

Exogenous salicylic acid alleviates the accumulation of pesticides and mitigates pesticide-induced oxidative stress in cucumber plants

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Abstract

Salicylic acid (SA) is an important signal molecule, regulating oxidative stress response in plants. In this study, we evaluated the influences of SA (1mg L⁻¹, 10mg L⁻¹ and 50mg L⁻¹) on the accumulation of clothianidin (CLO), dinotefuran (DFN) and difenoconazole (DFZ) (5mg L⁻¹) and pesticide-induced (CLO-10mg L⁻¹, DFN-20 mg L⁻¹, and DFZ-10mg L⁻¹) oxidative stress in cucumber plants. Exogenous SA at 10mg L⁻¹ significantly reduced the half-lives of three pesticides in nutrient solution and prevented the accumulation of pesticides in roots and leaves. And the role of SA in reducing residues was related to the major accumulation sites of pesticides. By calculating the root concentration factor (RCF) and translocation factor (TF), we found that SA at 10mg L⁻¹ reduced the ability of roots to absorb pesticides and enhanced the translocation ability from roots to leaves. Roots exposed to high concentrations of three pesticides could reduce biomass, low chlorophyll content, promote lipid peroxidation, and alter the activities of a range of antioxidant enzymes, respectively. Exogenous SA at low concentrations significantly mitigated these negative effects. Hence, we speculated that application of exogenous SA at 10 mg L⁻¹ could effectively alleviate the accumulation of pesticides and induce stress tolerance in cucumber planting systems.

ABSTRACT:

Salicylic acid (SA) is an important signal molecule, regulating oxidative stress response in plants. In this study, we evaluated the influences of SA (1mg L⁻¹, 10mg L⁻¹ and 50mg L⁻¹) on the accumulation of clothianidin (CLO), dinotefuran (DFN) and difenoconazole (DFZ) (5mg L⁻¹) and pesticide-induced (CLO-10mg L⁻¹, DFN-20 mg L⁻¹, and DFZ-10mg L⁻¹) oxidative stress in cucumber plants. Exogenous SA at 10mg L⁻¹ significantly reduced the half-lives of three pesticides in nutrient solution and prevented the accumulation of pesticides in roots and leaves. And the role of SA in reducing residues was related to the major accumulation sites of pesticides. By calculating the root concentration factor (RCF) and translocation factor (TF), we found that SA at 10mg L⁻¹ reduced the ability of roots to absorb pesticides and enhanced the translocation ability from roots to leaves. Roots exposed to high concentrations of three pesticides could reduce biomass, low chlorophyll content, promote lipid peroxidation, and alter the activities of a range of antioxidant enzymes, respectively. Exogenous SA at low concentrations significantly mitigated these negative effects. Hence, we speculated that application of exogenous SA at 10 mg L⁻¹ could effectively alleviate the accumulation of pesticides and induce stress tolerance in cucumber planting systems.

Keywords: Pesticides, Cucumber, Uptake, Translocation, Salicylic acid, Oxidative stress

Introduction

As a kind of plant protection product, pesticides play an important role in controlling diseases, insects, weeds, and increasing yield of crops (J. Liang & Tang, 2010). However, its misuse has caused a series of environmental safety and food safety problems. For example, some neonicotinoids have raised concerns about their toxicity to bees, which put them at risk of being banned (Jiang et al., 2018; Jiang, Zhang, Lin, Liu, & Mu, 2019). Moreover, overuse of pesticides may cause pesticide pollution in agricultural products as well as soil and water (K. Wang, Wu, & Zhang, 2012). And once the excessive accumulation of pesticides will cause phytotoxicity by injuring the physiological structure of plants (Mahapatra, De, Banerjee, & Roy, 2019). Therefore, it is necessary to investigate the uptake, translocation and accumulation behaviors of systemic pesticides in plants and make effective strategies to reduce pesticide stress.

The active expression of pesticides is closely related to its uptake and translocation behaviors in plants. On the one hand, the uptake and translocation behaviors of pesticides affect the efficacy and duration, which is beneficial to choose the application technology of pesticides (Huang et al., 2019); On the other hand, it is helpful to further understand the dynamic distribution and accumulation of pesticides in various parts of plants and clarify the dynamics residues of pesticides in the environment (Hingmire, Oulkar, Utture, Ahammed Shabeer, & Banerjee, 2015; Utture et al., 2011). Previous studies have reported that the uptake and translocation behaviors of systemic pesticides in plants are related to their physical and chemical properties, such as octanol/water partition coefficient ($\log K_{ow}$), water solubility and molecular weight (Y. Li, Long, et al., 2018; Namiki, Otani, Motoki, Seike, & Iwafune, 2018; Qiu et al., 2016), but also are related to the plant species, growth conditions, growth stage, application method and other factors (Ge et al., 2016; Hwang, Lee, & Kim, 2015; Y. Li, Yang, et al., 2018).

When plants subjected to various biological and abiotic stresses, a large number of reactive oxygen species (ROS) can accumulate in the cells, which can destroy the cell molecular structure and interfere with the physiological process of plants (Noctor, Mhamdi, & Foyer, 2016). For defense, plants have a variety of complex enzymatic and non-enzymatic antioxidant systems that they can resist oxidative stress including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and other enzymes and non-enzymatic substances they can remove different types ROS (Safari, Akramian, Salehi-Arjmand, & Khadivi, 2019). As a result of human production activities, many wastes remained in the environment for a long time, which can produce stress responses to plants such as heavy metals, pesticides, and engineered nanomaterials (Lian et al., 2020; Rodriguez-Serrano et al., 2006; Safari et al., 2019). Salicylic acid (SA) is a well-known endogenous signaling molecule related to plants stress resistance (Q. J. Wang et al., 2016; X. Y. Zhao et al., 2020; X. Y. Zhao et al., 2019). When plants are in adverse environments, SA can regulate various physiological and biochemical processes of plants to alleviate stress (W. Y. Song, Peng, Shao, Shao, & Yang, 2014; Q. Wang et al., 2013; Xu, Fan, Dong, Kong, & Bai, 2014). It is worth noting that SA can inhibit the accumulation of pesticides in plants, which is vital for reducing pesticide residues and ensuring the safety of agricultural products (Kaya & Yigit, 2014; C. Wang & Zhang, 2017). For phytotoxicity caused by pesticides, most previous studies focused on exogenous SA to reduce the toxicity of herbicides on nontarget plants (Akbulut, Yigit, Kaya, & Aktas, 2018; Kaya & Doganlar, 2016). However, the regulatory functions of SA under commonly used systemic pesticide stress and the effects of exogenous SA on the accumulation of pesticides in various parts of the plants were rarely investigated.

Cucumber (*Cucumis sativus L.*) is a widely cultivated cash crop in China. In the present study, the three pesticides, clothianidin (CLO), dinotefuran (DFN) and difenoconazole (DFZ) were selected as the representatives. CLO is a neonicotinoid insecticide with excellent systemic and osmotic activity that can be used as a soil, spray and seed treatment, for control of sucking and chewing insects (Jeschke, Nauen, Schindler, & Elbert, 2011). DFN is a systemic neonicotinoid insecticide with translaminar activity, and it can be applied on foliage, soil and nursery boxes by spray, drench, broadcast and pricking-in-hole treatments (Corbel, Duchon, Zaim, & Hougard, 2004). DFZ is a systemic fungicide of sterol demethylation inhibitors with a novel broad-range activity protecting the yield and crop quality by foliar application or seed treatment (Dong et al., 2013). The three pesticides all could be absorbed by plant roots, which can be used for soil treatment, but there are significant differences in water solubility and $\log K_{ow}$ of the three pesticides. The physical and chemical properties of these three pesticides are shown in **Table S1**. We investigated the

effects of exogenous SA on the uptake, translocation and accumulation behaviors of these three pesticides in cucumber plants by measuring the concentration of pesticides in various parts of the cucumber plants and calculating the root concentration factor (RCF) and translocation factor (TF) values. Moreover, the effects of SA on oxidative stress induced by these three pesticides to the cucumber plants were also studied by measuring biomass, photosynthetic pigment content, antioxidants content and antioxidant enzyme activities. This study provides a new perspective to understand the uptake and translocation behaviors of pesticides and pesticide pollution in plants.

