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Insecticidal effectiveness of *Prunus laurocerasus* leaf and seed extracts on *Halyomorpha halys* nymphs and adults

Prunus laurocerasus yaprak ve tohum ekstraktlarının *Halyomorpha halys* nifleri ve erişkinleri üzerindeki öldürücü etkinliği

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Anahtar Kelimeler: Kahverengi Kokarca, Karayemiş, Bitki ekstraktı, Toksisite

ABSTRACT

In this study, the toxicity of Cherry laurel, *Prunus laurocerasus* L. (Rosaceae) leaf and seed extracts at doses of 10, 15, and 20 mL/L against Brown Marmorated Stink Bug (*Halyomorpha halys*) adults and nymphs at 24., 48., 72., and 96. hours were investigated under laboratory conditions. All experiments were performed separately for five nymphal stages and adults and repeated three times for each dose. At the 96 hours of treatment, *P. laurocerasus* leaf extracts caused mortality between 21.5% and 100%, while the seed extract caused mortality between 30.3% and 100%. The highest mortality rate occurred in the N₁ and N₂ instar nymphs (100%) at the 20 mL/L dose, and the lowest mortality rate occurred in the adult stage (21.5%) at the 10 mL/L dose. The productivity rates of seed extracts were higher than those of leaf extracts. In all treatments, mortality rates increased with increasing dose and time. The results showed that *P. laurocerasus* leaf and seed extracts had a highly toxic effect against five nymphal stages and adult *H. halys*. The most toxic LD₅₀ values in leaf extract were kept as 0.45 and 3.64 mL/L for N₁ stage nymphs and juveniles, while the lowest LD₉₀ values were determined as 4.54 and 6.64 mL/L for N₅ instar nymphs and adults, respectively. The seed extract yielded the lowest LD₅₀ values of 0.29 and 1.84 mL/L for N₁ instar nymphs and adults, and the lowest LD₉₀ values of 2.89 and 3.15 mL/L for N₅ instar nymphs and adults, respectively. The results indicate that leaf and seed extracts of *P. laurocerasus* have excellent potential for the control of *H. halys*.

Öz

Bu çalışmada, Karayemiş bitkisinin, *Prunus laurocerasus* L. (Rosaceae) yaprak ve tohum ekstraktlarının 10,15 ve 20 mL/L dozlarının, 24., 48., 72. ve 96. Saatlerinde Kahverengi Kokarca (*Halyomorpha halys*) ergin ve nimflerine karşı laboratuvar şartlarında toksisiteyi incelenmiştir. Tüm deneyler beş nimf dönemi ve erginler için ayrı ayrı yapılmış, her bir doz için 3'er kez tekrarlanmıştır. Uygulamanın 96. saatinde, *P. laurocerasus* yaprak ekstraktları %21.5 ile %100 arasında, tohum ekstraktları %30.3 ile %100 arasında ölümlere sebep olmuştur. En yüksek ölüm oranı 20 mL/L dozunda N₁ ve N₂ nimf dönemlerinde (%100), en düşük ölüm oranı 10 mL/L dozunda ergin dönemde (%21.5) gerçekleşmiştir. Tohum ekstraktlarının toksisite oranları yaprak ekstraktlarından daha fazla olmuştur. Tüm uygulamalarda mortalite oranları artan doz ve zamanla birlikte artış göstermiştir. *P. laurocerasus* yaprak ve tohum ekstraktlarının beş nimf evresine ve *H. halys*'in yetişkinlerine karşı oldukça toksik bir etkiye sahip olduğu tespit edilmiştir. LD₅₀ ve LD₉₀ değerlerine göre, yaprak ekstraktında en toksik LD₅₀ değerleri N₁ dönem nimf ve yetişkinler için 0.45 ve 3.64 mL/L olarak kaydedilirken, en düşük LD₉₀ değerleri N₅ dönem nimf ve yetişkinler için sırasıyla 4.54 ve 6.64 mL/L olarak belirlenmiştir. Tohum ekstraktında ise bu değerler en toksik LD₅₀ değerleri N₁ dönem nimf ve yetişkinler için 0.29 ve 1.84 mL/L olarak kaydedilirken, en düşük LD₉₀ değerleri N₅ dönem nimf ve yetişkinler için sırasıyla 2.89 ve 3.15 mL/L olarak belirlenmiştir. Sonuçlar, *P. laurocerasus*'un yaprak ve tohum ekstraktlarının *H. halys* kontrolünde kullanılmak için iyi bir potansiyele sahip olduğunu işaret etmektedir.

