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Effect of plant phenolic compounds on the hemocyte concentration and antioxidant enzyme activity in *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) larvae infected by *Hyphantria cunea* granulovirus[1]

Hyphantria cunea granulovirus tarafından enfekte edilen *Hyphantria cunea* (Drury,1773) (Lepidoptera: Arctiidae) larvalarının hemosit konsantrasyonu ve antioksidan enzim aktivitelerine bitki fenolik bileşiklerinin etkisi

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Abstract

The aim of this study was to determine the effects of phenolic substances of four plants (apple, mulberry, plum and walnut) on hemocyte concentrations and antioxidant enzyme activity of *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) larvae infected with *Hyphantria cunea* granulovirus and uninfected. The plants used in this study were collected in Bafra, Samsun, Turkey in 2019. The phenolic concentrations of the leaves of these plants were determined. Then, the effect of these phenolic on hemocyte concentrations and antioxidant enzyme activity of infected and uninfected larvae were determined. The hemocyte concentrations of all groups increased with virus infection. The concentration of malondialdehyde decreased in all groups as a result of viral infection. The highest superoxide dismutase and catalase activities among both infected and uninfected larvae were in the plum groups with the highest concentration of chlorogenic acid, the lowest glutathione peroxidase activity was also in these groups. All this showed that different phenolic concentrations of host plants affected the hemocyte concentrations and antioxidant enzyme activity of *H. cunea* larvae.

Keywords: Antioxidant activity, granulovirus, hemocyte, *Hyphantria cunea*, phenolic compounds

Öz

Bu çalışmanın amacı, dört bitkide (elma, dut, erik ve ceviz) bulunan fenolik maddelerin *Hyphantria cunea* granulovirus ile enfekte ve enfekte olmamış *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) larvalarının hemosit konsantrasyonları ve antioksidan enzim aktivitesine olan etkilerini belirlemekti. Bu çalışmada kullanılan bitkiler Bafra, Samsun, Türkiye'de 2019 yılında toplandı. Bu bitkilerin yapraklarının fenolik konsantrasyonları belirlendi. Daha sonra, bu fenoliğin enfekte ve enfekte olmayan larvaların hemosit konsantrasyonlarına ve antioksidan enzim aktivitesine olan etkisi belirlendi. Tüm grupların hemosit konsantrasyonlarının virüs enfeksiyonu ile arttığı belirlendi. Viral enfeksiyon sonucu tüm gruplarda malondialdehit konsantrasyonu azaldı. Hem enfekte hem de enfekte olmayan larvalar arasında en yüksek süperoksit dismutaz ve katalaz aktiviteleri klorojenik asit konsantrasyonunun en yüksek olduğu erik gruplarında iken, en düşük glutatyon peroksidaz aktivitesi de bu gruplardaydı. Bütün bunlar, konukçu bitkilerin farklı fenolik konsantrasyonlarının *H. cunea* larvalarının hemosit konsantrasyonlarını ve antioksidan enzim aktivitesini etkilediğini göstermiştir.

Anahtar sözcükler: Antioksidan aktivite, granulovirüs, hemosit, *Hyphantria cunea*, fenolik bileşikler

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Introduction

Lepidopteran larvae consume a range of plants with varying nutritional content and allelochemical defenses. Plants contain metabolites known as plant secondary metabolites (PSMs), and insects take these metabolites into their bodies while feeding. PSMs are important in plant defense as they can disrupt the metabolic, biochemical, physiological functions and metabolic pathways of herbivorous insects (Alon et al., 2012). Phenolic compounds and flavonoids are PSMs that are widely present in different plant species. There are numerous studies on the effects of phenolic compounds and flavonoids on insects (Hafeez et al., 2019; Simmonds et al., 2019; Huang et al., 2020). Antioxidant activity is one of the most important effects of these metabolites in insects. Various studies have shown that tannic acid (Türkan et al., 2019), rutin and catechin (Iacopini et al., 2008), chlorogenic acid (Naveed et al., 2018), benzoic acid (Giannenas et al., 2014), protocatechuic acid (Girsang et al., 2020), and rosmarinic acid (Adomako-Bonsu et al., 2017) have antioxidant properties.

