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ORIGINAL ARTICLE / ORIGINALBEITRAG

The Impact of Green Shield Bug (*Palomena prasina* [Hemiptera: Pentatomidae]) Infestation on Antioxidant Enzyme Activities in Hazelnut (*Corylus avellana* L. cvs. 'Tombul,' 'Palaz' and 'Çakıldak')

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Abstract

This study was carried out to determine total protein and activity of the enzymes malondialdehyde (MDA), phenoloxidase (PO), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in healthy and necrotic kernels and in healthy parts of necrotic kernels of different hazelnuts (*Corylus avellana* L. cvs. 'Tombul,' 'Palaz' and 'Çakıldak'). The highest total protein was measured in the necrotic kernels of all cultivars, whereas healthy kernels of all cultivars except 'Çakıldak' had the lowest total protein. The highest MDA and GPX were noted in necrotic kernels of 'Tombul' cultivar, whereas the lowest values were determined for the healthy part of the necrotic kernels. Nevertheless, healthy kernels recorded the highest activities of CAT and PO compared to other kernel types. The highest values for MDA and CAT in 'Palaz' cultivar were measured in the healthy part of the necrotic kernels, while healthy kernels had the lowest values. Contrastingly, the highest and the lowest values of GPX were recorded in healthy kernels and healthy parts of the necrotic kernels, respectively. Necrotic kernels of 'Çakıldak' cultivar had higher values for MDA and PO than the rest of the kernel types. Likewise, the highest and the lowest values for GPX and SOD were recorded for healthy kernels and healthy parts of the necrotic kernels, respectively. It is concluded that damage caused by the green shield bug has significant impact on the activities of antioxidant enzymes in hazelnut kernels in relation to their defense mechanisms.

Keywords Catalase · Superoxide dismutase · Insect damage · Malondialdehyde · Protein

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Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
GPX	Glutathione peroxidase
GSB	Green shield bug
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
PO	Phenol oxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Turkey is one of the largest producers and exporters of hazelnut (*Corylus avellana* L.). The confectionery and chocolate industries prefer high-quality kernels as raw material, but kernel quality is significantly altered by irrigation, fertilization, and insect damage/infestation (Tuncer et al. 2005; Silvestri et al. 2021).

Stink bugs (Hemiptera: Pentatomidae) are one of the most important pests of hazelnut in the main production regions of Turkey and of Europe overall. Some bugs significantly reduce the kernel quality of hazelnuts and thereby cause serious economic damage (Ak et al. 2018; Moraglio et al. 2018). These bugs damage hazelnuts during the whole growing season in numerous ways, either by piercing or sucking the hazelnut kernels. This damage can result in fruit abortion during the early season, empty and grey–black nuts during the nut development stage, shriveled kernels during kernel development, and necrotic kernels during the kernel filling/development stage. Tuncer et al. (2005) reported that the necrotic kernel damage in Turkey was 20% in a 5-year sampling. Similarly, a recent study identified these losses as ranging between 3.79% and 11.53%, and significant economic losses were recorded due to necrotic kernels (Ak et al. 2018). Necrotic kernels differ from healthy kernels in shape, taste, and color, and they are generally used for flour production. Hazelnut exporters also have to bear the costs of decreased quality (Tuncer et al. 2005).

Although 17 stink bug species exist in Turkish hazelnut areas, the green shield bug (GSB; *Palomena prasina* L. [Hemiptera: Pentatomidae]) is considered one of the main hazelnut pests, with the highest distribution (85% hazelnut production areas) and occurrence above the economic threshold level (Saruhan, 2004; Tuncer et al. 2005). The GSB and other members of the order Hemiptera inject saliva secretions into the plants during their feeding activities. These secretions remove enzymes such as lipase, amylase, and esterase from the plants (Vaccino et al. 2008). Plants respond to this type of biotic stress through jasmonic acid signals, which contain consecutive enzymatic reactions leading to the formation of hydrogen peroxide (Fürstenberg-Hägg et al. 2013; Peiffer and Felton 2014). These oxidative compounds reduce consumer satisfaction by causing undesirable aroma and bitterness (Ghirardello et al. 2013).