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1. INTRODUCTION

Global warming and increased international trade and transport have facilitated the spread of plant pests to different continents and countries. Invasive species cause economic losses in a new ecosystem by negatively affecting biodiversity, natural and agricultural ecosystems (Simberloff et al., 2013). Typically, these pest species can precipitate epidemics rapidly due to the absence of natural predators, the emergence of new hosts, and their remarkable adaptability (Pimentel et al., 2000). The Brown Marmorated Stink Bug [*H. halys* (Stål, 1855) (Hemiptera: Pentatomidae)] is a prominent invasive pest.

Although it is native to the Far East (China, Japan, Korea, and Taiwan), it has spread to many countries around the world. In Turkey, it was detected in Artvin in 2017, and in the last 7 years it has spread all over the Eastern Black Sea Region and part of the Marmara Region (Göktürk, 2023).

H. halys has a wide host range and can feed on more than 300 plant species, including vegetables, nuts, agricultural crops, and ornamental plants (Nielsen & Hamilton, 2009). It has caused millions of dollars of losses in agricultural areas in the United States, Georgia, and many countries in Europe (Maistrello et al., 2017; Ak et al., 2019). Hazelnuts have been the most damaged plant in Turkey since the day of their discovery. According to Göktürk (2024), the damage in hazelnut orchards, particularly in Ordu and Giresun provinces, amounted to 30% in 2023-2024.

Adults and nymphs of *H. halys* inflict damage by directly injecting digestive enzymes into the fruit and sucking plant juice, a process made possible by their stinging and sucking mouth structures. Damaged crops greatly reduce their market value (Rice et al., 2014). It poses a significant urban issue due to its detrimental effects on human health, particularly the foul odors it emits, and its tendency to enter houses in large groups during the winter months (Inkley, 2012).

It has been shown that *H. halys* 4-6 generations annually in South Asia, but most countries within its distribution area only record 1 or 2 generations (Rice et al., 2014). After five nymphal stages, it becomes an adult and spends the winter among the plant residues on the ground, in holes, under the bark of trees, and in different residential areas (Lee et al., 2013).

Management of the *H. halys* is very difficult to control due to its rapid spread and excessive reproduction capacity. Different methods are used to control *H. halys*. Reports suggest that the pest's recruitment pheromones (PHER) serve as an important tool in biotechnical control (Weber et al., 2014). Turkey, along with many other countries worldwide, produces and releases *Trissolcus japonicus* (Hymenoptera: Scelionidae), the egg parasitoid of *H. halys*, into fields as part of biological control (Zhang et al., 2014; Göktürk, 2023). Most people prefer chemical control against the pest (Morrison et al., 2016). Most of the researchers prefer chemical control against the pest (Morrison et al., 2016) and broad-spectrum insecticides have been applied to control *H. halys* (Leskey et al., 2012; Leskey et al., 2014), but the control of *H. halys* requires alternative management methods due to their insufficient efficacy, short-term solution, negative environmental effects, and incompatibility with integrated pest management (IPM) strategies. Researchers have extensively investigated the potential application of plant extracts as alternatives to synthetic pesticides (Weber et al., 2014).

Plant extracts represent a non-chemical control alternative. Plant extracts contain secondary metabolites with known repellent, anti-feeding, egg, larval, nymphal, and adulticidal effects against insects (Tomczyk & Suszko, 2011). *Rhododendron ponticum* L. (Ericaceae), *Satureja spicigera* (C. Koch) Boiss, *Satureja hortensis* L., *Rosmarinus officinalis* L., (Lamiaceae), *Nerium oleander* L. (Apocynaceae), *Artemisia absinthium* L., *Tanacetum vulgare* L. (Asteraceae) and *Calotropis porcera* (Ait.) (Asclepiadaceae) are plants that have been tested many times and reported to be effective (Gokturk et al., 2021; Gokturk et al., 2023).

