



# Effects of Plant Phenolic Compounds and *Bacillus thuringiensis* subsp. *kurstaki* on Immune Responses and Antioxidant Enzyme Activities of *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) Larvae

Oğuzhan Yanar<sup>1\*</sup>, Elif Fatma Topkara<sup>1</sup>, Fatma Gönül Solmaz<sup>1</sup> & Sevcan Mercan<sup>2</sup>

<sup>1</sup> Department of Biology, Science and Art Faculty, Ondokuz Mayıs University, 55139 Samsun, Turkey

<sup>2</sup> Vocational School of Health Services, Ondokuz Mayıs University, 55139 Samsun, Turkey

**Abstract:** Studies on ecological immunology are crucial in determining how the immune responses of insects against both abiotic and biotic factors are affected. In this study, the effects of phenolic compounds in plants on the immune responses and antioxidant enzyme activities of *Hyphantria cunea* larvae infected by *Bacillus thuringiensis* subsp. *kurstaki* were investigated. For this purpose, phenolic compounds present in mulberry, apple, walnut and plum plants (preferred by *H. cunea* and having economic importance) were determined. *H. cunea* culture was obtained from larvae collected in Bafra, Samsun in 2019. It was found that the haemocyte counts of both control and infected larvae fed in the walnut plant containing the highest gallotannin amount were the highest. It was found that catechin and rutin flavonoids caused minimum phenoloxidase activity. It was determined that the highest superoxide dismutase activities were in the plum-fed groups and the lowest catalase activities were in the larvae fed in the walnut groups both in the control and infected groups. As a result, it was determined that immune responses and antioxidant enzyme activities can be changed due to both phenolic compounds present in plants and infection.

**Key words:** Phenolic compound, haemocyte, *Hyphantria cunea*, antioxidant activity, *Bacillus thuringiensis*, insect immunity

## Introduction

Plants are constantly faced with attacks by insect pests from a wide variety of taxonomic groups. To overcome these attacks, they defend themselves with both mechanical and chemical properties. They achieve this by producing chemical compounds called plant secondary metabolites (PSM) that deter or kill insects, along with mechanical properties such as trichomes, spines or waxy leaf coatings. PSMs act as both constituents and inducible substances. They can also act as antioxidants

or volatile attractants to predators (CARTEA et al. 2010, WAR et al. 2012). Phenolic compounds, a kind of PSM, play a crucial role in plant-herbivore interactions (HARBORNE & GRAYER 2017). Phenolic compounds have many biological activities. One of them is that these compounds serve as antioxidants (SCALBERT et al. 2005), which generally prevent the formation of free radicals (SHAHIDI & AMAROWICZ 1994). In studies, chlorogenic acid (OZBILGIN et al. 2015), rosmarinic acid (PETERSEN & SIMMONDS 2003), rutin (IBTISSEM et al. 2012), protocatechuic acid (SYAFNI et al. 2012) and tannic acid (ANDRADE

\*Corresponding author: oyanar46@gmail.com

et al. 2005) have been shown to exhibit antioxidant activities.

Previous studies on insect feeding generally evaluate insect performance in terms of development and survival (ROSA et al. 2018). However, the immune response of insects is equally important in shaping their performance when faced with pathogens and parasites. Insect immunity may vary in terms of both nutrient content (LEE et al. 2006) and concentration of PSMs (MARTEMYANOV et al. 2012, TAO et al. 2016), depending on the amount (SIVAJOTHY & THOMPSON 2002) and quality of the diet (LAMPERT 2012). While reactive oxygen species (ROS), which have effects on immune function, show beneficial effects with low or medium concentrations (FINKEL & HOLBROOK 2000), they damage cell structures such as lipids, proteins and DNA by causing oxidative stress with high concentration (SHARIFI-RAD et al. 2020). All aerobic organisms have antioxidant defence systems to balance these harmful effects caused by free radicals. Three antioxidant enzymes involved in antioxidant protection are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). While SOD reduces the superoxide radical ( $O_2^-$ ) to hydrogen peroxide ( $H_2O_2$ ), the  $H_2O_2$  formed by SOD is reduced to water with CAT or GPx (SHARIFI-RAD et al. 2020). In addition, changes in haemocyte counts in insects can be used to measure the immuno-suppressive or immuno-stimulatory effects of various compounds, including toxins (MOWLDS et al. 2010, CHAMPION et al. 2016). On the other hand, the melanization of insect haemolymph is a crucial response to the presence of infectious agents (microbes, viruses, or parasites), toxins, or abiotic stress factors (WHITTEN & COATES 2017). Early enzymatic steps that contribute to melanin formation are coordinated by phenoloxidases (POs), which are stored in certain haemocytes as inactive precursors.

The fall webworm *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) is a polyphagous herbivore that feeds on a large number of host plants, from a wide variety of forest and fruit trees to a variety of agricultural products. This insect, which is native to North America, has spread in Europe and Asia (ALBAYRAK-ISKENDER et al. 2017). However, *H. cunea* is a very harmful pest species around the world. As in many countries, it also causes critical damage to the crop in Turkey. There is still no sustainable method to control this pest (ALBAYRAK-ISKENDER et al. 2017). In our study, one of the most widely used species in biological control (RAYMOND et al. 2010), *Bacillus thuringiensis* subsp. *kurstaki* (Btk), was tested.

