

EFFECTS OF *PERILLA* STRAW FERMENTATION EXTRACT ON SEED GERMINATION AND SEEDLING GROWTH OF DIFFERENT FIELD CROPS

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Abstract

The disposal of medicinal plant residues represents a significant challenge of resource waste and environmental pollution. *Perilla frutescens* stalk ferment extract (PFSE) is a plant biostimulant obtained from fermented *P. frutescens* stalks, however, little is known about its dose response relationship and species-specific effects on major grain crops. The objective of this work was to determine the optimum PFSE concentration and compare species specific response in pea, maize, and wheat. The results showed the general “low-promotion, high-inhibition” phenomenon. Germination of pea and maize were greatly inhibited by 20 g/L while that of wheat was not so sensitive. Seedling morphology and root activity were optima at 5~10 g/L and 3~5 g/L, respectively. Physiological responses varied by crop: chlorophyll content peaked at 5 g/L in maize and pea, and at 3 g/L or 1 g/L in wheat. Proline increased at 5 g/L in all species, but the concentration optimum of soluble sugars and proteins were different. The levels of antioxidant enzymes (SOD, CAT, POD) and malondialdehyde (MDA) showed a concentration-dependent trend. At high concentrations of PFSE, oxidative damage was increased. We concluded that 5 g/L PFSE was the best dose to promote growth, with sensitivity in the order of pea>maize>wheat. These findings support PFSE as an efficient biofertilizer, which also suggests a sustainable method for using *Perilla* straw.

Key words: Straw fermentation; *Perilla frutescens*; Field crops; Seed germination; Seedling growth

Introduction

Despite China produces more than 800 million tonnes of crop straw each year, about 28% of which is still burnt or abandoned leading to serious resource waste and environment contamination, including the emission of about 1.1 Tg yr⁻¹ of PM_{2.5} (Mao *et al.*, 2023). The open burning of straw is not only source to the particulates, but also to greenhouse gases and toxic organic pollutants, posing a serious risk to the air quality or public health (Liu *et al.*, 2023). Straw is abundant with cellulose, hemicellulose, lignin and mineral nutrients, which have a huge potential of added-value utilization. In last ten years, straw valorization has been studied by researchers for different aspects; for example, co-fermentation of herb residues with straw to produce silage, provides good feedstock to be used in livestock farms, and at the same time solve the issue of medicinal waste disposal (Wu *et al.*, 2025; Bu *et al.*, 2025). Similarly, straw is used as a single carbon source for microbial fermentation and it is an important factor during the production process of bio-azotobacteria; these inoculants significantly speed up rice-straw decomposition, increasing the degradation rate by 4.9% and rice yield by 790 kg ha⁻¹ (Zhao *et al.*, 2024). All the above developments point toward a future where straw bioconversion will be an attractive, sustainable biotechnology with great potential. Notwithstanding this progress, most studies have focused on uses such as adding straw to soil or feeding it to animals, whereas straw-based bioactive fermentation product

utilization in crop priming, especially with medicinal plants, has received limited investigation.

Perilla frutescens (L.) Britt., belongs to the family Labiatae, is an annual herbaceous plant, which grows naturally in East Asia and is popularly used for food or medicinal purposes in China [Japan, Korea, Taiwan, and Vietnam (Ahmed, 2018)]. Its leaves, seeds and stem are widely used in traditional medicines with different applications (Ahmed, 2018). *Perilla* is grown over 60,000 ha in China, producing about 30,000 metric tons of the stem residues per year (Yang *et al.*, 2022) and these stems contain plenty of bioactive compounds, containing 2.8 % total polyphenols, 1.9 % flavonoids, 0.7 % rosmarinic acid and 0.6% essential oils (Yang *et al.*, 2022). Proper management of the residue has a huge opportunity to be used in resource recovery and its valorization. Recently, it has been reported that using *Lactobacillus plantarum* and *Rhizopus oligosporus* in the solid state fermentation process of *Perilla* stalk can transform these metabolites to low molecular weight phenolic acids, free amino acids, and phytohormones like IAA and trans-zeatin, which strongly increase the seed germination and antioxidant capacity in lettuce and tomato (Ezekiel *et al.*, 2024; Yaqoob *et al.*, 2025; Deng *et al.*, 2025; Yang *et al.*, 2025; Li *et al.*, 2025; Huang *et al.*, 2025). As for promoting sustainable agriculture worldwide, full exploitation of *P. frutescens* is receiving more and more attentions (Yi *et al.*, 2025; Huang *et al.*, 2025). These findings suggest that PFSE might serve as a novel bio-stimulator for promoting plant growth and production.

However, little is known about the dose–response relationship and species-specific effect of PFSE to seed vigour and seedling physiology for major grains crops. Thus, this study evaluated how various levels of PFSE from fermented *P. frutescens* stalks influenced germination parameters, morphogenetic process, antioxidant system, osmoregulation compound, lipid peroxidation of membranes with pea (*Pisum sativum* L.), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.) as the model plants. The aims of this work were (i) to find out which concentration of PFSE is most effective in promoting growth, and to understand the mechanism behind it; (ii) investigate whether stalk ferment extracts can partially replace chemical fertilizers and pesticides, thereby reducing agricultural non-point source pollution and promoting the development of green, ecological, and circular agriculture; and (iii) investigate an innovative “medicinal plant-crop” interaction model in order to synergistically attain green yield increase, stress tolerance, and better grain quality. This study can provide new ideas for sustainable agriculture input, as well as provide a theoretical and practical basis for high value utilization of medicinal plant straw in crop production system.

