini azaltmak için çaba harcanmalıdır. Bu çalışmanın amacı, Çanakkale ili tarım alanlarında çiftçilerin pestisit kullanımı konusundaki farkındalıklarını değerlendirmektir. Örnek büyüklüğü "Oran Ortalamalarına Dayalı Basit Rastgele Örnekleme" yöntemi kullanılarak hesaplanmıştır. Anket 270 çiftçi ile yapılmıştır. Katılımcı çiftçilerin %25.55'i 31-40 yıllık çiftçilik tecrübesine sahip, %47.78'i ilkokul mezunu ve %35.56'sı 55-65 yaşındadır. Çiftçilerin pestisit kullanımına ilişkin bilgi düzeyleri Likert Ölçeği ile değerlendirilmiştir. Çiftçilerin bilgi düzeylerinin eğitim, yaş, çiftçilik deneyimi, arazi büyüklüğü ve tarım türü ile ilişkisini araştırmak için Khi-Kare testi kullanılmıştır. Sadece eğitim düzeyi önemli bulunmuştur. Eğitim düzeyi arttıkça bilgi düzeyi de artmaktadır. Çiftçi davranışları açısından bakıldığında, çiftçilerin %78.89'u ilaçlamadan sonra kıyafetlerini değiştirdiğini, %46.67'si pestisit etiketlerindeki bilgileri önemsediğini, %45.18'i ilaçlama sırasında koruyucu ekipman kullandığını, %69.63'ü PHI (hasat aralığı/Son uygulama ile hasat arası geçmesi gereken süre)'yı önemsediğini, %15.92'si MRL (Maksimum Kalıntı Limiti) hakkında bilgi sahibi olduğunu belirtmiştir. %41.85'i kalan pestisit solüsyonlarını tarım alanlarının kenarlarına, %40.74'ü çöpe ve %6.3'ü çevreye atmaktadır. Bu bulgulara dayanarak, çiftçilerin pestisit kullanımı ve pestisitlerin insan sağlığı ve çevre üzerindeki potansiyel etkileri konusunda ciddi eğitime ihtiyaçları olduğu sonucuna varılmıştır.

Anahtar kelimeler: çiftçilerin bilinç düzeyi, Cronbach alfa katsayısı, çevre, Likert skalası, anket, pestisit

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Cite this article: Taşkır, U., & Tiryaki, O. (2024). Investigation of consciousness levels of Çanakkale farmers on environmental and toxicological risks of pesticides. Plant Protection Bulletin, 64-1. DOI: 10.16955/bitkorb.1338505

Atıf için: U., & Tiryaki, O. (2024). Çanakkale çiftçilerinin pestisitlerin çevresel ve toksikolojik riskleri konusundaki bilinç düzeylerinin araştırılması. Bitki Koruma Bülteni, 64-1. DOI: 10.16955/bitkorb.1338505

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Insecticidal activity of *Mentha piperita* L. (Lamiaceae) essential oil against two important stored product pests and its effect on wheat germination

Mentha piperita L. (Lamiaceae) uçucu yağının iki önemli depolanmış ürün zararlısına karşı insektisidal aktivitesi ve buğdayın çimlenmesi üzerine etkisi

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ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1382542
Received : 02-11-2023
Accepted : 17-12-2023

Keywords:
germination, contact activity, *Mentha piperita, Sitophilus granarius, Tribolium castaneum*

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ABSTRACT

This study aimed to evaluate the contact activity of the essential oil derived from *Mentha piperita* L. (Lamiaceae) against two significant stored product pests, namely *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae), in a controlled laboratory setting. For this purpose, concentrations of 0.05, 0.1, and 0.15 µl/insect of plant essential oil were applied to stored product pests using a microapplicator. Dead insects were counted at 24, 48, 72 and 96 hours after application. As a result of the study, the essential oil demonstrated contact activity at varying rates depending on the applied pest and dose. After 24 hours, the 0.15 µl/insect application dose was the most effective against *T. castaneum*, resulting in a 23.6% mortality rate; after 96 hours, this rate increased to 33.4%. The essential oil exhibited greater contact activity on *S. granarius*, resulting in a 93.4% mortality rate after 24 hours when administered at a concentration of 0.15 µl/insect. At the end of 96 hours, the mortality rate at the same dose was found for *S. granarius* to be 98.2%. In addition, the effect of essential oil on the germination power of wheat grain was examined at doses of 2, 5, 10 and 20 µl/Petri under laboratory conditions. 73.8% of the seeds germinated at the maximum dose of 20 µl/Petri, while 99.4% germinated at the minimum dose of 2 µl/Petri. The research findings indicate that the essential oil of *M. piperita* possesses the capacity to be employed for the management of *S. granarius*.

INTRODUCTION

A variety of conditions during storage cause agricultural products to lose significantly in terms of both quality and quantity (Kumar and Kalita 2017). Food losses during storage can be attributed to various factors, including biological damage caused by insects, rodents, and microbes, chemical damage resulting from the development of rancidity and modifications in flavor, as well as physical damage caused by crushing and breakage (Kumar and Rai 2013). It is commonly recognized that *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) seriously damage stored grain products worldwide and that these two species are the most prevalent pests in Türkiye. To limit the harm caused by these pests, various cultural, physical, and chemical management strategies are applied. Especially, synthetic insecticides are the most widely used to control these pests. However, intensive use of insecticides leads to the development of insecticide resistance, health problems in humans and animals and environmental contamination (Karaca and Gökçe 2014, Kumar and Rai 2013). Therefore, finding safe alternatives to these chemicals has become a necessity. Thus, in recent years, research has focussed on using plant oils as an alternative to synthetic chemical insecticides (Alkan 2020, Kedia et al. 2014, Kim et al. 2016, Saifi et al. 2023).

Bioinsecticides derived from plants are an important category of botanical insecticides. These substances exhibit diverse toxic and behavioural impacts on pests, in contrast to synthetic pesticides that have a direct lethal effect. Compared to synthetic pesticides, plant-based insecticides are cheaper, biodegradable and less hazardous to humans and the environment (Mamun and Ahmed 2011, Souta et al. 2021). Many studies were conducted to determine the potential of plant extracts and essential oils to control various pest groups using different approaches (Budak et al. 2022, Çam et al. 2012, Karakoç et al. 2013, Pazinato et al. 2014, Teke and Mutlu 2021). Essential oils, comprising volatile compounds, exhibit greater efficacy against pests through inhalation, hence research has largely focused on the fumigant mechanism of action, particularly to concerning storage pests (Alkan 2020, Becker 2003, Descamps et al. 2011).

