Plant Physiology and Biochemistry 135 (2019) 322–330

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Salicylic acid alleviates thiram toxicity by modulating antioxidant enzyme capacity and pesticide detoxification systems in the tomato (*Solanum lycopersicum* Mill.)

Elif Yüzbaşıoğlu*, Eda Dalyan

Department of Botany, Faculty of Science, Istanbul University, Vezneciler, Istanbul, Turkey

ARTICLE INFO

Keywords:
Tomato
Fungicide phytotoxicity
Antioxidant enzyme activity
Detoxification gene expression

In this study, we investigated how 6.6 mM thiram induces to stress response in tomato and evaluated the possible protective role of different concentration of salicylic acid (0.01, 0.1 and 1 mM SA) against thiram toxicity by analyzing tomato leaf samples taken on the 1st, 5th, 11th day of the treatment. The thiram treatment resulted in oxidative stress through an increase in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in a time-dependent manner and led to a decline in the total chlorophyll and carotenoid levels. However, thiram-treated plants induced antioxidant enzyme activities, including catalase (CAT; EC 1.11.1.6), glutathione reductase (GR; EC 1.6.4.2), and ascorbate peroxidase (APX; EC 1.11.1.11), as well as pesticide detoxification enzymes such as peroxidase (POX; EC 1.11.1.7) and glutathione S-transferase (GST; EC.2.5.1.18). In addition, three genes (*GST1*, *GST2*, *GST3*) that encode for glutathione S-transferase and one gene (*P450*) that encodes for cytochrome P-450 monooxygenases were upregulated. SA showed a positive effect on the plants treated with thiram thanks to the decrease in the H_2O_2 and MDA levels, the enhancement of photosynthetic pigments, and the regulation in antioxidant enzyme activities in the tomato leaves. In addition, the SA-pretreatment triggered the activity and expression of pesticide detoxification enzymes in the thiram-treated leaves. Particularly the pretreatment with 1 mM SA significantly improved the activity of GST and led to the upregulation of *GST1*, *GST2*, *GST3*, and *P450* expression levels. These results indicate that the application of thiram fungicide causes toxicity; however, the damaging effect could be mitigated through pretreatment with SA.

1. Introduction

Pesticides have become an important factor for preventing crop losses and increasing food production in modern agriculture. The use of these chemicals is considered to be necessary to prevent diseases and insect damage in the tomato (*Solanum lycopersicum*, formerly Mill.) cropping system. Tomato is a widely cultivated vegetable in the world and has become a model species for genetic studies on fruit quality, biotic and abiotic stress tolerance, and disease resistance. Although pesticides are regularly used, there are still many fungal diseases that cause severe yield losses of tomatoes worldwide (Panthee and Chen, 2010). Because of the susceptibility of tomato plants to major fungal diseases, different carbamate fungicides have been used widely in agricultural systems since 1931 to control fungus (Gupta et al., 2012). Thiram (tetramethylthiuram disulfide) is a dimethyl dithiocarbamate which is a broad-spectrum protectant fungicide for seeds, fruits, vegetables, and ornamental and turf crops against fungal diseases. In addition, thiram is used post-harvested crops during their storage and transportation (Gupta et al., 2012). According to EPA R.E.D. Facts (2004), thiram has been widely used fungicide for foliar application on fruit, vegetable, and ornamental plants since 1948, also thiram usage has an environmental risk about soil and aquatic life. However, there has been limited knowledge about thiram effect on plant physiology and biochemistry.

Although fungicides have been commonly used during plant growth to improve the quantity and quality of crops, they could be a major source of environmental contamination leading to potential public health threats (Dias, 2012). The effect of fungicides is not limited to the target pathogenic organisms. They also cause significant damages to the developmental and reproductive physiology of nontarget organisms, such as plants (Dias, 2012). Excessive use of fungicides results in oxidative stress on plants at the cellular level. Several studies have shown that pesticide toxicity reduces photosynthetic pigments (Petit et al., 2012), causes an accumulation of reactive oxygen species (ROS) (Fatma

* Corresponding author.
E-mail address: elifaytamka@hotmail.com (E. Yüzbaşıoğlu).

https://doi.org/10.1016/j.plaphy.2018.12.023
Received 25 October 2018; Received in revised form 19 December 2018; Accepted 21 December 2018
Available online 24 December 2018

et al., 2018), alters antioxidant and detoxification enzymes activities (Yıldıztekin et al., 2015), and enhances key genes involved in fungicide degradation (Wang et al., 2010) in nontarget plants after fungicide application.

Plants have a three-phase biotransformation strategy that has evolved to mitigate the negative effects of pesticides. In the first phase, the pesticides are converted into a less-toxic product by oxidation, reduction, and hydrolysis reactions that are catalyzed by cytochrome P450 monooxygenases and peroxidases. The second phase includes conjugation of a pesticide metabolite through glutathione-S-transferase (GST) and uridine diphosphate (UDP)-glycosyltransferase into a sugar, amino acid, or glutathione, which is less toxic and more water-soluble (Sun et al., 2018). In the third phase, these metabolites are transported from the plant's cytosol to the vacuoles and apoplasts (Sun et al., 2018).

Salicylic acid (SA) is a phenolic compound whose contribution to local and systemic plant defense responses against pathogens has been proven through detailed evidence (Cevahir et al., 2005). SA also plays a role during plant growth and development, such as germination, chlorophyll biosynthesis, vegetative growth, and flowering (San Vicente and Plasencia, 2011). There is some evidence to support that SA is an endogenous plant hormone for responses to abiotic stress factors, such as drought, salinity, cold, metals, and paraquat (Hayat et al., 2010). It regulates the oxidative burst during hypersensitive responses to stress and acts as an internal signal molecule to reactive oxygen species (ROS) in the signal transduction process. SA can trigger the accumulation of ROS as a result of blocking antioxidant enzyme activities under abiotic stress conditions, and the increasing ROS level functions as a secondary stress signal to activate cellular protective systems, such as enzymatic and nonenzymatic activities (Hayat et al., 2010). Recent studies have reported that some herbicide toxicity is alleviated by applying exogenous SA, which improves photosynthesis, enhances pigment content, induces antioxidant enzyme activities, and reduces the residual effects of herbicides in maize, sunflower, rice, and wheat (Radwan and Soltan, 2012; Kaya and Yiğit, 2014; Wang et al., 2016; Wang and Zhang, 2017; Akbulut et al., 2018). However, there is no information on exogenous SA application for fungicide toxicity in plants; and the effects of thiram on plant physiology and biochemistry remains ambiguous. Our study mainly focuses on (1) finding out the effects of thiram fungicide on stress parameters (lipid peroxidation and hydrogen peroxide content), photosynthesis (chlorophyll *a*, *b* and carotenoid), antioxidant enzyme activity (CAT, APX, GR), and pesticide detoxification enzymes (GST and POX); (2) evaluating the role of SA on fungicide application; and (3) assessing whether pesticide detoxification enzyme genes (*GST1*, *GST2*, *GST3*, and *P450s*) in tomato leaves are affected by the detoxification of thiram after pretreatment with or without SA.

2. Materials and methods

2.1. Plant material, growth condition and treatment

Lycopersicum esculentum Miller Narcan-8 (tomato) seeds were obtained from Balıkesir Küçükçiftlik Seed Corporation in Turkey. Tomato seeds were surface-sterilized in 1% sodium hypochlorite and then imbibed in deionized water for 24 h at room temperature. Seeds were planted in plastic pots containing a perlite irrigated by with ¼ Hoagland solution (Hoagland and Arnon, 1950). The seedlings were grown for 45 days in the growth chamber at following conditions, 16-8h photoperiod, 150 ìmol m^{-2} s^{-1} light intensity, 25/20 °C temperature, and 60%. relative humidity. Forty-five days after sowing, tomato plants were divided into two groups. Uniformly sized tomato plants were selected and sprayed with deionized water, 0.01, 0.1 and 1 mM salicylic acid (SA). Twenty-four hours after SA pretreatment, foliar parts of plants were sprayed with 6.6 mM thiram (Sigma-Aldrich, 45689) which was chosen according to Akpınar (2014). SA concentration was determined to literature which was commonly used. Experimental data have replicated three times and each replicated consisted of three plants. The leaves were harvested and completely randomize mix in the groups on 1, 5 and 11 days after treatment (DAT). Between the time period of two fungicide spraying was recommended 10–15 day by the manufacturer. So, we thought that 1, 5 and 11 days after thiram treatment was suitable for monitoring the changes in the leaves.

