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Germination and antioxidant response to butachlor in *Vallisneria natans* seeds

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Research Article

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Abstract

Butachlor is a herbicide extensively employed in rice (*Oryza sativa* L.) cultivation but historically under-investigated for its toxicological impacts on terrestrial vegetation. This study examines the dose-dependent effects of butachlor on the germination and antioxidant defense mechanisms in the seeds of Asian tape grass [*Vallisneria natans* (Lour.) H. Hara], an important submerged plant species widely distributed in the agricultural ponds. In a hydroponic setup, seeds were exposed to four concentrations of butachlor (0, 20, 200, and 2,000 μg ai L^{-1}), and cultivated under controlled light conditions to quantify germination rates and assess oxidative stress responses. Our findings showed that butachlor concentrations up to 20 μg L^{-1} had no effect on the germination rate of *V. natans* seeds, while germination rates decreased by 6.0% and 8.7% at 200 and 2,000 μg L^{-1}, respectively. At 2,000 μg L^{-1}, malondialdehyde (MDA) content increased by 5.7 nmol g^{-1} FW, and catalase (CAT) activity declined by 21%, indicating oxidative damage. Additionally, the antioxidants proline (Pro) and glutathione (GSH) were upregulated under 20 μg L^{-1} butachlor treatment after 12 h, contributing to reactive oxygen species (ROS) scavenging and cellular stability. This study highlights the nuanced interactions between butachlor exposure and the antioxidant defenses in *V. natans*, providing valuable insights into the ecological impacts of herbicide pollution. Understanding these interactions is crucial for development of sustainable agricultural practices and management of herbicide resistance in aquatic systems.

Introduction

Over the past few decades, herbicides have been widely used to enhance global agricultural productivity and ensure food security to meet the demands of a growing population (Tilman et al. 2002). However, the extensive use of herbicides has also led to considerable environmental challenges. China, as the largest user of herbicides worldwide, applies quantities that far exceed the global average (Sharma et al. 2019). In the agricultural ecosystem, herbicides do not degrade completely over time, leaving substantial residues in the soil (Froger et al. 2023; Zhang et al. 2022). These residues are frequently mobilized by rainfall, leading to their dispersion through environmental pathways, including atmospheric deposition, surface runoff, and leaching into groundwater, ultimately accumulating in freshwater ecosystems (He et al. 2023). The persistence and widespread presence of herbicides in aquatic ecosystems pose significant risks to the health and functioning of these systems. Non-target organisms, including microorganisms, aquatic plants, animals, and even humans, are vulnerable to herbicide exposure, which may disrupt ecological processes and biodiversity (Parrón et al. 2011). Specifically, herbicides can severely impair the germination and growth of aquatic plant seeds, threatening the vital ecological functions they provide (Zhang et al. 2024).

Submerged aquatic plants play a fundamental role in maintaining the ecological balance of lake ecosystems, particularly in shallow lakes. As primary producers, submerged plants enhance dissolved oxygen levels by releasing oxygen through photosynthesis, which is essential for sustaining aquatic life (Pedersen et al. 2013). Additionally, submerged plants possess excellent nutrient uptake ability, enabling them to accumulate heavy metals and degrade organic pollutants from lake water and sediment, thereby underscoring their high ecological value and widespread use in ecological restoration projects (Ersoy et al. 2020; Jiang et al. 2012; Sand-Jensen and Borum 1991). Moreover, submerged plant seeds are indispensable for maintaining plant populations and ensuring their resilience to environmental stressors, further amplifying their importance in lake ecosystems (O'Donnell et al. 2016; Zhao et al. 2017).

Butachlor, an acetanilide herbicide, is extensively used in rice (*Oryza sativa* L.) cultivation in China due to its high efficiency and cost-effectiveness (Lin et al. 2021). Despite its agricultural benefits, butachlor's strong hydrophobicity and low leaching potential result in its adsorption to soil particles, but it can still accumulate in water bodies through surface runoff or sediment resuspension. This characteristic, combined with its toxicity to non-target organisms, raises environmental concerns (Jolodar et al. 2021). Recent studies have shown that herbicides can induce hormetic effects in plants, stimulating growth at low concentrations and inhibiting it at higher doses (Belz and Duke 2014). These responses may affect plant physiology and species interactions, especially in variable aquatic environments. Chloroacetamide herbicides like butachlor are often applied with safeners such as fenclorim to reduce crop toxicity by enhancing detoxification via glutathione (GSH) *S*-transferases (Scarponi et al. 2006). However, the ecological effects of such safener–herbicide combinations on non-target aquatic plants remain poorly understood. Previous studies have highlighted the highly toxic mechanisms of butachlor toward various organisms (Abigail et al. 2015), yet its specific impacts on the germination and early growth of aquatic plants remain insufficiently understood. Given the ecological importance of aquatic plants in agro-aquatic systems, understanding how butachlor influences their germination is critical for assessing its broader environmental impact and informing sustainable herbicide management strategies.

The use of butachlor poses significant risks to freshwater vegetation. Even at minimal concentrations (as low as 0.0001 mg L^{-1}), butachlor has been shown to severely inhibit the growth of submerged plant species such as coontail (*Ceratophyllum demersum* L.), western waterweed [*Elodea nuttallii* (Planch.) H. St. John], and Asian tape grass [*Vallisneria natans* (Lour.) H. Hara] (Pan et al. 2009). At higher concentrations (>1.06 mg L^{-1}), butachlor becomes highly toxic, negatively affecting the dry matter accumulation and growth of floating aquatic plants such as water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and water lettuce (*Pistia stratiotes* L.) (Huang et al. 2024). Additionally, butachlor exposure reduces chlorophyll content in *V. natans* and *E. nuttallii*, severely impairing their photosynthetic efficiency (Abigail et al. 2015). This herbicide also inhibits enzymatic activities crucial for plant stress response, such as peroxidase activity in common duckweed (*Lemna minor* L.), which compromises the plant's ability to detoxify reactive oxygen species (ROS) under stress (Gomes et al. 2022). These findings illustrate the pervasive and detrimental impacts of butachlor on submerged plant physiology, raising concerns about its broader ecological implications in lake ecosystems.

Herbicides are known to affect non-target plant germination and growth, but their impacts vary depending on concentration, exposure duration, and species sensitivity. In aquatic systems, compounds like glyphosate have been shown to suppress seed germination and elevate oxidative stress markers such as malondialdehyde (MDA) and proline (Pro), while reducing antioxidant enzyme activities (Huang et al. 2020). In contrast, some terrestrial studies report stimulatory effects under certain conditions, reflecting species- and compound-specific responses (Shen et al. 2022; Yin et al. 2023).

