Autotoxic Effects of Rice Bran on Seed Germination, Growth and Yield Component

in Rice (*Oryza sativa* L.)

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ABSTRACT

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| The development of natural weed control methods using agricultural waste is gaining attention as part of sustainable farming practices. Rice bran is one such potential material; however, its application may adversely affect rice plants themselves. The aim of the research were to determine the concentration of rice bran water extract that inhibits rice germination and growth, determine the Inhibition Concentration (IC50)(50% inhibition concentration) of rice bran water extract in suppressing seedling growth and to evaluate the effect of rice bran extract concentration on rice growth and its impact on yield components.The research was carried out 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) was employed, in a single factor with five extract concentration treatments (0%, 2.5%, 5%, 7.5%, and 10%) and five replications. Observed variables included germination indicators such as radicle and plumule length, dry weight of radicle and plumule, and percentage of abnormal seedling, as well as growth and yield variables, including plant height, number of leaves, number of productive tillers, leaf area, panicle length, number of panicles per hill, leaf greenness, flowering time, grain weight, and dry weight of roots and shoots. The research showed that within the concentration range of 0-10%, higher rice bran extract concentrations significantly suppressed rice germination, as indicated by increased abnormal seedlings, reduced shoot-root length, and lower seedling weight compared to lower concentrations. A  3.54% rice bran extract concentration effectively inhibited rice test plant germination, with 50% of seedlings showing abnormalities, stunted radicles and plumules, and black spots on the grains. At 10% concentration, both vegetative growth and yield components of rice were more severely suppressed compared to lower extract concentrations.These results suggest that rice bran possesses autotoxic potential that could be harnessed as an eco friendly bioherbicide. However, proper management of rice residues in the field is essential to prevent negative impacts on crop productivity. |

*Keywords:* *allelopathy, autotoxicity, IC₅₀, rice germination, sustainable agriculture*

1. INTRODUCTION

Rice (*Oryza sativa* L.) serves as the primary food source for a large portion of the global population, especially in Asian countries (Fukagawa & Ziska, 2019; Megha et al., 2025). Its cultivation plays a crucial role in ensuring global food availability and sustaining the livelihoods of rural communities (Muthayya *et al.,* 2014). In Indonesia, rice is a vital commodity that underpins the national food security agenda, with ongoing research aimed at enhancing its productivity and long-term sustainability (Syuaib, 2016). Nevertheless, rice farming is confronted with several sustainability issues, such as soil fertility degradation, the emergence of herbicide-resistant weeds, and excessive reliance on agrochemicals (Gomiero, 2016). Rice are known to produce allelochemicals distributed throughout various plant parts (Abbas et al., 2015). These compounds have the potential to inhibit the growth of both weeds and rice themselves. Rice bran, in particular, has been identified as a rich source of allelopathic substances (Kato-Noguchi et al., 2005; Jung et al., 2004; Rayee et al., 2024).

Rice bran, a byproduct of the rice milling process accounting for approximately 8–10% of the grain's weight, is known for its valuable bioactive components but also poses a risk of autotoxicity. Autotoxicity refers to the suppression of a plant’s own growth due to chemical compounds released from the residues of the same species (Kong et al., 2018; Zhang et al., 2005). This byproduct contains various phenolic compounds, including coumarin, momilactone A, momilactone B, and flavonoids, which are the primary agents responsible for its allelopathic activity (Yulianto & Xuan, 2018). Coumarin has been reported to reduce seed germination and seedling development while enhancing weed suppression (Isda et al., 2013). Momilactones A and B interfere with seed germination by disrupting protein breakdown processes, whereas flavonoids influence plant development by modulating auxin transport mechanisms (Sultana et al., 2023; Shah & Smith, 2020). Although rice bran is frequently applied as organic material or used as livestock feed, its chemical constituents can trigger autotoxic effects that negatively affect seed germination and early plant growth. The extent of these allelopathic impacts depends on plant species, allelochemical concentration, and surrounding environmental factors (Rayee et al., 2024).