Plants not only saturate herbivorous insects, but also affect the immunological resistance of these insects to viruses that an important group of the entomopathogens. In this tritrophic interaction network, since different host plants contain different PSMs, their effects on the susceptibility of herbivorous insects to insect viruses can differ. For example, the cornworm caterpillars were found to be more susceptible to these viruses when fed corn rather than cotton (Farrar & Ridgway, 2000). *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) larvae fed on soybeans were found to be more susceptible to a virus than when fed on kale or water convolvulus (Wan et al., 2018). The phytochemicals of the host plants influence the susceptibility of herbivorous insects to insect viruses, and such differences in susceptibility are closely related to insect immunity (Wan et al., 2018). The immune response is a physiological process that protects the organism from natural enemies, and the strength of this response is affected by the PSMs of the plant consumed by herbivores (Gowler et al., 2015; Trowbridge et al., 2016). Feeding on various host plants has been suggest to enhance the immune response of herbivores, and in this case, herbivores may be better protected from pathogen infection, thereby increasing their probability of survival (Muller et al., 2015; Barthel et al., 2016).

Reactive oxygen species (ROS) have beneficial effects on immune function at low or moderate concentrations (Janssen-Heininger et al., 2008), while they cause oxidative stress and damage cell structures, such as proteins and lipids, and DNA at high concentrations (Kobayashi et al., 2019). Insects are constantly exposed to numerous environmental stressors such as ultraviolet radiation, bacteria and viruses, and agrochemicals. All of these can lead to ROS production, so insects are constantly exposed to ROS-induced oxidative stress. Insects, like other organisms, are equipped with antioxidant defense systems to reduce the harmful effects of free radicals. An effective antioxidant system promotes free radical scavenging activity and repairs damage to biomolecules required for life, making it necessary for organisms. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are antioxidant enzymes involved in antioxidant defense. SOD degrades the superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2), and then the resulting H_2O_2 is degraded to water by CAT or GSH-Px (Kamalakkannan & Prince, 2006). Scavenging free radicals and increasing antioxidant enzyme activity can directly suppress oxidative damage. In addition to antioxidant defense, hemocytes involved in phagocytosis, nodulation and encapsulation are important in cellular immunity. Also, the enzyme phenoloxidase (PO), a critical component of the immune system, is involved in infection defense.

Hyphantria cunea (Drury, 1773) (Lepidoptera: Arctiidae), originally from North America, is a common pest that has spread worldwide including Turkey. The larval stage of *H. cunea* can effectively defoliate a wide variety of crops and many plant species. Although various methods are used to control *H. cunea*, it continues to spread and cause damage around the world (Gencer et al., 2020). In the fight against *H. cunea*, it is more reliable to use eco-friendly and cost-effective entomopathogens rather than chemical insecticides.

Baculoviruses are thought to be one of the biocontrol methods for lepidopteran species including *H. cunea* (Gencer et al., 2018, 2020; Sayed et al., 2020). Various studies have shown that baculoviruses are effective against a wide variety of insects (Woestmann et al., 2018; Gencer et al., 2019; Sun et al., 2020).

In nature, herbivorous insects feed on a range of plants with different PSMs. These qualitative differences among plants may affect various mechanisms of insects, including immunological resistance (Smilanich et al., 2018). Also, baculovirus infection affects host immune responses (Ikeda et al., 2013), and viruses are also one of the sources of oxidative stress. Antioxidant enzyme activity, hemocyte concentrations, phenoloxidase activity and malondialdehyde (MDA) concentrations in insects change in response to oxidative stress. Determining these changes is critical in determining how insects respond to stress. The effects of PSMs on these parameters in *H. cunea* were investigated using apple, mulberry, plum and walnut plants, which are commonly eaten by *H. cunea* larvae and have economic value. In addition, the impact of *Hyphantria cunea* granulovirus (HycuGV) infection on these parameters was evaluated.

