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Biological Activity of Extracts from OM5930 Rice Components in Controlling Weedy Rice under Laboratory Conditions

Ho Le Thi[1*], Nguyen Gia Huy[1], Vu Nhat Vy[1], Le Nha Tran[1], Kieu Cong Vinh[1], and Nguyen The Cuong[2]

[1]*Faculty of Plant Protection, College of Agriculture, Can Tho University, Viet Nam*

[2]*Department of Agronomy, Cuu Long Delta Rice Research Institute, Viet Nam*

**Corresponding author (hlthi@ctu.edu.vn)*

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ABSTRACT

Weedy rice (Oryza sativa f. spontanea) poses significant challenges to rice production, reducing yield and commercial value. This study investigated the inhibitory effects of root, stem, and leaf extracts from a cultivated rice variety - OM5930 (60 days after sowing) - on two weedy rice lines, WR19 (short awn) and WR20 (long awn). The objectives were to (i) identify the most potent plant part for inhibition, (ii) determine the optimal treatment duration, and (iii) assess the resistance of the two weedy rice lines. Results demonstrated that extracts from all OM5930 plant parts suppressed seedling shoot and root growth in both weedy rice lines. Leaf extracts exhibited the strongest inhibition, achieving complete suppression (100%) at 0.3 g/mL across all time points (0, 48, and 96 hours after treatment). The 48-hour treatment showed the most stable inhibitory effect. Root and stem extracts displayed lower efficacy, reaching only 60-70% inhibition at the same concentration. WR20 was more susceptible than WR19, with a slight stimulation observed at lower concentrations (0.015 and 0.075 g/mL). The strong allelopathic potential of OM5930 leaf extracts suggests their application as an eco-friendly bioherbicide, reducing dependence on synthetic herbicides, minimizing production costs, and promoting sustainable rice cultivation.

1. INTRODUCTION

Weedy rice (*Oryza sativa* f. *spontanea*) has become a significant concern, increasing production costs and reducing rice yield, especially with the shift to direct-seeding rice (DSR) in Southeast Asian countries (Juliano et al., 2020). Weedy rice can adapt and co-evolve with cultivated rice, easily dispersing seeds into the soil seed bank before harvest (Thurber et al., 2010). The invasion of multiple weedy rice lines and the high genetic diversity within this species have led to increased competition, causing significant yield losses (Dai et al., 2014; Wedger & Olsen, 2018; Wu et al., 2022). Studies showed that weedy rice infestation in over 10% of paddy fields can reduce yield by up to 80% due to intense competition for nutrients and light, leading to severe damage to cultivated rice (Olajumoke et al., 2016; Vigueira et al., 2019).

Allelopathy, the phenomenon in which plants release chemical compounds that can inhibit or even stimulate the growth of other plant species, has been studied as an effective tool for weed control (Choudhary et al., 2023). Allelopathic compounds, released through roots, volatilized, or decomposed, can interact with and inhibit the growth of neighbouring plants without causing environmental

pollution (Ferreira & Reinhardt, 2016). It has been known for decades that allelochemicals could have practical values as herbicidal compounds. Recent studies suggest that the use of allelochemicals derived from plant extracts could be effective in controlling weeds without causing environmental pollution or promoting the development of herbicide resistance (Khan, 2011; Jabran et al., 2015).

Screening and analytical studies have confirmed that rice (*Oryza sativa*) releases various allelochemicals into the environment, including fatty acids, terpenes, indoles, phenylalkanoic acids, and phenolic acids (Kato-Noguchi & Peters, 2013), as well as momilactone A and B (Kato-Noguchi, 2004, 2009; Kato-Noguchi et al., 2010). These compounds primarily trigger induced allelopathy, playing a crucial role in biological defense, competition, and growth inhibition of other plant species (Kato-Noguchi & Ino, 2013). The weed-inhibitory effectiveness of allelochemicals varies among rice cultivars. For instance, extracts from the traditional Bangladeshi rice variety Kartikshail contain 9-hydroxy-4-megastigmen-3-one and 3-hydroxy-β-ionone, which can suppress *Echinochloa colonum*, *Lepidium sativum*, and *Digitaria sanguinalis* (Kato-Noguchi et al., 2011). Similarly, straw from the Basmati Super variety has been found to inhibit *Gossypium hirsutum*, *Ipomoea batatas*, *Rumex dentatus*, and *Convolvulus arvensis* (Afridi et al., 2014). Several Bangladeshi rice varieties (BRRI) have also demonstrated inhibitory effects against *Cyperus difformis*, *Cyperus iria*, *Echinochloa crus-galli*, *Fimbristylis miliacea*, and weedy rice (Alam et al., 2018).

Recently, our research analyzed the allelochemical composition of various Vietnamese rice varieties, including *Oryza sativa* L. cv. OM 2395, 5451, 6976, 380, 5930, 4498, 3536, N406, and 7347, revealing that all contained antagonistic compounds. Notably, the OM 5930 variety was rich in salicylic acid, vanillic acid, p-coumaric acid, 2,4-dimethoxybenzoic acid, and cinnamic acid, along with a newly identified compound, *N-trans-cinnamoyltyramine* (NTCT), which has promising weed-suppressing potential (Ho et al., 2020).

A recent study also indicated that extracts from OM 5930 strongly inhibit the growth of various weed species due to the presence of NTCT, with an ED_{50} of 1.6 µM against *Echinochloa crus-galli* and 0.24 µM against *Leptochloa chinensis* (Le Thi et al., 2014). However, no studies have yet assessed the inhibitory effects of this extract on weedy rice. Therefore, this study was conducted to determine the weed-suppressing potential of extracts from different parts of OM 5930 against two weedy rice lines, WR19 (short-awn) and WR20 (long-awn), which are among the most problematic weedy rice lines in the Mekong Delta, Viet Nam.

2. MATERIALS AND METHOD

2.1. Materials

The OM 5930 rice plants were harvested 60 days after planting from the greenhouse of the Faculty of Agriculture, Can Tho University. After harvesting, the plant samples were thoroughly washed to remove soil and dirt, then air-dried at room temperature (25°C) before the separation of roots, stems, and leaves.

The weedy rice seeds WR19 and WR20 were collected from Dong Thap province, then cultivated and harvested in the greenhouse of the Faculty of Agriculture, Can Tho University. The seeds were dried at 50°C for 1 hour to break seed dormancy (Le et al., 2024).

2.2. Experimental design

2.2.1. Objective of the experiment

The goals of the experiment were to extract allelopathic compounds from the OM 5930 rice plants 60 days after planting using MeOH solvent and to determine the optimal concentration and exposure time of the OM 5930 extract for inhibiting the seedling growth of two weedy rice lines, WR19 and WR20.