Materials and Methods

Plant materials: The microbial inoculum (purchased from Henan Shuanghui Agricultural Technology Development Co., Ltd.) was applied as the test agent. Seeds of three crop species were used in the study: pea (*Pisum sativum* L. var. ‘Wuxu-tiancui’), maize (*Zea mays* L. var. ‘Zhengdan 958’), and wheat (*Triticum aestivum* L. var. ‘Qimin 23’).

Preparation of fermentation broth: Aerial parts (stems and leaves) of ‘Yusu 6’ were harvested, air-dried in the shade, ground, and stored in airtight containers at room temperature. The following year, the dried powder was thoroughly mixed with an aqueous solution of a commercial biological starter (with *Bacillus subtilis*, *Bacillus licheniformis*, yeasts, lactic acid bacteria, and *Trichoderma* spp. as the main microbial components, and an effective viable count $\geq 2.0 \times 10^8$ CFU/g). The mixture was packed into plastic vats, pressed firmly, covered with plastic film, and anaerobically fermented for 40 days to yield a stock solution at 100 g/L. The stock solution was diluted with distilled water to obtain a series of five gradient concentrations: 1 g/L (T1), 3 g/L (T2), 5 g/L (T3), 10 g/L (T4), and 20 g/L (T5). Distilled water was used as the control (CK).

Experimental procedures

Germination indices: Seeds of the three crop species were soaked in solutions of fermented perilla extract at various concentrations for 24 h. Thereafter, they were evenly placed on double-layered filter paper in 10 cm-diameter petri dishes (100 seeds per replicate, three replicates per treatment). An appropriate volume of the fermented perilla solution was added to each dish such that the liquid level reached half the height of the seeds. The petri dishes were incubated in a lighted incubator at $25 \pm 1^\circ\text{C}$ under a 16/8 h (light/dark) photoperiod. The number of germinated seeds in each petri dish was recorded daily at 6:00 PM, and the fermented

solution of *P. frutescens* straw was replenished as needed. Germination was assessed after 7 days. The germination rate (GR) and germination energy (GE) were determined, and were calculated using the following formulas:

$$\text{GR (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of tested seeds}} \times 100$$

$$\text{GE (\%)} = \frac{\text{Number of germinated seeds in the first 4 days}}{\text{Total number of tested seeds}} \times 100$$

Measurement of growth and physicochemical indicators in seedlings:

Seeds of the three crops were soaked in differently diluted fermentation broths for 24 hours, using distilled water as the control. After soaking, the seeds were sown into pots with 5 seeds per pot and 50 pots per treatment. The seedlings were grown in a greenhouse for 40 days. During the cultivation period, the corresponding concentration of PFSE was applied at appropriate intervals: each pot received 80 mL applied to the root zone and 20 mL sprayed onto the leaf surface per application. After 40 days, 20 seedlings with uniform growth were selected from each group for measurement of morphological traits. For subsequent physiological and biochemical assays, the leaf samples were immediately frozen in liquid nitrogen and then transferred to a freezer for storage at -20°C .

Plant height was measured with a ruler and stem diameter with a vernier caliper. Roots were carefully washed free of substrate, blotted dry, and both roots and shoots were weighed on an electronic balance.

The following physiological and biochemical indices were measured according to the method described by Xu *et al.*, (2025).

Root oxidative activity was determined by measuring the α -naphthylamine (α -NA) depletion. Fresh roots (0.5 g) were incubated in 25 mL of 50 $\mu\text{g/mL}$ α -NA and 25 mL phosphate buffer (pH 7.0) at 25°C in the dark for 60 min, with a root-free control. Aliquots taken at 5 min and 60 min were reacted with sulfanilic acid and sodium nitrite, diluted to 25 mL, and measured at 510 nm after 25 min. α -NA consumption was quantified using a standard curve (0~40 $\mu\text{g/mL}$).

Chlorophyll content was measured spectrophotometrically. Fresh leaf tissue (0.5 g) was homogenized in 95% ethanol with quartz sand and calcium carbonate. The homogenate was supplemented with an additional 10 mL of ethanol, ground until the tissue turned white, allowed to stand for 3–5 min, and centrifuged at 4000 rpm for 10 min. The supernatant was diluted to 25 mL with 95% ethanol, and absorbance was read at 649 nm and 665 nm for quantification.

Proline contents were measured by the acid ninhydrin method. Fresh leaf sample of (0.2 g) was homogenized with 3 % sulfosalicylic acid, then extract was in a heated boiling water-bath for 10 min. After filtering, 1 mL of the extract was reacted with 2 mL glacial acetic acid and 2 mL of 2.5% acid ninhydrin at 100°C for 30 min. Chromophore was extracted by adding 4 mL toluene, and the absorbance was read at 520 nm. The amount of proline content was determined by plotting a calibration graph with a known concentration of 100 $\mu\text{g/mL}$ of proline.

The content of malondialdehyde (MDA) was determined by thiobarbituric acid (TBA) reaction. Fresh

leaves (1 g) were homogenized with 10% trichloroacetic acid (TCA) and centrifuged at 4000 rpm for 10 min. Then, 2 mL of the supernatant was mixed with 2 mL of 0.6% TBA, heated in a boiling water bath for 15 min, and centrifuged again after cooling. The absorbance of the supernatant at 450, 532 and 600 nm was measured.

