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Allelopathic Effects of Locally Cultivated Rice Varieties on Growth and Germination of Lettuce (*Lactuca sativa* L.) Using Sandwich and Seed Relay Assays

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RESEARCH ARTICLE INFORMATION	ABSTRACT

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This study aimed to evaluate the allelopathic potential of locally cultivated rice varieties in Isabela province, focusing on their effects on the germination and growth of lettuce (*Lactuca sativa* L.). The research employed two bioassay methods: the sandwich method, where crushed rice seeds were layered between agar with lettuce seeds, and the relay seeding method, which involved germinating rice seeds in petri dishes before co-culturing them with lettuce seeds. Using a Completely Randomized Design with five replications across eleven treatments, data on germination percentage, root length, shoot length, and dry weight were collected and analyzed. The results indicated significant allelopathic potential. The sandwich method revealed a notable decrease in lettuce germination, while the relay seeding method exhibited higher germination rates. The findings demonstrated that different rice varieties possess varying degrees of allelopathic potential, with NSIC Rc210H showing the most significant inhibitory effects on lettuce germination and growth.

Keywords: *Allelopathy, germination percentage, percent reduction, germination time, percent inhibition*

Introduction

Rice is the cornerstone of food security and economic stability in the Philippines, serving as the staple food for most Filipinos and the backbone of rural livelihoods (Mamiit et al., 2021). Nearly half of the nation's calorie intake comes from rice, and more than two million households are directly engaged in rice farming, with countless others depending on the rice value chain for employment and income (Radek, 2017). Rice availability and affordability are critical not only for nutrition but also for social and political stability in the Philippines. This was highlighted by the government's 2025 declaration of a food security emergency in response to soaring rice prices and supply concerns, prompting urgent measures to stabilize the market and protect consumers (USDA-FAS, 2025). Despite its central role, the Philippines remains the world's second-largest rice importer as of 2024, highlighting the persistent gap between domestic production and national demand. This reliance on imports exposes the country to global market volatility and underscores the urgent need to strengthen local rice production systems.

Isabela province is one of the Philippines' leading rice-producing regions, making a substantial contribution to both regional and national rice supplies. Improving rice productivity in Isabela has far-reaching implications for food security and economic resilience, particularly as weed infestation remains a major constraint. Weeds can reduce rice yields by up to 96% if not properly managed, increasing production costs and threatening the viability of smallholder farms (Khan et al., 2018; Olofsdotter, 2001). Traditional weed control methods—primarily reliant on chemical herbicides and labor-intensive mechanical interventions—are increasingly unsustainable due to their high costs and negative environmental impacts (Mai & Xuan, 2025; Rahaman et al., 2022).

This study addresses these challenges by investigating the allelopathic potential of locally grown rice varieties in Isabela—a promising but underutilized strategy for natural weed suppression. Allelopathy is the process by which plants release biochemicals (allelochemicals) that inhibit the growth and development of neighboring species, offering a potent, eco-friendly alternative to synthetic herbicides (Khan et al., 2018; Rahaman et al., 2022). Recent advances in molecular biology and plant breeding have accelerated the identification and enhancement of allelopathic traits in rice, with specific genetic markers and quantitative trait loci (QTLs) now mapped for use in breeding programs (Guo et al., 2020; Shehzad & Okuno, 2020). Notably, varieties such as MR439 and MR164, and genes like OsMYB57, have demonstrated strong allelopathic activity and regulatory control over allelochemical biosynthesis (Rahaman et al., 2022). By identifying and promoting rice varieties with strong allelopathic traits, this research aimed to provide farmers of Isabela with effective, sustainable weed management options that reduce input costs and environmental impact.

To rigorously quantify allelopathic effects, the study also employed lettuce as a model species in bioassays. Lettuce is highly sensitive to allelochemicals, making it a reliable indicator for evaluating and comparing the weed-suppressive capacity of different rice varieties (Cantila & Boholano, 2021; Olofsdotter, 2001). This dual focus strengthens the study's ability to generate actionable insights for both breeding and agronomic practices.

Furthermore, this work aligns with the principles of climate-smart agriculture (CSA) by promoting sustainable, low-input farming practices that enhance the resilience of rice systems to environmental stressors. By reducing reliance on synthetic herbicides and fostering resource-efficient production, the study supports the transition toward more sustainable and climate-resilient rice farming (Rahaman et al., 2022).

Hence, this research built on the foundational work of Olofsdotter (2001), Cantila and Boholano (2021), and recent advances in allelopathy and molecular breeding to offer a targeted, science-based solution to one of the most pressing constraints in Philippine rice production. By systematically evaluating the allelopathic potential of locally grown rice varieties and identifying those with the strongest weed-suppressive abilities, the study aimed to fill a critical research gap, guide breeding and management strategies, and ultimately contribute to food security and climate-smart agriculture in Isabela and beyond.

Methods

The study was conducted at the Science Laboratory of the Isabela State University-Echague campus from December 4, 2023, to February 24, 2024. It employed the following methods:

Preparation of the Test Organism

The lettuce (*Lactuca sativa* L.) was used as a test organism or the receiver organism in this study. This plant is a preferred choice for this study due to its sensitivity to allelopathic compounds, ease of cultivation, uniform growth characteristics, extensive research history, practical applications, availability, and utility as an indicator plant. Seeds of lettuce were procured from an agricultural supply in the locality. Before it is used in the study, a germination test using the ragdoll method was used to determine the viability of the seeds.

In the ragdoll method, two firm paper towels were moistened and laid flat on a surface. Twenty lettuce seeds were randomly selected from a pack and placed on one-half of the paper towel. The paper towel was then folded, rolled, and enclosed in a sealed plastic bag. This bag was placed in a warm room at room temperature. After four days, the paper towel was opened to observe germination. When 90% germination was achieved during the germination test, the remaining seeds in the pack were used for the allelopathy study.

The rice seeds used were the varieties that are popularly grown in the locality, which include both inbred and hybrid varieties. The rice seeds were procured in the locality. Before the conduct of the relay method bio-assay, the seeds had undergone a germination test through the ragdoll method. The same procedure was used as that of the lettuce seeds. When a 90% germination rate was observed, the rice seeds were eligible for bio-assay.

Preparation of the Rice Seeds

Seeds of different varieties of rice were procured locally. The varieties include both inbred and hybrid varieties. For the sandwich methods, the seeds were ground thoroughly and set aside for use in the bioassay. For the relay method, the viability of the seeds was tested through the ragdoll method.

