Autotoxicity of Rice Straw Extract on Germination, Growth, and Yield

of Rice (*Oryza sativa* L.)

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ABSTRACT

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| Rice is a major global staple, but its cultivation faces weed challenges, prompting herbicide use that harms the environment. Rice straw contains allelopathic compounds with bioherbicidal potential, yet these substances may also cause autotoxic effects on rice, impacting its growth and productivity. This study was conducted to assess the autotoxic effects of rice straw (*Oryza sativa* L.) extract on rice seed germination, plant growth, and yield, as well as to identify the concentration level that effectively inhibited these processes. The experiment took place at the Agronomy Laboratory and Greenhouse, Department of Crop Production, Faculty of Agriculture, University of Bengkulu, Indonesia from November 2024 to March 2025. A Completely Randomized Design (CRD) with a single factor was applied, involving five extract concentrations (0%, 2.5%, 5%, 7.5%, and 10%) and five replications. The observed variables included germination parameters such as radicle and plumule length, dry weight, and percentage of abnormal seedlings, along with growth and yield components such as plant height, leaf number, number of productive tillers, leaf length and width, panicle length, root and shoot dry weight, and grain weight. The study demonstrated that rice straw extract markedly suppressed seed germination and vegetative growth, while also diminishing rice yield. The highest inhibitory effect was observed at the 10% concentration, which led to a greater proportion of abnormal seedlings, shorter radicle length, and reduced dry weights of both radicles and plumules. Among the parameters analyzed, the percentage of abnormal seedlings exhibited the lowest Inhibition Concentration 50% (IC₅₀) value (4.35%), highlighting the heightened sensitivity of early plant development to allelopathic compounds in rice straw. These findings indicate rice straw has strong autotoxic properties, offering potential as an eco-friendly bioherbicide. However, proper management of rice straw waste is essential to minimize its adverse effects on crop yields. These findings highlight the potential of rice straw extract as an eco-friendly input that supports sustainable rice cultivation and promotes the proper utilization of agricultural residues.  |

*Keywords:* *autotoxicity, IC₅₀, rice straw, rice, germination*

1. INTRODUCTION

Rice serves as a primary food source for over half of the global population and a key provider of dietary energy, underscoring its vital role in ensuring food security worldwide (Jamal et al., 2023; Li et al., 2024b). However, modern rice cultivation encounters persistent challenges, including invasive weed infestations that significantly diminish crop yields. To mitigate these threats, farmers predominantly depend on synthetic herbicides, which may lead to the development of herbicide-resistant weeds while also environmental contamination risks and potential hazards to human health (Calha et al., 2023; Hamamura, 2018).

Rice produces allelopathic compounds, which exert direct or indirect inhibitory effects on neighboring plants through the release of bioactive chemicals into the surrounding environment (Rice, 1984). These allelochemicals include momilactones, phenolic acids, terpenoids, and flavonoids (Rahaman et al., 2022; Elzaawely et al., 2017). Research has demonstrated that rice allelopathy plays a significant role in weed suppression, with evidence showing reduced germination in *Echinochloa crus-galli* (barnyard grass), *Avena fatua* L., and lettuce (Anuar et al., 2015; Scavo & Mauromicale, 2021).

Rice straw can exhibit autotoxicity, a specialized form of allelopathy in which plants release phytochemicals that suppress the germination and growth of plants itself (Kato-Noguchi, 2024). This phenomenon has been observed in various weeds and crops, including rice (*Oryza sativa*) and alfalfa (*Medicago sativa*). Under anaerobic conditions, such as in flooded soils, the decomposition of plant residues releases toxic compounds that induce physiological stress, alter soil redox potential, and impair root development (Zhang et al., 2021). Among these allelochemicals, momilactones A and B inhibit proteolytic enzymes, disrupting nutrient mobilization during germination and hindering seedling establishment (Sultana et al., 2023). Additionally, flavonoids modulate auxin transport, affecting plant growth, while terpenoids contribute to defense mechanisms (Shah & Smith, 2020; Sultana et al., 2023).

Rice straw exhibits autotoxic properties that can suppress the growth of subsequent rice crops. Additionally, straw residues demonstrate potential as natural herbicides through allelopathic action (Mai & Xuan, 2024). Experimental studies by Anuar et al. (2015) revealed that 10% rice straw extract solution significantly reduced radicle elongation in *Echinochloa crus-galli*. These findings are supported by earlier work of Zhang et al (2025), who observed pronounced inhibition of radicle development by decomposing rice residue extracts. Elucidating these phenomena could advance the development of innovative, eco-friendly weed management strategies. As emphasized by Khamare et al. (2022), addressing global food security demands next-generation agricultural systems that prioritize sustainability while reducing dependence on synthetic agrochemicals.

While the allelopathic properties of rice straw have been extensively investigated, the mechanisms underlying its autotoxic effects particularly on germination, vegetative growth, and crop yield remain incompletely characterized. This study evaluated the efficacy of rice straw water extract on plant growth using the IC50 metric, defined as the extract concentration required to inhibit test organism activity by 50% within a specified timeframe (Hassan et al., 2023). Our objectives were to: (1) identify optimal extract concentrations for suppressing rice germination, growth, and yield, and (2) determine IC50 values through controlled germination bioassays.

2. methodologY

**2.1 Location Site and Research Design**

The experiment was conducted from November 2024 through February 2025 at the Agronomy Laboratory and Agriculture Experiment Station, University of Bengkulu, Indonesia.