Materials and Methods

Source of insect larvae and plants

Hyphantria cunea larvae were collected during field surveys in the Bafra District of Samsun, Turkey, in 2019 (41°30' N, 36°05' E). The larvae were kept in the laboratory at 25 ± 2°C and 60% RH under a 16:8 h L:D photoperiod. They were divided into four groups and fed on *Malus pumila* (Borkh, 1803) (Rosales: Rosaceae) (apple), *Morus alba* (L., 1753) (Rosales: Moraceae) (mulberry), *Prunus domestica* (L.) (Rosales: Rosaceae) (plum) and *Juglans regia* (L.) (Fagales: Juglandaceae) (walnut) until they reached the pupal stage. Adults emerged from the pupae were mated and females deposited eggs. The newly hatched larvae were divided into four groups to be fed on the four plant species. The leaves used in the study were collected daily, and the leaves was sterilized with 50% ethyl alcohol before being fed to the larvae.

Phenolic and gallotannin analysis of the leaves

The determination of phenolic compound was made with HPLC (Thermo-Finnigan Surveyor, Thermo Finnigan, San Jose, CA, USA). HPLC-UV analyses were performed on a reverse phase C18 column (150 mm × 4.6 mm id, 5 µm particle; Fortis, France) using a UV detector which is simultaneously operating dual-UV wavelength. Gradient elution was used for HPLC analyses. The mobile phase was 2% acetic acid in water (A) and 70:30 acetonitrile:water (B). The following gradient was used; 0-3 min 5% B; 3-8 min 5-15% B; 8-10 min 15-20% B; 10-12 min 20-25% B; 12-20 min 25-40% B; 20-30 min 40-80% B. The injection volume was 25 µl, the column temperature was 30°C and the flow rate was 1.2 ml/min.

The method used to determine the gallotannin contents of the leaf samples was described by Bate-Smith (1977). For gallotannin analysis, leaf samples from each plant species were taken daily and dried in an oven to constant weight and then were ground. Leaf samples (0.5 mg) were placed in 10-ml tubes with 1 ml of 5% KIO_3 solution added to three of the tubes and 1 ml of distilled water to the fourth tube (as a control). The tubs were kept on ice for 1 h before measuring their absorbance in a spectrophotometer at a wavelength of 550 nm. A standard curve was prepared with tannic acid solutions (0.1-0.7 mg/ml) to estimate the gallotannin concentrations of the samples.

Virus propagation

HycuGV-Hc1 was obtained from the entomopathogenic virus culture collection of the microbiology laboratory at Karadeniz Technical University (Bayramoglu et al., 2018). Propagation of virus was performed in healthy *H. cunea* larvae collected from the field. Mulberry leaves surface contaminated with 10 µl of viral stock culture were fed to *H. cunea* larvae. The infected larvae were homogenized in sterile distilled water and filtered through double-layer cheesecloth 7 days after treatment to remove larval debris. The filtrate was centrifuged at 5,000 g for 30 min. Pellets were suspended in 1 ml dH_2O, loaded onto 3 ml of 30%

sucrose and centrifuged at 5,000 g for 30 min (Bayramoglu et al., 2018). The resulting pellets were then washed with dH_2O and resuspended in 1 ml of dH_2O. The concentration of the virus was adjusted to 10^5 OB/ml by using a Neubauer hemocytometer.

Feeding experiments

Feeding experiments were conducted in two stages: uninfected and infected larvae. One hundred fifth instar larvae were used in both groups: 50 each for determination of enzyme activity and hemocyte concentration. The larvae in the uninfected groups were fed on uncontaminated leaves for 7 days. Five days after the start of the experiment, the larvae in the infected groups were infected by feeding leaves treated with 1 ml of HycuGV suspension for two more days. After 7 days for each group, hemolymph of the larvae in the infected groups was taken by cutting the third legs of the larvae.