However, most research has focused on terrestrial plants or non-target aquatic organisms, with limited attention to submerged macrophytes such as *V. natans* (Zhang et al. 2024). As a keystone species in shallow freshwater ecosystems, *V. natans* helps stabilize sediments, cycle nutrients, and purify water due to its strong adaptability and ability to absorb suspended particles and nutrients. It is widely distributed and plays an important role in reducing eutrophication. Despite its ecological value, little is known about how herbicides affect *V. natans* during seed germination. Butachlor, a common amide herbicide used in rice farming, is persistent in the environment (30 to 60 d half-life) and toxic to aquatic organisms (Abigail et al. 2015), but its impact on the antioxidant system of *V. natans* seeds remain poorly understood. Given that *V. natans* seeds have a narrow obovoid shape and glandular hairs, they may exhibit distinct responses to butachlor-induced stress, highlighting the need for further investigation. This study aims to fill this gap by addressing the following research questions: (1) Does butachlor reduce the germination rate of *V. natans* seeds? (2) How do varying concentrations of butachlor influence the activities of oxidative enzymes, antioxidants, and oxidative products in these seeds? (3) Is there a correlation between butachlor's impact on the antioxidant system and changes in germination rates? This work seeks to clarify the ecological impacts of butachlor in aquatic ecosystems, offering insights to support sustainable agricultural practices and strengthen environmental protection, ultimately promoting both ecological and agricultural sustainability.

Materials and Methods

The experiments were conducted using dormancy-broken seeds of *V. natans*. The seeds were procured in April 2023 from Xin Daye Yemucao Seed Industry, located in Hubei Province, China.

Germination Experiment

The experiment was conducted under controlled laboratory conditions in May 2023. Before the experiment, the *V. natans* seeds were surface sterilized using a 5% sodium hypochlorite solution for 5 min, followed by thorough rinsing with deionized water (4 to 5 times) to remove residual disinfectant. For the germination assay, 50 *V. natans* seeds were immersed in 10 ml of prepared butachlor solutions at concentrations of 2, 20, 200, and 2,000 μg L^{-1}, with a control treatment (0 μg L^{-1}) using deionized water. These concentrations represent a gradient of environmentally and toxicologically relevant exposure levels: 20 μg L^{-1} reflects typical butachlor concentrations detected in paddy field drainage water (Alhassan et al. 2025); 200 μg L^{-1} simulates peak runoff concentrations during intense rainfall events (Imaizumi et al. 2018); and 2,000 μg L^{-1} represents an extreme pollution scenario used in threshold-effect assessments, consistent with concentrations applied in worst-case ecotoxicological studies (Onwona-Kwakye et al. 2020). The germination experiment was carried out according to the method applied by Zhao et al. (2017). The seeds and the solutions were added to a 6-well transparent microplate (Corning Inc., Corning, NY, USA), which was placed in a constant-temperature incubator set to 25 ± 1 C with a 12-h:12-h light/dark cycle. Each treatment was replicated three times. Seed germination was recorded daily at 10:00 AM, and the experiment was terminated when no additional seed germination was observed for 3 consecutive days. In this study, germination ability was evaluated by germination percentage and germination index. Germination percentage was calculated using the following equation:

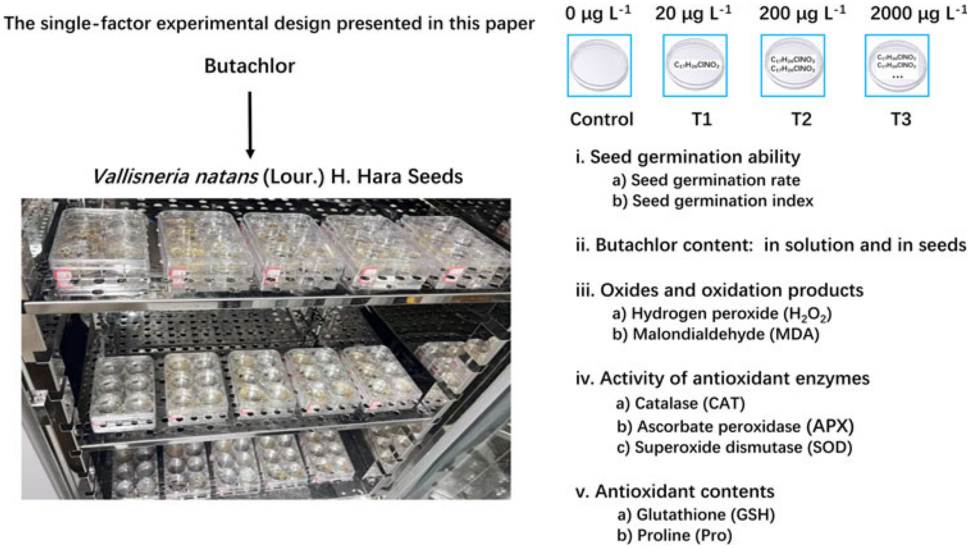

Figure 1. Experimental design.

$$\text{Germination percentage} = \frac{n}{N} \times 100\% \qquad (1)$$

where n is the total number of germinated seeds, and N is the total number of test seeds. Germination index was calculated using the following equation:

$$\text{Germination index} = \sum \frac{Gt}{Dt} \qquad (2)$$

where Gt is the number of germinated seeds at t days and Dt is the number of days required for germination.

Assessment of the Physiological Effects of Butachlor on Vallisneria natans Seeds

To investigate the physiological effects of butachlor on *V. natans* seeds, approximately 1.5 g of seeds (≈1,500 seeds) were immersed in 10 ml of butachlor solutions at concentrations of 0 (control), 20, 200, and 2,000 μg L^{-1}. The solutions were prepared using deionized water and distributed in a 6-well transparent microplate (Corning). Each treatment was replicated three times. Additionally, a seed-free control (without seeds) was included to assess butachlor degradation in the absence of plant material. All microplates were placed in a constant-temperature incubator set to 25 ± 1 C with a 12-h:12-h light/dark cycle for 3 d. At 12, 24, 48, and 72 h, microplates corresponding to each treatment were removed from the incubator. A subsample of seeds (approximately 0.2 g) was blotted dry with sterile filter paper and immediately weighed, with three replicates per concentration. The seeds were then transferred to 2-ml cryogenic tubes and stored at −80 C for subsequent biochemical analysis. Additionally, 5 ml of the culture solution was collected from each well, including those from the seed-free control group, for immediate quantification of butachlor concentration. From the preserved samples, one representative set was selected for determining butachlor accumulation within the seeds.

At 12, 24, 48, and 72 h, all samples were collected for the measurement of antioxidant indicators, including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), GSH, Pro, MDA, and hydrogen peroxide (H_2O_2). Each indicator was analyzed separately. A summary of the single-factor experimental design is presented in Figure 1.