Bio-Assays

Sandwich Method

The study employed the sandwich method, a laboratory-based donor-receiver bioassay, in screening the allelopathic potential of the seeds of rice varieties (donor plants) against the lettuce plant (receiver plant). The sandwich method, following the procedure of Fuji et al (2003), was used with slight modification.

In this study, 30 mL of 2% (*w/v*) agar solution was placed into a sterilized 250 mL mayonnaise bottle. The filled bottles were left undisturbed to solidify. Afterward, 150 mg of broken rice with husk from each variety was uniformly placed in each bottle on the solidified agar medium. The same amount of agar solution was poured again, and 20 lettuce seeds were placed on the agar medium in each bottle. The control sample was prepared with agar medium only.

All culture bottles were placed in the incubation chamber (25 ± 2 °C), and the experiment continued for 10 days. On the fourth day following germination, thinning was done to maintain 15 seedlings per bottle. After 10 days, parameters for germination and growth were gathered.

Relay Seeding Method

The relay seedling method followed the procedure developed by Navarez and Olofdotter (1996), who first used this laboratory bioassay approach to study the allelopathic potential of rice. Twenty (20) rice seeds from the different rice varieties were placed in each petri dish, lined with 9 cm Whatman No. 1 filter paper, and soaked in distilled water for 10 minutes.

Afterward, the petri dish was placed in the laboratory at room temperature (30 ± 2 °C) with a 12 h light period. After 12 days, the rice seedlings were reduced to ten (10) per petri dish, and twenty (20) lettuce seeds were placed as a co-culture together with the rice seedlings. In the control treatment, only 20 lettuce seeds were placed in petri dishes, and the experiment continued for up to 22 days or until the rice seedlings reached the four-leaf stage.To ensure proper hydration as well as absolute germination and development, 3 mL of distilled water was supplied to each petri dish every two days. Subsequently, the rice and lettuce seedlings were gently plucked and cleaned thoroughly for data collection.

Experimental Design and Layout

The experiment was laid out following the Completely Randomized Design (CRD) with five replications (Figure 1). The following treatments were used:

T0 – control (no donor plant)
T1 – NSIC Rc 222 (Tubigan 18)
T2 – NSIC Rc 436 (Tubigan 37)
T3 – NSIC Rc 480 (GSR 8)
T4 – NSIC Rc 508 (Tubigan 42)
T5 – NSIC Rc 512 (Tubigan 44)
T6 – PSB Rc 72H (Mestiso)
T7 – NSIC Rc 204H (Mestiso 20)
T8 – NSIC Rc 132H (Mestiso 6)
T9 – NSIC Rc 210H (Mestiso 23)
T10 – NSIC 2015 Rc404H (Mestiso 66)

Data Collection

In both methods, the following data were collected:

1. *Germination Percentage (GP)*. The number of lettuce seeds germinated was recorded daily starting on the fourth day of sowing. Earlier signs of germination, such as breaking of the seed coat, were considered germinated. This data was calculated using the formula:

$$GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2. *Mean Germination Time (MGT).* This was calculated using the following formula:

$$MGT = \frac{\sum nT}{\sum nt}$$

Where:

n = number of newly germinated seeds

T = time

t = days from sowing

3. *Root length (mm).* The seedling was laid flat on a clean surface. Using a ruler, the length was measured from the base of the stem to the longest root.
4. *Shoot length (mm).* Using a ruler, this was measured from the base of the aboveground part of the seedling up to the tip of the longest leaf.
5. *Dry Weight.* The lettuce seedlings were placed in paper bags and heated for 72 h in an electric oven set to 80 °C. After which, it was weighed using an analytical balance.
6. *Percent Reduction (PR).* To calculate the percent reduction in germination, mean germination time, root length, shoot length, and dry matter accumulation, the following mathematical equation used by Kabir et al. (2010) was employed as follows:

$$PR = \frac{C - T}{C} \times 100$$

Where:

C denotes the value under the control treatment

T denotes the value under the rice treatment

The percent reduction is used to determine the allelopathic potency of a treatment by calculating the percent reduction it causes in various growth parameters compared to a non-allelopathic control. The higher the percent reduction, the stronger the allelopathic effect.

7. *Average Percent Inhibition (API).* The API will be calculated following the formula of Karim et al (2014) as follows:

API = (% root reduction + % shoot reduction + % dry weight reduction + % germination rate reduction + % mean germination time reduction)/5.

A higher API value indicates a stronger inhibitory effect of the treatment on the plants, while a lower API value suggests less impact. If the API is negative, it would suggest that the treatment has a stimulatory effect rather than an inhibitory one.

Ethical Considerations

This study maintained ethical integrity by adhering to plant science research standards, excluding human/animal involvement. Locally sourced rice varieties were used legally, with no endangered species. Data confidentiality, originality, and proper citations were ensured. No conflicts of interest exist. The rights of contributors were acknowledged. The work upholds transparency and compliance for responsible scientific dissemination.

Results and Discussion

Germination Performance of Lettuce

Table 1 shows the germination performance of lettuce, the receiver plant, measured in terms of germination rate and mean germination time.

Table 1. Germination Performance of Lettuce as Affected by the Different Rice Varieties

The allelopathic effects of locally grown rice varieties on lettuce germination and growth were investigated using two bioassay methods: the sandwich method and the seed relay method. Both methods revealed varying impacts on germination rates and mean germination time (MGT), suggesting that the nature of plant interaction plays a key role in determining the strength of allelopathic effects.

Treatments	Germination Rate (%)		Mean Germination Time	
	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method
T_0 – control (no donor plant)	91.17[a]	95.67[a]	9.12[a]	10.50
T_1 – NSIC Rc 222 (Tubigan 18)	85.67[b]	90.08[abc]	8.61[b]	10.50
T_2 – NSIC Rc 436 (Tubigan 37)	83.00[e]	79.42[ab]	8.30[d]	10.50
T_3 – NSIC Rc 480 (GSR 8)	85.67[bc]	79.33[bc]	8.61[b]	10.50
T_4 – NSIC Rc 508 (Tubigan 42)	83.75[b]	76.33[b]	8.38[b]	10.50
T_5 – NSIC Rc 512 (Tubigan 44)	78.58[b]	91.67[b]	7.86[b]	10.50
T_6 – PSB Rc72H (Mestiso)	75.17[cd]	94.92[cd]	7.52[c]	10.50
T_7 – NSIC Rc204H (Mestiso 20)	73.08[de]	83.67[de]	7.31[cd]	10.50
T_8 – NSIC Rc132H (Mestiso 6)	72.50[e]	87.17[e]	7.25[d]	10.50
T_9 – NSIC Rc210H (Mestiso 23)	67.58[e]	75.58[e]	6.76[d]	10.50
T_{10} – NSIC 2015 Rc404H (Mestiso 66)	73.42[f]	91.92[f]	7.34[e]	10.50
Mean	79.05	85.98	7.91	10.50
ANOVA	**	**	**	ns

Note: Means with the same letter are not significantly different from each other at 1% level of significance
*** - highly significant*
ns – non-significant

In the sandwich method, where the lettuce plant was in close proximity to rice varieties, the control group (T_0), which was not exposed to any rice varieties, exhibited the highest germination rate at 91.17%. However, when rice varieties were introduced, a general decline in germination rates was observed.