Plants produce a complex mixture of PSMs from different structural classes for defence rather than a single compound (MASON & SINGER 2015, WINK 2015). The composition of these mixtures is not fixed and varies in both concentration and composition. Our aim in this study was to determine how the phenolic compounds present in the mulberry, apple, walnut and plum plants, which are the most preferred by *H. cunea* and economically important, affect the haemocyte counts, malondialdehyde (MDA) amounts, phenoloxidase (PO) and antioxidant activities (SOD, CAT and GPx) of larvae. Besides, we aimed to determine how *B. thuringiensis* subsp. *kurstaki* affects these parameters.

## Materials and Methods

*Hyphantria cunea* larvae were collected in field surveys in the borders of Bafra District of Samsun, Turkey, in June 2019 (N41°30'–E36°05'). The larvae brought to the laboratory were kept at 25±2°C, 16 hours light / 8 hours dark and 70% humidity. They were divided into four different groups until they reached the pupal stage and fed on *Morus alba* (mulberry), *Malus pumila* (apple), *Juglans regia* (walnut), and *Prunus domestica* (plum). The adults that emerged from the pupae mated and laid eggs. The hatched larvae were again divided into four groups and fed on the relevant plants. Plants used in the study were collected daily and each plant leaf was sterilized with 50% ethyl alcohol and then given to the larvae.

### Bacteria and culture conditions and infection of larvae with bacteria

The bacteria were grown overnight at 30°C in nutrient broth. The optical density of the growing culture was measured at 600 nm wavelength and set to  $OD_{600} = 1.89$  (DANISMAZOGLU et al. 2012). 1 ml of suspension was infected with each plant used for feeding the larvae. These plants were placed in plastic containers and larvae were placed in the specified amounts in accordance with the purpose of the study.

### Feeding experiments

Feeding experiments were carried out in two stages. In the first stage, larvae were not infected (controls). They were divided into four groups according to the diet. Each group contained 100 larvae to determine the enzyme activity and 50 larvae for haemocyte count. In the second stage, larvae were in the same number and division as in the first stage. Each group was infected with 1 ml of *Btk* and the larvae were allowed to consume the given plants for three more days.

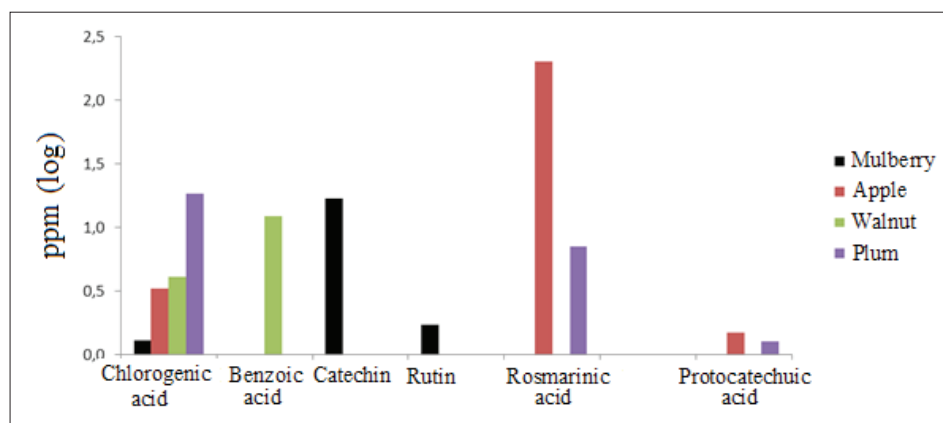


Fig. 1. The amounts of phenolic compounds in plants.

### Haemolymph collection

Haemolymphs of control larvae and infected larvae were removed by cutting their third legs. After the haemolymph of each group was placed in the Eppendorf tubes, the haemolymph tubes were kept at  $-80^{\circ}\text{C}$  until the enzyme analysis was performed.

### Giemsa staining

For the haemocyte count, 10  $\mu\text{l}$  of haemolymph was spread over each slide. After the haemolymphs spread on the lama were dried, the staining steps were started with Giemsa. After staining, fully preserved preparations were obtained and haemocytes were counted with a microscope.

### Enzyme analysis

In the study, protein concentration was determined according to the method of LOWRY et al. (1951), while superoxide dismutase activity was determined by the spectrophotometric method of MCCORD & FRIDOVICH (1969) and the method of FLOHÉ & OTTING (1984). Catalase activity was determined by the method of LUCK (1963) and glutathione peroxidase was determined by the method of LAWRENCE & BURK (1976). For phenoloxidase activity determination, the method of ASHIDA & SODERHÄLL (1984) was used and the amount of malondialdehyde was determined by the method of DRAPER & HADLEY (1990).

### Phenolic and gallotannin analysis

The phenolic compound determination was made with HPLC brand Thermo-Finnigan Surveyor. The method used to determine gallotannin contents of the leaf samples was described by BATE-SMITH (1977).