The study consisted of two stages: (1) a germination efficacy test of rice straw extract using petri dishes, and (2) a growth assay using buckets. A completely randomized single-factor design was employed with five concentrations: P0 = 0% (control), P1 = 2.5%, P2 = 5%, P3 = 7.5%, and P4 = 10%. Each treatment was replicated five times, totaling 25 experimental units, where each unit comprised two petri dishes and two buckets.

**2.2 Research Stage**

**2.2.1 Preparation of Rice Straw Extract**

Rice straw samples were collected from post-harvest rice fields located in Muara Bangkahulu, Bengkulu City, Indonesia. The collected straw was uniformly cut into 4-5 cm pieces and subsequently oven dried maintained at 50°C for 72 hours until a constant mass was attained. The dried material was then pulverized using an electric blender to produce a homogeneous fine powder.

The extraction process involved: (1) precisely weighing 100 g of the prepared straw powder, (2) mixing with 1000 ml of distilled water, and (3) agitating the mixture continuously at 150 rpm for 24 hours using an orbital shaker. The resulting suspension was subjected to dual filtration through Whatman No. 1 filter paper to yield a 10% (w/v) stock solution. Treatment solutions were prepared by serial dilution of the stock solution to obtain the following concentrations (total volume 500 ml each):

* Control (0%): 500 ml distilled water
* 2.5% : 125 ml stock + 375 ml distilled water
* 5% : 250 ml stock + 250 ml distilled water
* 7.5% : 375 ml stock + 125 ml distilled water
* 10% : 500 ml undiluted stock solution

**2.2.2 Laboratorium Test**

*2.2.2.1 Germination Bioassay*

The experimental procedure commenced with the sterilization and preparation of petri dishes (90 mm diameter) for the germination tests. All glassware was first sanitized with 5% sodium hypochlorite solution (commercial Bayclean®) followed by a 70% ethanol rinse to ensure aseptic conditions (Peranginangin et al., 2025). Each sterilized petri dish was lined with Whatman No. 1 filter paper to serve as the germination substrate.

For treatment application, 10 ml of the prepared rice straw extract was dispensed into each petri dish using a graduated pipette. Twenty-five surface-sterilized rice seeds (*Oryza sativa* L. cv. IR64) were then evenly distributed in each dish. The germination experiment was conducted under controlled laboratory conditions (25±2°C) with daily observations recorded for seven consecutive days.

**2.2.3 Greenhouse Test**

*2.2.3.1 Experimental Setup and Growth Medium Preparation*

The research used a planting medium with a weight ratio (1:1) of topsoil and organic compost. This homogeneous mixture was precisely measured, with each experimental container (plastic buckets) receiving 3.0 kg of the prepared medium.

Planting was conducted by making 1 cm-deep holes in the growing medium. Each hole was planted with three 7-day-old seedlings obtained from laboratory germination tests. Fertilization, watering, thinning, weeding, and pest control were conducted during growing period. Fertilization was performed once at planting date at a doses of 300 kg/ha urea (0.15 g/bucket), 100 kg/ha TSP (0.05 g/bucket), and 100 kg/ha KCl (0.05 g/bucket) (Ministry of Agriculture, 2007). Daily irrigation was applied. Thinning was conducted at 1 week after planting (WAP) by cutting two seedlings, leaving one plant per bucket. Rice was harvested at 116 days after planting (DAP).

**2.3 Observed Variables**

**Laboratorium test.** The variables observed in the germination test include the percentage of abnormal seedling (%), radicle length (cm), plumule length (cm), radicle dry weight (mg), plumule dry weight (mg), and total dry weight (mg). Observations were conducted on 10 seedling in each petri dish.

In the laboratory experiment, observations were conducted on the seventh day, focusing on the percentage of seedling growth includes the percentage of abnormal seedlings

Percentage abnormal seedling = $\frac{Number abnornal seedlings }{Total number of seeds}$ x100%

**Greenhouse test.** Observed variables include plant height (cm), number of leaves, leaf length (cm), leaf width (cm), leaf area (cm²), number of panicles per clump, panicle length (cm), weight of 1000 grains (g), shoot dry weight (g), and root dry weight (g).

Plant height, number of leaves, and number of tillers were conducted weekly from 1 to 7 weeks after planting (WAP). Leaf length and width were measured once, and leaf area was calculated using the formula LA = L × W × K (Setyowati et al., 2025). Leaf greenness was measured using a SPAD meter during the vegetative phase.

**2.4 Data Analysis**

The collected data were statistically analyzed using Analysis of Variance (ANOVA) at a 5% significance level. Orthogonal Polynomial (OP) regression analysis was applied to the laboratory data, while the Least Significant Difference (LSD) test was used for greenhouse data evaluation. The IC50 value of rice straw extract was determined through regression analysis.

Relative shoots length (RSL) = $\frac{shoot length in extract​ }{shoot length in control}$ x100% (Qi et al., 2019).

Relative Biomass of Roots and Shoots (RBRS) = $\frac{root dry weight + shoot dry weight in extract​ }{root dry weight + shoot dry weight in control​ }$ x100% (Qi et al., 2019).

Shoot-Root Ratio (SSR) = $\frac{shoot dry weight​ }{root dry weight​ }$ (Agathokleous *et al.,* 2019).

