Feng *et al. Chem. Biol. Technol. Agric.* (2024) 11:26
https://doi.org/10.1186/s40538-024-00545-5

Chemical and Biological
Technologies in Agriculture

RESEARCH **Open Access**

Mechanism of composite passivators to reduce cadmium absorption and accumulation in Chinese cabbage on cadmium-polluted soil

Jihong Feng[1], Ji He[1*], Lihong Song[1], Hongyan Liu[1], Chaoxuan Liao[2] and Chun Mao[1]

Abstract

Background The accumulation of heavy metals, including cadmium (Cd), in soil endangers the quality of agricultural products and can harm human health. At present, the application of passivators is a relatively efficient, quick, and economical way to address this problem. In the experimental site of the present study, the effects of different composite passivators (red mud + lime + phosphorite powder, red mud + lime + biochar, lime + humic acid + seafoam, seafoam + biochar + red mud, seafoam + biochar + phosphorite powder) on the physiology and biochemistry of Chinese cabbage were investigated.

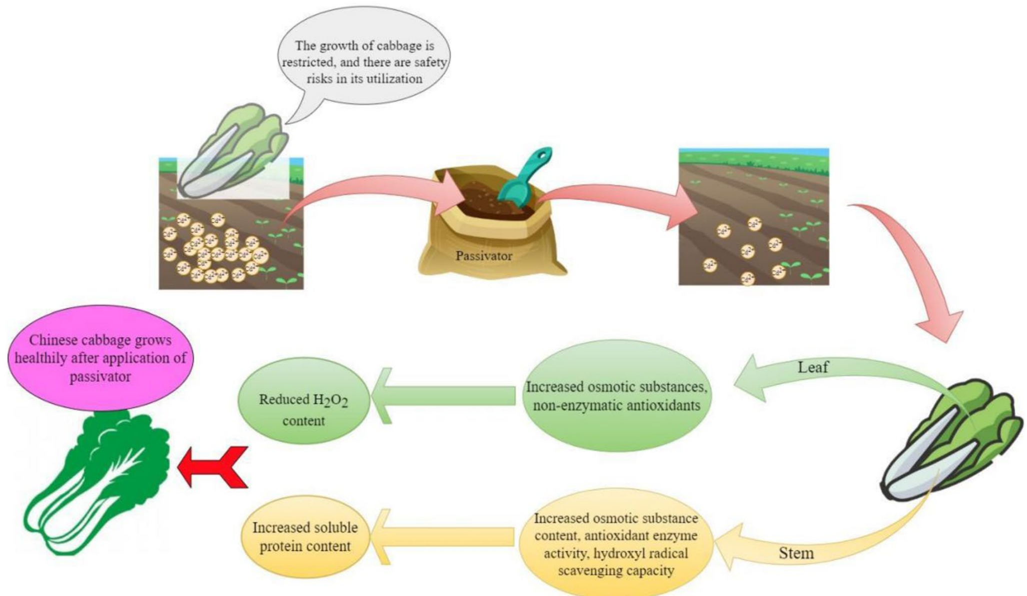
Results After passivator application, the soil's effective state Cd content was reduced, and the Cd content, bioconcentration factor (BCF), transfer coefficient (TF), oxidative stress, and antioxidant enzyme activity levels of Chinese cabbage leaves and stalks were reduced to different degrees. The reduction of reactive oxygen species content was mainly owing to passivator application, which reduced the degree of oxidative stress and increased the content of osmotic substances, the activity of antioxidant enzymes, and the ability to scavenge hydroxyl radicals. The soluble protein content of Chinese cabbage was mainly increased by an increase in the content of osmotic substances and non-enzymatic antioxidant substances and a reduction in the inhibition of protein synthesis.

Conclusions Our findings suggest that the reduction of reactive oxygen species was the main cause of the reduction of Cd accumulation, transport, and toxicity in leaves. The increase in soluble protein was the main cause of the reduction of Cd accumulation, transport, and toxicity in petioles.

Keywords Antioxidant system, Composite passivator, Moderately to mildly Cd-contaminated soil, Osmotic regulatory substances, Path analysis

*Correspondence:
Ji He
heji15@163.com
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Soil is a critical resource for plants and animals, and the gradual accumulation of heavy metals in soil affects the quality of soil and further endangers the safety of agricultural products through bioconcentration and biomagnification, which may eventually harm human health directly or indirectly. According to the National Soil Pollution Survey Bulletin of China, the exceedance rate of heavily Cd-contaminated soil sites in China is 0.5%, while those of moderately, mildly, and mildly to slightly Cd-contaminated soil sites are 0.5%, 0.8%, and 5.2%, respectively. It can be seen that, the current Cd pollution situation in China is relatively serious, and the treatment and remediation of Cd-contaminated soil is one of the key issues that urgently needs to be addressed. At present, the application of passivators is a relatively efficient, quick, and economical means of mitigating the problem. Common passivation materials, such as phosphorite powder, humic acid, seafoam, biochar, red mud, and lime, reduce the effectiveness of Cd in inducing toxicity by chelating with the heavy metal Cd and forming biologically inactive precipitates [16, 17]. When applied to Cd-contaminated soils, passivators can affect soil physico-chemical properties, microbial load, soil enzyme activity, and the physiological and biochemical properties of plants [13, 38]. In plants, reactive oxygen species are metabolic products that play an important role as signaling molecules in growth, development, and stress responses [21]. It has been shown that under Cd stress, excessive levels of oxygen radicals are formed in plants, causing oxidative stress reactions, damaging the plasma membrane system and biological macromolecules, and thus inhibiting plant growth [27]. When plants are poisoned by heavy metals, they activate their antioxidant defense systems to resist the damage caused by reactive oxygen species to cell membranes and enhance their ability to resist stress [12]. The combined action of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and the ascorbic acid–glutathione (AsA–GSH) cycle can scavenge excess reactive oxygen species and reduce the products of membrane peroxidation, thus decreasing the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and superoxide anions [1, 33]. Increases in the levels of the osmoregulatory substances proline, soluble sugar, and soluble proteins can reduce the osmotic potential of cells and enhance their water retention ability, thereby protecting cells from damage and maintaining their normal metabolism [11].

From 2014 to 2021, vegetable production in Guizhou Province, China, increased from 17.4051 million tonnes to 29.9087 million tonnes, and the planted area increased from 982.93 thousand hectares to 1511.33 thousand hectares. The vegetable industry, because of its short production cycle and high efficiency, is one of the 12 key advantageous industries of the Guizhou Provincial Party Committee and Provincial Government identified as priority for promoting the intended rural industrial revolution in depth. However, Liupanshui, Guizhou Province has a long history of coal mining that has caused pollution of the soil environment, seriously affecting the safe production of agricultural products. As Chinese cabbage (*Brassica rapa pekinensis*) is a vegetable that is sensitive to heavy metals, excessive soil Cd content not only affects the yield and quality of Chinese cabbage, but also affects human health through the food chain. Therefore, studying the safe production of Chinese cabbage is of great importance.

Recent studies on the safe production of Chinese cabbage have focused on risk assessment [18, 32], nutritional quality [10], and the effect of Cd contamination on plant growth [29]. However, the physiological mechanism of the reduction of Cd toxicity in Chinese cabbage after the application of passivators on moderately and mildly Cd-contaminated soil has been neglected. Based on previous studies, we predicted that the application of passivators would mainly reduce Cd toxicity in Chinese cabbage through the reduction of reactive oxygen species production rather than the enhancement of antioxidant capacity. To test this hypothesis, five composite passivators were selected for remediation of Cd-contaminated farmland soil. The effects of the five composite passivators on the available Cd content of the soil and the physiological properties of Chinese cabbage were studied. This study provides an empirical basis and critical guidance for the safe utilization and protection of moderately and mildly Cd-contaminated soil.

Materials and methods

Materials

The Chinese cabbage cultivar 'Jin Cai No.3' is characterized by its high yield, disease resistance, adaptability, high quality, and storage resistance. Additionally, for the present study, it was directly cultivated in Tianba, Miluo Town, Shuicheng District, Liupanshui City, Guizhou Province, China (104°59′51″–105°0′38″ E, 26°21′25″–26°23′6″ N) beginning on October 1, 2022.

The soil type at the study site was yellow loam. The physical and chemical properties of the soil in the cultivated soil layer are shown in Table 1.

The following passivators were utilized in this study: red mud (from Zunyi Aluminum Co. Ltd. [Zunyi, China], with a pH of 10.42 and a Cd content of 0.12 mg kg^{-1}), phosphorite powder (from Shandong Yutai Chemical Co. Ltd. [Jinan, China], with a pH of 9.76 and a Cd content of 0.09 mg kg^{-1}), lime (from Huifei Grey Industry Co. Ltd. [Xinyu, China], with a pH of 12.88 and a Cd content of 0.11 mg kg^{-1}), humic acid (from Shenzhen Dugao Biological New Technology Co. Ltd. [Shenzhen, China], with a pH of 9.86 and a Cd content of 0.15 mg kg^{-1}), biochar (from Gongyi Beishankou Hongchang Water Purification Material Factory [Gongyi, China], with a pH of 11.93 and a Cd content of 0.05 mg kg^{-1}), seafoam (from Tuoyi New Material Co. Ltd. [Guangzhou, China], with a pH of 10.12 and a Cd content of 0.10 mg kg^{-1}).