Soluble sugar content was determined by anthrone method. 0.5 g leaves were extracted with 10 ml 80% ethanol at 80°C for 40 min, then centrifuged at 6000 rpm for 15 min, and decolorized with activated carbon at 80°C for 30 min. After filtration, 5 ml of anthrone reagent was added to the filtrate and heated in a boiling water bath for 10 minutes. The absorbance was measured at 620 nm.

Soluble protein content was determined by Coomassie brilliant blue G-250 method. Leaves (0.5 g) were mixed it with 7 ml of distilled water, allowed to stand at 4°C for 10 minutes, and then centrifuged at 4000 rpm for 10 min. Next, 0.2 ml of the supernatant was mixed with 5 ml of Coomassie brilliant blue reagent and 0.8 ml of distilled water. After fully stirring, the absorbance is measured at 595 nm.

Superoxide dismutase (SOD) activity was assayed by the nitroblue tetrazolium (NBT) photochemical method. Leaf tissue (0.5 g) was homogenized in 5 mL of 50 mM phosphate buffer (pH 7.8) and centrifuged at 4000 rpm for 30 min at 4°C. Then, 0.3 mL supernatant was mixed with NBT and L-methionine solutions, illuminated under fluorescent light for 15 min, and absorbance was read at 560 nm.

Peroxidase (POD) activity was measured using guaiacol as substrate. Leaves (1 g) were homogenized in 25 mL of 0.1 M phosphate buffer (pH 6.0) and centrifuged at 4000 rpm for 15 min at 4°C. Then, 1 mL supernatant was added to a reaction mixture containing phosphate buffer (pH 6.0), 28 µL guaiacol, and 19 µL 30% H₂O₂. Absorbance change was recorded at 470 nm.

Catalase (CAT) activity was determined colorimetrically. Leaf samples (0.5 g) were homogenized in 25 mL ice-cold phosphate buffer (pH 7.8), incubated at 4°C for 10 min, and centrifuged at 4000 rpm for 15 min at 4°C. Then, 0.4 mL supernatant was mixed with 2 mL distilled water and 3 mL phosphate buffer (pH 7.8), heated at 100°C for 1 min, equilibrated at 25°C for 3 min, mixed with 0.3 mL 0.1 M H₂O₂, and absorbance was measured at 240 nm.

Data analyses: Data were processed using Microsoft Excel 2019 and SPSS 22.0 for analysis of variance (ANOVA) and correlation analysis, with statistical significance set at $p < 0.05$. Graphs were generated using Origin 2024.

Results

Effects of *Perilla frutescens* Stalk Fermented Extract (PFSE) on seed germination of the three crops: With the increasing concentration of PFSE, the germination rate of pea and maize kept decreasing while that of wheat first increased then decreased; The maximum germination rate of wheat was obtained when the concentration was 3 g/L (T2) or 5 g/L (T3), which were 1.93% and 2.70% greater than the control, respectively. In particular, germination was strongly inhibited by the high concentration (20 g/L) for all three species. The worst effect was observed in pea where seed germination decreased to less than 50% whereas for wheat it

was the smallest inhibition (Fig. 1a). Germination potential of both pea and wheat first increased and then decreased, although the intensity and sharpness of the peak differed: pea showed only a mild rise at 1 g/L (T1), while the highest germination capacity for wheat was recorded at 5 g/L (T3), which was 2.70% greater than the control. Conversely, the germination potential of maize decreased consistently as the PFSE level increased (Fig. 1b).

Effects of PFSE on the morphological characteristics and root activity of seedlings of the three crop species:

As the PFSE concentration increased, the plant height, stem diameter, and shoot fresh weight of pea, maize, and wheat all showed an initial increase followed by a decrease. Pea and wheat reached their maximum values in plant height, stem diameter, and shoot fresh weight under the 10 g/L (T4) treatment, with increases of 36.47%, 26.91%, and 74.80% for pea, and 41.67%, 111.68%, and 57.69% for wheat, respectively, compared to the control. In contrast, these parameters of maize peaked at 5 g/L (T3), which were 19.01%, 56.09% and 35.40% higher than the control group, respectively (Fig. 2a, b, c). The root fresh weight of pea and maize was increased first and then decreased with increasing PFSE concentration, peaking at 10 g/L (T4) and 5 g/L (T3), which were 120% and 36.62% higher than the control, respectively. In contrast, the root fresh weight of wheat showed a continuous upward trend (Fig. 2d). The root activity of pea, maize and wheat also increased first and then decreased with increasing PFSE concentration. Maize, pea, and wheat showed their highest root activity at 5 g/L (T3), with increases of 304.59%, 173.92%, and 186.81% over the control, respectively. However, maize also had high root activity at 3 g/L (T2), which was 36.82% higher than the control. Thus, by increasing root activity at optimal concentrations, PFSE enhanced water and nutrient use efficiency in all three crops. This improvement promoted shoot biomass production, reflected in greater plant height, stem thickness, and fresh weight.