3. RESULTS AND DISCUSSION

**3.1 Experiment Highlight**

In the Lab test, rice seed germination began on the fourth day, reaching approximately 95% by the seventh day in the control treatment (0% extract). However, as the concentration of rice straw extract increased (2.5–10%), there was a marked reduction in germination rates. The extract significantly influenced radicle and plumule lengths as well as seedling morphology. Higher extract concentrations led to more pronounced inhibitory effects, including shortened radicles, underdeveloped plumules, and a greater occurrence of abnormal seedlings (Figure 1A). These effects are thought to be caused by allelopathic compounds in the rice straw, which exhibit autotoxicity by disrupting enzyme activity during germination, inducing oxidative stress, and inhibiting both protein synthesis and cell elongation (Li et al., 2024a).

Greenhouse experiments indicate that seedlings exposed to high concentrations of rice straw extract exhibit suppressed growth during the early vegetative stage, particularly in terms of plant height and leaf development (Figure 1B). This observation supports the findings of Afridi et al. (2014), who reported that rice straw extract possesses allelopathic properties capable of inhibiting plant growth, including reductions in root and shoot length when applied at elevated concentrations.

 

(B)

(A)

**Figure 1. Effect of rice straw extract on seed germination (A) and rice growth (B)**

**3.2 Effectiveness of Rice Straw Extract in Laboratory Germination Tests**

Rice straw extract had a significant impact on the germination process, as evidenced by the suppressed growth of rice seedlings. These inhibitory effects suggest the presence of autotoxicity caused by allelopathic compounds in the rice straw. Observations revealed irregular germination patterns and abnormal sprout development. The different concentrations of rice straw extract had a highly significant influence on all measured parameters (Table 1).

**Table 1. Analysis of variance on the autotoxicity effect of rice straw on rice germination**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **F‑calc** | **CV (%)** | **F-table (5 %)** | **F-table (1%)** |
| Percentage of Abnormal Seedlings t | 6899.05\*\* | 1.430 | 2.87 | 4.43 |
| Radicle Length  | 106.63\*\* | 8.91 |
| Plumule Length | 41.95\*\* | 10.00 |
| Radicle Dry Weight | 33.03\*\* | 12.66 |
| Plumule Dry Weight | 37.86\*\* | 12.07 |
| Total Dry Weight | 43.81\*\* | 11.00 |

*Note: \* = significantly different; \*\* highly significantly different; ns = not significant different, t = data transformed √x + 0,5. CV = Coefficient of Variation*

The data revealed that rice straw extract exerted a significant influence on multiple seedling growth parameters, including the percentage of abnormal seedling, radicle and plumule lengths, as well as the dry weights of the radicle, plumule, and overall seedling. These results suggest that rice straw exhibits allelopathic activity capable of disrupting physiological processes during the early stages of plant development.

**3.2.1 Abnormal Seedling Percentage**

At the 0% concentration (control), seedlings exhibited normal growth characterized by elongated radicles, the emergence of secondary roots, and upright, uniform plumules. At 2.5%, initial signs of growth inhibition appeared, including shorter, curved radicles and reduced plumule size. A 5% concentration led to more pronounced effects, such as stunted growth, blackened and shortened radicles, yellowing plumules, and the appearance of black spots on the seed surface. These symptoms intensified at 7.5%, evidenced by further reduction in radicle and plumule length, discoloration to brown or black hues, and necrotic lesions on the grains. The highest inhibition occurred at 10%, where radicles were extremely short and blackened, plumules failed to develop, and seed grains showed substantial damage. These inhibitory effects are presumed to result from allelopathic compounds present in rice straw such as ferulic acid, sinapate, and p-hydroxybenzoate which interfere with key enzymes and growth regulators, thereby disrupting cell elongation and expansion during the germination process (Li et al., 2024a).

Rice (1984) stated that allelopathic interactions can induce physiological alterations in plants, such as embryo deformation and a higher frequency of abnormal seedling formation. Supporting this, Li et al. (2024a) stated that phenolic compounds present in rice straw interfere with seed hormonal regulation, hinder developmental processes, and elevate stress levels in plant tissues. The progressive increase in rice straw extract concentration was directly correlated with a rise in the proportion of abnormal seedlings, highlighting the presence of autotoxic effects from the earliest stages of seed development (Figure 2).

**Figure 2. Percentage of abnormal seedlings 7 days after treatment**

**3.2.2 Radicle and Plumule Length**

The plumule, which is the embryonic structure that later forms the shoot and leaf system, serves as a key indicator of successful germination. Impaired plumule development indicates physiological stress, often resulting from the presence of allelochemical compounds that are toxic to emerging plant tissues. Analytical results demonstrate that rice straw extract had a highly significant inhibitory effect on both radicle and plumule growth (Figure 3).

(B)

(A)

**Figure 3. Effect of Rice Straw Extract Concentration on Radicle Length (A) and Plumule Length (B)**

Regression analysis revealed a negative linear correlation between rice straw extract concentration and both radicle and plumule lengths. In the control (0%), radicle length was 6.31 cm, decreasing to around 2 cm at 10%. Each 2.5% increase in concentration reduced radicle length by approximately 1.03 cm. Similarly, plumule length declined from 4.83 cm (control) to about 2 cm at 10%, with each 2.5% increment causing a 0.62 cm reduction.

The use of rice straw extract commonly causes visible alterations in seeds, including darkened color, swelling, shortened radicles, and little to no development of root hairs. The reduction in radicle length is attributed to allelopathic compounds like ferulic acid, p-coumaric acid, and sinapic acid, which disrupt the seeds' physiological functions (Li et al., 2024a). This compound can suppress the function of enzymes involved in cell division and elongation, disturb osmotic regulation, and impair cell membrane integrity (Šoln et al., 2022). These effects demonstrate that allelopathic compounds hinder both cell proliferation and elongation, as well as the differentiation of root tissues during early growth stages.