The OM 5930 rice plants (including roots, stems, and leaves) were harvested from the Faculty of Agriculture greenhouse at Can Tho University, washed, and air-dried at room temperature (25°C).

2.2.2. Preparation and extraction method

The extraction of plant parts was performed using the MeOH solvent method (Le Thi et al., 2008): One hundred grams of fresh rice plant samples, after preparation, were chopped and soaked in 600 mL MeOH and 400 mL distilled water (6:4 ratio) for 48 hours. The extract was filtered once using a Buchner funnel, and the residue was then soaked again in 500 mL of pure MeOH for 2 more days, mixed well, and filtered again. The extracts from the two soaking steps were combined, and the solvent was evaporated using a rotary evaporator at 42°C and 80 rpm to recover 300 mL of extract containing the compounds to be isolated. The extract dosage was calculated based on the fresh weight of OM 5930

rice plants and used to evaluate inhibitory properties at four concentrations: 0.015, 0.075, 0.15, and 0.3 g/mL.

2.2.3. Experimental layout

The experiment was conducted using a completely randomized design with two factors: soaking duration (0h, 48h, and 96h) and extract concentration (0.015, 0.075, 0.15, and 0.3 g/mL), along with a control treatment using distilled water. These soaking durations were carefully chosen to simulate practical agronomic conditions commonly observed in the Mekong Delta, Viet Nam. In this region, herbicides are often applied during land preparation under flooded conditions using a "drip method," in which a bottle of herbicide solution is attached to a tiller and released directly onto the soil during mixing. This immediate exposure scenario is mimicked in the laboratory by the 0h treatment, where dry seeds are placed directly onto filter paper pre-moistened with the extract solution without prior soaking.

The 48h and 96h treatments simulate alternative herbicide application timings in the field. For the 48h condition, seeds were soaked in distilled water for 2 days before exposure to the extract, representing a situation in which herbicides are applied a couple of days after flooding the field. The 96h treatment involved soaking seeds for four days before applying the extract, simulating herbicide application just before field drainage and seedbed preparation. These time intervals were selected to correspond with physiologically meaningful pre-germination stages and different levels of seed water uptake and metabolic activation (Matsushima & Sakagami, 2013; El-Mowafy & Kishk, 2017; Ota et al., 2024). By aligning laboratory treatments with real-world practices, this experimental approach provides practical insights into the allelopathic sensitivity of seeds and enhances the relevance and reproducibility of the findings for potential application in rice production systems.

A total of 15 treatments were used, with each treatment repeated three times. Each replicate contained 10 seeds of either WR19 or WR20 in a Petri dish. After dormancy-breaking, the weedy rice seeds were soaked in distilled water and then replaced with the OM 5930 extract at the prepared concentrations.

Each Petri dish contained 10 seeds and was kept in a dark incubator for 48 hours. After this period, the extract was rinsed off with distilled water, and the seeds were soaked in distilled water for the specified time.

2.2.4. Data collection

The shoot and root lengths were measured on days three and seven. The inhibitory effect of the OM 5930 extract was calculated using Abbott's formula:

$$I\ (\%) = [(L1 - L2)/L1] \times 100.$$

Where: I = Inhibitory effect, L1 = Shoot or root length in the control group, L2 = Shoot or root length in the treatment group.

2.3. Data analysis

The data were entered and processed using Microsoft Excel 2016, and statistical analysis was performed using SPSS version 26. The mean values were compared using ANOVA, followed by Duncan's Multiple Range test, with means separated at the 5% significance level.

3. RESULTS AND DISCUSSION

3.1. The inhibitory ability of root extracts of OM 5930 rice on weedy rice line WR19 under laboratory conditions

Table 1 shows that the stem development of WR19 is inhibited by the root extract of OM 5930 rice at most concentrations and treatment times, except for the 0.015 g/mL concentration at 96h, which shows a slight stimulation (-2.41%). The inhibition increases significantly from 16.49% to 100% as the concentration rises from 0.015 g/mL to 0.3 g/mL, especially at 0.15 g/mL, where 100% inhibition is achieved at both 48h and 96h. Similarly, the 0.3 g/mL concentration also achieves complete inhibition at all three treatment times. The inhibition of WR19 root development at 3 DAT increases with increasing concentration and treatment time. At 48h and 96h, the inhibition is significantly higher, especially at concentrations between 0.15 and 0.3 g/mL, reaching 100% inhibition.

The inhibition of WR19 stem development by the root extract increases with concentration (from 15.45% to 100%) (Figure 1). At 0.3 g/mL, complete inhibition is achieved at all three treatment times, and at 0.075 g/mL, 100% inhibition is observed at both 48h and 96h. The most effective treatment times are 48h (61.84%) and 96h (61.75%).

Table 1. Inhibition of root extract of OM 5930 rice on the growth of WR19 weedy rice at 3 và 7 DAT

Extract concentration (g/ mL) (A)	Shoot Inhibition (%)				Root Inhibition (%)			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
				3 DAT				
0,015	25.78c	26.11c	-2.41c	**16.49^{D}**	11.39c	11.32c	9.13c	**10.61^{C}**
0,075	33.47c	48.46b	54.83b	**45.59^{C}**	17.05c	26.67b	31.25b	**24.99^{C}**
0,15	52.54b	100.00a	100.00a	**84.18^{B}**	30.70b	100.00a	100.00a	**76.90^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**52.95^{B}**	**68.64^{A}**	**63.10A^{B}**		**39.78^{B}**	**59.50^{A}**	**60.10^{A}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*0.58*	*0.49*	*0.72*		*0.93*	*0.72*	*0.71*	
				7 DAT				
0,015	14.45d	17.74c	14.16c	**15.45^{C}**	12.74c	4.79c	12.65c	**10.06^{D}**
0,075	22.97c	29.60b	32.85b	**28.48^{C}**	29.29b	23.27b	28.93b	**27.17^{C}**
0,15	32.51b	100.00a	100.00a	**77.50^{B}**	33.56b	100.00a	100.00a	**77.85^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**42.48^{B}**	**61.84^{A}**	**61.75^{A}**		**43.90^{B}**	**57.01^{A}**	**60.40^{A}**	
Significance level	**F(A)**; F(B)**; F(AxB)****				**F(A)**; F(B)*; F(AxB)****			
CV (%)	*0.83*	*0.65*	*0.66*		*0.80*	*0.80*	*0.69*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

Data were transformed to arcsin (x) before statistical processing.