2.2. Analysis of photosynthetic pigments

Leaves were extracted in 100% acetone in mortar and pestle. The homogenate was centrifuged at 3000g 4 °C for 15 min. The supernatant was measured spectrophotometrically with the wavelength of 661.6, 644.8, and 470 nm. Chlorophyll *a*, *b* and carotenoid contents were calculated in μg/ml, by describing Lichtenthaler and Buschmann (2001) method.

2.3. Analysis of hydrogen peroxide level

Fresh leaves sample (0.5 g) was extracted in 0.1% (w/v) TCA (Trichloroacetic acid) buffer and centrifuged at 12,000 × *g* for 15 min. The supernatant was mixed with 10 mM potassium phosphate buffer (pH 7.0), 1 M potassium iodide. The absorbance was determined at 390 nm by using a spectrophotometer (Epoch 2 Microplate Spectrophotometer, Winooski, USA). H_2O_2 content was given on a standard curve (Velikova et al., 2000).

2.4. Analysis of lipid peroxidation

Lipid peroxidation was indicated according to malondialdehyde (MDA) content which was analyzed using Jiang and Zhang (2001) method's. Fresh leaves sample (0.5 g) was extracted with 10 ml of 0.25% TBA (Thiobarbituric acid) + 10% TCA. The homogenate was boiled at 95 °C for 30 min. The cooled homogenate was then centrifuged at 5000 × *g* for 10 min. The absorbance of the supernatant was measured at 532 and 600 nm by using a Epoch 2 Microplate Spectrophotometer (Winooski, USA). MDA content was calculated as μmol g^{-1} fresh weight (FW) using the extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5. Analysis of antioxidant enzymes activities

Leaves (0.5 g) was homogenated in extraction buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA and 1% PVPP in a cold mortar with pestle. The homogenate was centrifugated at 13,000 × *g*, 4 °C for 40 min. The supernatant was collected for determining the content of total protein and the enzyme activity. The protein concentration was analyzed according to Bradford (1976) method. All spectrophotometric measurements were performed by using Epoch 2 microplate spectrophotometer.

CAT (EC 1.11.1.6) enzyme activity was determined by monitoring the decomposition of H_2O_2 for 2 min at 240 nm extinction coefficient (extinction coefficient 39.4 mM^{-1} cm^{-1}) (Bergmeyer, 1970). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.3% H_2O_2, and 12 μl enzyme extraction a 200 μl total volume.

APX (EC 1. 11.1. 11) activity was determined by monitoring the linear decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) for 2 min (Nakano and Asada, 1981). The reaction mixture was contained 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA-Na_2, 0.12 mM H_2O_2 and 20 μl of enzyme extract in a 200 μl total volume.

POX (EC 1.11.1.7) activity was determined by the increase in the absorbance at 465 nm (extinction coefficient 2.47 mM-1 cm-1) for 3 min. The reaction mixture contained DAB solution (0.15 M sodium phosphate-citrate buffer (pH 4.4) and 50% (w/v) gelatin), 0.6% H_2O_2 and 5 μl enzyme extract in a 200 μl total volume (Herzog and Fahimi,

Table 1
Time-dependent effect of foliar application of salicylic acid (SA) on photosynthetic pigments in tomato plants under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and then sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences between the control and thiram treatment groups.

Time	Treatment	Chl a (μg/mL)	Chl b (μg/mL)	Carotenoid (car) (μg/mL)	Total chl/car … … …
1 DAT	Control	527.08 ± 4.00	181.27 ± 2.62	129.29 ± 0.97	5.48 ± 0.02
	0.01 mM SA	380.22 ± 3.57^{ab}	136.26 ± 7.33^{ab}	102.79 ± 0.83^{ab}	5.03 ± 0.12^{a}
	0.1 mM SA	354.75 ± 3.40^{ab}	127.77 ± 1.25^{ab}	91.94 ± 1.09^{ab}	5.25 ± 0.03^{a}
	1 mM SA	325.31 ± 4.72^{ab}	115.91 ± 2.46^{a}	87.63 ± 1.62^{ab}	5.04 ± 0.06^{a}
	6.6 mM Thiram	306.47 ± 8.65^{a}	112.14 ± 5.45^{a}	81.25 ± 2.04^{a}	5.15 ± 0.04^{a}
	0.01 mM SA with Th	392.89 ± 2.63^{ab}	143.84 ± 1.48^{ab}	105.65 ± 0.78^{ab}	5.08 ± 0.01^{a}
	0.1 mM SA with Th	345.72 ± 5.00^{a}	127.77 ± 3.49^{ab}	92.76 ± 1.36^{ab}	5.10 ± 0.06^{a}
	1 mM SA with Th	407.06 ± 5.61^{ab}	154.78 ± 6.86^{ab}	113.86 ± 1.40^{ab}	4.94 ± 0.08^{ab}
5 DAT	Control	385.07 ± 3.55	138.28 ± 2.34	102.86 ± 1.17	5.09 ± 0.03
	0.01 mM SA	422.86 ± 12.11^{ab}	154.94 ± 5.49^{ab}	110.42 ± 0.62^{ab}	5.33 ± 0.03^{a}
	0.1 mM SA	346.99 ± 2.55^{a}	123.18 ± 1.86^{a}	92.99 ± 0.32^{a}	5.06 ± 0.03
	1 mM SA	216.23 ± 3.52^{ab}	79.43 ± 3.94^{ab}	58.23 ± 0.67^{ab}	5.08 ± 0.11
	6.6 mM Thiram	345.53 ± 6.23^{a}	128.33 ± 6.75	96.28 ± 1.20^{a}	4.92 ± 0.10
	0.01 mM SA with Th	427.72 ± 3.61^{ab}	157.73 ± 1.45^{ab}	109.36 ± 0.68^{ab}	5.35 ± 0.10^{ab}
	0.1 mM SA with Th	358.12 ± 9.72^{ab}	134.77 ± 6.42^{a}	96.49 ± 0.05^{a}	5.11 ± 0.11^{b}
	1 mM SA with Th	294.31 ± 6.89^{ab}	114.73 ± 5.25^{b}	84.56 ± 2.64^{ab}	4.84 ± 0.11^{a}
11 DAT	Control	319.77 ± 1.80	112.99 ± 2.03	90.46 ± 1.13	4.78 ± 0.04
	0.01 mM SA	266.70 ± 2.58^{ab}	96.86 ± 2.76^{ab}	75.52 ± 1.07^{ab}	4.81 ± 0.05
	0.1 mM SA	432.65 ± 1.07^{ab}	151.78 ± 2.15^{ab}	114.69 ± 0.71^{ab}	5.09 ± 0.05^{ab}
	1 mM SA	349.20 ± 2.56^{ab}	123.88 ± 0.68^{ab}	101.07 ± 0.88^{ab}	4.70 ± 0.05
	6.6 mM Thiram	231.10 ± 0.32^{a}	84.77 ± 1.04^{a}	65.98 ± 0.44^{a}	4.79 ± 0.03
	0.01 mM SA with Th	370.20 ± 1.63^{ab}	132.92 ± 0.74^{ab}	94.29 ± 0.45^{ab}	5.34 ± 0.01^{ab}
	0.1 mM SA with Th	216.25 ± 2.36^{ab}	80.64 ± 4.39^{a}	62.31 ± 1.11^{ab}	4.77 ± 0.12^{b}
	1 mM SA with Th	356.15 ± 4.98^{ab}	130.18 ± 4.35^{ab}	94.99 ± 1.11^{ab}	5.12 ± 0.01^{ab}

Notes: DAT, day(s) after treatment; Chl, chlorophyll.