2. Experimental Section

2.1. Chemicals and materials

Certified pesticide standards of CLO (97%), DFN (98%), DFZ (97%), and standards of SA (99.5 %) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China)

Reagents: Sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Sinopharm Chemical Reagent (Beijing, China). PSA (40-63 μm) was obtained from Tianjin Bonna-Agela Technology Co., Ltd. (Tianjin, China). Acetonitrile (HPLC grade) was obtained from Fisher Chemicals (Fair Lawn, NJ, USA).

2.2. Experiment design

Plant cultivation: The seeds of cucumber were provided by the Xintai Yuyuan Seed Industry Co., Ltd (Shandong, China). Cucumber seeds were wrapped in wet gauze and placed in a petri dish to accelerate germination. When the seeds germinating, we transferred the seeds into a seedling tray and put one seed into each hole. The temperature of the greenhouse was 25 in the day and 20 at night. After growth 21 days, the young plants were used in pesticides exposure trials.

2.2.1. Pesticide exposure experiments-uptake and translocation

Hydroponic experiments: The concentration of three pesticides was set at 5 mg L⁻¹, and the concentrations of SA were set at 1 mg L⁻¹, 10 mg L⁻¹, and 50 mg L⁻¹, respectively. CLO, DFN or DFZ was first dissolved in acetonitrile to form the stock standard solution, respectively. And then the standard stock solutions were diluted to the required concentration by ultrapure water. SA was dissolved and diluted with 20 % methanol-water.

After germination, the cucumber seedlings were removed from the seedling tray, and the roots were washed by deionized water before transferred to hydroponic cultivation buckets with 0.25 L modified Hoagland nutrient solution in the greenhouse. The composition of the nutrient solution can be found in **Table S2**. The growth conditions were the same as the plant cultivation conditions. And a total of 12 treatments were set in the experiment with 27 cucumber seedlings per treatment, the processing information for each test can be found in **Table 1**. The hydroponic cultivation buckets were covered with aluminum foil to prevent algae growth and water evaporation. The nutrient solution was supplemented to the initial volume at 11 am every day.

Control experiments: Two control groups were included: nutrient solution with cucumber seedlings without pesticides was as the treatment of C1 group; and nutrient solution with CLO, DFN and DFZ but without cucumber seedlings were as the treatments of CLO-C2, DFN-C2 and DFZ-C2 groups, respectively.

Sample collection: Cucumber seedlings were grown for 28 days, nutrient solution and plant samples were collected at 0.25, 1, 3, 5, 7, 10, 14, 21, 28 days after the transfer. The plants were divided into roots, stems and leaves for pesticide analysis. At each sampling time, three replicate samples were randomly taken. All samples were stored at -20 until analysis.

2.2.2. Pesticide exposure experiments- oxidative stress in cucumber plants

Single root exposure of CLO, DFN and DFZ: Hydroponic experiment conditions and plant growth conditions were the same as the “uptake and translocation.” Given the tendency for repeated application

of pesticides, a series of preliminary experiments using various concentrations of pesticides were carried out to evaluate the cucumber growth parameters. When the root application of CLO at 10mg L⁻¹, DFN at 20mg L⁻¹ and DFZ at 10mg L⁻¹, the fresh biomass of cucumber plants significantly decreased, respectively. We estimated that at these concentrations, the three pesticides caused significant stress responses, respectively (**Fig S1**). Therefore, we set the concentration of CLO at 10 mg L⁻¹, DFN at 20 mg L⁻¹ and DFZ at 10 mg L⁻¹, and with SA supplementation at 1mg L⁻¹, 10mg L⁻¹ and 50mg L⁻¹, the root, stem and leaf samples were collected after 10 days of growth, respectively. A total of 12 treatments were set in this part of experiment. The plants were grown in blank nutrient solution as a control group. After the root, stem and leaf samples were weighed, the root samples were stored at -80 until measured.

2.3. Determination of CLO, DFN and DFZ in cucumber plants

For “**pesticide exposure experiments- uptake and translocation**” part, the contents of pesticides in plant tissue samples were analyzed. The QuEChERS method was used to prepare nutrient solution and plant tissue samples (Ge et al., 2017). The specific sample preparation steps and instrumental analysis conditions are shown in the **supplementary materials**.

The recoveries of three pesticides in nutrient solution and plant tissues were used to verify the feasibility of the analytical method. External standard method was used for quantitative analysis. The results of quality assurance and quality control are shown in the **supplementary materials**.

2.4. Determination of antioxidant enzyme activity

The extraction of enzyme liquid was carried out according to the method described by Andrews et al. (2005). The method of SOD determination referred to Farouk and Al-Amri (2019). APX activity was determined by the reduction value of absorbance at 290 nm per unit time using the method of Kaya and Doganlar (2016). GST activity was tested according to Habig, Pabst, and Jakoby (1974). The specific measurement steps are shown in the **supplementary materials**.

2.5. Determination of the total chlorophyll and H₂O₂ content in cucumber plants

The pigment was extracted by methanol extraction following the method of L. Zhao et al. (2019). The specific measurement steps are shown in the **supplementary materials**. The content of H₂O₂ in cucumber roots was determined by H₂O₂ kits.

2.6. Determination of the malondialdehyde (MDA) content

The level of lipid peroxidation was evaluated by the determination of malondialdehyde (MDA) content based on the method of Heath and Packer (1968). The specific measurement steps are shown in the **supplementary materials**.

2.7. Determination of proline content

The content of proline in plant roots was determined according to the method of Bates, Waldren, and Teare (1973). The specific measurement steps are shown in the **supplementary materials**.

2.8. Data processing and statistical analysis

In order to compare the difference in the behaviors of three pesticides between supplementing different concentrations of SA and without SA, the root concentration factor (RCF) and translocation factor (TF) were calculated. The root concentration factor (RCF) was used to indicate the ability to absorb target compounds by plant roots in nutrient solution, RCF > 1 indicates that the compound is easily absorbed by the roots, and calculated as follows (Qiu et al., 2016): (Eqn 1)

$$RCF = \frac{\text{concentration in root (mg/kg)}}{\text{concentration in nutrient solution (mg/L)}}$$

Translocation factors (TF) was used to indicate the ability of leaves and stems to transport target compounds from roots, TF > 1 indicates that the excellent capacity for stems and leaves to translocate the pesticides from roots, and calculated as follows (Ge et al., 2016): (Eqn 2 and Eqn 3)

$$TF_{\text{stem}} = \frac{\text{concentration in stem (mg/kg)}}{\text{concentration in root (mg/kg)}}$$

$$TF_{\text{leaf}} = \frac{\text{concentration in leaf (mg/kg)}}{\text{concentration in root (mg/kg)}}$$

The concentration of pesticides in nutrient solution was calculated using the first-order equation $C_t = C_0 e^{-kt}$. The half-life was calculated by the equation $t_{1/2} = \ln 2/k$, where C_t is the concentration of target pesticides at time t (days), C_0 is the concentration of target pesticides at the initial time, and k is the first-order rate constant (day^{-1}).

An independent sample t-test was used to compare the differences between different treatments and pesticides. All statistical analyses were statistically significant at the 0.05 level.

3. Results

3.1 Dissipation dynamics of pesticides in nutrient solution.

The dissipation dynamic of CLO, DFN and DFZ in nutrient solution during the experimental period were shown in **Fig 1 A**, and the half-lives ($t_{1/2}$) of the pesticides in nutrient solution were shown in **Table 2**. None of the target pesticides were detected in the control group C1. The three pesticides showed the same dissipation trend, and all of them followed the first-order kinetics. Approximately 80-90% of pesticides were degraded in nutrient solution during the 28-day experimental period, and the dissipation half-lives of CLO, DFN and DFZ were 6.3, 11.5 and 13.8 days, respectively. However, in unplanted nutrient solution, the dissipation half-lives of CLO, DFN and DFZ were 12.4, 15.8 and 18.2 days, respectively.