There are very few studies on the trial of herbal products against *H. halys*. Gokturk (2021) tested *Satureja spicigera* essential oil against *H. halys* adults and nymphs and found that it has a high killing effect, especially in the N1 and N2 instar nymph. Zhang et al. (2014) found that lemongrass oil (Poaceae), ylang-ylang oil (Annonaceae), peppermint oil (Lamiaceae), and clove oil (Myrtaceae) almost completely prevented *H. halys* from entering the host, while ennyroyal, wintergreen, rosemary, and geranium oils showed 50–85% repellency.

Thomas (2004) suggests using Cherry laurel, *P. laurocerasus* L. (Rosaceae) as a pesticide. *P. laurocerasus* is known as 'Taflan', 'Georgian Cherry', 'Karamış', 'Kattak', 'Laz Grape', and 'Tahnal' (Eminağaoğlu & Anşin,

2005; Akbulut et al., 2007; Akpulat et al., 2019). It is an evergreen tree and a very characteristic part of the Black Sea Region, especially in Northern and Southern Anatolia (Işık & Eminağaoğlu, 2023; Kolaylı et al. 2003). *P. laurocerasus* grows naturally in many areas in Artvin province (Eminağaoğlu, 2012; Eminağaoğlu, 2015; Eminağaoğlu, 2023). It grows up to six meters high, and its fruits are oval, 8-20 mm in diameter, dark purple, and black when ripe. The leaves are dark green, thin, and 16.3 cm long on average (Islam & Deligöz, 2012; Yüksel & Eminagaoglu, 2017).

P. laurocerasus has a high morphological diversity but also has a high content of substances that support human health, such as anthocyanins, flavonoids, and phenolic acids (Gecer et al., 2020; Grygorieva et al., 2021). The fruits of the cherry laurel are a rich source of monosaccharides, dietary fibers, minerals, vitamins (Ca, Fe, K, Mg, Cr, Na, Zn, Cu, Ni, P, Mo, Co), and phenolic compounds (benzoic acids, chlorogenic, vanillic, and caffeic), and are noted for their significant antioxidant activity (Demir et al., 2017; Erguney et al., 2017).

Ertürk et al. (2004) demonstrated the harmful impact of *P. laurocerasus* leaf extract on *Plutella xylostella* L. (Lepidoptera: Plutellidae). Akyazı et al. (2015) examined the poisonous and repellent properties of *P. laurocerasus* extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae) and determined that the seed extract exhibited an efficacy of 96.56%-100%.

This study aimed to assess the lethal efficacy of seed and leaf extracts of *P. laurocerasus* on *H. halys* nymphs and adults in a laboratory conditions.

2. MATERIAL AND METHOD

2.1. Collecting insect

H. halys adults were collected from Arhavi (Artvin) from pheromone traps hung in kiwifruit fields in May-June 2024. The collected insects were brought to the laboratory, given fresh cucumber slices as food, and kept in net cages for 48 hours at 25±2°C temperature, 65±5% humidity, and a 14:10 (L:D) lighting period. Afterwards, some of the healthy and active-looking adults were taken directly into the treatment, and some of them were placed in cages with dimensions of 60 x 60 x 60 cm and covered with tulle, specially made of wood and containing bean plants, with 10 males and females in 10 cages. In these cages, they were expected to lay eggs on

the leaves. The nymphs of *H. halys* that emerged from the eggs were fed with strawberry fruit and were taken into plastic containers at the 1st, 2nd, 3rd, 4th, 5th nymph stages and used in the experiments.

2.2. Collection of plant samples

The leaves and seeds of *P. laurocerasus* plant used in this study were collected from Artvin province before the flowering period and during the ripening stages of the fruits. The collected leaves were dried at room temperature in a sun-free environment. The seeds of the fruits were removed, and the seeds were dried in a cool place.