The peppermint, *Mentha piperita* L. (Lamiaceae), is an important medicinal plant that is a perennial with a strong fragrance. Temperate regions of Asia, North Africa, Europe, , and North America, among others, are where it is widely cultivated. Numerous industries utilize the essential oil extracted from this plant, including food, beverage, pharmaceutical, cosmetic, health, and tobacco sectors. Menthol, menthone, and menthofuran are the major components of peppermint oil. Moreover, there exists substantial data suggesting that peppermint oil may exhibit a range of advantageous characteristics, such as its potential anti-spasmodic, anti-inflammatory, and antibacterial capabilities (Mahieu et al. 2007, Papathanasopoulos et al. 2013). Insecticidal and antifeedant effects of peppermint oil derived from *M. piperita* have been reported against a wide variety of pests, including *S. oryzae* (Khani et al. 2012, Khani et al. 2017, Klys 2012, Lashgaria et al. 2014) and *T. castaneum* (Lashgaria et al. 2014, Lee et al. 2002). Herein, we searched the contact activity of the essential oil obtained from *M. piperita* against two significant stored product pests, *T. castaneum* and *S. granarius*, under laboratory conditions. Additionally, the impact of the essential oil on the germination of wheat seeds was evaluated.

MATERIALS AND METHODS

Extraction of essential oils

The essential oil was obtained by hydrodistillation of dried and crushed above ground parts (100 g) of *M. piperita* for three hours in a Clavenger apparatus. The condenser part of the Clavenger apparatus was connected to the microhiller so that the cooling water was kept at 4 °C. The isolated essential oil was purified from water on Na_2SO_4 and transferred to amber-coloured bottles and stored at -20 °C until the day of application.

Insect rearing

The insects used in this study were individuals of both male and female of *S. granarius* and *T. castaneum*. These insects were cultivated in glass jars with a capacity of one liter, and the rearing process took place in an environment with ambient conditions, namely at a temperature of 25±1 °C and a relative humidity of 65±5%. For *T. castaneum*, each jar had an average of 250 g of broken wheat and 100 g of wheat flour, while *S. granarius* jars contained 250 g of whole wheat. The jar apertures were sealed with rubber-secured cheesecloth. A total of 200-300 adult beetles from both sexes of *S. granarius* and *T. castaneum* were placed in separate jars and provided with a conducive environment for reproduction for 48 hours. Following oviposition, adult beetles of *S. granarius* and *T. castaneum* were eliminated from their feeding habitats. A new culture was formed with the laid eggs and the 7-21 day-old adults obtained from this culture were used in the experiments.

Contact toxicity assay

To conduct the contact activity tests, solutions of essential oils were created by diluting them with acetone at concentrations of 0.05 µl/insect, 0.1 µl/insect, and 0.15 µl/

insect. These doses were then administered to the ventral of each insect's abdomen using a micro applicator (Hamilton, GR, Switzerland). In the experimental design, the insects in the control group were subjected to an equivalent dosage of acetone. To serve as a positive control, a group of insects was subjected to treatment with K-Obiol® (Bayer). This pesticide [K-Obiol®- Deltamethrin (25 g/l) + Piperonyl Butoxide (250 g/l)] was applied at the dosage indicated by the manufacturer. The treated insects were placed in Petri dishes with a diameter of 6 cm containing a food source. Each replication consisted of a sample size of 10 adults. The mortality rates were documented at intervals of 24, 48, 72 and 96 hours. To feed adult *S. granarius*, 20 whole pieces of wheat were added to the medium, while adult *T. castaneum* was fed 0.2 g of cracked wheat. The study was carried out using a completely randomized design, with a total of nine replicates. It was conducted in a climate-controlled cabinet (NUVE, ID-501) at a temperature of 25±1 °C, a relative humidity of 60±10% and a light/dark cycle of 16:8 hours.

The effect of essential oil on wheat germination

The wheat variety used for germination testing in this study was *Triticum aestivum* cv. Eser (Poaceae), sourced from the Fields Crops Central Research Institute in Ankara, Türkiye. Before the experiments were carried out, it was preliminarily determined that the germination capacity of wheat seeds was above 95%. For surface sterilization, the seeds were treated with NaOCl (5%) and ethanol (96%) solutions for 5 and 30 minutes, respectively. Afterwards, the seeds were then rinsed thoroughly with distilled water. To examine the effect of *M. piperita* essential oil on the germination process of wheat seeds was carried out using Petri dishes with a diameter of 9 cm. In this experiment, a total of 20 seeds were evenly placed within Petri dishes containing two layers of Whatman filter paper (No:1). The drying paper was adequately hydrated using distilled water. Due to the limited water solubility of the essential oil, it was used in gaseous form during the test procedure. To achieve the intended objective, a fragment of filter paper was affixed to the lids of the Petri dishes using adhesive. The essential oil was then carefully dispensed onto the blotting paper using a micropipette. Next, the Petri dishes were securely sealed and tightly wrapped with parafilm. In the experiment, different amounts of essential oils were applied, including a control group with no application (0 µl/Petri dishes), as well as doses of 2, 5, 10, and 20 µl/Petri dishes. The Petri dishes were subjected to a seven-day incubation period, during which they were alternately exposed to 12 hours of light and 12 hours of darkness while maintained at a temperature of 23±2 °C (Kadioglu and Yanar 2004, Kordali et al. 2009, Sadeghi et al. 2010). After the designated time period, the germination rates were assessed. The seeds were counted as germinated when both shoot and root growth had reached a size corresponding to half of the original seed. The experiments were carried out in four replicates with twice.

Statistical analysis

The raw data was initially converted into mortality percentages and subsequently underwent an ArcSin transformation to standardize the proportional data, following the according to of Zar (1999) and Warton and Hui (2011). The mortality data was evaluated using analysis of variance (ANOVA). Tukey's multiple comparison test with a significance threshold of 5% was used to evaluate the differences among the treatments. The statistical analysis was performed utilizing the "General Linear Model" within the MINITAB Release 18.1 software package, following the methods described by McKenzie and Goldman (2005).

RESULTS

Contact toxicity of essential oil

In contact toxicity bioassays, mortality rates increased depending on dose and time. The essential oil of *M. piperita* showed an important contact activity against *S. granarius*. This effect was more than 90% at both 0.1 µl/insect and 0.15 µl/insect doses after 24 hours and these two application doses were statistically in the same group with the positive control group after 48 hours. However, at the lowest dose (0.05 µl/insect), the mortality rate could only reach 30.11% after 96 hours ($F= 123.12$; $df=4.49$; $P <0.05$) (Table 1).