For instance, lettuce exposed to Tubigan 18 - NSIC Rc 222 (T_1) and GSR 8 - NSIC Rc 480 (T_3) showed slightly reduced germination rates of 85.67%, but these were still the highest among the treatments. In contrast, certain rice varieties, such as Mestiso 23 - NSIC Rc210H (T_9), had a more pronounced allelopathic effect, resulting in the lowest germination rate of 67.58%, indicating the release of substances that inhibited lettuce germination.

The mean germination time (MGT) for the control group was relatively long at 9.12 days. However, several rice varieties accelerated the germination process, with Mestiso 23-NSIC Rc210H (T_9) showing the shortest germination time at 6.76 days. This suggests that some rice varieties may promote faster germination in lettuce, likely due to specific allelopathic interactions.

In the seed relay method, the control group (T_0) again showed the highest germination rate at 95.67%, which was similar to the sandwich method. However, unlike the sandwich method, where several rice varieties significantly reduced germination rates, the seed relay method demonstrated generally higher germination rates across treatments. For example, lettuce plants planted with Tubigan 44-NSIC Rc512 (T_5) exhibited a high germination rate of 91.67%, and Mestiso-PSB Rc72H (T_6) reached 94.92%, both of which were nearly identical to the control group.

Despite the higher germination rates, the MGT across all treatments and the control group remained consistent at 10.50 days. This consistency suggests that the seed relay method did not effectively capture variations in germination time caused by rice varieties, indicating that it might be less sensitive to allelopathic effects compared to the sandwich method.

The results from both methods indicate that the allelopathic effects of rice varieties on lettuce germination were influenced by the method used. The sandwich method, with close physical contact between rice and lettuce, demonstrated a more pronounced influence on germination rates and time, making it more sensitive to detecting allelopathic interactions. The seed relay method, on the other hand, showed higher germination rates overall and less variation in MGT, suggesting that it might reduce the direct impact of allelopathic compounds due to physical separation, which could mitigate the effects of these compounds.

These findings imply that the allelopathic potential of rice varieties is more noticeable in scenarios where plants are in closer contact, as simulated by the sandwich method. In contrast, in environments where physical separation exists (as in the seed relay method), the allelopathic effects may be less pronounced, resulting in better growth performance for companion plants like lettuce. This has practical implications for rice farming systems, particularly in managing crop interactions and optimizing weed suppression strategies while considering the potential impacts on desirable crops.

The findings from this study aligned with or diverged from previous research in several ways. For instance, the reduction in germination rates observed in the sandwich method, particularly for Mestiso 23 (T_9), is consistent with prior studies that show varying allelopathic effects of rice varieties on lettuce germination, ranging from 5% to 40% (Karim et al., 2012). Furthermore, the shorter MGT of lettuce exposed to certain rice varieties—such as Mestiso 23 (T_9)—aligns with findings that allelopathic substances can influence seed germination dynamics by affecting hormonal and biochemical pathways (Rahaman et al., 2022).

The sensitivity of the sandwich method in detecting allelopathic interactions is supported by previous studies, which show that closer proximity between donor and recipient plants enhances the transfer of allelopathic compounds. This is in contrast to the seed relay method, which demonstrated higher germination rates and less variation in MGT across treatments, consistent with findings that physical separation or dilution of allelopathic compounds can reduce their inhibitory effects (Kim & Shin, 2003; Wang et al., 2022).

Interestingly, some studies have observed hormesis effects, where low concentrations of allelopathic compounds promote growth while high concentrations inhibit it (Wang et al., 2022). While this phenomenon was not explicitly noted in the current study, it could explain why certain rice varieties had less pronounced inhibitory effects in some cases. The findings emphasize the importance of bioassay methods in detecting and interpreting allelopathic interactions. The sandwich method appears more effective at identifying strong interactions due to close plant contact, while the seed relay method may better simulate field conditions where physical separation reduces the effects of allelopathy.

These results are valuable for developing strategies to manage crop-weed interactions and optimize intercropping systems. By selecting rice varieties with desirable allelopathic traits, researchers and farmers can better utilize rice for weed suppression while minimizing negative impacts on companion crops like lettuce. Understanding how different methods capture these interactions will help in designing more effective intercropping systems and in selecting rice varieties that enhance crop yields while controlling unwanted vegetation.

The findings of the study underscore the importance of bioassay methods in evaluating allelopathic potential and suggest that methods like the sandwich method, which mimic close plant interactions, may be more effective for detecting allelopathic effects. By understanding the variations in allelopathic interactions across different methods, researchers and farmers can better utilize rice varieties for weed suppression while minimizing negative impacts on companion crops like lettuce.

Growth Performance of Lettuce

Table 2 shows the growth performance of lettuce, measured by root length, shoot length, and dry weight, as affected by the different rice seed varieties using the sandwich method and the seed relay method. These measurements were taken using two bioassay methods, the sandwich method and the seed relay method.

As gleaned from the table, the results indicate significant variations in lettuce root length, shoot length, and dry weight depending on the rice variety and method used, highlighting the complex nature of allelopathic interactions.

In the sandwich method, where direct contact between plants is enhanced, lettuce in the control group (T_0) exhibited the most robust root growth, measuring 10.77 mm, indicating optimal growth conditions without allelopathic interference. Root length was significantly reduced in most treatments, with the lettuce plant exposed to Mestiso 23-NSIC Rc210H (T_9) showing the most pronounced reduction at 4.62 mm. Lettuce plants exposed to other varieties, such as Tubigan 18-NSIC Rc222 (T_1) and Tubigan 37-NSIC Rc436 (T_2), also exhibited shorter root lengths compared to the control, measuring 7.78 mm and 6.60 mm, respectively.