Examination and counting of hemocytes by Giemsa staining

Hemolymph was placed in Eppendorf tubes and 10 µl spread on a glass slide and air-dried for 20-30 min to allow hemocytes to adhere to the glass. The cells were fixed in methanol: acetic acid solution (3:1) for 10 min. The slides were stained for 10 min with Giemsa (Merck, Darmstadt, Germany) and then washed with distilled water. After air-drying, the slides were treated with xylene and then mounted in Entellan. A Zeiss Primo Star microscope was used to count the hemocytes. The hemocytes were counted in twenty randomly selected areas on each slide. The hemocyte concentrations were calculated (number per 10 µl) by multiplying the mean cell concentrations by the microscope factor determined from the microscope sight field (Fitts & Laird, 2004).

Enzyme analysis

Hemolymph samples collected from the larvae were homogenized with an ultrasonic processor (VCX 130 Sonics, Newtown, CT, USA). The homogenates (20 ml), were transferred to Eppendorf tubes and centrifuged at +4°C for 20 min at 15,000 rpm in a refrigerated centrifuge (model 3500, Kubota, Tokyo, Japan). After centrifugation, the supernatant was kept at -80°C until total protein determination and enzyme activity analyses. Protein determination was made according to the method of Lowry et al. (1951). Solution A containing 2% Na_2CO_3, 1% $CuSO_4$, and 2% Na-K-tartrate mixture and solution B containing a 1:1 diluted Folin-Ciocalteu phenol reagent were prepared for protein determination. A 10-µl aliquot was then added to 2.5 ml of solution A and vortexed. After that, 250 µl of solution B was added and vortexed again. The mixture was incubated in the dark for 30-60 min. Spectrophotometric measurement was performed at 595 nm. Standards were prepared with bovine serum albumin and plotted. The absorbance values obtained from the samples were calculated by proportioning by the standard. The activity of superoxide dismutase was determined using the method of Flohé & Ötting (1984) and the spectrophotometric method of McCord & Fridovich (1969). A 0.76 mg (5 µl) xanthine solution in 10 ml 0.001 N NaOH was mixed with a 24.8 mg (2 µmol) cytochrome c solution in 100 ml 50 Mm pH 7.8 phosphate buffer containing 0.1 M EDTA. A freshly prepared 0.2 U/ml xanthine oxidase solution in 0.1 mM EDTA was used in this experiment. The reduction of cytochrome c by the xanthine/xanthine oxidase system was spectrophotometrically measured at 550 nm to determine SOD activity. Catalase activity was determined by the Luck (1963) method. $Na_2HPO_4.H_2O$-KH_2PO_4 buffer (67 mM) was prepared at pH 7 to determine CAT activity. For each activity measurement, 160 µl of H_2O_2 was added to 100 ml of Na-K buffer. When the samples were added to the mixture, the CAT activity was determined spectrophotometrically with the decrease in absorbance at 240 nm due to H_2O_2 degradation. Glutathione peroxidase activity was determined by the method of Lawrence & Burk (1976). GSH-Px catalyzes the oxidation of glutathione by Cumene hydroperoxide. $NADP^+$ oxidation occurs in the reaction medium during the conversion of oxidized glutathione to reduced glutathione with the cofactors glutathione reductase and NADPH. Potassium phosphate buffer (50 mM) was prepared at pH 7 to measure GSH-Px activity. The decreases in absorbance were measured using a spectrophotometer at 340 nm.

Activity of PO were determined according to Ashida & Söderhäll (1984). MDA concentrations were determined according to Draper & Hadley (1990). The activity was measured in terms of µmol of oxidized NADPH per min. T70 UV/VIS spectrophotometer was used to determine the enzyme activity.

