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Proceeding Paper

Sulfur Application Amends Detoxification Processes in Eggplant in Response to Excessive Doses of Thiacloprid †

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Abstract: Sulfur is considered an essential macronutrient during plant growth and is found to play critical roles in xenobiotic detoxifying processes in plants. In the present study, the effects of exogenous sulfur treatment as additional fertilization on detoxifying enzyme activities and plant health indicators were investigated in eggplant (*Solanum melongena*) seedlings exposed to excessive doses of thiacloprid. Eggplant seedlings (cultivar Hansel F1) were irrigated with ammonium sulfate (140 mg L^{-1}) 14 days after sowing in combination with the spraying of a 4-fold recommended dose of thiacloprid. In another treatment, seedlings received ammonium sulfate (70 mg L^{-1}) as a minimum sulfur need in their growth in combination with a mentioned dose of thiacloprid. After 14 days of treatment, leaves were collected to determine their physiological parameters. Based on results, plant health indicators including malondialdehyde, hydrogen peroxide, and electrolyte leakage index were significantly lower in treatments that received additional amounts of sulfur than other ones. Moreover, the activities of glutathione S-transferase, glutathione reductase, glutathione peroxidase, thioredoxin reductase, and cytochrome P450 monooxygenase were higher in them. Our findings suggest that sulfur can decrease membrane permeability and increase cell viability as well as magnify their detoxification capacity which consequently leads to the reduction of oxidative damage in plants. It can be concluded that the sulfur supply in eggplant farms where thiacloprid is intensively used against sap feeder insects should be considered because it can lead to reducing potential risk to the environment by decreasing pesticide damage to host plants as non-target organisms.

Keywords: detoxifying enzymes; eggplant; health indicator; sulfur; thiacloprid

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1. Introduction

Sulfur is an essential macronutrient that is necessary for plants' growth and development. Changes in plants' sulfur content disrupt their tolerance against biotic and abiotic stresses [1]. Moreover, sulfur is the main element of xenobiotic detoxification processes in plants, especially in thiolic compound-based pathways [2]. Glutathione, as a non-proteinous thiol, plays a key role in xenobiotic detoxification processes [3]. Of note, glutathione S-transferase, glutathione reductase, and glutathione peroxidase enzymes are involved in glutathione production, consumption, and detoxification processes [4]. Moreover, thioredoxin reductase acts as a reducer for oxidized thioredoxin [5] and helps the glutathione oxidation-reduction cycle to incorporate with glutathione reductase and glutathione peroxidase [6]. Cytochrome monooxygenase P450 is found to be another important detoxifying enzyme that catalyzes oxidation reactions and changes chemical compounds of pesticides to secondary metabolites [7].

In the present study, we hypothesized that ammonium sulfur can mitigate the phytotoxicity effects of thiacloprid in high doses in eggplant seedlings. To investigate this,

detoxifying enzymes activity was examined in response to the 4-fold recommended dose of thiacloprid in combination with ammonium sulfate. Furthermore, plant health indices including malondialdehyde, hydrogen peroxide, and electrolyte leakage indexes were evaluated to scrutinize plant health status and gain more understanding of oxidative stress situations.

2. Materials and Methods

Eggplant seeds (cultivar Hansel F1) were planted in 15 cm diameter plastic pots of sterilized soil composed of 1:1:2 cocopeat:peat moss:perlite. Plants were grown in a greenhouse under controlled conditions of a L16:D8 photoperiod, with a temperature of 26 ± 2 °C, and 30–40% relative humidity.

The eggplant seedlings were irrigated with ammonium sulfate (140 mg L^{-1}) 14 days after growth. At the same time, they were sprayed with a 4-fold recommended dose (0.4 gr a.i./L) of thiacloprid (Actara® 25 WG, Syngenta, Switzerland). Simultaneously, a group of control plants was irrigated with 70 mg L^{-1} ammonium sulfate (the crucial sulfur content for eggplants growth), in combination with the same dose of thiacloprid spray. Eggplant leaves were collected 14 days after treatment to determine their physiological parameters.