The effects of exogenous SA on the dissipation dynamics of pesticides are shown in **Fig 1 B, C, D** and the half-lives ($t_{1/2}$) are shown in **Table 2**. For CLO, the half-lives decreased by 8% and 35% with supplementing SA at 1 mg L^{-1} and 10 mg L^{-1} , respectively, compared with the without SA treatments. For DFN, the half-lives decreased by 14% and 40% with supplementing SA at 1 mg L^{-1} and 10 mg L^{-1} , respectively, compared with the without SA treatments. For DFZ, the half-lives decreased by 8%, 61% and 28% with supplementing SA at 1 mg L^{-1} , 10 mg L^{-1} and 50 mg L^{-1} , respectively, compared with the without SA treatments.

3.2 Dynamic distribution, translocation and accumulation of pesticides in cucumber plants

The dynamic distribution and accumulation of CLO, DFN and DFZ in cucumber plants are shown in **Fig. 2A** under hydroponic experiments. For CLO and DFN, the concentrations of pesticides in leaves were much higher than those in roots and stems, indicating that CLO and DFN were mainly accumulated in leaves, and these have great upward conductivity, respectively. For DFZ, the concentration of pesticide in roots was much higher than that in stems and leaves, indicating that DFZ could be mainly accumulated in roots, and its upward conductivity was poor.

The effects of exogenous SA on the distribution of three pesticides in the roots, stems and leaves of cucumber plants are shown in **Fig. 2 B, C, D**. From the charts, it can be seen that with SA supplementation the concentrations of three pesticides in roots, stems and leaves of cucumber plants basically showed a decreasing trend during the 28 days of growth. To assess the overall effects of SA on the distribution of three pesticides in different parts of cucumber plants, the mean residues of three pesticides in different parts of cucumber plants after 28 days of growth were calculated, respectively (**Fig. 3**). With SA supplementation at 1 mg L^{-1} , the concentration of CLO and DFN in roots and leaves significantly decreased by 27%, 18% and 23%, 19%, respectively, compared with the treatments of CLO and DFN alone; with SA supplementation at 10 mg L^{-1} , the concentration of CLO and DFN in roots and leaves significantly decreased by 56%, 28% and 46%, 25%, respectively, compared with the treatments of CLO and DFN alone. With SA supplementation at 50 mg L^{-1} , the concentration of CLO in roots and leaves significantly decreased by 23% and 20%, respectively, compared with the treatment of CLO alone. For DFZ, the concentration in roots significantly decreased by 11% and 40% after addition of SA at 1 mg L^{-1} and 10 mg L^{-1} , respectively, compared with the treatment of DFZ alone.

3.3 Root concentration factor and translocation concentration factor

To further explore the difference in the uptake and translocation of three pesticides with SA or without SA in the cucumber plants, the root concentration factor (RCF) and translocation factor (TF) were analyzed. The average RCF values of three pesticides are summarized in **Table 3**, and the dynamic RCF values of three pesticides during 28-days of exposure are shown in **Table S5**. The RCF value ranged from 0.15 to 3.12 for CLO, from 0.2 to 1.11 for DFN, from 26.10 to 148.30 for DFZ during 28-days of exposure. The average RCF values for CLO, DFN, DFZ were 1.07, 0.64 and 52.19, respectively. Whereas with SA supplementing, the average RCF values for the three pesticides were all decreased. For CLO, the average RCF values were significantly decreased by 49%, 35% and 26% with addition of SA at 1mg L⁻¹, 10 mg L⁻¹ and 50 mg L⁻¹, respectively, compared with the treatment of CLO alone. For DFN, the average RCF values were decreased by 6.25% and 11% with addition of SA at 1 mg L⁻¹ and 10mg L⁻¹, respectively, compared with the treatment of DFN alone. For DFZ, the average RCF value was significantly decreased by 16% with addition of SA at 10mg⁻¹ compared with the treatment of DFZ alone.

The average TF values of three pesticides are summarized in **Table 4**, and the dynamic TF values of three pesticides during 28-days of exposure are shown in **Table S6** and **S7**. The average TF_{stem} and TF_{leaf} of CLO, DFN, DFZ were 0.98, 0.69, 0.02 and 8.81, 8.03, 0.005, respectively. In addition to CLO, no significant differences were found for TF_{stem} between the treatment groups with SA supplementation and the groups without SA supplementation, which again demonstrated that pesticides migrated up via the transpiration stream and eventually accumulated in the leaves, and the stems were just a bridge for pesticides upward conduction and reached equilibrium with transpiration stream (Volpe, Marani, Albertson, & Katul, 2013). For CLO, the TF_{leaf} values were significantly increased by 102%, 130% and 31% after adding SA at 1 mg L⁻¹, 10 mg L⁻¹ and 50 mg L⁻¹, respectively, compared with the treatment of CLO alone. For DFN, the TF_{leaf} values were significantly increased by 77% and 43% after adding SA at 10 mg L⁻¹ and 50 mg L⁻¹, respectively, compared with the treatment of DFN alone. For DFZ, the TF_{leaf} values were significantly increased by 160% and 120% after adding SA at 10 mg L⁻¹ and 50 mg L⁻¹, respectively, compared with the treatment of DFZ alone.

3.4 Effects of exogenous salicylic acid on oxidative stress in cucumber plants

3.4.1 Fresh biomass and total chlorophyll content

Single root exposure of CLO (10 mg L⁻¹), DFN (20 mg L⁻¹) and DFZ (10 mg L⁻¹) significantly affected the fresh biomass of plants and total chlorophyll content (**Fig. 4 A** and **B**). Compared with the controls, for CLO, DFN and DFZ, the values of fresh biomass of plants decreased by 15.4 %, 24.5 % and 53.8% after single root exposure, respectively (**Fig. 4 A**); the values of total chlorophyll content decreased by 26.2 %, 28 %, 31 %, respectively (**Fig. 4 B**). As shown in **Fig. 4 A** and **B**, among the treatments with SA supplementation, the fresh biomass of plants increased compared with the without SA treatments. With SA supplementation at 1mg L⁻¹, the fresh biomass of plants increased by 25.5 %, 1.18 %, 66.7 % compared to the single root exposure of CLO, DFN and DFZ treatments, respectively. With SA supplementation at 10 mg L⁻¹, the fresh biomass of plants significantly increased by 33.8 %, 18.9 %, 74.6 % compared to the single root exposure of CLO, DFN and DFZ treatments, respectively. (**Fig. 4 A**). Similarly, compared with the treatments with CLO, DFN or DFZ alone, SA at 1mg L⁻¹ and 10 mg L⁻¹ significantly improved the total chlorophyll content, which increased by 47. 1%, 124 %, 144 % and 41.1 %, 150 %, 113 % (**Fig. 4 B**).

3.4.2 ROS generation and proline content

The values of H₂O₂ content and proline content are shown in **Fig. 4 C, D**. From the data in **Fig. 4 C**, it could be seen that after single root exposure to CLO, DFN and DFZ, the values of H₂O₂ content increased by 313 %, 300 % and 424 %, respectively, compared with the controls. Also, the low concentration (1mg L⁻¹ and 10 mg L⁻¹) of SA have significant effects on reducing the H₂O₂ content of roots. With SA supplementation at 1mg L⁻¹, the H₂O₂ content in roots significantly decreased by 74%, 62% and 52%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments. With SA supplementation at 10mg L⁻¹, the H₂O₂ content in roots significantly decreased by 78%, 82% and 62%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments (**Fig. 4 C**).