As with other biotic and abiotic stresses, insect infestation also causes oxidative damage to plants (Bi and Felton, 1995; Orozco-Cardenas and Ryan 1999). Oxidative stress occurs with the formation of free radicals, which cause oxidative damage because of oxidative phosphorylation. Free radicals are necessary for plant life and are produced under normal conditions; however, their quantity remains in balance with the antioxidant system. Once this balance is disturbed under stress conditions, these radicals cause oxidative damage such as protein denaturation, lipid peroxidation, and DNA mutations (War et al. 2011). Malondialdehyde (MDA) results from lipid peroxidation; therefore, one of the ways to measure the extent of oxidative damage is to determine MDA content under stressful environments. Plants possess an antioxidant defense system consisting of different compounds and enzymes to protect them from the harmful effects of free radicals. Plants capable of using this system more actively are more tolerant of insect attack and other stresses. The antioxidant defense system in plants can be stimulated by stress or externally applied by different elicitors (Gulsen et al. 2010). Some elicitors are used to increase the resistance of plants against various biotic and abiotic stresses. However, to choose an appropriate elicitor, it is necessary to know the defense mechanism of the plant against any stress. Therefore, this study was designed to determine the changes in activities of the oxidative stress–reducing enzymes phenol oxidase (PO), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), as well as in the protein and MDA levels in hazelnut plants against GSB infestation.

Materials and Methods

Hazelnut Caging, Insect Release, and Harvesting

In this study, 'Tombul,' 'Palaz' and 'Çakıldak' cultivars of hazelnut (*Corylus avellana* L.), widely grown in Turkey, were selected as plant materials. Hazelnut orchards (41°08′26.07″ N latitude, 36°78′06.66″ E longitude, and 50 m altitude) with the above-mentioned cultivars were selected for caging to obtain necrotic and healthy hazelnut kernels. The climate data of the study region are pre-

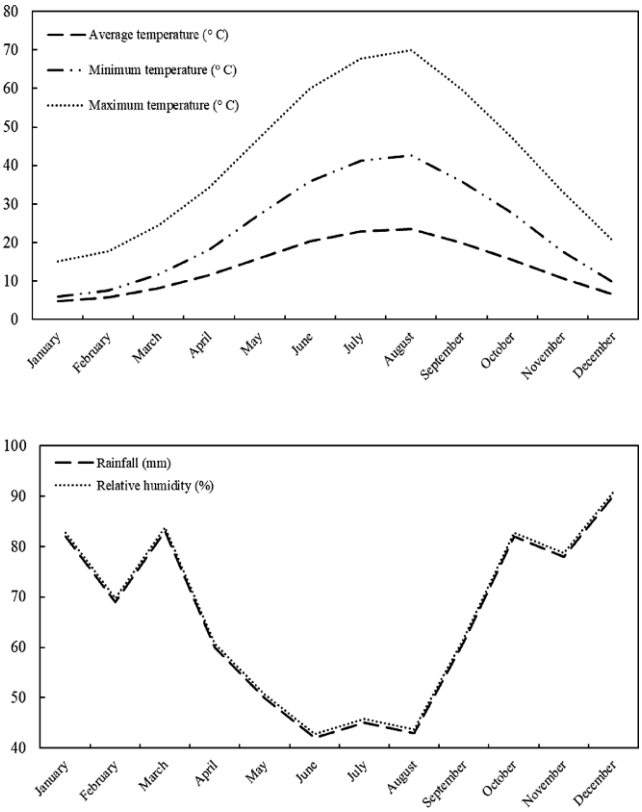

Fig. 1 Climate data of the study region

sented in Fig. 1. The soil of the orchards was clay–loam (22%), with little lime (0.65%) and sufficient organic matter (3.30%).

Experimental Design

The experiment was laid out according to a randomized complete block design with three replications for all treatments. For each cultivar, 20 trees were determined in each block. A branch of each tree containing at least 10 husks was enclosed in the cage (May 1, 2018). As of June 25, 10 of the 20 cages installed for each cultivar were classified as controls (for the healthy/no insect-damaged kernels) and 10 of them were regarded as insect cages (for the necrotic kernels). The nymphs of the GSB collected from the hazelnut orchard in Samsun in Çarşamba district (41°07′98.49″ N latitude, 36°78′27.30″ E longitude, and 51 m altitude) were placed in each cage (five nymphs in each insect cage) and kept in the cages until harvest. The hazelnuts in the cages were hand-harvested at commercial harvest time (on August 10 for 'Tombul' and 'Palaz' and on August 30 for 'Çakıldak') for each cultivar, and the cages were transferred to the laboratory and labeled as healthy or insect-affected for each cultivar.