Higher concentrations of rice straw extract resulted in stronger suppression of plumule growth in rice seeds. While the control treatment exhibited normal and healthy plumule development, treatments with 2.5% to 10% concentrations showed a notable decline in growth. Earlier research supports these findings, indicating that rice straw extracts from different varieties can impede germination and early seedling growth, affecting both plumule and radicle formation. This inhibitory effect intensifies with rising extract concentrations, as observed in studies on species such as *Echinochloa crus-galli* and *Medicago sativa* (Anuar et al., 2015; Zhang et al., 2021).

**3.2.3** **Radicle, Plumule and Total Seedling Dry Weight**

Regression analysis revealed a highly significant (p<0.01) inhibitory effect of rice straw allelochemicals on radicle, plumule, and total dry weights (Figure 4). The observed biomass reduction exhibited a concentration-dependent response, demonstrating a linear decrease pattern with increasing extract concentration.

(A)

(B)

(C)

**Figure 4. Effect of Rice Straw Extract Concentration on Radicle (A), Plumule (B) and Total Dry Weight (C).**

Regression analysis showed a linear decrease in radicle, plumule, and total dry weights with increasing rice straw extract concentration. At 0% concentration, radicle dry weight was 2.63 mg, dropping to 2.25 mg at 2.5% a reduction of 0.38 mg per 2.5% increase. Similarly, plumule dry weight declined from 2.90 mg at 0% to 2.48 mg at 2.5%, showing a 0.42 mg decrease per 2.5% concentration increment. The reduction in radicle dry weight is likely due to allelopathic compounds, particularly phenolic acids and their derivatives, which may inhibit key enzymatic activity, alter root cell membrane permeability, and impair water and nutrient uptake. Asaduzzaman & Pramanik (2005) reported that rice straw residues release toxic compounds that hinder root growth. The reduced dry weight of the plumule is attributed to allelopathic substances in the straw, which, according to Li et al. (2024a), disrupt enzyme activity, induce oxidative stress, and inhibit protein synthesis and cell elongation. Higher extract concentrations lead to greater inhibition of plumule growth and biomass accumulation.

Total dry weight, consisting of radicle and plumule dry weights, was 5.54 mg at 0% extract and dropped to 4.74 mg at 2.5%, indicating a 0.80 mg decrease for every 2.5% increase in concentration. This reduction is likely due to impaired mobilization of endosperm food reserves caused by allelopathic compounds in rice straw extract. Such stress inhibits α-amylase activity by disrupting gibberellin biosynthesis, limiting the use of stored nutrients (Li et al., 2024a; Sansenya et al., 2021). According to Kumar et al. (2024), environmental stress, including allelopathy, can slow seed metabolism and hinder nutrient transfer to developing tissues.

**3.3 Inhibition Concentration (IC50) of Rice Straw Autotoxicity**

The IC₅₀ value was derived from linear regression of various rice seedling growth parameters, including abnormal seedling percentage, radicle and plumule lengths, and dry weights. This value indicates the concentration of rice straw extract that inhibits 50% of seedling growth in petri dish tests. Table 2 showed IC50 of rice germination variables.

**Table 2. IC50 of rice germination variables**

|  |  |  |
| --- | --- | --- |
| **Variables** | **Regression Equation** | **IC50 (%)** |
| Abnormal Seedling Percentage | y = 86.344x + 3.2542 | 4.35 |
| Radicle Length | y = -41.533x + 6.3105 | 13.99 |
| Plumule Length | y = -25.071x + 4.8392 | 17.31 |
| Radicle Dry Weight | y = -15.109x + 2.6365 | 14.14 |
| Plumule Dry Weight | y = -17.016x + 2.9093 | 14.15 |
| Total Dry Weight | y = -32.125x + 5.5459 | 15.71 |

Based on the IC₅₀ regression analysis, rice straw extract significantly affected several rice seedling growth parameters. The most sensitive response was the percentage of abnormal seedling, with an IC₅₀ of 4.35%, indicating a 50% increase in abnormalities at this concentration (Table 2. This suggests that allelopathic compounds in rice straw can disrupt seedling development even at low levels, likely by affecting antioxidant enzymes, protein synthesis, and hormonal balance (Mai & Xuan, 2024).

The IC₅₀ values for radicle length and dry weight are 13.99% and 14.14%, respectively, while plumule length has a slightly higher IC₅₀ of 17.31%. This suggests that the radicle is more sensitive to allelopathic compounds. Its higher sensitivity is likely due to its role as the first organ to emerge and directly interact with the extract, making it more vulnerable. In contrast, the plumule develops later, reducing its early exposure. Rice (1984) also noted that roots are more prone to allelopathic effects than plumules, as they are the first to encounter allelochemicals in the growth medium.

The IC₅₀ value for total dry weight was 15.71%, indicating that at this concentration, rice straw extract reduced biomass accumulation by 50% compared to the control. This value is higher than those for radicle length and abnormal sprout percentage, suggesting greater tolerance to allelopathic effects, likely due to unutilized food reserves in the endosperm. According to Einhellig (1994), phenolic compounds can inhibit endosperm breakdown and enzyme activity involved in carbohydrate metabolism. Compounds like ferulic acid, sinapic acid, and p-coumarate in rice straw may disrupt reserve mobilization and seed physiology (Li et al., 2024a). These findings confirm the autotoxic effect of rice straw extract, with germination being the most sensitive stage.