Effects of PFSE on chlorophyll contents of the three crops:

The total chlorophyll content of maize and wheat showed a similar unimodal response to increasing PFSE concentration, peaking at 5g/L (T3), 3g/L (T2) by an increase of 38.85%, and 112.21% over the control, respectively (Fig. 3a), while their chlorophyll b content went down steadily. For pea, on the other hand, total chlorophyll content was reduced in all treatments, with the smallest decrease at 5 g/L (T3, Fig. 3b). In contrast to the latter, pea's chlorophyll b was increased first and then declined with increasing concentrations up to the maximal increase value of 49.83% at 5 g/L (T3, Fig. 3b).

Effects of PFSE on osmotic adjustment and MDA levels in seedlings of three crops:

With the increase of PFSE concentration, the contents of proline and soluble sugar in these three crops increased first and then decreased (Fig. 4a, b). Pea and maize reached the highest proline content at a concentration of 5 g/L (T3), which was increased by 107.25% and 90.93%, respectively, compared with the control (Fig. 4a). However, wheat peaked at 3 g/L (T2) and 5 g/L (T3), which increased by 61.20% and 60.34%, respectively, compared with the

control (Fig. 4a). For soluble sugar, peas and maize reached the maximum at the concentration of 3 g/L (T2), which was increased by 46.35% and 4.59%, respectively, compared with the control. Wheat reached at its peak at a concentration of 10 g/L (T4), which was increased by 64.96% compared with the control (Fig. 4b). With the increase of PFSE concentration, the soluble protein content in peas and wheat increased at first and then decreased, while that in maize showed a continuous downward trend. Specifically, the soluble protein content of peas was increased significantly under T1 to T3 treatment conditions (1~5 g/L). Under T2 and T3 treatment conditions (3 ~ 5 g/L), the soluble protein

content of wheat reached the peak. In contrast, the soluble protein content of maize reached a maximum at 1 g/L (T1), which was 24.70 % higher than that of the control group (Fig. 4c). In pea and wheat, the content of MDA decreased at first, then increased with the increase of PFSE concentration, and finally reached the lowest value at 3 g/L (T2) and 5 g/L (T3), which was 9.02% and 52.31% lower than that of the control, respectively. In contrast, the MDA content in maize showed a continuous upward trend. It is worth noting that under the high concentration treatment (20 g/L), the increase of MDA content in wheat relative to the control was the smallest, followed by pea, and the increase of maize was the largest (Fig. 4d).

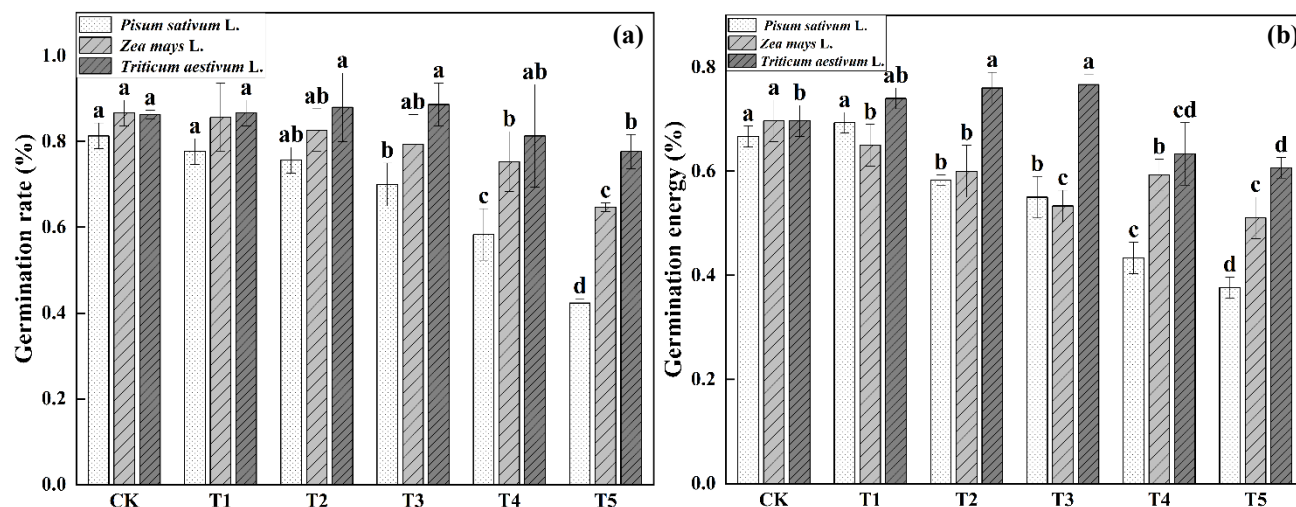


Fig. 1. Effects of different concentrations of PFSE on germination rate (a) and germination energy (b) of the three crops.