*Values in the same column followed by the same letter are not significantly different by Duncan's Multiple Range Test; ** Significant difference at 1% level; * Significant difference at 5% level.*

Table 2. Inhibition of stem extract of OM 5930 rice on the growth of WR19 weedy rice at 3 and 7 DAT

Extract concentration (g/ mL) (A)	Shoots				Roots			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
				3 DAT				
0,015	25.78c	26.11c	-2.41c	**16.49^{D}**	8.91c	20.18c	9.38c	**12.82^{C}**
0,075	33.47c	48.46b	54.83b	**45.59^{C}**	16.43bc	26.06b	22.14b	**21.54^{C}**
0,15	52.54b	100.00a	100.00a	**84.18^{B}**	24.19b	100.00a	100.00a	**74.73^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**52.95^{B}**	**68.64^{A}**	**63.10A^{B}**		**37.38^{B}**	**61.56^{A}**	**57.88^{A}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)*; F(AxB)****			
CV (%)	*0.58*	*0.49*	*0.72*		*1.03*	*0.65*	*0.76*	
				7 DAT				
0,015	15.30d	20.48c	12.37c	**16.05^{D}**	10.47c	11.13c	11.44c	**11.01^{C}**
0,075	20.63c	27.54b	32.48b	**26.88^{C}**	15.81c	26.46b	24.06b	**22.11^{C}**
0,15	31.32b	100.00a	100.00a	**77.11^{B}**	30.52b	100.00a	100.00a	**76.84^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**41.81^{B}**	**62.01^{A}**	**61.21^{A}**		**39.20^{B}**	**59.40^{A}**	**58.87^{A}**	
Significance level	**F(A)**; F(B)**; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*0.85*	*0.64*	*0.67*		*0.96*	*0.72*	*0.74*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

Data were transformed to arcsin (x) before statistical processing.

*Values in the same column followed by the same letter are not significantly different by Duncan Multiple Range Test; ** Significant difference at 1% level; * Significant difference at 5% level.*

All concentrations at all treatment times inhibited WR19 root development at 7 DAT. Similar to the stem development results, the inhibition of root development increases with higher concentrations (from 10.06% to 100%). At 0.3 g/mL, complete inhibition was achieved at all treatment times, and at 0.075 g/mL, 100% inhibition was observed at 48h and 96h. The longer the treatment time, the higher the inhibition: 0h (43.90%), 48h (57.01%), and 96h (60.40%).

Similar to the results from other plant parts, the root extract of OM 5930 rice also inhibits the development of both the stem and root of WR19 rice weed at all treatment times and concentrations tested. All concentrations cause inhibition of WR19's stem and root development, with stronger inhibition as the concentration increases. The most effective treatment times are 48h and 96h, with the most effective concentrations being 0.15 to 0.3 g/mL for inhibiting the development of both the stem and root of WR19.

3.2. The inhibitory ability of stem extracts of OM 5930 rice on weedy rice line WR19 under laboratory conditions

Table 2 shows the evaluation of the inhibition effect of OM 5930 stem extract on the shoot growth of WR19 at 3 DAT. At all-time points and all concentrations, WR19 shoot growth was inhibited, with concentrations ranging from 0.015 to 0.3 g/mL showing inhibition rates of 16.49% to 100%. At time points 0h, 48h, and 96h, the highest inhibition rates were 52.95%, 68.64%, and 63.10%, respectively. Notably, at a concentration of 0.3 g/mL (100%) at all time points and at 0.15 g/mL (84.18%). The inhibition of WR19 root growth by the stem extract of OM 5930 increased with higher concentrations at all treatment times. Inhibition ranged from 12.82% to 100% at concentrations of 0.015 to 0.3 g/mL. The most effective treatment times were at 48h (61.56%) and 96h (57.88%).

Table 3. Inhibition of leaf extract of OM 5930 rice on the growth of WR19 weedy rice at 3 and 7 DAT

Extract concentration (g/ mL) (A)	Shoots				Roots			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
3 DAT								
0,015	24.37d	26.68c	11.03c	**20.70^{D}**	0.52d	6.79c	11.95c	**6.42^{D}**
0,075	43.67c	54.27b	69.55b	**55.83^{C}**	13.18c	27.42b	43.90b	**28.16^{C}**
0,15	63.31b	100.00a	100.00a	**87.77^{B}**	34.60b	100.00a	100.00a	**78.20^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**57.84^{B}**	**70.24^{A}**	**70.14^{A}**		**37.07^{B}**	**58.55^{A}**	**63.96^{A}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*0.51*	*0.47*	*0.55*		*1.08*	*0.75*	*0.62*	
7 DAT								
0,015	12.54c	17.08c	15.90c	**15.17^{D}**	10.84c	7.24c	16.13c	**11.41^{D}**
0,075	20.05c	31.31b	70.69b	**40.68^{C}**	18.29c	27.71b	65.36b	**37.12^{C}**
0,15	45.60b	100.00a	100.00a	**81.87^{B}**	41.18b	100.00a	100.00a	**80.39^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**44.55^{B}**	**62.09^{A}**	**71.65^{A}**		**42.58^{B}**	**58.74^{A}**	**70.37^{A}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*0.81*	*0.64*	*0.50*		*0.86*	*0.74*	*0.51*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

Data were transformed to arcsin (x) before statistical processing.

*Values in the same column followed by the same letter are not significantly different by Duncan test; ** Significant difference at 1% level; * Significant difference at 5% level.*

Inhibition of WR19 shoot growth by the stem extract of OM 5930 at 7 DAT showed that all concentrations at all treatment times inhibited WR19 shoot development, with higher concentrations leading to greater inhibition (16.05% to 100%) (Figure 2). The most effective treatment times were at 48h (62.01%) and 96h (61.21%). Among the concentrations, 0.15 g/mL and 0.3 g/mL were the most effective at these two treatment times. As in the previous results, inhibition of WR19 root growth was observed at all concentrations and treatment times. Higher concentrations resulted in

more effective inhibition. From 0.15 g/mL to 0.3 g/mL, the inhibition reached its highest effectiveness (76.84% to 100%). The results indicate that the most effective treatment times for WR19 weed seed inhibition were at 48h and 96h, with inhibition rates of 59.40% and 58.87%, respectively.

From the above results, it can be concluded that the extract from the OM 5930 rice stem has a complete inhibitory effect on the development of both the stem and root of WR19 rice weed seedlings (Figure 2). Concentrations of 0.15 to 0.3 g/mL showed near-total inhibition at all treatment times, with the most effective treatment time being 48 hours, demonstrating the strongest inhibitory effects on both stem and root development at both 3 and 7 DAT (Days After Treatment.