2.3. Obtaining of plant extracts

P. laurocerasus leaf and seed extracts were obtained by the Soxhlet extraction method (Luque de Castro & García-Ayuso, 1998). Plant leaves were first washed with ultrapure water, filtered, and dried. We crushed the dried leaves in a mortar, placed them in a cellulose cartridge, and then placed them in a Soxhlet extractor. The ethanol solvent was placed in a single-neck flask with a round bottom, and the Soxhlet extractor and recoler system were set up. The extraction process was carried out above the boiling temperature of ethanol (>78 °C). A rotary evaporator removed the ethyl alcohol after the extraction process was complete. Leaf extracts were dried completely with a vacuum system and stored in a refrigerator at 4 °C for use in the tests. The same procedures were carried out for seed extracts. The extracts obtained were then diluted with distilled water and adjusted to target concentrations (10, 15, and 20 mL/L) for use in the experiments.

2.4. Bioassays using plant extracts

Adult and nymphs (N1, N2, N3, N4, and N5) of *H. halys* were placed in 20 transparent plastic containers (20 x 15 cm). Three different concentrations (10, 15, and 20 mL/L) of leaf and seed extracts were sprayed onto the insects using a spray tower, 5 mL for each concentration. Live and dead insects were counted at 24, 48, 72, and 96 hours. Nymphs and adults were deemed deceased when no leg or antennal movements were seen following stimulation with a fine brush. Sterile water combined with ethanol served as the negative control, whereas the commercial pesticide Nimbecidine® EC (Neem-Oil Based Herbal pesticide, 10% Azadirachtin) functioned as the positive control. Each test was conducted in triplicate for every nymphal stage, adult stage, and dosage. The

average fatality rates observed in adults and nymphs due to plant extract poisoning were represented as a percentage. All toxicity assessments were conducted under controlled laboratory circumstances of 25°C (±2), 65% (±5) relative humidity, and a 14:10 h light/dark photoperiod. The experiments were completed in 5 months (May-September) under laboratory conditions.

2.5. Statistical analyses

An analysis of variance (ANOVA) test using the SPSS 17.0 software package determined differences between the contact toxicities of *P. laurocerasus* leaf and seed extracts. Mortality rates were shown as mean (percentage) ± standard error. Duncan's test was employed to assess differences between means, with values of $P < 0.05$ deemed statistically significant. LD50 and LD90 values at 96 hours were determined by probit regression analysis utilizing SPSS (Finney, 1971). Probit analysis of dose-mortality data was performed to determine the LD50 and LD90 values, along with their respective 95% confidence intervals for each treatment.

3. RESULTS AND DISCUSSION

P. laurocerasus leaf and seed extracts were used in the study at 3 different doses (10, 15, and 20 mL/L respectively). These doses caused different death rates in the adults and 5 instar nymphs of *H. halys* compared to the controls. Table 1 summarizes the results of the leaf extract application. It was determined that the mortality rates for all individuals ranged from 30.7% to 100% 96 hours after the applications. As a result of the application, statistical differences were determined in mortality rates among nymphal stages at 24, 48, 72, and 96 hours in *P. laurocerasus* leaf extract application. It has been determined that the most effective dose for N1, N2, N3, N4, and N5 instars is 20 mL/L. During the nymph stages, we observed the highest mortality rates in N1 and N2 stage nymphs at a dose of 20 mL/L of extract 96 hours later (78.7-100%), and the lowest mortality rates in N5 stage nymphs at a dose of 10 mL/L of extract 24 hours later (21.5%). In adults, the highest mortality rate was observed at 96 hours after a dose of 20 mL/L of extract, at 59.5%, while the lowest mortality rate was recorded at 24 hours after a dose of 10 mL/L of extract, at 20.8% (Table 1; $P < 0.05$).