Statistical analysis

In this study, SPSS 21.0 software was used for statistical analysis. The effects of phenolic compounds in plants on hemocyte concentrations and antioxidant enzyme activity of *H. cunea* larvae were determined using the ANOVA and Tukey's test. Two independent samples t-test was used to determine the relationship between these parameters based on PSMs. All analyzes were performed in three replicates.

Results

Plant phenolic compounds

Phenolic compounds in the plants are given in Table 1. Plum leaves contained the most chlorogenic acid and mulberry the least. Benzoic acid was only found in walnut leaves, and catechin and rutin were only found in mulberry leaves. Rosmarinic acid and protocatechuic acid were only found in apple and plum leaves with apple leaves having the higher concentration. For gallotannin (Table 1), the highest concentration of was found in walnut leaves and the least in the mulberry leaves.

Table 1. Phenolic compounds and gallotannin (µg/mg) in the plant leaves used in this study

Plant	Benzoic acid	Catechin	Chlorogenic acid	Gallotannin	Protocatechuic acid	Rosmarinic acid	Rutin
Apple	0	0	3.3	58	1.5	200	0
Mulberry	0	16.7	1.3	16	0	0	1.7
Plum	0	0	18.4	84	1.1	7	0
Walnut	12.3	0	4.1	116	0	0	0

Hemocyte concentrations

Hemocyte concentrations are given in Table 2. In the uninfected groups, larvae fed on apple leaves had the lowest hemocyte concentration and those fed on walnut leaves had the highest. The mean hemocyte concentration of all virus-infected groups increased compared to the uninfected groups. In the infected groups, larvae fed on mulberry leaves had the lowest hemocyte concentration and those fed on walnut larvae had the highest.

Table 2. Hemocyte concentrations (no./µl; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	275 ± 0.25	450 ± 0.51	-59.2	<0.001	4.48 a	4.14 c
Mulberry	284 ± 0.30	310 ± 0.40	-12.4	<0.001	2.23 b	4.00 a
Plum	281 ± 0.33	363 ± 0.30	-22.4	<0.001	5. 25 b	5.41 b
Walnut	359 ± 0.59	528 ± 0.17	-56.8	<0.001	2.48 c	4.33 d

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Phenoloxidase activity

PO activity is given in Table 3. In the uninfected groups, larvae fed on apple leaves had the lowest PO activity and those fed on walnut leaves had the highest activity. In the infected groups, larvae fed on mulberry leaves had the lowest PO activity and those fed on plum leaves had the highest activity.

Table 3. Phenoloxidase activity (IU; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	26 ± 0.6	35 ± 0.7	-11.6	<0.001	1.24 a	1.17 b
Mulberry	33 ± 0.9	15 ± 0.5	23.3	<0.001	1.41 b	0.89 a
Plum	33 ± 0.9	36 ± 0.9	-3.5	<0.001	1.21 b	1.28 b
Walnut	40 ± 0.1	36 ± 0.1	4.5	<0.001	1.40 c	1.43 b

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Malondialdehyde concentrations

MDA concentrations are given in Table 4. In the uninfected groups larvae fed on mulberry leaves had the lowest MDA concentration and those fed on apple leaves had the highest. The MDA concentrations of all groups decreased in virus infection compared to the uninfected groups. In the infected groups, larvae fed on apple leaves had the highest MDA concentration and those fed on mulberry leaves had the least.

Table 4. Malondialdehyde concentrations (IU; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	418 ± 0.1	354 ± 0.3	12	<0.001	5.80 c	9.83 c
Mulberry	236 ± 0.7	210 ± 0.2	8.6	<0.001	4.51 a	4.49 a
Plum	365 ± 0.3	335 ± 0.3	4.8	<0.001	11.16 b	6.43 b
Walnut	371 ± 0.6	353 ± 0.9	3.1	<0.001	9.40 b	9.01 c

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Superoxide dismutase activity

SOD activity is given in Table 5 with activity with larvae fed on mulberry leaves having highest and those fed on plum leaves the lowest. In the infected groups, larvae fed on walnut had the lowest SOD activity and those fed on plum leaves-the highest.