Autotoxicity takes place as rice bran undergoes decomposition in the soil, during which allelochemical compounds are released into the surrounding environment. These compounds can disrupt key physiological processes such as mitochondrial activity, cell division, nutrient uptake, and can induce oxidative stress (Mahayaning & Darmanti, 2013). A study by Kayode and Ayeni (2009) demonstrated that rice bran extract had a significant inhibitory effect on corn seed germination. Supporting results were reported by Yong-in (2001), who found that applying rice bran extract at concentrations ranging from 1% to 5% with the strongest effect observed at 5% was effective in suppressing the germination and growth of barnyard grass (*Echinochloa crus-galli* L.).

One method to evaluate the toxicity of a substance is through a toxicity assay, which is a biological approach used to measure how toxic a compound is. A chemical is considered acutely toxic if it produces harmful effects within a short period. In allelopathic studies, a key indicator of inhibitory activity is the IC₅₀ value, the concentration needed to reduce free radical activity by 50% (Katrin et al., 2015; Cortés et al., 2001). A lower IC₅₀ signifies a higher level of toxicity or inhibition. This value represents the concentration at which 50% of the target organisms growth is suppressed, and it is typically determined through graphical methods and calculations conducted at specific observation intervals (Handayani et al., 2018; Fadhillah et al., 2024).

While the phytotoxic properties of rice bran are widely recognized, the threshold for its safe application in agriculture and the influence of soil microorganisms in mitigating its toxicity remain insufficiently understood. Previous research has primarily focused on individual compounds like ferulic acid or momilactone, often at concentrations that do not reflect realistic field conditions (Kato-Noguchi et al., 2010). In contrast, rice bran naturally contains a complex mixture of more than 20 phenolic compounds that may interact synergistically or antagonistically (Ho et al., 2020). Proper handling of autotoxicity can optimize the use of rice bran, for example by composting or enzymatic detoxification to suppress weed growth and improve soil health without sacrificing crop productivity. Effective management strategies, such as composting or enzymatic detoxification, could help reduce its toxicity, allowing rice bran to be used as a natural weed suppressant and soil enhancer without negatively impacting crop performance. Such practices support sustainable agriculture by minimizing waste and reducing dependence on synthetic inputs in rice cultivation. The aim of the study were to determine the concentration of rice bran water extract that inhibits rice seedling and vegetative growth, determine the IC50 of rice bran water extract in suppressing seedling growth and to assess the effect of rice bran extract concentration on rice growth and yield components.

2. material and methods

2.1 Location SITE and Research Design

The study was carried out from November 2024 to March 2025 at the Agronomy Laboratory and the Experimental Station of the Faculty of Agriculture, University of Bengkulu. It consisted of two main phases: (1) a laboratory experiment using Petri dishes to investigate the effect of rice bran extract on seed germination and early growth, and (2) a greenhouse experiment using buckets to evaluate its impact on rice growth and yield. The experimental design followed a Completely Randomized Design (CRD) with a single factor rice bran concentration comprising five treatment levels: P0 = 0%, P1 = 2.5%, P2 = 5%, P3 = 7.5%, and P4= 10%. Each treatment was replicated five times, yielding a total of 25 experimental units. Each unit included two Petri dishes and two buckets.

* 1. Research Stages

2.2.1 preleminary research

Rice bran was collected from local rice farmers. Rice brand was then oven-dried at a temperature of 50 °C for three days (Yong-in, 2001). The dried material was then ground using a blender to produce fine bran powder. A total of 100 g of the powdered bran was mixed with 1000 ml of distilled water and agitated on a shaker at 150 rpm for 24 hours (Yong-in, 2001). The mixture was subsequently filtered twice using Whatman No. 1 filter paper to obtain a 10% stock extract. This stock solution was then diluted to prepare different treatment concentrations as follows: P0 (control) - 0 ml extract + 100 ml distilled water; P1 (2.5%) - 25 ml extract + 75 ml distilled water; P2 (5%) - 50 ml extract + 50 ml distilled water; P3 (7.5%) - 75 ml extract + 25 ml distilled water; and P4 (10%) - 100 ml extract with no dilution.