Determination of Butachlor in Solution and Seed

The concentration of butachlor in the culture solution and *V. natans* seeds was determined using gas chromatography, following the method of Tsuda et al. (1997). For solution analysis, 5 ml of the sample was mixed with 5 ml of *n*-hexane and vortexed for 5 min, and the upper phase was collected. The solvent was evaporated to dryness using a solvent evaporation workstation, reconstituted with 1 ml of *n*-hexane, vortexed for 30 s, and filtered before being injected into a 2-ml chromatography vial for gas chromatographic analysis. For seed analysis, a 0.2-g seed sample was ground in a mortar with 5 ml of water and transferred into a centrifuge tube, and then extracted with 5 ml of *n*-hexane by vortexing for 5 min. The upper phase was processed as described for solution samples before gas chromatographic analysis.

Determination of H_2O_2 and MDA Contents

After exposure to butachlor for 12, 24, 48, and 72 h, 0.2 g of seeds from each treatment were harvested, thoroughly rinsed with deionized water, and blotted dry. To determine H_2O_2 content, the seeds were homogenized in 2 ml of 1% trichloroacetic acid (TCA) on an ice bath, and the resulting extract was centrifuged at 12,000 × *g* for 15 min at 4 C. The supernatant was collected and mixed with 0.5 ml of phosphate-buffered saline and 1 ml of potassium iodide. The absorbance at 390 nm was measured using a spectrophotometer, and the H_2O_2 concentration was calculated by comparing the absorbance values to a standard calibration curve (Velikova et al. 2000).

To determine MDA content, a total of 0.2 g seeds were homogenized in a prechilled mortar with 2 ml of 10% TCA on an ice bath to form a uniform homogenate. The homogenate was transferred to a centrifuge tube and centrifuged at 12,000 × *g* for 10 min at 4 C, and the resulting supernatant was collected as the enzyme extract. For the assay, 1.5 ml of enzyme extract was mixed with 1.5 ml of 0.6% thiobarbituric acid (TBA), thoroughly vortexed, and incubated in a boiling water bath for 15 min. The total MDA

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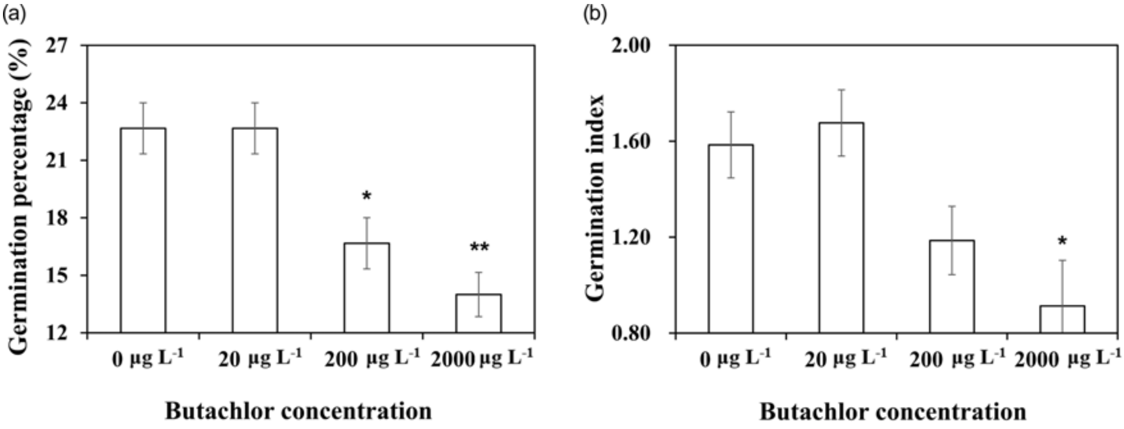

Figure 2. The germination ability of seeds of *Vallisneria natans* under different concentrations of butachlor after 14 d: (A) germination percentage and (B) germination index. Significant differences from the control are indicated by * ($P < 0.05$) and ** ($P < 0.01$). $n = 3$. Error bars represent the standard deviation.

content was determined using the TBA method (Heath and Packer 1968).

Determination of Antioxidant Enzymes

To determine the activities of antioxidant enzymes, a total of 0.2 g of seeds was homogenized in a prechilled mortar with 2 ml of ice-cold 50 mmol L^{-1} sodium phosphate buffer (pH 7.8). The homogenate was then transferred to a centrifuge tube and centrifuged at 12,000 × *g* for 20 min at 4 C. The supernatant was collected as the enzyme extract. The 0.2 ml of the enzyme extract was used to determine CAT activity by monitoring the changes in H_2O_2 concentration at 240 nm according to the method outlined by Zhang and Kirkham (1996). For the SOD activity, 0.05 ml of enzyme extract was used to determine the enzyme's capacity to inhibit the photochemical reduction of nitroblue tetrazolium at 560 nm, as described by Zhang and Kirkham (1996). To assess APX activity, 0.1 ml of enzyme extract was used, and the change in ascorbic acid (ASA) concentration was measured at 290 nm, following the method of Nakano and Asada (1981).

Determination of Pro and GSH Contents

To determine the Pro and GSH contents, a total of 0.2 g of seeds was homogenized in a prechilled mortar with 2 ml of 5% sulfosalicylic acid on an ice bath to obtain a uniform homogenate. The homogenate was transferred to a centrifuge tube and centrifuged at 12,000 × *g* for 20 min at 4 C. The resulting supernatant was collected as the enzyme extract. For GSH quantification, 0.05 ml of enzyme extract was analyzed based on the reaction of ferric ion, ASA, and bathophenanthroline at 412 nm, following the method of Griffith (1980). Similarly, Pro content was determined using 0.05 ml of enzyme extract, based on its reaction with triketohydrindene hydrate at 520 nm, following the method of Bates et al. (1973).

Data Analyses

Statistical analyses were conducted in IBM SPSS Statistics (Armonk, NY, USA). A one-way ANOVA was used to assess the differences in butachlor accumulation in *V. natans* seeds, residual butachlor concentration in solution, seed germination parameters, and physiological indicators among different butachlor concentrations (0, 20, 200, and 2,000 μg L^{-1}) at each time point (12, 24, 48, and 72 h). A repeated-measures ANOVA was conducted to evaluate the temporal variations in butachlor accumulation, residual butachlor concentration, germination parameters, and physiological indicators under different butachlor treatments (0, 20, 200, and 2,000 μg L^{-1}) throughout the experimental period. Additionally, Pearson correlation analysis was performed to examine the relationships among butachlor accumulation in seeds, residual butachlor concentration in solution, seed germination parameters, and physiological indicators. The confidence limits were set at 95%.