### Statistical analysis

In this study, SPSS 21.0 software was used for statistical analysis. The effects of phenolic compounds

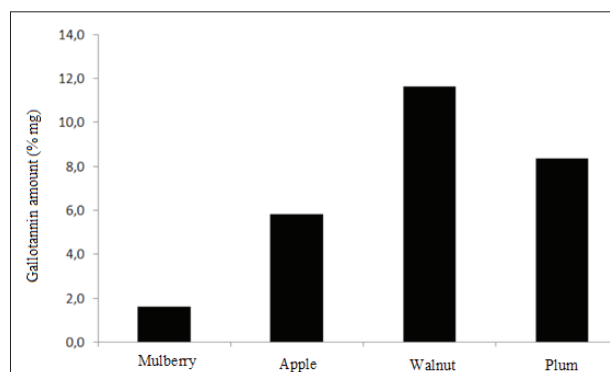


Fig. 2. The gallotannin amounts in plants.

in plants on immune responses and antioxidant enzyme activities of *H. cunea* larvae were determined using the ANOVA Duncan Test. Two independent samples t-test was used to determine the relationship between these parameters according to PSM. Analyses of both haemocytes counts and all enzyme activities were performed in three replicates.

## Results

The amounts of phenolic compounds in plants Among all plants, the highest chlorogenic acid amount was in plum and the lowest one was in the mulberry plant. Benzoic acid was detected only in the walnut plant, while catechin and rutin were detected only in the mulberry plant. Rosmarinic acid and protocatechuic acid were present only in apple and plum, with the highest amount in apple and the lowest one in plum (Fig. 1). Among all plants, the highest amount of gallotannin was in walnut and the lowest one was in the mulberry plant (Fig. 2).

### Haemocyte counts

Among the control groups, while the lowest mean haemocyte count was found in larvae fed on apple

**Table 1.** Mean haemocyte counts of *Hyphantria cunea* larvae.

Plants	Groups	Mean ± standard error (n/10µl)	t	P
Mulberry	Control	3151±3.8	28	<0.001
	Infected	3757±4.1		
Apple	Control	2923±2.7	46	<0.001
	Infected	3733±5.3		
Walnut	Control	3639±6.1	28	<0.001
	Infected	4212±1.9		
Plum	Control	3340±3.5	29	<0.001
	Infected	4008±3.0		

**Table 2.** Phenoloxidase activities of *Hyphantria cunea* larvae.

Plants	Groups	Mean ± standard error (IU/ml)	t	P
Mulberry	Control	10±0.2	-4.6	<0.001
	Infected	8±0.2		
Apple	Control	30±0.6	18	<0.001
	Infected	46±0.5		
Walnut	Control	44±1.0	-3.3	<0.001
	Infected	40±1.0		
Plum	Control	39±0.9	-3.7	<0.001
	Infected	35±0.6		

(2923±2.7, t=46, P<0.001), the highest one was in larvae fed on walnut (3639±6.1, t=28, P<0.001). The mean haemocyte counts of all groups infected with bacteria increased compared to the control groups. Among the infected groups, the lowest haemocyte count was in the apple-fed group (3733±5.3, t=46, P<0.001) and the highest one was in the walnut-fed group (4212±1.9, t=28, P<0.001) (Table 1).

**Phenoloxidase activities**

Among all control groups, the lowest phenoloxidase activity was found in the mulberry-fed group (10±0.2 IU, t=-4.6, P<0.001) and the highest activity was in the walnut-fed group (44±1.0 IU, t=-3.3, P<0.001). In the infected groups, while the lowest PO activity was in the mulberry-fed group (8±0.2 IU, t=-4.6, P<0.001), the highest one was in the apple-fed group (46±0.5 IU, t=18, P<0.001). A decrease in PO activities of all groups (except apple) with bacterial infection was observed compared to the control ones (Table 2).

**Malondialdehyde (MDA) amounts**

MDA amounts in control groups were 235±1.8 IU, t=17, P<0.001 in mulberry, 296±1.5 IU, t=197, P<0.001 in apple, 344±2.3 IU, t=44, P<0.001 in walnut and 374±4.0 IU, t=-51, P<0.001 in plum. Among the infected groups, while the lowest amount of MDA was found in the plum-fed group (144±1.9 IU, t=-51, P<0.001), the highest one was in the apple-fed group (934±2.7 IU, t=197, P<0.001). Also, an increase in the amounts of all groups (except plum) with bacterial infection was noted compared to the control groups (Fig. 3).

**Superoxide dismutase (SOD) activities**

SOD activities in control groups were plum (802±5.9 IU, t=15, P<0.001) > apple (230±2.7 IU, t=-38, P<0.001) > mulberry (189±2.7 IU, t=145, P<0.001) > walnut (152±2.4 IU, t=68, P<0.001), respectively. In the infected groups, SOD activities were 892±4.1 IU, t=145, P<0.001 in mulberry, 113±1.9 IU, t=-38, P<0.001 in apple, 407±2.8 IU, t=68, P<0.001 in walnut and 917±3.9 IU, t=15, P<0.001 in plum (Fig. 4).