3.3. The inhibitory ability of leaf extracts of OM 5930 rice on weedy rice line WR19 under laboratory conditions

Based on Table 3, regarding the development of WR19 coleoptile at 3 DAT under the inhibition of the OM 5930 rice leaf extract, the higher the concentration of the extract, the stronger the inhibition on WR19 coleoptile development. The inhibition effect ranged from 20.70% to 100% for concentrations from 0.015 to 0.3 g/mL. At a concentration of 0.3 g/mL, complete inhibition (100%) occurred at all three time points. The inhibition of the rice leaf extract on WR19 radicle growth at 3 DAT for all four concentrations (0.015; 0.075; 0.15; and 0.3 g/mL) were 6.42%, 28.16%, 78.20%, and 100%, respectively, at three time points (0h, 48h, and 96h), which were 37.07%, 58.55%, and 63.96%, respectively. The results show that WR19 radicle growth was completely inhibited at 0.3 g/mL at all time points, and at 0.15 g/mL, complete inhibition occurred at 48h and 96h.

The leaf extract inhibited WR19 coleoptile growth at 7 DAT, as evidenced by reduced coleoptile length across all concentrations and time points (Figure 3). Specifically, at 0.15 g/mL, complete inhibition occurred at 48h and 96h, while at 0.3 g/mL, complete inhibition was observed at all time points. The leaf extract's inhibition of WR19 radicle growth at 7 DAT resulted in complete inhibition (100%) at all time points for the 0.3 g/mL concentration, and a similar result was achieved at 48h and 96h for 0.15 g/mL. The inhibition ranged from 11.41% to 100%, with the highest inhibition observed at 48h (58.74%) and 96h (70.37%). The above results demonstrate that the leaf extract from OM 5930 rice strongly inhibits the development of both the coleoptile and radicle of WR19. All concentrations and time points tested showed inhibition, with particularly strong effects at concentrations between 0.075 and 0.3 g/mL at both 3 and 7 DAT. Treatment at 48h and 96h provided the highest inhibition, particularly at 96h (Figure 3).

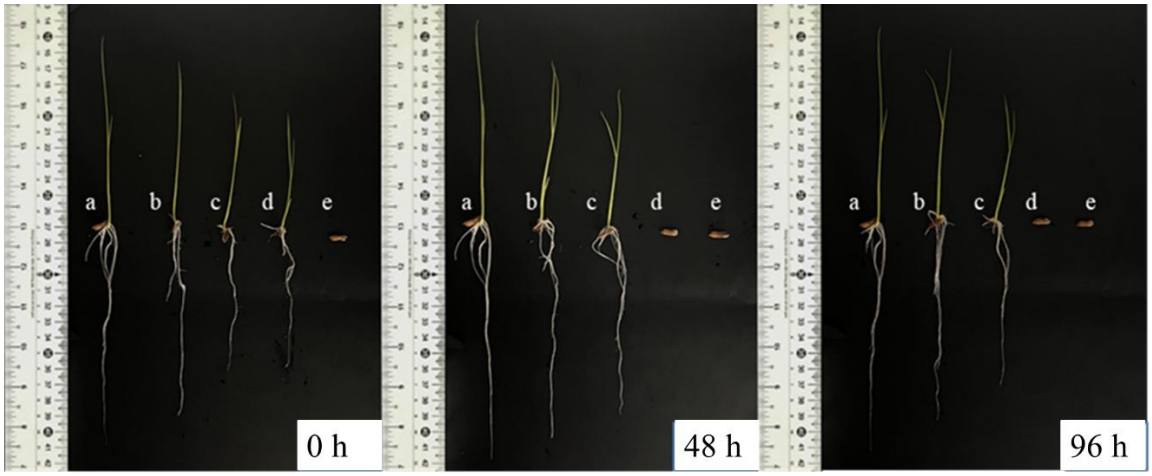

Figure 1. Effect of root extract of OM 5930 on the shoot and root development of WR19 weedy rice at 7 days after treatment

The results show that all parts of the OM5930 rice variety, including roots, stems, and leaves, exhibited a significant inhibitory effect on the WR19 weedy rice. However, the inhibitory effects varied at different time points. At 0h, a 100% inhibition was observed at a concentration of 0.3 g/mL, while at 48 hours and 96 hours, a high level of inhibition

(100%) was achieved at both 0.15 and 0.3 g/mL concentrations.

This phenomenon is attributed to the allelochemicals present in plant tissues or released into the roots through various mechanisms such as exudation, volatilization, or decomposition of waste products (Sangeetha & Baskar, 2015), which in our study refers to the accumulation of antagonistic compounds in the roots, stems, and leaves of OM5930 rice. In particular, the formation of secondary metabolites in plants typically falls into various chemical groups, such as alkaloids, fatty acids, indoles, phenolics, and terpenes. However, phenolics are the dominant group of allelochemicals implicated in allelopathic phenomena (Zohaib et al., 2016).

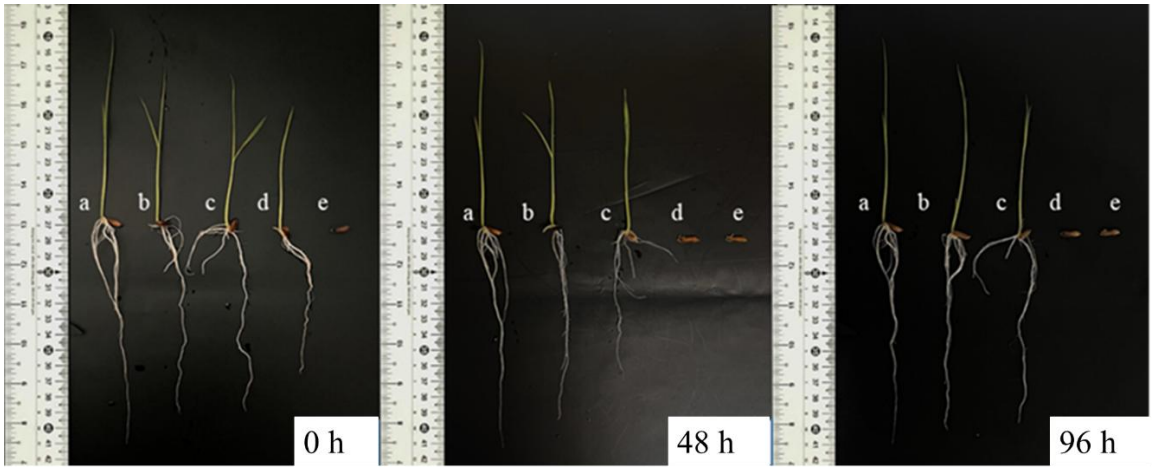

Figure 2. Effect of stem extract of OM 5930 on the shoot and root development of WR19 weedy rice at 7 days after treatment

