Investigating the Impact of Acid Precipitation Adaptation on Plant Species Across pH Gradients

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ABSTRACT

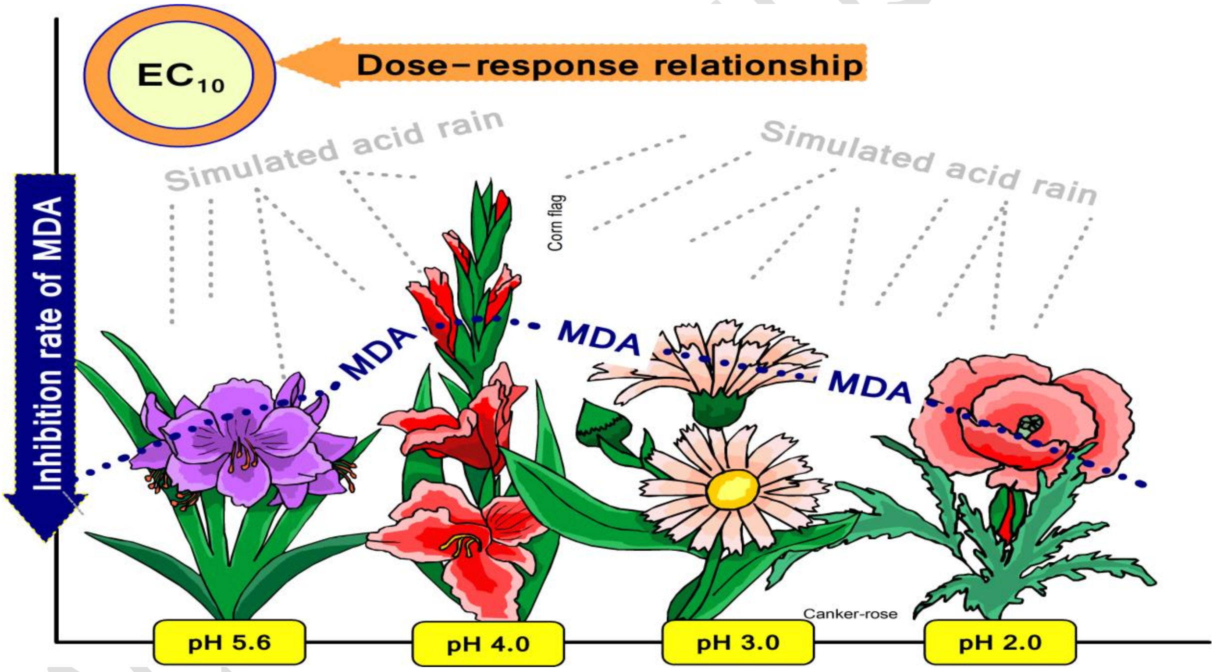
This study investigates the dose-response relationship between acid precipitation and malondialdehyde (MDA) accumulation in plants, utilizing sophisticated curve-fitting or linear-fitting techniques to pinpoint toxicity thresholds arising from simulated acid rain (SAR) stress. Four distinct types of dose-response relationship patterns emerged: promotion, non-affection, low promotion-high suppression, and full suppression. The research meticulously examined the dose-response relationship of the inhibition rate of MDA content in plant leaves after 50 days of exposure to SAR across 18 plant species, with pH levels of 5.6 (control), 4.0, 3.0, and 2.0. By applying precise dose-response relationship fitting, the EC_{10} value—reflecting the SAR pH that elicits a 10% increase in MDA levels—was calculated, unveiling critical toxicity thresholds. The results revealed striking variations in species-specific MDA responses, providing vital insights into the ecological impact of acid rain and establishing key toxicity benchmarks for future studies.

Keywords: Acid rain, Dose-response relationship, Malondialdehyde, Plant species

HIGHLIGHTS

- Toxic effects on different plants were observed.
- MDA accumulation in plant leaves was used to assess stress from SAR.
- The dose-response relationship and EC_{10} are jointly used to determine toxicity thresholds.
- The toxic thresholds for SAR on 18 plant species have been determined.