In the present study, it was found that the three pesticides all enhanced accumulation of proline and more increased when the SA at 1mg L⁻¹ and 10 mg L⁻¹ were supplemented. With SA supplementation at 1mg L⁻¹, the proline content in roots significantly increased by 35%, 29% and 35%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments. With SA supplementation at 10mg L⁻¹, the proline content in roots significantly increased by 44%, 52% and 83%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments (**Fig. 4 D**).

3.4.3. Antioxidant enzymes assays and lipid peroxidation

The activities of SOD, APX and GST in roots were markedly affected under CLO, DFN and DFZ treatments compared with the controls, respectively. The SOD activity increased by 150 %, 191 % and 210 % at the single root exposure of CLO, DFN, and DFZ treatments, respectively, compared with the controls, while APX activity was significantly inhibited (**Fig. 4E, F**). Similarly, single root exposure to CLO, DFN, and DFZ also led to increasing GST activity, respectively (**Fig. 4 G**). From the dates in **Fig. 4 E, F, G**, exogenous SA also could alter the antioxidant enzyme activities. The SOD activity significantly decreased in the roots of cucumber plants treated with 1mg L⁻¹ and 10 mg L⁻¹ SA compared with the treatments with pesticides alone, and it was similar to the control groups (**Fig. 4 E**). Similarly, SA at 1 mg L⁻¹ and 10 mg L⁻¹ also increased the APX activity, suggesting that the phytotoxicity caused by three pesticides were alleviated, respectively (**Fig. 4 F**). With supplementing exogenous SA (1mg L⁻¹ and 10mg L⁻¹), GST activity in roots was elevated, which was beneficial to promoting pesticide metabolism and alleviating phytotoxicity (Han, Mhamdi, Chaouch, & Noctor, 2013; Kaya & Doganlar, 2016).

As for single root of CLO, DFN, and DFZ exposure, MDA contents in cucumber roots significantly increased by 40%, 59% and 67%, respectively, compared with the unstressed plants (**Fig. 4H**). With SA supplementation at 1mg L⁻¹, the MDA content in roots significantly decreased by 56%, 57% and 37%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments. With SA supplementation at 10mg L⁻¹, the MDA content in roots significantly decreased by 61%, 61% and 56%, respectively, compared with the single root exposure of CLO, DFN and DFZ treatments.

4. Discussion

4.1. The dissipation and accumulation behaviors of pesticides

In this study, the half-lives of individual pesticides in planted nutrient solution were significantly shorter than that in unplanted nutrient solution. Plant intake was an important factor, and previous studies have shown that the half-lives of pesticides were relevant to the factors as the cultivation modes of plants, the existence of plants, and soil types (Lockridge, Verdier, & Schopfer, 2019; Šudoma et al., 2019). Lv et al. (2016) found that during the experimental period, the pH level of the unplanted systems keep constant, but the pH of the planted systems significantly decreased. Plants can secrete secondary metabolites during the growth period, including low-molecular-weight substances (e.g., organic acids, amino acids, and fatty acids) and high-molecular-weight substances (e.g., polysaccharides and proteins) (L. Zhao et al., 2016). Substances that produced by plant roots metabolism might be responsible for changes in pH of nutrient solutions. Therefore, the pH level of nutrient solution might be one reason for the change of half-lives.

The CLO and DFN were mainly accumulated in leaves, and the DFZ was mainly accumulated in roots, which could be attributed to the water solubility and partition coefficient octanol/water (logK_{ow}) of the pesticides and differences in physiological structure between organs (Ge et al., 2016; Qiu et al., 2016). This finding was in agreement with the previous result, which showed that the concentrations of imidacloprid and thiamethoxam detected in rice leaves were higher than roots, but for difenoconazole, the concentration detected in rice roots was higher than leaves (Ge et al., 2017).

The RCF values of DFZ was highest, followed by CLO and DFZ. The larger the logK_{ow} of pesticide, the easier it is to accumulate in the organs with more lipids. The order of lipid content of root, stem and leaf was root > stem > leaf (Qiu et al., 2016). Thus, DFZ was more easily accumulated in the roots of cucumber plants. Previous studies have shown that the RCF values increased with increasing logK_{ow} values

of pesticides, which was in accord with our results (Namiki et al., 2018; Qiu et al., 2016). Moreover, among the three pesticides, the highest TF value was the CLO followed in declining order by DFN and DFZ. The higher the solubility of pesticide, the easier it is to accumulate in the leaves (Qiu et al., 2016). The $\log K_{ow}$ of DFZ was higher, while water solubility was poorer than CLO and DFN. Thus, the TF_{stem} and TF_{leaf} of DFZ were all lower than CLO and DFN, which again indicating that DFZ mainly accumulated in roots, and its upward migration ability was weak. The $\log K_{ow}$ of CLO and DFN was comparative, and the TF_{stem} and TF_{leaf} was similar, respectively. For CLO and DFN, TF_{leaf} were much higher than TF_{stem} , indicating that both had stronger ability to migrate to the leaves, which could possibly be related to the higher water content in the leaves.

4.2. The effect of exogenous SA on the behaviors of pesticides

The effect of exogenous SA on pesticides half-lives depended on its concentrations. In the present study, when the exogenous SA was 10 mg L⁻¹, it had the greatest impacts on the half-lives of the three pesticides. In addition, among the three pesticides, addition of SA at three concentrations all reduced the half-lives of DFZ in nutrient solution. And with supplementing SA at 10 mg L⁻¹, the half-lives were reduced by more than half compared with the without SA treatments, which indicating that exogenous SA has the strongest effects on promoting the degradation of DFZ in nutrient solution. This might be related to the physical and chemical properties of pesticides. Importantly, the lower half-lives of individual pesticides mean that they keep shorter time in the environment, which can alleviate environmental pressure (Mohapatra et al., 2019).

Addition of SA at three concentrations all could block the accumulation of three pesticides in various parts of cucumber plants except for DFZ with SA supplementation at 50mg L⁻¹. When the SA was 1mg L⁻¹ and 10mg L⁻¹, the inhibition of accumulation of the three pesticides was the most significant. Therefore, exogenous SA could prevent the accumulation of pesticides in plants, but had a dose effect. Previous study had shown that SA could reduce residues by promoting the intracellular herbicides catabolism (Y. C. Lu, Zhang, & Yang, 2015). Noteworthy, addition of SA at 1mg L⁻¹ and 10mg L⁻¹ significantly reduced the concentrations of CLO and DFN in roots and leaves, respectively; but for DFZ, the concentration was significantly reduced in roots. For these phenomena, we speculated that it might be related to the accumulation behaviors of three pesticides in various parts of cucumber plants. And the effects of SA suppressing the pesticide entry into plant tissues might be related to the concentrations of pesticides (Kaya & Yigit, 2014). Exogenous SA played the strongest role in promoting the degradation of pesticides in the sites with high concentration of pesticides. This idea could be proved again by showing that when SA was 10mg L⁻¹, the concentration of DFZ in the root was 18.70mg kg⁻¹, equivalent to 60% of the without SA treatments, which also indicated that exogenous SA had the greatest effect on reducing the DFZ residue in cucumber plants. In summary, the role of SA in reducing pesticide residues in a particular plant might be related to the major sites of accumulation, which provided a basis for selecting the application site of SA on the plants.

Addition of SA at 10 mg L⁻¹, the RCF values of three pesticides all decreased, indicating that SA can inhibit pesticide uptake by roots and impaired the ability to accumulate pesticides. The TF_{leaf} values of three pesticides all improved after application of SA, indicating that the pesticides moved faster in cucumber plants than the without SA treatments. In our study, we found that SA can improve the upward migration ability of three pesticides. It was difficult to explain this result, but it might be related to the change of pesticide behavior and pesticide metabolism level in plants after the addition of SA (Kong, Dong, Xu, Liu, & Bai, 2014). This finding also accorded with the earlier research, which showed that with SA supplementation, the TF value of isoproturon in wheat plants was higher than the without SA treatments (Yi Chen Lu et al., 2014).