1973).

GR (EC 1.6.4.2) activity was determined by the decrease in the absorbance at 340 nm (extinction coefficient 6.2 mM^{-1} cm^{-1}) for 2 min. The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPH Na_4 and 20 μl enzyme extract in a 200 μl volume (Foyer and Halliwell, 1976).

GST (EC.2.5.1.18) activity was determined the increase in the absorbance at 340 nm (extinction coefficient 9.6 mM^{-1} cm^{-1}) for 5 min (Habig and Jacoby, 1981). The reaction mixture was comprised of 50 mM potassium phosphate buffer (pH 6.5), 1 mM EDTA, 1 mM CDNB, 5 mM GSH and 10 μl enzyme extract in a 200 μl volume.

2.6. The total RNA extraction, cDNA synthesis, and quantitative real-time PCR

Total RNA was isolated from tomato leaves by using Innuprep Plant RNA (Analytik Jena) according to the manufacturer's instruction. The genomic DNA eliminated with 10 U RNase-free DNaseI (Thermo Scientific) for 20 min at 37 °C and purified and quantified according to the method described previously (Yüzbaşıoğlu et al., 2017).

The first strand cDNA was synthesized by using cDNA synthesis kit reagent (Bioline Sensifast) according to the manufacturer's instructions. Gene-specific primers for quantitative real-time RT-PCR were designed based on mRNA sequences, GST1 (AY007558.1, F: 5′GGATTGATGAG TTCTTGTGCTGT3′, R: 5′GCTGGATGCCTTTTGAGTTG3′), GST2 (EF409975.1, F:5′TCTACTCGTTTTTGGGCTCGT3′, R:5′ GTAATCCCTC TACCACCGATTCA 3′), GST3 (AF193439.1 F:5′CCTTTCCTCCCTTCTGA TCCT 3′, R: 5′ CTTGCTCTCCTCCCTTTGTTG 3′), P450 (GQ370622.1, F:5′TCAGGTTTTCTTCGGTCTAGCGA3′, R:5′CGCCTGTGTCTCCGTCA GTA 3′) as well as the elongation factor 1 alpha gene (X14449.1, F: 5′ ACCCCTCCGTCTTCCACTTC 3′, R: 5′ TGGAGAGCTTCGTGGTGCAT 3′), used as an housekeeping. Primers were constructed based on Primer 3 online software according to Thornton and Basu (2011).

Quantitative real-time PCR was applied with three replicates in 96-well plates with a Light Cycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany). The reaction was performed by LightCycler® 480 SYBR Green I Master (Roche, Mannheim, Germany).

The reaction mixture contained 5 u SYBR Green I master mix, 100 ng cDNA, and 10 pmol of each gene-specific primer in a final volume of 20 μl. The qRT-PCR program was consisted of following conditions: denaturation at 94 °C for 3 min, followed by40 cycles each comprising of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, and the final extension at 72 °C for 10 min. In all of the experiments, we can use negative control containing no template cDNA to avoid possible contamination. All experiment included three technical and biological replicates. Primers specificity were controlled with melting curve analysis. The quantification of mRNA level calculated comparative CT analysis method (Yüzbaşıoğlu et al., 2017). Firstly, ΔCt value calculated by using the following formula: $\Delta CT = CT_{gene} - CT_{housekeeping}$. Then, we calculated relative expression level (ΔΔCt) of the target gene by subtracting the control ΔCt values from the sample. Finally, the fold changes in expression level were calculated as $2^{-\Delta\Delta Ct}$.

2.7. Statistical analysis

Each treatment included three replicates and each experiment was carried out at least three at different times. All data obtained were performed by one-way analysis of variance (ANOVA) followed by Bonferroni Post Hoc Test analysis at $p < 0.05$, with the mean and standard deviation (SD) of each treatment calculated. All statistical analyses were made by GraphPad Prism version 5.2 software (GraphPad Software, San Diego, CA).

3. Results

3.1. The effects of SA pretreatment on photosynthetic pigments in tomato leaves with or without thiram

The chlorophyll *a*, chlorophyll *b*, and carotenoid content changed in the tomato leaves that were sprayed with 0.01, 0.1 and 1 mM SA in a time- and concentration-dependent manner (Table 1). Compared to the

control group, all SA pretreatment concentrations caused a significant reduction in the chlorophyll *a*, chlorophyll *b*, and carotenoid contents on the 1st day after treatment (DAT). Pretreatment with 0.01 mM SA led to a significant increase and pretreatment with 0.1 and 1 mM SA led to a significant decrease in the chlorophyll *a*, chlorophyll *b*, and carotenoid contents 5 DAT. Pretreatment with 0.1 mM SA increased chlorophyll *a*, chlorophyll *b*, and carotenoid contents by 35%, 30%, and 26%, respectively, compared to the control group on the 11 DAT. The ratio of total chlorophylls to carotenoids (total chl/car) reduced after the SA pretreatment on the 1 DAT compared to the control group. Pretreatment with 0.01 mM SA significantly increased the total chl/car ratio, and pretreatment with 0.1 and 1 mM SA showed similar total chl/car ratios compared to the control group on the 5 DAT. Pretreatment with 0.1 mM SA increased the chl/car ratio by 6.5% compared to the control group on the 11 DAT (Table 1).

The concentration of photosynthetic pigments in the tomato plant was negatively affected by the application of thiram fungicide. On the first day after the thiram treatment, the content of chlorophyll *a*, chlorophyll *b*, and carotenoids (41%, 36% and 36%, respectively) significantly reduced in tomato leaves and further reduced by 10%, 7%, and 6.5%, respectively, on the 5 DAT and by 28%, 25%, and 27%, respectively, on the 11 DAT compared to the control group. The only statistically significant reduction was seen in the total chl/car ratio in the thiram-treated leaves on the 1 DAT compared to the control group (Table 1).

As shown in Table 1, pretreatment with different concentrations of SA augmented the chlorophyll *a*, chlorophyll *b*, and carotenoid contents in the thiram-treated leaves in a time- and concentration-dependent manner. All concentrations of SA pretreatment remarkably increased the amount of chlorophyll *a*, chlorophyll *b*, and carotenoids on the 1 DAT. The pretreatment of 0.01 mM SA led to an increase by 24%, 23%, and 14% in chlorophyll *a*, chlorophyll *b*, and carotenoid contents, respectively on the 5 DAT. Similarly, the highest increment increases in the chlorophyll *a*, chlorophyll *b*, and carotenoid contents were 60%, 57%, and 43%, respectively, on the 11 days after pretreatment with 0.01 mM SA. Pretreatment with 1 mM SA significantly reduced the total chl/car ratio in the tomato leaves on the 1 DAT; however, pretreatment with 0.01 and 0.1 mM SA significantly increased the total chl/car ratio on the 5 DAT. All SA concentrations increased the total chl/car ratio on the 11 DAT.

3.2. The effects of SA pretreatment on the H_2O_2 and malondialdehyde contents in tomato leaves with or without thiram

Pretreatment with 0.1 and 1 mM SA resulted in H_2O_2 levels similar to those of the control group; however, pretreatment with 0.01 mM SA increased the H_2O_2 content to 19%; higher than that of the control group on the 1 DAT (Table 2). Although pretreatment with 0.1 mM SA significantly increased the H_2O_2 content, pretreatment with 1 mM SA remarkably decreased the H_2O_2 content compared to the control group the 5 DAT (Table 2).