GRAPHICAL ABSTRACT



INTRODUCTION

An unintended consequence of industrial development has been the increased frequency of acid precipitation, primarily driven by elevated emissions of sulfur dioxide (SO_2) and nitrogen oxides (NO_x), which negatively impact plant and tree health (Sani *et al*.2024; Debnath *et al*.2020; Odiyi *et al*.2015). China, as a large nation, has experienced considerable challenges with acid rain, especially in regions experiencing rapid economic growth Monitoring data from 2003 to 2023 across 17 stations in the southwest of Chengdu—one of the country's fastest-growing economic areas—revealed that 24.53% of stations recorded rainwater with a pH below 4.0, 18.65% with pH below 5.0, and 45.06% with pH less than 5.6 (Xuan, C..*et al*.2021; Wang et al.2025; Zhang et al.2025). Additionally, approximately 42.19% of the stations reported an acid rain frequency exceeding 50%, with certain areas surpassing 90%. These results suggest that the region is characterized by frequent acid rain events, heightened acidity, and high pollution (Prakash *et al*.2023; Qin *et al*.2001). Acid precipitation can cause visible leaf damage, including chlorotic and necrotic spots, while also negatively affecting plant physiological processes. It inhibits photosynthesis, depletes essential nutrients, disrupts water balance, and diminishes enzyme activity (Ahmad *et al*.2021; Gou et al.2025; Gou et al.2021). Studies show that acid rain contributes to the degradation of vegetation and green spaces (Liu *et al*.2021; Ren *et al*.1997). In the Sichuan Basin, China, acid precipitation poses a threat to approximately 275.6 thousand square kilometers of vegetation, accounting for 32.2% of the region's total green space. This reduction in forest cover has resulted in an estimated annual economic loss of RMB 0.14 billion in the timber industry (Li *et al*.2016; Ahirwal *et al*.2016). In response to this challenge, plant species resistant to acid precipitation for ecological restoration can mitigate vegetation damage while enhancing biodiversity as well as ecosystem productivity in the region (Tong et al. 2025; Lamers *et al*.2015).In this context, numerous studies (Selvaraj et al.2025; Selvanarayanan et al.2024) have been conducted to identify species with significant ecological value in relation to pollution levels in their environments, emphasizing their capacity to tolerate specific environmental pollutants (Fig. 1).

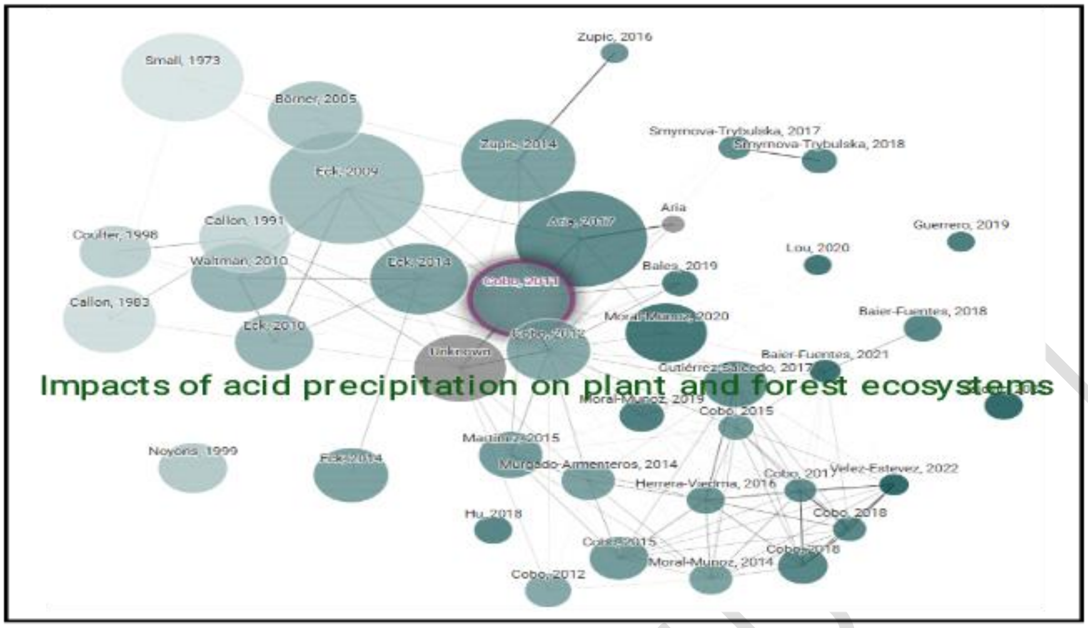


Figure 1. Co-citation addressed the examination of acid precipitation impacts on plant ecosystems.

For instance, Thomas et al.(2022) investigate acid mine drainage (AMD) and its effects on impacted environments, offering a critical analysis of strategies for plant protection and restoration. Indeed, the mentioned approach was proposed to optimize resource use, support income generation, mitigate biodiversity loss, and enhance carbon sequestration, thereby addressing significant climate change challenges and promoting sustainable agricultural practices. Du et al. (2024) examined the adverse impacts of excessive nitrogen deposition on soil chemistry and plant communities, highlighting significant negative effects that necessitate intervention. The study also assessed ecosystem responses to reduced nitrogen deposition to identify critical processes requiring modification and proposed specific management strategies to alleviate the consequences of excessive nitrogen deposition and facilitate ecosystem recovery. McGivney et al. (2019) proposed a dynamic model, HD-MINTEQ (Husby Dynamic MINTEQ), to evaluate the potential long-term effects of two hypothetical harvest scenarios projected for 2020. The model was employed to compare the impacts on soil chemistry and weathering rates across three forest sites in Sweden—Aneboda, Gårdsjön, and Kindla—investigating how these factors affect crop yield in relation to acid rain. Campos da Silva et al. (2005) conducted prediction research to assess the impact of acid precipitation on plant growth. They classified toxicity threshold values based on the proportion of visible chlorotic and necrotic spots observed on leaf surfaces, categorizing these into diverse levels.