Results and Discussion

Changes in Germination Ability of Vallisneria natans Seeds

Germination percentages dropped to 23%, 23%, 17%, and 14% at 0, 20, 200, and 2,000 μg butachlor L^{-1}, respectively (Figure 2A). The germination index also decreased to 1.6, 1.7, 1.2, and 0.9 at the same herbicide concentrations (Figure 2B). The greatest reductions in these parameters compared with the control were observed at 200 and 2,000 μg L^{-1}. The seed germination percentage was lower than that of the control (0 μg L^{-1}) at butachlor concentrations of 200 and 2,000 μg L^{-1}. Similarly, the seed germination index at butachlor concentrations of 2,000 μg L^{-1} was lower than that of the control.

Changes in Butachlor Content in Vallisneria natans Seeds and in the Solution

Butachlor concentrations in *V. natans* seeds increased to 109, 330, and 267 μg kg^{-1} after 72-h exposure to 20, 200, and 2,000 μg L^{-1}, respectively (Figure 3A). Residual butachlor in the solution decreased to 2.3, 5.5, and 39 μg L^{-1} at the same exposure levels (Figure 3B). The highest accumulation in seed tissue and corresponding decline in aqueous butachlor levels were observed at 200 and 2,000 μg L^{-1}. Seed butachlor content was markedly higher than in the control (0 μg L^{-1}) at 200 and 2,000 μg L^{-1}, while the residual concentrations in solution were lower than initial levels.

Changes in H_2O_2 and MDA Contents in Vallisneria natans Seeds

After 12-h exposure, H_2O_2 content in seeds was 0.6, 0.5, 0.6, and 0.7 μmol g^{-1} FW at 0, 20, 200, and 2,000 μg butachlor L^{-1},

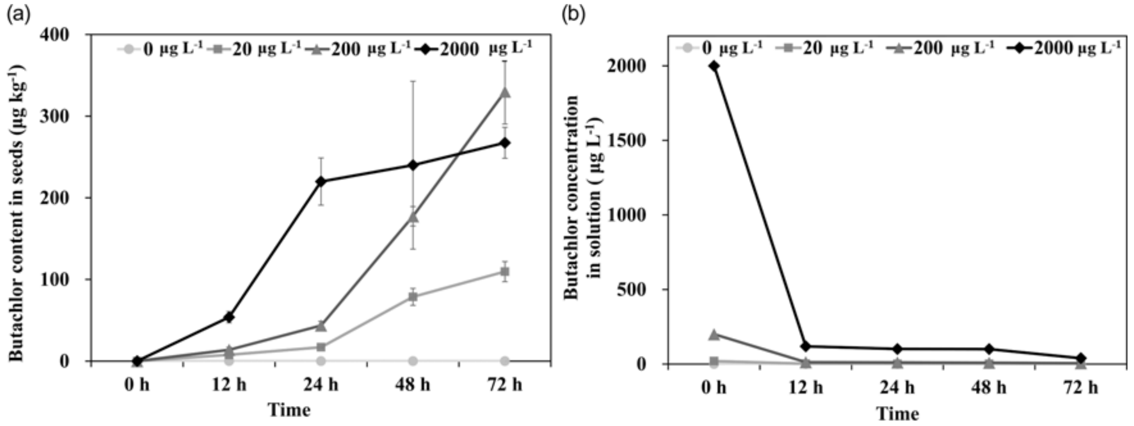

Figure 3. The butachlor content in *Vallisneria natans* seeds and in the solution under different butachlor concentrations: (A) butachlor content in seeds (μg kg^{-1}) and (B) butachlor concentration in solution (μg L^{-1}). Significant differences from the control are shown by * ($P < 0.05$) level compared with control; ** ($P < 0.01$). $n = 3$. Error bars represent the standard deviation.

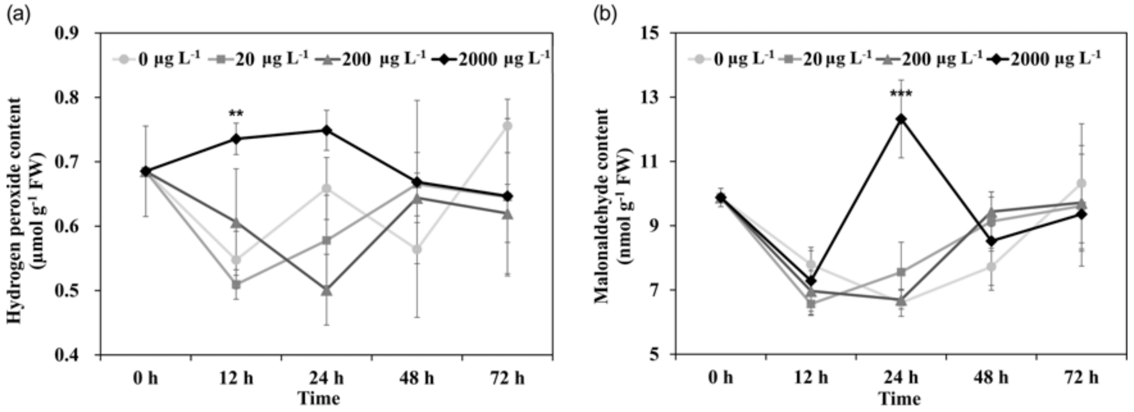

Figure 4. The hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in *Vallisneria natans* seeds under different concentrations of butachlor and exposure time: (A) H_2O_2 content (μmol g^{-1} FW) and (B) MDA content (nmol g^{-1} FW). Significant differences from the control are shown by * ($P < 0.05$) level compared with control; ** ($P < 0.01$). $n = 3$. Error bars represent the standard deviation.

respectively (Figure 4A). MDA content after 24 h exposure was 6.6, 7.6, 6.7, and 12.3 nmol g^{-1} FW at the same concentrations (Figure 4B). The greatest increases in both parameters compared with the control were observed at 2,000 μg L^{-1}. H_2O_2 content at 2,000 μg L^{-1} was 0.1 μmol g^{-1} FW higher than the control. Similarly, MDA content under 2,000 μg L^{-1} was 5.7 nmol g^{-1} FW higher than that of the control.

Changes in Activities of Antioxidant Enzymes in Vallisneria natans Seeds

The activity of CAT in *V. natans* seeds after 12 h was 31, 31, 33 and 9.7 U min^{-1} g^{-1} FW at 0, 20, 200, and 2,000 μg butachlor L^{-1}, respectively (Figure 5A). The activity of APX in *V. natans* seeds also changed to 48, 47, 58, and 16 U $min^{-1}g^{-1}$ FW under the same treatment conditions (Figure 5B). The greatest reductions in both enzyme activities compared with the control were observed at 2,000 μg L^{-1}. In contrast, SOD activity showed no differences across butachlor concentrations (Figure 5C).