**3.4 Rice Straw Extract Effectivity in Greenhouse Test**

The rice straw extract significantly affected most rice growth variables. Of the 15 variables measured, 12 showed a highly significant effect (\*\*), one was significant (\*), and two showed no significant difference (ns) (Table 3).

**Table 3. Analysis variance of the effects of rice straw extract on rice growth**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **F-Value** | **CV (%)** | **F-table****(5 %)** | **F-table****(1 %)** |
| Plant Height | 4.83 \*\* | 4.98 | 2.87 | 4.43 |
| Number of Leaves | 5.15 \*\* | 14.28 |
| Number of Tillers | 7.16 \*\* | 11.79 |
| Leaf Length | 5.42 \*\* | 4.63 |
| Leaf Width | 6.61 \*\* | 4.53 |
| Leaf Area | 13.62 \*\* | 14.90 |
| Leaf Greenness | 4.57 \*\* | 3.93 |
| Panicle Length | 4.27 \* | 5.63 |
| Number of Productive Tillers | 10.75 \*\* | 11.00 |
| 1000-Grain Weight | 4.54 \*\* | 3.94 |
| Shoot Dry Weight | 36.63 \*\* | 12.00 |
| Root Dry Weight | 20.26 \*\* | 12.00 |
| Shoot-to-Root Ratio | 2.13 ns | 14.37 |
| Relative Shoots Length | 1,64 ns | 6,16 | 3.24 | 5.29 |
| Relative Biomass of Roots and Shoots | 7,40 \*\* | 6,97 |

*Note: \* = significantly different; \*\* highly significantly different; ns = not significantly different. CV= Coefficient of Variation.*

Some variables including plant height, leaf number, tiller number, leaf dimensions, leaf area, leaf greenness, number of productive tillers, 1000-grain weight, shoot and root dry weights were significantly influenced by the treatment (Table 3).

**3.4.1 Rice straw extract effectivity on rice growth**

Rice straw extract significantly affected rice vegetative growth. LSD analysis at different concentrations showed significant effects on all observed variables in the bucket planting experiment (Table 4).

**Table 4. Effect of rice straw extract on rice growth components.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Extract Concentration(%)** | **PH (cm)** | **LN** | **TN** | **LL (cm)** | **LW (cm)** | **LA (cm²)** | **LG** |
| 0.0 | 105.50 a | 156.13 a | 46.27 a | 72.50 a | 1.12 a | 9620.54 a | 34.25 a |
| 2.5 | 101.73 ab | 150.47 a | 45.07 a | 69.87 ab | 1.06 ab | 8488.81 a | 32.72 ab |
| 5.0 | 96.80 bc | 124.87 b | 38.13 b | 66.73 bc | 1.03 bc | 6494.80 b | 32.05 b |
| 7.5 | 94.77 c | 121.73 b | 37.53 b | 65.33 c | 1.01 bc | 6080.73 b | 31.50 b |
| 10.0 | 94.37 c | 111.73 b | 32.73 b | 64.73 c | 0.97 c | 5377.21 b | 31.18 b |

*Note:* *Values followed by different letters within the same column indicate significant differences according to the LSD test.* *PH=Plant Height,* *LN=Leaves Number,* *TN=Tillers Number,* *LL=Leaf Length,* *LW=Leaf Width,* *LA=Leaf Area,* *LG=Leaf Greenness.*

At 5% concentration of rice straw extract marks the threshold where vegetative growth decline becomes statistically significant, although early symptoms appeared at 2.5%. For plant height, the control (0%) had the highest value, significantly different from the 5%, 7.5%, and 10% treatments, while 2.5% was not significantly different from the control. This suggests that allelopathic effects begin at 2.5% but become significant at 5%.

Leaf number, tiller number, and leaf area declined with increasing extract concentration. The 10% treatment showed the lowest values, significantly different from the 0% and 2.5% treatments, but not from 5% and 7.5%. This suggests that significant growth inhibition begins at 5%, likely due to metabolic disruption. Allelopathic compounds like momilactone B, phenolic acids, and flavonoids in rice straw can inhibit cell expansion and key enzyme activities (Khanh et al., 2007; Rayee et al., 2024).

The leaf length variable showed a significant difference starting at the 5% treatment compared to the control, while the 2.5% treatment did not show a significant difference. The leaf width variable only showed a significant difference between the 0% and 10% treatments, while the 2.5% to 7.5% treatments were not significantly different from each other or from the control. The 5% concentration can be identified as the initial threshold for leaf growth inhibition, marked by the start of a significant difference in leaf length compared to the control treatment.

Leaf length showed a significant reduction starting at the 5% treatment, while the 2.5% treatment remained similar to the control. For leaf width, a significant difference was only observed between the 0% and 10% treatments, with no significant changes between 2.5% to 7.5%. Thus, 5% is identified as the threshold concentration where leaf growth inhibition begins, as indicated by the significant decline in leaf length.

Leaf greenness declined with higher extract concentrations. A significant drop occurred at 5%, while 2.5% showed no significant difference from the control. Although chlorophyll reduction began at 2.5%, it became significant at 5%, indicating physiological stress. This is likely due to phenolic compounds that inhibit enzymes and damage cell membranes (Reigosa et al., 2006), reducing photosynthesis and energy production, and ultimately affecting growth and yield (Hussain & Reigosa, 2021).

