EFFECTS OF IMIDACLOPRID APPLICATION ON ANTIOXIDANT SYSTEM IN SUNFLOWER LEAVES

KIRECCI, O. A.[1] – KUSTILI, Y.[2] – KIRMIZIKAYA, G.[3] – OZSAHIN, D.[4*]

[1]*Bitlis Eren University, Hizan Vocational School, Bitlis, Turkey*

[2]*Bitlis Eren University, Department of Biology, Graduate Education Institute, Bitlis, Turkey*

[3]*Firat University, Faculty of Science, Department of Biology, Elazig, Turkey*

[4]*Bitlis Eren University, Faculty of Arts and Sciences, Department of Biology, Bitlis, Turkey*

[*]*Corresponding author*
e-mail: molekuler@gmail.com; phone: +90-506-381-0661

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Abstract. Imidacloprid, one of the latest members of the neonicotinoids, is a widely used synthetic organic insecticide. In this study, it was aimed to reveal the effects of 2 ppm, 4 ppm, and 8 ppm imidacloprid on antioxidant enzymes and lipid peroxidation in *Helianthus annuus* L. Tarsan-1018 oilseed sunflower leaves. As a result of the applications, sunflower leaves were collected and frozen in liquid nitrogen, then stored at -40 °C until analyses were conducted. Sunflower seeds were a registered variety and were obtained from Trakya Agricultural Research Institute. Imidacloprid were applied as foliar to the Sunflower plant grown under controlled conditions and samples were taken at 24. 48. and 72. hours. Superoxide dismutase (SOD), catalase (CAT), glutathione s-transferase (GST), glutathione (GSH), oxidized glutathione (GSSG) and malondialdehyde (MDA) analyzes were performed in the samples taken after the applications. As a result, it was determined that imidacloprid had different effects on the biochemical parameters of sunflower plant leaves. It was understood that imidacloprid used in ppm doses stimulated antioxidant defense and created stress responses in the plant, and as with all plant protection products, the importance of proper dosage and controlled use must not be overlooked.
Keywords: *antioxidant, enzyme, Helianthus annuus, oxidative stress, pesticide*

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), pesticides are all substances used to eliminate and control all types of damage and other negative effects that may occur during the production, processing, transportation, storage and marketing of food, agricultural products, wood and animal feed. Because of their specific formulation to affect the biology of insects, numerous research efforts have focused on the effects of insecticides on insects (Dirilgen et al., 2023). Similarly, they can be applied to the products before and after the harvest to prevent potential deterioration during storage and transportation. The agents that regulate the plant growth are defoliants, desiccants or fruit reducers, that prevent the shedding of raw fruits from falling off before ripenning (Altikat et al., 2009).

Neonicotinoids are synthetic compounds with a structure similar to that of nicotine and dinotefuran (DIN), thiamethoxam (THM), thiacloprid (THD), clothianidin (CLO), imidacloprid (IMI), nitenpyram, and cetamiprid (ACE) are types of neonicotinoids (Zhang et al., 2023). They are water-soluble and are easily absorbed by plants through their roots or leaves, then transported through the plant tissue. Neonicotinoids are fourth-generation pesticides and are widely used to control insect pests on vegetables, fruits,

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cotton, rice and other industrial crops (Zhang et al., 2023). Neonicotinoids are a widely used class of systemic pesticides and are synthetically derived from nicotinoids (Annoscia et al., 2020). They offer many benefits to human in pest control as they protect all parts of the plant. For example, they are effective against boring and root-feeding insects, where foliar sprays with non-systemic compounds fail. Imidacloprid is one of the most widely used neonicotinoid insecticides in the world (Motauna, 2020). Neonicotinoids are known to be used in control of against pests in sugar beet, vegetables, fruit, cotton, rice and other industrial crops and seed spraying is the most common method (Katic et al., 2021). Sunflowers are a short-season plant belonging to the family *Asteraceae* and the genus *Helianthus* – of which 70 species are known worldwide (Vilvert et al., 2018). Studies have revealed that sunflower seeds are rich foods and they contain various phytochemicals that contribute to the improving human health, such as antioxidants, flavonols, phenolic acids, procyanidins, phytosterols, amino acids, dietary fiber, potassium, arginine, monounsaturated and polyunsaturated fatty acids (Alagawany et al., 2015). Xenobiotic compounds, such as pesticides, which are similar to heavy metals, also trigger ROS production and lead to oxidative stress (Sharma et al., 2019).