2.2.2 Laboratory test

Sterile Petri dishes were prepared by lining them with Whatman No. 1 filter paper. Prior to use, the Petri dishes were sterilized using a 5% Bayclin solution (bayclin contains sodium hypochlorite which is used as a disinfectant to kill germs and bacteria), followed by rinsing with 70% ethanol (Peranginangin et al., 2025). Each dish received 10 ml of rice bran extract according to the designated treatment concentration. Twenty-five rice seeds were then arranged in each petri dish and incubated at room temperature (27˚C) for a period of seven days.

2.2.3 Greenhouse test

The planting medium consisted of a 1:1 (w:w) mixture of soil and organic cow manure. Each bucket was filled with 3 kg of this mixture. Three seedlings, each seven days old and previously test in the laboratory experiment, were transplanted into every bucket. Throughout the growth period, standard agronomical practices were implemented, including watering, thinning, fertilization, manual weed removal, and pest management. Fertilizer was applied twice: once at one week after transplanting and at three weeks after transplanting. The fertilizer dosage per bucket was equivalent to field application rates: 0.3 g of Urea (300 kg/ha), 0.01 g of TSP (100 kg/ha), and 0.01 g of KCl (100 kg/ha) (Ministry of Agriculture, 2007).

2.2.4 Harvesting

Harvesting was carried out during the generative phase, specifically at 120 days after planting (DAP). The process involved cutting open one side of the bucket, followed by rinsing the planting medium with running water to completely remove the soil

2.2 Observations in the Laboratory and Greenhouse

2.2.1 LABORATORY EXPERIMENT

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%

The percentage of abnormal seedlings is calculated from day 1 to 7 after sowing. Radicle and plumule lengths are measured on day 7 using a ruler. Radicle, plumule, and total dry weight were obtained after oven-drying at 70°C until a constant weight, then weighed using a digital scale.

2.2.1 GREENHOUSE EXPERIMENT

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 (Tripathi et al., 2023). Panicle length was measured after grain formation. The number of panicles was counted based on productive tillers. Flowering time was recorded from planting to the emergence of the first flower. The weight of one thousand grains was obtained by weighing 1,000 dry grains. Shoot and root dry weights were measured after drying in an oven at 70°C until constant weight.

* 1. Data Analysis

The collected data were statistically analyzed using analysis of variance (Anova) at a 5% and 1% significance level. For the significant result, further analysis was performed using Orthogonal Polynomial tests. The 50% inhibition concentration (IC₅₀) of the rice bran-based bioherbicide was determined through regression analysis. While the Least Significant Difference (LSD) test was used for greenhouse data evaluation.

Relative shoot length (RSL) was calculated using the formula

 RSL = $\frac{extract shoot length }{control shoot length}$ x 100%

Relative root and shoot weight (RRSW):

 RRSW = $\frac{extract roots + shoot dry weight  }{control roots + shoot dry weight }$x 100%

The shoot root ratio (SRR):

 SRR = $\frac{extract roots + shoot dry weight  }{control roots + shoot dry weight }$x 100%

3. results and discussion

3.1 Effectiveness of rice bran extract on rice seed germination

The application of rice bran extract had a highly significant impact on seed germination and suppressed the growth of rice seedlings. Prior to analyzing the percentage of abnormal seedlings, the data were transformed using the formula √𝑥 + 0.5 (Peranginangin et al., 2025). The rice bran concentration treatments significantly affected all variables (Table 1).

**Table 1. Variance Analysis of Rice Seed Germination Variables**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **F-calc** | **CV (%)** | **F-table 5%** | **F-table 1%** |
| Abnormal Seedling Percentage | 88.26 \*\* | 1.24 | 2.87 | 4.43 |
| Plumule Length | 6.62\*\* | 12.61 |
| Radicle Length | 21.71\*\* | 20.4 |
| Plumula Dry Weight | 22.08\*\* | 11.89 |
| Radicle Dry Weight | 33.4\*\* | 13.39 |
| Total Dry Weight | 9.99\*\* | 19.09 |

*\*= significant different; \*\*= highly significant different, t= transformed data √x 0.5*

The results of the variance analysis indicate the presence of allelopathic effects on several variables, including the percentage of abnormal seedlings, radicle length, plumule length, radicle dry weight, plumule dry weight, and total dry weight.