Table 2. Growth Performance of Lettuce as Affected by the Different Rice Varieties

Treatments	Root Length (mm)		Shoot Length (mm)		Dry Weight (mg)	
	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method
T_0 – control (no donor plant)	10.77[a]	30.70[abc]	21.23[a]	45.60[ab]	11.30	24.77[a]
T_1 – NSIC Rc 222 (Tubigan 18)	7.78[b]	18.52[d]	12.22[d]	28.02[de]	6.33	18.53[ab]
T_2 – NSIC Rc 436 (Tubigan 37)	6.60[c]	26.73[d]	13.40[cd]	27.72[cde]	5.97	17.57[b]
T_3 – NSIC Rc 480 (GSR 8)	6.75[bc]	34.03[bcd]	13.72[cd]	53.10[de]	7.23	15.83[ab]
T_4 – NSIC Rc 508 (Tubigan 42)	7.28[bc]	18.57[ab]	12.72[c]	24.68[a]	5.90	20.63[b]
T_5 – NSIC Rc 512 (Tubigan 44)	6.45[bc]	21.40[d]	13.55[cd]	35.48[e]	5.73	14.80[ab]
T_6 – PSB Rc72H (Mestiso)	6.57[c]	40.03[cd]	13.43[cd]	39.17[bcd]	6.53	16.50[b]
T_7 – NSIC Rc204H (Mestiso 20)	6.92[bc]	20.50[a]	13.08[cd]	26.83[bc]	6.07	16.43[b]
T_8 – NSIC Rc132H (Mestiso 6)	6.85[bc]	21.93[cd]	13.15[cd]	25.00[de]	6.03	20.97[b]
T_9 – NSIC Rc210H (Mestiso 23)	4.62[bc]	19.65[cd]	15.38[cd]	30.40[e]	5.63	14.54[ab]
T_{10} – NSIC 2015 Rc404H (Mestiso 66)	6.35[d]	20.07[d]	13.65[b]	28.88[cde]	6.30	15.10[b]
Mean	6.99	27.21	14.14	33.17	7.30	17.79
CV (%)	6.04	14.70	10.69	3.55	15.56	14.13
ANOVA	**	**	**	**	ns	**

Note: Means with the same letter are not significantly different from each other at 1% level of significance
*** - highly significant*
ns – non-significant

Similarly, the shoot length of lettuce was longest in the control group at 21.23 mm, with those exposed in Tubigan 18-NSIC Rc222 (T_1) and Tubigan 42-NSIC Rc508 (T_4) showing the most substantial reductions, measuring 12.22 mm and 12.72 mm, respectively. Meanwhile, lettuce plants exposed to Mestiso 23-NSIC Rc210H (T_9) had a slightly longer shoot length of 15.38 mm, suggesting less severe inhibition. Dry weight measurements also revealed that the control group had the highest biomass at 11.30 mg, whereas lettuce exposed to Tubigan 44-NSIC Rc512 (T_5) and Tubigan 37-NSIC Rc436 (T_2) had the lowest, at 5.73 mg and 5.97 mg, respectively.

These findings align with Raihan et al. (2019), who observed that root growth is particularly sensitive to allelochemicals in soil-based assays, and are consistent with studies showing that radicle elongation in lettuce was more inhibited than hypocotyl elongation because roots are the first organs to absorb allelochemicals from the environment. Anjum et al. (2010) and Villa et al. (2019) also found that aqueous plant extracts can reduce lettuce radicle elongation by disrupting nutrient uptake and metabolic processes, supporting the hypothesis that allelochemicals disrupt nutrient uptake and cellular processes in roots.

The seed relay method, which involves a longer exposure period of lettuce without direct contact with rice, generally showed longer root lengths across treatments. The lettuce in the control group again performed well with a root length of 30.70 mm, but lettuce in Mestiso-PSB Rc72H (T_6) and GSR 8-NSIC Rc480 (T_3) treatments demonstrated enhanced root growth at 40.03 mm and 34.03 mm, respectively. However, Tubigan 18-NSIC Rc222 (T_1) and Tubigan 42-NSIC Rc508 (T4) resulted in shorter root lengths of 18.52 mm and 18.57 mm, respectively.

For shoot length, lettuce in GSR 8-NSIC Rc480 (T_3) led to the highest values at 53.10 mm, while Tubigan 37-NSIC Rc436 (T_2) and Tubigan 42-NSIC Rc508 (T_4) showed reduced shoot lengths of 27.72 mm and 24.68 mm, respectively. Dry weight of lettuce was highest in the control group at 24.77 mg, followed by lettuce plants co-planted with

Tubigan 42-NSIC Rc508 (T_4) and Tubigan 18-NSIC Rc222 (T_1) at 20.63 mg and 18.53 mg, respectively, but Mestiso 23-NSIC Rc210H (T_9) exhibited the lowest dry weight at 14.54 mg.

These results are consistent with Nath et al. (2016) and Wang et al. (2022), who noted that allelochemical effects depend on concentration and exposure time, with some compounds potentially promoting shoot growth to compensate for impaired root function, enhancing photosynthesis and resource allocation. The observation that some varieties like GSR 8-NSIC Rc480 (T_3) promoted shoot growth of lettuce aligns with findings that allelochemicals can either inhibit or stimulate shoot development depending on their concentration and exposure duration.

Analysis of variance (ANOVA) results showed significant differences ($p < 0.01$) across treatments in both methods. In the sandwich method, the control group had significantly longer roots than most treatments, with the shortest root length observed in lettuce plants exposed to Mestiso 23-NSIC Rc210H (T_9). Similar trends were noted in the seed relay method, where lettuce plants co-planted with Mestiso-PSB Rc72H (T_6) and GSR 8-NSIC Rc480 (T_3) had significantly longer roots than those exposed to Tubigan 18-NSIC Rc222 (T_1) and Tubigan 42-NSIC Rc508 (T_4).

The inhibitory effects of Tubigan 18-NSIC Rc222 (T_1) and Mestiso 23-NSIC Rc210H (T_9) highlight their potential as natural weed suppressants. These rice varieties exhibit strong allelopathic properties, which can reduce reliance on chemical herbicides, promoting sustainable and eco-friendly agricultural practices. This aligns with findings by Morikawa (2012), who emphasized the role of allelopathy in controlling invasive species and enhancing crop productivity.

The use of bioassay methods, such as the sandwich and seed relay techniques, provides valuable insights into how specific rice varieties influence lettuce growth through biochemical interactions. These methods demonstrate that direct biochemical interactions and prolonged exposure to allelochemicals significantly impact plant growth and biomass accumulation. This reinforces existing knowledge about the dual nature of allelopathy—both inhibitory and stimulatory—and its significance in shaping plant interactions. Consequently, allelopathy could serve as an eco-friendly alternative for controlling invasive species and improving crop productivity.