**Catalase (CAT) activities**

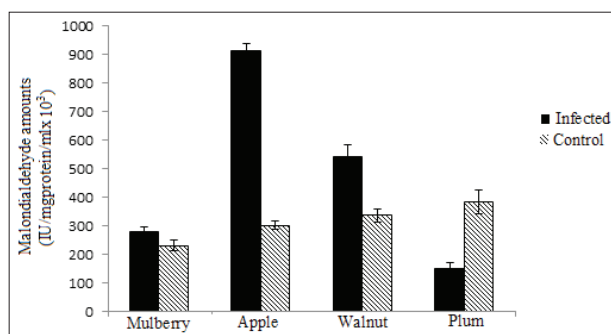
Among the control groups, the lowest CAT activity was found in the walnut-fed group (121±1.8 IU, t=9, P<0.001) and the highest one was in the apple-fed group (1866±2.7 IU, t=-18, P<0.001). While there was an increase in the enzyme activities of the mulberry-fed and walnut-fed groups with the bacterial infection compared to the control groups, it was found that the activities of the larvae fed on apple and plum decreased (Fig. 5).

**Glutathione peroxidase (GPx) activities**

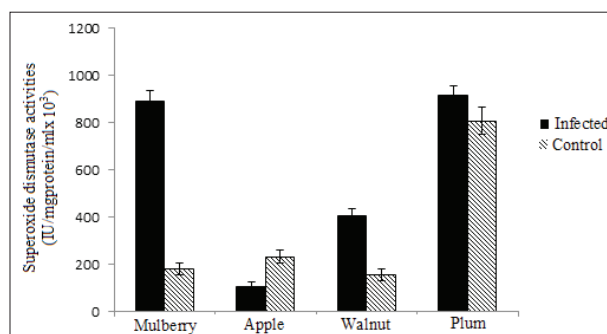
In control groups, GPx activities were 277±4.6 IU, t=-47, P<0.001 in mulberry, 124±2.7 IU, t=18, P<0.001 in apple, 106±1.0 IU, t=-62, P<0.001 in walnut and 59±0.8 IU, t=35, P<0.001 in plum. In infected groups, these activities were 55±0.7 IU, t=-47, P<0.001 in mulberry, 189±2.4 IU, t=18, P<0.001 in apple, 38±0.4 IU, t=-62, P<0.001 in walnut and 170±3.1 IU, t=35, P<0.001 in plum (Fig. 6).

**Discussion**

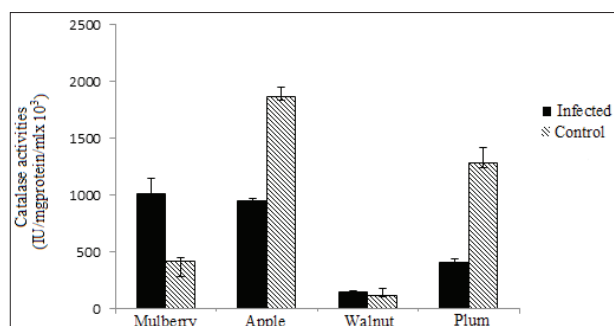
The activity of the immune system in insects is related to the haemocyte count (BERGER & SLAVIČKOVÁ 2008). Among both the control and infected groups, the lowest haemocyte counts of *H. cunea* larvae were found to be in individuals fed on apple groups and the highest ones were in walnut-fed groups. Compared to the control groups, it may be thought that the rich content of rosmarinic acid in apple plant may be responsible for the minimum haemocyte



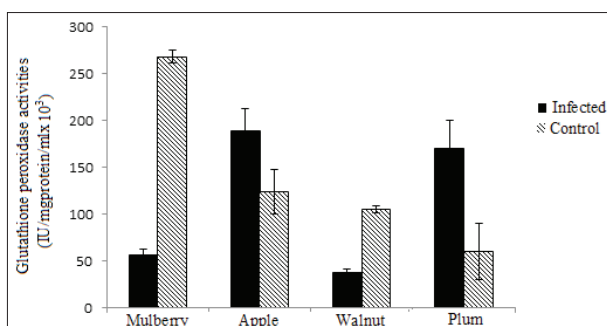
**Fig. 3.** Average malondialdehyde amounts of *Hyphantria cunea* larvae ( $\pm$ S.E.) in the infected and control groups. Two independent samples t-test,  $P < 0.001$ .



**Fig. 4.** Average superoxide dismutase activities of *Hyphantria cunea* larvae ( $\pm$ S.E.) in the infected and control groups. Two independent samples t-test,  $P < 0.001$ .



**Fig. 5.** Average catalase activities of *Hyphantria cunea* larvae ( $\pm$ S.E.) in the infected and control groups. Two independent samples t-test,  $P < 0.001$ .