Changes in GSH and Pro Contents in Vallisneria natans Seeds

The GSH content in seeds at the conditions of 0, 20, 200 and 2,000 μg L^{-1} after 72 hours was 14, 21, 17 and 15 μmol g^{-1} FW respectively (Figure 6A). The greatest increase compared with the control was observed at 20 μg L^{-1}, where GSH content was 6.5 μmol g^{-1} FW higher. There was no difference in the Pro content of *V. natans* seeds in response to different concentrations of butachlor (Figure 6B).

Relationship between Physiological Indexes and Germination Ability in Vallisneria natans Seeds

Positive relationships were observed between APX activity and *V. natans* seed germination percentage (Figure 7A), as well as between APX activity and germination index (Figure 7B). The strongest positive relationships were observed at higher APX activity levels. MDA content was positively correlated with butachlor concentration in the seeds (Figure 7C) and the solution (Figure 7D). In contrast, a negative correlation was observed between H_2O_2 content and CAT activity in *V. natans* seeds (Figure 7E).

Effects of Butachlor on the Germination of Vallisneria natans Seeds

Seed germination rate is a critical indicator for assessing seed viability and the capacity of seeds to establish under varying

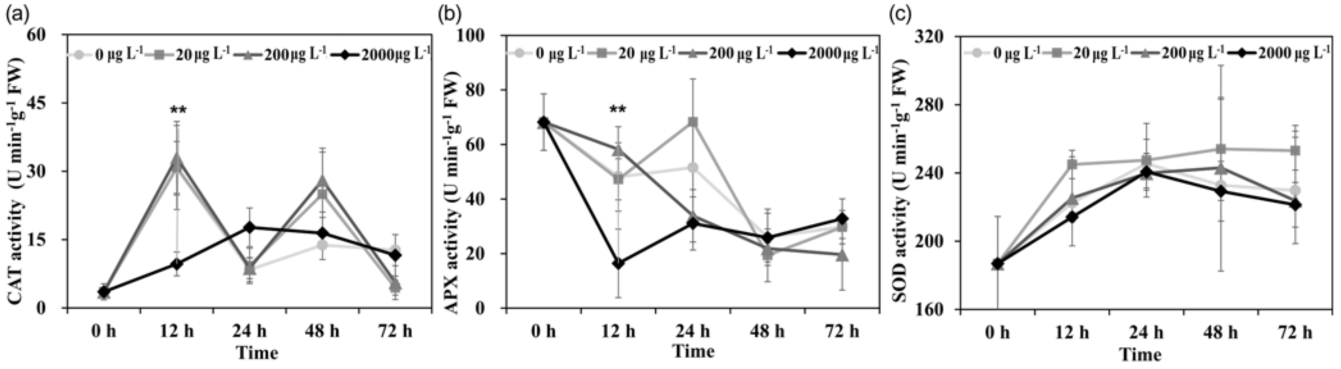

Figure 5. Activities of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) in *Vallisneria natans* seeds under different concentrations of butachlor: (A) CAT activity (U $min^{-1}g^{-1}$ FW); (B) APX activity (U $min^{-1}g^{-1}$ FW); and (C) SOD activity (U $min^{-1}g^{-1}$ FW)). Significant differences from the control are shown by * ($P < 0.05$) level compared with control; ** ($P < 0.01$). $n = 3$. Error bars represent the standard deviation.

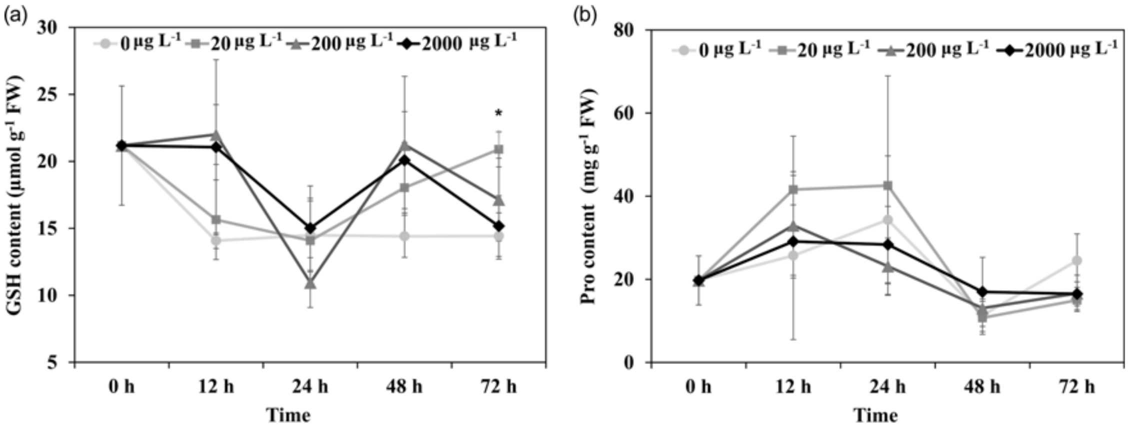

Figure 6. The glutathione (GSH) and proline (Pro) contents in *Vallisneria natans* seeds under different concentrations of butachlor: (A) GSH content (μmol g^{-1} FW) and (B) Pro content (mg g^{-1} FW). Significant differences from the control are shown by * ($P < 0.05$) level compared with control; ** ($P < 0.01$). $n = 3$. Error bars represent the standard deviation.