The results emphasize the potential application of allelopathic rice varieties for natural weed management. By reducing reliance on chemical herbicides, these varieties can contribute to more sustainable farming practices. Similar conclusions have been drawn in studies suggesting that allelopathy could serve as an eco-friendly alternative for controlling invasive species and improving crop productivity (Morikawa et al., 2012; Villa et al., 2019). Overall, the integration of such rice varieties into agricultural systems offers a promising approach to reducing environmental impact while maintaining or enhancing crop yields.

Percent Reduction (%) on the Germination of Lettuce as Affected by the Different Rice Variables

Table 3 shows the percent reduction in lettuce germination as influenced by various rice varieties.

Table 3. Percent Reduction (%) on the Germination of Lettuce as Affected by the Different Rice Varieties

Treatments	Percent Reduction (%) on the Germination Rate		Percent Reduction (%) on Mean Germination Time	
	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method
T_0 – control (no donor plant)	7.26^{c}	4.76^{a}	1.03^{f}	1.03^{f}
T_1 – NSIC Rc 222 (Tubigan 18)	25.29^{ab}	4.12^{ab}	7.82^{e}	7.82^{e}
T_2 – NSIC Rc 436 (Tubigan 37)	22.71^{bc}	2.53^{ab}	10.70^{bc}	10.70^{bc}
T_3 – NSIC Rc 480 (GSR 8)	17.12^{ab}	2.47^{cd}	7.00^{de}	7.00^{de}
T_4 – NSIC Rc 508 (Tubigan 42)	21.89^{abc}	1.67^{cd}	9.86^{e}	9.86^{e}
T_5 – NSIC Rc 512 (Tubigan 44)	25.63^{ab}	4.30^{d}	15.29^{e}	15.29^{e}
T_6 – PSB Rc72H (Mestiso)	24.21^{ab}	4.68^{ab}	18.49^{cd}	18.49^{cd}
T_7 – NSIC Rc204H (Mestiso 20)	23.07^{ab}	3.22^{a}	20.45^{bc}	20.45^{bc}
T_8 – NSIC Rc132H (Mestiso 6)	21.63^{ab}	3.75^{bc}	20.87^{b}	20.87^{b}
T_9 – NSIC Rc210H (Mestiso 23)	26.44^{ab}	1.56^{abc}	26.21^{b}	26.21^{b}
T_{10} – NSIC 2015 Rc404H (Mestiso 66)	15.13^{a}	4.34^{d}	19.33^{a}	19.33^{a}
Mean	20.94	3.40	14.28	14.28
CV (%)	18.21	14.05	11.48	11.48
ANOVA	**	ns	**	**

Note: Means with the same letter are not significantly different from each other at 1% level of significance
*** - highly significant*
ns – non-significant

In terms of the percent reduction in germination rate, a significant variation is observed between treatments when employing the sandwich method. The lettuce plants in the control (T_0) had the lowest percent reduction in germination rate at 7.26%, indicating minimal allelopathic effect. In contrast, lettuce plants exposed to Mestiso 23-NSIC Rc210H (T_9) exhibited the highest reduction at 26.44%, suggesting a strong allelopathic potential. Lettuce plants in other treatments, such as Tubigan 44-NSIC Rc512 (T_5) and Tubigan 18-NSIC Rc222 (T_1), also showed high reductions, with 25.63% and 25.29%, respectively.

For the seed relay method, the reductions are generally lower compared to the sandwich method, with values ranging from 1.56% Mestiso 23-NSIC Rc210 (T_9) to 4.76% (T_0). The control (T_0) still had the least reduction, indicating the absence of allelopathic interaction. Mestiso 66-NSIC 2015 Rc404H (T_{10}) (4.34%) and Tubigan 44-NSIC Rc 51 (T_5) (4.30%) showed slightly higher reductions, but overall, the variations were not statistically significant (as indicated by the non-significant ANOVA result).

In terms of percent reduction in mean germination time, both the sandwich and seed relay methods showed significant variations, as evidenced by the significant ANOVA result ($p < 0.01$). The control (T_0) had the lowest reduction at 1.03% for both methods, confirming minimal influence on the lettuce plant germination.

In the sandwich method, lettuce plants exposed to Mestiso 23-NSIC Rc210H (T_9) exhibited the highest reduction (26.21%), followed by Mestiso 20-NSIC Rc204H (T_7) and Mestiso 6-NSIC Rc132H (T_8), with reductions of 20.45% and 20.87%, respectively. These values suggest that these varieties significantly delayed lettuce germination, implying strong allelopathic effects on seedling development.

Similarly, in the seed relay method, the reductions in the germination of the lettuce plants closely mirrored, with Mestiso 23-NSIC Rc210H (T_9) still displaying the greatest reduction (26.21%), followed by Mestiso 20-NSIC Rc204H (T_7) (20.45%), and Mestiso 6-NSIC Rc132H (T_8) (20.87%). This alignment between methods reinforces the strength of allelopathic effects on germination time for these varieties.

The ANOVA results indicate that the percent reduction in germination rate using the sandwich method is significantly influenced by the different rice varieties, while the seed relay method showed no significant differences. This indicates that the sandwich method is more sensitive in detecting allelopathic interactions between rice residues and lettuce seeds.

As for mean germination time, both methods showed significant effects, implying that rice varieties have a more pronounced allelopathic influence on the germination of the lettuce plants. Varieties such as Mestiso 23 (NSIC Rc210H), Mestiso 20 (NSIC Rc204H), and Mestiso 6 (NSIC Rc132H) exhibited the strongest allelopathic potential against the test organism (lettuce), delaying germination, which could reduce the competitive ability of lettuce in agricultural settings.

The results suggest that certain rice varieties possess strong allelopathic properties, which can hinder the rate of lettuce seed germination. Specifically, Mestiso 23-NSIC Rc210H, Mestiso 6-NSIC Rc132H, and Mestiso 20-NSIC Rc204H possess strong allelopathic properties that can delay lettuce seed germination. This could reduce the competitive ability of lettuce in agricultural settings, potentially influencing crop rotation strategies and weed management in rice-based cropping systems.

The findings of previous studies largely support the observed allelopathic effects of rice varieties on lettuce germination, as highlighted in the current research. For instance, allelochemicals such as phenolics and momilactones, which are key contributors to allelopathic activity, have been identified in rice varieties and shown to disrupt weed growth by inhibiting biochemical and physiological processes (Chung et al., 2006; Rahaman et al., 2022; Serra et al., 2021).