**Fig. 6.** Average glutathione peroxidase activities of *Hyphantria cunea* larvae ( $\pm$ S.E.) in the infected and control groups. Two independent samples t-test,  $P < 0.001$ .

count. In a study conducted with *Vanessa atalanta* larvae (YANAR et al. 2018), it was found that as the amount of tannic acid in the diet content increased, the haemocyte count of the larvae decreased. In contrast, in the current study, we determined that the larvae fed on walnut with the most gallotannin amount had the highest haemocyte count. In studies on *Lobesia botrana*, it was found that feeding on the alternative host plant caused an increase in haemocyte count (MULLER et al. 2014). The results of both that study and the current study show that feeding on different plants can affect the haemocyte count of insects due to diverse plant ingredients. Haemocytes play a central role in the cellular immune responses of insects against foreign invaders, including pathogens and parasitoids (STOEPLER et al. 2013). Insecticides are another factor affecting the haemocyte count. In studies (SHARMA et al. 2003, KURT & KAYIS 2015), it was determined that the haemocyte counts decreased with an insecticide application. On the contrary, we found that the mean haemocyte counts of all groups increased with the *B. thuringiensis* application, which we used as a bioinsecticide. This result proves that different insecticides show different effects on the cellular immunity of insects.

Phenoloxidase is an enzyme that plays a critical role in immunity (GORMAN et al. 2007) and is an essential factor of insect immunity with its crucial roles in coagulation, melanization and wound healing processes (AJAMHASSANI et al. 2012). In this study, we determined that the lowest PO activities among both the control and infected groups were in individuals fed on mulberry. It can be assumed that catechin and rutin flavonoids present only in the mulberry plant may be essential factors in the low PO activity of the larvae. Also, in a study (SLINN et al. 2018), it was hypothesized that caterpillars grown in plants with high phytochemical diversity had lower PO activity, which might be a general consequence of feeding on various plants phytochemically. Since the PO is an enzyme involved in cellular defence (GASMI et al. 2019), haemocyte count and PO activity are related. The phytochemical content of the walnuts, which has the highest amount of gallotannin and benzoic acid not present in other plants, may have caused the haemocyte count to be high in the control group, resulting in maximum PO activity. The fact that PSMs consumed by caterpillars induce changes in immune responses (SMILANICH et al. 2009) and the larvae feeding on different plants have different PO

activities (MULLER et al. 2014) supports the results of our study. One of the first responses of the insect immune system to infection is the activation of the PO system in the haemolymph. PO activity leads to the production of melanin, which contributes to the elimination of pathogens (STAÇZEK et al. 2020) and melanin formation is initiated by the conversion of inactive prophenoloxidase (PPO) into active PO in the presence of serine proteinases (STAÇZEK et al. 2017). Insecticides can interfere with the melanization process by interfering with the PO cascade or by disrupting serine protease activity (JAMES & XU 2012). In our study, it was found that *Btk* caused a decrease in PO activities (except apple) compared to the control groups.

Malondialdehyde is one of the main products of lipid peroxidation and an indicator of the level of lipid peroxides (ZHANG et al. 2011). According to our results, the lowest amount of MDA among the control groups was in the mulberry-fed group. Flavonoids prevent the production of free radicals, which are increased by the oxidation of saturated lipids (KHAN et al. 2020). In this case, catechin and rutin have a positive effect on larvae against lipid peroxidation. Among the infected groups, the highest amount of MDA was in individuals fed on apple. The phytochemical content of apple (rich in rosmarinic acid and protocatechuic acid) was thought to cause high lipid peroxidation. MDA amounts increased with *Btk* application (except plum). It is evidence that *Btk* increases lipid peroxidation. The increase in lipid peroxidation is an indicator of ROS production. Oxidative stress caused by ROS damages lipids (SHARIFI-RAD et al. 2020) and this causes the structural and functional integrity of the cells to deteriorate. In polyphagous insects, lipid peroxidation is particularly detrimental because lipids are not only components of the cell membrane, but also play an important role in the development and reproductive physiology of insects (SUGANYA et al. 2016).

Plant phenolic compounds serve as antioxidants due to the hydrogen donor properties of the phenolic hydroxyl groups (LINDSAY & ASTLEY 2002) and thus protect cells against the harmful effects of ROS. It was found that the highest SOD activities in both control and infected groups were in larvae fed on plum containing the highest chlorogenic acid amount. Since chlorogenic acid has a strong antioxidant and free radical scavenging activities (OZBILGIN et al. 2015), it is not surprising that this phenolic compound causes SOD activity to be up-regulated to eliminate ROS. An increase in SOD activities of all groups (except apple) with *Btk* in-

fection was observed. It indicates that the bacterial infection increases the formation of free radicals and the larvae increase their SOD activities in response to this. The increase observed as a result of the bacterial infection was mostly in the larvae fed on mulberry plants. Flavonoids can scavenge ROS (KHAN et al. 2020). It can be said that flavonoids present in mulberry cause a high SOD increase to overcome oxidative stress caused by *Btk*.