**3.1.1 Percentage of Abnormal Seedling**

Abnormal seedlings are those that lack the ability to grow and develop into healthy, normal plants. They typically exhibit morphological abnormalities, such as shortened or decayed radicles, underdeveloped plumules, unusual coloration, or inhibited growth.

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**Figure 1. Allelopathic effects of rice bran on germination (A) and growth of rice (B)**

Allelopathic substances found in rice plant residues, such as momilactone A and momilactone B, have been reported to possess autotoxic properties that can inhibit rice seed germination (Kato-Noguchi & Ota, 2013). In this experiment, in the control treatment (0%), seedlings exhibited normal development, marked by well elongated radicles, plumules and the formation of secondary roots. However, at a 2.5% concentration, signs of growth inhibition began to appear, including shortened radicles, abnormal radicle orientation (e.g., radicle avoidance), shortened plumules, and the presence of black spots on the seed surface. When the concentration increased to 5%-10%, the seedlings became severely stunted, with shortened and darkened radicles and plumules, along with more pronounced black spotting on the seed surface. These symptoms suggest that autotoxic compounds like momilactone A and B can interfere with key physiological processes during germination such as enzyme activity, cell elongation, and cell expansion leading to metabolic disturbances and abnormal seedling development (Estiati, 2019).

**3.1.2 Radicle and Plumule Length**

The radicle is an embryonic part of a seed that serves as the initial root structure and is the first organ to emerge during the germination process. Its main role is to anchor the seedling into the growing medium and begin absorbing water and essential nutrients needed for early plant development.

The plumule is the embryonic structure within the seed that gives rise to the shoot system, which grows above the soil surface and eventually forms the plant’s stems and leaves. Its development plays a crucial role in the initial stages of plant growth, as it influences the successful formation of the shoot and the efficiency of subsequent photosynthesis processes.

 **A B**

**Figure 2. Effect of rice bran extract concentration on radicle (A) and plumule length (B)**

At a 0% concentration, the radicle length reached 5.18 cm, while at 2.5%, it decreased to 4.27 cm. This suggests that for every 2.5% increase in concentration, radicle length was reduced by approximately 0.19 cm. The increase in rice bran allelopathic concentration led to a progressive reduction in radicle elongation in rice seeds. This inhibition is likely due to interference in the mobilization of food reserves, particularly the suppression of hydrolytic enzymes needed during germination. Radicle development depends on enzymes like amylase and protease, which are essential for breaking down starch and protein stored in the endosperm (Thu & Xuan, 2018). Phenolic compounds present in the radicle may disrupt early metabolic activities, while oxidative stress is also believed to contribute to growth inhibition (Butsat & Siriamornpun, 2010).

At 0% concentration, the average plumule length was 4.15 cm, while at 2.5% concentration, it decreased to 3.86 cm. For every 2.5% increase in concentration, there was a reduction of 0.29 cm in plumule length. These findings suggest that allelopathic compounds in rice bran have a strong inhibitory effect on early plant growth, particularly on the plumule, which plays a vital role during the initial stages of plant development. This result aligns with the study by Ahluwalia et al. (2016), which reported that rice residues release allelopathic substances, such as phenolic acids, that can suppress the growth of both radicles and plumules. The inhibition mechanism involves disruption of enzymatic activity, alteration of membrane permeability, and suppression of the synthesis of growth hormones like auxin and gibberellin. Similarly, research by Kayode and Ayeni (2009) found that extracts from rice residues suppressed corn seedling growth, including plumule and radicle development. In the present study, it was consistently observed that plumule length was greatest in the control treatment (0%) and decreased progressively with increasing concentrations of rice bran extract (2.5%, 5%, 7.5%, and 10%).