The H_2O_2 in 6.6 mM thiram-treated leaves remarkably reduced by 64% compared to that in the control group on the 1 DAT; however, it increased by 8% and 34% compared to that in the control group on the 5 and 11 DAT, respectively (Table 2).

Pretreatment with SA less than 6.6 mM thiram significantly reduced the H_2O_2 production in a dose- and time-dependent manner. For example, pretreatment with 0.01 mM SA under the application of 6.6 thiram decreased the H_2O_2 content by 32% and 20% compared to the treatment with thiram alone on the 5 and 11 DAT, respectively (Table 2). 0.1 and 1 mM SA pretreatment mitigated H_2O_2 content by 19 and 15% spray with thiram 11 DAT (Table 2).

The malondialdehyde (MDA) content was higher in the leaves pretreated with 0.01 mM SA than in the control group on the 1, 5 and 11 DAT. No significant difference was found in the MDA content of the two groups on the 1 and 5 DAT with 1 mM SA; however, all SA concentrations increased the MDA contents compared to the control group on the 11 DAT (Table 2).

Table 2
Time-dependent effect of foliar application of salicylic acid (SA) on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents of tomato plants under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences from the control and the thiram treatment groups.

Time	Treatment	MDA content (μMmol/g FW)	H_2O_2 content (μmol/mL)
1 DAT	Control	5,85 ± 0,32	36,87 ± 0,91
	0.01 mM SA	7,29 ± 0,45^{ab}	43,77 ± 1,45^{ab}
	0.1 mM SA	6,06 ± 0,22^{b}	37,43 ± 0,85^{b}
	1 mM SA	6,55 ± 0,15^{b}	35,93 ± 0,47^{b}
	6.6 mM Thiram	4,32 ± 0,11^{a}	13,40 ± 0,20^{a}
	0.01 mM SA with Th	6,41 ± 0,20^{b}	48,24 ± 0,54^{ab}
	0.1 mM SA with Th	6,79 ± 0,52^{b}	48,24 ± 0,54^{ab}
	1 mM SA with Th	5,31 ± 0,17	51,50 ± 1,05^{ab}
5 DAT	Control	4,63 ± 0,14	33,90 ± 1,51
	0.01 mM SA	5,39 ± 0,16^{ab}	34,12 ± 0,82
	0.1 mM SA	6,38 ± 0,15^{a}	37,70 ± 0,75^{a}
	1 mM SA	4,46 ± 0,13^{b}	31,18 ± 1,44^{ab}
	6.6 mM Thiram	6,72 ± 0,53^{a}	36,73 ± 0,80^{a}
	0.01 mM SA with Th	4,66 ± 0,69^{b}	25,10 ± 0,40^{ab}
	0.1 mM SA with Th	4,91 ± 0,12^{b}	34,07 ± 0,67
	1 mM SA with Th	4,81 ± 0,36^{b}	50,03 ± 0,83^{ab}
11 DAT	Control	4,79 ± 0,34	42,77 ± 0,70
	0.01 mM SA	6,32 ± 0,31^{a}	40,60 ± 1,47^{b}
	0.1 mM SA	6,26 ± 0,12^{a}	39,90 ± 0,46^{b}
	1 mM SA	6,37 ± 0,42^{ab}	46,23 ± 0,31^{ab}
	6.6 mM Thiram	5,66 ± 0,36^{a}	57,37 ± 0,64^{a}
	0.01 mM SA with Th	3,75 ± 0,28^{ab}	45,70 ± 1,13^{b}
	0.1 mM SA with Th	5,46 ± 0,35	46,07 ± 2,38^{b}
	1 mM SA with Th	4,52 ± 0,48^{b}	48,00 ± 0,87^{ab}

Notes: DAT, day(s) after treatment.

On the contrary, MDA content decreased by 26% after the first day of thiram treatment compared the control (Table 2). However, 6.6 mM thiram sprayed on the leaves triggered the accumulation of MDA content by 45% and 18% on the 5 and 11 days after treatment, respectively. On the first DAT, the MDA content significantly increased in the leaves pretreated with 0.01 and 0.1 mM SA, although this increase was not significantly different from the increase in the leaves pretreated with 1 mM SA. On the 5 DAT, all SA concentrations remarkably reduced the MDA content in the tomato leaves by approximately 30%. On the 11 DAT, pretreatment with 0.01 and 1 mM SA significantly decreased the MDA content in the tomato leaves by 34% and 20%, respectively (Table 2).

3.3. The effects of SA pretreatment on antioxidant enzyme activities in tomato leaves under thiram application

Tomato leaves sprayed with thiram showed highly significant CAT activity compared to the control plants. Thiram treatments increased the CAT activity in tomato leaves by 100%, 60%, and 60% on the 1, 5, and 11 DAT, respectively (Fig. 1A), while all SA concentrations significantly decreased the CAT activity in tomato leaves sprayed with 6.6 mM thiram on the 1 DAT. However, CAT activity remarkably enhanced by 30% and 50% with the pretreatment of 0.01 and 0.1 mM SA

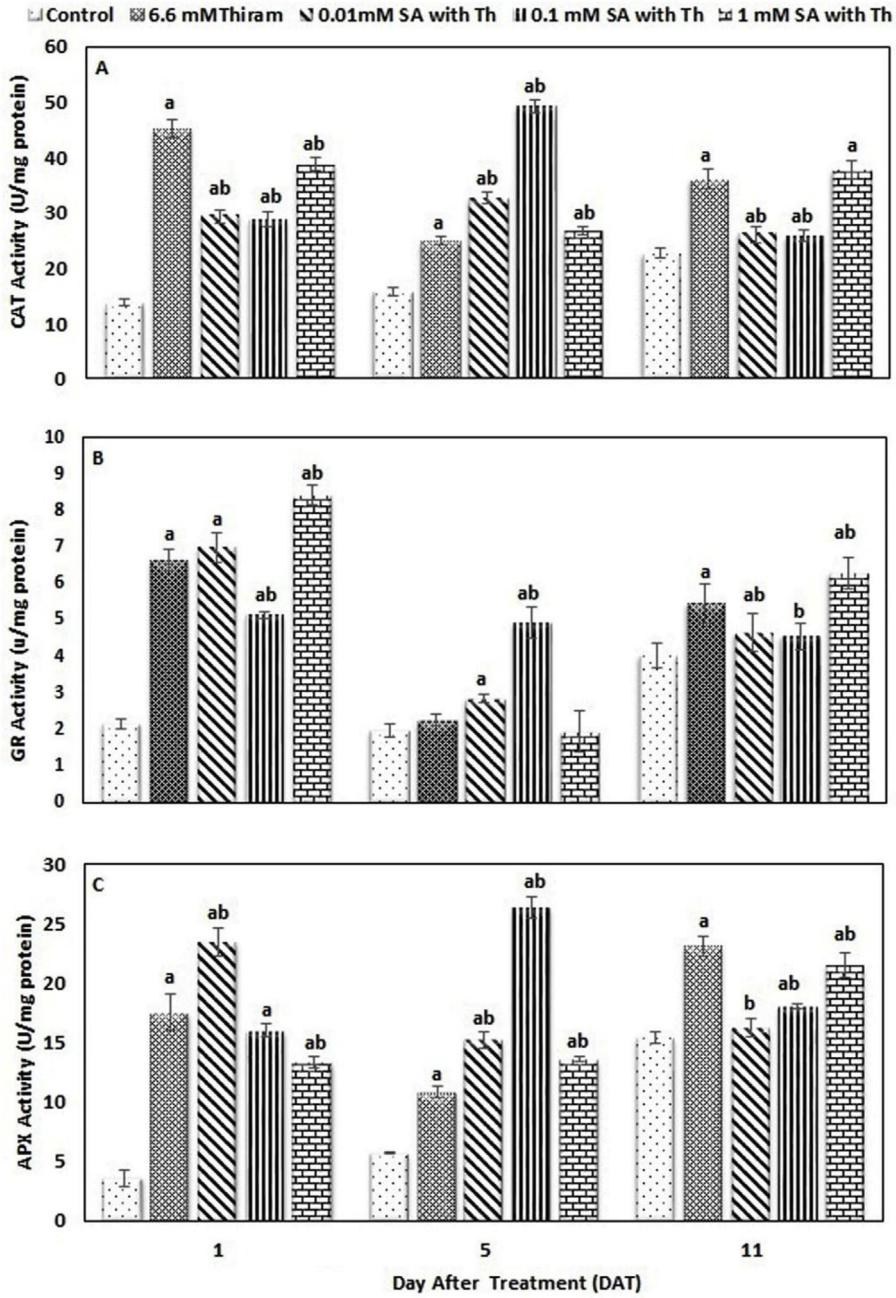

Fig. 1. Time-dependent effect of foliar application of salicylic acid (SA) on catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) activities in the tomato plant under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and then sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences from the control and the thiram treatment groups.