Methods

Based on the results of soil culture and pot experiments, the five composite passivators in Table 2 were selected, because they had the best reduction effect on effective

Table 1 Physicochemical properties of soil in the experimental area

pH	Total Cd	Organic matter g kg^{-1}	Total nitrogen g kg^{-1}	Total potassium g kg^{-1}	Total phosphorus g kg^{-1}	Alkali-hydrolyzable nitrogen mg kg^{-1}	Available potassium mg kg^{-1}	Available phosphorus mg kg^{-1}
4.64	0.81	34.21	2.51	10.34	0.3	165.44	128	19.02

Table 2 Composite passivator and their addition ratios

Numbering	Composite passivator	Add proportions	Application rate (kg hm^{-2})
CK	0	0	0
T1	Red M (1.5%): Lime (1.5%): PMP (1.5%)	1:1:1	5000
T2	Red M (1.5%): Lime (1.5%): BB (2.5%)	3:3:5	5000
T3	Lime (1.5%): HA (1.5%): Sep (1.5%)	1:1:1	5000
T4	Sep (1.5%): BB (2.5%): Red M (1.5%)	3:5:3	5000
T5	Sep (1.5%): BB (2.5%): PMP (1.5%)	3:5:3	5000

Red M, red mud; PMP, phosphorite powder; BB, biochar; HA, humic acid; Sep, seafoam

Cd (i.e., passivator rate of soil Cd > 40%), the soil Cd content after passivator application was below the screening value (0.3 mg kg^{-1}) (GB 15618-2018), and the Cd content of each part of sampled cabbage plants was within a limited range (0.2 mg kg^{-1}) (GB 2762-2017). Therefore, this study established the following six treatments in the field: CK, control without any passivators; T1, red mud + lime + phosphorite powder; T2, red mud + lime + biochar; T3, lime + humic acid + seafoam; T4, seafoam + biochar + red mud; T5, seafoam + biochar + phosphorite powder. Each treatment had three replicates. For each plot of 18 m^2, 750 kg hm^{-2} of potassium sulfate compound fertilizer ($N:P_2O_5:K_2O$, 15: 15: 15) and compound passivator (with the compound passivator of each treatment mixed proportionally) were applied. For each treatment, 5000 kg hm^{-2} of weighed compound fertilizer and compound passivator were applied to the tillage layer, which was then tilled and mixed 7 d before cabbage direct seeding. Under the unified management of the field, soil samples and cabbage samples were collected on December 6, 2022. Cabbage samples were placed in an insulated box after collection and stored in a – 80 ℃ ultra-low temperature freezer within 6 h on the same day.

Soil samples were collected according to the S-shaped sampling method after first removing debris and dead leaves from the soil, and samples were placed in bags. Then, the collected soil samples were brought back to the laboratory and dried in a ventilated and cool place.

Chinese cabbage samples were also collected according to the S-shaped sampling method. Whole Chinese cabbages were put into mesh bags and brought back to the laboratory, washed with distilled water, patted dry, divided into roots, petioles, and leaves, and then dried in an oven at 60 °C.

Measurements and methods

The effective Cd content of soil was extracted by leaching soil samples with diethylenetriaminepentaacetic acid (DTPA) solution, and soil Cd morphology was determined by the Community Bureau of Reference (BCR) method. Additionally, Chinese cabbage Cd content was extracted by combined HNO_3-H_2O_2 decoction, and the Cd content of each of the above extracts was determined by inductively coupled plasma mass spectrometry (ICP-MS). Quality control of the Cd analysis of soil samples and cabbage samples was ensured by use of standard substances (GBW-07410 and GB W10049, respectively). The recoveries of Cd elements were controlled between 95 and 105%. The chlorophyll content of Chinese cabbage leaves was determined by use of a SPAD-502 portable chlorophyll meter (Beijing Sunshine Yisda Technology Co., Ltd, Beijing, China) [8]. The content of soluble sugar was determined by the anthrone colorimetric method [34]. The content of soluble protein was determined by the colorimetric method using Thomas Brilliant Blue [34]. The content of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) colorimetric method [34]. The content of superoxide anion was determined by the hydroxylamine method [23]. The content of hydrogen peroxide (H_2O_2) was determined by spectrophotometry [30]. Hydroxyl radical scavenging capacity was determined by the fluorometric method [35]. Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) photochemical reduction method [34]. Catalase (CAT) activity was determined by the potassium permanganate titration method [34]. Peroxidase (POD) activity was determined by the guaiacol colorimetric method [34]. Dehydroascorbic acid (DHA) content was determined by the fluorometric method [22]. Ascorbic acid (AsA) content was determined by the colorimetric method [22]. The content of glutathione (GSH) was determined by the fluorometric method [6]. The content of proline was determined by the colorimetric method using ninhydrin [34].

Data processing

Excel 2019 (Microsoft Corp., Redmond, WA, USA) and SPSS27 software (IBM Corp., Armonk, NY, USA) were used for data processing, correlation analysis, and path analysis [9]. The significance of differences was tested by the least significant difference (LSD) method. Graphing was performed using Origin 2022 software (OriginLab, Northampton, MA, USA).

$$\begin{aligned}&\text{Bioconcentration factor (BCF)}\\&= \text{Cd content in plant / Cd content in the soil,}\end{aligned} \tag{1}$$

$$\begin{aligned}\text{Transport factor (TF)} &= \text{Cd content of above} - \text{ground plant /}\\&\quad \text{Cd content of below} - \text{ground plant.}\end{aligned} \tag{2}$$

Results

Effect of composite passivator on the Cd content of soil and Chinese cabbage

As shown in Fig. 1a, compared with the CK treatment, the T2, T3, T4, and T5 treatments significantly reduced the soil available Cd content by 12.03%, 12.17%, 13.90%, and 16.21%, respectively ($P < 0.05$). Compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly reduced the Cd content in leaves by 22.01%, 22.15%, 14.54%, 21.96%, and 17.00%, respectively ($P < 0.05$). The T1, T4, and T5 treatments significantly reduced the Cd content in petioles by 14.77%, 23.37%, and 38.13%, respectively ($P < 0.05$). The T1, T2, T3, T4, and T5 treatments significantly reduced the BCF of

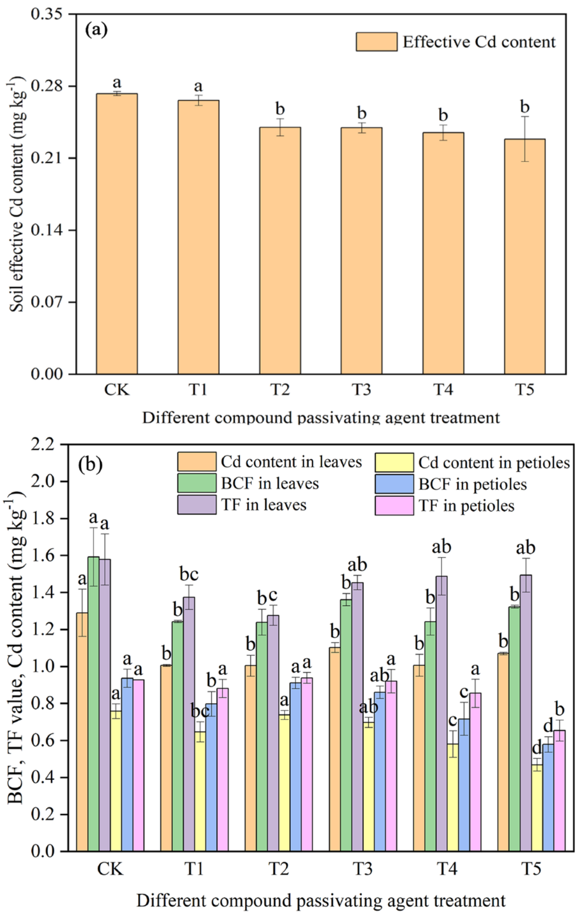


Fig. 1 Effects of composite passivator on the effective Cd content (**a**) in the soil, Cd content in different parts of Chinese cabbage, BCF, and TF (**b**)

leaves by 22.01%, 22.15%, 14.53%, 21.96%, and 17.00%, respectively ($P<0.05$). Compared with the CK treatment, the T1, T4, and T5 treatments significantly reduced the BCF of petioles by 14.78%, 23.96%, and 17.00%, respectively ($P<0.05$). The TF of leaves under T1 and T2 treatments was 12.93% and 19.14% lower than that under the CK treatment, respectively ($P<0.05$). The TF of petioles under the T5 treatment was 29.54% lower than that under the CK treatment ($P<0.05$, Fig. 1b).