The aforementioned studies have mainly explored the impact of acid rain on green spaces and crops, highlighting growing concerns about the detrimental environmental effects of this phenomenon. t is essential to recognize that plant responses to sustained changes in acidity are ongoing processes. Estimating toxicity threshold values based on predetermined pH levels may introduce systematic errors due to the design of experimental gradients, which can compromise the accuracy of these estimations. In this sense, this study makes the following contributions to the current literature:

1- The inhibitory rate of MDA concerning SAR is assessed across 18 plant species, through adosage-response relationship.
2- Plant species are classified based on their sensitivity to acid precipitation, providing a foundation for the selection of appropriate species for ecological restoration in the studied area.

MATERIALS AND METHODS

Experimental design and establishment of dose-response relationship

This study assesses the effects of acid precipitation on plants by investigating 18 plant species in the Chengdu Plain, China. The evaluation specifically involved 2-year-old potted trees, each measuring 25 cm in height and 23 cm in diameter. Four pH levels were established for the investigation: pH 5.6 (benchmark), pH 4.0, pH 3.0, and pH 2.0. Each level is tested with four replicates, comprising four trees per species. Also, to prevent cross-exposure during acid precipitation, the trees are cultivated in separate, isolated enclosures. Additionally, to ensure data accuracy, the pH of the simulated acid rain solution is measured before each application (Pandey *et al*.2009). If discrepancies arise between the intended and measured pH values, a new solution is prepared accordingly (Fig. 2).

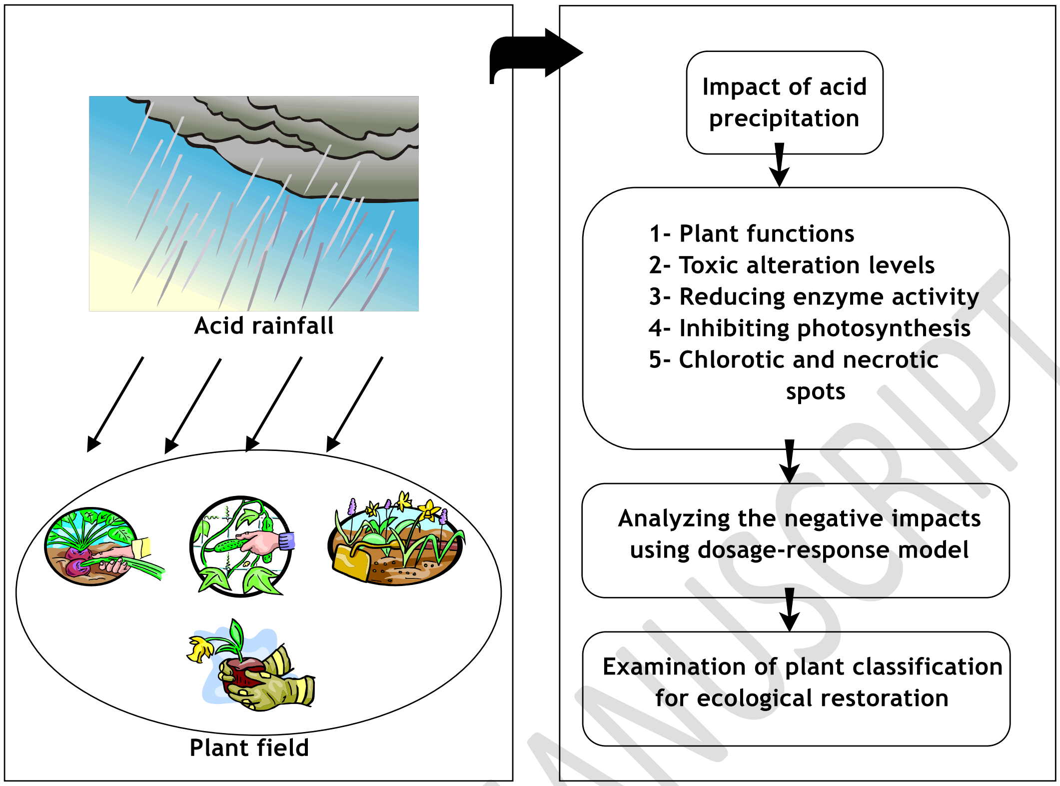


Figure 2. Conceptual framework.

Beyond that, to analyze the impact of acid precipitation, an agriculture hand knapsack sprayer is implemented to ensure that droplets are formed on the leaf surfaces. Accordingly, spraying was performed once every 10 days, with a total of five applications administered over a 50-day period of acid precipitation stress.

The MDA content was determined using the thiobarbituric acid (TBA) method (Zhang Zhiliang *et al*.2009) and analyzed with a UV-Vis spectrophotometer (Model: TU-1810, Purkinje General Instrument Co., China).

Fitting of dose-response relationships and identification of threshold values

The inhibition rate of MDA is calculated using the method provided by Sun et al (Sun *et al*.2010). The dose-response relationship picture is plotted with '7 - pH value' on the x-axis and the inhibition rate on the y-axis. Also, a linear fit is conducted using two acid precipitation treatment points with inhibition rates near -10% to identify the EC_{10} value.

$$\lambda = z.(7 - PH) + u \quad (1)$$

Where, λ is the inhibition rate of MDA, and z and u are coefficients.

Data Processing and Analysis

Data calculations were performed using Excel 2024. Analysis of variance (ANOVA) was conducted using DPS 7.05, a data processing system designed for statistical analysis and experimental design. The least significant difference (LSD) method was applied for multiple comparisons to determine whether the differences between group means were statistically significant. Dose–response relationship fitting was carried out using Origin 2023.