5 DAT. In addition, pretreatment with 0.01 and 0.1 mM SA significantly decreased the CAT activity, but CAT activity after pretreatment with 1 mM SA was not significantly different from the activity under thiram toxicity on the 11 DAT (Fig. 1A).

The highest activity of GR (3 times higher) in thiram-treated leaves was determined on the 1 DAT, which decreased approximately to the levels in the control group 5 DAT. The thiram-treated leaves showed an increase by 15% in the GR activity compared to the control group 11 DAT (Fig. 1B). SA pretreatment remarkably augmented the GR activity on the 1, 5, and 11 DAT in a time- and concentration-dependent manner. Among all concentrations of SA pretreatment, 1 mM SA increased the GR activity by 27% and 15% compared to thiram application on the 1 and 11 DAT, respectively. In addition, pretreatment with 0.01 mM SA triggered the GR activity by 2-fold compared to thiram application on the 5 DAT (Fig. 1B).

Thiram-treated leaves showed a significantly enhanced APX activity, which increased by 6-fold, 2-fold, and 50% on the 1, 5 and 11 DAT, respectively, compared to the control group (Fig. 1C). Pretreatment with 0.01 mM SA led to an increase by 30% while pretreatment with 1 mM SA caused a decrease by 23% in APX activity 1 DAT. On the 5 DAT, pretreatment of 0.1 mM SA showed the highest level of APX activity with a 2.5-fold increase in the tomato leaves compared to the thiram-treated leaves with no pretreatment. In addition, pretreatment with 0.01 and 1 mM SA significantly increased APX activity by 41% and 26% on the 5 DAT, respectively. However, all concentrations of SA pretreatment led to a remarkable decrease in APX activity under thiram toxicity on the 11 DAT (Fig. 1C).

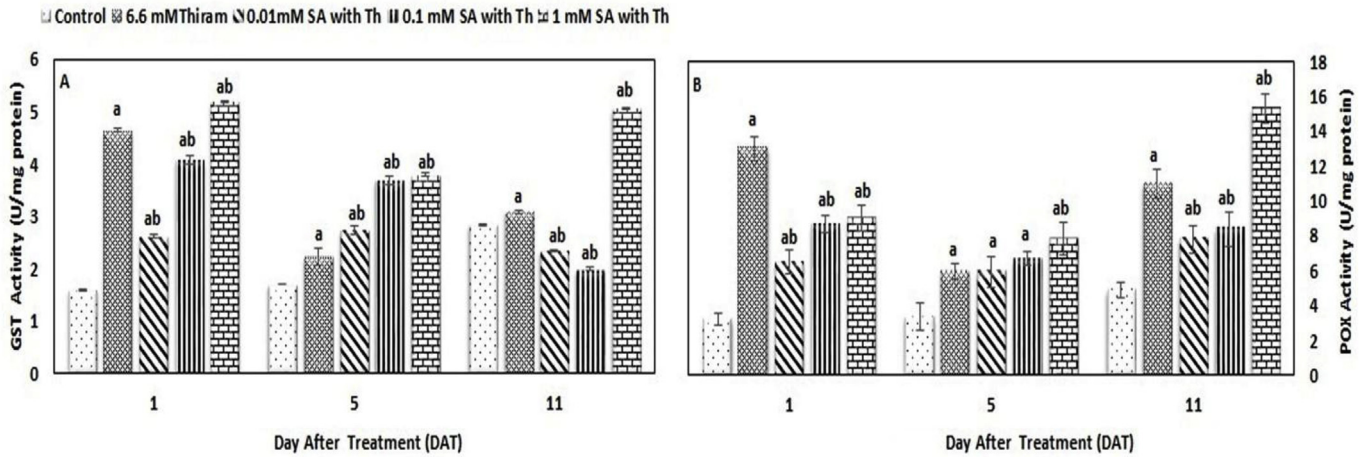

Fig. 2. Time-dependent effect of foliar application of salicylic acid (SA) on peroxidase (POX) and glutathione S-transferase (GST) activities in the tomato plant under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and then sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences from the control and the thiram treatment groups.

3.4. The effects of SA pretreatment on pesticide detoxification enzyme activities in tomato leaves under thiram application

Treatment with 6.6 mM thiram had a significant effect on the GST activity in tomato leaves, which increased by 3-fold, 32%, and 9% on the 1, 5, and 11 DAT, respectively, compared to the control group (Fig. 2A). Among all concentrations of SA pretreatment, 1 mM significantly increased the GST activity by 12%, 69%, and 64% on the 1, 5, and 11 DAT, respectively, under thiram toxicity. All concentrations of SA pretreatment remarkably enhanced the GST activity on the 5 DAT (Fig. 2A).

POX activity was triggered by the application of 6.6 mM thiram on the 1, 5, and 11 DAT compared to the control plants. The highest POX activity (4 times higher) was observed on the 1 DAT (Fig. 2B). All concentrations of SA decreased the POX activity under thiram toxicity on the 1 DAT. On the 5 and 11 DAT, the POX activity increased by 32% and 39% in the leaves pretreated with 1 mM SA compared to the thiram-treated leaves (Fig. 2B).

3.5. Effects of SA pretreatment on the expression of the pesticide detoxification genes in tomato leaves under thiram application

GST1, *GST2*, and *GST3* displayed higher expression levels in tomato leaves exposed to 6.6 mM thiram than in the control group on the 1, 5, and 11 DAT (Fig. 3A–C). Among the three GST transcripts, the expression level of *GST2* was approximately 3-fold on the 1, 5 and 11 DAT (Fig. 3B). The relative expression levels of *GST1* and *GST3* were approximately 100% and 70% higher on the 5 DAT (Fig. 3A, C). Pretreatment with SA led to higher expression levels of all *GST*s under thiram application, depending on the duration of toxicity and the thiram concentration. The highest expression levels of *GST1*, *GST2*, and *GST3* were observed with 1 mM SA pretreatment on the 5 DAT, which were approximately 5-fold, 50%, and 3-fold, respectively (Fig. 3A-–C). In addition, the relative expression levels of *GST1* and *GST2* were approximately 3- and 2-fold in 1 mM SA on the 11 DAT (Fig. 3A and B).

Thiram treatment caused significant alterations on the expression level of *P450* in tomato leaves on the 1 DAT, but this level gradually declined to less than that of the control on the 5 and 11 DAT. The relative expression level of *P450* increased in SA-pretreated leaves in a dose-dependent manner on the 5 and 11 DAT. Particularly the pretreatment with 0.1 and 1 mM SA induced the expression level of P450 by 4-fold and 8-fold on the 5 DAT compared to the control group. In addition, pretreatment with 1 mM SA increased the *P450* expression level by 50% on the 11 DAT (Fig. 4).