Effect of composite passivator treatments on the content of different forms of Cd

As shown in Fig. 2, after passivator application, the Cd content in the weak acid extraction state, i.e., the reducible Cd content, showed a tendency to decrease. Compared with the CK treatment, T5 significantly reduced ($P<0.05$) the Cd content in the weak acid extraction state and the Cd content in the reducible state by 42.43% and 24.99%, respectively. The oxidizable

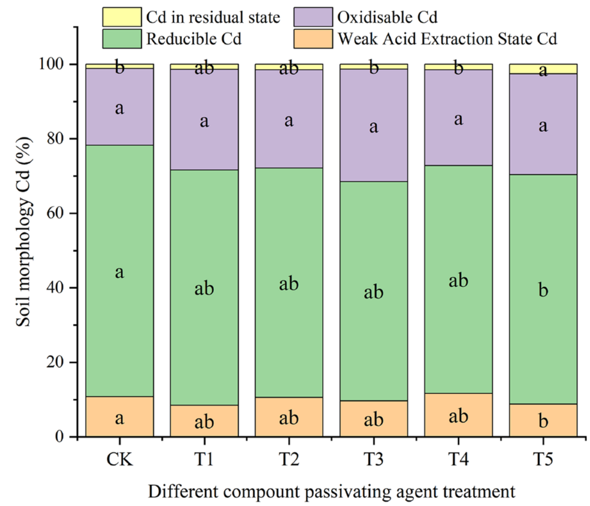


Fig. 2 Effect of composite passivator on cadmium in soil morphology

Cd content and residual Cd content showed an increasing trend; however, the difference in oxidizable Cd content did not reach significance ($P>0.05$) under any of the treatments compared to the CK treatment, but the T5 treatment significantly increased the residual Cd content by 82.61% ($P<0.05$).

Effect of composite passivator treatments on the contents of chlorophyll, soluble sugar, soluble protein, and proline in Chinese cabbage

Compared with the CK treatment, the soluble sugar content of petioles under the T2, T3, T4, and T5 treatments were 25.28%, 23.61%, 43.13%, and 64.91% higher than that of the CK treatment, respectively ($P<0.05$, Fig. 3a). The T2, T3, T4, and T5 treatments significantly increased the proline content in leaves by 18.61%, 80.11%, 167.15%, and 20.08%, respectively ($P<0.05$). The T3 and T5 treatments significantly increased the proline content in petioles by 91.76% and 54.45%, respectively ($P<0.05$, Fig. 3b). Compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly increased the soluble protein content in leaves by 299.77%, 245.21%, 239.04%, 74.89%, and 295.66%, respectively ($P<0.05$). The T1, T2, T3, T4, and T5 treatments significantly increased the soluble protein content in petioles by 189.50%, 339.51%, 351.11%, 326.85%, and 489.95%, respectively ($P<0.05$, Fig. 3c). The T2, T3, T4, and T5 treatments significantly increased the chlorophyll content of leaves by 17.7%, 44.4%, 39.8%, and 46.0%, respectively ($P<0.05$, Fig. 3d).

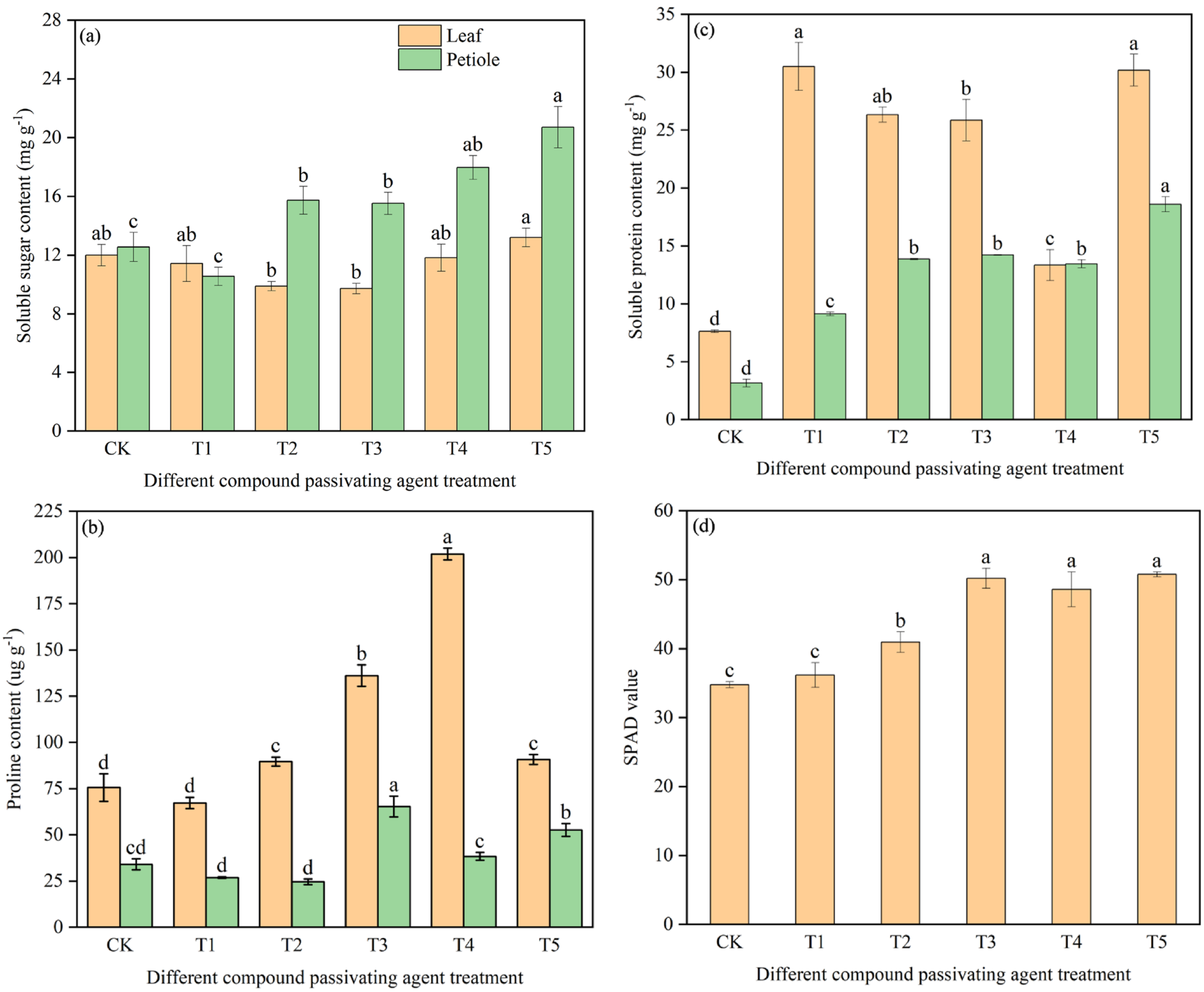


Fig. 3 Effects of composite passivator on soluble sugar (**a**), proline (**b**), soluble protein (**c**), and SPAD (**d**) contents in Chinese cabbage. Note: Different lowercase letters indicate significant differences between treatments ($p<0.05$)

Effect of composite passivator treatments on indicators of oxidative stress in Chinese cabbage

As shown in Fig. 4, compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly reduced the MDA content of leaves by 20.68%, 22.48%, 25.94%, 42.14%, and 34.11%, respectively ($P<0.05$). The T2, T3, T4, and T5 treatments significantly reduced the MDA content of petioles by 51.10%, 30.92%, 66.67%, and 68.42%, respectively ($P<0.05$, Fig. 4a). Compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly reduced the superoxide anion content of leaves by 11.38%, 10.15%, 11.66%, 11.43%, and 9.32%, respectively ($P<0.05$). The T1 and T5 treatments significantly reduced the superoxide anion content of petioles by 5.30% and 7.65%, respectively ($P<0.05$, Fig. 4b). Compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly reduced the H_2O_2 content of leaves by 46.66%, 46.06%, 50.00%, 59.41%, and 63.24%, respectively ($P<0.05$). The T1, T2, T3, T4, and T5 treatments significantly reduced the H_2O_2 content of petioles by 43.59%, 33.63%, 38.63%, 29.26%, and 47.34%, respectively ($P<0.05$, Fig. 4c). Compared with the CK treatment, the T1, T2, T3, T4, and T5 treatments significantly increased the hydroxyl radical scavenging capacity of leaves by 49.79%, 50.30%, 51.83%, 37.96%, and 51.02%, respectively ($P<0.05$). The T1, T2, T4, and T5 treatments significantly increased the hydroxyl radical scavenging capacity of petioles by 61.86%, 117.05%, 60.28%, and 152.90%, respectively ($P<0.05$, Fig. 4d).