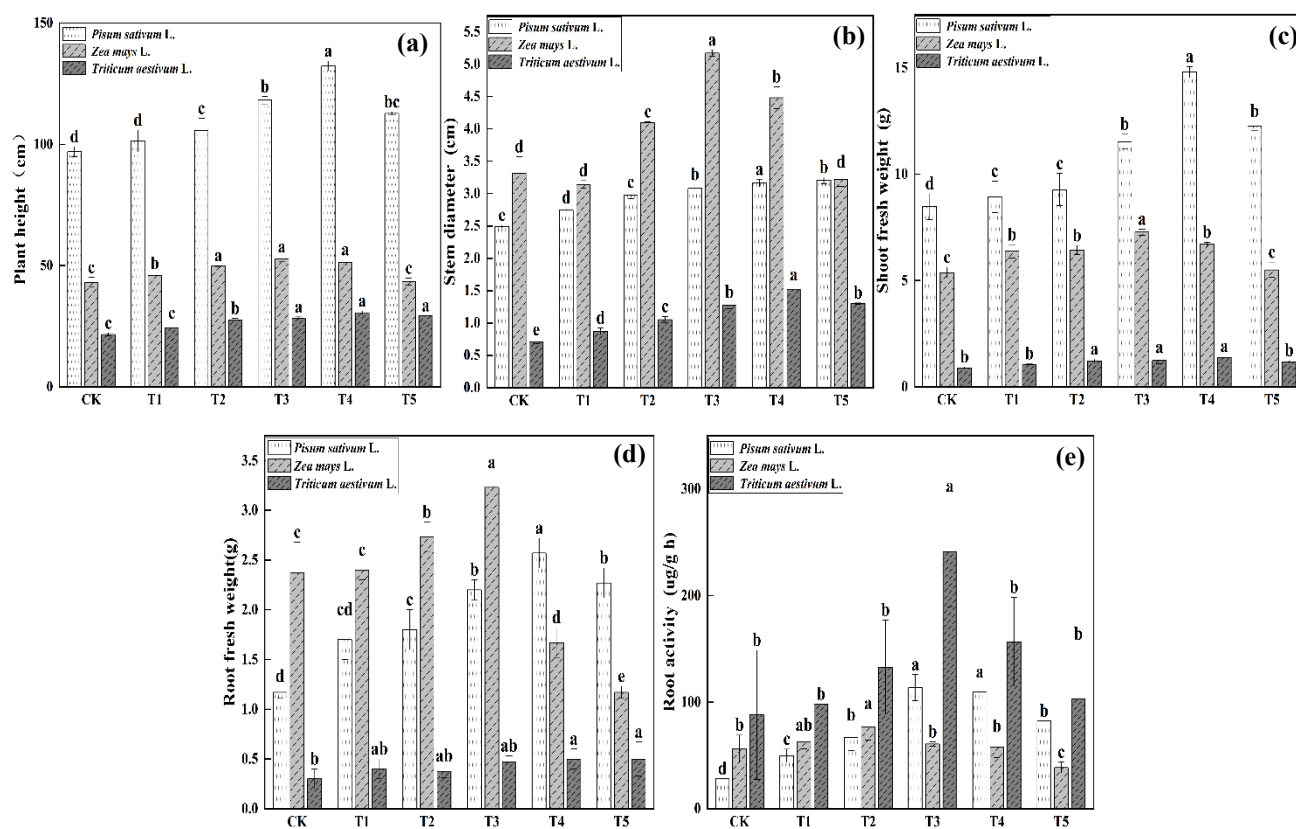


Fig. 2. Effects of different concentrations of PFSE on the morphological traits of the three crops. a. Plant height. b. Stem diameter. c. Shoot fresh weight. d. Root fresh weight. e. Root activity.

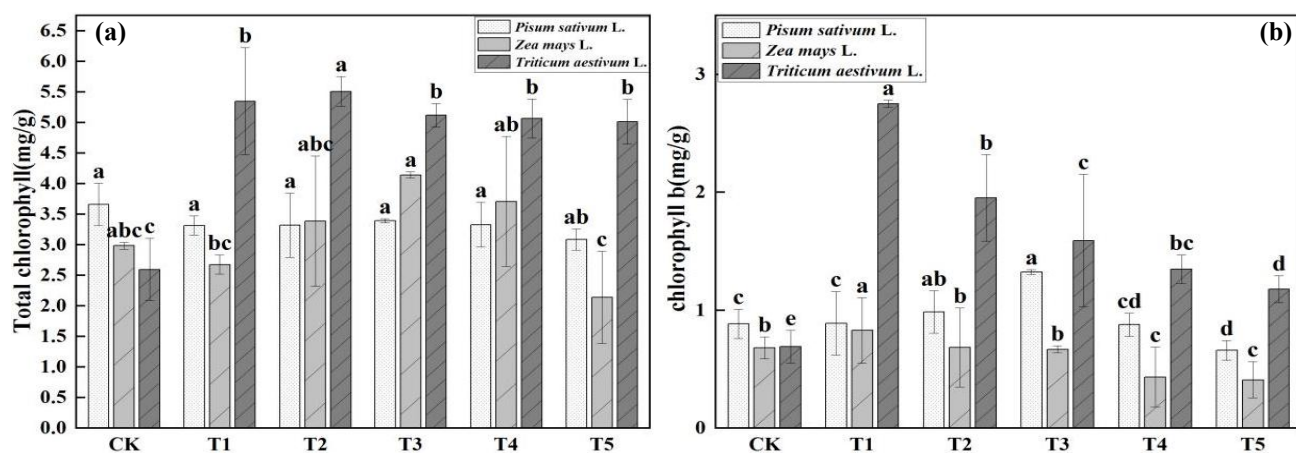


Fig. 3. Effects of different concentrations of PFSE on total chlorophyll (a) and chlorophyll b (b) contents in the three crops.