Table 1. The effectiveness of *P. laurocerasus* leaf extracts on *H. halys*

		Dose (%)	Mortality (%) Exposure Time (hours)			
			24	48	72	96
N ₁ Instar Nymph						
<i>P. laurocerasus</i>	10		37.4 ± 2.15 ^d	50.7 ± 2.44 ^d	59.8 ± 1.48 ^d	74.5 ± 1.12 ^c
	15		53.5 ± 1.43 ^c	64.5 ± 3.12 ^c	73.5 ± 1.44 ^c	84.7 ± 3.24 ^b
	20		70.7 ± 1.49 ^b	81.6 ± 1.22 ^b	91.3 ± 4.26 ^b	100 ± 0.0 ^a
Positive Control	10		100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control			0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^d
N ₂ Instar Nymph						
<i>P. laurocerasus</i>	10		32.8 ± 1.86 ^d	46.9 ± 3.22 ^d	48.5 ± 1.21 ^d	67.2 ± 1.12 ^d
	15		45.5 ± 3.52 ^c	57.4 ± 1.15 ^c	62.5 ± 1.23 ^c	72.4 ± 2.52 ^c
	20		61.2 ± 2.55 ^b	69.2 ± 4.11 ^b	80.6 ± 3.22 ^b	89.6 ± 1.21 ^b
Positive Control	10		100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control			0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
N ₃ Instar Nymph						
<i>P. laurocerasus</i>	10		28.9 ± 2.22 ^d	37.4 ± 1.24 ^d	43.3 ± 2.73 ^d	51.3 ± 3.21 ^d
	15		37.6 ± 2.13 ^c	41.5 ± 1.56 ^c	51.8 ± 1.15 ^c	62.5 ± 2.25 ^c
	20		50.5 ± 3.35 ^b	55.6 ± 2.22 ^b	69.7 ± 2.44 ^b	78.7 ± 2.44 ^b
Positive Control	10		100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control			0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
N ₄ Instar Nymph						
<i>P. laurocerasus</i>	10		24.2 ± 1.25 ^d	30.5 ± 2.33 ^d	37.2 ± 1.33 ^d	45.5 ± 1.12 ^d
	15		31.4 ± 2.17 ^c	37.1 ± 3.42 ^c	42.4 ± 1.44 ^c	58.4 ± 3.23 ^c
	20		41.1 ± 2.25 ^b	40.6 ± 2.13 ^b	57.5 ± 3.12 ^b	69.2 ± 1.43 ^b

Positive Control	10	95.6 ± 1.95 ^a	98.7 ± 3.32 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
N ₅ Instar Nymph					
<i>P. laurocerasus</i>	10	21.5 ± 3.33 ^d	30.2 ± 1.13 ^d	32.8 ± 1.22 ^d	36.6 ± 4.12 ^d
	15	29.2 ± 2.71 ^c	34.5 ± 2.11 ^c	36.5 ± 2.41 ^c	50.2 ± 1.78 ^c
	20	35.4 ± 1.83 ^b	37.6 ± 7.12 ^b	49.1 ± 1.15 ^b	62.4 ± 3.41 ^b
Positive Control	10	85.7 ± 1.95 ^a	89.5 ± 3.53 ^a	94.3 ± 2.12 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
Adult					
<i>P. laurocerasus</i>	10	20.8 ± 1.12 ^d	26.3 ± 2.27 ^d	28.8 ± 1.51 ^d	30.7 ± 1.72 ^d
	15	26.4 ± 2.45 ^c	30.1 ± 2.15 ^c	33.2 ± 3.43 ^c	45.3 ± 1.83 ^c
	20	30.5 ± 3.33 ^b	36.5 ± 1.15 ^b	50.4 ± 1.23 ^b	59.5 ± 3.41 ^b
Positive Control	10	80.5 ± 4.73 ^a	86.4 ± 3.41 ^a	94.5 ± 1.12 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e

^a Values followed by various letters in the same column differ crucially at $P \leq 0.05$ according to the Duncan Multiple tests.

^b Mean ± SE of three replicates, each set up with 20 adult/nymph

The experiments observed the highest mortality rate of 70.7% in the N₁ instars nymph of *H. halys* at a dose of 20 mL/L on the 24th hour. On the other hand, the lowest nymph toxicity of the *P. laurocerasus* leaf extract at a dose of 10 mL/L was determined to be 21.5% at the N₅ nymph nymph. Similarly, adult toxicity was found to be highest (59.5%) at a dose of 20 mL/L, while the lowest (20.8%) was at a dose of 10 mL/L. In all applications, mortality rates have increased with higher doses and over time. The mortality rates in the N₁, N₂, N₃, N₄, and N₅ nymph stages were determined to be 37.4-100%, 32.8-89.6%, 28.9-78.7%, 24.2-69.2%, and 21.5-62.4% at the 24th, 48th, 72nd, and 96th hours, respectively.

In positive controls (Nimbecidine® EC (10 mL/L), 100% toxicity was observed in both adult and nymph stages after 96 hours. Additionally, in all applications, no deaths were observed in the negative control for nymphs and adults (Table 1; $P < 0.05$).