**3.1.3 Radicle, Plumule and Total Dry Weight**

Radicle dry weight serves as an important variable for evaluating root system development. Greater radicle dry weight typically signifies robust root growth, reflecting the plant’s ability to efficiently absorb water and nutrients. This measurement also helps assess the impact of different treatments, including the application of rice bran allelopathic extracts, on root development. Similarly, plumule dry weight is measured as an indicator of overall plant growth and vitality. A higher plumule dry weight usually corresponds to improved vegetative growth and enhanced physiological function. This variable is also useful for evaluating the influence of various treatments and determining the effectiveness of photosynthesis during plant development.

**A B**

**C**

**Figure 3. Effect of Rice Bran Extract on Radicle (A), Plumule (B) and Total Dry Weight (C)**

The radicle dry weight was recorded at 2.63 mg for the 0% concentration and decreased to 2.23 mg at 2.5% concentration, indicating a reduction of 0.4 mg with every 2.5% increase in concentration. Similarly, the plumule dry weight declined from 2.61 mg at 0% concentration to 2.30 mg at 2.5%, showing a decrease of 0.31 mg. These results suggest that the allelopathic compounds present in rice bran exert toxic effects on seedling growth in a concentration dependent manner.

The reduction in radicle and plumule dry weight was associated with the increasing concentration of rice bran allelopathic compounds. This decline is thought to be caused by the presence of allelochemicals, particularly phenolic acids and their derivatives, which can interfere with enzymatic activity in the radicle, alter root cell membrane permeability, and hinder nutrient uptake (John & Sarada, 2012). According to Kato-Noguchi et al. (2010), the compound momilactone B exhibits strong allelopathic activity by restricting root tissue expansion and reducing water and nutrient absorption, thereby decreasing biomass production and radicle dry weight. Further research by Kato-Noguchi and Peters (2013) confirmed that momilactone B is the primary compound responsible for the allelopathic effects of rice, showing significant inhibitory influence on plant growth. This growth suppression also extends to the plumule, which plays a crucial role in early plant development. Hence, the allelopathic influence of momilactone B in rice bran affects not only root development but also significantly impairs plumule biomass by disrupting physiological processes during the early stages of growth.

The total dry weight was measured to evaluate the impact of rice bran allelopathic extracts on rice seed development. This measurement provides insights into the growth performance and vigor of the seedlings, as it combines the dry weight of both the plumule and the radicle. Analysis results indicated that the allelopathic compounds in rice bran significantly influenced total dry weight. At 0% concentration, the total dry weight was 4.92 mg, while at 2.5% concentration it declined to 4.31 mg, showing a reduction of 0.60 mg with every 2.5% increase in concentration. These findings are consistent with previous observations on both the length and dry weight of the plumule and radicle. Higher extract concentrations were found to suppress seed germination and seedling growth. The strong inhibitory effects observed can be attributed to allelopathic compounds present in rice bran, such as flavonoids, momilactone A and B, methylamine, and siloxane derivatives (Yulianto & Xuan, 2018; Quan et al., 2019).

**3.2 Inhibition Concentration 50% (IC50 )**

The IC50 value in this study was used to assess the concentration of rice bran allelopathic compounds required to inhibit 50% of rice seed growth. The IC50 was determined using regression equations based on several variables, including the percentage of abnormal seedlings, radicle and plumule length, and radicle, plumule, and total dry weight.

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

|  |  |  |
| --- | --- | --- |
| **Variables** | **Regression Equation** | **IC50** |
| Abnormal Seedling Percentage | y = 84.131x + 3.4754 | 3.54 |
| Radicle Length | y = -36.181x + 5.184 | 12.95 |
| Plumule Length | y = -11.789x + 4.1573 | 31.02 |
| Radicle Dry Weight | y = -16.117x + 2.6389 | 13.27 |
| Plumule Dry Weight | y = -12.341x + 2.614 | 17.10 |
| Total Dry Weight | y = -24.28x + 4.9245 | 18.22 |

The most sensitive indicator of rice bran extract toxicity was the percentage of abnormal seedling, with an IC50 of 3.54%, suggesting early morphological disruption. Radicle growth was also more affected (IC50 = 12.95%) than plumule growth (IC50 = 31.02%), and this pattern was consistent in dry weight measurements of radicle (IC50 = 13.27%) vs. plumule (IC50 = 17.10%). This suggests that the radicle is more vulnerable due to its direct exposure and simpler tissue structure (Susilo et al., 2022; Dora et al., 2025; Peranginangin et al., 2025), supporting evidence that allelochemicals interfere with seed physiology.