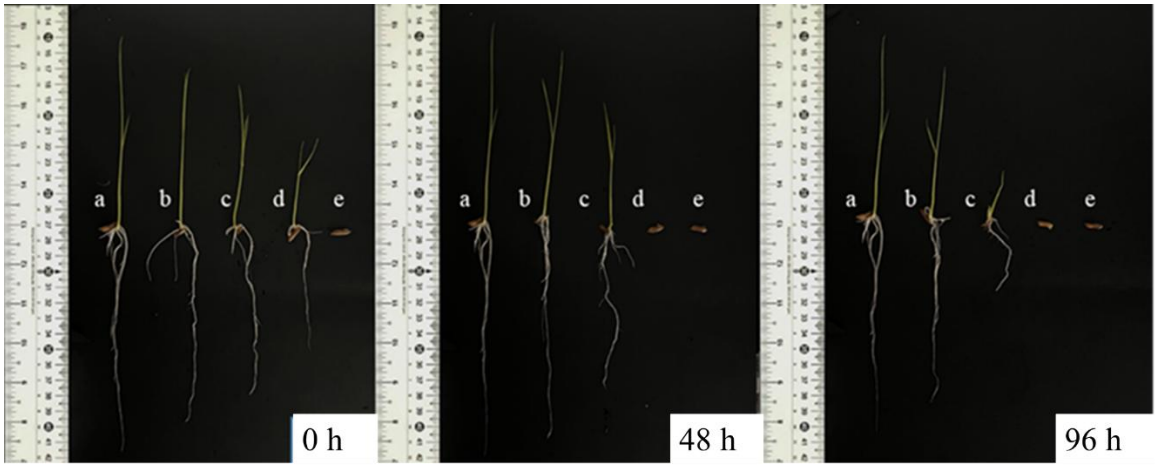

Figure 3. Effect of leaf extract of OM 5930 on the shoot and root development of WR19 weedy rice at 7 days after treatment

Note: a: Control; b: 0.015 g/mL; c: 0.075 g/mL; d: 0.15 g/mL; e: 0.3 g/mL. 0h, 48h, and 96h are the durations of seed soaking before applying the extract treatment.

3.4. The inhibitory ability of root extracts of OM 5930 rice on weedy rice line WR20 under laboratory conditions

Based on Table 4, the inhibition of WR20 coleoptile development by the OM 5930 rice root extract shows that concentrations ranging from 0.075 to 0.3 g/mL resulted in inhibition levels of 31.92% to 100%. However, at 0.075 g/mL, at the 0h treatment time, there was a slight stimulation of coleoptile growth (-2.27%), and at 0.015 g/mL, coleoptile growth was stimulated at all three treatment times

(up to -12.78%). The conclusion indicates that, for optimal treatment, WR20 grass seed should be treated at 48h (57.77%) and 96h (60.22%) with concentrations of 0.15 to 0.3 g/mL to achieve absolute inhibition (100%). The inhibition of WR20 radicle growth at the three treatment times (0h, 48h, and 96h) with concentrations of 0.015 to 0.3 g/mL resulted in inhibition ranging from 10.44% to 100%. Absolute inhibition (100%) was achieved with concentrations of 0.15 to 0.3 g/mL. The results show that treatment at all three time points (0h, 48h, and 96h) achieved inhibition of 56.04%, 61.40%, and 60.14%, respectively.

Table 4. Inhibition of root extract of OM 5930 rice on the growth of WR20 weedy rice at 3 and 7 DAT

Extract concentration (g/ mL) (A)	Shoots				Roots			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
				3 DAT				
0,015	-12.27b	-15.52c	-10.55c	**-12.78^{C}**	13.72b	10.61c	7.01c	**10.44^{C}**
0,075	-2.27b	46.60b	51.44b	**31.92^{B}**	10.46c	34.98b	33.56b	**26.33^{B}**
0,15	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**46.37^{B}**	**57.77^{A}**	**60.22^{A}**		**56.04^{B}**	**61.40^{A}**	**60.14^{A}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)*; F(AxB)****			
CV (%)	*1.22*	*0.87*	*0.79*		*0.82*	*0.67*	*0.71*	
				7 DAT				
0,015	-2.22b	4.37c	-1.98c	**0.06^{C}**	16.43b	14.10b	6.85b	**12.46^{B}**
0,075	1.60b	16.31b	17.71b	**11.87^{B}**	16.42b	16.19b	17.26b	**16.62^{B}**
0,15	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**49.85^{B}**	**55.17^{A}**	**53.93^{A}**		**58.21**	**57.57**	**56.03**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)^{ns}; F(AxB)****			
CV (%)	*1.05*	*0.85*	*0.90*		*0.75*	*0.77*	*0.83*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

Data were transformed to acrsin(x) before statistical processing.

*Values in the same column followed by the same letter are not significantly different by Duncan's Multiple Range test; ** Significant difference at 1% level; * Significant difference at 5% level.*

The inhibition of WR20 coleoptile development at 7 DAT by the root extract at concentrations ranging from 0.015 to 0.3 g/mL resulted in inhibition ranging from 0.06% to 100%. However, at 0.015 g/mL, coleoptile growth was stimulated at 0h (-2.22%) and 96h (-1.98%). The results indicate that for optimal treatment, WR20 grass seed should be treated at 48h (55.17%) and 96h (53.93%) with concentrations of 0.15 to 0.3 g/mL for absolute inhibition (100%). The inhibition of WR20 radicle growth at 0h, 48h, and 96h by the root extract at concentrations of 0.015 to 0.3 g/mL resulted in inhibition ranging from 12.46% to 100%. Absolute inhibition (100%) was achieved with concentrations of 0.15 to 0.3 g/mL. The results show that treatment at all three time points (0h, 48h, and 96h) produced similar inhibition effects: 58.21%, 57.57%, and 56.03%, respectively (Figure 4).

Overall, the results show that treating WR20 grass seed with the OM 5930 rice root extract at concentrations ranging from 0.15 g/mL to 0.3 g/mL at the three treatment times (0h, 48h, and 96h) resulted in effective inhibition and absolute treatment effectiveness on both the coleoptile and radicle growth at both 3 DAT and 7 DAT. However, at 3 DAT, WR20 coleoptile growth was stimulated at lower concentrations (especially 0.015 g/mL), whereas higher concentrations exerted stronger inhibitory effects.