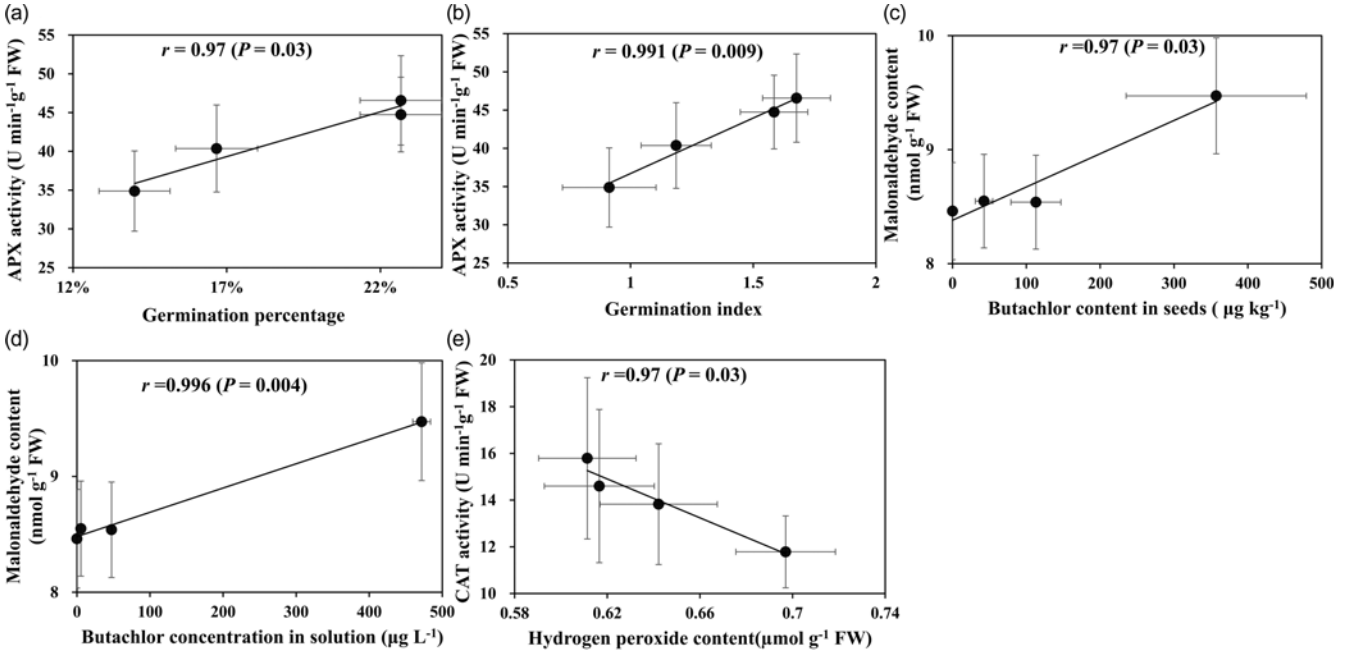

Figure 7. Relationship between physiological indices and germination ability in *Vallisneria natans* seeds: (A) ascorbate peroxidase (APX) activity vs. germination percentage; (B) APX activity vs. germination index; (C) malondialdehyde (MDA) content vs. butachlor content in seeds; (D) MDA content vs. butachlor content in solution; and (E) hydrogen peroxide (H_2O_2) content vs. catalase (CAT) activity in seeds.

environmental conditions (Wang et al. 2023). Our germination experiments revealed that seed germination rates declined to 23%, 23%, 17%, and 14%, and the germination index to 1.6, 1.7, 1.2, and 0.9, at 0, 20, 200, and 2,000 μg L^{-1} butachlor, respectively. Both indicators were inhibited at 200 and 2,000 μg L^{-1}. This dose-dependent suppression indicated that butachlor residues could impair the early developmental stages of aquatic plants. Our findings were consistent with previous studies that have documented similar herbicide-induced inhibitory effects on seed germination in terrestrial plants. For example, Kumar and Jagannath (2014) observed a reduction in both germination rates and early growth parameters in wheat (*Triticum aestivum* L.) when exposed to butachlor, with germination percentages decreasing proportionally with increasing herbicide concentrations. Further supporting evidence came from Zhang et al. (2021), who demonstrated that the germination rate, vigor, and vitality index of seeds from perennial ryegrass (*Lolium perenne* L.), alfalfa (*Medicago sativa* L.), and sainfoin (*Onobrychis viciifolia* Scop.) were suppressed following exposure to glufosinate and glyphosate. Our results demonstrated that butachlor can inhibit the germination of *V. natans* seeds, particularly at higher concentrations (200 and 2,000 μg L^{-1}). This dose-dependent response aligned with previous findings in terrestrial species, indicating that butachlor exerts a broad phytotoxic effect across plant types. The sensitivity observed in *V. natans* suggested that early developmental stages of submerged macrophytes were especially vulnerable to butachlor exposure. Such inhibition of germination under herbicide stress reduced seedling recruitment and regeneration capacity, ultimately affecting population persistence in aquatic habitats.

Effects of Butachlor on Antioxidant Product and the Antioxidant Defense System in Vallisneria natans Seeds

Plants under stress conditions produce ROS that exhibit dual roles: acting as signaling molecules at low concentrations while causing oxidative damage upon excessive accumulation (Mittler 2002). Our study demonstrated that butachlor exposure increased H_2O_2 content (0.1 μmol g^{-1} FW higher than control) and MDA levels (5.7 nmol g^{-1} FW elevation) in *V. natans* seeds, indicating oxidative stress as a key mechanism for germination inhibition. These findings align with reports on ROS-mediated seed viability reduction (McDonald 1999), while the observed activation of antioxidant enzymes such as SOD, CAT, and APX can be explained by H_2O_2-induced lipid peroxidation (Lehner et al. 2008). As a biomarker of lipid peroxidation, MDA accumulation directly reflects the degree of membrane lipid peroxidation in plants (Tripthi et al. 2020). Our study revealed that butachlor exposure disrupted redox homeostasis in *V. natans* seeds through two distinct phases: concentration-dependent effects and temporal dynamics. For concentration-dependent effects, high butachlor concentrations (2,000 μg L^{-1}) rapidly induced H_2O_2 and MDA accumulation, consistent with findings in gramineous plants (Wang et al. 2013). In contrast, low concentrations (200 μg L^{-1}) transiently activated antioxidant enzymes, leading to H_2O_2 scavenging during early exposure. For temporal dynamics, prolonged exposure (48 h) overwhelmed the antioxidant defense system, corroborating Jin et al.'s (2010) report on herbicide-induced enzymatic disruption. However, the observed late-phase MDA decline (Liu et al. 2015) suggests partial ROS homeostasis recovery capacity.

APX activity in *V. natans* seeds declined within 12 h of butachlor exposure, indicating that this herbicide inhibits APX and impairs H_2O_2 metabolism. In contrast, CAT and SOD activities increased with higher butachlor concentrations and prolonged exposure, suggesting a compensatory response to oxidative stress. These enzymes are part of the antioxidant defense system that regulates ROS levels and protects against cellular damage (Jiang et al. 2014; Passardi et al. 2004). Additionally, nonenzymatic antioxidants such as GSH also responded to butachlor stress, particularly at low concentrations, consistent with their role in maintaining redox homeostasis and mitigating oxidative damage (Mostofa et al. 2015; Singh et al. 2013). These enzymatic and nonenzymatic antioxidants function cooperatively, partly via the ascorbate–GSH cycle, to regulate ROS levels and maintain redox balance (Arias-Baldrich et al. 2017; Foyer and Noctor 2011).

Our findings showed that Pro and GSH levels increased in *V. natans* seeds in response to butachlor exposure, indicating their role in maintaining redox balance under oxidative stress. Pro levels rose after 12 h of exposure to 20 μg L^{-1} butachlor, suggesting that Pro contributes to early stress adaptation by stabilizing cellular structures and mitigating ROS-induced damage (Sharma and Dietz 2006). Similarly, GSH levels increased at 200 μg L^{-1} and higher concentrations after 12 h, supporting its role in ROS scavenging and redox regulation (Dresler et al. 2020; Noctor et al. 2012). Together, these responses reflect the involvement of both enzymatic and nonenzymatic antioxidant systems in protecting *V. natans* seeds from butachlor-induced oxidative stress.