Glutathione S-transferase activity was evaluated using CDNB as a substrate [8]. The absorbance of the reaction mixture that consisted of GSH (5 mM), CDNB (1 mM), and phosphate buffer (50 mM, pH 7) was measured at 340 nm and its activity was calculated using the extinction coefficient equal to 9.6 mM^{-1} cm^{-1}. Glutathione reductase activity was measured according to Homayoonzadeh et al. [8]. An increase in absorbance at 412 nm was observed due to the formation of TNB resulting from the reaction of DTNB (1 mM) with GSH (10 mM) (ε = 14.15 mM^{-1} cm^{-1}), as a measure of enzyme activity. The glutathione peroxidase activity was analyzed based on the Herbette et al. method [9], which was measured by monitoring NADPH oxidation at 340 nm. The reaction mixture contained Tris-HCl (100 mM, pH 7.5), EDTA (5 mM), NADPH (0.2 mM), and GSH (3 mM) (ε = 6220 mM^{-1} cm^{-1}). Thioredoxin reductase activity was measured following the method of Holmgren and Bjornstedt [10]. This enzyme reduces DTNB (8 mM) to TNB by NADPH (0.25 mM) and has an absorbance maximum of 412 nm (ε = 13,600 M^{-1} cm^{-1}). The activity of cytochrome P450 monooxygenase was investigated according to the method described by Guengerich et al. [11]. The reaction mixture consisted of phosphate buffer (100 mM, pH 7.1), EDTA (1 mM), glycerol (20%, *v*/*v*), and sodium cholate (0.5%, *w*/*v*). The optical density of the mixture was recorded at 450 nm (ε = 91 mM^{-1} cm^{-1}).

Malondialdehyde content was quantified using the TBA test as described by Homayoonzadeh et al. [12]. The spectrophotometric measurement was performed at 532 nm using an extinction coefficient equal to 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was estimated according to the method of Homayoonzadeh et al. [13], which is based on KI oxidation by H_2O_2 in an acidic medium. The absorbance of the reaction mixture including phosphate buffer (10 mM, pH 7), KI (1 M), and TCA (0.1%, *w*/*v*) was measured at 390 nm while using a standard curve of hydrogen peroxide. Moreover, the electrolyte leakage index was estimated according to the method of Homayoonzadeh et al. [14]. The electrolyte leakage index was estimated as the percentage of initial to final conductivity after placing leaf discs at 25 °C for 3 h and then boiling at 105 °C for 4 min.

The experiments were designed and carried out in a completely randomized design using five independent biological replicates. An unpaired *t*-test was used to compare the results between treatments. The trait means were compared with the Tukey test at the 0.05 probability level. All analyses were performed in GraphPad Prism version 8.2.0.

3. Results

Results demonstrated that detoxifying enzyme activities were higher in plants treated with the 4-fold recommended dose of thiacloprid combined with ammonium sulfate (140 mg L^{-1}) than in the control. Specific activities of glutathione S-transferase, glu-

tathione reductase, and glutathione peroxidase were significantly higher in treated eggplant seedlings compared to the control in 1.35-, 1.39-, and 1.40-fold, respectively. In addition, the specific activities of thioredoxin reductase and cytochrome P450 monooxygenase experienced a similar trend with 1.60- and 1.62-fold significant increases, respectively, in treated eggplant seedlings (Table 1).

Table 1. Mean (±SE) specific activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPX), thioredoxin reductase (TrxR), and cytochrome P450 monooxygenase (CYT P450) (μmol min^{-1} mg^{-1} protein) in tomato seedlings after exposure to ammonium sulfate (70 mg^{-1} L) + a 4-fold recommended dose of thiacloprid (control) and ammonium sulfate (140 mg^{-1} L) + a 4-fold recommended dose of thiacloprid (treated). Asterisks were used to show statistically significant differences between treated and non-treated plants.