The 5% concentration is identified as the most effective in significantly inhibiting rice vegetative growth, marking the threshold of visible and statistical allelopathic effects. Although 10% showed the lowest growth, it was not always significantly different from 5%, likely due to early-stage physiological damage that already suppressed further growth. Beyond this point, increasing the dose adds little effect, as key traits like plant height, leaf area, chlorophyll, and biomass are already reduced (Li et al., 2024a; Dora et al., 2025).

**3.4.2 Rice straw extract effectivity on rice yield components**

The application of rice straw extract significantly affected rice yield components. At various concentrations, rice straw extract, showed highly significant differences across all observed variables (Table 5).

**Table 5. The effect of rice straw extract on rice yield components.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Extract Conc.(%)** | **PL (cm)** | **PT** | **TGW (g)** | **SDW (g)** | **RDW (g)** | **SRR** | **RSL (%)** | **RBRS (%)** |
| 0.0 | 28.30 a | 27.60 a | 29.80 a | 129.81 a | 111.37 a | 1.18 a | – | – |
| 2.5 | 27.40 ab | 25.10 ab | 28.81 a | 109.78 b | 99.33 a | 1.10 ab | 0.97 a | 230.12 a |
| 5.0 | 25.93 bc | 22.40 bc | 28.72 a | 93.63 c | 85.56 b | 1.10 ab | 0.92 a | 216.22 ab |
| 7.5 | 25.63 bc | 19.80 cd | 28.62 a | 73.02 d | 75.68 b | 0.96 b | 0.90 a | 206.15 bc |
| 10.0 | 24.93 c | 18.70 d | 26.83 b | 53.40 e | 57.45 c | 0.95 b | 0.90 a | 187.74 c |

*Note: Values followed by different letters within the same column indicate significant differences according to the LSD test. PL=Panicle Length, PT=Productive Tiller, TGW=1000-Grain Weight,* *SDW=Shoot Dry Weight,* *RDW=Root Dry Weight,* *SRR=Shoot-Root Ratio, RSL= Relative Shoot Length,* *RBRS= Relative Biomass of Roots and Shoots.*

Table 5 shows that rice straw extract significantly affected most yield components. Allelopathic effects appeared at low concentrations and intensified with higher doses. Panicle length was first significantly reduced at 5%, with the strongest inhibition at 10%, indicating 5% as the threshold and 10% as the most effective level.

The productive tiller number demonstrated notable sensitivity, exhibiting significant suppression starting at 2.5% concentration with progressive reduction up to 10%. This response suggests rice straw allelochemicals substantially impair reproductive organ development. Bioactive components like momilactones A and B likely disrupt critical growth hormones (cytokinins and gibberellins) essential for shoot initiation and panicle formation (Kato-Noguchi & Ota, 2013). Consequently, allelopathic effects systemically influence all growth phases, spanning both vegetative and reproductive stages.

A significant reduction in 1000-grain weight was observed at the 10% concentration, which resulted in the lowest values among all treatments. This decline likely results from impaired photosynthetic capacity associated with diminished leaf area and chlorophyll content. Such physiological constraints limit photoassimilate allocation to grain filling, ultimately compromising both grain quality and weight (Zang et al., 2022; Cheng & Cheng, 2015).

The 10% extract treatment caused the most pronounced reduction in root dry weight, showing significant differences from all other concentrations. Shoot dry weight followed a similar pattern, with maximum values in the control treatment and progressive decreases at higher concentrations. Treatments of 2.5% and 5% already demonstrated significant reductions compared to the control, while 7.5% and 10% concentrations yielded the lowest biomass values, differing significantly from all other treatments. These results suggest that rice straw allelochemicals interfere with fundamental energy metabolism pathways in plants.

The 0% treatment had the highest shoot-root ratio, significantly different from the 10% treatment, while other treatments showed no significant differences. RSL values remained relatively unchanged across treatments. For RBRS, the highest value was at 2.5%, significantly higher than 10% and similar to 5%. RBRS decreased with increasing concentrations, indicating greater disruption to root and stem biomass accumulation at higher extract levels.

Phenolic compounds can alter cell structure, inhibit cell division, and disrupt ATPase enzyme activity involved in mobilizing food reserves, leading to impaired plant growth (Li et al., 2010). Growth inhibition may also cause anatomical and morphological changes in root tips. Coumarin and similar phenolic acids can deform root morphology (Kumar et al., 2020), while ferulic and p-coumaric acids hinder leaf expansion. Additionally, p-hydroxybenzoic and vanillic acids from red pepper roots have autotoxic effects, suppressing root growth in monocultures (Rice, 1984; Elzaawely et al., 2017). This study confirms that rice straw extract negatively impacts rice growth and yield, likely due to phenolic compounds like p-coumaric, vanillic, and ferulic acids, which have been shown to inhibit the growth of crops such as upland rice, peas, *Medicago sativa*, and sugarcane (Ciulu et al., 2018; Zhang et al., 2021).

4. Conclusion

The effective concentration of rice straw extract that inhibits rice seed germination is above 4.35%, based on the IC₅₀ value for the abnormal sprout percentage variable. At this level, more than 50% of the sprouts exhibit morphological abnormalities, such as increased abnormal seedling numbers, shortened radicles and plumules, and the appearance of black spots on the grains.

The most effective concentration of rice straw extract for suppressing plant growth during the vegetative stage is 5%, which significantly reduces plant height, leaf number, leaf length, and leaf greenness. In the yield formation stage, a concentration of 10% shows the strongest effect by decreasing dry weight of both shoots and roots, the number of productive tillers, panicle length, and 1000-grain weight.