4.3. The role of exogenous SA in pesticide-induced oxidative stress in cucumber plants

Previous studies indicated that excessive long-term application of pesticides could produce phytotoxicity once it exceeds the limits of non-target plant, which would affect plant growth by decreasing biomass and chlorophyll content and so on (Kaya & Yigit, 2014; C. Wang & Zhang, 2017). The roots system could absorb and transport water and nutrients to ensure the growth of the plants (Yu et al., 2020). When the plant roots

are exposed to excessive pesticides, the physiological structure of the roots can be damaged, resulting in slow growth (C. Wang & Zhang, 2017). Notably, compared with CLO and DFN, the fresh biomass of plants decreased by more than half when single root exposure of DFZ, which possibly due to the more residue of DFZ in the roots. After single root exposure of DFZ, the root morphology of cucumber plants changed compared with the control groups, which mainly showed that the lateral root decreased, the root volume increased, the diameter increased, and the root length became shorter (**Fig. S2**). In this study, the fresh biomass and total chlorophyll content significantly increased with SA supplementation at 1mg L^{-1} and 10mg L^{-1} compared with the treatments of pesticides alone, respectively. Previous studies have demonstrated that low concentration of SA promoted plants growth, while high concentration of SA inhibited plants growth and low concentration of SA also could increase chlorophyll content (Kong, Dong, Zhang, et al., 2014; Pasternak et al., 2019; Y. Song et al., 2016).

Single root exposure of CLO, DFN and DFZ, the H_2O_2 content in cucumber roots significantly increased, indicating that the ROS balance in the plants was out of balance and might have a toxic effect on the plants (J. Xu et al., 2014). Similarly, it was also reported that some herbicides markedly increased the H_2O_2 content in non-target plants (Q. Li et al., 2019; Spormann, Soares, & Fidalgo, 2019). However, the ROS imbalance of cucumber plants was alleviated by exogenous SA (1mg L^{-1} and 10mg L^{-1}). Similarly, the research showed that application of 5 mg L^{-1} exogenous SA significantly eased the phytotoxicity of wheat inducing by isoproturon and also reduced the abundance of O_2^- and H_2O_2 (L. Liang, Lu, & Yang, 2012). Consistent with our results, exogenous SA had also been reported to increase the content of free proline in a variety of stressed plants (Q. Li et al., 2019; S. Liu, Dong, Xu, & Kong, 2013; Safari et al., 2019). Proline is a small molecular organic solvent, which is widely found in plants in a free state. It can regulate cell osmotic pressure, stabilize sub-cellular structures such as membranes and proteins, remove reactive oxygen species and other (Ashraf & Foolad, 2007). It also has been proved that free proline can remove reactive oxygen species caused by heavy metal Hg contamination (F. Wang, Zeng, Sun, & Zhu, 2008). This fact was in accord with our study that as low concentration of SA-induced the increasing level of free proline coincided with the decreasing trend of H_2O_2 content in pesticides-stressed plants. Hence, increasing proline accumulation appeared to be an adaptive response which enhanced plants resistance to the pesticide stress.

Adverse environmental conditions stimulated the plants to induce oxidative stress and increased the activity of antioxidant enzymes to withstand stress (Akbulut et al., 2018; Lian et al., 2020). Exogenous SA at low concentrations also regulated antioxidant enzyme activities to resist stress (Kong, Dong, Xu, et al., 2014). In this study, after addition of SA at 1mg L^{-1} and 10mg L^{-1} , the activities of APX and GST increased compared with the treatments of pesticides alone, respectively, and the SOD activity returned to the control level. Malondialdehyde (MDA) is the primary substance produced during lipid peroxidation, serving as an indicator of oxidative damage of membrane lipids. Under pesticide stress, cucumber plants treated with exogenous SA had lower MDA content than untreated ones. These results indicated the critical role of SA in plants resistance to stress and reduction of oxidative damage. This finding was further verified by the positive role of exogenous SA on the antioxidant enzyme activities and proline content. It has also been reported that exogenous SA can lower MDA content in other plants under stress of pesticides, heavy metals and nanomaterials (Safari et al., 2019; C. Wang & Zhang, 2017).

4.4. Mechanisms of alleviates phytotoxicity by exogenous SA

In our study, three concentrations of exogenous SA were supplemented to assess the effects of SA on pesticides-induced oxidative stress in cucumber plants. Based on the above results, we found that low concentration of SA (1mg L^{-1} and 10mg L^{-1}) had significant effects on relieving oxidative stress of cucumber plants induced by the three pesticides. Whereas, high concentration of exogenous SA (50 mg L^{-1}) had little effect on alleviating oxidative stress of cucumber plants. Therefore, the effects of exogenous SA on reducing oxidative stress depends on its concentrations. In accordance with the present results, previous studies have demonstrated that low concentration of SA can improve the tolerance of plants to adversity. In contrast, high concentration of SA induced a higher level of oxidative stress, leading to a decrease in tolerance to environmental stress (Bai, Dong, Kong, Xu, & Liu, 2014; Z. Liu, Ding, Wang, Ye, & Zhu, 2016; Pasternak

et al., 2019). In the previous part of this study, we found that exogenous SA could impair the absorption capacity of roots to pesticides and thus reduce the accumulation of pesticides in cucumber plants. These results matched those observed in earlier studies. SA could reduce the accumulation of chlorpyrifos in wheat plants by decreasing uptake and translocation abilities of chlorpyrifos (C. Wang & Zhang, 2017). SA could reduce the accumulation of isoproturon in wheat by promoting its degradation (L. Liang et al., 2012; Yi Chen Lu et al., 2014). SA could reduce the residual of flurochloridone in sunflower leaves by the foliar treatment (Kaya & Yigit, 2014). SA was able to reduce the concentration of thiamethoxam, hymexazol and chlorantraniliprole in roots and leaves of cucumber plants and the uptake ability of roots, the bioconcentration ability of plants, and the translocation ability from roots to leaves were weakened (T. Liu et al., 2020). Taken together, we speculated that SA mitigated phytotoxicity by weakening the uptake ability of pesticides in plants and enhancing the capacity of antioxidative stress. However, the exact mechanism of SA on plant stress resistance needs to be further illuminated.

5. Conclusions

This study showed that exogenous SA (10mg L^{-1}) could promote the degradation of three pesticides in nutrient solution and suppress the accumulation of three pesticides in roots and leaves. Noteworthy, the role of SA in reducing pesticide residues was related to the accumulation sites of cucumber plants. Through the calculation of root concentration factor (RCF) and translocation factor (TF), it was found that exogenous SA (10mg L^{-1}) significantly weakened the absorption capacity of roots to the three pesticides. Whereas, the translocation capacity of the three pesticides in cucumber plants were enhanced with three concentrations of SA supplementation. According to our study, single root exposure of three pesticides all caused phytotoxicity, which showed that the plants biomass decreased, chlorophyll synthesis was inhibited, proline accumulation increased, lipid peroxidation was promoted, and the activities of antioxidant enzyme (SOD, APX and GST) was destroyed, respectively. However, these adverse effects could be alleviated by supplementing with appropriate concentration of exogenous SA (1mg L^{-1} and 10mg L^{-1}). This present study provided evidence that SA could reduce the accumulation of pesticides in plants and provided a basis for the application of exogenous SA in agricultural production. However, the exact interaction mechanism between pesticides and SA in plants needs to be further elucidated.