Table 5. Superoxide dismutase activity (IU; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	237 ± 0.4	186 ± 0.1	10.2	<0.001	7.56 c	7.32 c
Mulberry	198 ± 0.5	149 ± 0.6	10	<0.001	7.03 b	6.98 b
Plum	791 ± 0.3	470 ± 0.9	51.7	<0.001	7.56 c	8.55 d
Walnut	170 ± 0.8	137 ± 0.4	7.8	<0.001	9.95 d	6.03 a

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Catalase activity

CAT activity is shown in Table 6 with the uninfected groups ranked from the highest to the lowest as plum > mulberry > walnut > apple. In the infected groups, larvae fed on plum had the highest CAT activity and those fed on apple leaves had lowest.

Table 6. Catalase activity (IU; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	130 ± 0.8	202 ± 0.8	-15.6	<0.001	6.64 a	6.94 a
Mulberry	606 ± 0.3	580 ± 0.3	4.6	<0.001	9.23 c	8.20 c
Plum	1112 ± 0.8	804 ± 0.2	27.5	<0.001	17.05 d	9.75 d
Walnut	329 ± 0.1	244 ± 0.2	15.4	<0.001	7.78 b	8.73 b

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Glutathione peroxidase activity

GSH-Px activity is shown in Table 7 with uninfected groups ranked from highest to lowest as walnut > mulberry > apple > plum. For the infected groups, the ranking was walnut > apple > mulberry > plum.

Table 7. Glutathione peroxidase activity (IU; mean ± SE) of *Hyphantria cunea* larvae in the uninfected and infected groups

Plant	Uninfected	Infected	t	P	Uninfected SD (Tukey's test)*	Infected SD (Tukey's test)*
Apple	229 ± 0.8	235 ± 0.3	-1.6	<0.001	7.48 b	6.82 c
Mulberry	299 ± 0.9	129 ± 0.3	35	<0.001	8.13 c	6.24 b
Plum	55 ± 0.7	91 ± 0.9	-13.6	<0.001	2.82 a	4.57 a
Walnut	418 ± 0.3	287 ± 0.2	26.8	<0.001	7.60 d	7.02 d

* Values within the column followed by the same letter are not significantly different (P < 0.001).

Discussion

The hemocyte concentrations of insects is related to the effectiveness of the immune system (Ghosh et al., 2018). Larvae fed on apple leaves had the lowest mean hemocyte concentration in the uninfected groups. The highest concentration of rosmarinic acid was present in the apple leaves, and in this case, a high concentration of rosmarinic acid can be assumed to reduce the hemocyte concentrations. Also, we found that larvae fed on walnut, the species containing the highest gallotannin concentration, had the highest hemocyte concentration. Both the findings of this study and the results of other studies (Pandey et al., 2012; Smilanich et al., 2018) show that feeding on different plants can affect the hemocyte concentrations of insects due to differences in plant constituents. The hemocyte concentration is an important indicator that reflects an insect's ability to resist entomopathogens. Viral infections induce cellular defense reactions (Millanta et al., 2019). It was found that the hemocyte concentrations of *S. exigua* larvae infected with the virus varied with the plant species consumed (Wang et al., 2021). Povey et al. (2013) found that the virus-treated *Spodoptera exempta* (Walker, 1856) (Lepidoptera: Noctuidae) larvae had higher hemocyte concentrations than control larvae. In this study, it was determined that the mean hemocyte concentrations of all virus-infected groups increased compared to the uninfected groups. It was found that the highest mean hemocyte concentration in the infected groups was in larvae fed on walnut leaves, which was the only plant with benzoic acid among the plants. Additionally, the concentration of gallotannin was the greatest in the walnut leaves. Hemocytes have an active role in clearing viruses from the hemolymph (Mahmoud & Soliman, 2015), and they do so by increasing their numbers, usually in response to infection. A higher hemocyte concentration means stronger resistance to insect virus infection as well as immunity. In this case, the presence of benzoic acid and high gallotannin may give the larvae an advantage in cellular defense.

