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Detection of bioactive compounds in methanol *Kalanchoe pinnata* leaf extracts capable of reducing rice bacterial leaf blight

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ABSTRACT

Bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae severely damages rice production, particularly under climate change conditions. This study detected bioactive compounds from leaf extracts of Kalanchoe pinnata (Lam.) Pers. and tested their disease-reducing effects against BLB. Methanol leaf extracts of K. pinnata were prepared by liquid-liquid extraction combined with ultrasonication. Using gas chromatography–mass spectrometry, 27 compounds were identified in the extracts. Based on their abundances and reported involvements in plant defense responses against pathogens, three compounds were selected for further tests, i.e., 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester; ethyl linolenate and 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. To have adequate amounts of these compounds for subsequent bioassays under net house conditions, their commercially available analogs were employed, i.e., dimethyl furan-2,5-dicarboxylate (FDME), water-soluble linoleic acid (LinA) and bis(2-ethylhexyl) phthalate (DEHP). Rice seeds were soaked in six different concentrations of each compound (3, 5, 10, 15, 20, and 25 ppm) for 24 h before sowing. Seed soaking with either FDME or DEHP significantly reduced BLB lesion lengths after pathogen inoculation. The effects of FDME were maintained until 21 DAI. Moreover, seed soaking using a mixture of FDME and DEHP provided similar protection as that of the chemical control using Starner 20WP.

1. INTRODUCTION

Rice bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), remains one of the most destructive diseases affecting rice production, particularly in tropical and subtropical regions of Asia (Noda et al., 1996; Quibod et al., 2016; Tuan et al., 2018; Khwanngam et al., 2024). In recent decades, the combination of intensive rice farming and climate change has contributed to more frequent and severe BLB outbreaks (Kumar et al., 2020; Manzoor et al., 2024; Zhang et al., 2024). Although genetic resistance has been widely adopted, the rapid evolution of *Xoo* populations has led to the breakdown of resistance in several commercially important rice varieties, thereby reducing the long-term effectiveness of this strategy (Oliva et al., 2019). Consequently, sustainable and complementary disease management approaches are increasingly required.

Chemical bactericides have been used to mitigate BLB severity; however, their application is associated with ecological risks, potential impacts

on non-target organisms, and the development of pathogen resistance (Solekha et al., 2020). In this context, induced resistance has emerged as a promising alternative strategy that enhances the plant's innate defense capacity rather than directly targeting the pathogen. Induced resistance involves the activation of complex defense signaling networks, including reactive oxygen species (ROS) production, antioxidant regulation, and the accumulation of defense-related metabolites (Pieterse et al., 2012; Walters et al., 2014). In rice–*Xoo* interactions, increased activities of antioxidant and defense enzymes such as peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) have been consistently associated with reduced disease severity (Yu et al., 2016; Khoa et al., 2017; Solekha et al., 2020; Xa et al., 2023a).

Plant-derived extracts have attracted considerable interest as resistance-inducing agents due to their natural origin, chemical diversity, and relatively low environmental impact. Among these, *Kalanchoe pinnata* (Lam.) Pers. is a medicinal plant known to contain a wide range of secondary metabolites, including flavonoids, phenolic acids, and other antioxidant compounds that are potentially involved in plant defense regulation (Mierziak et al., 2014). Previous studies have demonstrated that aqueous extracts of *K. pinnata* leaves can reduce BLB severity in rice when applied as seed treatments or foliar sprays, primarily through the enhancement of defense enzyme activities following *Xoo* infection (Khoa et al., 2017; Huong et al., 2018; Xa et al., 2023b). More recently, methanol extracts of *K. pinnata* leaves were reported to exhibit greater efficacy in suppressing BLB than aqueous extracts, suggesting that the extraction solvent plays a critical role in isolating bioactive compounds responsible for resistance induction (Xa et al., 2023a; Duy et al., 2024).

Despite these findings, the specific bioactive compounds present in methanol *K. pinnata* leaf extracts that contribute to BLB suppression have not been sufficiently characterized. Most existing studies have focused on disease reduction and physiological responses of rice plants, while the phytochemical profiles underlying these effects remain largely unexplored. This represents a critical knowledge gap, as the identification of key compounds is essential for understanding the mechanistic basis of induced resistance and for the potential development of standardized, plant-based resistance elicitors. Therefore, the objective of the present study was to detect and characterize bioactive compounds in methanol extracts of *K. pinnata* leaves associated with the reduction of rice bacterial leaf blight. The results are expected to provide insight into the chemical components linked to resistance induction and to support the rational use of plant-derived extracts in sustainable BLB management strategies.

2. MATERIALS AND METHOD

2.1. Plant materials and bacterial strain

Whole healthy and mature plants of *K. pinnata* (Lam.) Pers. (approximately one year old) were collected from the net house of Vinh Long University of Technology Education, Vinh Long Province, Viet Nam. Harvesting was conducted at 7:00 a.m. to minimize diurnal variation in secondary metabolite accumulation. Uniform, fully expanded leaves (approximately 10 cm in length) free from visible damage or disease symptoms were selected, thoroughly washed with distilled water, air-dried under laboratory conditions, and used immediately for extract preparation.