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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. *Ecotoxicology and Environmental Safety*, *148*, 901-909. doi:10.1016/j.ecoenv.2017.11.053
- Andrews, C. J., Cummins, I., Skipsey, M., Grundy, N. M., Jepson, I., Townson, J., & Edwards, R. (2005). Purification and characterisation of a family of glutathione transferases with roles in herbicide detoxification in soybean (*Glycine max* L.); selective enhancement by herbicides and herbicide safeners. *Pesticide Biochemistry and Physiology*, *82* (3), 205-219. doi:10.1016/j.pestbp.2004.11.009
- Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59* (2), 206-216. doi:10.1016/j.envexpbot.2005.12.006
- Bai, X., Dong, Y., Kong, J., u, L., & Liu, S. (2014). Effects of application of salicylic acid alleviates cadmium toxicity in perennial ryegrass. *Plant Growth Regulation*, *75* (3), 695-706. doi:10.1007/s10725-014-9971-3
- Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid Determination of Free Proline for Water-Stress Studies. *plant soil*, *39* (1), 205-207.

- Corbel, V., Duchon, S., Zaim, M., & Hougard, J. M. (2004). Dinotefuran: a potential neonicotinoid insecticide against resistant mosquitoes. *J Med Entomol*, *41* (4), 712-717. doi:10.1603/0022-2585-41.4.712
- Dong, F., Li, J., Chankvetadze, B., Cheng, Y., Xu, J., Liu, X., . . . Zheng, Y. (2013). Chiral triazole fungicide difenoconazole: absolute stereochemistry, stereoselective bioactivity, aquatic toxicity, and environmental behavior in vegetables and soil. *Environ Sci Technol*, *47* (7), 3386-3394. doi:10.1021/es304982m
- Farouk, S., & Al-Amri, S. M. (2019). Exogenous melatonin-mediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. *Ecotoxicol Environ Saf*, *180* , 333-347. doi:10.1016/j.ecoenv.2019.05.021
- Ge, J., Cui, K., Yan, H., Li, Y., Chai, Y., Liu, X., . . . Yu, X. (2017). Uptake and translocation of imidacloprid, thiamethoxam and difenoconazole in rice plants. *Environ Pollut*, *226* , 479-485. doi:10.1016/j.envpol.2017.04.043
- Ge, J., Lu, M., Wang, D., Zhang, Z., Liu, X., & Yu, X. (2016). Dissipation and distribution of chlorpyrifos in selected vegetables through foliage and root uptake. *Chemosphere*, *144* , 201-206. doi:10.1016/j.chemosphere.2015.08.072
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. J. J. b. c. (1974). Glutathione S-transferase, The first enzymatic step in mercapturic acid formation. *249* (22), 7130-7139.
- Han, Y., Mhamdi, A., Chaouch, S., & Noctor, G. (2013). Regulation of basal and oxidative stress-triggered jasmonic acid-related gene expression by glutathione. *Plant Cell Environ*, *36* (6), 1135-1146. doi:10.1111/pce.12048
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. II. Role of electron transfer. *Arch Biochem Biophys*, *125* (3), 850-857. doi:10.1016/0003-9861(68)90523-7
- Hingmire, S., Oulkar, D. P., Utture, S. C., Ahammed Shabeer, T. P., & Banerjee, K. (2015). Residue analysis of fipronil and difenoconazole in okra by liquid chromatography tandem mass spectrometry and their food safety evaluation. *Food Chem*, *176* , 145-151. doi:10.1016/j.foodchem.2014.12.049
- Huang, X. P., Luo, J., Song, Y. F., Li, B. X., Mu, W., & Liu, F. (2019). Favorable Bioactivity of the SDHI Fungicide Benzovindiflupyr Against *Sclerotinia sclerotiorum* Mycelial Growth, Sclerotial Production, and Myceliogenic and Carpogenic Germination of Sclerotia. *Plant Dis*, *103* (7), 1613-1620. doi:10.1094/PDIS-05-18-0729-RE
- Hwang, J. I., Lee, S. E., & Kim, J. E. (2015). Plant Uptake and Distribution of Endosulfan and Its Sulfate Metabolite Persisted in Soil. *PLoS One*, *10* (11), e0141728. doi:10.1371/journal.pone.0141728
- Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem*, *59* (7), 2897-2908. doi:10.1021/jf101303g
- Jiang, J., Ma, D., Zou, N., Yu, X., Zhang, Z., Liu, F., & Mu, W. (2018). Concentrations of imidacloprid and thiamethoxam in pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (*Apis mellifera* L.). *Chemosphere*, *201* , 159-167. doi:10.1016/j.chemosphere.2018.02.168
- Jiang, J., Zhang, Z., Lin, J., Liu, F., & Mu, W. (2019). The minimally effective dosages of nitenpyram and thiamethoxam seed treatments against aphids (*Aphis gossypii* Glover) and their potential exposure risks to honeybees (*Apis mellifera*). *Sci Total Environ*, *666* , 68-78. doi:10.1016/j.scitotenv.2019.02.156
- Kaya, A., & Doganlar, Z. B. (2016). Exogenous jasmonic acid induces stress tolerance in tobacco (*Nicotiana tabacum*) exposed to imazapic. *Ecotoxicol Environ Saf*, *124* , 470-479. doi:10.1016/j.ecoenv.2015.11.026
- Kaya, A., & Yigit, E. (2014). The physiological and biochemical effects of salicylic acid on sunflowers (*Helianthus annuus*) exposed to flurochloridone. *Ecotoxicol Environ Saf*, *106* , 232-238. doi:10.1016/j.ecoenv.2014.04.041

- Kong, J., Dong, Y., Xu, L., Liu, S., & Bai, X. (2014). Effects of Exogenous Salicylic Acid on Alleviating Chlorosis Induced by Iron Deficiency in Peanut Seedlings (*Arachis hypogaea* L.). *Journal of Plant Growth Regulation*, *33* (4), 715-729. doi:10.1007/s00344-014-9418-0
- Kong, J., Dong, Y., Zhang, X., Wang, Q., Xu, L., Liu, S., . . . Fan, Z. (2014). Effects of Exogenous Salicylic Acid on Physiological Characteristics of Peanut Seedlings under Iron-Deficiency Stress. *Journal of Plant Nutrition*, *38* (1), 127-144. doi:10.1080/01904167.2014.920391
- Li, Q., Wang, G., Wang, Y., Dan, Y., Guan, C., & Ji, J. (2019). Foliar application of salicylic acid alleviate the cadmium toxicity by modulation the reactive oxygen species in potato. *Ecotoxicol Environ Saf*, *172* , 317-325. doi:10.1016/j.ecoenv.2019.01.078
- Li, Y., Long, L., Yan, H., Ge, J., Cheng, J., Ren, L., & Yu, X. (2018). Comparison of uptake, translocation and accumulation of several neonicotinoids in komatsuna (*Brassica rapa* var. *perviridis*) from contaminated soils. *Chemosphere*, *200* , 603-611. doi:10.1016/j.chemosphere.2018.02.104
- Li, Y., Yang, L., Yan, H., Zhang, M., Ge, J., & Yu, X. (2018). Uptake, translocation and accumulation of imidacloprid in six leafy vegetables at three growth stages. *Ecotoxicol Environ Saf*, *164* , 690-695. doi:10.1016/j.ecoenv.2018.08.082
- Lian, J., Zhao, L., Wu, J., Xiong, H., Bao, Y., Zeb, A., . . . Liu, W. (2020). Foliar spray of TiO₂ nanoparticles prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays* L.). *Chemosphere*, *239* , 124794. doi:10.1016/j.chemosphere.2019.124794
- Liang, J., & Tang, S. (2010). Optimal dosage and economic threshold of multiple pesticide applications for pest control. *Mathematical and Computer Modelling*, *51* (5-6), 487-503. doi:10.1016/j.mcm.2009.11.021
- Liang, L., Lu, Y. L., & Yang, H. (2012). Toxicology of isoproturon to the food crop wheat as affected by salicylic acid. *Environmental Science and Pollution Research*, *19* (6), 2044-2054. doi:10.1007/s11356-011-0698-7
- Liu, S., Dong, Y., Xu, L., & Kong, J. (2013). Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regulation*, *73* (1), 67-78. doi:10.1007/s10725-013-9868-6
- Liu, T., Yuan, C., Gao, Y., Luo, J., Yang, S., Liu, S., . . . Zou, N. (2020). Exogenous salicylic acid mitigates the accumulation of some pesticides in cucumber seedlings under different cultivation methods. *Ecotoxicology and Environmental Safety*, *198* . doi:10.1016/j.ecoenv.2020.110680
- Liu, Z., Ding, Y., Wang, F., Ye, Y., & Zhu, C. (2016). Role of salicylic acid in resistance to cadmium stress in plants. *Plant Cell Rep*, *35* (4), 719-731. doi:10.1007/s00299-015-1925-3
- Lockridge, O., Verdier, L., & Schopfer, L. M. (2019). Half-life of chlorpyrifos oxon and other organophosphorus esters in aqueous solution. *Chem Biol Interact*, *311* , 108788. doi:10.1016/j.cbi.2019.108788
- Lu, Y. C., Zhang, S., Miao, S. S., Jiang, C., Huang, M. T., Liu, Y., & Yang, H. (2014). Enhanced Degradation of Herbicide Isoproturon in Wheat Rhizosphere by Salicylic Acid. *Journal of Agricultural and Food Chemistry*, *63* (1), 92-103. doi:10.1021/jf505117j
- Lu, Y. C., Zhang, S., & Yang, H. (2015). Acceleration of the herbicide isoproturon degradation in wheat by glycosyltransferases and salicylic acid. *J Hazard Mater*, *283* , 806-814. doi:10.1016/j.jhazmat.2014.10.034
- Lv, T., Zhang, Y., Casas, M. E., Carvalho, P. N., Arias, C. A., Bester, K., & Brix, H. (2016). Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. *Chemosphere*, *148* , 459-466. doi:10.1016/j.chemosphere.2016.01.064
- Mahapatra, K., De, S., Banerjee, S., & Roy, S. (2019). Pesticide mediated oxidative stress induces genotoxicity and disrupts chromatin structure in fenugreek (*Trigonella foenum - graecum* L.) seedlings. *J Hazard Mater*, *369* , 362-374. doi:10.1016/j.jhazmat.2019.02.056