Table 1. Mean mortality percentage (±SE) of *Sitophilus granarius* adults exposed on treated with different *Mentha piperita* essential oils dosages and time

Treatment	24 h	48 h	72 h	96 h
Control	0.29±0.24d*	0.80±0.51c	1.15±0.57c	2.03±0.63c
0.05 µl/insect	19.55±1.50c	27.04±1.92b	27.04±1.92b	30.11±2.25b
0.1 µl/insect	91.08±1.96b	93.45±2.68a	95.64±2.60a	96.27±2.39a
0.15 µl/insect	93.40±2.68b	96.53±1.81a	98.16±1.54a	98.20±1.54a
K-Obiol	100.00±0.0a	100.00±0.00a	100.00±0.00a	100.00±0.00a

*Means followed by the same lowercase letter within each column are not significantly different using Tukey test at $P<0.05$.

Table 2. Mean mortality percentage (±SE) of *Tribolium castaneum* adults exposed on treated with different *Mentha piperita* essential oils dosages and time

Treatment	24 h	48 h	72 h	96 h
Control	0.00±0.00d*	0.03±0.14c	0.03±0.14c	0.03±0.14c
0.05 µl/insect	7.74±0.96c	18.66±2.47b	23.68±1.73b	27.94±0.75b
0.1 µl/insect	17.14±1.53bc	24.35±2.42b	24.35±2.42b	26.48±2.75b
0.15 µl/insect	23.60±3,09b	30.11±5.06b	30.11±5.06b	33.40±3.99b
K-Obiol	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

*Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

The effect of *M. piperita* essential oil against *T. castaneum* was much lower than that of *S. granarius*. Even at the highest dose (0.15 µl/insect), the effect was less than 35% at the end of 96th hour (F= 90.84; df=4, 49; P <0.05) and this dose were in statistically different group with the positive control group. The lowest dose (0.05% v/v) caused only 27.94% effect at the end of 96th hour (F= 90.84; df=4.49; P <0.05) (Table 2).

As a result of the interaction analysis, the insect * dose interaction was found to be statistically significant. On the other hand, dose*time, dose*time*insect interactions were found to be statistically insignificant (Table 3).

Table 3. ANOVA parameters for main effects of variables for the adults of *Sitophilus granarius*, and *Tribolium castaneum* in the study

Source	DF	F-Value	P-Value
Insect	1	351.08	P<0.05
Dose	3	395.29	P<0.05
Time	3	4.56	P<0.05
Insect x Dose	3	81.31	P<0.05
Insect x Time	3	0.05	P>0.05
Dose x Time	9	0.35	P>0.05
Insect x Dose x Time	9	0.23	P>0.05
Error	328		
Total	359		

The effect of the essential oil on wheat germination

It was concluded that *M. piperita* essential oil affected wheat germination depending on the application dose (Table 4). In 2 µl application dose, a 99.4% germination rate and in 5 µl application dose, a 95.6% germination rate was determined. These two application doses were statistically in the same group with the control group (F=66.64; df=4, 39; P<0.05). However, when the application dose was increased to 10 and 20 µl, a significant decrease in germination rates was found and a germination rate of 85.0% was determined at 10 µl dose and 73.8% at 20 µl dose. These two application doses were in statistically different groups from the control group (Table 4).

Table 3. ANOVA parameters for main effects of variables for the adults of *Sitophilus granarius*, and *Tribolium castaneum* in the study

Doses (µl/Petri)	Germination (%±StDev)
2	99.4±1.8a*
5	95.6±5.0a
10	85.0±6.0b
20	73.8±3.5c
Control	100.0±0.0a

*Means followed by the same lowercase letter within the column are not significantly different using Tukey test at P<0.05.

DISCUSSION

In this study, the insecticidal activity of the essential oil varied according to the insect species. *S. granarius* was much more sensitive to essential oil than *T. castaneum*. Especially, doses 0.1 µl/insect and 0.15 µl/insect showed more than 90% activity in *S. granarius* after 24 hours (Table 1, 2). The insecticidal properties of essential oil derived from *M. piperita* against storage insects have been the subject of several investigations. Lashgari et al. (2014) found that the fumigant effect of *M. piperita* essential oil was 82% against *S. oryzae* and 68% against *T. castaneum*. According to Vendan et al. (2017), the efficacy of *M. piperita* essential oil was notably demonstrated in *S. oryzae* when administered at an air concentration of 400 µl/l. This led to mortality rates of 83% and 100% in conditions both with and without food, respectively, within a 72-hour exposure period. Lee et al. (2002) determined the activity of essential oils of different plants against *T. castaneum* and emphasized that the highest activity was in *Rosmarius officinalis* L. (LD50: 7.8 µl/air) and the activity of *M. piperita* was also important (LD_{50}: 25.8 µl/air). There are also studies showing that the essential oils of some other plants are low effective against *T. casteneum*. Stefanazzi et al. (2011), reported that the contact effect of *Tagetes terniflora* Kunth essential oil was less in *T. castaneum* than in *S. oryzae*. Descamp et al. (2011) stated that the essential oils obtained from the leaves and fruits of *Schinus areira* caused 63% and 20% mortality against *T. castaneum* adults at the highest doses, respectively. The difference in this

effect between insect species may be due to several reasons. These include differences in weight of various insect species, cuticle thickness, and cuticle content (Stefanazzi et al. 2011). It is also known that the effect of essential oils derived from the same plants species differ based on the species of the insect, the insect's developmental stage and the plant's (Lashgari et al. 2014).

There are many studies on the effect of plant essential oils on the germination capacity of wheat (Dudai et al. 2000, El-Bakry et al. 2016, Marichali et al. 2014, Rozhkova et al. 2021). Plant essential oils can have various effects on the germination of wheat seeds and these effects may alter depending on the type of oil, concentration and exposure time. It is known that the main components of essential oils are monoterpenes. These monoterpenes may cause anatomical and physiological changes in plants and may cause accumulation of lipid globules in the cytoplasm and decrease in organelles such as mitochondria and nucleus depending on exposure (Azirak and Karaman 2008). In a previous study, it was revealed that *M. piperita* essential oil inhibited the germination of different wheat seeds by 0-53% (Turgut and Coskun 2021). Although the results of that study and our study are generally similar, differences were observed in terms of upper limits. This is thought to be due to the fact that plants may have different chemical compositions.

ACKNOWLEDGEMENTS

The authors would like to thank to H. Bilal TAŞLIOĞLU for his assistance in laboratory studies. A part of this article was presented as an oral presentation at the 6th International Anatolian Agriculture, Food, Environment and Biology Congress (TARGID 2022) held on 7-9 October 2022 at Kütahya/Türkiye and published as an abstract.