3.5. The inhibitory ability of stem extracts of OM 5930 rice on weedy rice line WR20 under laboratory conditions

Table 5 shows the inhibition of coleoptile growth of WR20 by the extract from the stem part of OM 5930 rice at 0.075 g/mL across three time points, resulting in coleoptile inhibition (21.10-100%). The extract achieved absolute inhibition (100%) at concentrations of 0.15 g/mL at 48h, 96h, and 0.3 g/mL at all time points.

At a concentration of 0.015 g/mL, coleoptile stimulation was observed across all three treatment time points, with a maximum of -5.03% recorded. This low-dose enhancement suggests a hormetic effect, in which subinhibitory levels of allelopathic compounds may temporarily boost early seedling growth. However, this effect was transient and diminished at higher concentrations. Among all time points, the 48h treatment emerged as the most effective in suppressing shoot elongation, with 60.39% inhibition recorded, reinforcing its potential as the optimal application timing for OM 5930 extract. Regarding WR20 root growth, the stem extract from OM 5930 consistently inhibited development across all concentrations (0.015 to 0.3 g/mL) and exposure durations, with inhibition ranging from 5.03% to 100%. These findings align with previous studies reporting a biphasic response in plant tissues to allelopathic stress, in which lower doses induce mild stimulation before higher doses exert inhibitory effects (Chon & Nelson, 2010; Li et al., 2011). The consistency of root inhibition across treatment windows suggests that root tissues may be more susceptible to allelopathic compounds than coleoptiles, and reinforces the strategic value of OM 5930 extract as a bioherbicidal agent, particularly when applied at the pre-germination stage (48h) to maximize its efficacy.

Table 5. Inhibition of stem extract of OM 5930 rice on the growth of WR20 weedy rice at 3 và 7 DAT

Extract concentration (g/ mL) (A)	Shoots				Roots			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
				3 DAT				
0,015	-2.27b	-1.61c	-11.30c	**-5.03^{D}**	7.28d	23.66c	-15.85c	**5.03^{C}**
0,075	9.93c	43.17b	10.20b	**21.10^{C}**	13.40c	49.27b	-8.40b	**18.09^{C}**
0,15	33.75b	100.00a	100.00a	**77.92^{B}**	25.96b	100.00a	100.00a	**75.32^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**35.38^{B}**	**60.39^{A}**	**49.72^{A}**		**36.66^{B}**	**68.23^{A}**	**43.94^{B}**	
Significance level	F(A)**; F(B)**; F(AxB)**				F(A)**; F(B)**; F(AxB)**			
CV (%)	*1.18*	*0.74*	*1.07*		*1.06*	*0.51*	*1.34*	
				7 DAT				
0,015	-3.49c	13.79c	10.44b	**6.91^{C}**	7.14c	21.03b	20.58b	**16.25^{C}**
0,075	4.29c	19.19b	11.54b	**11.67^{C}**	5.91c	25.36b	21.84b	**17.70^{C}**
0,15	21.91b	100.00a	100.00a	**73.97^{B}**	21.53b	100.00a	100.00a	**73.84^{B}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**30.68^{B}**	**58.25^{A}**	**55.50^{A}**		**36.66^{B}**	**68.23^{A}**	**43.94^{B}**	
Significance level	F(A)**; F(B)**; F(AxB)**				F(A)**; F(B)**; F(AxB)**			
CV (%)	*1.40*	*0.75*	*0.84*		*1.21*	*0.66*	*0.68*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

*Data were transformed to arcsin (x) before statistical processing. Values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range test; ** Significant difference at 1% level; * Significant difference at 5% level.*

The inhibition of coleoptile growth of WR20 from the stem extract of OM 5930 at most concentrations at three different time points, resulting in coleoptile inhibition from 6.91 to 100%. However, at a concentration of 0.015 g/mL at the 0h time point, coleoptile stimulation occurred (-3.49%). The results indicate that weedy rice seeds with concentrations from 0.015 to 0.3 g/mL exhibited coleoptile inhibition at the 96h time point (10.44 to 100%) and achieved the highest inhibition at 48h (13.79 to 100%). In most concentrations at the three time points, root inhibition of WR20 ranged from 16.25% to 100%. This result shows that at concentrations of 0.15 to 0.3 g/mL at the 48h time point, the treatment efficiency ranged from 21.03 to 100%, with absolute inhibition achieved with 0.15 g/mL at 48h, 96h, and 0.3 g/mL at all three time points (Figure 5).

In summary, the results show that treatment of WR20 seeds with the stem extract of OM 5930 at the 48h and 96h time points with concentrations ranging from 0.15 g/mL to 0.3 g/mL resulted in both inhibition and absolute treatment efficiency at both evaluation time points (day 3 and day 7). On the other hand, at lower concentrations (0.015 to 0.075 g/mL) at 0h and 96h, stimulation occurred at the 3-day evaluation time point, indicating that treatment

at 48h provides the most optimal results for coleoptile and root growth inhibition of WR20 weedy rice, Allelochemicals often exert a strong impact on the physiological processes of plants, including the inhibition or alteration of enzyme activity, protein synthesis, photosynthesis, respiration, cell division, and cell growth (Zohaib et al., 2016). Although families such as Brassicaceae, Polygonaceae, Asteraceae, Brassicaceae, etc., contain allelochemicals (Mahé et al., 2022), Poaceae has certain advantages in mitigating the effects of rice varieties when influenced by extracts from other rice varieties or closely related species within Poaceae.

3.6. The inhibitory ability of leaf extracts of OM 5930 rice on weedy rice line WR20 under laboratory conditions

Table 6 shows the inhibition of coleoptile growth of WR20 from the leaf extract of OM 5930 rice at three time points. At concentrations of 0.075 to 0.3 g/mL, coleoptile inhibition occurred with effectiveness ranging from 47.79 to 100%. However, at a concentration of 0.015 g/mL, coleoptile stimulation was observed at all treatment times (-18.41%), with a strong stimulation at 96h (-42.10%). The results suggest that at concentrations of 0.15 to 0.3 g/mL, absolute inhibition (100%) was achieved at all three time points (0h, 48h, and 96h). At three time points (0h, 48h, and 96h), the leaf extract of OM 5930 caused root inhibition of WR20 weedy rice, with concentrations ranging from 0.015 to 0.3 g/mL achieving inhibition from 10.20 to 100%. Absolute inhibition (100%) was achieved at concentrations of 0.15 to 0.3 g/mL. The optimal treatment effectiveness occurred at 48h (16.25 to 100%).