RESULTS AND DISCUSSION

Evaluation of the dose-response relationship of SAR on MDA in plants

MDA is a key product of membrane lipid peroxidation, and its accumulation can worsen membrane damage. Consequently, MDA content is frequently utilized as an indicator in studies of plant aging physiology and resistance physiology. It serves to assess the extent of membrane lipid peroxidation and indirectly gauge the level of damage to the membrane system and the plant's stress resistance. After 50 days of SAR stress, the effects on MDA levels in 18 plant species were exhibited as varying patterns, which could be categorized into four types: promotion, non-affection, low promotion-high suppression, and suppression.

In promotional type, the exposure to SAR resulted in a reduction of MDA by over 10% in 4 plant species—*K. japonica, C. indica, L. lucidum,* and *P. heterocycla*—compared to the control, with statistically significant differences (as shown in Fig. 3). Based on the results, the aforementioned plants demonstrate a degree of stress resistance and can actively cope with adverse conditions under the SAR tests (pH>2.0). They have elevated the activity of antioxidant enzymes or synthesized more non-enzymatic antioxidants to enhance membrane system stability and mitigate the adverse effects of membrane lipid peroxidation on plants, leading to a decrease in MDA content in leaves.

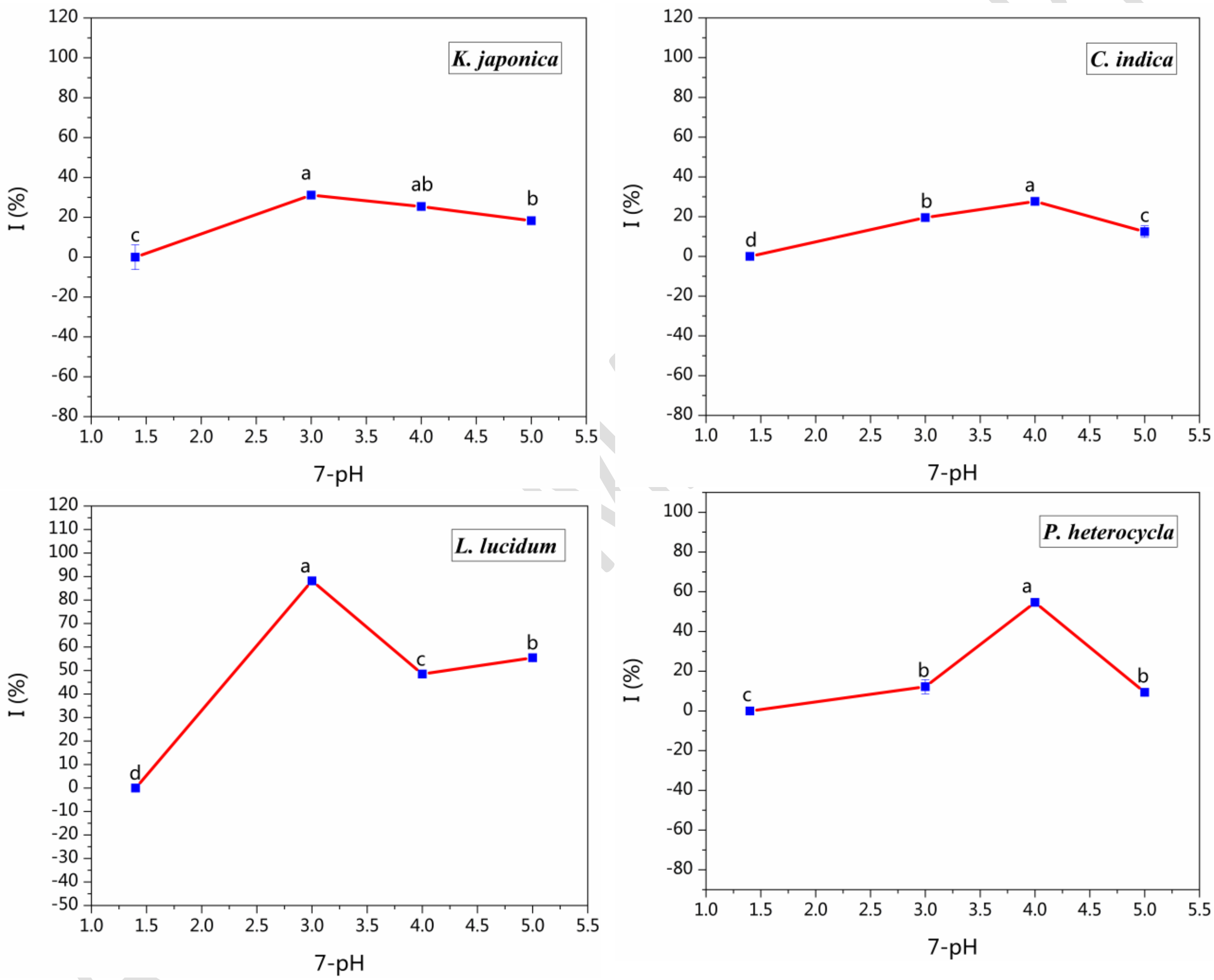
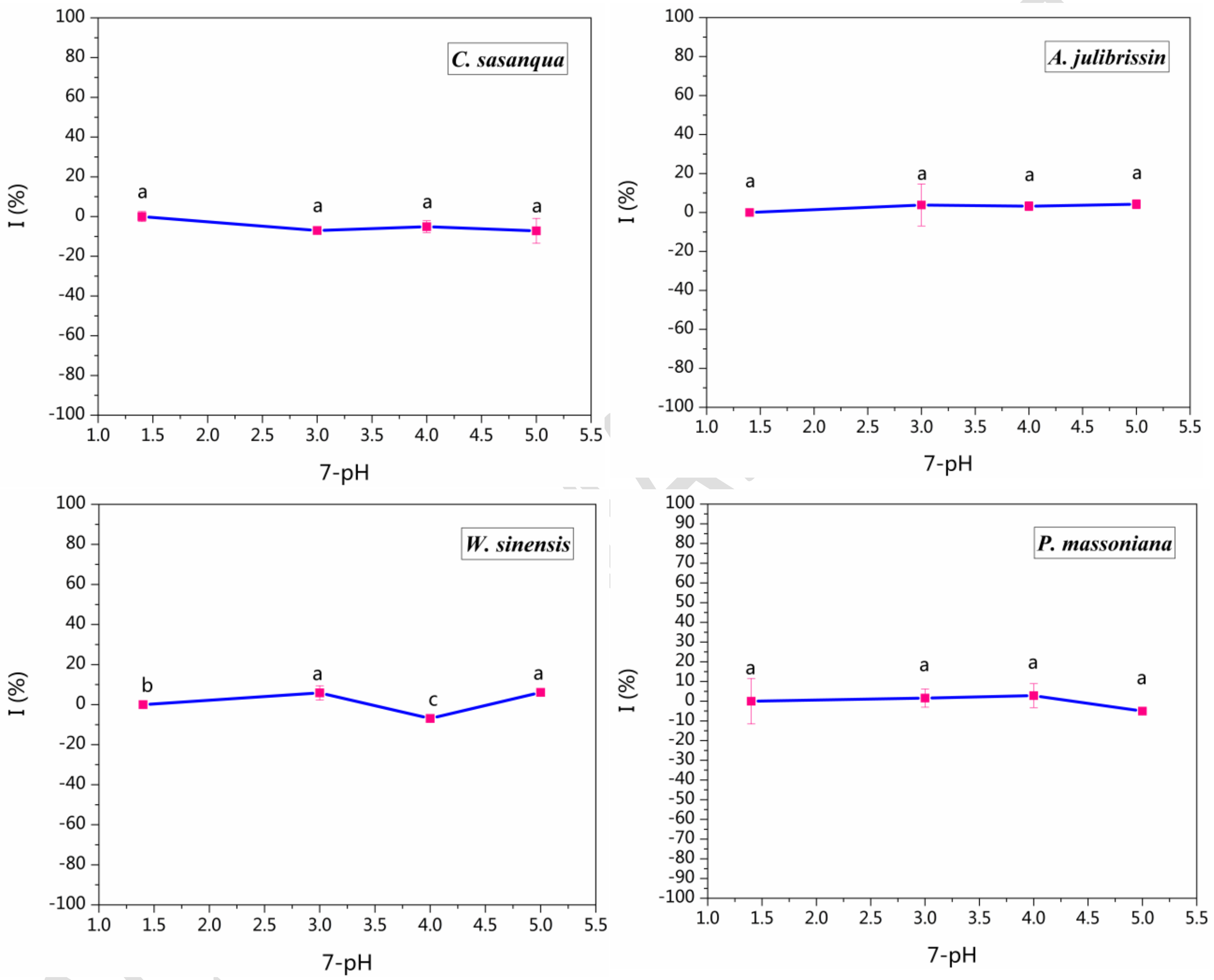


Figure 3. Dosage–response relationship showing promotion of SAR on MDA.

In the unaffected category, the MDA content of 8 plant species— *C. sasanqua, A. julibrissin, W. sinensis, P. massoniana, M. denudata, R. simsii, C. camphora,* and *B. papyrifera*—under SAR conditions showed no significant variation compared to the control (Fig. 4).

These results indicate that, within the SAR pH levels used in this study (pH≥2.0), SAR does not significantly affect the MDA content in the leaves of these species. Alternatively, plants may actively activate protective mechanisms and promptly eliminate adverse effects.