Studies have demonstrated that rice cultivars with strong allelopathic traits can suppress weed growth significantly, with intermediate-maturing varieties exhibiting higher inhibition rates compared to early or late-maturing ones (Belz, 2007; Li et al., 2019). Traditional varieties like Siam and Jambok, as well as newer types such as MR77 and MR84, have also shown considerable allelopathic activity on lettuce and barnyard grass seedlings (Li et al., 2022; Yan et al., 2023).

Research on allelopathic rice cultivars such as PI312777 and Huagan-1 further confirms their strong weed-suppressive effects, particularly when integrated with cultural management practices like planting density and flooding depth. These methods enhance the weed suppression ability of allelopathic rice varieties while maintaining grain yield, suggesting that such strategies could reduce herbicide use in paddy fields (Kong et al, 2008). Additionally, the spatial distribution of allelopathic rice roots has been found to significantly impact weed suppression, with traits like larger root surface area density correlating strongly with the presence of phenolic acids responsible for allelopathy (Li et al., 2022).

The successful breeding of commercially viable allelopathic rice cultivars, such as Huagan-3 in China, demonstrates the practical application of these traits in reducing herbicide use without compromising yield. Huagan-3 exhibited strong weed suppression even under slight infestations, reinforcing the potential for integrating allelopathic rice into sustainable agricultural systems (Kong et al., 2011). Furthermore, large-scale screenings have identified hundreds of rice cultivars capable of inhibiting weed growth under controlled conditions, underscoring the widespread potential for using allelopathy in eco-friendly weed management strategies (Ho et al., 2020).

Overall, these studies validate the strong allelopathic effects observed in Mestiso 23-NSIC Rc210H (T_9), Mestiso 6-NSIC Rc132H (T_8), and Mestiso 20-NSIC Rc204H (T_7) in the current research. They also highlight the importance of further exploring allelochemicals and optimizing cultivation practices to harness these effects for sustainable agriculture.

The allelopathic potential of these rice varieties may be harnessed for sustainable weed management, minimizing reliance on synthetic herbicides. However, further studies are necessary to evaluate the long-term ecological impacts and practical applications of allelopathy in diversified cropping systems. Understanding these effects can contribute to more efficient and environmentally friendly agricultural practices, enhancing crop productivity while reducing weed pressure.

Percent Reduction in the Growth Performance of Lettuce as Affected by the Different Rice Varieties

Table 4 presents the percent reduction in the growth performance of lettuce, specifically measuring root length, shoot length, and dry weight, as influenced by various rice varieties using the sandwich and seed relay methods.

Table 4. Percent Reduction in the Growth Performance of Lettuce as Affected by the Different Rice Varieties

Treatments	Root Length (mm)		Shoot Length (mm)		Dry Weight (mg)	
	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method	Sandwich Method	Seed Relay Method
T_0 – control (no donor plant)	2.38^{c}	4.08^{abc}	4.99^{d}	4.91^{ab}	4.99	3.54^{a}
T_1 – NSIC Rc 222 (Tubigan 18)	33.03^{b}	1.95^{d}	52.47^{a}	2.52^{cde}	52.47	2.54^{ab}
T_2 – NSIC Rc 436 (Tubigan 37)	40.95^{b}	3.53^{bcd}	37.85^{b}	2.48^{cde}	37.86	2.32^{b}
T_3 – NSIC Rc 480 (GSR 8)	38.39^{b}	4.46^{abcd}	37.53^{b}	5.64^{cde}	37.53	1.90^{ab}
T_4 – NSIC Rc 508 (Tubigan 42)	33.62^{b}	2.10^{ab}	42.60^{bc}	2.81^{a}	42.60	2.91^{b}
T_5 – NSIC Rc 512 (Tubigan 44)	41.84^{b}	2.41^{cd}	37.65^{b}	3.73^{de}	37.65	2.08^{ab}
T_6 – PSB Rc72H (Mestiso)	39.93^{b}	5.10^{bcd}	39.04^{bc}	4.21^{abcd}	39.04	1.95^{ab}
T_7 – NSIC Rc204H (Mestiso 20)	36.82^{b}	2.51^{a}	40.57^{b}	2.32^{abc}	40.57	2.98^{ab}
T_8 – NSIC Rc132H (Mestiso 6)	37.78^{b}	2.72^{bcd}	39.91^{b}	1.42^{cde}	39.91	1.54^{ab}
T_9 – NSIC Rc210H (Mestiso 23)	58.30^{b}	2.38^{bcd}	29.42^{b}	3.02^{e}	29.42	1.39^{b}
T_{10} – NSIC 2015 Rc404H (Mestiso 66)	40.09^{a}	2.45^{bcd}	39.84^{c}	2.75^{bcde}	30.84	2.25^{b}
Mean	36.65	35.65	36.53	3.17	36.53	24.53
CV (%)	10.84	23.37	7.76	20.76	7.76	7.76
ANOVA	**	**	**	**	ns	**

Note: Means with the same letter are not significantly different from each other at 1% level of significance
*** - highly significant*
ns – non-significant

The data on the percent reduction in the growth performance of lettuce, using rice varieties as treatments, reveals significant allelopathic effects, particularly on root length, shoot length, and dry weight. The ANOVA results indicate significant differences ($p < 0.01$) for percent reduction in root and shoot length across both the sandwich and seed relay methods, showing that different rice varieties have a strong impact on lettuce growth.

In the sandwich method, the lettuce plants in the control treatment (T_0) registered the shortest root length, with a minimal reduction (2.38 mm), while Mestiso 23-NSIC Rc210H (T_9) exhibited the most significant reduction at 58.30 mm, showing its strong allelopathic potential. Other varieties, such as Tubigan 37-NSIC Rc436 (T_2) and NSIC Rc512–Tubigan 44 (T_5), also demonstrated notable reductions, suggesting that these varieties contain allelochemicals that inhibit root growth.

In the seed relay method, lettuce plants co-planted with Mestiso-PSB Rc72H (T_6) recorded the longest root length at 5.10 mm, while lettuce planted with Tubigan 18-NSIC Rc222 (T_1) had the most pronounced reduction (1.95 mm), reflecting its strong inhibitory effect.