4. Discussion

In recent years, crop productivity has significantly increased because of the widespread use of fungicides, insecticides, and herbicides; however, the residues from these chemicals have accumulated in many ecosystems and become a significant source of pollutants in farmland and surrounding water (Cui et al., 2010). Thus, it is important to understand fungicides regulation pathways in intact plants and the mechanism by which they work. In this study, we discussed the direct effects of thiram fungicide on photosynthetic pigment, oxidative injuries, antioxidant enzyme activities, and the pesticide detoxification system and discussed the amelioration effect of SA against thiram toxicity in tomato leaves. SA is a well-known signal molecule in disease resistance and induces an oxidative burst through the accumulation of ROS, such as H_2O_2 (Radwan, 2012). Exogenous SA application at low concentrations can increase the levels of H_2O_2 as a second messenger for stress signaling, and this signal is similar to the stress-acclimating process. A rapid transient increase in the oxidative status stimulates the antioxidative mechanism (Horváth et al., 2007). SA is also known as an inhibitor of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene (Wang et al., 2016). Thus, SA application can protect plants from the adverse effects of biotic and abiotic stress factors.

The major symptom of fungicide toxicity in plants is observed in the photosynthetic process (Petit et al., 2012). Photosynthetic pigments are important factors in evaluating the plant responses to environmental stress. Chlorophyll *a*, the main photosynthetic pigment in the photosystems I and II, converts the optical energy of light. Chlorophyll *b* is an accessory pigment that absorbs the energy of light (Petit et al., 2012). Carotenoids function as phytoprotectants by quenching ROS. Chlorophyll *a*, chlorophyll *b*, and carotenoids bind to light-harvesting complex (LHC) proteins through weak noncovalent bonds, and the energy of light is captured by the pigments in LHC proteins and transferred to the reaction centers in the chloroplast thylakoid membrane (Petit et al., 2012). Thiram application causes a significant reduction in the photosynthetic pigments in tomato leaves in a time-dependent manner. In this study, the highest reduction of chlorophylls and carotenoids was observed on the 1 DAT. Chlorophyll *a* was more affected than chlorophyll *b* and carotenoids in the presence of thiram. This finding indicates that thiram may have a harmful effect on photosynthetic pigments by destroying chlorophyll *a*, chlorophyll *b*, and carotenoids. The reduction in chlorophylls might be the result of the degradation of LHC proteins (Wang and Zhang, 2017), the changes in chlorophyll fluorescence (Xia et al., 2009), and the disruption in the breakdown of the thylakoid and chloroplast envelopes (Wang et al., 2016). Singh and

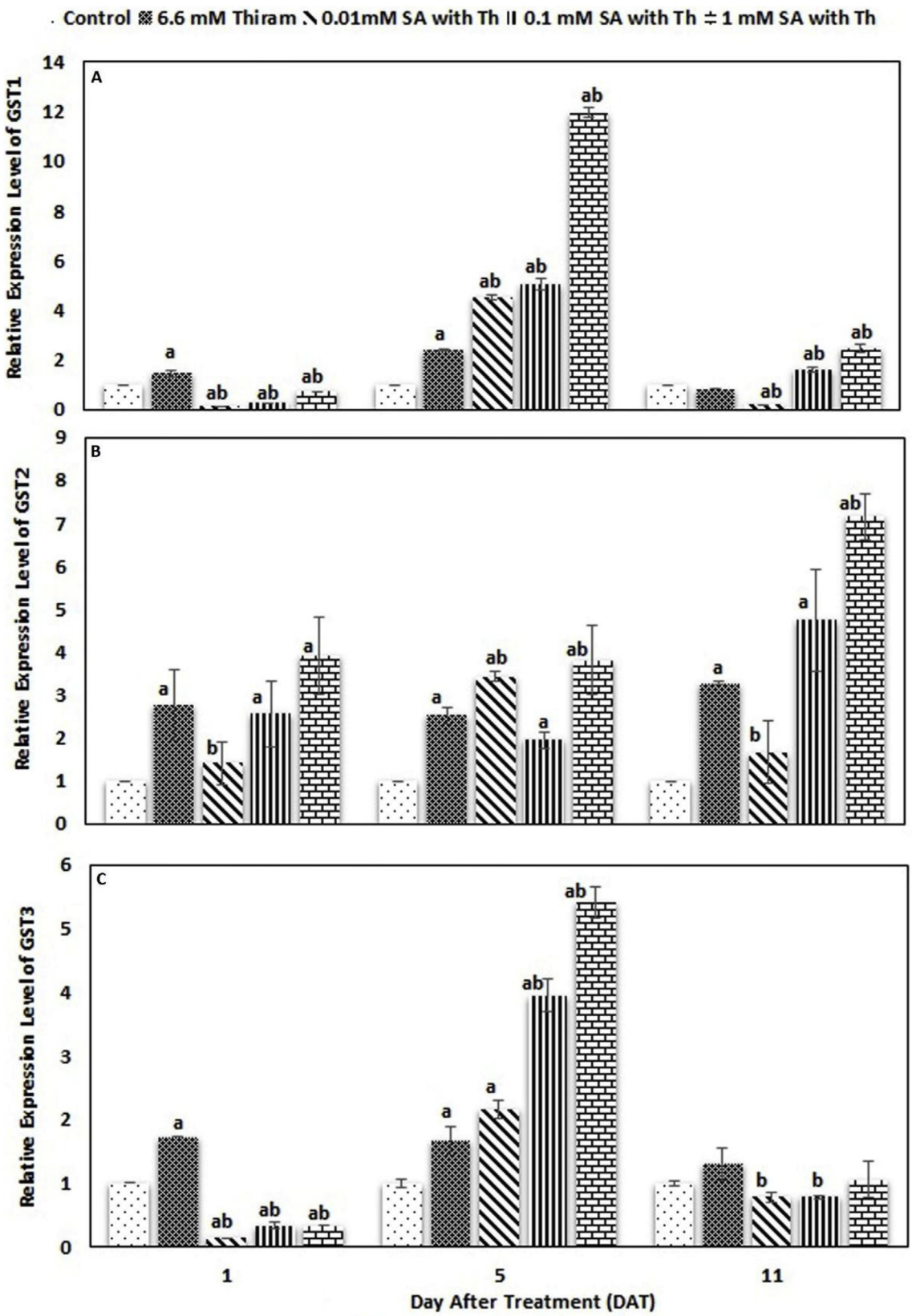

Fig. 3. Time-dependent effect of foliar application of salicylic acid (SA) on the expression level of glutathione S-transferase (GST)–related genes (*GST1*, *GST2*, *GST3*) in the tomato plant under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and then sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences from the control and the thiram treatment groups.

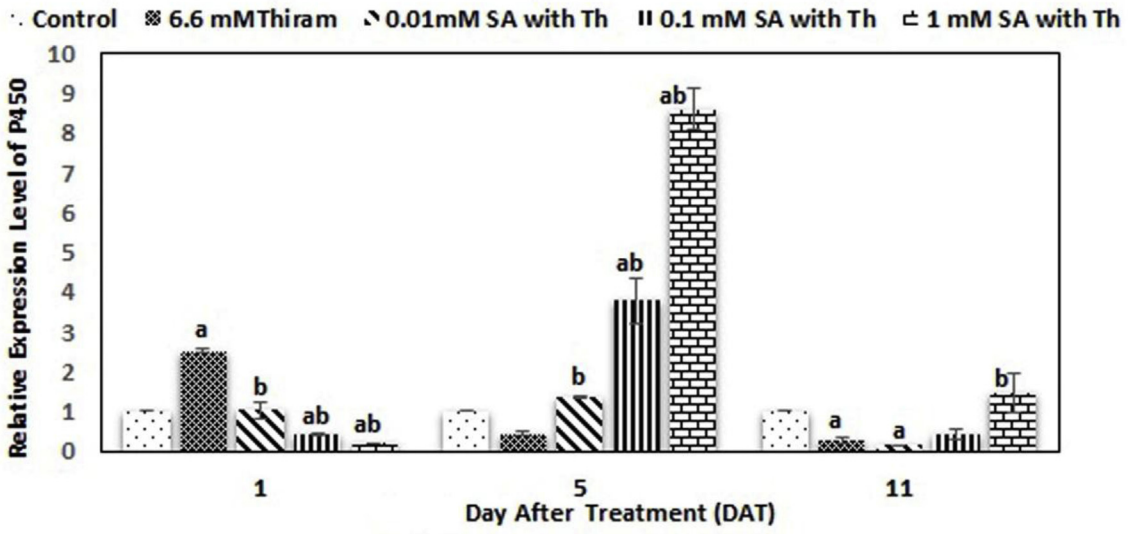

Fig. 4. Time-dependent effect of foliar application of salicylic acid (SA) on the expression level of *P450* in the tomato plant under 6.6 mM thiram treatment. Tomato leaves were pretreated with 0.01, 0.1, or 1 mM SA and then sprayed with 6.6 mM thiram fungicide. The different letters represent the statistically significant differences from the control and the thiram treatment groups.