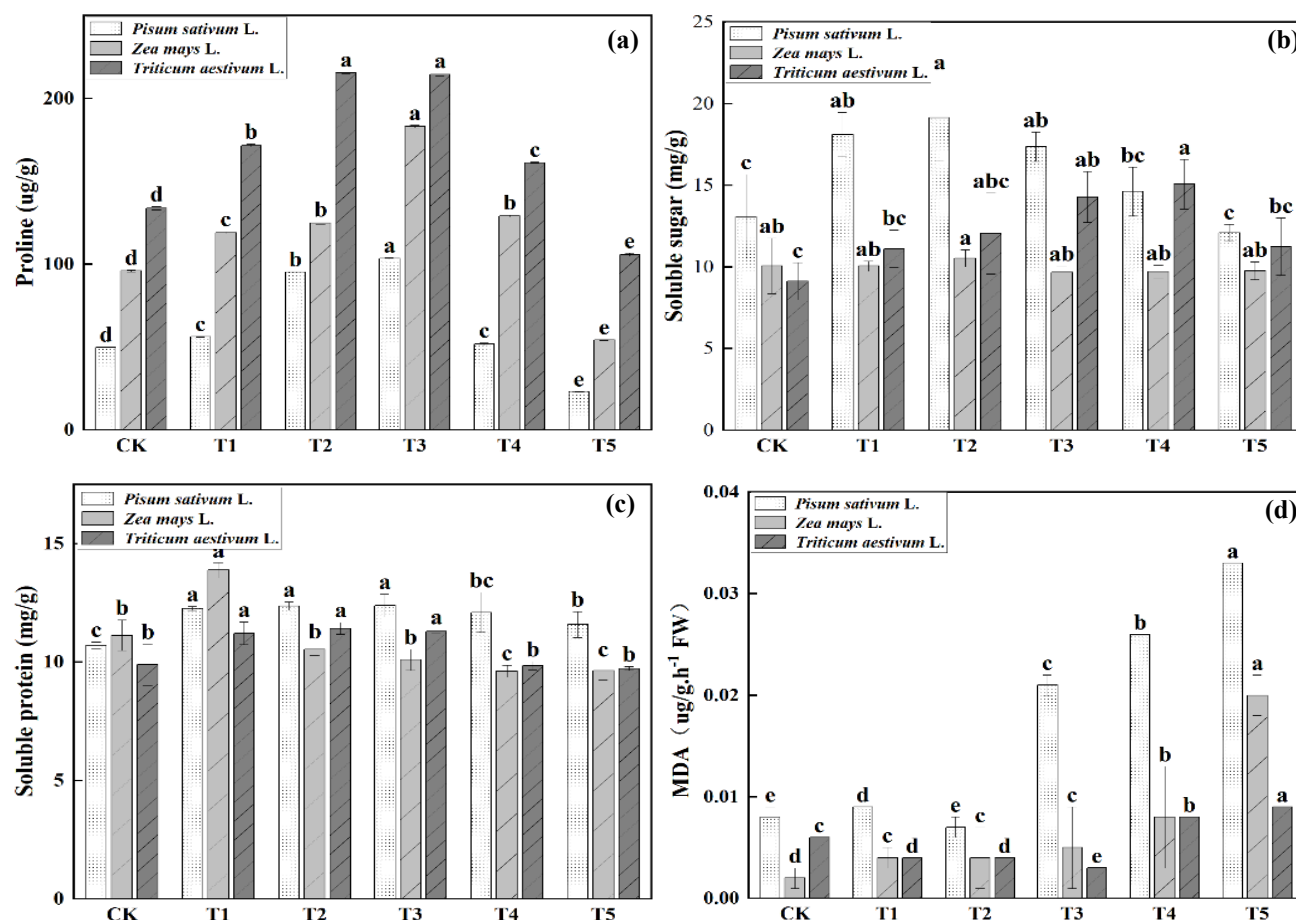


Fig. 4. Effects of different concentrations of PFSE on osmotic regulators and MDA content in three crop species a. Proline contents. b. Soluble sugar contents. c. Soluble protein contents. d. MDA content.

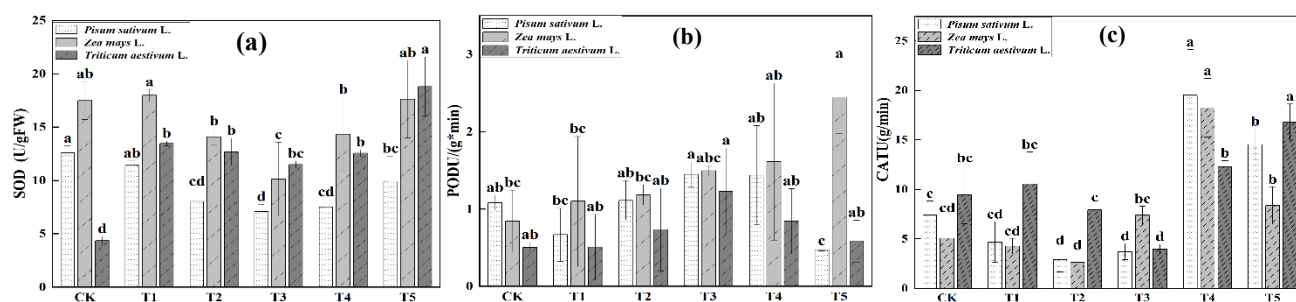


Fig. 5. Effects of different concentrations of PFSE on antioxidant enzyme activities of the three crops a. SOD (Superoxide Dismutase). b. POD (Peroxidase). c. CAT (Catalase).