Phenoloxidase is an enzyme that plays a critical role in the immune response (Wang et al., 2020) and is one of the main factors in insect immunity due to its role in the melanization process (Dudzic et al., 2015). Larvae fed on apple leaves had the lowest PO activity in the uninfected groups. In this case, the high concentration of rosmarinic acid found in the apple leaves may be effective. Also, the maximum PO activity was in larvae fed on walnut leaves containing a high concentration of gallotannin. Our findings show that different PSMs at high concentrations have different effects on the PO activity of *H. cunea* larvae. In addition, the fact that the larvae feeding with various plants have different PO activity (Ebrahimi & Ajamhassani, 2020) supports our findings. Since PO is the main component of hemocytes (Akita & Hoshi, 1995), the correlation between hemocyte concentration and PO activity was also confirmed by our findings in uninfected groups. The PO activity in the groups with the highest and lowest hemocyte concentrations (walnut and apple uninfected groups, respectively) also showed parallel results. The activation of the PO system in the hemolymph is one of the first responses of the insect immune system to infection. Phenoloxidase is present in the hemolymph as prophenoloxidase (proPO) and is activated by a serine proteinase (Nakhleh et al., 2017). The proPO activation cascade may facilitate in the protection of host larvae from baculovirus infection (Jiang et al., 2010). Studies have reported that hemolymph PO activity of lepidopteran larvae are affected by viral infection. For example, Millanta et al. (2019) found that virus-treated honeybees had higher PO activity than the control. Li et al. (2021) reported that the PO activity of *S. exigua* larvae infected with the virus was significantly increased. In this study, the PO activity of larvae fed on apple and plum leaves increased with viral infection compared to the uninfected larvae, which is consistent with the findings of these studies. Rosmarinic acid and protocatechuic acid, which were only found in the apple leaves and the plum leaves, could have been effective. In contrast, the PO activity of larvae fed on mulberry and walnut leaves were found to decrease relative to the uninfected larvae. The decrease in PO activity of the virus-infected larvae was somewhat unexpected, but it is consistent with the findings of previous studies (Rao et al., 2010; Yuan et al., 2017; Wang et al., 2020).

Malondialdehyde is an indicator of free radical-induced lipid peroxidation and acts as a marker of oxidative stress (Schuessel et al., 2006). The MDA concentration was lowest in larvae fed on mulberry leaves in both the uninfected and infected groups. Flavonoids prevent the formation of free radicals increased by the oxidation of saturated lipids (Oboh et al., 2015). Rutin can remove lipid peroxidation products (Zhu et al., 2019). In the study of Iacopini et al. (2008), rutin and catechin were shown to have antiradical and antioxidant properties. Our findings are consistent with these studies, and we conclude that catechin and rutin flavonoids, which are only found in the mulberry leaves, protect larvae from lipid peroxidation. Lipid peroxidation as a result of oxidative stress during the viral infection has been established in insect cell lines (Wang et al., 2001); however, we found that the concentrations of MDA with viral infection were lower than those of the uninfected groups. This situation can be explained by PSMs having antioxidant properties (Zhu et al., 2019) that protect larvae from infection against lipid peroxidation.