According to Yulifrianti et al. (2015), allelopathic compounds can enter seeds as water-soluble secondary metabolites that function like natural herbicides, inhibiting the activity of key growth hormones such as gibberellic acid (GA) and indole acetic acid (IAA). Suppression of gibberellin synthesis disrupts the production of amylase enzymes, limiting glucose availability needed for growth. This ultimately restricts cell division and elongation, hindering seed germination and seedling development. Similarly, Einhellig (1994) reported that phenolic compounds absorbed by seeds can interfere with endosperm metabolism and reduce the effectiveness of germination enzymes, especially those involved in carbohydrate breakdown. These findings align with the view that allelopathic substances in rice bran such as ferulic acid, cinnamic acid, and p-coumarate disturb seed physiology and the mobilization of food reserves.

**3.3 Effectiveness of Rice Bran Extract on the Vegetative Growth of Rice**

The application of rice bran extract has been shown to influence the vegetative growth and yield component of rice. The different extract concentration treatments had a highly significant impact on all observed variables (Table 3).

**Table 3. Analysis Variance of the Effect of Rice Bran Extract on Rice Vegetative Growth and Yield Component**

\*=significant different; \*\*=highly significant different

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **F-calc** | **CV (%)** | **F-table 5%** | **F-table 1%** |
| Plant Height | 18.55\*\* | 5.26 | 2.87 | 4.43 |
| Number of Leaf | 17.29\*\* | 13.50 |
| Number of Tillers | 91.81\*\* | 8.70 |
| Leaf Area | 84.55\*\* | 13.53 |
| Leaves Greeness | 129.85\*\* | 3.65 |
| Panicles Length  | 98.69\*\* | 3.21 |
| Number of Panicles  | 19.92\*\* | 17.10 |
| Flowering Time | 6.36\*\* | 3.06 |
| 1000 Grain Weight | 47.05\*\* | 2.77 |
| Shoot Root Ratio | 3.49\*\* | 12.81 |
| Relative Shoot Length | 5.89\*\* | 5.99 | 3.24 | 5.29 |
| Relative Shoot Root Weight | 151.11\*\* | 2.16 |

Table 3 showed, variables such as plant height, number of leaves, number of tillers, leaf area, leaf greenness, panicle length, number of panicles, flowering time, weight of one thousand grains, shoot root ratio and relative shoot root weight highly significantly affected by rice bran extract concentration.

**Table 4. Effect of Rice Bran Extract on RiceGrowth**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Extract Concentration (%)** | **PH (cm)** | **NL** | **LL (cm)** | **NT** | **LA (cm)** | **LG** |
| 0 | 103.82a | 148,8a | 69.52a | 43.46a | 9928,88a | 42.21a |
| 2.5 | 91.81b | 121.26b | 64.18b | 34.93b | 59952.14b | 38.40b |
| 5 | 89.31b | 106.2bc | 59.13c | 30.26c | 5792.99b | 37.12b |
| 7.5 | 86.56b | 98.73c | 54.40d | 24.40d | 3634.18c | 33.15c |
| 10 | 78.73c | 74.33d | 45.84e | 14.26e | 186.28d | 24.984d |

*Note: numbers followed by different letters in the same column are significantly different in the LSD test. PH = Plant height (cm), NL = Number of leaves, LL = Leaf length (cm), NT = Number of tillers, LA = Leaf area (cm2), LG = Leaf greenness.*

Varying concentrations of rice bran significantly influenced plant height, leaf area, leaf greenness, number of leaves, and number of tillers (Table 4). The control treatment (0%) resulted in significantly greater plant height compared to all other treatments (2.5%, 5%, 7.5%, and 10%). However, the 2.5% concentration did not differ significantly from the 5% and 7.5% levels, suggesting that the inhibitory effect at lower concentrations is still relatively weak. Significant differences in leaf number emerged between 2.5% and 5% treatments, while 5% and 7.5% remained comparable. The 10% treatment yielded the fewest leaves, showing significant differences versus all other treatments. Leaf length and tiller number progressively decreased with increasing extract concentration, reaching minimal values at 10% treatment that differed significantly from 0-7.5% treatments. This demonstrates concentration dependent growth suppression from rice bran phytotoxicity.