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

Bu çalışmada *Mentha piperita* L. (Lamiaceae) bitkisinde elde edilen uçucu yağın iki önemli depo zararlısı *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) ve *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae)'a karşı kontakt aktivitesi laboratuvar koşullarında test edilmiştir. Bu amaçla bitki uçucu yağı 0.05, 0.1 ve 0.15 µl/böcek dozunda depolanmış ürün zararlılarına mikroaplikatör yardımı ile uygulanmıştır. Uygulamadan 24, 48, 72 ve 96 saat sonra ölü bireyler kaydedilmiştir. Çalışma sonucunda uçucu yağ uygulama yapılan zararlı ve doza bağlı olarak değişen oranlarda kontakt aktivite göstermiştir. *T. castaneum* için 24 saat sonunda en yüksek aktivite %23.6 ölüm oranı ile 0.15 µl/böcek uygulama dozunda belirlenirken bu oran 96 saat sonunda en yüksek %33.4 olarak belirlenmiştir. *S. granarius* için ise bu yağın kontakt aktivitesi daha yüksek olmuş ve 24 saat sonunda 0.15 µl/böcek uygulama dozunda %93.4 ölüm oranı belirlenmiştir. 96 saat sonunda ise aynı dozdaki ölüm oranının *S. granarius* için %98.2 olduğu saptanmıştır. Ayrıca uçucu yağın buğday tanesinin çimlenme gücü üzerine etkisi 2, 5, 10 ve 20 µl/Petri dozlarında laboratuvar koşullarında araştırılmıştır. En yüksek uygulama dozu olan 20 µl/Petri dozunda buğdayların çimlenme oranı %73.8 olarak belirlenirken en düşük uygulama dozu olan 2 µl/Petri dozunda buğdayların %99.4'ü çimlenmiştir. Çalışma sonuçları *M. piperita* uçucu yağının *S. granarius* mücadelesinde kullanılma potansiyelinin olduğunu ortaya koymaktadır.

Anahtar kelimeler: çimlenme, kontakt aktivite, *Mentha piperita*, *Sitophilus granarius*, *Tribolium castaneum*

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Cite this article: Atay, T., Alkan, M., Tarhanacı, B., Alkan, F. R. (2024). Insecticidal activity of *Mentha piperita* L. (Lamiaceae) essential oil against two important stored product pests and its effect on wheat germination. Plant Protection Bulletin, 64-1. DOI: 10.16955/bitkorb.1382542

Atıf için: Atay, T., Alkan, M., Tarhanacı, B., Alkan, F. R. (2024). *Mentha piperita* L. (Lamiaceae) uçucu yağının iki önemli depolanmış ürün zararlısına karşı insektisidal aktivitesi ve buğdayın çimlenmesi üzerine etkisi. Bitki Koruma Bülteni, 64-1. DOI: 10.16955/bitkorb.1382542

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Genetic diversity of *Sinapis arvensis* L. (wild mustard) in Türkiye determined by microsatellite markers

Mikrosatellit marker ile Türkiye'de *Sinapis arvensis* L. (yabani hardal)'ın genetik çeşitliliğinin belirlenmesi

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ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1378621
Received : 06-08-2023
Accepted : 14-11-2023

Keywords:
SSR, wild mustard, molecular marker, genetic diversity

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ABSTRACT

Wild mustard (*Sinapis arvensis* L.) is a self-fertilizing weed species that exerts negative impacts on wheat production and herbicides are intensively used to manage it. Cross-fertilization may lead to genetic differentiation in this species. Therefore, this study investigated genetic diversity among wild mustard populations collected from wheat fields across various regions in Türkiye. Genetic variation was evaluated using 5 simple sequence repeat (SSR) markers in populations collected from 30 different locations. Populations were analyzed using UPGMA (unweighted pair group method with arithmetic mean) and principal component analysis (PCA). The mean genetic diversity (GD) and polymorphism information content (PIC) values were 0.752 and 0.844, respectively. High genetic variability was recorded among populations within geographic locations. The populations were categorized into two major groups by UPGMA. There was no apparent geographic isolation among tested populations, which displayed a high degree of variability. The primary source of this variability is thought to be the adaptability of wild mustard seeds dispersed through various methods across diverse locations. Despite being a predominantly self-pollinating species, wild mustard may also employ some cross-pollination mechanisms. In conclusion, SSR markers proved useful in determining genetic diversity in outcrossing species, especially where no prior genotypic information is available. The study suggests that genetic diversity is maintained in wild mustard populations even with rotational farming practices and intensive use of herbicides.

INTRODUCTION

Wheat is the staple food of many countries since it ranks first in terms of cultivation and second in production in the world among the cultivated plants used in human nutrition, has wide adaptation ability, the appropriate nutritional value of wheat grain, and the ease of storage and processing. Wheat is the second most-produced cereal after maize and the global trade of wheat is more than all other crops combined (FAO 2023). World cereal production was 2.8 billion tons in 2021-

2022, 28% of which is wheat. Globally, wheat was cultivated on 220 million hectares during 2020-2021, which produced 770 million tons of wheat grains and Türkiye accounted for 3.3% of the global wheat cultivation area and ranked 10th with ~20 million tons production (FAO 2023).

Cereals are ranked first in terms of cultivation area and production amount among field crops in Türkiye. Several factors adversely affect wheat production, which has such a large cultivation area and production potential, and cause significant losses. Weeds reduce the yield and quality of wheat by creating competition for water, light, and nutrients on the one hand, and by being intermediate hosts of diseases and pests on the other hand, are the most important of these factors. Crop loss in cereals due to competition of weeds varies between 20-40% on average in the world (Güncan 2010).

The problematic weed species in wheat cultivation areas are; *Alopecurus myosuroides* Huds., *Avena* spp., *Lolium* spp., *Bromus tectorum* L., *Hordeum murinum* L., *Phalaris* spp., *Secale cereale* L., *Setaria viridis* (L.) P.Beauv., *Sinapis arvensis* L., *Bifora radians* M. Bieb., *Galium* spp., *Chenopodium album* L., *Boreava orientalis* Jaub. & Spach, *Ranunculus arvensis* L., *Papaver rhoeas* L., *Centaurea depressa* Bieb., *Convolvulus arvensis* L., *Cirsium arvense* (L.) Scop., *Acroptilon repens* (L.) D.C., *Vicia* spp., *Capsella bursa-pastoris* (L.) Medik., *Stelleria media* (L.) Vill., *Lamium amplexicaule* L., and *Rumex crispus* L. (Anonymous 2023).

There are differences among the genotypes/populations of a species in terms of various morphological, anatomical, physiological, biochemical, and behavioral characteristics. Differences among populations are due to different alleles of a gene and different frequency distributions of these alleles among populations. Genetic diversity refers to all variations in gene composition within a species. Genetic diversity is one of the components of biological diversity and its determination is one of the most important conditions for ecosystems to be healthy and efficient and for their sustainable operation. High intra-species genetic diversity is a guarantee for adaptation to changing environmental conditions. Species and races with high genetic diversity can adapt more successfully to environmental conditions changing according to time and place (Işık 1997). Genetic diversity in weeds, which is a major problem in wheat agricultural areas, is undesirable (Kaya 2008).