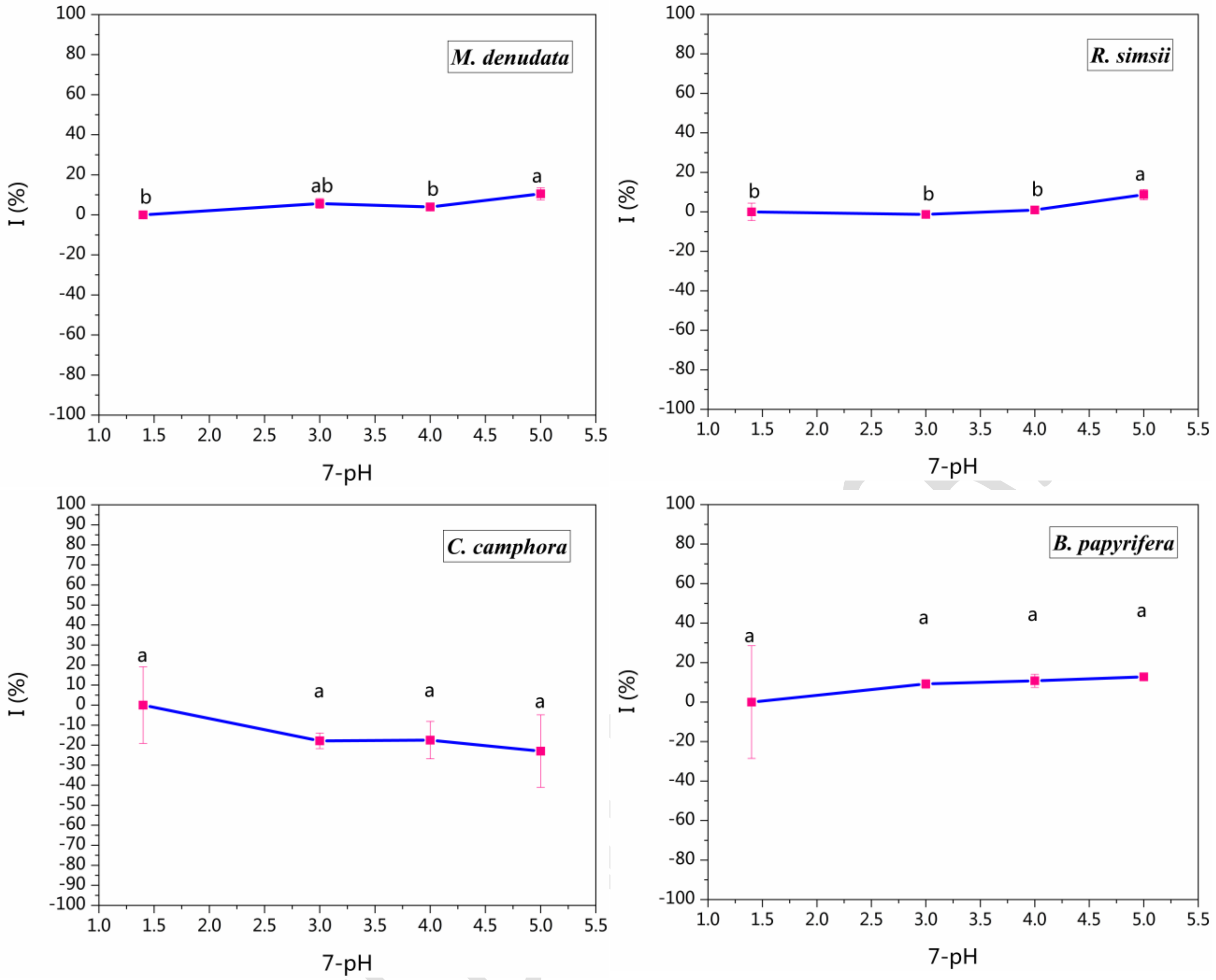


Figure 4. Dosage-response relationship showing non-affection of SAR on MDA.

In the low promotion-high suppression type, SAR at pH≥3.0 significantly reduced the content of MDA in leaves compared to the control, as shown in the inhibition rate (Fig. 5). Conversely, SAR at pH<3.0 resulted in an increase in MDA content in the plants compared to the control, causing the inhibition rate to shift from positive to negative. These findings indicate that SAR with lower acidity enhances the self-regulation ability of plants, resulting in a significant reduction in MDA levels in the tested plants. On the other hand, SAR with higher acidity exacerbates lipid peroxidation and elevates MDA content in plant cell membranes.