Sahota (2018) suggested that chlorophyll content decreased in chickpea cultivars under fungicide stress. On the other hand, SA pretreatment enhanced the photosynthetic pigments under the application of 6.6 mM thiram in a time- and concentration-dependent manner in this study. The total chl/car ratio was higher than that of the control group in SA pretreatment on the 5 and 11 DAT. The cause of the lower total chl/car ratio is indicated to be the stress-related damage to the photosynthetic apparatus, which shows a faster breakdown of chlorophyll than carotenoid (Lichtenthaler and Buschmann, 2001). Therefore, SA can have a protective effect against thiram toxicity on photosynthetic pigments in tomato leaves. Wang et al. (2016) showed that quinclorac herbicide stress caused significant damage on the nuclear membrane, the cell wall, and the chloroplasts; however, exogenous SA application significantly improved the structure of cell organelles under quinclorac herbicide stress.

It is well-known that ROS can rapidly increase plant cells under stressful conditions. Excessive accumulation of ROS results in oxidative damage, which consequently causes lipid peroxidation and membrane leakage at cellular level in plants. MDA, a metabolic product of lipid peroxidation, is an important indicator of membrane lipid peroxidation. Plants have developed various mechanisms to protect themselves from the harmful effects of oxidative stress. One of these mechanisms is the antioxidant defense machinery which includes the CAT, GR, and APX enzymes. In the present study, H_2O_2 and MDA content decreased on the 1 DAT. This decrease may be explained by the rapid increases in antioxidant enzymes during this period. In this case, the higher activity observed in the antioxidant enzymes was not sufficient to explain the decrease in oxidative stress because we found that antioxidant enzyme activities (CAT, GR, and APX) and the H_2O_2 and MDA content increased on the 5 and 11 DAT in the thiram-treated leaves compared to those in the control group, which showed the presence of oxidative stress in the tomato leaves under thiram application. This result indicated that the significant effect of thiram toxicity was observed in tomato leaves on the 5 and 11 DAT. Similar studies have shown that pesticide-induced oxidative stress triggered antioxidant enzyme activities in tomato seedlings (Shakir et al., 2018). In the present study, SA pretreatment decreased the H_2O_2 and MDA levels under thiram toxicity on the 5 and 11 DAT. Singh et al. (2017) have obtained similar findings, reporting that exogenous SA application decreased lipid peroxidation and H_2O_2 production under glyphosate-based herbicide stress. Moreover, exogenous SA application regulates the antioxidant capacity by inhibiting or activating antioxidant enzyme activities in tomato leaves exposed to thiram. SA pretreatment inhibited the CAT activity as well as GR and APX activities that were stimulated under thiram application in a time- and concentration-dependent manner. SA can modulate ROS accumulation, which is related to the inhibition of H_2O_2 detoxifying enzymes, such as CAT and POX. Ananieva et al. (2004) reported that SA treatment did not cause any change in the APX activity, but it decreased the CAT activity and increased the POX and GR activities in barley plants exposed to paraquat stress.

Plants can generate various phases to transform and metabolize pesticides in order to diminish their toxicity. GST is an important part of the three-phase pesticide detoxification system that converts pesticides into less-toxic molecules in plants. Another antioxidant enzyme, POX, similar to the GSTs, plays a role in the response to pesticides by detoxifying the pesticides and eliminating the H_2O_2 in plants. In this study, GST and POX enzyme activities were induced in the tomato leaves after thiram application. This result indicated that thiram application triggered the pesticide detoxification enzymes in the plants. A similar study showed that GST and POX enzyme activities increased after propazine herbicide application in wheat, maize, and rapeseed (Zhang et al., 2018). Pretreatment with SA in the presence of thiram increased GST and POX enzyme activities in the tomato leaves. In particular, 1 mM SA, increasing GST and POX activity, is the most effective concentration against thiram toxicity. These results show that SA may have reduced thiram toxicity by stimulating the pesticide detoxification enzymes in the tomato leaves. Cui et al. (2010) indicated that SA might develop plant resistance by increasing the GST activity under napropamide toxicity in rapeseed.

There is a limited information on how fungicides change the transcriptional levels of pesticide detoxification enzymes. Cytochrome P450 is known as a first-phase enzyme which catalyzes the initial metabolic reactions to the pesticides. In this study, relative expression levels of *P450* were induced on the 1 DAT; however, *P450* was downregulated on the 5 and 11 DAT. In addition, pretreatment with 1 mM SA significantly stimulated the *P450* transcription levels in the tomato leaves exposed to thiram on the 5 and 11 DAT. Wang et al. (2017) showed that transcriptional levels of *P450* increased in a time-dependent manner in response to chlorothalonil fungicide in grapevines. The present study found that GST isoenzymes had a different transcript level under thiram stress in the tomato. The relative expression level of *GST2* was more abundant than that of *GST1* and *GST3* in the thiram-treated leaves.

Wang et al. (2010) indicated that GST isoenzymes had different detoxification capacity for several pesticides. The present study also showed that SA pretreatment significantly increased the transcriptional level of GST isoenzymes under thiram toxicity in tomato leaves. The *GST1*, *GST2*, and *GST3* transcription levels were highly induced after pretreatment with 1 mM SA on the 5 DAT in the tomato leaves. It is remarkable that the expression level of both GST isoenzymes and *P450* were highly activated after SA pretreatment on the 5 DAT; however, there is no information on how SA regulates *GST* and *P450* expression under pesticide toxicity in plants. Herrera-Vásquez et al. (2015) indicated that SA may have played an antioxidant role in concert with glutathione (GSH) in the response to stress.

As a result of this study, pretreatment with SA remarkably diminishes thiram toxicity, which was explained by the significantly regulated antioxidant enzyme activities (CAT, GR, and APX), enhanced pesticide detoxification enzyme activity (POX and GST), and upregulated pesticide detoxification enzyme genes (*GST1*, *GST2*, *GST3*, and *P450*) in the tomato leaves.

5. Conclusion

In recent years, excessive use of pesticides has become a major threat to agricultural production. A better understanding of the adverse effect of pesticide on the nontarget plant can help to improve new approach for using pesticides on the agricultural systems. Important physiological advances provide basic knowledge for the future manipulation methods against pesticide toxicity in plants. Salicylic acid is a pivotal molecule as phytohormone in biotic and abiotic stress response in plants so, it is a critical role for promoting sustainable agriculture production. Our findings reveal that SA alleviates the adverse effect of thiram fungicide in tomato. Consequently, we can recommend that SA might use with pesticide application for improving crop yield and production in agriculture.

CRediT authorship contribution statement

Elif Yüzbaşıoğlu: Formal analysis, Writing - original draft. **Eda Dalyan:** Formal analysis.

Acknowledgements

This study was funded by the Research Fund of Istanbul University [grant number 41364 and FBA-2016-3745].