Genetic variation studies are not only considered in an evolutionary context. It also helps in eradication and weed control as an important part of research (Sun 1997). By knowing the genetic diversity of rapidly spreading species, the geographical origin of these plants can be determined (Meekins et al. 2001) and in light of this information, it is possible to select biocontrol agents for biological control (Nissen et al. 1995). To determine the biological control agents to be applied against species that have developed resistance, genetic variation should be determined. To choose the biological control agents to use against strains that have evolved resistance, genetic variation should be evaluated. This genetic heterogeneity in plants is shown using several molecular DNA marker techniques (Kaya 2008, Yalım 2005, Yılmaz 2021). Simple sequence repetition (SSR) markers are employed in a variety of domains, including genetic linkage mapping, plant evolution, and genetic diversity research (Özden Çiftçi and Altınkut Uncuoğlu 2019, Yorgancılar et al. 2015). In a study conducted by Ash et al. (2003), the genetic variation of *Carthamus lanatus* (L.) (safflower) was determined and it was reported that effective control was achieved by applying mycoherbicide to varieties that developed resistance to herbicides. This situation is also important because it sheds light on biological control applications against weeds and at the same time allows the use of herbicides to be reduced. Populations showing genetic diversity can develop higher rates of resistance to control agents and make control possibilities difficult (Meekins et al. 2001).

Nowadays, with the development of molecular techniques, studies on the genetic diversity of weed genotypes have allowed new openings in Weed Science (Leon et al. 2021, Ye et al. 2004). Markers that detect phenotypic and/or genotypic characteristics of an individual are defined as markers If there is more than one gene or phenotypic trait in a population, it can be said that that genotype is polymorphic, and markers can determine the polymorphism rate. An ideal marker should be polymorphic, reproducible, codominant, uniformly distributed throughout the genome, not subject to environmental influences, neutral, and economical (de Vicente and Fulton 2004). Markers are divided into three groups: phenotypic, biochemical, and molecular markers (Kaya 2008).

Microsatellites, also known as simple sequence repeats (SSR), are the smallest repeated units in DNA sequences, and repeat motifs vary between 1-6 bp. If the sequences of the flanking regions surrounding microsatellites (flanking region) are known, appropriate primers (usually 20-25 bp in length) can be designed for those regions and amplified by polymerase chain reaction (PCR). In addition, SSR primers between related species can be used in different organisms. Sequence skipping, incorrect base pairing, and unequal crossing-over events that occur during DNA replication are the main events that cause differences in microsatellite numbers and are determined by gel electrophoresis (Matsuoka et al. 2002). Microsatellite markers can be used effectively in population genetics and gene mapping studies because they require less DNA, are codominant and stable marker systems, are abundant and distributed in the genome, are reproducible and suitable for automation, contain high polymorphism, and are an informative marker system (Filiz and Koç 2011, Powell et al. 1996). Gıdık (2016) researched the determination of yield and

quality elements of *S. arvensis* and *S. nigra* species collected from the Thrace Region, employing ISSR and SSR methods to investigate the genetic diversity of these species. As a result of the study, it was identified 9 genotypes of *S. arvensis*, observing polymorphism in all 10 ISSR primers used in the research. The ISSR method revealed that the polymorphism rate within the *S. arvensis* population ranged between 58.33% and 14.58%. The study emphasized the limited number of molecular genetic characterization studies related to the intraspecific variations of *S. arvensis* and underscored the necessity of expanding such studies. Similarly, Erden (2018) investigated the phylogenetic relationships of species within the Brassicaceae family, including 1 biotype of *S. arvensis,* by amplifying the nrDNA ITS region through PCR for a total of 43 taxa belonging to 28 genera from the Brassicaceae family. The study revealed that the Brassicaceae family is a phylogenetically paraphyletic group, indicating that it is a group where a common ancestor is known, but the species belonging to this family are not fully discovered, or the relationships between species are not completely resolved. In another study, in analyses based on rDNA and cpDNA gene regions to investigate intraspecific variations of *Sinapis arvensis*, it was found that the ITS4 and ITS5 regions of the rDNA gene did not allow for the determination of subspecies. However, these regions were considered highly suitable for species diagnosis and determining species relationships. On the other hand, analyses relying on the matK region of cpDNA (trnK-710F and trnK-2R) were found to be a successful method for distinguishing subspecies of *S. arvensis*. Furthermore, the results of phylogenetic analyses based on rDNA and cpDNA regions suggested that taxonomically, *Sinapis arvensis* should be considered within the *Brassica* genus rather than the *Sinapis* genus (Ateş 2022).

Sinapis arvensis is a species that is mostly self-pollinated but it can also exhibit some cross-pollination (Stewart 2002). A review of *Brassica* species, cross-pollination, and implications for pure seed production in New Zealand. Cross-pollination may cause genetic differentiation of this species. To determine all these differences, this study aims to determine the genetic diversity of *S. arvensis* by using the Microsatellite (SSR-Simple Sequence Repeats) method, one of the PCR-based marker techniques, and to contribute to the determination of strategies for control.

MATERIALS AND METHODS

Sampling and breeding of populations

The seeds of *Sinapis arvensis* were collected from 48 locations in Amasya, Balıkesir, Bilecik, Bursa, Çorum, and Samsun provinces and from 5 different parts of each field to represent the field at the end of the summer period. A 5-10 km distance between the locations was ensured while taking samples from locations in the same province (Barret 1982). For pre-germination, *S. arvensis* seeds of each population were placed in Petri dishes 9 cm in diameter with a double layer of moistened blotting paper and placed in an incubator at +22 ^{0}C with a 12/14 light period. The germinated populations were transferred to pots and grown in sterile soil in a greenhouse under controlled conditions. For further research, 30 populations were selected (11 from Amasya (AMS-1, AMS-2, AMS-3, AMS-4, AMS-5, AMS-6, AMS-7, AMS-8, AMS-9, AMS-10, AMS-11) 4 from Samsun (SAM-1,- SAM-2, SAM-10, SAM-12) 1 from Çorum (ÇOR-1) 5 from Bursa (BUR-1, BUR-2, BUR-5, BUR-9, BUR-10) 7 from Balıkesir (BAL-1, BAL-2, BAL-3, BAL-4, BAL-5, BAL-6, BAL-7) and 2 from Bilecik (BİL-1, BİL-3)) (Figure 1).

Figure 1. Seed collection sites of *Sinapis arvensis* in Türkiye

DNA extraction and PCR application

For extraction, genomic DNA was extracted from leaf samples when the plants grown under greenhouse conditions reached the 4-6 leaf stage using a DNeasy DNA extraction kit (Qiagen, Germany) according to the kit protocol. Genomic DNA extraction was performed from 100 mg of fresh leaf tissue. Following the DNA protocol, the DNAs obtained were stored at -80 ^{o}C until SSR-PCR application.