Table 2 displays the L_D values (LD₅₀ and LD₉₀ for leaf extract) of the study. The study found that the *P. laurocerasus* leaf extract exhibited LD₅₀ values of 0.55, 0.61, 0.79, 1.52, and 2.49 mL/L for the N₁, N₂, N₃, N₄, and N₅ stages, respectively, 96 hours after application. The LD₉₀ values of the *P. laurocerasus* leaf extract 96 hours after application were found to be 1.63, 2.24, 2.95, 3.81, and 4.54 mL/L for each nymph stage, respectively. When the LD₅₀ and LD₉₀ values of the *P. laurocerasus* leaf extract were checked for adults 96 hours after application, they were found to be 3.64 and 6.64 mL/L, respectively (Table 2). The leaf extract of *P. laurocerasus* has caused significant toxicity in the five nymph and adult stages of *H. halys*. (Table 1, 2). In conclusion, when comparing the mortality rates of the N₁, N₂, N₃, N₄, and N₅ stages of *H. halys* 96 hours after treatment, the adult and N₅ stages of *H. halys* are the most resistant to the *P. laurocerasus* leaf extract, while the N₁ and N₂ stages are the most sensitive (Table 1, 2).

Table 2. LD₅₀ and LD₉₀ values of *P. laurocerasus* leaf extracts on *H. halys*

Stage of <i>H. halys</i>	LD ₅₀ ^a	LD ₉₀ ^b	X ² ^c	Slope ± SE ^d
N ₁ Instar Nymph	0.45	1.63	4.58	3.75 ± 1.12
N ₂ Instar Nymph	0.61	2.24	6.13	2.34 ± 0.83
N ₃ Instar Nymph	0.79	2.95	6.92	2.53 ± 1.14
N ₄ Instar Nymph	1.52	3.81	9.34	3.26 ± 2.55
N ₅ Instar Nymph	2.49	4.54	12.26	4.66 ± 1.29
Adult	3.64	6.64	14.43	2.84 ± 0.94

^a The lethal dose causing 50% mortality after 96 h, ^b The lethal dose causing 90% mortality after 96 h, ^c Chi-square value $P \leq 0.01$,

^d Slope of the concentration-mortality regression line ± standard error.

Table 3 summarizes the application results of the seed extracts. It was determined that the mortality rates were

between 30.3% and 100% for all individuals 96 hours after the applications (Table 3).