The IC₅₀ values of rice straw extract on rice sprout growth differ across parameters, with the lowest value found in the percentage of abnormal sprouts and the highest in plumule length and plumule dry weight.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

During the manuscript preparation, AI tools (e.g., Grammarly, QuillBot, ChatGPT) were employed under strict limitations ie. enhancing technical language (such as grammar and spelling corrections) and aiding readability through carefully controlled paraphrasing. Importantly, the scientific integrity of the research remained uncompromised, as AI was entirely excluded from critical aspects of the study, including data analysis, result generation, and any decision-making processes related to the research.

References

Afridi, A. R., Khan, A. M., Gul, H., & Khan, D. M. (2014). Allelopathic influence of rice extracts on phenology of various crops and weeds. Pakistan Journal of Botany, 46(4), 1211–1215.

Agathokleous, E., Belz, R. G., Kitao, M., Koike, T., & Calabrese, E. J. (2019). Does the root to shoot ratio show a hormetic response to stress? An ecological and environmental perspective. Journal of Forestry Research, 30(5), 1569–1580. <https://doi.org/10.1007/s11676-018-0863-7>

Anuar, F. D. K., Ismail, B. S., & Ahmad, W. J. W. (2015). Allelopathy effect of rice straw on the germination and growth of *Echinochloa crus-galli* (L.) P. Beauv. AIP Conference Proceedings, 1678, 020014. <https://doi.org/10.1063/1.4931199>

Asaduzzaman, M., & Pramanik, M. H. R. (2005). Allelopathic effect of rice straw in soil on nutrient and chlorophyll contents of transplanted aman rice. Bangladesh Journal of Environmental Science, 11(2), 359–363.

Calha, I., Oliveira, M. de F., & Reis, P. (2023). Weed management challenges in rice cultivation in the context of pesticide use reduction: a survey approach. Sustainability (Switzerland), 15(1). <https://doi.org/10.3390/su15010244>

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Frontiers in Plant Science, 6(11), 1–16. <https://doi.org/10.3389/fpls.2015.01020>

Ciulu, M., de la Luz Cádiz-Gurrea, M., & Segura-Carretero, A. (2018). Extraction and analysis of phenolic compounds in rice: A review. Molecules, 23(11), 1–20. <https://doi.org/10.3390/molecules23112890>

Dora, Z. A., Nurjanah, U., Setyowati, N., Marlin, & Hairani, P. M. (2025). Allelopathic effects of mexican sunflower (*Tithonia diversifolia* (Hemsl) A. Gray) aqueous extract on rice (*Oryza sativa* L.) test plants germination and early growth. Journal of Agriculture and Ecology Research International, 26(3), 43–55. <https://doi.org/10.9734/jaeri/2025/v26i3674>

Einhellig, F. A. (1994). Allelopathy: Current Status and Future Goals. American Chemical Society, 1–24.

Elzaawely, A. A., Maswada, H. F., El-Sayed, M. E. A., & Ahmed, M. E. (2017). Phenolic compounds and antioxidant activity of rice straw extract. International Letters of Natural Sciences, 64, 1–9. <https://doi.org/10.18052/www.scipress.com/ilns.64.1>

Hamamura, K. (2018). Development of herbicides for paddy rice in Japan. Weed Biology and Management, 18(2), 75–91. <https://doi.org/10.1111/wbm.12147>

Hassan, M., Shahzadi, S., & Kloczkowski, A. (2023). Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: a review. Molecules, 28(1). <https://doi.org/10.3390/molecules28010378>

Hussain, M. I., & Reigosa, M. J. (2021). Secondary metabolites, ferulic acid and p-hydroxybenzoic acid induced toxic effects on photosynthetic process in rumex acetosa l. Biomolecules, 11(2), 1–13. <https://doi.org/10.3390/biom11020233>

Jamal, M. R., Kristiansen, P., Kabir, M. J., & Lobry de Bruyn, L. (2023). Challenges and adaptations for resilient rice production under changing environments in Bangladesh. Land, 12, 1–21. <https://doi.org/10.3390/land12061217>

Kato-Noguchi, H. (2024). Isolation and identification of allelochemicals and their activities and functions. Journal of Pesticide Science, *49*(1), 1–14. <https://doi.org/10.1584/JPESTICS.D23-052>

Kato-Noguchi, H., & Ota, K. (2013). Biological activities of rice allelochemicals Momilactone A and B. Rice Research: Open Access, 1(2), 1–5. <https://doi.org/10.4172/jrr.1000108>

Khamare, Y., Chen, J., & Marble, S. C. (2022). Allelopathy and its application as a weed management tool: A review. Frontiers in Plant Science, 13, 1–17. <https://doi.org/10.3389/fpls.2022.1034649>

Khanh, T. D., Xuan, T. D., & Chung, I. M. (2007). Rice allelopathy and the possibility for weed management. Annals of Applied Biology, 151(3), 325–339. <https://doi.org/10.1111/j.1744-7348.2007.00183.x>

Kumar, N., Singh, H., Giri, K., Kumar, A., Joshi, A., Yadav, S., Singh, R., Bisht, S., Kumari, R., Jeena, N., Khairakpam, R., & Mishra, G. (2024). Physiological and molecular insights into the allelopathic effects on agroecosystems under changing environmental conditions. Physiology and Molecular Biology of Plants, 30(3), 417–433. <https://doi.org/10.1007/s12298-024-01440-x>

Kumar, S., Abedin, M. M., Das, S., & Singh, A. K. (2020). Role of phenolic compounds in plant-defensive mechanisms. Plant Phenolics in Sustainable Agriculture, 1, 517–532. <https://doi.org/10.1007/978-981-15-4890-1>