PCR for SSR molecular marker testing was performed in a total volume of 25 µl. The SSR-PCR reaction mixture consisted of; 2 µl (1.0 ng/µl-1) Genomic DNA, 3 µl (25 ng) of each forward and reverse primers (Table 1), 0.5 µl 10 mM dNTP, 0.2 µl Taq DNA Pol, 2.5 µl 10XPCR buffer, 13.7 µl sdH_2O. The temperature values and times to be applied for PCR were established as follows: (1) 94 ^{o}C→ 3 min (Initial denaturation), 35 cycles (2) 94 ^{o}C→ 1 min (Separation of DNA strands), (3) 38 ^{o}C→ 1 min (Primer adhesion to the strands), (4) 72 ^{o}C→ 1 min (Completion of the strand), and (5) 72 ^{o}C→ 10 min (Final incubation). For the analysis of DNA fragments formed after PCR, 3.5% agarose gel was run on a horizontal maxi electrophoresis device (BioRad) using 1 x TBE buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.3), 2 g agarose (Serva Agarose) (Serva, Germany), 1.5 g microporous

agarose (Nusseive GTG Agarose) (Combrex, USA). The bands obtained with reference to a 1 Kb DNA marker (New England Biolabs®UK) were photographed with the help of a gel imaging device (Vilber Lourmat).

Evaluation of the bands

The determination of SSR bands was based on the appearance or absence of the band in the gel analyzed after electrophoresis. In this study, optimal PCR conditions were established by repeating the amplifications several times, and conditions that gave a stable band profile for each primer were selected. To obtain the phenogram, monomorphic and polymorphic bands in the gels were detected.

Statistical analysis

The genetic diversity was assessed by using 11 microsatellite (SSR) primers. As a result of the optimization studies, a total of 5 SSR primers were found to work. The band images obtained from the gel were evaluated with reference to the marker sizes, and band matrices were created. The band sizes of the polymorphic bands were entered as present (1) or absent (0). Thus, the band matrices to be used in the following stages were created. The number of alleles, mean number of alleles per locus (NA), expected heterozygosity (HE), observed heterozygosity (HO), gene diversity (GD), and polymorphism information content (PIC) was calculated using the genetic analysis program NTSYSpc2.1. Further processing of data was done by carrying out sequential agglomerative hierarchical non-overlapping clustering (SAHN) on a squared Euclidean distance matrix. Dissimilarity matrices were used to construct the UPGMA (Unweighted Pair Group Method with Arithmetic average) dendrogram. In addition, the genetic relationships among genotypes were represented using a PCA (Backhaus et al. 1989) analysis with SPSS 21.0 software (IBM Corp. Released 2012)

RESULTS AND DISCUSSION

SSR markers were used to determine the genetic diversity within and between populations in *S. arvensis* and it was determined that the populations of this species have high genetic diversity.

In this study, 11 SSR markers were tested to determine the genetic diversity of *S. arvensis*. The binding temperature of each SSR primer was determined firstly by using the formula and then by using a thermal cycling device. Other PCR parameters were also optimized separately and it was determined which primers work in *S. arvensis*. As a result of the optimization studies, a total of 5 SSR primers were found to work in *S. arvensis*. The SA1 showed banding in the range of 155-185 bp (base pair). The expected heterozygosity was 0.626 and the observed heterozygosity was 0.9. SA2 showed banding in the range of 150-200 bp (base pair). The expected heterozygosity was 0.356 and the observed heterozygosity was 0.62. SA3 showed banding in the range of 200-315 bp (base pair). The expected heterozygosity was 0.874 and the observed heterozygosity was 0.543. SA4 showed banding in the range of 120-160 bp (base pair). The expected heterozygosity was 0.456 and the observed heterozygosity was 0.123. SA5 showed banding in the range of 150-185 bp (base pair). The expected heterozygosity was 0.325 and the observed heterozygosity was 0.675. As a result of the analyses, the mean genetic diversity value was determined 0.752 and the mean PIC value was 0.844. While the SA1 locus showed the highest genetic diversity, the SA4 locus was at the lowest value, 0.954 and 0.486 respectively (Table 1).

Table 1. The primers used in SSR application and amplification results.

Locus	Repeatmotif	Primer sequence (5′–3′)	Amplification					
			Size range (bp)	*NA*	*HO*	*HE*	*GD*	*PIC*
SA1	(A)7–10	F: TCAATTGCACATTCTAGAATTCTAAG R: CAATTCAATATGGTTATATATTAGAG	157–185	5	0.900	0.626	0.954	0.966
SA2	(T)8–13	F: GGTTCCGTCGTTCCCATCGC R: CATAATAATTAGATAAATCTGTTCC	150-200	7	0.620	0.356	0.568	0.679
SA3	(T)7–10	F: AATGGTATGACTAGCTTATAAGG R: CTTAACAATGAGATGAGGCAATC	273–311	8	0.543	0.874	0.879	1.090
SA4	(C)3–8 (T)6–12 (T)7–9	F: CGGATCTATTATGACATATCC R: GAAATATGAATACACTAGATTAGG	127–155	8	0.123	0.456	0.486	0.598
SA5	(A)7–8(T)5–6	F: GAAGGAATAGTCGTTTTCAAG R: CATAAATAGAGTTCCATTTCGG	155–164	5	0.675	0.325	0.874	0.886
Mean				6.6	0.572	0.527	0.752	0.844

Here, NA = number of alleles, HO = observed heterozygosity, HE = expected heterozygosity, GD = gene diversity, and PIC = polymorphism information content

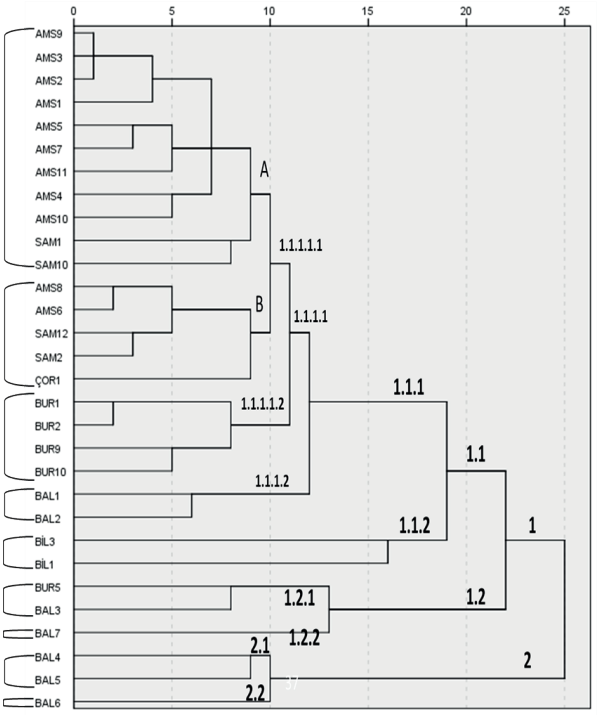

Figure 2. Dendrogram indicating genetic relationship among *Sinapis arvensis* populations generated by hierarchical clustering

The dendrogram based on the presence or absence of band indices divided the populations into two main groups (Figure 2). Based on the genotypes studied, the family tree is divided into 2 main groups. According to UPGMA analysis, high similarity (96%) was determined between AMS-2/ Amasya-Merkez and AMS-9/Amasya-Suluova populations.