Mohapatra, S., Siddamallaiah, L., Matadha, N. Y., Udupi, V. R., Raj, D. P., & Gadigeppa, S. (2019). Dissipation of neonicotinoid insecticides imidacloprid, indoxacarb and thiamethoxam on pomegranate (*Punica granatum* L.). *Ecotoxicol Environ Saf*, *171* , 130-137. doi:10.1016/j.ecoenv.2018.12.070

Namiki, S., Otani, T., Motoki, Y., Seike, N., & Iwafune, T. (2018). Differential uptake and translocation of organic chemicals by several plant species from soil. *J Pestic Sci*, *43* (2), 96-107. doi:10.1584/jpestics.D17-088

Noctor, G., Mhamdi, A., & Foyer, C. H. (2016). Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. *Plant Cell Environ*, *39* (5), 1140-1160. doi:10.1111/pce.12726

Pasternak, T., Groot, E. P., Kazantsev, F. V., Teale, W., Omelyanchuk, N., Kovrizhnykh, V., . . . Mironova, V. V. (2019). Salicylic Acid Affects Root Meristem Patterning via Auxin Distribution in a Concentration-Dependent Manner. *Plant Physiol*, *180* (3), 1725-1739. doi:10.1104/pp.19.00130

Qiu, J., Chen, G., Xu, J., Luo, E., Liu, Y., Wang, F., . . . Ouyang, G. (2016). In vivo tracing of organochloride and organophosphorus pesticides in different organs of hydroponically grown malabar spinach (*Basella alba* L.). *J Hazard Mater*, *316* , 52-59. doi:10.1016/j.jhazmat.2016.05.024

Rodriguez-Serrano, M., Romero-Puertas, M. C., Zabalza, A., Corpas, F. J., Gomez, M., Del Rio, L. A., & Sandalio, L. M. (2006). Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. *Plant Cell Environ*, *29* (8), 1532-1544. doi:10.1111/j.1365-3040.2006.01531.x

Safari, F., Akramian, M., Salehi-Arjmand, H., & Khadivi, A. (2019). Physiological and molecular mechanisms underlying salicylic acid-mitigated mercury toxicity in lemon balm (*Melissa officinalis* L.). *Ecotoxicol Environ Saf*, *183* , 109542. doi:10.1016/j.ecoenv.2019.109542

Song, W. Y., Peng, S. P., Shao, C. Y., Shao, H. B., & Yang, H. C. (2014). Ethylene glycol tetra-acetic acid and salicylic acid improve anti-oxidative ability of maize seedling leaves under heavy-metal and polyethylene glycol 6000-simulated drought stress. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, *148* (1), 96-108. doi:10.1080/11263504.2013.878408

Song, Y., Dong, Y., Kong, J., Tian, X., Bai, X., & Xu, L. (2016). Effects of root addition and foliar application of nitric oxide and salicylic acid in alleviating iron deficiency induced chlorosis of peanut seedlings. *Journal of Plant Nutrition*, *40* (1), 63-81. doi:10.1080/01904167.2016.1201491

Spormann, S., Soares, C., & Fidalgo, F. (2019). Salicylic acid alleviates glyphosate-induced oxidative stress in *Hordeum vulgare* L. *J Environ Manage*, *241* , 226-234. doi:10.1016/j.jenvman.2019.04.035

Šudoma, M., Neuwirthová, N., Hvězdová, M., Svobodová, M., Bílková, Z., Scherr, K. E., & Hofman, J. (2019). Fate and bioavailability of four conazole fungicides in twelve different arable soils – Effects of soil and pesticide properties. *Chemosphere*, *230* , 347-359. doi:10.1016/j.chemosphere.2019.04.227

Utture, S. C., Banerjee, K., Dasgupta, S., Patil, S. H., Jadhav, M. R., Wagh, S. S., . . . Adsule, P. G. (2011). Dissipation and distribution behavior of azoxystrobin, carbendazim, and difenoconazole in pomegranate fruits. *J Agric Food Chem*, *59* (14), 7866-7873. doi:10.1021/jf200525d

Volpe, V., Marani, M., Albertson, J. D., & Katul, G. (2013). Root controls on water redistribution and carbon uptake in the soil-plant system under current and future climate. *Advances in Water Resources*, *60* , 110-120. doi:10.1016/j.advwatres.2013.07.008

Wang, C., & Zhang, Q. (2017). Exogenous salicylic acid alleviates the toxicity of chlorpyrifos in wheat plants (*Triticum aestivum*). *Ecotoxicology and Environmental Safety*, *137* , 218-224. doi:10.1016/j.ecoenv.2016.12.011

Wang, F., Zeng, B., Sun, Z., & Zhu, C. (2008). Relationship Between Proline and Hg²⁺-Induced Oxidative Stress in a Tolerant Rice Mutant. *Archives of Environmental Contamination and Toxicology*, *56* (4), 723-731.

doi:10.1007/s00244-008-9226-2

Wang, K., Wu, J. X., & Zhang, H. Y. (2012). Dissipation of difenoconazole in rice, paddy soil, and paddy water under field conditions. *Ecotoxicol Environ Saf*, *86*, 111-115. doi:10.1016/j.ecoenv.2012.08.026

Wang, Q., Liang, X., Dong, Y., Xu, L., Zhang, X., Kong, J., & Liu, S. (2013). Effects of Exogenous Salicylic Acid and Nitric Oxide on Physiological Characteristics of Perennial Ryegrass Under Cadmium Stress. *Journal of Plant Growth Regulation*, *32* (4), 721-731. doi:10.1007/s00344-013-9339-3

Wang, Q. J., Sun, H., Dong, Q. L., Sun, T. Y., Jin, Z. X., Hao, Y. J., & Yao, Y. X. (2016). The enhancement of tolerance to salt and cold stresses by modifying the redox state and salicylic acid content via the cytosolic malate dehydrogenase gene in transgenic apple plants. *Plant Biotechnol J*, *14* (10), 1986-1997. doi:10.1111/pbi.12556