Preparation of Harvested Hazelnuts for Analysis

Biochemical analyses were conducted from healthy/no insect-damaged kernels, necrotic kernels, and healthy parts of the necrotic kernels (Fig. 2). The hazelnuts transferred to the laboratory were separated by hand from their husks and placed on the bench under ventilated laboratory conditions to dry for 10 d at 25 ± 1 °C and 70% humidity. After drying (moisture content 6.45%), the samples were stored at –80 °C until analysis. The kernels of each cultivar were divided into three different groups (i.e., control/healthy kernel, necrotic kernel, and healthy part of the necrotic kernel) as described above. A total of 50 nuts of each cultivar in each group were used for the biochemical analysis. The target parts of the hazelnut kernels were scraped with a scalpel and put into sterile Eppendorf tubes for analysis.

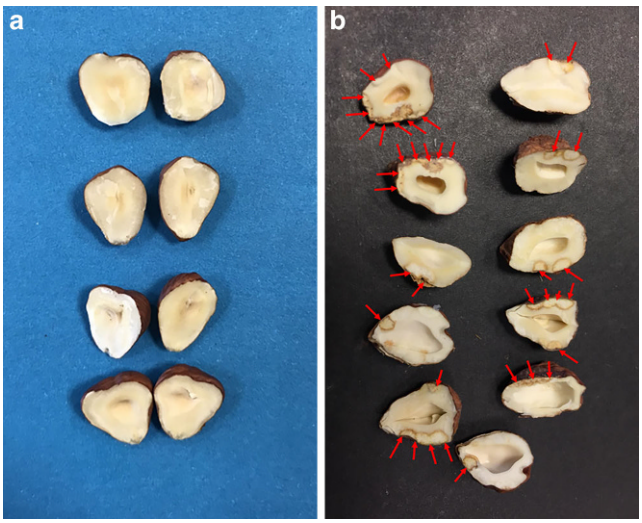

Fig. 2 **a** Healthy kernel. **b** Necrotic kernel with healthy part (necrosis is shown by *red arrows*, and the healthy part is the rest of the tissue in the kernel)

Homogenization and Sonication of Hazelnut Kernels

The samples were weighed on a sensitive scale and diluted with 0.9% NaCl solution in a 1:2 ratio and were subjected to sonication (Vibra-Cell VCS 130, Sonics & Materials, Newton, CT, USA) for 20-s and 10-s intervals six times at 25% amplitude.

Total Protein

The sonicated samples were poured into centrifuge tubes and centrifuged at +4 °C at 15,000 rpm for 20 min. After centrifugation, the supernatant was put in clean sample bottles, and enzymatic activity and protein values were measured from this supernatant. Total protein was determined as described by Lowry et al. (1951), and results were stated as μg mL^{-1}.

Malondialdehyde

The MDA forms a pink complex because of incubation with thiobarbituric acid at 90 °C under aerobic conditions. The absorbance of this complex was read on a spectrophotometer at a wavelength of 532 nm. Analysis and calculations were performed according to the methods of Draper and Hadley (1990) and Hammouda et al. (1995). The results were expressed in μmol mL^{-1}.

Catalase Activity

The protocol of Luck (1963) was followed to determine CAT activity. For this determination, 1/15 M $Na_2HPO_4.H_2O$ -KH_2PO_4 buffer was prepared at pH 7. Samples were diluted by adding distilled water in a 1:10 ratio. One unit of CAT activity is defined as the amount of enzyme that catalyzes cleavage of 1 μmol H_2O_2 per minute. The results were expressed in international unit (IU) mL^{-1}.

Superoxide Dismutase Activity

The SOD activity was determined by the spectrophotometric method of McCord and Fridovich (1969) and the method of Flohe and Otting (1984). One unit of SOD is defined as the amount of enzyme required for 50% inhibition of the re-

duction of cytochrome c with superoxide anion produced by the xanthine/xanthine oxidase system. It was spectrophotometrically monitored at 550 nm. The results were expressed in IU mL^{-1}.

Glutathione Peroxidase Activity

The activity of GPX enzyme was determined by the method of Lawrence and Burk (1976). Absorbance at 340 nm was recorded for 5 min. The activity was expressed in IU mL^{-1} of NADPH oxidized per minute.

Phenol Oxidase Activity

The PO activity was determined according to the methods of Cotter and Wilson (2002) and Lee et al. (1991). Here, an enzyme unit is the amount of enzyme required to increase the absorbance by 0.001 per minute. The slope of the ribbon, which reflects the absorbance–time relationship, was calculated in terms of absorbance × min^{-1} × mL^{-1} and is specified as activity level. The activity was expressed in IU mL^{-1}.