The aim of this study is to investigate the effects of next-generation pesticides on the leaves of the sunflower plant, a representative living organism. Also, the effects of synthetic pesticides are not fully understood to date. In this study, we tried to explain the oxidative damage caused by imidacloprid (insecticide), which belongs to the neonicotinoid group, in sunflower plants. In this way, it will provide ideas for future studies on living models and provide resources for further studies on the effects of pesticides on plants.

Material and methods

Plant material

In the present study, sunflower oil plant (*Helianthus annuss* L. cv. TARSAN – 1018) was obtained from Thrace Agricultural Research Institute (Turkey). The samples (sunflower leaves) were frozen in liquid nitrogen and stored in a deep freezer at -40°C until analysis.

Cultivation of plants and pesticide application

After surface sterilization, the seeds were left to soaked for 24 hours in water provided by an aquarium pump. Next, we planted the seeds in pots, and allowed them to grow at 25±2 °C under controlled conditions with 60-65% humidity. The experimental materials were grown in pots with a diameter of 21 cm and depth of 18 cm. The study was to include 10 pots for each concentration and time, and one seed per pot. Due to the possibility of insufficient germination, 5 pots were prepared as replacements for each application group and time. After applications, analyzes were performed with 5 randomly selected plants from concentration and time and 5 randomly collected leaves from each of these plants. The seeds were irrigated only with Hoagland culture solution until the pesticide application (8 weeks). At the end of the 8th week, we applied pesticide via foliar application, and watered them with Hoagland culture solution (Hoagland and Arnon, 1938). Then, we took samples after 24, 48, and 72 hours. And the applications and analysis were repeated three times. To establish an appropriate ratio between doses, preliminary applications were conducted 2 ppm, and its multiples were performed on

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germinated seeds. The effects on the seedlings were monitored. As a result of these applications, and since it is not in the literature, doses of 2 ppm, 4 ppm, and 8 ppm were chosen to observe the effects of the dose.

Superoxide dismutase (SOD) enzyme activity

The Superoxide dismutase enzyme activity was performed with reference to the method specified by Sairam et al. (2002). The SOD activity was measured by recording a decrease in optical density of nitroblue tetrazolium (NBT). 3 ml of assay mixture consisted of 13 mM methionine, 25 mM nitroblue tetrazolium chloride, 0.1mM EDTA, 50mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.1 ml enzyme. Reaction was initiated with the addition of riboflavin and kept under two 15W fluorescent lamps for 15 minutes. Reaction was stopped by closing the light and using assay mixture without enzyme, giving the maximum coloration, as control. Complete assay mixture, which was not illuminated, was used as blank. SOD activity of one unit was defined as the amount of enzyme needed to cause 50% inhibition of NBT at 560 nm and was expressed in Unit/g.

Catalase (CAT) enzyme activity

The determination of the catalase activity was made according to the method outlined by Aebi (1984). Plant tissues were homogenized within a solution including 50 mM Tris-HCl (pH 7.8) buffer with 0.1 mM EDTA, 0.2% (v/v), 0.1 mM EDTA, 0.2% (v/v) Triton X-100, 5 mM DTT and 0.2% PVPP (w/v), after grinding in liquid nitrogen. Homogenate was centrifuged at 13.000 g 4^{o}C for 25 minutes. Supernatant was used to measure the enzyme activity. Catalase activity is based on measurement of decrease occurring in absorbance associated with degradation of H_2O_2 in the presence of enzyme. Assay was conducted via spectrophotometer according to decrease in H_2O_2 concentration at 240 nm by adding 1 mL phosphate buffer with 1 mL hydrogen peroxide onto 2 mL sample taken from supernatant. The measurement continued for 60 seconds with 15 seconds of intervals. Enzyme activity was determined as μg/g/min.