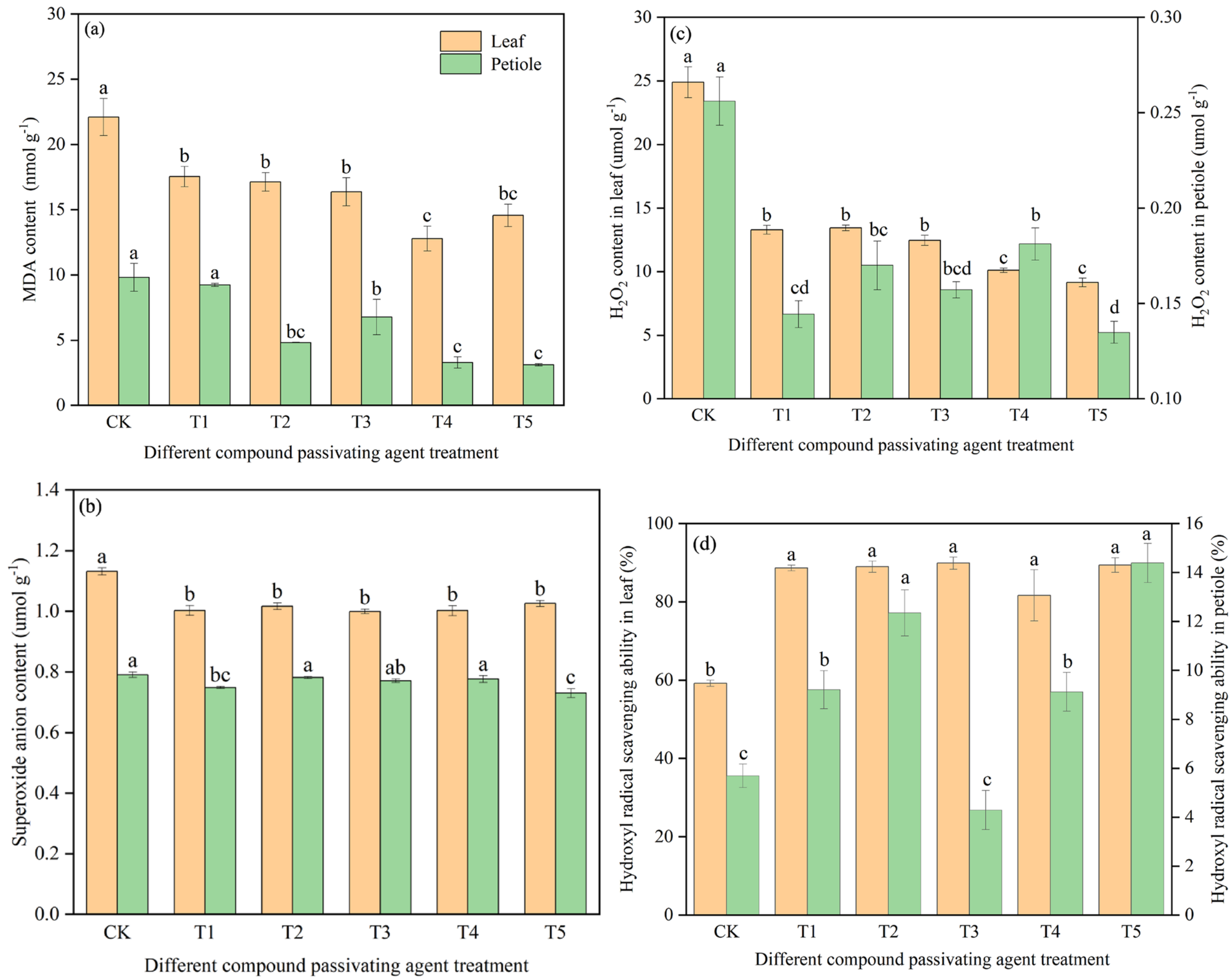


Fig. 4 Effects of composite passivator on MDA (**a**), superoxide anion (**b**), H_2O_2 (**c**), and hydroxyl radical scavenging ability (**d**) in Chinese cabbage. Note: Different lowercase letters indicate significant differences between treatments ($p<0.05$)

Effect of composite passivator treatments on the activity of antioxidant enzymes and the content of non-enzymatic antioxidant substances

As shown in Fig. 5, the T1, T2, and T5 treatments significantly increased the CAT activity in leaves by 181.43%, 158.89%, and 70.03%, respectively ($P<0.05$). The T1, T2, T4, and T5 treatments significantly increased the CAT activity in petioles by 66.77%, 106.21%, 103.42%, and 37.58%, respectively ($P<0.05$, Fig. 5a). Compared with the CK treatment, the T1 and T3 treatments significantly increased the SOD activity in leaves by 40.44% and 50.34%, respectively ($P<0.05$). The T4 and T5 treatments significantly increased the SOD activity in petioles by 106.43% and 85.13%, respectively ($P<0.05$, Fig. 5b). The T1, T2, T3, T4, and T5 treatments significantly increased the POD activity in leaves by 88.89%, 91.56%, 78.22%, 178.67%, and 70.22%, respectively ($P<0.05$). The T1, T3, T4, and T5 treatments significantly increased the POD activity in petioles by 61.15%, 103.18%, 50.96%, and 98.09%, respectively ($P<0.05$, Fig. 5c). The T4 and T5 treatments significantly reduced the DHA content in leaves by 35.59% and 35.24%, respectively ($P<0.05$). The T2, T3, T4, and T5 treatments significantly reduced the DHA content of petioles by 26.11%, 30.07%, 77.86%, and 72.84%, respectively ($P<0.05$, Fig. 5d). The T2 and T5 treatments significantly increased the AsA content of leaves by 38.44% and 164.17%, respectively ($P<0.05$). The T2 and T5 treatments significantly increased the AsA content of petioles by 65.22% and 56.92%, respectively ($P<0.05$, Fig. 5e). The T1, T2, T3, and T4 treatments significantly increased the GSH content of leaves by 27.88%, 52.83%, 83.57%, and 47.35%, respectively ($P<0.05$). The T1, T2, T3, T4, and T5 treatments significantly increased the GSH content in petioles by 134.89%, 197.16%, 73.62%, 218.87%, and 200.17%, respectively ($P<0.05$, Fig. 5f).

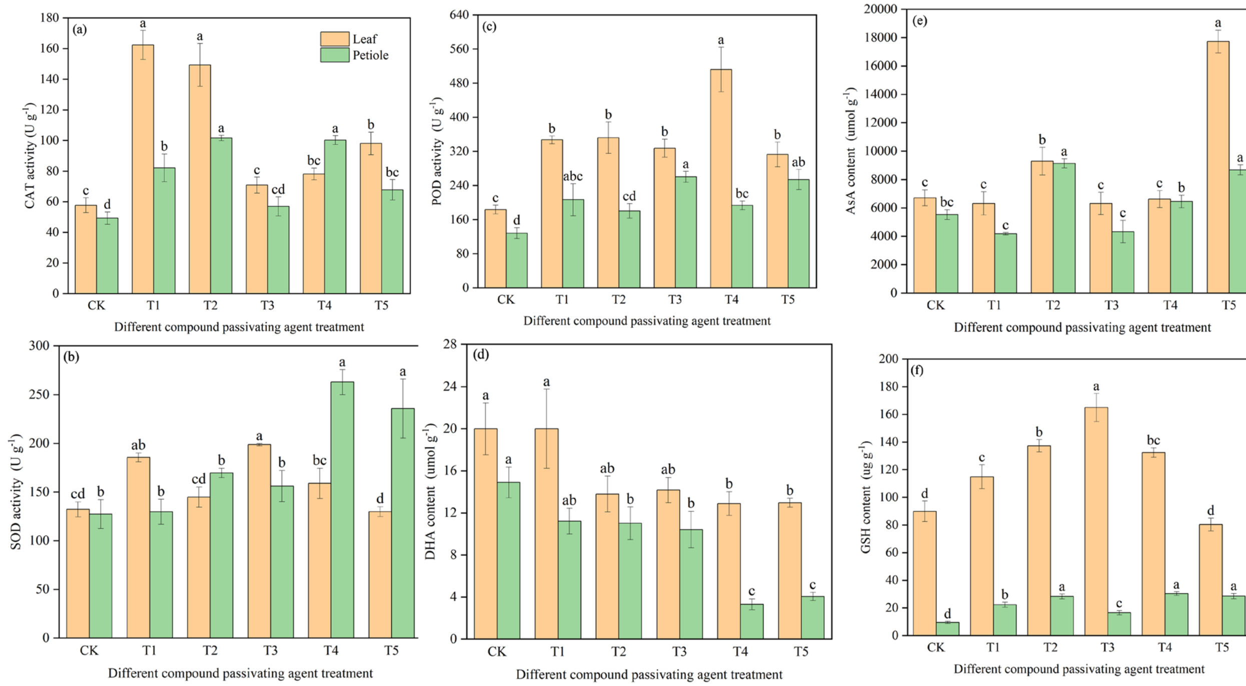


Fig. 5 Effects of composite passivator on CAT (**a**), SOD (**b**), POD (**c**), DHA (**d**), AsA (**e**) and GSH (**f**) in Chinese cabbage. Note: Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