Statistical Analysis

The normality assumption of the data was examined using the Shapiro–Wilk test, and normal distribution was observed ($P > 0.05$). In addition, variance homogeneity was examined by the Levene test, and the variance of the data was homogeneous ($P > 0.05$). Analysis of variance was used to test the significance in the data. The least significant difference post hoc test at 5% probability was used to separate the means where analysis of variance indicated significant differences.

Results

Overall, the highest total protein was recorded for necrotic kernels. Different kernel types of 'Tombul' and 'Palaz' cultivars significantly differed in total protein content, and the lowest values were recorded for healthy kernels. However, the protein contents of healthy kernels and healthy parts of the necrotic kernels were similar in 'Çakıldak' cultivar (Fig. 3). The MDA contents significantly differed among kernel types of all cultivars. The highest MDA contents were recorded for necrotic kernels of 'Tombul' and 'Çakıldak' cultivars. However, in 'Palaz' cultivar, the highest and the lowest MDA contents were observed for healthy parts of the necrotic kernels and for healthy kernels, respectively. The lowest MDA content in 'Tombul' cultivar was noted for the healthy parts of the necrotic kernels, whereas healthy

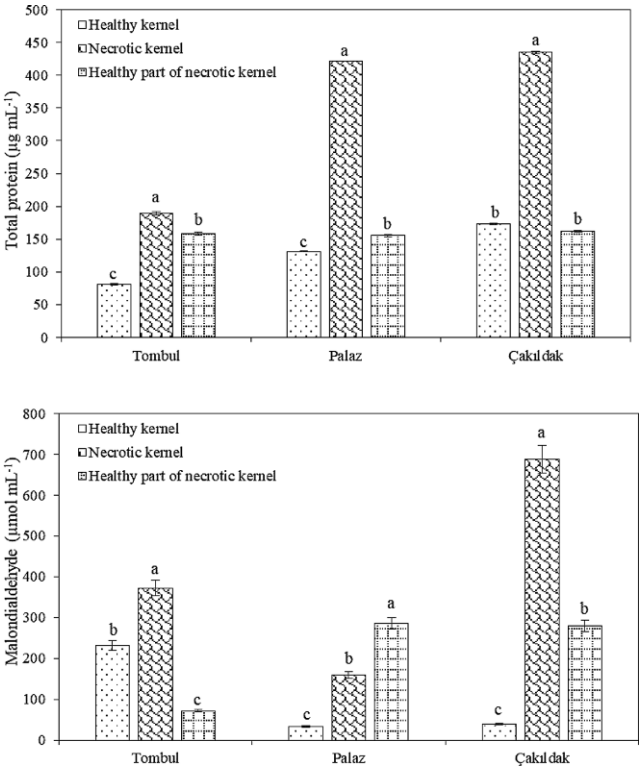

Fig. 3 Associations of healthy/no insect-damaged kernels, necrotic kernels, and healthy parts of necrotic kernels with total protein and malondialdehyde content in hazelnut cultivars. The means shown with the same lowercase letter on the bar are not different according to the least significant difference test at $P < 0.05$

kernels of 'Çakıldak' cultivar had the lowest MDA content (Fig. 3).

Various kernel types of 'Tombul' cultivar significantly differed for PO activity. The highest and the lowest PO activity levels were recorded for healthy kernels and for healthy parts of the necrotic kernels, respectively. Healthy kernels and healthy parts of the necrotic kernels of 'Palaz' and 'Çakıldak' cultivars had similar PO activity; however, necrotic kernels recorded lower PO activity. The highest SOD activity in 'Palaz' cultivar was noted for healthy parts of the necrotic kernels. However, healthy kernels of 'Çakıldak' cultivar recorded the highest SOD activity. The lowest SOD activity in 'Palaz' cultivar was recorded for necrotic kernels, whereas healthy parts of the necrotic kernels in 'Çakıldak' cultivar had the lowest SOD activity. Healthy and necrotic kernels of 'Tombul' cultivar had similar SOD activities; however, healthy parts of the necrotic kernels had lower SOD activity (Fig. 4).

The GPX and CAT activities in different kernel types of all cultivars significantly differed from each other. The highest GPX activity was recorded for healthy kernels of 'Palaz' and 'Çakıldak' cultivars and for necrotic kernels of 'Tombul' cultivar. The lowest GPX activity was recorded for healthy parts of the necrotic kernels in all cultivars.