In the study, among the control groups, the CAT activity was the highest in the larvae fed on apple with the maximal rosmarinic acid. It is known that apples contain high amounts of rosmarinic acid (AMZAD HOSSAIN et al. 2009) and that rosmarinic acid also has antioxidant properties (VUKOVIC et al. 2013). As a result of our study, the fact that the maximum CAT activity was in larvae on the apple-fed group suggests that the content of apple may be crucial for the larvae. In a study (GULCIN et al. 2010), it was shown that tannic acid has the free radical scavenging effect and increases antioxidant levels. In the current study, we found that the larvae fed on walnuts containing the highest gallotannin amount had minimal CAT activity. There was an increase in CAT activities of individuals on mulberry-fed and walnut-fed groups with bacterial infection compared to the control group. Since  $H_2O_2$  formed as a result of SOD activity will be reduced by CAT (SHARIFI-RAD et al. 2020), there must be a significant relationship between SOD and CAT. It explains that the increase in SOD activities with infection in these groups causes an increase in CAT activity. In a study where WANG et al. (2020) applied rutin to *Calliptamus abbreviatus* grasshoppers, they found that SOD and CAT activities were increased, which is consistent with our current result that individuals fed on mulberry had increased SOD activity as a result of infection and therefore had maximum CAT activity.

Flavonoids can reduce the production of ROS and increase the production of antioxidant enzymes, which leads to a decrease in oxidative stress (KHAN et al. 2020). The rich content of mulberries in flavonoids may suggest that they caused the highest GPx activity among the control groups. Hydrogen peroxide is detoxified by conversion to water by either CAT or GPx enzymes. In cases where  $H_2O_2$  formation increases, CAT has a significant effect (KOC & USTUN 2008) and GPx acts in lower  $H_2O_2$  concentrations. In this study, an increase in CAT activities and a decrease in GPx activities of larvae fed on mulberry and walnut with *Btk* application were observed.

## Conclusion

The main purpose of ecological immunology studies is to understand the ecological and evolutionary sources of variation underlying the immune response (SCHULENBURG et al. 2009). In our study, the effects of both phenolic compounds present in various plants and bacterial infection on the immune responses and antioxidant enzyme systems of *H. cunea* larvae were observed. The utility of both host plants whose phytochemical content was determined and bacterial infection in biological control with this species was shown. Understanding what factors the insect immune system is affected by will play a principal role in pest control (particularly microbial control).

**Acknowledgement:** We thank Prof. İsmail Demir (Karadeniz Technical University) for his contributions. This study was supported by the Ondokuz Mayıs University Research Foundation (PYO.FEN.1904.18.001).

## References

- AJAMHASSANI M., SENDI J. J., FARSI M. J. & ZIBAAE A. 2012. Purification and characterization of phenoloxidase from the hemolymph of *Hyphantria cunea* (Lepidoptera: Arctiidae). *Invertebrate Survival Journal* 9: 64–71.
- ALBAYRAK-ISKENDER N., ORTUCU S. & AKSU Y. 2017. Insecticidal activity of isolated bacteria from *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae). *Artvin Coruh University Journal of Forestry Faculty* 18 (1): 55–61.
- AMZAD HOSSAIN M., SALEHUDDIN S. M., KABIR M. J., RAHMAN S. M. M. & VASANTHA RUPASINGHE H. P. 2009. Sinensetin, rutin, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of the skin of apple fruit. *Food Chemistry* 113: 185–190.
- ANDRADE R. G., DALVI L. T., SILVA J. M. C., LOPES G. K. B., ALONSO A. & HERMES-LIMA M. 2005. The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. *Archive of Biochemistry and Biophysics* 437: 1.