Glutathione S-transferase (GST) enzyme activity

The analysis of glutathione S-transferase was conducted following the methods outlined by Habig et al. (1974). 100 mM potassium phosphate (pH 6.5), 1 mM EDTA, 1 mM CDNB (1-Chloro 2.4-Dinitro Benzene), 2 mM GSH were used as the reaction medium. 20 mM of stocks were prepared for CDNB and GSH. Enzyme activity was identified measuring the amount of enzyme which catalyzes 1 micro mole of dinitrophenyl glutathione occurring per minute by using GSH and CDNB at 344 nm and 37 ^{o}C. Enzyme activity was determined as μg/g/min.

Measurement MDA amounts

MDA levels were measured using the HPLC method. Proteins were precipitated by taking 1 mL of the supernatant homogenized with Tris buffer and treated with 10% perchloric acid ($HClO_4$) (Karatas et al., 2002).

The measurement of GSH and GSSG amounts

The measurement of GSH and GSSG amounts was analysed in the HPLC device (Klejdus et al., 2004). 1 mL of homogenate was taken, 1 mL of 10% TCA was added and it was deproteinized. After centrifugation at 6000 rpm, 1 mL was taken into autosampler

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vials. In quantitative measurements, analysis was performed using Shimadzu brand fully automatic HPLC device at 214 nm, and LC-10 ADVP UV-visible pump, SPD-M10AVP, PDA detector, CTO-10ASVP column oven, SIL-10ADVP autosampler, DGU-14A degasser unit and Class VP 6.26 operating program (Shimadzu, Kyota Japan).

Statistical analyses

All experimental data were repeated three times under some conditions. A comparative analysis of variance was performed between the control group and the experimental group. The statistical analyses of the data were carried out by using the SPSS 15.0 software program. Analysis of variance (ANOVA) and least significant difference (LSD) test were also used to compare of groups with the control group. Data were shown as mean ± SD. Each group was compared with its own control group.

Results

Antioxidant enzyme activities

Table 1 shows the effects of the pesticides on antioxidant enzymes at 24th hour. Catalase (CAT) enzyme activity was 273.006±0.5 μg/g/min in the control group. It decreased to 240.93±0.7 μg/g/min at 2 ppm ($p<0.05$), decreased significantly at 4 ppm (179.42±0.9 μg/g/min) ($p<0.001$) and increased at 8 ppm (221.43±0.8 μg/g/min) ($p<0.01$). Glutathione S-Transferase (GST) enzyme activity decreased compared to the control group at 2 ppm ($p>0.05$). A similar decrease was observed at 4 ppm concentration (19.56±0.77 μg/g/min) ($p<0.05$). There was an increase in enzyme activity at 8 ppm concentration, but it was determined to be lower than the control group (22.69±0.99 μg/g/min) ($p<0.01$). The activity values of the Superoxide Dismutase (SOD) enzyme increased at 2 ppm compared to the control group (40.55±0.15 U/g) ($p<0.001$). SOD enzyme activities decreased at 4 ppm concentration (29.05±0.36 U/g) ($p<0.001$) and 8 ppm concentration (26.36±0.15 U/g) ($p<0.01$).