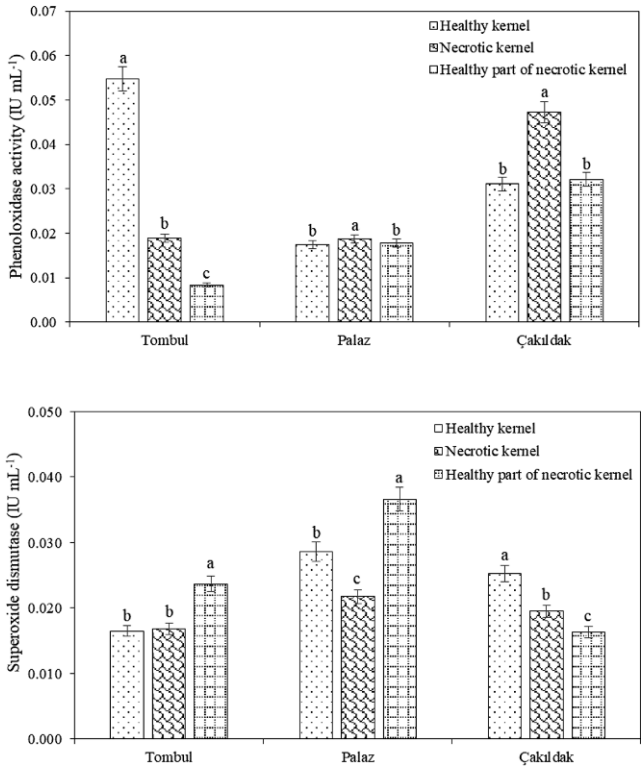

Fig. 4 Associations of healthy/no insect-damaged kernels, necrotic kernels, and healthy parts of necrotic kernels with phenol oxidase activity and superoxide dismutase in hazelnut cultivars. The means shown with the same lowercase letter on the bar are not different according to the least significant difference test at $P < 0.05$

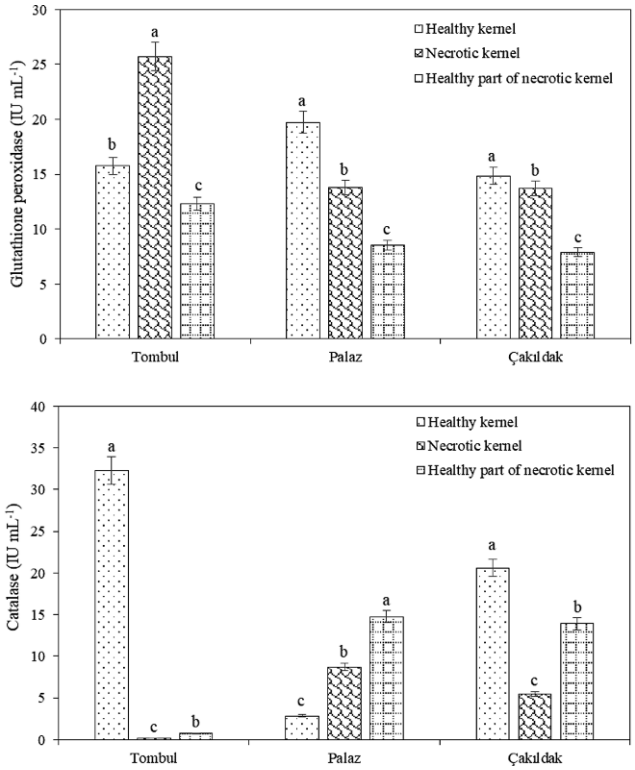

Fig. 5 Associations of healthy/no insect-damaged kernels, necrotic kernels, and healthy parts of necrotic kernels on glutathione peroxidase and catalase of hazelnut cultivars. The means shown with the same lowercase letter on the bar are not different according to the least significant difference test at $P < 0.05$

'Tombul' and 'Çakıldak' cultivars recorded the highest CAT activity in healthy kernels, while 'Palaz' cultivar had the highest CAT activity in healthy parts of the necrotic kernels. The lowest CAT activity in 'Tombul' and 'Çakıldak' cultivars was noted for necrotic kernels, while healthy kernels of 'Palaz' cultivar had the highest CAT activity (Fig. 5).