Mechanisms by which composite passivators reduced the absorption and accumulation of Cd in leaves and petioles of cabbage

Correlations of Cd content, BCF, and TF with physiological and biochemical indicators in cabbage

As shown in Fig. 6, Cd content in Chinese cabbage leaves exhibited a highly significant positive correlation with leaf MDA, superoxide anion, and H_2O_2 contents ($P < 0.01$), a highly significant negative correlation with leaf hydroxyl radical scavenging capacity, CAT activity, and POD activity ($P < 0.01$), and a significant negative correlation with leaf soluble protein content ($P < 0.05$, Fig. 6a). The Cd BCF of Chinese cabbage leaves showed a highly significant positive correlation with leaf MDA, superoxide anion, and H_2O_2 contents ($P < 0.01$), a highly significant negative correlation with leaf hydroxyl radical scavenging capacity, CAT activity, and POD activity ($P < 0.01$), and a significant negative correlation with leaf soluble protein content ($P < 0.05$, Fig. 6b). The Cd TF of Chinese cabbage leaves showed a highly significant negative correlation with leaf CAT activity ($P < 0.01$) and a significant negative correlation with leaf hydroxyl radical scavenging capacity ($P < 0.05$, Fig. 6c). Cd content in Chinese cabbage petioles exhibited a highly significant positive correlation with petiole superoxide anion and DHA contents ($P < 0.01$), a significant positive correlation with petiole MDA and H_2O_2 contents ($P < 0.05$), a highly significant negative correlation with petiole SOD activity and soluble protein content ($P < 0.01$), and a significant negative correlation with petiole soluble sugar content, hydroxyl radical scavenging capacity, POD activity, and GSH content ($P < 0.05$, Fig. 7a). The Cd BCF of Chinese cabbage petioles exhibited a highly significant positive correlation with petiole superoxide anion and DHA contents ($P < 0.01$), a significant positive correlation with petiole MDA and DHA contents ($P < 0.05$), a highly significant negative correlation with petiole SOD activity and soluble protein content ($P < 0.01$), and significant negative correlation with petiole soluble sugar content, hydroxyl radical scavenging capacity, POD activity, and GSH content ($P < 0.05$, Fig. 7b). The Cd TF of Chinese cabbage petioles exhibited a highly significant positive correlation with petiole superoxide anion content ($P < 0.01$), a significant positive correlation with petiole MDA and H_2O_2 contents ($P < 0.05$), a highly significant negative correlation with petiole SOD activity ($P < 0.01$), and a significant negative correlation with petiole soluble sugar content, soluble protein content, and hydroxyl radical scavenging capacity ($P < 0.05$, Fig. 7c).

Path analysis of Cd content, BCF, and TF with physiological and biochemical indicators in Chinese cabbage

Path analysis was performed based on regression analysis. As shown in Fig. 8a, the direct effects on Cd content

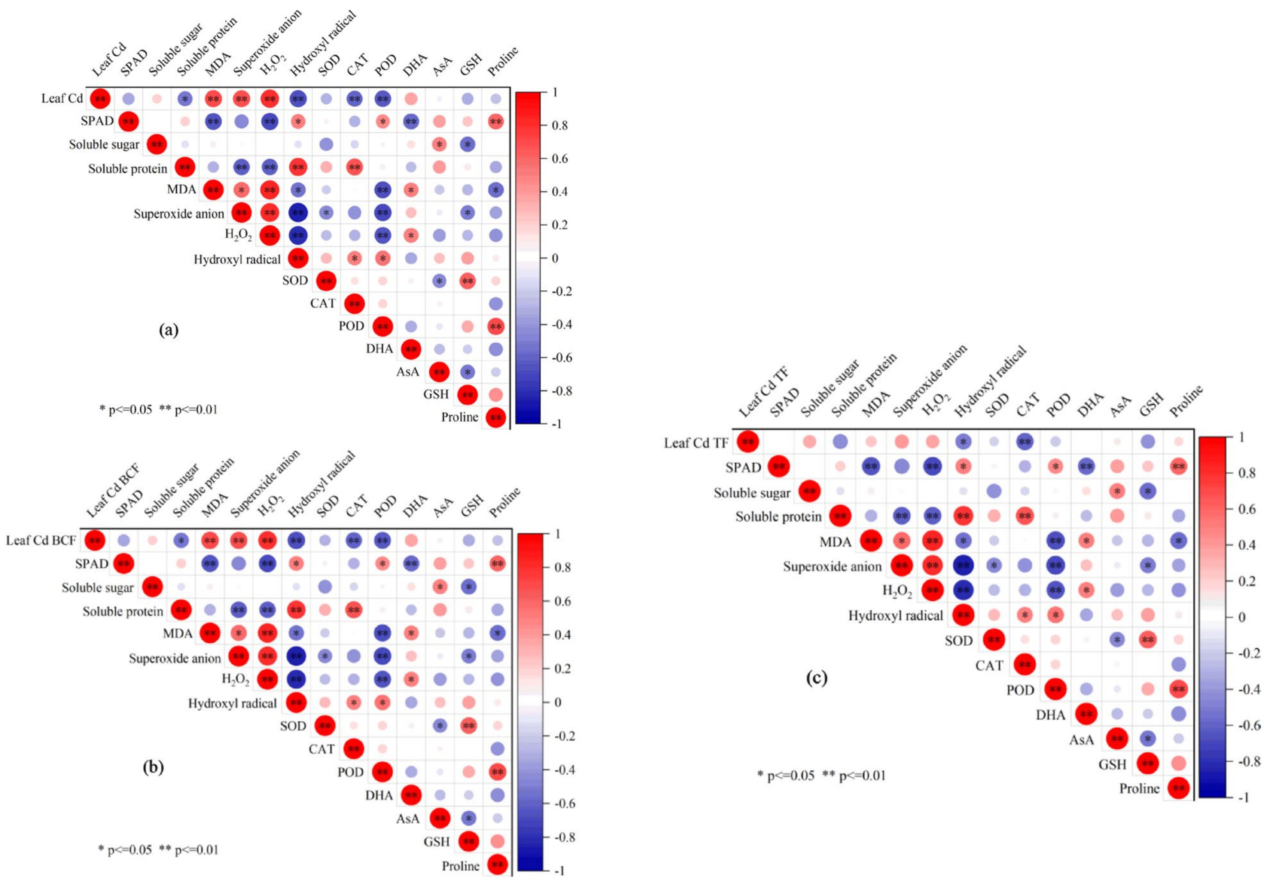


Fig. 6 Correlation analysis of Cd content (**a**), BCF (**b**), and TF (**c**) in Chinese cabbage leaves with physiological and biochemical indicators

in Chinese cabbage leaves were highest for H_2O_2 content (3.261), followed by proline (1.111), hydroxyl radical scavenging capacity (1.042), and then MDA content (− 1.016). The indirect effects on leaf Cd content were greatest for H_2O_2 content (− 2.458), followed by hydroxyl radical scavenging capacity (− 1.739), MDA content (1.717), superoxide anion content (1.676), and then proline content (− 1.368). MDA content, superoxide anion content, hydroxyl radical scavenging capacity, and proline content had greater indirect effects on the Cd content of leaves through H_2O_2. The direct effect on the Cd BCF of Chinese cabbage leaves was greatest for H_2O_2 content (3.264), followed by proline content (1.112), hydroxyl radical scavenging capacity (1.042), and then MDA (− 1.019). The indirect effects on the leaf Cd BCF were greatest for H_2O_2 content (-2.461), followed by hydroxyl radical scavenging capacity (− 1.738), MDA content (1.719), superoxide anion content (1.682), and then proline content (− 1.368). MDA content, superoxide anion content, hydroxyl radical scavenging capacity, and proline content had greater indirect effects on the Cd BCF of leaves through H_2O_2 content (Fig. 8b). The direct effect on Cd TF in Chinese cabbage leaves was greatest for H_2O_2 content (5.734), followed by proline content (3.394), hydroxyl radical scavenging capacity (2.595), MDA content (− 2.387), and then POD content (− 2.250). The indirect effects on the leaf Cd TF were greatest for H_2O_2 content (− 5.367), followed by proline content (− 3.229), hydroxyl radical scavenging capacity (-3.114), MDA content (2.633), and then POD activity (2.036). MDA content, hydroxyl radical scavenging capacity, POD activity, and proline content acted with a greater indirect effect on the Cd TF of leaves through H_2O_2 content (Fig. 8c).