Table 3. The effectiveness of *P. laurocerasus* seed extracts on *H. halys*

	Dose (%)	Mortality (%) Exposure Time (hours)			
		24	48	72	96
N ₁ Instar Nymph					
<i>P. laurocerasus</i>	10	45.8 ± 1.63 ^d	56.5 ± 1.55 ^d	62.9 ± 1.22 ^c	79.6 ± 3.22 ^b
	15	64.2 ± 2.56 ^c	73.8 ± 1.22 ^c	87.5 ± 1.47 ^b	100 ^a
	20	88.4 ± 3.25 ^b	100 ^a	100 ^a	100 ^a
Positive Control	10	100 ^a	100 ^a	100 ^a	100 ^a
Negative Control		0.0 ^e	0.0 ^e	0.0 ^e	0.0 ^c
N ₂ Instar Nymph					
<i>P. laurocerasus</i>	10	43.5 ± 1.22 ^d	54.5 ± 1.12 ^d	61.6 ± 2.22 ^c	75.5 ± 1.12 ^c
	15	57.2 ± 1.55 ^c	65.2 ± 2.34 ^c	78.4 ± 2.29 ^b	90.7 ± 1.22 ^b
	20	74.5 ± 2.26 ^b	87.6 ± 1.51 ^b	100 ± 0.0 ^a	100 ± 0.0 ^a
Positive Control	10	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^d	0.0 ± 0.0 ^e
N ₃ Instar Nymph					
<i>P. laurocerasus</i>	10	39.5 ± 1.22 ^d	45.2 ± 1.12 ^d	49.5 ± 2.22 ^d	60.9 ± 1.21 ^d
	15	48.9 ± 2.33 ^c	52.4 ± 2.34 ^c	61.4 ± 1.33 ^c	71.7 ± 3.55 ^c
	20	59.5 ± 1.27 ^b	65.7 ± 2.22 ^b	72.7 ± 2.77 ^b	87.5 ± 2.43 ^b
Positive Control	10	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
N ₄ Instar Nymph					
<i>P. laurocerasus</i>	10	35.7 ± 1.12 ^d	40.2 ± 1.33 ^d	46.5 ± 1.65 ^d	52.4 ± 1.64 ^d
	15	43.5 ± 3.14 ^c	48.5 ± 2.22 ^c	54.7 ± 2.44 ^c	63.5 ± 1.55 ^c
	20	52.3 ± 2.25 ^b	56.3 ± 2.35 ^b	63.4 ± 1.42 ^b	75.3 ± 3.22 ^b
Positive Control	10	96.7 ± 1.12 ^a	97.8 ± 1.22 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
N ₅ Instar Nymph					
<i>P. laurocerasus</i>	10	30.8 ± 1.34 ^d	34.6 ± 1.22 ^d	40.4 ± 1.22 ^d	46.1 ± 1.28 ^d
	15	38.5 ± 1.18 ^c	42.5 ± 2.15 ^c	48.2 ± 3.12 ^c	54.2 ± 1.33 ^c
	20	44.1 ± 1.22 ^b	48.3 ± 4.23 ^b	54.5 ± 1.24 ^b	63.4 ± 3.28 ^b
Positive Control	10	86.6 ± 1.99 ^a	90.1 ± 3.87 ^a	95.2 ± 1.54 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e
Adult					
<i>P. laurocerasus</i>	10	30.3 ± 1.28 ^d	33.2 ± 1.72 ^d	37.5 ± 1.19 ^d	42.6 ± 2.23 ^d
	15	36.5 ± 2.33 ^c	39.4 ± 1.12 ^c	42.7 ± 2.21 ^c	49.9 ± 1.37 ^c
	20	42.4 ± 2.56 ^b	45.9 ± 1.22 ^b	51.6 ± 1.63 ^b	60.1 ± 2.42 ^b
Positive Control	10	83.1 ± 4.24 ^a	85.6 ± 4.18 ^a	93.7 ± 1.22 ^a	100 ± 0.0 ^a
Negative Control		0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e

^a Values followed by various letters in the same column differ crucially at P≤0.05 according to the Duncan Multiple tests.

^b Mean±SE of three replicates, each set up with 20 adult/nymph

It was determined that there were statistical differences in the mortality rates of *P. laurocerasus* seed extract 24, 48, 72, and 96 hours after the application, especially between the nymph stages. The most effective dose for

N₁, N₂, N₃, N₄, and N₅ instars was determined to be 20 mL/L. In nymphal stages, the highest mortality rates were observed in N₁ and N₂ instar nymphs at 20 mL/L extract dose after 96 hours (100%), while the lowest

mortality rates were observed in N₅ instar nymphs at 10 mL/L extract dose after 24 hours (30.8%). In adults, the highest mortality rate was 60.1% at the 20 mL/L extract dose after 96 hours, while the lowest mortality rate was 30.3% at the 10 mL/L extract dose after 24 hours (Table 3; $P < 0.05$).

At the 24th hour of the experiments, the highest mortality rate of 88.4% was observed in N₁ instars of *H. halys* at 20 mL/L dose. On the other hand, the lowest nymph toxicity of *P. laurocerasus* seed extract at 10 mL/L dose was 30.8% at the N₅ instar nymph. Similarly, adult toxicity was highest (60.1%) at 20 mL/L dose and lowest (30.3%) at 10 mL/L dose. Mortality rates increased with increasing dose and time in all treatments. Mortality rates of N₁, N₂, N₃, N₄, and N₅ instar nymphs were 45.8-100%, 43.5-100%, 39.5-87.5%, 35.7-75.3%, and 30.8-63.4% at 24, 48, 72, and 96 hours, respectively. In positive controls (Nimbecidine® EC (10 mL/L)), 100% toxicity was determined in both adults and nymphs after 96 hours. In all treatments, no mortality was observed

for nymphs and adults in the negative control (Table 3; $P < 0.05$).