As the concentration of the extract increased, both leaf area and greenness gradually decreased. The 0% treatment had notably higher values compared to the 2.5%, 5%, 7.5%, and 10% treatments, while there was no significant difference between the 2.5% and 5% treatments. A decrease in chlorophyll content was initially detected at the 2.5% concentration, but statistically significant effects became apparent only at 5% and higher concentrations.

Leaf area is vital for capturing light needed for photosynthesis. A reduction in leaf area leads to decreased photosynthetic efficiency, which in turn lowers plant height, leaf number, and tiller formation. This condition also limits biomass production, including root dry weight, due to reduced photosynthates being transported to the lower plant parts (Maisura et al., 2020). These findings are consistent with Kayode and Ayeni (2009), who found that rice bran extract significantly suppressed corn seed germination, with the allelopathic effect intensifying as the extract concentration increased. Hughes et al. (2017) reported that various allelochemicals are capable of suppressing plant growth, including that of rice. In cereal crops like rice, flavonoids especially those found in rice bran play a significant role. Rice bran contains flavonoids such as tricin, apigenin, and luteolin. These compounds have been shown to exhibit autotoxic effects, meaning they can negatively affect rice growth when applied at high concentrations (Kong et al., 2007).

**Table 5. Effect of Rice Bran Extract on Rice Yield Component**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Extract Concentration (%)** | **PL (cm)** | **NP** | **FT**  | **WOG (g)** | **RST** | **RSL** | **RRSW**  |
| 0 | 27.45a | 26.50a | 72a | 34.86a | 1,08a | - | - |
| 2.5 | 26.40bc | 22.3ab | 71.1ab | 31.40b | 1,03ab | 0.88a | 218.79a |
| 5 | 23.90c | 20.70bc | 70.7ab | 29.78c | 0.99ab | 0.86a | 208.49b |
| 7.5 | 21.53d | 16.60c | 68.3bc | 29.08cd | 0.05b | 0.83a | 186.39c |
| 10 | 19.27e | 9.30d | 66.1c | 28.26d | 0.82c | 0.76b | 166.83d |

*Note: numbers followed by different letters in the same column are significantly different in the LSD test. PL = Panicle Length (cm), NP = Number of Panicles, FT = Flowering Time, WOG = Weight of 100 Grains (g), RST = Root-shoot ratio, RLS = Relative Shoot Length, RRSW = Relative Root Shoot Weight.*

The 0% concentration of rice bran extract produced the highest values for panicle length, number of panicles, and one thousand grain weight, significantly outperforming treatments with concentrations from 2.5% to 10%. At 10%, these yield components were notably lower than those of control treatment.These findings suggest that rice bran extract negatively affects the generative phase of rice growth, leading to reduced panicle number, shorter panicles, and lower grain weight. This effect is likely due to the inhibition of cell division and elongation in meristematic tissues of stems and roots (Reigosa et al., 1999; Chung et al., 2001), limiting stem elongation and panicle development. Consequently, tiller transformation into productive panicles is disrupted, reducing grain weight. These results are consistent with findings by Alam et al. (2018), who reported that rice plant water extracts significantly suppressed the growth of monocot weeds such as *Echinochloa crus-galli, Cyperus difformis, Cyperus iria, Fimbristylis miliacea,* and weedy rice.