Shoot length also showed significant differences in both methods. In the sandwich method, the control (T_0) had the smallest shoot length at 4.99 mm, while lettuce plants exposed to Tubigan 18-NSIC Rc222 (T_1) demonstrated the highest reduction, with 52.47 mm, indicating a strong allelopathic effect on shoot growth. Other varieties, like Tubigan 42 - NSIC Rc508 (T_4) and Mestiso-PSB Rc72H (T_6), also caused substantial reductions in shoot length of the lettuce plant, further highlighting the presence of allelochemicals that hindered lettuce growth. In the seed relay method, lettuce plants co-planted with GSR 8-NSIC Rc480 (T_3) recorded the longest shoot length at 5.64 mm, while Mestiso 6-NSIC Rc132H (T_8) had the shortest at 1.42 mm. These variations suggest that the allelopathic effect of rice varieties on shoot length is significant, with some varieties allowing lettuce to grow taller while others, such as Mestiso 6-NSIC Rc132H, strongly suppress shoot growth.

For dry weight, the results were not significant in the sandwich method, indicating that allelopathic effects on lettuce biomass accumulation were less consistent. However, in the seed relay method, there were significant differences ($p < 0.01$) across treatments. The lettuce plants at the control (T_0) had the highest dry weight (3.54 mg), while those co-planted with Mestiso 23-NSIC Rc210H (T_9) exhibited the most substantial reduction at 1.39 mg, further suggesting that this variety may inhibit lettuce growth. Other varieties also demonstrated reduced dry weight, but the variability was less pronounced compared to root and shoot length.

The results suggest that certain rice varieties, particularly Mestiso 23-NSIC Rc210H and Tubigan 18-NSIC Rc222, possess strong allelopathic properties that significantly inhibit the growth of lettuce, especially in terms of root and shoot development. The results of the current study, which demonstrate significant allelopathic effects of rice varieties on lettuce growth, particularly in terms of root length, shoot length, and dry weight, are supported by findings from previous research.

Studies conducted at Bangladesh Agricultural University revealed that certain rice varieties exhibit strong allelopathic potential, causing considerable inhibition in lettuce growth. For instance, BR5615-9-1-2 reduced lettuce germination by 40%, while BR23 caused a 31.07% reduction in shoot length and a 30.26% reduction in root length (Karim et al., 2012). In addition, Masum et al. (2016) identified the varieties Boterswar, Goria, Biron, and Kartiksail have been identified as having high allelopathic potential among Bangladeshi rice varieties. Similarly, Kato-Noguchi et al. (2022) claimed that the red rice cultivar Tsushima-akamai exhibited significant inhibitory effects on lettuce growth. These findings align with the current observations that varieties like Mestiso 23-NSIC Rc210H and Tubigan 18-NSIC Rc222 significantly inhibit lettuce root and shoot development.

Biochemical studies further support these results by identifying phenolics and momilactones as key allelochemicals responsible for the herbicidal effects in rice (Jabran, 2017; Kato-Noguchi & Peters, 2013; Mai & Xuan, 2025; Rahaman et al., 2022). Research has shown that intermediate-maturing rice cultivars exhibit higher inhibition rates (59.3%) compared to early or late-maturing varieties, suggesting that certain genetic traits enhance allelopathic activity (Belz, 2007; Li et al., 2019; Li et al., 2022; Olofsdotter, 2001). Traditional varieties such as Siam and Jambok, as well as newer types like MR77 and MR84, have demonstrated substantial inhibitory effects on lettuce seedlings, reinforcing the strong allelopathic potential observed in the current study (Rahaman et al., 2022).

Genetic studies provide additional evidence for these inhibitory effects. Quantitative Trait Loci (QTL) analysis has identified specific regions on chromosome 8 associated with allelopathic traits in rice. For example, the QTLs qISL-8 and qITL-8 explained 20.38% and 14.93% of the phenotypic variation for inhibition of shoot length and total length, respectively (Chung et al., 2020; Ebana et al., 2001; Okuno & Ebana, 2003). These findings confirm that allelopathy is genetically influenced and that certain rice varieties are predisposed to exert strong inhibitory effects on receiver plants like lettuce (Chung et al., 2020).

However, some studies highlighted variability in allelopathic effects depending on experimental conditions. For instance, while the current study observed consistent reductions in dry weight using the seed relay method, other research had reported negligible or even stimulatory effects under certain conditions. The sensitivity of bioassays to allelopathic effects can vary based on the test species, growth conditions, and methods used (Inderjit & Weston, 2000; Wu et al., 2001). Hormesis effects, where low concentrations of allelochemicals promote plant growth, have also been documented, suggesting that allelopathic interactions can vary based on concentration and environmental factors (Chung et al., 2006; Rahaman et al., 2022).

The consistency between previous studies and the current findings underscores the strong allelopathic properties of certain rice varieties like Mestiso 23-NSIC Rc210H and Tubigan 18-NSIC Rc222. These results have significant implications for sustainable agriculture by highlighting the potential use of allelopathic rice varieties as natural weed suppressants to reduce reliance on chemical herbicides.

Average Percent Inhibition on the Germination and Growth Performance of the Lettuce

The average percent inhibition on the germination and growth performance of lettuce using the sandwich method and seed relay method is shown in Table 5.

Table 5. Average Percent Inhibition (API) on the Germination and Growth Performance of Lettuce

Treatments	Sandwich Method	Seed Relay Method
T_0 – control (no donor plant)	130.16	3.53[a]
T_1 – NSIC Rc 222 (Tubigan 18)	153.77	2.02[cde]
T_2 – NSIC Rc 436 (Tubigan 37)	152.16	1.81[cde]
T_3 – NSIC Rc 480 (GSR 8)	147.37	3.08[de]
T_4 – NSIC Rc 508 (Tubigan 42)	99.36	1.02[abc]
T_5 – NSIC Rc 512 (Tubigan 44)	159.93	2.35[e]
T_6 – PSB Rc72H (Mestiso)	152.91	3.34[bcd]
T_7 – NSIC Rc204H (Mestiso 20)	153.76	1.50[ab]
T_8 – NSIC Rc132H (Mestiso 6)	153.58	2.02[de]
T_9 – NSIC Rc210H (Mestiso 23)	158.33	1.05[cde]
T_{10} – NSIC 2015 Rc404H (Mestiso 66)	151.85	2.02[e]
Mean	146.65	2.16
CV (%)	18.12	17.66
ANOVA	ns	**

Note: Means with the same letter are not significantly different from each other at 1% level of significance
*** - highly significant*
ns – non-significant

The data on the average percent inhibition (API) of germination and growth performance of lettuce reveals different levels of inhibitory effects among rice varieties, particularly when comparing the sandwich method to the seed relay method. The ANOVA results indicate no significant differences in API across treatments for the sandwich method, while significant differences ($p < 0.01$) are observed in the seed relay method, suggesting that the inhibitory effects of rice varieties on lettuce performance are more pronounced and measurable under the seed relay method.