Table 1. *Antioxidant enzyme activities in leaf tissues after 24 hours*

GROUPS	CAT (μg/g/min)	GST (μg/g/min)	SOD (Unit/g)
CONTROL at 24th Hour	273.006±0.5	24.3±0.21	30.65±0.19
2 ppm at 24th Hour	240.93±0.7^{b}	22.54±0.43^{a}	40.55±0.15^{d}
4 ppm at 24th Hour	179.42±0.9^{d}	19.56±0.77^{b}	29.05±0.36^{d}
8 ppm at 24th Hour	221.43±0.8^{c}	22.69±0.99^{c}	26.36±0.15^{c}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Table 2 shows antioxidant enzyme activities in the samples after 48 hours. It was determined that the CAT enzyme activity increased at 2 ppm compared to the control group (314.66±0.9 μg/g/min) and continued to increase by 4 ppm and 8 ppm ($p<0.001$). The GST enzyme activity reached its peak value at 2 ppm compared to the control group (43.85±0.98 μg/g/min) ($p<0.001$). At concentrations of 4 ppm (32.17±0.88 μg/g/min; $p<0.01$) and 8 ppm (27.95±0.87 μg/g/min; $p<0.05$), increased GST enzyme activities were determined compared to the control group (*Table 2*). The SOD enzyme activity also

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increased at 2 ppm (45.58±0.87 Unit/g; $p<0.01$). When *Table 2* is examined, it is seen that SOD enzyme activities decrease at 4 ppm and 8 ppm concentrations as compared to the control group. It was determined that the SOD enzyme activity, which was 24.17±3.07 Unit/g ($p<0.01$) at 4 ppm concentration, was 28.43±0.15 Unit/g ($p<0.05$) at 8 ppm concentration. When *Table 2* is examined, it is seen that CAT enzyme activity increases depending on the dose. GST enzyme activity decreased. While SOD enzyme activity increased at 2 ppm concentration, it decreased at 4 ppm and 8 ppm concentrations compared to the control group, but unlike the decrease at 4 ppm concentration, an increase was observed at 8 ppm concentration. This activity increase value is lower than the control group.

Table 2. *Antioxidant enzyme activities in leaf tissues after 48 hours*

GROUPS	CAT (µg/g/min)	GST (µg/g/min)	SOD (Unit/g)
CONTROL 48th Hour	271.49±0.9	24.55±0.36	32.66±0.41
2 ppm 48th Hour	314.66±0.9^{d}	43.85±0.98^{d}	45.58±0.87^{c}
4 ppm 48th Hour	329.35±0.6^{d}	32.17±0.88^{c}	24.17±3.07^{c}
8 ppm 48th Hour	348.56±0.8^{d}	27.95±0.87^{b}	28.43±0.15^{b}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Table 3 shows the effects of application concentrations on antioxidant enzyme activities at 72nd hour. Here, the CAT enzyme activity increased at 2 ppm ($p<0.01$), and it continued to increase at 4 ppm and 8 ppm concentrations and reached a peak at 8 ppm concentration. (410.42±0.9 µg/g/min) ($p<0.001$). The activity of the GST enzyme increased at concentrations of 2 ppm (38.22±0.66 µg/g/min), 4 ppm (47.28±1.77 µg/g/min), and 8 ppm (54.09±0.93 µg/g/min). When it came to the SOD enzyme activity, we noticed three increases: one at 2 ppm, having reached 48.94±0.87 Units/g, the second at 53.35±0.77 Units/g at 4 ppm, and the third at 65.75±0.95 Units/g at 8 ppm. All three increases are statistically significant ($p<0.001$). When the activities of antioxidant enzymes are evaluated as a whole; activities of CAT, GST and SOD increased at 24th hour. At 48th hour, CAT enzymes activity improved but GST and SOD enzyme activities reduced compared to 24th hour. In the 72nd hour applications, there were significant increases in the activities of all three enzymes. The results showed that pesticide application had a stimulating effect on the antioxidant enzyme system over time.