The activity of antioxidant enzymes in insect tissues is related to the host plant spectrum (Dampc et al., 2020). According to a study (Durak et al., 2018), *Aphis pomi* (de Geer, 1773) (Hemiptera: Aphididae), an oligophagous species, had higher SOD activity than *Cinara tujafilina* (Del Guercio, 1909) (Hemiptera: Aphidoidea), which has a broad host plant spectrum. Lukasik (2007) found that the SOD activity of *Rhopalosiphum padi* (L., 1758) (Hemiptera: Aphididae) and *Sitobion avenae* (Fabricius, 1775) (Hemiptera: Aphididae) were influenced by their host plants, which is consistent with our findings. These differences in SOD activity are most probably due to the different PSMs of the host plants. Larvae fed on walnut leaves had the lowest SOD activity in both the uninfected and infected groups. In this case, the benzoic acid found in the walnut leaves may have been effective. Velika & Kron (2012) found that benzoic acid has antioxidant properties against superoxide radicals, which is in contrast to our findings. In addition, it was determined that the highest SOD activity in both the uninfected and infected groups were in larvae fed on plum leaves, which contained the highest concentration of chlorogenic acid. Given that chlorogenic acid is a potent

antioxidant and has free radical scavenging properties (Kim et al., 2018), it is not surprising that this phenolic compound causes SOD activity to increase to eliminate ROS. SOD activity decreased in all groups with viral infection compared to the uninfected groups. It is counterintuitive because, in the face of a stress factor such as infection, the larvae are expected to upregulate their SOD activity to eliminate ROS.

Perić-Mataruga et al. (2014) reported that *Lymantria dispar* (L., 1758) (Lepidoptera: Lymantriidae) larvae exhibited different CAT activity when fed on various plants. It has been determined that *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) fed on various host plants had varying CAT activity (Abdelsalam et al., 2016). In this study, we recorded that *H. cunea* larvae fed on various plants had different CAT activity. The lowest CAT activity in both the uninfected and infected groups were in larvae fed on apple leaves. Possibly the maximum concentration of rosmarinic acid present in the apple leaves may have caused CAT activity to be minimal. Larvae fed on plum leaves had the highest CAT activity in both the uninfected and infected groups. In this case, the highest concentration of chlorogenic acid found in plum leaves may have been effective. Also, the maximum SOD activity were in larvae fed on plum leaves. Since the H_2O_2 formed as a result of SOD activity is degraded by CAT (Kaushal et al., 2018), this explains the maximum CAT activity of the larvae fed on plum leaves having the highest SOD activity. Catalase activity is affected by virus application. For example, Wang et al. (2001) determined that virus application changed the CAT activity of larvae over time. It was found that CAT activity in all groups (except the apple group) with the viral infection were lower than those of the uninfected groups in this study.

The highest GSH-Px activity in both the uninfected and infected groups were in larvae fed on walnut leaves. Since tannins have powerful antioxidant properties (Payab et al., 2019), the highest concentration of gallotannin in walnut leaves may have given the larvae an advantage in GSH-Px activity. Hydrogen peroxide is detoxified by either CAT or GSH-Px enzymes converting it to water. When there is a high H_2O_2 concentration, CAT acts, whereas GSH-Px acts when there is a low H_2O_2 concentration (Baud et al., 2004). The fact that the larvae fed on plum leaves (both the uninfected and infected) had the highest CAT activity and the lowest GSH-Px activity confirms that these two enzymes work in a coordinated manner. GSH-Px plays a role in defense against pathogenic organisms (Brigelius-Flohé & Maiorino, 2013). The enzyme activity of larvae fed on apple and plum leaves increased due to viral infection. In this case, the rosmarinic acid and protocatechuic acid found in the apple leaves and the plum leaves may have given the larvae an advantage in overcoming the cytotoxic effect of H_2O_2.

In this study, in which we correlated the phenolic content of host plants with the concentrations of hemocytes and antioxidant enzyme activity, it was observed that the responses of *H. cunea* larvae to the various parameters measured differed depending on the phenolic compounds present. In addition, our findings determined that when *H. cunea* larvae infected with *Hyphantria cunea* granulovirus, they gave priority to cellular defense with increased hemocyte concentration rather than antioxidant activity. Plant-herbivore-entomopathogen interactions are quite complex. Identifying any immune changes should be considered as a significant issue in insecticide application in agroecosystems. Understanding which factors affect the insect immune system will be critical in combating harmful insects (especially microbial control).

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