In the sandwich method, the API values indicate varying levels of inhibition on lettuce germination and growth, though no significant differences were found among the treatments. The control (T_0) recorded an API of 130.16, providing a baseline comparison for the inhibitory effects of the rice treatments. Several varieties, such as Tubigan 44-NSIC Rc512 (T_5) with 159.93, and Mestiso 23-NSIC Rc210H (T_9) with 158.33, showed the highest inhibition percentages, suggesting that these varieties have a strong allelopathic effect against the lettuce plants. Other varieties, such as Tubigan 18-NSIC Rc222 (T_1) and Mestiso-PSB Rc72H (T_6), also exhibited high API values, indicating their potential in suppressing lettuce germination and growth. On the other hand, Tubigan 42-NSIC Rc508 (T_4) had the lowest API at 99.36, suggesting a weaker allelopathic effect in comparison to the other varieties.

The lack of significant differences in the sandwich method may indicate that the allelochemicals released by rice varieties were not uniformly potent enough to cause substantial variations in inhibition, or that this method is less sensitive in detecting allelopathic effects compared to the seed relay method.

In contrast, the seed relay method showed significant differences in API among treatments, suggesting that this method is more sensitive to detecting the allelopathic potential of rice varieties on lettuce. The lettuce plants in the control (T_0) recorded a low

API of 3.53, indicating minimal inhibition in the absence of donor rice plants. The treatments with the highest inhibition were Mestiso-PSB Rc72H (T_6) with 3.34, and GSR 8-NSIC Rc480 (T_3) with 3.08, showing that these varieties had a considerable inhibitory effect on lettuce germination and growth. In contrast, lettuce plants co-planted with Tubigan 42-NSIC Rc508 (T_4) and Mestiso 23-NSIC Rc210H (T_9) exhibited much lower API values of 1.02 and 1.05, respectively, suggesting that these varieties had a weaker inhibitory effect on lettuce performance under the seed relay method.

The significant differences observed in the seed relay method indicate that rice varieties vary in their ability to release allelochemicals that inhibit the germination and growth of lettuce. The lower inhibition values in some treatments may be due to the lesser release or activity of allelopathic compounds in those specific varieties, while the higher API values in other varieties imply stronger allelopathic activity.

The results suggest that certain rice varieties, such as Tubigan 44-NSIC Rc512, Mestiso 23-NSIC Rc210H, and Mestiso-PSB Rc72H, possess stronger allelopathic potential as they cause greater inhibition of lettuce germination and growth, particularly in the seed relay method. This has important implications for sustainable agriculture, as these varieties could be utilized for natural weed suppression. However, the varying levels of inhibition among the treatments also highlight the need for further research to identify and characterize the specific allelochemicals responsible for these effects. Understanding the mechanisms behind these interactions could lead to the development of rice varieties with enhanced allelopathic traits for integrated weed management, potentially reducing the need for chemical herbicides and promoting more eco-friendly farming practices.

The findings of the study on differences observed between the sandwich and seed relay methods are supported and contrasted by previous research. Karim et al. (2012) and Kato-Noguchi et al. (2013) demonstrated that allelopathic effects of rice varieties on lettuce germination and growth were significant under laboratory conditions using a relay seeding technique, with average percent inhibition (API) values ranging from 22% to 40%. Rice varieties such as BR23 exhibited high inhibition levels, similar to Tubigan 44 and Mestiso 23 in the current study, suggesting strong allelopathic activity. The sensitivity of the relay seeding method in detecting allelopathy aligns with the present study's observation that the seed relay method was more effective in identifying significant varietal differences in inhibitory effects.

Further supporting evidence comes from studies by Kato-Noguchi (2011) and Toyomasu et al. (2014), who identified momilactones A and B as key allelochemicals responsible for rice allelopathy. These compounds were found to inhibit weed growth effectively, particularly in barnyard grass and lettuce, reinforcing the notion that specific rice varieties possess potent allelopathic traits. Varieties with higher concentrations of momilactones or other allelochemicals, such as Mestiso-PSB Rc72H in the current study, could serve as natural weed suppressors in sustainable agriculture.

Contrastingly, Azmi et al. (2000) noted variability in dry matter accumulation reduction among rice varieties, with some exhibiting weaker inhibitory effects. For instance, Lalparija showed minimal inhibition compared to other varieties like BR23, which parallels the weaker effects observed for Tubigan 42 in the sandwich method of the present study. This highlights that not all rice varieties uniformly exhibit strong allelopathic activity, suggesting genetic or environmental factors influencing their effectiveness.

Additionally, research by Islam (2010) and Karim et al. (2012) found positive correlations between laboratory and greenhouse experiments for API values of rice varieties, reaffirming the reliability of controlled methods like relay seeding in detecting allelopathy. However, other studies caution against over-reliance on laboratory methods alone, as field conditions may yield different results due to environmental complexities.

Conclusion and Future Works

Based on the results of the study, it can be concluded that different locally-grown rice varieties exhibited varying degrees of allelopathic potential, significantly affecting the germination and growth performance of lettuce. The NSIC Rc210H variety showed the strongest allelopathic effects, with a germination rate dropping from 91.17% in the control group to 67.58%. While the seed relay method generally resulted in higher germination rates, it still revealed substantial growth inhibition. Other varieties, like PSB Rc72H and NSIC Rc480, also exhibited notable allelopathic potential.

Building on the findings of this study, future research may prioritize the identification and characterization of specific allelochemicals responsible for growth inhibition in lettuce through advanced biochemical analyses, including investigations into their stability and bioavailability under diverse environmental conditions to assess their agricultural viability. The practical application of allelopathic rice varieties, such as NSIC Rc210H and PSB Rc72H, may also be explored through their integration into crop rotation or intercropping systems to reduce dependence on synthetic herbicides. Expanding the scope of research to evaluate the allelopathic effects of additional locally cultivated rice varieties on a wider range of crops and weeds will further enhance their potential as bio-herbicides. Field trials are likewise essential to validate the efficacy, yield outcomes, and soil health impacts of these varieties in real-world agricultural settings, ensuring scalability and farmer adoption. Finally, developing frameworks to integrate allelopathic rice into sustainable farming practices will promote biodiversity, minimize chemical inputs, and advance long-term environmental stewardship, bridging laboratory insights with practical agricultural innovation.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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