Xu, J., Yang, K. Y., Yoo, S. J., Liu, Y., Ren, D., & Zhang, S. (2014). Reactive oxygen species in signaling the transcriptional activation of WIPK expression in tobacco. *Plant Cell Environ*, *37* (7), 1614-1625. doi:10.1111/pce.12271

Xu, L. L., Fan, Z. Y., Dong, Y. J., Kong, J., & Bai, X. Y. (2014). Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of two peanut cultivars under cadmium stress. *Biologia Plantarum*, *59* (1), 171-182. doi:10.1007/s10535-014-0475-9

Yu, R. P., Zhang, W. P., Yu, Y. C., Yu, S. B., Lambers, H., & Li, L. (2020). Linking shifts in species composition induced by grazing with root traits for phosphorus acquisition in a typical steppe in Inner Mongolia. *Sci Total Environ*, *712*, 136495. doi:10.1016/j.scitotenv.2020.136495

Zhao, L., Huang, Y., Hu, J., Zhou, H., Adeleye, A. S., & Keller, A. A. (2016). (1)H NMR and GC-MS Based Metabolomics Reveal Defense and Detoxification Mechanism of Cucumber Plant under Nano-Cu Stress. *Environ Sci Technol*, *50* (4), 2000-2010. doi:10.1021/acs.est.5b05011

Zhao, L., Zhang, H., White, J. C., Chen, X., Li, H., Qu, X., & Ji, R. (2019). Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. *Environmental Science: Nano*, *6* (6), 1716-1727. doi:10.1039/c9en00137a

Zhao, X. Y., Qi, C. H., Jiang, H., Zhong, M. S., You, C. X., Li, Y. Y., & Hao, Y. J. (2020). MdWRKY15 improves resistance of apple to *Botryosphaeria dothidea* via the salicylic acid-mediated pathway by directly binding the MdICS1 promoter. *J Integr Plant Biol*, *62* (4), 527-543. doi:10.1111/jipb.12825

Zhao, X. Y., Qi, C. H., Jiang, H., Zhong, M. S., Zhao, Q., You, C. X., . . . Hao, Y. J. (2019). MdWRKY46-Enhanced Apple Resistance to *Botryosphaeria dothidea* by Activating the Expression of MdPBS3.1 in the Salicylic Acid Signaling Pathway. *Mol Plant Microbe Interact*, *32* (10), 1391-1401. doi:10.1094/MPMI-03-19-0089-R

Figure captions

Figure. 1 The dissipation dynamic curves of CLO, DFN and DFZ after application of exogenous SA in nutrient solution. The dissipation dynamic curves of CLO, DFN and DFZ in nutrient solution without SA supplementation were shown in fig.1(A); the dissipation dynamic curves of CLO in nutrient solution with SA supplementation were shown in fig.1(B); the dissipation dynamic curves of DFN in nutrient solution with SA supplementation were shown in fig.1(C); the dissipation dynamic curves of DFZ in nutrient solution with SA supplementation were shown in fig.1(D).

Figure. 2 The dynamic distribution and accumulation of CLO, DFN and DFZ in roots, stems and leaves of cucumber plants. The dynamic distribution and accumulation of CLO, DFN and DFZ in roots, stems and leaves of cucumber plants without SA supplementation were shown in fig.2(A); the dynamic distribution and accumulation of CLO in roots, stems and leaves of cucumber plants with SA supplementation were shown in fig.2(B); the dynamic distribution and accumulation of DFN in roots, stems and leaves of cucumber plants

with SA supplementation were shown in fig.2(C); the dynamic distribution and accumulation of DFZ in roots, stems and leaves of cucumber plants with SA supplementation were shown in fig.2(D).

Figure. 3 The effects of exogenous SA on the mean concentrations of pesticides in roots, stems and leaves of cucumber plants 28-days of exposure. The effects of exogenous SA on the mean concentrations of CLO in roots, stems and leaves of cucumber plants 28-days of exposure were shown in fig.3(A); the effects of exogenous SA on the mean concentrations of DFN in roots, stems and leaves of cucumber plants 28-days of exposure were shown in fig.3(B); the effects of exogenous SA on the mean concentrations of DFZ in roots, stems and leaves of cucumber plants 28-days of exposure were shown in fig.3(C).

Figure. 4 Effects of exogenous SA on fresh biomass (A), chlorophyll content (B), H₂O₂ content (C), proline content (D), SOD activity (E), APX activity (F), GST activity (G) and MDA content (H) of cucumber plants after roots exposure to CLO (10mg L⁻¹), DFN (20mg L⁻¹) and DFZ (10mg L⁻¹), respectively. Asterisks (*) indicate significant differences between the control groups and the treatment groups without supplementing SA (p < 0.05).

Tables

Table 1

Test designs and experimental treatments code of “pesticides uptake and translocation part”

Analyte	Without SA	SA-1mg L ⁻¹	SA-10mg L ⁻¹	SA-50mg L ⁻¹
CLO	CLO-C	CLO-SA-1	CLO-SA-2	CLO-SA-3
DFN	DFN-C	DFN-SA-1	DFN-SA-2	DFN-SA-3
DFZ	DFZ-C	DFZ-SA-1	DFZ-SA-2	DFZ-SA-3

Table 2

The half-life (t_{1/2}; d) of three pesticides in nutrient solution. Values represent the mean ± SD (n=3). The different letters are significantly different between the treatments (P<0.05) for each pesticide according to independent samples T test.

Analyte	t _{1/2} (d)				
	C2	C	SA-1	SA-2	SA-3
CLO	12.4±0.98b	7±0.7c	5.78±0.55cd	4.07±0.92d	17.3±2.23a
DFN	15.8±0.56b	11.5±0.85c	9.9±0.80c	6.93±0.45d	23.1±2.14a
DFZ	18.2±0.8a	13.8±0.98b	12.6±0.78b	5.33±0.61d	9.9±0.79c

Table 3

Mean RCF values of CLO, DFN and DFZ in cucumber plants cultivated under hydroponic conditions during 28-days of exposure. Values represent the mean ± SD (n=3). The different letters are significantly different between the treatments (P<0.05) for each pesticide according to independent samples T test.

Analyte	Mean root concentration factor (RCF)	Mean root concentration factor (RCF)	Mean root concentration factor (RCF)
	C	SA-1	SA-2
CLO	1.07±0.14a	0.55±0.053d	0.7±0.02c
DFN	0.64±0.015b	0.6±0.017c	0.57±0.022d
DFZ	52.19±3.29b	57.4±4.76a	43.92±5.77c

Table 4

Mean TF_{stem} and TF_{leaf} values of CLO, DFN and DFZ in cucumber plants cultivated under hydroponic conditions during 28-days of exposure. Values represent the mean \pm SD (n=3). The different letters are significantly different between the treatments ($P < 0.05$) for each pesticide according to independent samples T test.

Translocation factor (TF)	Analyte	C	SA-1	SA-2	SA-3
TF_{stem}	CLO	0.98 \pm 0.15b	1.57 \pm 0.19a	1.44 \pm 0.28a	1.1 \pm 0.19b
	DFN	0.69 \pm 0.14a	0.76 \pm 0.07a	0.88 \pm 0.095a	0.85 \pm 0.095a
	DFZ	0.02 \pm 0.003a	0.03 \pm 0.002a	0.05 \pm 0.003a	0.03 \pm 0.002a
TF_{leaf}	CLO	8.83 \pm 0.84d	17.35 \pm 1.77b	20.27 \pm 1.81a	11.6 \pm 0.83c
	DFN	8.01 \pm 0.64c	9.05 \pm 0.67bc	14.16 \pm 0.67a	11.53 \pm 0.79b
	DFZ	0.005 \pm 0.0003b	0.007 \pm 0.0004b	0.013 \pm 0.001a	0.011 \pm 0.003a