The most distant genetic similarity (2%) was determined between BAL-1/Balıkesir-Bandırma and BAL-6/Balıkesir-Gönen, BAL-2-BAL-6/Balıkesir-Gönen-1,2 BAL-2/ Balıkesir-Gönen and BAL-5/Balıkesir-Manyas, AMS-9/ Amasya-Suluova and BAL-6/Balıkesir-Gönen and AMS-11/ Amasya-Merkez and BAL-6/Balıkesir-Gönen populations. Moderate similarity was found between the other populations (Table 2).

SSR markers were used to determine the genetic diversity within and between populations in *S. arvensis* and it was determined that the populations of this species have high genetic diversity.

As a result of the optimization studies, a total of 5 SSR primers were found to work in *S. arvensis*. In the dendrogram created by using hierarchical clustering analysis of molecular parameters, the sampled genotypes were taxonomically classified into a total of 9 groups, including 2 main groups.

Table 2. Correlation matrix of *Sinapis arvensis* populations based on SSR-PCR band results

	BAL6	BAL4	BAL5	BAL7	BİL3	BUR5	BAL3	BAL1	BAL2	AMS8	AMS6	SAM1	AMS1	ÇOR1	AMS9	AMS2	AMS4	AMS5	AMS7	AMS3	AMS10	1	SAM10	BUR1	BUR2	BUR9	BİL1	0	SAM12	SAM2
BAL6	100%																													
BAL4	70%	100%																												
BAL5	66%	70%	100%																											
BAL7	33%	36%	49%	100%																										
BİL3	49%	46%	43%	62%	100%																									
BUR5	21%	15%	36%	70%	62%	100%																								
BAL3	20%	15%	27%	43%	41%	73%	100%																							
BAL1	2%	6%	10%	27%	39%	39%	57%	100%																						
BAL2	2%	11%	2%	33%	49%	38%	46%	79%	100%																					
AMS8	23%	19%	16%	25%	39%	32%	39%	72%	66%	100%																				
AMS6	23%	28%	16%	17%	39%	23%	30%	64%	66%	91%	100%																			
SAM1	19%	15%	11%	29%	44%	17%	25%	59%	70%	79%	70%	100%																		
AMS1	6%	19%	7%	24%	41%	29%	37%	62%	74%	83%	83%	79%	100%																	
ÇOR1	23%	36%	24%	7%	23%	12%	29%	54%	57%	75%	83%	62%	83%	100%																
AMS9	2%	23%	19%	19%	37%	33%	49%	66%	70%	71%	71%	66%	87%	79%	100%															
AMS2	6%	28%	24%	24%	41%	29%	46%	62%	66%	66%	66%	70%	83%	74%	96%	100%														
AMS4	15%	28%	32%	15%	32%	21%	29%	54%	57%	58%	66%	62%	66%	74%	79%	83%	100%													
AMS5	11%	15%	28%	19%	29%	33%	32%	58%	62%	71%	71%	66%	70%	70%	74%	70%	87%	100%												
AMS7	7%	19%	23%	23%	34%	36%	44%	61%	66%	76%	76%	71%	83%	75%	87%	83%	75%	87%	100%											
AMS3	6%	19%	15%	15%	41%	29%	46%	71%	74%	75%	75%	70%	83%	74%	96%	91%	83%	79%	83%	100%										
AMS10	6%	19%	24%	33%	41%	38%	46%	62%	66%	58%	58%	62%	66%	57%	79%	83%	83%	70%	66%	83%	100%									
AMS11	2%	7%	20%	29%	36%	35%	34%	59%	62%	70%	61%	74%	70%	53%	75%	79%	79%	83%	79%	79%	79%	100%								
SAM10	19%	24%	20%	39%	34%	29%	36%	69%	71%	70%	61%	74%	71%	62%	67%	71%	71%	76%	72%	71%	71%	74%	100%							
BUR1	23%	28%	24%	41%	49%	38%	37%	54%	57%	66%	58%	70%	66%	49%	62%	66%	66%	70%	66%	66%	74%	79%	71%	100%						
BUR2	24%	28%	23%	40%	43%	36%	36%	44%	49%	59%	59%	62%	58%	49%	53%	58%	66%	70%	66%	58%	66%	71%	63%	92%	100%					
BUR9	28%	41%	20%	31%	52%	20%	27%	51%	62%	61%	61%	66%	71%	53%	67%	71%	62%	58%	63%	71%	62%	66%	74%	79%	72%	100%				
BİL1	23%	20%	25%	27%	47%	26%	24%	30%	32%	31%	31%	44%	41%	23%	37%	41%	41%	37%	34%	41%	49%	53%	43%	67%	60%	69%	100%			
BUR10	28%	41%	20%	39%	61%	29%	36%	60%	71%	70%	70%	74%	71%	53%	67%	71%	62%	58%	63%	71%	62%	66%	65%	79%	72%	83%	52%	100%		
SAM12	28%	41%	20%	20%	44%	26%	34%	59%	62%	79%	87%	57%	70%	70%	66%	62%	62%	66%	71%	70%	53%	57%	57%	70%	71%	74%	44%	83%	100%	
SAM2	32%	28%	16%	25%	48%	41%	48%	64%	58%	83%	74%	61%	66%	58%	62%	58%	49%	62%	67%	66%	49%	61%	61%	75%	67%	70%	48%	78%	87%	100%

This is a similarity matrix

When the genotypes were analyzed based on geographical locations, genotypes from the same locations were grouped into the same subgroups.

Freville et al. (2001) investigated the genetic structure of *Centaurea corymbosa*, a narrow endemic plant, using six microsatellite loci and compared their results with allozyme analysis. Microsatellite analysis revealed that there is a wide differentiation among populations. It was also reported that allozyme loci are less powerful than microsatellites in determining the extent of gene flow (Çağlar 2010). Naghavi et al. (2009) used 21 SSR primers to determine the genetic relationship between 52 *Triticum aestivum* and 13 *Aegilops* species collected from various regions of Iran using SSR markers.