As shown in Fig. 9a, the direct effects on the Cd content of Chinese cabbage petioles were greatest for soluble protein content (1.218), followed by GSH content (− 0.646), superoxide anion content (0.561), and then proline content (− 0.561). The indirect effects on petiole Cd content were greatest for soluble protein (− 1.841), followed by proline content (0.224), and then DHA content (0.188). DHA and proline contents had greater indirect effects on the Cd content of petioles through soluble protein content. The direct effect of the Cd BCF on Chinese cabbage petioles was greatest for soluble protein content (1.218), followed by GSH content (− 0.648),

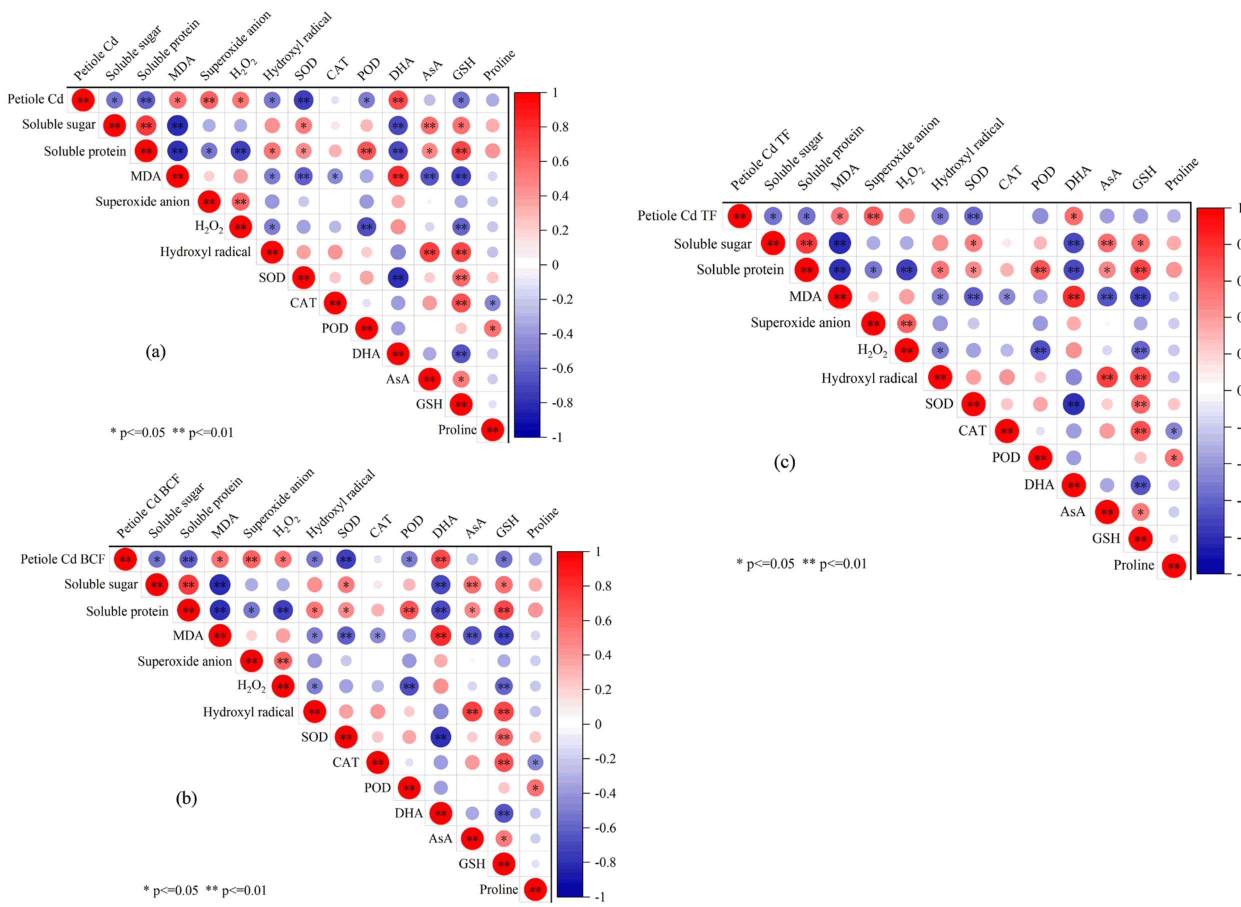


Fig. 7 Correlation analysis of Cd content (**a**), BCF (**b**), and TF (**c**) in Chinese cabbage petioles with physiological and biochemical indicators

proline content (− 0.562), and then superoxide anion content (0.559). The indirect effect on Cd BCF in petioles was greatest for soluble protein content (− 1.842), followed by proline content (0.224), and then DHA content (0.189). DHA and proline contents mainly had greater indirect effects on the BCF of petioles through soluble protein content (Fig. 9b). The direct effect of the Cd TF on Chinese cabbage petioles was greatest for soluble protein content (1.092), followed by MDA content (0.857), and then proline content (− 0.648). The indirect effect on Cd TF of petioles was greatest for soluble protein content (− 1.638), followed by proline content (0.331), and then MDA content (− 0.316). MDA had a greater indirect effect on the TF of petioles through soluble protein content (Fig. 9c).

Discussion

It has previously been shown that the application of passivators to Cd-contaminated soil can protect plants from Cd contamination by decreasing the effective state of Cd content in the soil while also both increasing the activity of the antioxidant enzymes and reducing the production of reactive oxygen species in plants [39]. In the present study, the tested composite passivators reduced the soil effective state Cd content, leaf and petiole Cd content, Cd BCF, and Cd TF to different degrees, and the T5 treatment had the greatest effect on the conversion of Cd from its dissolved to its non-dissolved state. The other four treatments, although they also reduced the bioavailability of soil Cd to some extent, were not as effective as the T5 treatment, probably because the T5 passivator combination of seafoam, biochar, and phosphorite powder was better suited to the nature of the soil, the degree of Cd contamination, and the plant species at this site. Seafoam and biochar in the T5 treatment have high porosity and high specific surface area, which helps to adsorb heavy metals, and phosphorite powder can form insoluble phosphorite powder precipitates with heavy metals in the soil. All of these factors can reduce the bioefficacy of heavy metals, thus improving plant tolerance and enabling better physiological functioning in heavy-metal-polluted environments. In addition, phosphorite powder dust provides plants with the phosphorus they need to grow. When the three passivators seafoam,

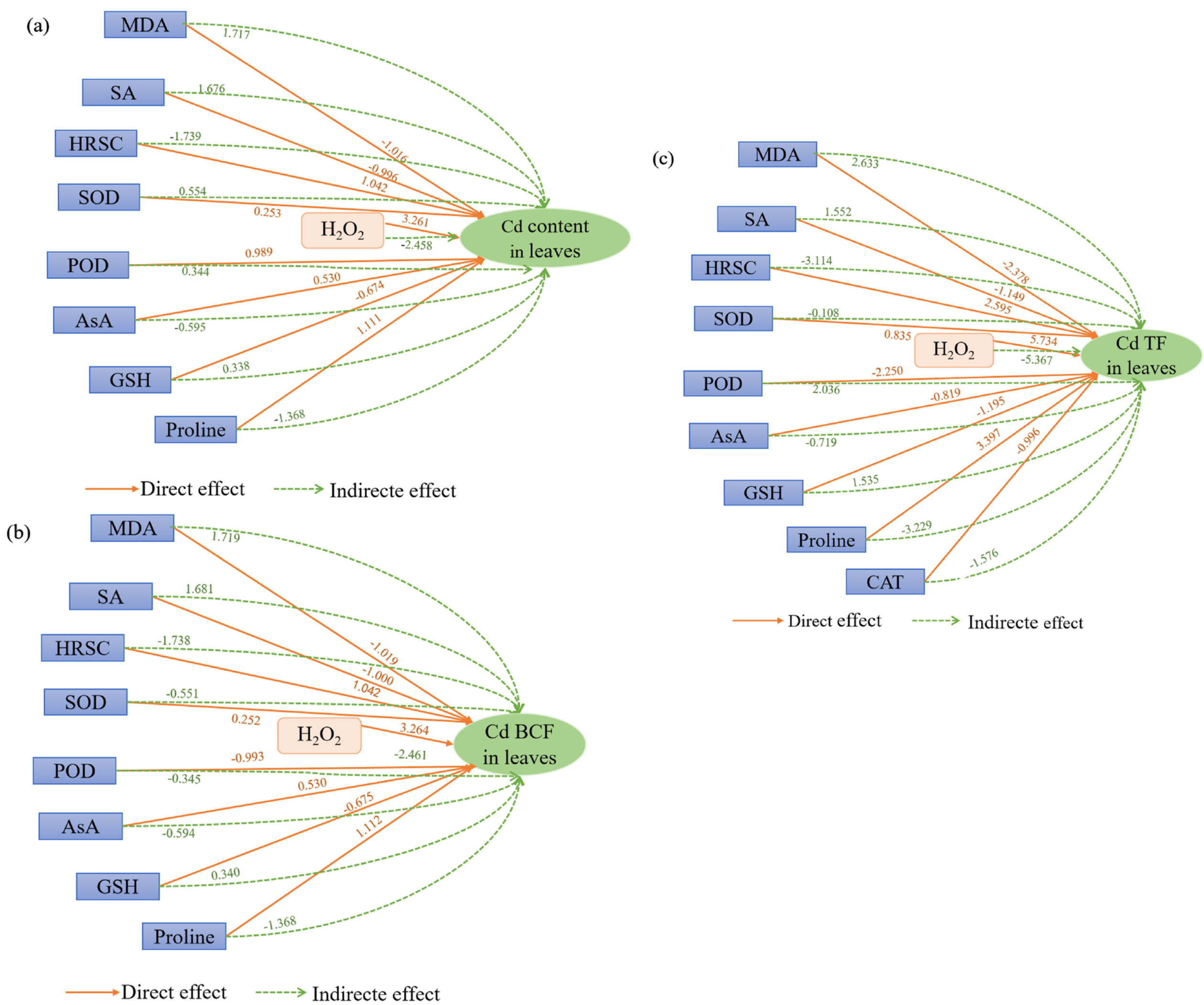


Fig. 8 Path analysis of Cd content (**a**), BCF (**b**) and TF (**c**) in Chinese cabbage leaves with physiological and biochemical indicators