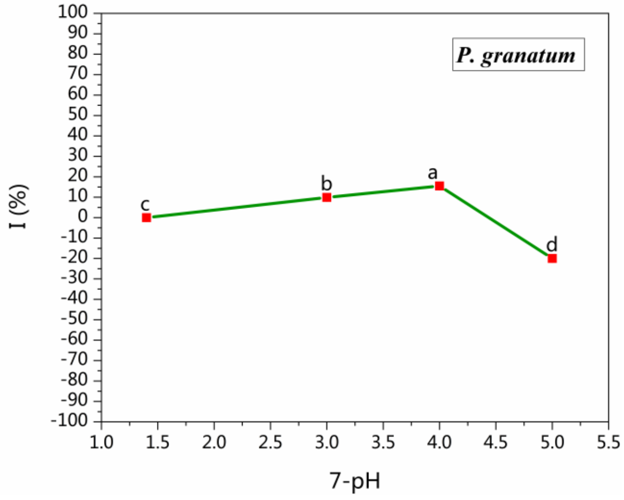
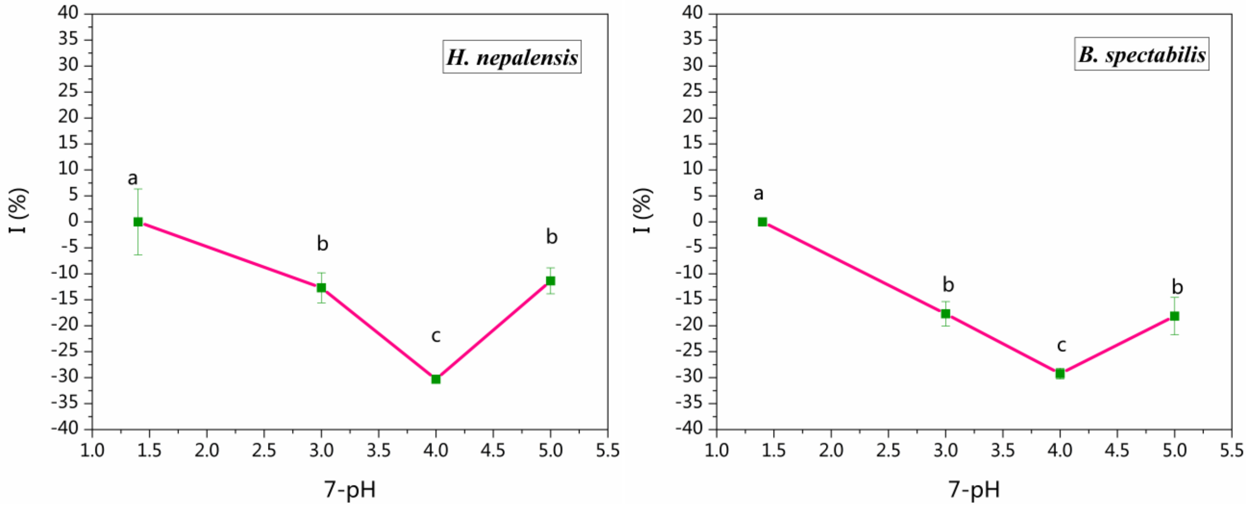


Figure 5. Dosage–response relationship showing low promotion-high suppression of SAR on MDA.

In the suppression category, treatment with SAR led to a significant increase in the suppression of MDA content in the leaves of 5 plant species—*H. nepalensis, B. spectabilis, G. biloba, P. fortuneana*, and *M. basjoo*,—compared to the control, as evidenced by negative inhibition rates (Fig. 6). These findings indicate that SAR contributes to aggravating lipid peroxidation in the cell membrane of the aforementioned plants, severely damaging the cell membrane and increasing MDA content.