The inhibition of coleoptile growth of WR20 from the leaf extract of OM 5930 at the 7-day time point. Results show coleoptile inhibition at all three time points (0h, 48h, and 96h) with concentrations of 0.015 to 0.3 g/mL resulting in inhibition ranging from 6.53 to 100%. Absolute inhibition (100%) was observed at concentrations of 0.15 to 0.3 g/mL. The highest inhibition effectiveness occurred at 48h (73.58%) and 96h (64.45%). The inhibition of root growth of WR20 from the leaf extract of OM 5930 at the 7-day time point. Root inhibition occurred at all three time points (0h, 48h, and 96h) with concentrations of 0.015 to 0.3 g/mL, resulting in inhibition of 16.80 to 100%. Absolute inhibition (100%) was achieved at concentrations of 0.15 to 0.3 g/mL. The optimal treatment effectiveness occurred at 48h (75.49%) and 96h (67.61%) (Figure 6).

Based on the results from the tables above, the leaf extract of OM 5930 showed coleoptile and root inhibition at all three time points (0h, 48h, and 96h) with concentrations ranging from 0.015 g/mL to 0.3 g/mL, and this inhibition was observed at both evaluation times (day 3 and day 7). However, at a concentration of 0.015 g/mL on day 3, coleoptile stimulation was observed in WR20 seeds. To achieve optimal inhibition and absolute treatment effectiveness, WR20 weedy rice seeds should be treated with concentrations ranging from 0.15 g/mL to 0.3 g/mL.

The findings of this study highlight the significant allelopathic potential of cultivated rice (*Oryza sativa* cv. OM 5930) in suppressing the growth and development of weedy rice (*Oryza sativa* f. *spontanea*). This is particularly relevant given that both cultivated and weedy rice share the same scientific origin, belonging to the *Oryza sativa* species, and coexist within the same agroecosystem. The ability of cultivated rice to inhibit the growth of weedy rice through allelopathic interactions offers an environmentally sustainable approach to weed management, reducing reliance on synthetic herbicides and promoting eco-friendly rice production.

The experimental results demonstrated that extracts from different parts of OM 5930 exhibited varying degrees of inhibitory effects on weedy rice lines. The root and leaf extracts of OM 5930 showed 100% inhibition across all treatment periods, while the stem extract also exhibited complete inhibition at higher concentrations (0.3 g/mL at 0h and 0.15 to 0.3 g/mL at 48h and 96h). These results align with previous findings by Thi et al. (2014), which identified *N-trans*-Cinnamoyltyramine in OM 5930, a compound known for its allelopathic properties. The differential response observed between the two weedy rice lines is also noteworthy. WR20 showed greater sensitivity to OM 5930 extracts, whereas WR19 showed stronger resistance. At lower concentrations (0.015 and 0.075 g/mL), WR20 exhibited a stimulation effect at 0h, a phenomenon often associated with hormesis in plant allelopathy.

Table 6. Inhibition of leaf extract of OM 5930 rice on the growth of WR20 at 3 and 7 DAT

Extract concentration (g/mL) (A)	Shoots				Roots			
	Processing time (h) (B)				Processing time (h) (B)			
	0h	48h	96h	Average (A)	0h	48h	96h	Average (A)
				3 DAT				
0,015	-1.49b	-11.64b	-42.10c	**-18.41^{C}**	8.76b	16.25b	5.58c	**10.20^{C}**
0,075	5.34b	92.92a	45.12b	**47.79^{B}**	8.04b	93.55a	41.68b	**47.76^{B}**
0,15	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**50.96^{B}**	**70.32^{A}**	**50.75^{B}**		**54.20^{B}**	**77.45^{A}**	**61.82^{B}**	
Significance level	**F(A)**; F(B)*; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*1.01*	*0.71*	*1.20*		*0.88*	*0.48*	*0.69*	
				7 DAT				
0,015	1.48b	10.66c	7.47c	**6.53^{C}**	5.05b	17.15c	28.18c	**16.80^{C}**
0,075	2.89b	83.67b	50.32b	**45.63^{B}**	0.52b	84.80b	42.24b	**42.52^{B}**
0,15	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
0,3	100.00a	100.00a	100.00a	**100.00^{A}**	100.00a	100.00a	100.00a	**100.00^{A}**
Average (B)	**51.09^{B}**	**73.58^{A}**	**64.45^{A}**		**51.39^{B}**	**75.49^{A}**	**67.61^{A}**	
Significance level	**F(A)**; F(B)**; F(AxB)****				**F(A)**; F(B)**; F(AxB)****			
CV (%)	*1.00*	*0.52*	*0.63*		*0.99*	*0.47*	*0.51*	

Note: 0h, 48h and 96h are the germination times before extract treatment. DAT: days after treatment.

Data were transformed to arcsin (x) before statistical processing.

*Values in the same column followed by the same letter are not significantly different by Duncan's Multiple Range test; ** Significant difference at 1% level; * Significant difference at 5% level*

Previous studies have established that certain cultivated rice varieties release phytochemicals that suppress the growth of competing plant species, including weedy rice (Kong et al., 2019; Kong et al., 2024). These allelochemicals, such as phenolic acids, flavonoids, and momilactones, are exuded from rice roots, leaves, or decomposing plant residues, affecting seed germination and seedling development in nearby plant species (Eroğlu et al., 2022; Khamare et al., 2022). The inhibitory effects observed in this study further support the role of cultivated rice as a natural bioherbicide, particularly in direct-seeded rice systems, where weedy rice presents a significant agronomic challenge (Chauhan, 2013). The competition between cultivated and weedy rice is complex due to their shared ecological niche, similar growth patterns, and physiological characteristics (Motmainna et al., 2021). Weedy rice often exhibits high genetic variability, greater tillering ability, and strong competitive traits, leading to substantial yield losses in rice fields (Chauhan, 2013; Nadir et al., 2017). However, some cultivated rice varieties may have evolved defensive mechanisms, including allelopathy, to counteract weedy rice infestations (Kato-Noguchi & Ino, 2001).

These findings highlight the potential of allelopathic rice varieties, such as OM 5930, for sustainable weed management. The integration of allelopathic crops aligns with sustainable agriculture goals, reducing herbicide dependency and fostering agroecosystem resilience (Eroğlu et al., 2022; Khamare et al., 2022). Further research should focus on identifying and quantifying the full specific allelochemicals present in OM 5930, optimizing their effectiveness, and assessing their field-scale applications for controlling weedy rice under various environmental conditions. Given the growing concerns about herbicide resistance and environmental sustainability, utilizing allelopathic rice varieties as a natural weed suppression tool presents a promising avenue for integrated weed management programs.