References

Akbulut, G.B., Yigit, E., Kaya, A., Aktas, A., 2018. Effects of salicylic acid and organic selenium on wheat (*Triticum aestivum* L.) exposed to fenoxaprop-p-ethyl. Ecotoxicol. Environ. Saf. 148, 901–909.

Akpınar, I., 2014. The Physiological Effects of Thiram on Tomato (*Lycopersicum esculentum* Miller) Plants. Istanbul University Graduate School of Science and Engineering Biology Master Thesis.

Ananieva, E.A., Christov, K.N., Popoya, L.P., 2004. Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. J. Plant Physiol. 161 319-38.

Bergmeyer, N., 1970. Methoden der enzymatischen, Analyse, vol. 1. Akademie Verlag, Berlin, pp. 636–647.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Cevahir, G., Yentür, S., Aytamka, E., Eryılmaz, F., Yılmazer, N., 2005. The effect of nitric oxide, salicylic acid and hydrogen peroxide on the pigment content in excised cotyledons of red cabbage (*Brassica oleraceae* L.). Fresenius Environ. Bull. 14, 591–598.

Cui, J., Zhang, R., Wu, G.L., Zhu, H.M., Yang, H., 2010. Salicylic acid reduces napropamide toxicity by preventing its accumulation in rapeseed (*Brassica napus* L.). Arch. Environ. Contam. Toxicol. 59, 100–108.

Dias, M.C., 2012. Phytotoxicity: an overview of the physiological responses of plants exposed to fungicides. J. Bot., Le 135479. https://doi.org/10.1155/2012/135479.

Fatma, F., Kamal, A., Srivastava, A., 2018. Exogenous Application of Salicylic Acid Mitigates the Toxic Effect of Pesticides in *Vigna radiata* (L.) Wilczek J Plant Growth Regul. https://doi.org/10.1007/s00344-018-9819-6.

Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Planta 133, 21–25.

Gupta, B., Rani, M., Kumar, R., 2012. Degradation of thiram in water, soil and plants: a study by high-performance liquid chromatography. Biomed. Chromatogr. 26, 69–75.

Habig, W.H., Jacoby, W.B., 1981. Assays for differentiation of glutathione S-transferases. Methods Enzymol. 77, 398–405.

Hayat, Q., Hayat, S., Irfana, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 68, 14–25.

Herrera-Vásquez, A., Salinas, P., Holuigue, L., 2015. Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Front. Plant Sci. 6, 171. https://doi.org/10.3389/fpls.2015.00171.

Herzog, V., Fahimi, H., 1973. Determination of the activity of peroxidase. Anal. Biochem. 55, 554–562.

Hoagland, D.R., Arnon, D.I., 1950. The water culture method for growing plants without soil. Circ. Calif. Agric. Exp. Stn. 347, 32.

Horváth, E., Szalai, G., Janda, T., 2007. Induction of abiotic stress tolerance by salicylic acid signaling. J. Plant Growth Regul. 26, 290–300.

Jiang, M., Zhang, J., 2001. Effect of abscisic acid on active oxygen species, antioxidative defense system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. 42, 1265–1273.

Kaya, A., Yiğit, E., 2014. The physiological and biochemical effects of salicylic acid on sunflowers (*Helianthus annuus*) exposed to flurochloridone. Ecotoxicol. Environ. Saf. 106, 232–238.

Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry 1 F4.3.1-F4.3.8.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Panthee, D.R., Chen, F., 2010. Genomics of fungal disease resistance in tomato. Curr. Genom. 11, 30–39.

Petit, A.N., Fontaine, F., Vatsa, P., Clement, C., Vaillant-Gaveau, N., 2012. Fungicide impacts on photosynthesis in crop plants. Photosynth. Res. 111, 315–326.

Radwan, D.E.M., 2012. Salicylic acid induced alleviation of oxidative stress caused by clethodim in maize (*Zea mays* L.) leaves. Pestic. Biochem. Physiol. 102, 182–188.

Radwan, D.E.M., Soltan, D.M., 2012. The negative effects of clethodim in photosynthesis and gas-exchange status of maize plants are ameliorated by salicylic acid pretreatment. Photosynthetica 50 (2), 171–179.

San Vicente, M.R., Plasencia, J., 2011. Salicylic acid beyond defence: its role in plant growth and development. J. Exp. Bot. 62 (10), 3321–3338.

Shakir, S.K., Irfan, S., Akhtar, B., Rehman, S., Daud, M.K., Taimur, N., Azizullah, A., 2018. Pesticide-induced oxidative stress and antioxidant responses in tomato (*Solanum lycopersicum*) seedlings. Ecotoxicology 27, 919–935.

Singh, G., Sahota, H.K., 2018. Impact of benzimidazole and dithiocarbamate fungicides on the photosynthetic machinery, sugar content and various antioxidative enzymes in chickpea. Plant Physiol. Biochem. 132, 166–173.

Singh, H., Singh, N.B., Singh, A., Hussain, I., 2017. Exogenous application of salicylic acid to alleviate glyphosate stress in *Solanum lycopersicum*. Int. J. Veg. Sci. 23 (6), 552–566.

Sun, L., Xua, H., Su, W., Xue, F., An, S., Lu, C., Wu, R., 2018. The expression of detoxification genes in two maize cultivars by interaction of isoxadifen-ethyl and nicosulfuron. Plant Physiol. Biochem. 129, 101–108.

Thornton, B., Basu, C., 2011. Real-time PCR (qPCR) primer design using free online software. Biochem. Mol. Biol. Educ. 39 (2), 145–154.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66.

Wang, C., Zhang, Q., 2017. Exogenous salicylic acid alleviates the toxicity of chlorpyrifos in wheat plants (*Triticum aestivum*). Ecotoxicol. Environ. Saf. 137, 218–224.

Wang, J., Lv, M., Islam, F., Gill, R.A., Yang, C., Ali, B., Yan, G., Zhou, W., 2016. Salicylic acid mediates antioxidant defense system and ABA pathway related gene expression in *Oryza sativa* against quinclorac toxicity. Ecotoxicol. Environ. Saf. 133, 146–156.

Wang, J.T., Jiang, Y.P., Chen, S.C., Xia, X.J., Shi, K., Zhou, Y.H., Yu, Y.L., Yu, J.Q., 2010. The different responses of glutathione-dependent detoxification pathway to fungicide chlorothalonil and carbendazim in tomato leaves. Chemosphere 79, 958–965.

Wang, Z., Jiang, Y., Peng, X., Xu, S., Zhang, H., Gao, J., Xi, Z., 2017. Exogenous 24-epibrassinolide regulates antioxidant and pesticide detoxification systems in grapevine after chlorothalonil treatment. Plant Growth Regul. 81, 455–466.

Xia, X.J., Zhang, Y., Wu, J.X., Wang, J.T., Zhou, Y.H., Shi, K., Yu, Y.L., Yu, J.Q., 2009. Brassinosteroids promote metabolism of pesticides in cucumber. J. Agric. Food Chem. 57, 8406–8413.

Yıldıztekin, M., Kaya, C., Tuna, A.L., Ashraf, M., 2015. Oxidative stress and antioxidative mechanisms in tomato (*Solanum lycopersicum* L.) plants sprayed with different pesticides. Pakistan J. Bot. 47, 717–721.

Yüzbaşıoğlu, E., Dalyan, E., Memon, A., Öz, G., Yüksel, B., 2017. Functional specialization of Arf paralogs in nodules of model legume, *Medicago truncatula*. Plant Growth Regul. 81, 501–510.

Zhang, J.J., Wang, Y.K., Zhou, J.H., Xie, F., Guo, Q.N., Lu, F.F., Jin, S.F., Zhu, H.M., Yang, H., 2018. Reduced phytotoxicity of propazine on wheat, maize and rapeseed by salicylic acid. Ecotoxicol. Environ. Saf. 162, 42–50.