- ASHIDA M. & SODERHÄLL K. 1984. The prophenoloxidase activating system in crayfish. *Comparative Biochemistry and Physiology-Part B- Biochemistry & Molecular Biology* 77: 21–26.
- BATE-SMITH E. C. 1977. Astringent tannins of *Acer* species. *Phytochemistry* 16: 2331–2336.
- BERGER J. & SLAVIČKOVÁ K. 2008. Morphological characterization of haemocytes in the adult linden bug, *Pyrrhocoris apterus* (L.) (Heteroptera). *Zoological Studies* 47 (4): 466–472.
- CARTEA M. E., FRANCISCO M., SOENGAS P. & VELASCO P. 2010. Phenolic compounds in Brassica vegetables. *Molecules* 16: 251–280.
- CHAMPION O. L., WAGLEY S. & TITBALL R. W. 2016. *Galleria mellonella* as a model host for microbiological and toxin research. *Virulence* 7: 840–845.
- DANISMAZOGLU M., DEMIR İ., SEVİM A., DEMIRBAG Z. & NALCA-CIOGLU R. 2012. An investigation on the bacterial flora of *Agriotes lineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. *Crop Protection* 40: 1–7.
- DRAPER H. H. & HADLEY M. 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology* 186: 421–431.
- FINKEL T. & HOLBROOK N. J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239–247.
- FLOHÉ L. & OTTING F. 1984. Superoxide dismutase assays. *Methods in Enzymology* 105: 93–104.
- GASMI L., MARTÍNEZ-SOLÍS M., FRATTINI A., YE M., COLLADO M. C., TURLINGS T. C. J., ERB M. & HERRERO S. 2019. Can herbivore-induced volatiles protect plants by increasing the herbivores' susceptibility to natural pathogens? *Applied and Environmental Microbiology* 85:e01468–18.
- GORMAN M. J., AN C. & KANOST M. R. 2007. Characterization of tyrosine hydroxylase from *Manduca sexta*. *Insect Biochemistry and Molecular Biology* 37: 1327–1337.
- GULCIN I., HUYUT Z., ELMASTAS M. & ABOUL-EENEIN H. Y. 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry* 3: 43–53.
- HARBORNE J. B. & GRAYER R. J. 2017. *The flavonoids advances in research since 1986*. Routledge: Abingdon, UK. 676 p.
- IBTISSEM B., CHEDLY A. & SFAR S. 2012. Antioxidant and antibacterial properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* extracts. *Advances in Chemical Engineering and Science* 2: 359–365.
- JAMES R. R. & XU J. 2012. Mechanisms by which pesticides affect insect immunity. *Journal of Invertebrate Pathology* 109: 175–182.
- KHAN H., ULLAH H., TUNDIS R., BELWAL T., DEVKOTA H. P., DAGLIA M., SAYGILI I., CETIN Z., da GRACA-CAMPOS M., CAPANOGLU E., DU M., DAR P. & XIAO J. 2020. Dietary flavonoids in the management of Huntington's disease: mechanism and clinical perspective. *eFood* 1: 38–52.
- KOC E. & USTUN A. S. 2008. Defence against pathogens in plants and antioxidants. *Erciyes University Journal of Institute of Science and Technology* 24 (1-2): 82–100.
- KURT D. & KAYIS T. 2015. Effects of the pyrethroid insecticide deltamethrin on the haemocytes of *Galleria mellonella*. *Turkish Journal of Zoology* 39: 452–457.
- LAMPERT E. C. 2012. Influences of plant traits on immune responses of specialist and generalist herbivores. *Insects* 3: 573–592.
- LAWRENCE R. A. & BURK R. F. 1976. Glutathione peroxidase activity in selenium deficient rat liver. *Biochemical and Biophysical Research Communications* 71: 952–958.
- LEE K. P., CORY J. S., WILSON K., RAUBENHEIMER D. & SIMPSON S. J. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings of the Royal Society B: Biological Sciences* 273: 823–829.
- LINDSAY D. G. & ASTLEY S. B. 2002. European research on the functional effects of dietary antioxidants-EUROFEDA. *Molecular Aspects of Medicine* 23: 1–38.
- LOWRY O. H., RSEBROUGH N. T., FARR A. L. & RANDALL R. J. 1951. Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry* 193: 265–275.
- LUCK H. 1963. Methods of enzymatic analysis. In: BERGMAYER H. U. (Ed.): *Methods of Enzymatic Analysis*. New York: Weinheim and Academic Press, pp. 885–888.