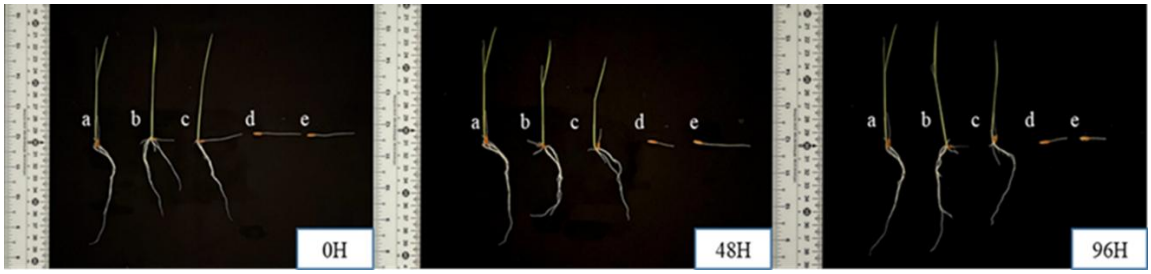

Figure 4. Effect of root extract of OM 5930 on the shoot and root development of WR20 weedy rice at 7 days after treatment

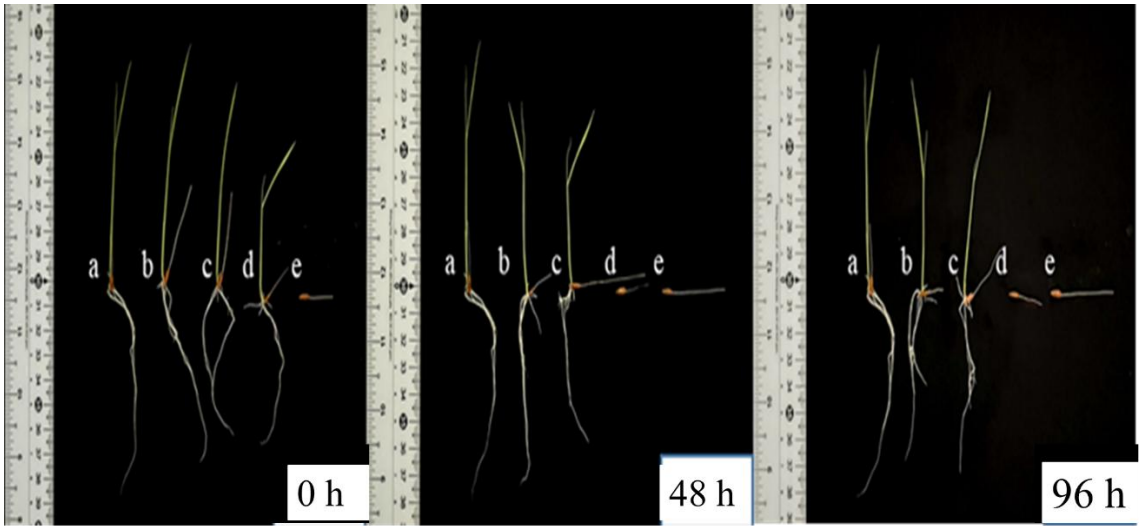

Figure 5. Effect of stem extract of OM 5930 on the shoot and root development of WR20 weedy rice at 7 days after treatment

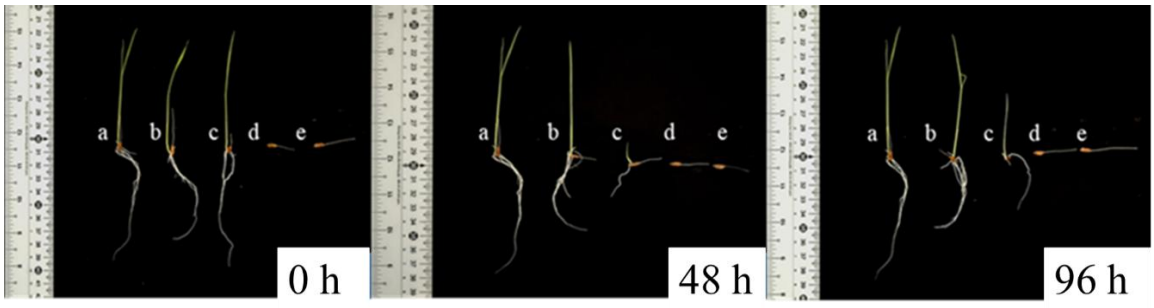

Figure 6. Effect of leaf extract of OM 5930 on the shoot and root development of WR20 weedy rice at 7 days after treatment

Note: a: Control; b: 0.015 g/mL; c: 0.075 g/mL; d: 0.15 g/mL; e: 0.3 g/mL. 0h, 48h, and 96h are the durations of seed soaking before applying the extract treatment.

4. CONCLUSION

Extracts from different parts (roots, stems, and leaves) of OM5930 rice showed inhibitory effects on the growth of shoot and root seedlings of both WR19 and WR20 weedy rice lines. The inhibitory effect increased with extract concentration, and all extract samples achieved complete inhibition at 0.3 g/mL across all testing times for both weed lines. The 48-hour treatment time proved to be the most effective, providing greater stability compared to

the other two time points (0h and 96h) for both WR19 and WR20 weedy rice lines.

Methanol extracts from the stems and roots of OM5930 also inhibited the growth of shoot and root seedlings in both weed lines, although the effect was not as strong as the leaf extract. Despite varying concentrations, the extracts from the stems and roots showed increasing inhibition as the treatment time and concentration increased, although they did not reach the maximum effect observed with the leaf extract.

The WR20 line exhibited weaker resistance to extracts from various parts of OM5930 than the WR19 line. However, at low concentrations (0.015 and 0.075 g/mL), the WR20 line showed a stimulatory effect at 0.015 g/mL. In contrast, the WR19 line, despite showing better resistance, did not exhibit this stimulation effect. This suggests that the WR19 line (short awn) has better resistance to extracts from OM5930 compared to the WR20 line (long awn).

Although both lines belong to the same species, their resistance to the OM5930 extracts differs, indicating the need for further research into how OM5930 extracts can be processed to inhibit multiple weedy rice lines, while also considering the effective pre-germination treatment time, which in this study was 48 hours.

From these laboratory-based findings, OM5930 rice shows potential as a source of allelopathic compounds that could be leveraged to support sustainable weed management in rice fields. Nonetheless, these extracts should not yet be considered "selective bioherbicides" until validated through comprehensive chemical, ecological, and agronomic evaluations. Moreover, if OM5930 meets essential criteria for yield performance, grain quality, and adaptability, its broader cultivation in the Mekong Delta could contribute not only to rice production but also to limiting the proliferation of weedy rice through natural suppression. This integrated benefit positions OM5930 as a promising dual-purpose variety pending further research to support weed management efforts within ecologically based rice farming systems.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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