Physiological Mechanisms of Butachlor Affecting Vallisneria natans Seed Germination

Although further studies are needed to confirm the specific signaling pathways involved, our results suggested that butachlor inhibited *V. natans* seed germination by disrupting redox homeostasis and impairing antioxidant defenses. The initial increase in SOD activity, followed by significant inhibition of APX and CAT activities, indicates an imbalance in ROS detoxification. This sequential response may lead to excess H_2O_2 accumulation, forming a "H_2O_2 trap" where ROS production exceeds scavenging capacity.

As oxidative stress intensifies, lipid peroxidation occurs, as evidenced by increased MDA levels. MDA is a marker of membrane damage, and its accumulation suggests compromised cellular integrity during early germination. Antioxidant enzyme inhibition and membrane lipid peroxidation together explain the reduced germination observed under butachlor exposure (Figure 8).

Butachlor disrupts the physiological processes of *V. natans* seeds by inhibiting key antioxidant enzymes, particularly APX and CAT, leading to the accumulation of H_2O_2. The excess H_2O_2 induces oxidative damage through membrane lipid peroxidation, as indicated by increased levels of MDA. Prolonged oxidative stress from butachlor exposure compromises membrane integrity, reduces metabolic activity, and ultimately decreases germination rates. These findings highlight the critical role of the antioxidant defense system in protecting aquatic plants from herbicide-induced oxidative damage. Future research should focus on understanding the long-term ecological impacts of butachlor and other herbicides on aquatic ecosystems, particularly their effects on plant community dynamics and ecosystem stability. Prospective studies could incorporate a more comprehensive dose range to

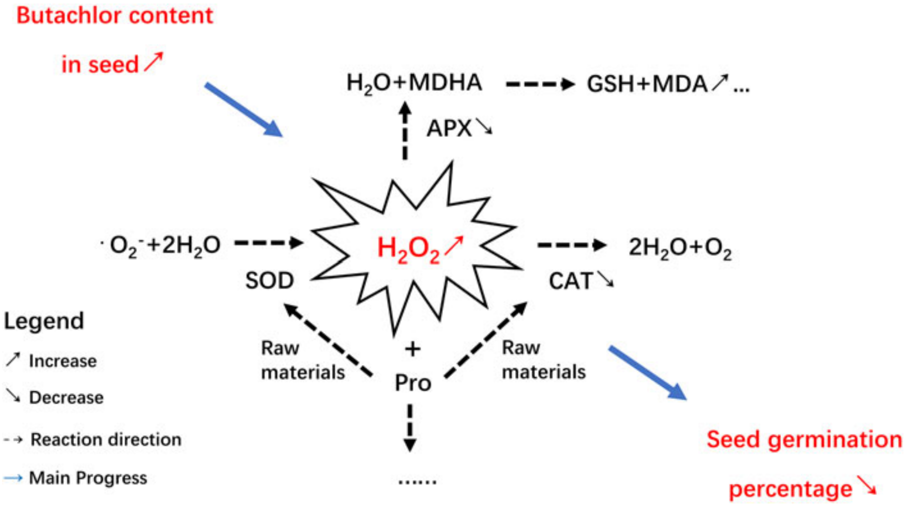

Figure 8. Mechanism of butachlor's effect on the germination of *Vallisneria natans* seeds. Abbreviations: APX, ascorbate peroxidase; CAT, catalase; GSH, glutathione; MDA, malondialdehyde; MDHA, monodehydroascorbate; O_2^-, superoxide anion; Pro, proline; SOD, superoxide dismutase.

better characterize the dose–response relationship, particularly within the 20 to 200 μg L^{-1} transition zone, while concurrently employing tetrazolium viability assays and seed histological analysis to assess impacts on seed vigor and internal structures.

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References

Abigail MEA, Samuel SM, Ramalingam C (2015) Addressing the environmental impacts of butachlor and the available remediation strategies: a systematic review. Int J Environ Sci Technol 12:4025–4036

Alhassan M, Ashong GW, Ababio BA, Kwaansa-Ansah EE (2025) Butachlor contamination in surface water and sediment: assessing human health, ecological risks, and environmental implications of the Bontanga irrigation scheme in the Northern region of Ghana. Environ Nanotechnol Monit Manag 23:101073

Arias-Baldrich C, de la Osa C, Bosch N, Ruiz-Ballesta I, Monreal JA, Garcia-Maurino S (2017) Enzymatic activity, gene expression and posttranslational modifications of photosynthetic and non-photosynthetic phosphoenolpyruvate carboxylase in ammonium-stressed sorghum plants. J Plant Physiol 214:39–47

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Belden JB, Gilliom RJ, Lydy MJ (2007) How well can we predict the toxicity of pesticide mixtures to aquatic life? Integr Environ Assess Manag 3:364–372

Belz RG, Duke SO (2014) Herbicides and plant hormesis. Pest Manag Sci 70:698–707

Dresler S, Strzemski M, Kovacik J, Sawicki J, Staniak M, Wojciak M, Sowa I, Hawrylak-Nowak B (2020) Tolerance of facultative metallophyte *Carlina acaulis* to cadmium relies on chelating and antioxidative metabolites. Int J Mol Sci 21:2828

Ersoy Z, Scharfenberger U, Baho DL, Bucak T, Feldmann T, Hejzlar J, Levi EE, Mahdy A, Noges T, Papastergiadou E, Stefanidis K, Sorf M, Sondergaard M, Trigal C, Jeppesen E, Beklioglu M (2020) Impact of nutrients and water level changes on submerged macrophytes along a temperature gradient: a pan-European mesocosm experiment. Global Chang Biol 26:6831–6851

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Froger C, Jolivet C, Budzinski H, Pierdet M, Caria G, Saby NPA, Arrouays D, Bispo A (2023) Pesticide residues in French soils: occurrence, risks, and persistence. Environ Sci Technol 57:7818–7827

Gomes MP, Kitamura RSA, Marques RZ, Barbato ML, Zamocky M (2022) The role of H_2O_2-scavenging enzymes (ascorbate peroxidase and catalase) in the tolerance of *Lemna minor* to antibiotics: implications for phytoremediation. Antioxidants 11:151

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione-reductase and 2-vinylpyridine. Anal Biochem 106:207–212

He J, Li J, Gao Y, He X, Hao G (2023) Nano-based smart formulations: a potential solution to the hazardous effects of pesticide on the environment. J Hazard Mater 456:131599

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. 2. Role of electron transfer. Arch Biochem Biophys 125:850