Table 3. *Antioxidant enzyme activities in leaf tissues after 72 hours*

GROUPS	CAT (µg/g/min)	GST (µg/g/min)	SOD (Unit/g)
CONTROL 72nd Hour	272.99±0.7	25.02±0.36	31.05±0.98
2 ppm 72nd Hour	363.99±0.6^{c}	38.22±0.66^{d}	48.94±0.87^{d}
4 ppm 72nd Hour	373.33±0.5^{d}	47.28±1.77^{d}	53.35±0.77^{d}
8 ppm 72nd Hour	410.42±0.9^{d}	54.09±0.93^{d}	65.75±0.95^{d}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

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Malondialdehyde glutathione and oxidized glutathione amounts

Table 4 shows the effects of the application groups on MDA, GSH and GSSG after 24 hours. The malondialdehyde (MDA) content increased at 2 ppm (28.74±0.2 nmol/g), 4 ppm (32.81 nmol/g), and 8 ppm (52.59 nmol/g) ($p<0.001$) compared to the control group. The data showed that MDA content increased as the concentration increased. The amount of glutathione (GSH) decreased at all three concentrations in the 24th hour ($p<0.001$). Oxidized glutathione (GSSG) content also increased compared to the control group at all three concentrations ($p<0.001$).

Table 4. *MDA, GSH and GSSG amounts in leaf tissues after 24 hours*

GROUPS	MDA(nmol/g)	GSH (µg/g)	GSSG (µg/g)
CONTROL 24th Hour	21.55±0.39	8.32±0.23	7.48±0.02
2 ppm 24th Hour	28.74±0.2^{d}	6.12±0.05^{d}	9.42±0.01^{d}
4 ppm 24th Hour	32.81±0.6^{d}	4.5±0.1^{d}	10.07±0.1^{d}
8 ppm 24th Hour	52.59±0.2^{d}	3.56±0.01^{d}	10.57±0.05^{d}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Table 5 shows the effects of the application groups on MDA, GSH, and GSSG after 48 hours. MDA amount was at 21.36±0.24 nmol/g in the control group. MDA amount increased at 2 ppm (39.28±0.25 nmol/g), 4 ppm (41.16±0.11 nmol/g) and 8 ppm (nmol/g). All of these increases are statistically significant ($p<0.001$). The GSH content increased compared to the control group at 2 ppm ($p<0.001$), at 4 ppm ($p<0.001$), and at 8 ppm ($p<0.001$). GSSG levels were determined to be lower than the control group at 2 ppm (6.97±0.02) ($p<0.05$) and 4 ppm (5.62±0.05) ($p<0.001$). The amount of GSSG increased at 8 ppm, but this increase was not statistically significant ($p>0.05$).

Table 5. *MDA, GSH and GSSG amounts in leaf tissues after 48 hours*

GROUPS	MDA (nmol/g)	GSH (µg/g)	GSSG (µg/g)
CONTROL 48th Hour	21.36±0.24	8.36±0.02	7.55±0.05
2 ppm 48th Hour	39.28±0.25^{d}	10.56±0.11^{d}	6.97±0.02^{b}
4 ppm 48th Hour	41.16±0.11^{d}	13.61±0.05^{d}	5.62+0.05^{d}
8 ppm 48th Hour	59.74±0.1^{d}	17.93±0.05^{d}	7.96±0.05^{a}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the own treatment groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Table 6 shows the effects of the application groups on MDA, GSH and GSSG at 72nd hour. MDA level increased at all of the concentrations ($p<0.001$) compared to the control group. GSH content increased at 2 ppm (10.19±0.01 µg/g) ($p<0.01$), 4 ppm (15.42±0.05 µg/g), and 8 ppm (19.08±0.05 µg/g) ($p<0.001$). By contrast, GSSG decreased somewhat at 2 ppm (6.19±0.05) ($p<0.01$), 4 ppm (5.43±0.01 µg/g) and 8 ppm (4.08±0.05 µg/g) ($p<0.001$). When we look at the effects of pesticide applications on MDA in general; It is understood that the amount of MDA increases in parallel with the

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application dose and duration. The amount of GSH decreased in the 24th hour and increased in the 48th and 72nd hours. GSSG amount increased the 24th hour and decreased in the 48th and 72nd hours.