biochar and phosphorite powder are mixed in certain proportions, the biochar and phosphorite powder synergistically reduce the bioavailability of heavy metals while improving the soil quality, which is further enhanced by the excellent adsorption capacity of seafoam, which makes this combination of passivators so effective. The above results indicate that the applied composite passivator treatment had chelation, adsorption, and organic matter effects on Cd ions in the soil to form inactive substances, which reduced the content of Cd in the weak acid extraction state and the content of Cd in the reducible state, while increasing the content of Cd in its residual state and limiting the migration of Cd in the soil effective state to the plant body, thus mitigating Cd toxicity in cabbage. Thus, the applied composite passivator chelated, adsorbed, and had organic matter effects with Cd ions in the soil that induced the formation of inactive substances, which reduced the effectiveness of Cd in the soil and restricted the uptake of Cd in its effective state into the plant, thereby mitigating Cd toxicity in cabbage.

Chlorophyll is the main pigment enabling photosynthesis. In the present study, the chlorophyll content of Chinese cabbage leaves was significantly increased under T2, T3, T4, and T5 treatments. The application of composite passivators was able to promote the synthesis of photosynthetic pigments and photosynthetic carbon assimilation in Chinese cabbage leaves, enhance photosynthesis and the accumulation of organic matter, and ultimately slow down the inhibitory effect of Cd on photosynthesis to improve the growth and development of plants [5, 25].

Plants can also adjust their osmotic pressure to resist heavy metal toxicity in response to heavy metal stress through the production of osmoregulatory substances such as soluble sugars, soluble proteins, and proline. The

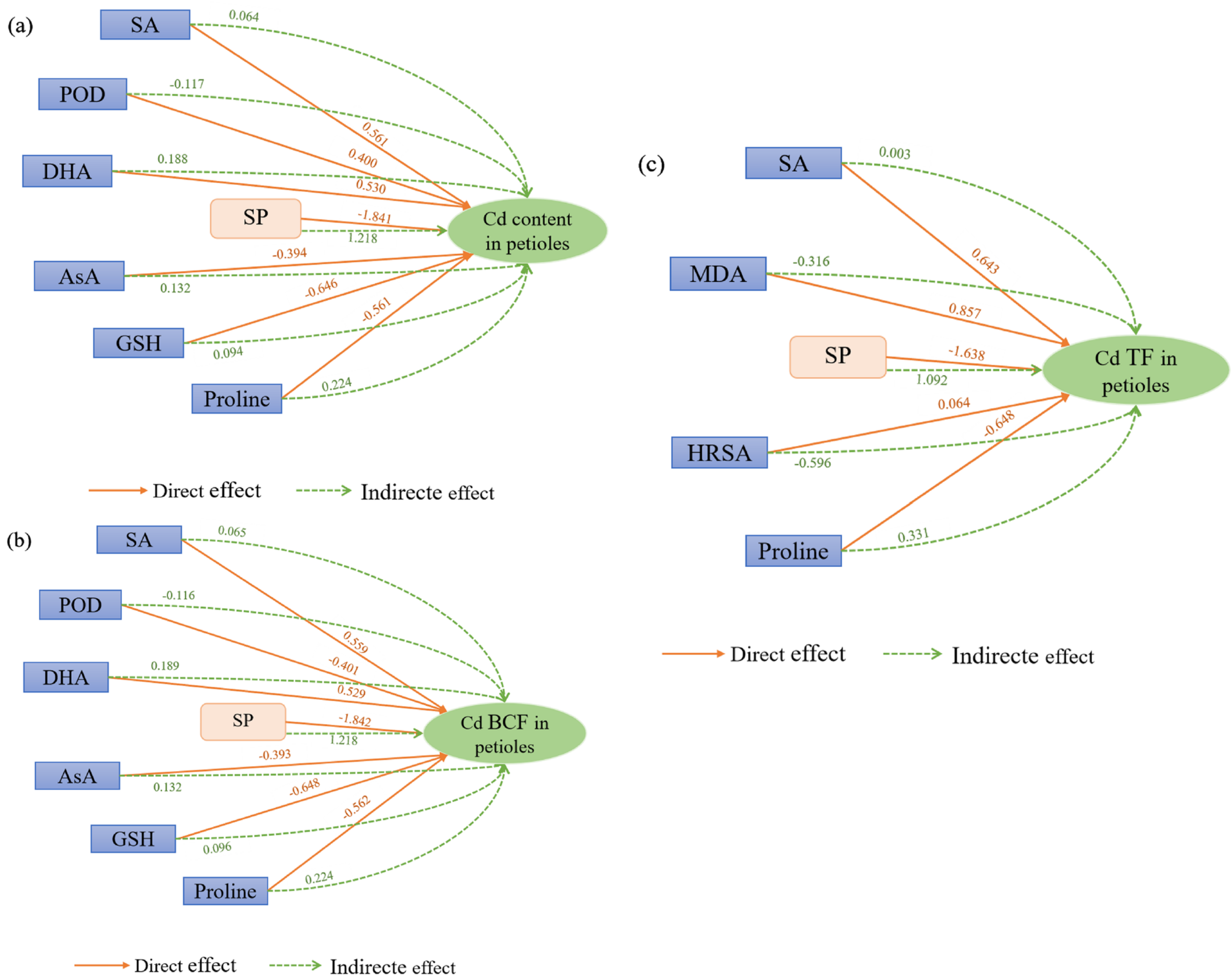


Fig. 9 Path analysis of Cd content (**a**), BCF (**b**) and TF (**c**) in Chinese cabbage petioles with physiological and biochemical indicators

results of the present study showed that the soluble sugars of Chinese cabbage leaves were not significant under all treatments compared to the CK treatment. This may be related to the different effects of the different treatments on sugar metabolism pathways. However, determining the specific underlying mechanisms requires further analysis. The soluble sugar content of Chinese cabbage petioles was significantly higher in the T2, T3, T4, and T5 treatments, which may be attributed to the fact that the passivator mitigated the toxic effect of Cd on the petioles and promoted the growth and metabolism of the petioles, thus increasing the soluble sugar content of the petioles [28]. All treatments significantly increased the soluble protein content of Chinese cabbage leaves and petioles, while T3 and T5 treatments significantly increased proline content in the leaf and petiole cells, which may be attributed to the fact that an increase in proline content can reduce the absorption of Cd or enhance its complexation in roots, such that the cabbage absorbed and accumulated less Cd, which may have promoted protein synthesis [7, 40]. After the application of composite passivator treatments, there were increases in soluble sugar, soluble protein, and proline contents, such that the osmotic potential of Chinese cabbage leaves and petioles was reduced and osmotic adjustment ability was increased, which protected the integrity and water balance of cells while reducing Cd accumulation, thereby enhancing the plant's ability to physiologically adapt to the environment and maintain its normal metabolism [15].

The MDA content, reactive oxygen species (i.e., superoxide anion, H_2O_2) content, and hydroxyl radical scavenging capacity in the leaf and petiole cells of Chinese cabbage were key factors that were able to respond to the oxidative stress faced by the plants, and they play important roles in several biological processes, including plant growth, development, and stress responses. Among these factors, MDA, as one of the products of membrane lipid peroxidation, can cause cross-linking polymerization of biological macromolecules, such as proteins and nucleic acids, and thus poison cells. In the present study, T3, T4, and T5 treatments significantly reduced the MDA content in the leaf and petiole cells, indicating that passivator application can reduce the degree of oxidative stress in plants, reduce the possibility of Cd triggering lipid peroxidation, and thus mitigate the toxic effects of the heavy metal Cd on Chinese cabbage. When under stress, plants produce excessive reactive oxygen species, which can lead to oxidative damage of biomolecules and ultimately cell death [31]. In Cd-contaminated soils, the main source of reactive oxygen species is Cd ions interfering with various intracellular metabolisms, including the mitochondrial respiratory chain and NADPH oxidase [20]. Hydroxyl radical scavenging capacity refers to the ability of plants to scavenge hydroxyl radicals, which can reflect the degree of oxidative stress and the ability of plants to physiologically adapt to environmental stress. H_2O_2 has high transmembrane permeability characteristics, and its accumulation in large quantities may cause adverse reactions, including cell damage, cell death, oxidative stress, and growth inhibition, which is one of the main consequences of heavy metal toxicity in plants [36].