It was observed that SSR analysis demonstrated significant gene expression in the investigations aimed at identifying genetic diversity in different species (Guo et al. 2022, Randazzo et al. 2019, Singh et al. 2020, Xiong et al., 2019). It was reported that when the genetic composition of the indigenous plant *Centaurea corymbosa* was analyzed, SSR analysis employing six microsatellite loci indicated a significant divergence across populations. According to Lopez-Vinyallonga et al. (2011), these microsatellites were found to be more effective than allozyme loci at detecting gene flow coverage.

In this study, 11 SSR primers were used to determine the genetic relationship between 30 *S. arvensis* populations. In the study of Çağlar (2010), 3 SSR primers were used and it was observed that the genetic similarity rate in *Centaurea nivea* biotype varied between 26% and 76%. Kaya-Altop (2012) determined the genetic relationship between *Cyperus difformis* populations, which is a problem in paddy cultivation areas, using RAPD primers and determined that genetic similarity varied between 0.01% and 96%. In addition, Gıdık (2016) identified 9 genotypes of *S. arvensis*, observing polymorphism in all 10 ISSR primers used in the research. The ISSR method revealed that the polymorphism rate within the *S. arvensis* population ranged between 58.33% and 14.58%. In another study, in analyses based on rDNA and cpDNA gene regions to investigate intraspecific variations of *Sinapis arvensis*, it was found that the ITS4 and ITS5 regions of the rDNA gene did not allow for the determination of subspecies. However, these regions were considered highly suitable for species diagnosis and determining species relationships. On the other hand, analyses relying on the matK region of cpDNA (trnK-710F and trnK-2R) were found to be a successful method for distinguishing subspecies of *S. arvensis*. Furthermore, the results of phylogenetic analyses based on rDNA and cpDNA regions suggested that taxonomically, *Sinapis arvensis* should be considered within the *Brassica* genus rather than the *Sinapis* genus (Ateş 2022). In another study with 5 SSR primers, the genetic diversity rate among 62 *E. oryzoides* populations was found to support the present study (Altop et al. 2018). In another study, genetic diversity studies were carried out on 40 different *Alopecurus myosuroides* populations with 5 different SSR primers, and it was determined that genetic diversity was detected at a high rate and had similar results and backing up to the current study (Boylu and Kaya Altop 2021). For *S. arvensis*, while the genetic similarity rate varied between 2% and 96%, it was determined that the closest populations to each other genetically were AMS-2/ Amasya-Merkez and AMS-9/ Amasya-Suluova populations with 96% similarity rate. The most distant genetic similarity was determined between BAL-1/Balıkesir-Bandırma and BAL-6/Balıkesir-Gönen, BAL-2-BAL-6/Balıkesir-Gönen-1,2 BAL-2/Balıkesir-Gönen and BAL-5/Balıkesir-Manyas, AMS-9/Amasya-Suluova and BAL-6/Balıkesir-Gönen and AMS-11/Amasya-Merkez and BAL-6/ Balıkesir-Gönen populations with a value of 2%.

The variation among populations was highly diverse, and the clarity of geographical isolation was remarkable. This can be interpreted primarily in terms of adaptation to geographical areas and the concept that even if there are self-fertilized species, some cross-fertilization may also be present in them. Other important factors may include the transport of seeds between regions by humans and tools and the resistance developed by the weed against herbicides used in weed control methods. The high rate of genetic diversity in the findings obtained points to the potential of the species to come up with the problem of resistance. The possibility of gene escape between the resistant forms and wild or susceptible populations is considered a strong possibility. The present study may shed light on the next researchers and issues.

ACKNOWLEDGEMENTS

This study was carried out in Plant Protection Department of Agricultural Faculty of Ondokuzmayis University.

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

Yabani hardal (*Sinapis arvensis* L.), buğday üretimi üzerinde olumsuz etkiler yaratan, kendi kendine döllenen bir yabancı ot türüdür ve bu türle mücadelede yoğun olarak herbisitler kullanılmaktadır. Yabancı döllenme bu türde genetik farklılaşmaya yol açabilir. Bu nedenle bu çalışmada Türkiye'nin çeşitli bölgelerindeki buğday tarlalarından toplanan yabani hardal popülasyonları arasındaki genetik çeşitlilik araştırılmıştır. Yabani hardal popülasyonlarının genetik varyasyon derecesi, Türkiye'nin 30 farklı lokasyonundan

alınan örneklerde 5 basit dizi tekrarı (SSR) işaretleyici kullanılarak değerlendirilmiştir. Popülasyonlar hiyerarşik kümeleme analizi (UPGMA) ve temel bileşen analizi (PCA) kullanılarak analiz edilmiştir. Ortalama genetik çeşitlilik (GD) ve polimorfizm bilgi içeriği (PIC) değerleri sırasıyla 0.752 ve 0.844 olarak bulunmuştur. Sonuçlar, coğrafi konumlar içinde bireysel genotipler arasında yüksek genetik değişkenlik göstermiştir. Popülasyonlar UPGMA dendrogramı tarafından gösterildiği gibi, iki ana grupta kategorize edilmiştir. İncelenen yabani hardalın genotipleri arasında belirgin coğrafi izolasyon belirlenememiş ve yüksek derecede değişkenlik göstermiştir. Bu değişkenliğin ana kaynağının, farklı lokasyonlara çeşitli yöntemlerle dağıtılan yabani hardal tohumlarının adaptasyonu olduğu düşünülmektedir. Çoğunlukla kendi kendine döllenme yapan bir tür olmalarına rağmen, bazı yabancı döllenme mekanizmalarını da kullanabilirler. Sonuç olarak, SSR belirteçlerinin, özellikle önceden genotipik bilginin mevcut olmadığı durumlarda, geçiş yapan türlerdeki genetik çeşitliliğin belirlenmesinde yararlı olduğu belirlenmiştir. Çalışma, rotasyonel tarım uygulamaları ve yoğun herbisit kullanımına rağmen yabani hardal popülasyonlarında genetik çeşitliliğin korunduğunu göstermektedir.

Anahtar kelimeler: SSR, yabani hardal, moleküler marker, genetik çeşitlilik

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Cite this article: Güzel, O., & Kaya Altop, E. (2024). Genetic diversity of *Sinapis arvensis* L. (wild mustard) in Türkiye determined by microsatellite markers. Plant Protection Bulletin, 64-1. DOI: 10.16955/bitkorb.1378621

Atıf için: Güzel, O., & Kaya Altop, E. (2024). Mikrosatellit marker ile Türkiye'de *Sinapis arvensis* L. (yabani hardal)'ın genetik çeşitliliğinin belirlenmesi. Bitki Koruma Bülteni, 64-1. DOI: 10.16955/bitkorb.1378621

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