Table 6. *MDA, GSH and GSSG amounts in leaf tissues after 72 hours*

GROUPS	MDA(nmol/g)	GSH (µg/g)	GSSG (µg/g)
CONTROL 72nd Hour	21.33±0.1	8.44±0.05	7.44±0.05
2 ppm 72nd Hour	46.45±0.2^{d}	10.19±0.01^{c}	6.19±0.05^{c}
4 ppm 72nd Hour	51.48±0.4^{d}	15.42±0.05^{d}	5.43±0.01^{d}
8 ppm 72nd Hour	68.67±0.1^{d}	19.08±0.05^{d}	4.08±0.05^{d}

** The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the own treatment groups. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Discussion

MDA is an important indicator of membrane lipid peroxidation and an important member of plant defence mechanism, thereby helping us understand the effects of stress (Heidarvand and Maali-Amiri, 2013). One of the most important aldehydes among these is malondialdehyde (MDA) (Girotti, 1998; Bolanos et al., 2009). All biological membranes consist of polyunsaturated fatty acids combined with amphipathic lipids and membrane proteins. Plants can give off various physiological, biochemical (e.g. chlorosis, lipid peroxidation), and antioxidant responses when applied with herbicides (Doganlar, 2012; Kaya and Yigit, 2014). Pesticides reduce plant growth, reduce photosynthetic efficiency, trigger molecular changes, increase ROS production and alter antioxidant status (Sharma et al., 2019; Yuzbasioglu et al., 2019). Liu et al. (2021) showed that excessive application of Imidacloprid induces oxidative stress in plants and leads to membrane lipid peroxidation by increasing H_2O_2 and O_2^- content. All of these affect the accumulation of MDA. One study conducted on the Oryza sativa plant reported an increase in MDA levels following the application of Imidacloprid (Sharma et al., 2015). Likewise, in another study, Sharma et al. (2013) observed an increase in the amount of MDA in seeds 12 days after the application of imidacloprid. Many studies about different pesticide derivatives have been published to date. Increases in MDA content have been reported in studies on different plants treated with pesticides from the amide group, such as Acetochlor, Napropamide, Rac-metolachlor, and S-metolachlor. For example, Cui et al. (2010) found that MDA levels in *Brassica napus* seeds increased after they were treated with Napropamide, which is consistent with the findings of the present study. In our study, MDA content increased dramatically after 24, 48, and 72 hours, depending on the time and amount of concentration. In other words, imidacloprid applications cause lipid peroxidation and damage plant membranes even at ppm doses. Glutathione protects plants from free radicals and peroxides (Marcel, 2013). It predominantly exists in the reduced form as GSH, serving as an effective antioxidant through various mechanisms. In the ascorbate-glutathione cycle, GSH plays a role in reducing both enzymatic and non-enzymatic dehydroascorbate (DHA), and it itself is oxidized to GSSG (oxidized glutathione). Glutathione functions as a non-enzymatic antioxidant. According to a study, the GSH content increased in *Brassica juncea* after exposure to 300 mg/kg of imidacloprid for 60 and 65 days (Sharma et al., 2016). Alla and Hassan (2014) applied