In the present study, T1, T2, T4, and T5 treatments significantly increased the hydroxyl radical scavenging capacity of Chinese cabbage leaf and petiole cells, while T1 and T5 treatments significantly decreased the superoxide anion content of Chinese cabbage leaf and petiole cells; moreover, all treatments significantly reduced H_2O_2 content in Chinese cabbage leaf and petiole cells. Thus, the application of passivators reduced the Cd toxicity of Chinese cabbage. On the one hand, passivators promote the conversion of Cd ions from their active form to their inactive form, thus reducing the absorption and transport of Cd in all parts of the Chinese cabbage as well as the degree of damage to the Chinese cabbage by oxidative stress, which leads to a reduction in the content of reactive oxygen species, enhances the ability of plants to maintain oxidative balance, and ultimately promotes normal plant growth and development. On the other hand, passivators help to maintain cellular integrity, which reduces the production of MDA and ultimately reduces the degree of oxidative stress caused by Cd in various tissues of Chinese cabbage.

To protect cells from damage caused by reactive oxygen species, a complete antioxidant defense system has evolved in plants. The antioxidant defense system includes antioxidant enzymes (e.g., SOD, CAT, POD) and non-enzymatic antioxidant substances (e.g., ASA, GSH, DHA). Thus, plants can eliminate reactive oxygen species through various defense pathways and reaction mechanisms [37]. The main function of SOD is to catalyze the disproportionation of superoxide anion radicals into H_2O_2 and O_2, thereby scavenging excess superoxide anions [41], and the resulting H_2O_2 is broken down into H_2O and O_2 by CAT, inhibiting the formation of highly reactive hydroxyl radicals, among toxic reactive substances [14]. POD can independently catalyze the reaction between H_2O_2 and phenolic compounds to scavenge H_2O_2, or it can act together with CAT to catalyze the formation of H_2O from H_2O_2, thus effectively preventing the excess accumulation of superoxide anion radicals and H_2O_2 and thereby maintaining the normal physiological metabolism of the plant itself [26]. In the present study, T1 and T3 treatments significantly increased SOD activity in leaves. T4 and T5 treatments significantly increased SOD activity in petioles. T1, T2, and T5 treatments significantly increased both CAT and POD activity levels in leaves. T1, T3, T4, and T5 treatments significantly increased CAT and POD activity levels in petioles. These findings indicate that the application of the composite passivator treatments was able to increase the activity level of the antioxidant enzymes to different degrees, and the ability to scavenge reactive oxygen species in the plant body was improved, thus, the oxidative damage and antioxidant defense of the plant were in a dynamic equilibrium under Cd stress, which protected the stability of the plant cells.

Non-enzymatic antioxidants can also directly react with reactive oxygen species and thus play an important role in scavenging reactive oxygen species [19]. AsA is mainly found in the chloroplast stroma of plants, where it can not only scavenge reactive oxygen species, but also serve as a substrate for enzymes involved in scavenging reactive oxygen species [2]. DHA and AsA jointly form a redox system that acts as electron acceptors [4]. The AsA-GSH cycle is an important pathway for clearing ROS and an important defense mechanism against oxidative stress caused by heavy metals in plants [24]. As a major scavenger, GSH has a strong affinity with metal ions and can effectively bind with them to form non-toxic compounds, thereby playing a detoxifying role [3]. In the present study, T4 and T5 treatments significantly reduced the DHA content in the leaf and petiole cells. T2 and T5 treatments significantly increased AsA content in leaves and petioles. T1, T2, T3, and T4 treatments significantly increased the GSH content in the leaf and petiole cells. These findings indicate that the application of the composite passivators was able to reduce oxidative stress, thus protecting DHA from oxidation and at the same time promoting the synthesis of AsA and GSH antioxidant substances in plants, thereby increasing scavenging capacity for reactive oxygen species as well as reducing toxic effects on the leaves and petioles of Chinese cabbage.

Interactions between Chinese cabbage chlorophyll content, osmotic substances, reactive oxygen species, antioxidant enzymes activities, and non-enzymatic antioxidants collectively influenced the effects of Cd on Chinese cabbage. Analyzing the direct and indirect effects of each factor on the uptake and accumulation of Cd in Chinese cabbage through path analysis clarified how Chinese cabbage regulated its physiological function and metabolic activity to physiologically adapt to new environmental conditions. In the present study, MDA content, H_2O_2 content, hydroxyl radical scavenging capacity, and proline content were the main direct factors influencing leaf Cd content, BCF, and TF. The direct and indirect effects of H_2O_2 on leaf Cd content, BCF, and TF were greater than those of other factors. Thus, the application of composite passivator treatments was able to reduce the oxidative stress response of Chinese cabbage and promote the content of osmotic substances and hydroxyl radical scavenging capacity, which was able to protect biomacromolecules from oxidative damage, reduce the absorption and transport of Cd, and thereby reduce the toxic effect of Cd on Chinese cabbage leaves. The direct and indirect effects of soluble protein content on petiole Cd content, BCF, and TF were greater than those of other factors. Thus, the application of composite passivator treatments increased the antioxidant capacity and osmoregulatory capacity of petioles by increasing the content of non-enzymatic antioxidant substances and osmoregulatory substances, which was able to protect vital cellular substances and membranes from damage, thus reducing the inhibition of protein synthesis and promoting the production of soluble proteins. Collectively, these effects reduced the absorption and transport of Cd, ultimately reducing the toxic effect of Cd on the petiole cells of Chinese cabbage.

In summary, this study evaluated the use of mildly and lightly Cd-contaminated farmland soil for Chinese cabbage cultivation to provide empirical support for the remediation of such soil through the application of passivators; additionally, leaves were able to reduce the toxic effect of Cd mainly by decreasing the production of reactive oxygen species, and petioles mitigated the toxic effect of Cd mainly by increasing the production of osmotic substances, which is at variance with the initial hypothesis of the study. Accordingly, this study not only informs the safe cultivation of Chinese cabbage, but also provides insights into the selection and identification of passivators under moderate and light cadmium contamination, as well as the safe production of other crops.

Conclusions

The present study has deepened the current understanding of both passivation to mitigate the toxicity of the heavy metal Cd in Chinese cabbage and its physiological and biochemical mechanisms. All evaluated passivator treatments were able to reduce the degree of oxidative stress induced by the heavy metal Cd to a certain extent, reduce the contents of MDA, superoxide anion, H_2O_2, and DHA, increase the contents of proline and soluble protein and hydroxyl radical scavenging capacity, and promote the production of SOD, POD, CAT, GSH, and AsA, thus protecting plants from Cd toxicity. However, different composite passivators mitigated Cd toxicity to different degrees, and, overall, the T5 treatment (seafoam [1.5%] + biochar [2.5%] + phosphorite powder [1.5%]) was the most effective. We also found that the direct and indirect effects of H_2O_2 and soluble protein content on the leaf and petiole Cd content, Cd BCF, and Cd TF, respectively, were greater than those of other factors. These findings indicate that after the application of composite passivators, on the one hand, Chinese cabbage exhibited increased antioxidant capacity and hydroxyl radical scavenging capacity by reducing the content of reactive oxygen species, which reduced oxidative stress and protected biomolecules from oxidative damage, thus reducing the toxic effects of Cd on Chinese cabbage. On the other hand, by improving osmoregulatory capacity, the passivator treatments protected essential cellular structures and membranes from damage and promoted the production of soluble proteins, which in turn increased the ability of Chinese cabbage to physiologically adapt to changing environmental conditions. However, both H_2O_2 and soluble sugars can act as regulators or signaling factors in plants, guiding them to make more adaptive responses by modulating Cd-related signaling pathways. Therefore, to further clarify the specific effects of H_2O_2 and soluble sugars on the physiology and biochemistry of Chinese cabbage, we plan to conduct an in-depth follow-up study to provide more targeted guidance for agricultural production.

Acknowledgements

We are grateful to Guizhou University and Guizhou Testing Research Institute for providing us with the experimental conditions. We would like to thank MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

Author contributions

FJH was mainly responsible for experiments, data analysis, and paper writing. HJ was mainly responsible for experimental design, the modification and the improvement of papers. SLH and LHY were mainly responsible for solving technical issues in the experiment and revising the paper. LCX was mainly responsible for completing part of the indoor experiment. MC was mainly responsible for completing some sample collection work. All authors reviewed the manuscript.

Funding
This work was funded by Guizhou Provincial Basic Research Program (Natural Science), grant number Qiankehejichu-ZK [2021] YB133; Guizhou Provincial Scientific and Technological Program, grant number Qiankehehoubuzhu [2020] 3001; and National Natural Science Foundation of China-Guizhou Provincial People's Government Karst Science Research Centre (U1612442).

Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]College of Agriculture, Guizhou University, Guiyang 550000, China. [2]Guizhou Academy of Testing and Analysis, Guiyang 550014, China.

Received: 27 September 2023 Accepted: 7 February 2024
Published online: 19 February 2024

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