Huang Y, Deng C, Li J, Ye T, Wei H, Sang T, Xie W (2020) Effects of herbicides on seed germination and seedling physiological characteristics of *Festuca arundinacea* and *Dichondra repens.* Pratacultural Sci 37:872–881. Chinese

Huang Y, Zhao S, Xian L, Li W, Zhou C, Sun J (2024) Negative effects of butachlor on the growth and physiology of four aquatic plants. Plants 13:304

Imaizumi Y, Suzuki N, Shiraishi F, Nakajima D, Serizawa S, Sakurai T, Shiraishi H (2018) Development and validation of a simulation method, PeCHREM, for evaluating spatio-temporal concentration changes of paddy herbicides in rivers. Environ Sci Process Impacts 20:120–132

Jiang H-S, Li M, Chang F-Y, Li W, Yin L-Y (2012) Physiological analysis of silver nanoparticles and $AgNO_3$ toxicity to *Spirodela polyrhiza*. Environ Toxicol Chem 31:1880–1886

Jiang HS, Qiu XN, Li GB, Li W, Yin LY (2014) Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. Environ Toxicol Chem 33:1398–1405

Jin ZL, Zhang F, Ahmed ZI, Rasheed M, Naeem MS, Ye QF, Zhou WJ (2010) Differential morphological and physiological responses of two oilseed *Brassica* species to a new herbicide ZJ0273 used in rapeseed fields. Pest Biochem Physiol 98:1–8

Jolodar NR, Karimi S, Bouteh E, Balist J, Prosser R (2021) Human health and ecological risk assessment of pesticides from rice production in the Babol Roud River in northern Iran. Sci Total Environ 772:144729

Kumar NKH, Jagannath S (2014) Phytotoxic effect of butachlor 50 EC on early seed growth and biochemical parameters of Triticum aestivum L. Int J Adv Life Sci 6:425–433

Lehner A, Mamadou N, Poels P, Come D, Bailly C, Corbineau F (2008) Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. J Cereal Sci 47:555–565

Lin Z, Pang S, Zhou Z, Wu X, Bhatt P, Chen S (2021) Current insights into the microbial degradation for butachlor: strains, metabolic pathways, and molecular mechanisms. Appl Microbiol Biotechnol 105:4369–4381

Liu H, Mu P, Zhao G, Zhou X (2015) The impact of herbicides on production and antioxidant properties of oats. Acta Prataculturae Sin 24:41–48. Chinese

McDonald MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol 27:177–237

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mostofa MG, Hossain MA, Fujita M, Tran L-SP (2015) Physiological and biochemical mechanisms associated with trehalose-induced copper-stress tolerance in rice. Sci Rep 5:11433

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

Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484

O’Donnell J, Fryirs KA, Leishman MR (2016) Seed banks as a source of vegetation regeneration to support the recovery of degraded rivers: a comparison of river reaches of varying condition. Sci Total Environ 542:591–602

Onwona-Kwakye M, Hogarh JN, Van den Brink PJ (2020) Environmental risk assessment of pesticides currently applied in Ghana. Chemosphere 254:126845

Pan H, Li X, Xu X, Gao S (2009) Phytotoxicity of four herbicides on *Ceratophyllum demersum*, *Vallisneria natans* and *Elodea nuttallii.* J Environ Sci 21:307–312

Parrón T, Requena M, Hernández AF, Alarcón R (2011) Association between environmental exposure to pesticides and neurodegenerative diseases. Toxicol Appl Pharmacol 256:379–385

Passardi F, Penel C, Dunand C (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci 9:534–540

Pedersen O, Colmer TD, Sand-Jensen K (2013) Underwater photosynthesis of submerged plants—recent advances and methods. Front Plant Sci 4:140

Sand-Jensen K, Borum J (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate fresh-waters and estuaries. Aquat Bot 41:137–175

Scarponi L, Quagliarini E, Del Buono D (2006) Induction of wheat and maize glutathione S-transferase by some herbicide safeners and their effect on enzyme activity against butachlor and terbuthylazine. Pest Manag Sci 62:927–932

Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD, Dar OI, Singh K, Jasrotia S, Bakshi P, Ramakrishnan M, *et al.* (2019) Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 1:1446

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726

Shen L, Zhang S, Peng Z, Zhang X, He W (2022) Effects of glyphosate on germination and growth of *Vallisneria natans*. Asian J Ecotoxicol 17:418–426. Chinese

Singh VP, Srivastava PK, Prasad SM (2013) Nitric oxide alleviates arsenic-induced toxic effects in ridged *Luffa* seedlings. Plant Physiol Biochem 71:155–163

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Tripthi DK, Varma RK, Singh S, Sachan M, Guerriero G, Kushwaha BK, Bhardwaj S, Ramawat N, Sharma S, Singh VP, Prasad SM, Chauhan DK, Dubey NK, Sahi S (2020) Silicon tackles butachlor toxicity in rice seedlings by regulating anatomical characteristics, ascorbate-glutathione cycle, proline metabolism and levels of nutrients. Sci Rep 10:14078

Tsuda T, Kojima M, Harada H, Nakajima A, Aoki S (1997) Herbicides in water and fish from rivers flowing into lake Biwa. Toxicol Environ Chem 61:243–249

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

Wang H, Wang X, Li Y, Gao R, Rao MPN, Song J, Li Q (2023) Effect of environmental factors on seed germination and seedling emergence of *Viola prionantha*, a cleistogamous plant. J Plant Res 136:631–641

Wang S, Li H, Lin C (2013) Physiological, biochemical and growth responses of Italian ryegrass to butachlor exposure. Pest Biochem Physiol 106:21–27

Yin M, Yan K, Zhuang D, Fang D, Gu M (2023) Effect of isopropyl-methylthio-carbamate on seed germination and seedling growth of *Chenopodium quinoa*. South-Central Agricultural Science and Technology 44:248–250. Chinese

Zhang J, He J, Zhang X, Zhang S, Zhang H, Li Y (2021) Three forage extracts on seed germination and seedling growth of recipient plants under herbicide stress. Acta Agrestia Sin 29:1719–1728. Chinese

Zhang JX, Kirkham MB (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol 132:361–373

Zhang Y, Hu M, Li A (2024) Review of the occurrence of herbicides in environmental waters of Taihu Lake basin and its potential impact on submerged plants. Water 16:726

Zhang Z, Yan X, Jones KC, Jiao C, Sun C, Liu Y, Zhu Y, Zhang Q, Zhai L, Shen Z, Chen L (2022) Pesticide risk constraints to achieving sustainable development goals in China based on national modeling. NPJ Clean Water 5:59

Zhao S, Zhang R, Liu Y, Yin L, Wang C, Li W (2017) The effect of storage condition on seed germination of six Hydrocharitaceae and Potamogetonaceae species. Aquat Bot 143:49–53