Li, B., Wu, W., Shen, W., Xiong, F., & Wang, K. (2024a). Allelochemicals released from rice straw inhibit wheat seed germination and seedling growth. Agronomy, 14(10). <https://doi.org/10.3390/agronomy14102376>

Li, J., Xiao, Q., Wu, H., & Li, J. (2024b). Unpacking the global rice trade network: centrality, structural holes, and the nexus of food insecurity. Foods, 13(4), 1–26. <https://doi.org/10.3390/foods13040604>

Li, Z. H., Wang, Q., Ruan, X., Pan, C. De, & Jiang, D. A. (2010). Phenolics and plant allelopathy.Molecules,15(12),8933–8952. <https://doi.org/10.3390/molecules15128933>

Mai, N. P., & Xuan, T. D. (2024). A review on the utility potential of rice derived products in weed management. Weed Research, 65(1), 1–18. <https://doi.org/10.1111/wre.12678>

Ministry of Agriculture. (2007). Site-Specific Fertilizer Recommendations for N, P, and K on Lowland Rice. Regulation of the Minister of Agriculture Number 40/Permentan/OT.140/4/2007, pp. 1–34.

Peranginangin, P. H., Setyowati, N., Nurjanah, U., Supanjani, Chozin, M., & Anggraini, S. (2025). Cogongrass (*Imperata cylindrica* L.) Allelopathy and its influence on cucumber test plant seedling development and productivity. Asian Journal of Agricultural and Horticultural Research, 12(2), 272–285. <https://doi.org/10.9734/ajahr/2025/v12i2385>

Qi, Y., Wei, W., Chen, C., & Chen, L. (2019). Plant root-shoot biomass allocation over diverse biomes: A global synthesis. Global Ecology and Conservation, 18(18), e00606. <https://doi.org/10.1016/j.gecco.2019.e00606>

Rahaman, F., Shukor Juraimi, A., Rafii, M. Y., Uddin, K., Hassan, L., Chowdhury, A. K., Karim, S. M. R., Yusuf Rini, B., Yusuff, O., Bashar, H. M. K., & Hossain, A. (2022). Allelopathic potential in rice - a biochemical tool for plant defence against weeds. Frontiers in Plant Science, 13(12), 1–14. <https://doi.org/10.3389/fpls.2022.1072723>

Rayee, R., Anh, L. H., Khanh, T. D., & Xuan, T. D. (2024). Potential momilactones in rice stress tolerance and health advantages. Agronomy, 14(3), 1–21. <https://doi.org/10.3390/agronomy14030405>

Reigosa, M., Pedrol, N., & Luis Gonzalez. (2006). Allelopathy: a physiological process with ecological implications. Springer Science.

Rice, E. (1984). Allelopathy (2nd ed.). Academic Press.

Sansenya, S., Payaka, A., Wannasut, W., Hua, Y., & Chumanee, S. (2021). Biological activity of rice extract and the inhibition potential of rice extract, rice volatile compounds and their combination against α-glucosidase, α-amylase and tyrosinase. International Journal of Food Science and Technology, 56(4), 1865–1876. <https://doi.org/10.1111/ijfs.14816>

Scavo, A., & Mauromicale, G. (2021). Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. Agronomy, *11*(11). <https://doi.org/10.3390/agronomy11112104>

Setyowati, N., Siregar, D. F. A., Nurjanah, U., & Muktamar, Z. (2025). Allelochemicals of goatweed ( *Ageratum conyzoides* L .): potential as a natural herbicide for weed control. American Journal of Multidisciplinary Research & Development, 07(03), 1–8

Shah, A., & Smith, D. L. (2020). Flavonoids in agriculture: Chemistry and roles in, biotic and abiotic stress responses, and microbial associations. Agronomy, 10(8). <https://doi.org/10.3390/agronomy10081209>

Šoln, K., Klemenčič, M., & Koce, J. D. (2022). Plant cell responses to allelopathy: from oxidative stress to programmed cell death. Protoplasma, 259(5), 1111–1124.

Sultana, M. H., Alamin, M., Qiu, J., Fan, L., & Ye, C. (2023). Transcriptomic profiling reveals candidate allelopathic genes in rice responsible for interactions with barnyardgrass. Frontiers in Plant Science, 14(2), 1–15. <https://doi.org/10.3389/fpls.2023.1104951>

Zang, Y., Yao, Y., Xu, Z., Wang, B., Mao, Y., Wang, W., Zhang, W., Zhang, H., Liu, L., Wang, Z., Liang, G., Yang, J., Zhou, Y., & Gu, J. (2022). The relationships among “STAY-GREEN” trait, post-anthesis assimilate remobilization, and grain yield in rice (*Oryza sativa* L.). International Journal of Molecular Sciences, 23(22). <https://doi.org/10.3390/ijms232213668>

Zhang, C., Suo, W., Pan, Y., & Feng, Y. (2025). Effects of bt rice straw extract on seed germination and plant growth of pakchoi: novel variables of cropping system. Plants, 14(12), 1–15. <https://doi.org/10.3390/plants14121797>

Zhang, X. Y., Shi, S. L., Li, X. L., Li, C. N., Zhang, C. M., Yun, A., Kang, W. J., & Yin, G. L. (2021). Effects of autotoxicity on alfalfa (*Medicago sativa*): Seed germination, oxidative damage and lipid peroxidation of seedlings. Agronomy, 11(6). <https://doi.org/10.3390/agronomy11061027>