Table 4 displays the LD values (LD₅₀ and LD₉₀ for seed extract) of the study. The LD₅₀ values of *P. laurocerasus* seed extract 96 hours after application were 0.29, 0.35, 0.43, 1.15, and 1.84 mL/L for N₁, N₂, N₃, N₄, and N₅ instar nymph, respectively. The LD₉₀ values of *P. laurocerasus* seed extract 96 hours after application were 1.12, 1.64, 1.92, 2.43, and 2.89 mL/L for each nymphal stage, respectively. The LD₅₀ and LD₉₀ values of *P. laurocerasus* seed extract 96 hours after application were 1.84 and 3.15 mL/L, respectively, for adults (Table 4). *P. laurocerasus* seed extract caused significant toxicities in five nymph and adult stages of *H. halys* (Table 3, 4). As a result, when the mortality rates of N₁, N₂, N₃, N₄, and N₅ instar nymph of *H. halys* were compared 96 hours after application, the adult and N₅ instar nymph of *H. halys* were the most resistant to *P. laurocerasus* seed extract, while the N₁ and N₂ instar nymphs were the most sensitive (Tables 3, 4).

Table 4. LD₅₀ and LD₉₀ values of *P. laurocerasus* seed extracts on *H. halys*

Stage of <i>H. halys</i>	LD ₅₀ ^a	LD ₉₀ ^b	X ² ^c	Slope ± SE ^d
N ₁ Instar Nymph	0.29	1.12	7.22	3.21 ± 1.22
N ₂ Instar Nymph	0.35	1.64	8.19	2.89 ± 1.87
N ₃ Instar Nymph	0.43	1.92	8.25	4.32 ± 1.24
N ₄ Instar Nymph	1.15	2.43	10.63	2.65 ± 1.83
N ₅ Instar Nymph	1.68	2.89	11.44	3.12 ± 1.45
Adult	1.84	3.15	13.62	3.45 ± 1.12

^a The lethal dose causing 50% mortality after 96 h, ^b The lethal dose causing 90% mortality after 96 h, ^c Chi-square value $P \leq 0.01$, ^d Slope of the concentration-mortality regression line ± standard error.

There are many studies on the effects of plant extracts and essential oils on insect pests, but studies on *H. halys* are very limited. Gokturk (2021), in his study to determine the toxicity of *Satureja spicigera* (C. Koch) Boiss (Lamiaceae) essential oil on *H. halys* nymphs and adults, determined that mortality rates were between 2.1% and 87.5%. In this study, mortality rates increased up to 100% in leaf and seed extracts of *P. laurocerasus*.

In another study to determine the killing effects of *P. laurocerasus* leaf, flower, and seed extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae) eggs, it was determined that 10% dose of leaf extracts was 55.57%, 10% dose of flower extracts was 79.22%, and 10% dose of seed extracts was 96.56% effective (Akyazi et al., 2015). When the lethal effects of leaf and seed

extracts of *P. laurocerasus* on *H. halys* were analyzed, it was observed that seed extract was more effective than leaf extract. It is thought that this effect may be due to the difference in the chemical composition of leaf and seed extracts of *P. laurocerasus*.

In another study conducted to determine the efficiency of *P. laurocerasus* leaf extract on *Ricania simulans* (Ricanidae: Hemiptera) nymphs and adults, a 100% killing effect on nymphs and an 86% killing effect on adults was observed (Tanyel & Ramoğlu, 2015).

In a study conducted by Dursun (2010) it was reported that blackcurrant leaves contain prunasin and amygdalin substances called cyanogenic glycosides. These substances release cyanide in their composition as

hydrocyanic acid (HCN) (Akyüz et al., 2018). HCN compounds inhibit enzymes that transport oxygen in the blood to tissues (Dursun, 2010). This explains why *P. laurocerasus* leaf extract causes high mortality rates in all instar nymphs and adults.

Essential oils and plant extracts derived from therapeutic aromatic herbs possess insecticidal effects. They serve as an exceptional substitute for chemical pesticides, mitigating the adverse effects of synthetic insecticides while safeguarding crops from pests. *P. laurocerasus* leaf and seed extracts can also be considered as a herbal insecticide to supplement other control methods used against *H. halys*. However, their open field use should also be investigated. However, their open field application should also be investigated in detail as there are going to be combination of various ecological conditions.

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