Momilactone B, a rice specific diterpenoid, has been shown to suppress the synthesis of key growth hormones like gibberellin (GA) and auxin (IAA) (Kato-Noguchi & Ino, 2003). Disruption in hormone production or transport can interfere with flowering and grain-filling stages, leading to reduced grain weight per panicle. Flavonoids present in rice bran such as tricin, apigenin, and luteolin can impair photosynthesis, inhibit antioxidant enzyme activity, induce reactive oxygen species (ROS) accumulation, and cause oxidative stress in leaf and floral tissues, ultimately hindering proper grain development (Hughes et al., 2017). Additionally, phenolic compounds like ferulic acid and p-coumarate are known to inhibit key enzymes in primary metabolism, including nitrate reductase and enzymes involved in amino acid biosynthesis. Collectively, the physiological impact of these allelopathic compounds may disrupt critical stages of generative growth from panicle initiation to flowering and grain filling leading to fewer panicles per cluster, shorter panicles, lighter grains, and ultimately lower rice yield (Chung etal., 2001).

The flowering time variable showed that the 0% concentration was significantly different from the 10% treatment but not from the 2.5% and 5% levels. Notably, plants in the 10% treatment flowered earlier than those in other treatments, except for 7.5%. This early flowering at higher concentrations may be due to stress induced by allelopathic compounds, triggering an escape strategy a survival response where plants accelerate their reproductive phase under stress conditions. This physiological adaptation often involves increased ethylene production and sometimes elevated gibberellin levels, promoting a quicker shift from vegetative to generative growth (Cheng, 2015). Therefore, although high concentrations of rice bran extract can cause physiological stress, they may also prompt earlier flowering as a genetic survival mechanism.

The root-to-shoot ratio represents the balance between the above ground (shoot) and below-ground (root) biomass of the plant. Observations showed that the 0% concentration yielded the highest root shoot ratio, significantly different from the 7.5% and 10% treatments. The 7.5% and 10% concentrations resulted in lower biomass compared to the 0%–5% treatments. This decline is likely due to the higher levels of allelopathic compounds in those treatments, which induced metabolic disturbances in rice plants. During earlier exposure in the Petri dish phase, the roots had already encountered these compounds, leading to a shift in photosynthate allocation toward the shoot. Consequently, root growth was more severely inhibited than shoot growth, reducing nutrient uptake efficiency and negatively affecting overall plant development. According to Li et al. (2010), allelochemicals can impair the absorption of water, oxygen, and nutrients, as well as disrupt photosynthesis.

For the relative shoot length (RSL) and relative root and shoot weight (RRSW), the 0% concentration serves as the baseline or reference point, so no values are assigned to it in these variables. In the RSL analysis, the 2.5% concentration produced the highest value and was significantly different from the 10% concentration. The 10% treatment showed a significantly lower RSSL value compared to the 2.5%–7.5% treatments. In the RRSW variable, the 2.5% treatment also recorded the highest value, significantly different from the 5%–10% concentrations, with 10% yielding the lowest. This reduction in RRSW suggests that increasing allelopathic concentrations negatively affect biomass distribution, with root growth being more suppressed than shoot growth, thus lowering the root-to-shoot ratio. Yaseen (2014) emphasized that allelopathic inhibition is strongly influenced by extract concentration and plant origin. Additionally, phenolic compounds penetrating cell membranes may disrupt vital enzyme activities such as ATPase and peroxidase, leading to suppressed root cell division and elongation (Li et al., 2010; Weston & Duke, 2003). These disruptions impair root development and directly influence relative root-to-shoot biomass, meaning higher allelopathic concentrations result in greater inhibition of both root and shoot growth in rice.

4. Conclusion

The research showed that within the concentration range of 0–10%, higher rice bran extract concentrations significantly suppressed rice germination, as indicated by increased abnormal seedlings, reduced shoot and root length, and lower seedling weight compared to lower concentrations. A 3.54% rice bran extract concentration effectively inhibited rice test plant germination, with 50% of seedlings showing abnormalities, stunted radicles and plumules, and black spots on the grains. At 10% concentration, both vegetative growth and yield components of rice were more severely suppressed compared to lower rice bran extract concentrations.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Throughout the writing phase, artificial intelligence tools (including but not limited to Grammarly, QuillBot, and ChatGPT) were utilized solely for language refinement through grammar and spell checking, suggested wording improvements to increase clarity while maintaining factual accuracy. All research content, data analysis, and final determinations were exclusively produced by the authors, with AI playing no role in study outcomes or scientific judgments.

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