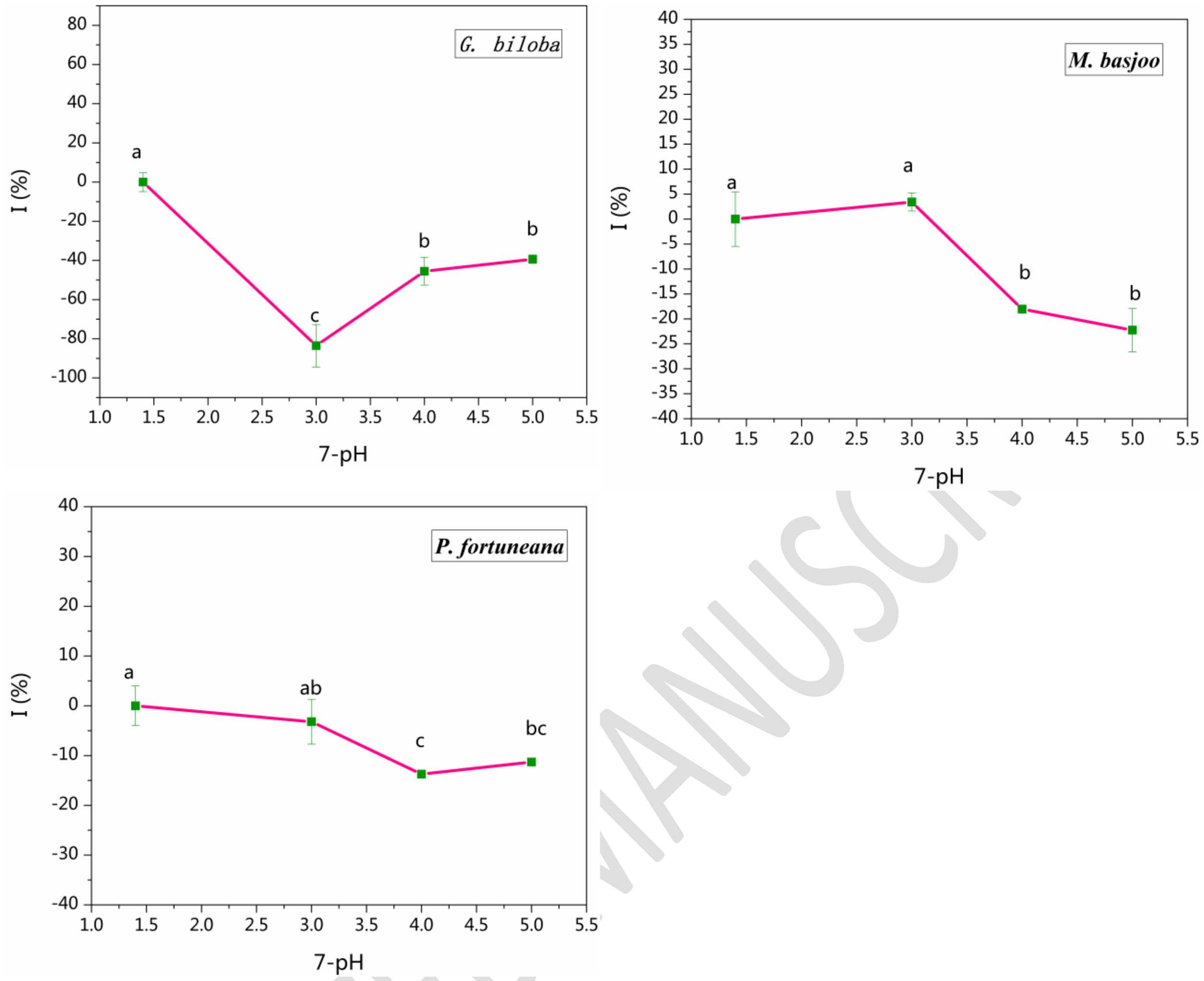


Figure 6. Dosage–response relationship showing suppression of SAR on MDA.

Evaluation of the toxic threshold of SAR on MDA content in plants

This study employed a pH value of SAR that induces a 10% increase in MDA content relative to the control (EC_{10}) as the toxicity threshold. EC_{10} values were established based on the dose-response relationships associated with SAR stress (Table 1). For the 12 plant species categorized as exhibiting promotion or non-affection responses, which did not exhibit suppression effects within the study's pH range, the EC_{10} was set to pH<2.0. For plants in the low promotion-high suppression category—such as *P. granatum*—and those in the suppression category—such as *M. basjoo, H. nepalensis, P. fortuneana, B. spectabilis,* and *G. biloba*— EC_{10} values were calculated using the methods and formula outlined in this study.

Table 1. EC_{10} of SAR on MDA of 18 Types of Plants.

Plants Name	EC_{10} pH level	PlantsName	EC_{10} pH level
K. japonica	<2.0	*R. simsii*	<2.0
C. indica	<2.0	*C. camphora*	<2.0
L. lucidum	<2.0	*B. papyrifera*	<2.0
P. heterocycla	<2.0	*P. granatum*	2.3
C. sasanqua	<2.0	*M. basjoo*	3.4
A. julibrissin	<2.0	*P. fortuneana*	3.4
W. sinensis	<2.0	*H. nepalensis*	4.3
P. massoniana	<2.0	*B. spectabilis*	4.7
M. denudata	<2.0	*G. biloba*	5.4

This study proposed the toxicity threshold of plants to SAR stress by measuring the content of MDA in plants and employing dose-response relationship fitting analysis. This approach provides a novel method for evaluating plant tolerance to acid rain.

The significant correlation between MDA content and acid rain resistance provides a basis for further investigation into plant response mechanisms. However, as a single indicator, MDA does not fully reflect the plant's tolerance to acid rain, as the plant's ability to resist acid rain is influenced by multiple factors.

In general, the analysis results for *C. sasanqua, C. camphora,* and *B. papyrifera* were consistent among the seven overlapping plant species when compared to the net photosynthetic rate. Additionally, the results for

K. japonica and *C. indica* were consistent among the twelve overlapping plant species when compared to total chlorophyll content.

In summary, the observed dose-response relationships were generally in line with those reported in this study, further confirming that SAR significantly affects total MDA content in plants.

The present study not only aligns with but also extends previous research on plant responses to acid rain. For instance, the strong tolerance of *K. japonica* and *C. indica* corroborates the dose-response patterns in chlorophyll content reported by Gou et al. (2021). Conversely, the marked sensitivity of *G. biloba* and *H. nepalensis*, indicated by their high EC_{10} values, is consistent with previous observations of visible leaf damage under acidic conditions (Silva et al., 2005). Furthermore, the variability in MDA response types among species underscores the importance of a multi-indicator approach for a comprehensive assessment of acid rain tolerance, as has been emphasized in previous research (Gou et al., 2025).

Building upon this rationale for a multi-faceted assessment, given that different indicators have varied responses to acid rain stress, future research needs to combine a comprehensive analysis using multiple indicators to provide a more accurate theoretical basis for predicting plant toxicity thresholds under acid rain stress.

CONCLUSION

This study robustly assessed the effects of acid precipitation on different plant species across a range of pH levels. Key insights derived from this way include: 1) MDA content in plant leaves serves as an effective indicator for assessing the dose-response relationship between SAR and plants; 2) SAR causes both increases and decreases in MDA content in plant leaves, indicating that plants adaptive response mechanisms to the effects of SAR.; 3) among the 18 plant species exposed to SAR, the toxicity thresholds (EC_{10}) for *K. japonica, C. indica, L. lucidum, P. heterocycla, C. sasanqua, A. julibrissin, W. sinensis, P. massoniana, M. denudata, R. simsii, C. camphora,* and *B. papyrifera* were determined to be pH<2.0. These species are identified as tolerant to acid rain and are therefore recommended for use in ecological restoration or greening projects in acid rain-affected regions of southwestern China.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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