Discussion

Different kernel types significantly differed for the studied biochemical attributes in the current study. The higher protein content in GSB-damaged fruits of all hazelnut cultivars used in the current study might be linked to the plant's defense response. Increased synthesis of stress proteins has been reported as a typical response of plants to insect infestation (Golan et al. 2017). Our results are in agreement with those of Turan (2021), who reported that protein content in hazelnut fruits was increased in response to GSB infestation. Several other studies conducted on different plants other than hazelnuts also reported increased protein content in response to insect infestation (Usha Rani and Jyothsna 2010; War et al. 2013). Peumans and Van Damme (1995) reported that lectins, known as defense proteins, increase during insect infestation and prevent digestion by binding to the epithelial cells that cover the digestive system of the insects.

The MDA is a product of lipid peroxidation and is used as a suitable biomarker to assess oxidative stress (Rael et al. 2004). Production of reactive oxygen species caused by oxidative stress results in lipid peroxidation and, consequently, accumulation of MDA. The MDA is an important index for all stresses (Sytykiewicz et al. 2019; Sachdev et al. 2021). Higher MDA contents were recorded in necrotic kernels than in healthy kernels of all cultivars used in the current study, indicating that GSB infestation caused oxidative stress in all cultivars. The GSB infestation–mediated increase in MDA content was higher in 'Çakıldak' cultivar than in the rest of the cultivars included in our study. The increased MDA contents indicate that 'Çakıldak' cultivar observed higher oxidative damage than the rest of the cultivars. It has been reported by several researchers that biotic stresses such as insect infestation increase MDA content in plants (Broz et al. 2010; War et al. 2012; Wei et al. 2007).

Numerous earlier studies have reported that biotic stresses, such as insect infestation, increased PO activity in several plant species (Broz et al. 2010; War et al. 2012). It has been suggested that PO plays a pivotal role

in the defense against insect herbivory by reducing the digestibility and palatability of plant tissues (Mahanil et al. 2008; Bhonwong et al. 2009; Zhou et al. 2009). Furthermore, PO increases cell wall resistance against insects and pathogens by promoting the formation of melonin (Zhou et al. 2009). The PO activity in response to GSB infestation significantly differed among the studied hazelnut cultivars. Raj et al. (2006) reported a positive correlation between PO contents and cultivars' resistance to pathogens. However, no such findings were obtained in our study. The highest PO activity in response to GSB infestation was recorded in 'Çakıldak' cultivar, which demonstrated higher oxidative stress according to MDA contents. Hence, these results reveal that cultivars' resistance cannot be evaluated solely by PO activity. Constabel and Barbehenn (2008) reported great variability in the expression patterns and activity levels of PO depending on the species. They further indicated that similar variability in PO activity is expected during defense response to pathogens.

Several researchers have reported that SOD, GPX, and CAT enzymes are important elements of the defense system of plants against various biotic and abiotic stresses and that insect infestation results in increased activities of these enzymes (Łukasik and Goławska 2013; Kmieć et al. 2022). There are some reports indicating that cultivars capable of increasing the activities of antioxidant enzymes, e.g., SOD, GPX, and CAT, are more resistant to insect attack (Sabljic et al. 2020). In this study, GPX activity was increased in 'Tombul' cultivar, whereas CAT activity was increased in 'Palaz' cultivar upon GSB infestation. On the other hand, no significant increase in SOD activity was recorded in any cultivar in response to GSB infestation. These results suggest that the hazelnut cultivars used in the current study do not possess an adequate defense system against GSB infestation. Furthermore, the defense systems of plants may differ according to species and cultivar.

Conclusion

Green shield bug damage led to significant increases in total protein and MDA contents in necrotic kernels. This finding indicates that GSB infestation caused significant oxidative stress in the studied hazelnut cultivars. Although GSB infestation increased enzyme activities in some cases, overall enzyme activities indicated that all of the hazelnut cultivars used in the current study do not possess effective antioxidant defense systems against GSB infestation. On the other hand, considering that plants have different defense systems that may vary according to species and variety, further studies are needed to explore the defense mechanisms against GSB infestation in hazelnuts.

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Author Contribution **Ismail Oguz Ozdemir**: Methodology, investigation, conceptualization, validation, writing—original draft, visualization, formal analysis. **Celal Tuncer**: methodology, investigation, conceptualization, validation, review, editing. **Fatma Gonul Solmaz**: investigation, validation, formal analysis. **Burhan Ozturk**: validation, writing—original draft, review, editing.

Conflict of interest I.O. Ozdemir, C. Tuncer, F.G. Solmaz, and B. Ozturk declare that they have no competing interests.

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