Enzyme	Control	Treated	*t*-Value	*p*-Value
GST	1.320 ± 0.086	1.789 ± 0.127 *	3.546	0.025
GR	1.111 ± 0.072	1.546 ± 0.203 *	2.643	0.048
GPX	1.006 ± 0.058	1.414 ± 0.313 *	3.619	0.034
TrxR	1.239 ± 0.095	1.986 ± 0.441 *	4.217	0.043
CYT P450	0.452 ± 0.032	0.735 ± 0.043 *	4.186	0.024

The analyses of plant health indicators illustrated significantly more accumulation of malondialdehyde (1.66-fold), hydrogen peroxide (1.11 fold), and electrolyte leakage percent (1.23-fold) in control plants than in treated ones (Table 2).

Table 2. Mean (±SE) contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) (μg g^{-1} fresh weight), and electrolyte leakage index (ELI) (%) in tomato seedlings after exposure to ammonium sulfate (70 mg^{-1} L) + a 4-fold recommended dose of thiacloprid (control) and ammonium sulfate (140 mg^{-1} L) + a 4-fold recommended dose of thiacloprid (treated). Asterisks were used to show statistically significant differences between treated and non-treated plants.

Parameter	Control	Treated	*t*-Value	*p*-Value
MDA	0.578 ± 0.037	0.347 ± 0.046 *	2.766	0.014
H_2O_2	8.565 ± 0.407	7.653 ± 0.291 *	4.619	0.025
ELI	19.42 ± 0.924	15.72 ± 0.804 *	3.802	0.037

4. Discussion

This study revealed that the sulfur application in eggplants could induce detoxifying enzyme activity and then mitigate phytotoxicity in response to high doses of thiacloprid as a common insecticide in sap feeder insects' control. Sulfur, as the most abundant element in thiol groups, is essential in redox reactions and also acts as the modulator of detoxifying enzymes' structure [15]. When sulfur is taken up by plants, it is inverted to the amino acids synthesis cycle, especially methionine and cysteine, which act as the intersection of primary metabolism to form S-containing defense compounds. Moreover, excessive sulfur is transported to leaves and stored in vacuoles to make a sulfur reserve for plant metabolism such as detoxification processes [16].

Thiacloprid, as a neonicotinoid insecticide, is metabolized in plants by a sulfoxidation reaction that results in producing the SO metabolite [17]. Thus, it seems that sulfur application enhances eggplant seedlings to overcome the detrimental impacts of thiacloprid high doses.

In this study, the specific activity of cytochrome P450 monooxygenase was increased in eggplant seedlings that received an additional amount of sulfur in combination with a high dose of thiacloprid. It is clear that cytochrome P450 monooxygenase is found to be one of the key detoxifying enzymes in phase one and catalyzes the oxidation reaction to make products of phase one reactions that are more hydrophilic than the parent xenobiotic [18].

Thus, it seems that the additional sulfur application in eggplants results in an activated phase one reaction.

Then, phase one products are detoxified through conjugation with plant metabolites such as glutathione [18]. Glutathione reductase, glutathione peroxidase, and thioredoxin reductase are enzymes that make a glutathione redox system to provide GSH for glutathione S-transferase activity. Glutathione S-transferase catalyzes the conjugation of the GSH with phase one electrophilic compounds [19]. Demonstrating increased content of glutathione reductase, glutathione peroxidase, thioredoxin reductase, and glutathione S-transferase activity, the present study indirectly reflects phase two reactions induction in eggplants.

Based on observed results, plant health indices including malondialdehyde, hydrogen peroxide, and electrolyte leakage index were improved in response to additional sulfur access. Thus, it can be concluded that sulfur application is able to decrease mem-brane permeability as well as increase cell viability.

To put it in a nutshell, sulfur application in eggplant farms where the thiacloprid is used intensively should receive more attention. The sulfur solution can be used to mitigate the deleterious effects of high doses of neonicotinoid insecticides on host plants. This may benefit moderating pesticide potential risk to the environment, especially to the non-target plants.

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