Effects of PFSE on antioxidant enzyme activities in seedling leaves: The activity of SOD in pea, maize and wheat showed a biphasic change pattern of decreasing first and then increasing with the increase of PFSE concentration. Under all treatment conditions, SOD activity of wheat was higher than that of the control group, and the increase was the smallest under the treatment of 5 g/L (T3). In contrast, SOD activity was inhibited in maize (from T2 to T4) and pea (in all treatments). For maize and pea, the most significant inhibitory concentration was 5 g/L (T3), and its activity was decreased by 42.07% and 43.70%, respectively, compared with the control (Fig. 5a). The activity of POD in pea and wheat increased at first and then decreased with the increase of PFSE concentration, while the activity of maize showed a continuous upward trend and peaked at 20 g/L. In peas, POD activity was significantly enhanced under T3 (5 g/L) and T4 (10 g/L) treatments, which was 33.94% and 33.09% higher than that of the control, respectively. The POD activity of wheat reached to its highest level under T3 (5 g/L) treatment, which was 143.83% higher than that of the control (Fig. 5b). With the increase of PFSE concentration, CAT activity in all three crops decreased first and then increased. CAT activity of pea and corn was the lowest under T2 treatment (3 g/L), which was 61.02% and 47.59% lower than that of the control, respectively. On the contrary, their CAT activity reached the peak under T4 treatment (10 g/L), which was increased by 163.55% and 260.00%, respectively, compared with the control. For wheat, CAT activity reached the lowest value at T3 (5 g/L), which was decreased by 58.20% compared with the control, and reached the highest value at the highest concentration (20 g/L), which was increased by 77.00% compared with the control (Fig. 5c).

Discussion

Straw fermentation broth (SFB) has a variety of effects on crop growth, including stimulation, inhibition, biphasic response or no significant effect, depending on the concentration and plant species. This study showed that *Perilla frutescens* stalk fermentation extract (PFSE) significantly affected seed germination, seedling growth and physiological responses of pea, maize and wheat in a concentration- and species-dependent manner. Our results show a typical "low-promotion, high-inhibition" pattern, which are consistent with previous studies on plant extracts or fermentation products (Gassama *et al.*, 2025; Wang *et al.*, 2023). Particularly, the high concentrations of PFSE (20 g/L) severely inhibited the germination of peas and maize, while the germination rate of wheat remained relatively high, suggesting a tolerance specific to each species. In terms of seedlings growth, the moderate concentration range of PFSE (5~10 g/L) significantly increased plant's height, thickness of stem, and biomass for all three crops, with higher responses recorded for pea and wheat at 10 g/L concentrations, which is consistent to reports where extracts from plants enhanced their growth through promoting nutrition absorption or enhancing root health (Osug *et al.*, 2019; Yue *et al.*, 2023). Of particular interest was the fact that root activity, an important parameter reflecting nutrient and water uptake capacity,

reached its maximum value at 3~5 g/L PFSE, thereby corroborating our hypothesis that PFSE can improve seedlings' early vigour.

Chlorophyll contents, as an important parameter for the photosynthesis ability, were also tested. The total chlorophylls and chlorophyll b of maize and wheat were increased at up to 5 g/L and 3 g/L, respectively, before decline indicating a possible dependence on concentration for regulation of the photosynthetic machinery. While pea total chlorophyll did not change much between the treatments, chlorophyll b was maximal with 5 g/L, suggesting specific induction of chlorophyll b production at this level, and these increased photosynthetic parameters could be due to the higher nutrient concentrations, as fermentation broths with high levels of organic material may enhance the soil fertility, and plant nutrients absorption (Xu *et al.*, 2014; Yue *et al.*, 2023) providing good conditions to synthesize chlorophylls. Under stress condition plants accumulate the compatible osmolytes such as soluble protein, sugar and proline for maintaining cell homeostasis. In this study, soluble sugar, and proline contents all showed significant increases in the moderate PFSE treatment (3~5 g/L), which indicates that the plants have a stronger ability of osmotic adjustment; soluble protein content also increased, especially in pea and wheat, that could be involved in the stability of enzymes and tolerance under stressful conditions. Such results are in accordance with former studies reporting on the ability of straw extracts to regulate osmotic balance and increase stress resistance (Li *et al.*, 2019; Sun *et al.*, 2019).

The antioxidant system showed a complicated regulation at different concentrations. MDA content, which can reflect the level of membrane lipid peroxidation, increased sharply at 20 g/L in pea and maize whereas in wheat first decrease was observed followed by an increase. This suggests a strong membrane damage with the high PFSE concentration, especially for pea and maize. It was consistent with the findings of Wan (2019), which found increased ROS and MDA contents in wheat root when the concentration of wood vinegar was higher than a certain level. In addition, antioxidant enzyme activities including SOD or CAT were decreased for the three plants with low concentrations (1~5g/L) while they were increased with high concentrations, while POD activity of maize was constantly increasing along the concentration range, and reached its maximum value at 5 g/L for pea and wheat then decreased. These profiles indicate that POD is mobilized in response to mild oxidative challenge while SOD and CAT respond to greater levels of oxidative stress. This coordinated but specific response reflects the complex nature of activating antioxidants (Shao *et al.*, 2005). Early induction of POD might be associated with its function in cell wall remodelling and H₂O₂ scavenging whereas late up-regulation of SOD and CAT represents a latter phase detoxification process (Mittler *et al.*, 2022).