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Atrazine to *Zea mays* plants of Hybrid 351 and Giza 2 cultivars, and observed that after 8 days, the GSH content increased in the Hybrid 351 cultivar but decreased in the Giza 2 cultivar. This demonstrates that results can vary significantly even within the same species. When examining *Tables 4, 5, and 6*, we observed that the GSH content increased to varying degrees after 48 and 72 hours of pesticide exposure. However, decreases were observed at the 24th hour. What this tells us is that it is necessary to exercise caution in the application of pesticides, even at ppm dosages. GSSG and GSH content are closely interconnected. The GSH/GSSG ratio provides insights into how plants respond to stress. A lower GSH/GSSG ratio indicates greater oxidative stress (Schafer and Buettner, 2001). In the findings of the present study, the GSH/GSSG ratio was high at the 24th hour, but low at the 48th and 72nd hours. We noticed that as the GSH ratio decreased, the GSSG ratio increased. Referring to *Tables 5 and 6*, GSH increased and GSSG decreased. Since the ratio began to increase from 1, we can deduce that the plant's antioxidant defense mechanism had taken effect. The reaction catalyzed GSSG to GSH via glutathione reductase. Therefore, it is expected that GSSG will decrease while GSH increases. The results of the present study are consistent with Kasnak and Palamutoglu's findings (2015) and demonstrate that when imidacloprid was applied at certain ppm concentrations, it promotes antioxidant defense in the plant. It has been stated that application of imidacloprit in sublethal doses causes an increase in SOD enzyme activity and MDA amount, and decreases in GPx, GST, GR enzyme activities and GSH content in sweet potato weevil plant (Vinod and Jayaprakas, 2024). The mentioned results are partially compatible with our results. This may be due to the difference in the dose used, application time and experimental material. Because it is difficult to obtain similar results in living organisms Pesticides affect plants in different ways (Jones et al., 1986). One reported found that SOD, CAT and APX enzyme activities in cucumber plants exposed to imidacloprid were higher than those of the control group (Homayoonzadeh et al., 2020). Enzymatic and non-enzymatic antioxidants play unique roles in plants' defence systems. Gonias et al. (2008) discovered that catalase, glutathione reductase, and peroxidase increased slightly upon the application of imidacloprid although increasing temperature decreased enzyme activities. Another study showed that soil containing imidacloprid induced elevated antioxidative enzyme activities (e.g. SOD, CAT, APOX, GPOX and DHAR) in the leaves of *the Brassica juncea* (Sharma et al., 2017). A separate study focusing on fluorochloridone demonstrated that when 11 mM of pesticide was sprayed to *Helianthus annuus* leaves, the APOX and CAT activity decreased. That same study also found that GR, GST and SOD enzyme activities increased after 15 days after the application (Sharma et al., 2015). Kurshid et al. (2024) investigated the physiological and molecular effects of Thiamethoxam on potato plants. According to the results of the study, Thiamethoxam caused an increase in SOD, CAT and POD enzyme activities. At the cellular level, pesticides evoke oxidative stress (reactive oxygen species such as hydrogen peroxide, superoxide, and hydroxyl radicals) and therefore contribute to toxicity in the form of ROS. ROS molecules are highly toxic and can oxidize lipids, proteins, and nucleic acids, thereby causing cell death via lipid peroxidation, membrane damage, and enzyme deactivation. Shakir et al. (2018) reported that pesticide applications in tomato plants caused an increase in MDA level, SOD, POD, CAT, GR and APX enzyme activities. In turn, oxidative stress can damage biological systems and impair antioxidant defence systems. As a result of stress, increased antioxidant enzyme activities cause high levels of antioxidant in plants. Our data revealed that pesticide application caused oxidant stress in sunflower plants. The results are supported by literature.

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Conclusion

Antioxidant enzymes can counteract free radicals, and plants activate defence mechanisms in response to stress conditions triggered by imidacloprid applications. Likewise, antioxidant enzyme results also prove to be significant. When all the values are considered together, it is understood that pesticide application creates oxidative stress in the plant.

Upon reviewing the literature, it becomes evident that there are no comparable studies involving imidacloprid. However, it is worth noting that studies involving various other pesticide derivatives have been conducted. Understanding the long-term effects of these substances is important for plant health and productivity. A life without plants is unthinkable. Therefore, the long-term effects of pesticides need to be examined. This work has been revealed that the effects of long-term pesticide use on yield and product quality in sunflower should be investigated. In the literature review, it is seen that there are few pesticide studies on plants. Therefore, it is important to conduct similar studies and reveal the effects on product yield and product quality.

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Researchers contribution rate declaration summary. The authors declared that they contributed equally to the article.

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