- MARTEMYANOV V. V., DUBOVSKIY I. M., BELOUSOVA I. A., PAVLUSHIN S. V., DOMRACHEV D. V., RANTALA M. J., SALMINEN J. P., BAKHVALOV S. A. & GLUPOV V. V. 2012. Rapid induced resistance of silver birch affects both innate immunity and performance of gypsy moths: the role of plant chemical defenses. *Arthropod-Plant Interactions* 6: 507–518.
- MASON P. A. & SINGER M. S. 2015. Defensive mixology: combining acquired chemicals towards defence. *Functional Ecology* 29: 441–450.
- McCORD J. M. & FRIDOVICH I. 1969. Superoxide dismutase. An enzymic function for erithrocuprein (Hemocuprein). *Journal of Biological Chemistry* 244 (22): 6049–6055.
- MOWLDS P., COATES C., RENWICK J. & KAVANAGH K. 2010. Dose-dependent cellular and humoral responses in *Galleria mellonella* larvae following  $\beta$ -glucan inoculation. *Microbes and Infection* 12: 146–153.
- MULLER K., VOGELWEITH F., THIÉRY D., MORET Y. & MOREAU J. 2014. Immune benefits from alternative host plants could maintain polyphagy in a phytophagous insect. *Oecologia* 177: 467–475.
- OZBILGIN S., ERGENE B., ALTUN M. L., SEVER-YILMAZ B., SALTAN G. & YUKSEL E. 2015. HPLC method for the analysis of chlorogenic acid of *Viburnum tinus* L. and *Viburnum orientale* Pallas. *Turkish Journal of Pharmaceutical Sciences* 12 (2): 130–136.
- PETERSEN M. & SIMMONDS M. S. J. 2003. Molecules of interest: rosmarinic acid. *Phytochemistry* 62: 121–125.
- RAYMOND B., JOHNSTON P. R., NIELSEN-LEROUX C., LERECLUS D. & CRICKMORE N. 2010. *Bacillus thuringiensis*: an impotent pathogen? *Trends in Microbiology* 18: 189–194.
- ROSA E., WOESTMANN L., BIÈRE A. & SAASTAMOINEN M. 2018. A plant pathogen modulates the effects of secondary metabolites on the performance and immune function of an insect herbivore. *Oikos* 127: 1539–1549.
- SCALBERT A., MANACH C., MORAND C. & REMESY C. 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* 45: 287–306.
- SCHULENBURG H., KURTZ J., MORET Y. & SIVA-JOTHY M. T. 2009. Ecological immunology. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 364: 3–14.
- SHAHIDI F. & AMAROWICZ R. 1994. Antioxidant activity of green tea catechins in a  $\beta$ -carotene-linoleate Model System. *Journal of Food Lipids* 2: 47–56.
- SHARIFI-RAD M., KUMAR N.V.A., ZUCCA P., VARONI E. M., DINI L., PANZARINI E., RAJKOVIC J., FOKOU P. V. T., AZZINI E., PELUSO I., MISHRA A. P., NIGAM M., RAYESS Y. E., BEYROUTHY M. E., POLITO L., IRITI M., MARTINS N., MARTORELL M., DOCEA A. O., SETZER W. N., CALINA D., CHO W. C. & SHARIFI-RAD J. 2020. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology* 11: 694.
- SHARMA P. R., SHARMA O. P. & SAXENA B. P. 2003. Effect of neem gold on haemocytes of the tobacco armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Current Science* 84: 690–695.
- SIVA-JOTHY M. T. & THOMPSON J. J. W. 2002. Short-term nutrient deprivation affects immune function. *Physiological Entomology* 27: 206–212.
- SLINN H. L., RICHARDS L. A., DYER L. A., HURTADO P. J. & SMILANICH A. M. 2018. Across multiple species, phytochemical diversity and herbivore diet breadth have cascading effects on herbivore immunity and parasitism in a tropical model system. *Frontiers in Plant Science* 9: 656.
- SMILANICH A. M., DYER L. A., CHAMBERS J. Q. & BOWERS M. D. 2009. Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters* 12: 612–621.
- STĄCZEK S., GRYGORCZUK K., ZDYBICKA-BARABAS A., SIEMIŃSKA-KUCZER A., VERTYPOROKH L., ANDREJKO M., WODA I. & CYTRYŃSKA M. 2017. Different faces of phenoloxidase in animals (Polish). *Postępy Biochemii* 63: 315–325.
- STĄCZEK S., ZDYBICKA-BARABAS A., PLESZCZYŃSKA M., WIATER A. & CYTRYŃSKA M. 2020. *Aspergillus niger*  $\alpha$ -1,3-glucan acts as a virulence factor by inhibiting the insect phenoloxidase system. *Journal of Invertebrate Pathology* 171: 107341.
- STOEPLER T. M., CASTILLO J. C., LILL J. T. & ELEFThERIANOS I. 2013. Haemocyte density increases with developmental stage in an immune-challenged forest caterpillar. *Plos One* 8 (8): e70978.
- SUGANYA M., KARTHI S. & SHIVAKUMAR M. S. 2016. Effect of cadmium and lead exposure on tissue specific antioxidant response in *Spodoptera litura*. *Free Radicals and Antioxidants* 6: 90–100.
- SYAFNI N., PUTRA D. P. & ARBAIN D. 2012. 34-Dihydroxybenzoic acid and 34-dihydroxybenzaldehyde from the fern *Trichomanes chinense*; isolation antimicrobial and antioxidant properties. *Indonesian Journal of Chemistry* 12: 273–278.
- TAO L., HOANG K. M., HUNTER M. D. & de ROODE J. C. 2016. Fitness costs of animal medication: antiparasitic plant chemicals reduce fitness of monarch butterfly hosts. *Journal of Animal Ecology* 85: 1246–1254.
- VUKOVIC R., BAUER N. & CURKOVIC-PERICA M. 2013. Genetic elicitation by inducible expression of cryptogein stimulates secretion of phenolics from *Coleus blumei* hairy roots. *Plant Science* 199–200: 18–28.
- WANG Y., HUANG X., CHANG B. H. & ZHANG Z. 2020. Growth performance and enzymatic response of the grasshopper, *Calliptamus abbreviatus* (Orthoptera: Acrididae), to six plant-derived compounds. *Journal of Insect Science* 20 (3): 1–8.
- WAR A. R., PAULRAJ M. G., AHMAD T., BUHROO A. A., HUSSAIN B., IGNACIMUTHU S. & SHARMA H. C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior* 7: 1306–1320.
- WHITTEN M. & COATES C. J. 2017. Re-evaluation of insect melanogenesis research: views from the dark side. *Pigment Cell & Melanoma Research* 30 (4): 386–401.
- WINK M. 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicines* 2: 251–286.
- YANAR O., ALIYEVA R., TOPKARA E. F., SEZER-TUNCSOY B. & OZALP P. 2018. Effects of diet quality on survival of *Vanessa atalanta* (L., 1758) (Lepidoptera: Nymphalidae) larvae infected by *Bacillus thuringiensis* subsp. *kurstaki*. *Acta Zoologica Bulgarica* 70 (2): 241–246.
- ZHANG Y., SUN G., YANG M., WUA H., ZHANG J., SONG S., MAE E. & GUO Y. 2011. Chronic accumulation of cadmium and its effects on antioxidant enzymes and malondialdehyde in *Oxya chinensis* (Orthoptera: Acridoidea). *Ecotoxicology and Environmental Safety* 74 (5): 1355–1362.