It should be noted that these bioactive components of PFSE including phenolic acid, free amino acids, and phytohormones were released from solid-state fermentation and probably responsible for their biological activity (Ezekiel *et al.*, 2024; Yang *et al.*, 2025). They might even be the signal itself to trigger cascades that result in enhanced germination and root growth, and stress adaptation. It is

worth discussing the potential mechanism underlying the strong inhibition observed at the highest concentration (20 g/L). PFSE fermentation processes have been shown to enhance the content of total phenolics and bioactive peptides (Ezekiel *et al.*, 2024; Yi *et al.*, 2025). At elevated concentrations, these phenolic compounds may exert allelopathic effects on seed germination and seedling growth. Phenolic compounds can disrupt membrane integrity, interfere with hormone balance, and induce oxidative stress, ultimately suppressing root elongation and shoot development (Li *et al.*, 2019). In our study, the marked increase in MDA content and the up-regulation of antioxidant enzymes (SOD, CAT) at 20 g/L PFSE in pea and maize (Figs. 4d, 5a, 5c) support the occurrence of phenolic-induced oxidative damage. The fact that wheat was less affected suggesting species-specific tolerance to high phenolic loads, possibly due to more efficient detoxification pathways. Although we did not quantify individual phenolic acids in PFSE, the concentration-dependent inhibition pattern is consistent with a phenolic-mediated allelopathic effect, as previously reported for other plant extracts (Wang *et al.*, 2023; Gassama and Kanneh., 2025). Species-dependent effects also imply that the choice of plant species, as well as the concentration to be applied, is important when using PFSE in agriculture.

The observed species-specific sensitivity (pea > maize > wheat) may be partly explained by fundamental differences between dicots and monocots. Pea possesses a taproot system, whereas wheat and maize develop fibrous root systems (Sparks & Benfey, 2017), which may lead to higher local PFSE concentrations per unit root surface area in pea. Regarding cell wall composition, monocot cell walls contain higher levels of esterified phenolic acids—particularly ferulic and p-coumaric acids—than dicot cell walls (Lozovaya *et al.*, 2000), and exogenous application of these phenolics can inhibit cell growth by reducing cell wall extensibility (Tan *et al.*, 1992). These intrinsic differences may pre-adapt monocots to tolerate higher exogenous phenolic loads. Additionally, previous studies have shown that dicot species are generally more sensitive to phenolic allelochemicals than monocots (Weidenhamer *et al.*, 2023; El-Khawas & Shehata, 2005). Collectively, these physiological and structural disparities likely underlie the differential responses observed in our study.

The present investigation took place in a greenhouse setting, which focused only on the effect of PFSE during germination and early growth stages; several key agronomic aspects were therefore not tested, including the variation of soil types, microbe communities, straw degradation rate, and soil C/N ratio. Thus, it's necessary to conduct a field test to validate the effectiveness of PFSE in nature as well as give more practical instructions on its agricultural usage and straw resources exploitation. Metabolomic and transcriptomic analysis in future work would provide insight into how PFSE promotes growth as well as adapts to environmental stress at a molecular level.

Conclusions

Overall, the present work has shown that PFSE obtained by fermenting *P. frutescens* stalks affects germination of seeds in a dose-dependent manner, seedling development, and physiological reactions in pea (*Pisum sativum* L. var.

waxu-tianai), corn (*Zea mays* L. var. *zhengdan* 958), and wheat (*Triticum aestivum* L. var. *qimin* 23). Low-moderate levels (3 ~ 10 g/L) of PFSE generally enhanced morphological characters, increased root activity, and enhanced chlorophyll synthesis, osmoregulation, and antioxidant enzyme activities. On the other hand, higher concentration (20 g/L) significantly inhibited germination and growth while increasing lipid peroxidation, especially for pea and maize. A total growth promoting effect of PFSE showed its highest activity at a dose rate of 5 g/L. The most effective among the tested species, pea was most sensitive to PFSE treatment, maize responded with an intermediate level of sensitivity while wheat was more tolerant, especially at higher levels. This study shows that PFSE could be used as an efficient bio-stimulator in sustainable crops cultivation, and also provides a scientific basis for high value utilization of medicinal plants straw. Further studies are needed to validate this effect under field conditions and reveal the mechanism behind these species-specific reactions.

Data availability: The datasets generated during and/or analysed during the current study are presented in the Tables and Figures found in the main text.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author's Contribution: **Xuying Wang:** Writing - original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization; **Yuyin Pan:** Conceptualization, Data Curation, Writing-Original Draft; **Tao Lu:** Conceptualization, Resources, Investigation; **Chunyan Yang:** Validation, Investigation, Visualization; **Yihui Wang:** Software, Validation, Investigation, Visualization; **Bo Wei:** Conceptualization, Supervision, Formal analysis, Writing - Review & Editing, Project administration, Funding acquisition.

Funding: This work was supported by the Yunnan Provincial Key Laboratory of Cross-Border Chinese Herbal Materials (20254916CE340074). Scientific Research Fund Project of Yunnan Provincial Education Department (Grant No. 2024J1017) and the Honghe University Research Fund Special Project (Grant No. XJ22B11). The Key Laboratory of Medicinal Plant Biology of Yunnan Province, Yunnan Agricultural University (2018DG009).

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