Rice (*Oryza sativa* L.) cultivar Jasmine 85, which is known to be susceptible to BLB, and virulent isolates of *Xoo* were provided by the Plant Pathology Group, Institute of Food and Biotechnology, Can Tho University, Viet Nam. Bacterial isolates were maintained on nutrient agar medium at 28 ± 2°C and subcultured prior to experimental use following standard phytopathological procedures.

2.2. Preparation of *K. pinnata* leaf extracts

Methanol extracts of *K. pinnata* leaves were prepared using a liquid-liquid extraction method with slight modifications based on Kagale et al. (2004) and Xa et al. (2023a). Briefly, 300 g of fresh leaves were homogenized for 5 min using a laboratory blender containing 1500 mL of absolute methanol (100%, polarity index 0.762; Sigma-Aldrich, Germany), corresponding to a ratio of 1:5 (w/v). The homogenate was subjected to ultrasonication in an Elmasonic S300 ultrasonic bath (Elma Co., Germany) at 120 W for 60 min to enhance the release of intracellular bioactive compounds.

Following ultrasonication, the mixture was salted out with anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, then filtered through Whatman filter paper (Ø 110 mm). The resulting filtrate was concentrated under reduced pressure

using a rotary vacuum evaporator (IKA, Calgon Scientific Co., India) at 50°C and 5000 rpm. The concentrated extract was further dried at 50°C to obtain a crude methanol extract, which was stored at −4°C in amber glass bottles until further chemical analysis and bioassays.

2.3. Gas chromatography–mass spectrometry (GC–MS) analysis of bioactive compounds

The chemical composition of the methanol *K. pinnata* leaf extract was analyzed using gas chromatography–mass spectrometry (GC–MS) following protocols described by Syed-Ab-Rahman et al. (2020) and Xa (2024), with minor adjustments. Analyses were performed on a GCMS-QP2010 Ultra system (Shimadzu, Japan) equipped with an HP-5 MS capillary column (5% phenyl methyl siloxane) measuring 30 m × 250 µm × 0.25 µm.

The injection temperature was set at 250°C, while the ion source temperature was maintained at 230°C with an ionization energy of 70 eV. Helium was used as the carrier gas at a constant flow rate. The oven temperature program was initiated at 50°C and held for 10 min, followed by a gradual increase to 310°C at a rate of 5°C min^{-1}. Mass spectra were recorded over a scan range appropriate for phytochemical profiling.

Identification of compounds was achieved by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST 09) spectral library. The retention time, molecular weight, and fragmentation patterns were used to confirm compound identities. The relative abundance of each compound was calculated as the percentage of the total peak area in the chromatogram.

2.4. Selection of major bioactive compounds

GC–MS analysis detected a total of 27 bioactive compounds in the methanol extract of *K. pinnata* leaves. Among these, eight compounds were consistently present in the methanol fraction. Three compounds were selected for further investigation based on their high relative abundance (> 5%) and reported roles in plant defense induction and stress signaling. These compounds included:

(1) 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester
(2) linoleic acid
(3) 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester.

These compounds were selected because their chemical analogs are commercially available, allowing the preparation of sufficient quantities for subsequent biological assays and mechanistic studies. Dimethyl furan-2,5-dicarboxylate (FDME) was used as an equivalent of 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester; water-soluble linoleic acid (LinA) was used as an equivalent of linoleic acid and ethyl linolenate; and bis(2-ethylhexyl) phthalate (DEHP) was used as an equivalent of 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. The selection was further supported by previous studies reporting the involvement of fatty acid derivatives and aromatic esters in the activation of plant defense responses and induced resistance (Walters et al., 2014; Solekha et al., 2020; Manzoor et al., 2024).

2.5. Evaluation of disease-reducing effects of selected bioactive compounds using seed soaking

The disease-reducing effects of selected bioactive compounds against rice BLB were evaluated using a seed soaking method under net house conditions. Three commercially available compounds, namely FDME, LinA, and DEHP, were used in this experiment.

Rice seeds were surface-sterilized and soaked for 24 h in aqueous solutions of each compound at concentrations of 3, 5, 10, 15, 20, and 25 ppm. Seeds soaked in sterile distilled water served as the untreated control. After soaking, the seeds were incubated at 28°C for 48 hours prior to sowing. The experiment was arranged in a completely randomized design with three replications, each consisting of five plants.

The bacterial leaf blight pathogen was cultured on modified Wakimoto's agar medium at 28°C for 72 h. The bacterial suspension was prepared at a concentration of 10^9 CFU/mL. At 45 days after sowing, three fully expanded leaves per plant were inoculated using the leaf-clipping method (Kauffman et al., 1973). Disease severity was assessed by measuring lesion length at 7, 14, and 21 days after inoculation (DAI) (Khoa et al., 2017; Xa et al., 2023a).

2.6. Data analyses

GC–MS data were processed using the instrument's integrated software, and relative peak areas were expressed as mean percentages. Descriptive statistics were used to summarize the chemical

profiles of the extracts. BLB lesion length data were analyzed by analysis of variance (ANOVA) without data transformation using PC-SAS® version 9.1 (SAS Institute Inc., Cary, NC, USA). All experiments followed a completely randomized design with three replications and were independently repeated three times.

3. RESULTS AND DISCUSSION

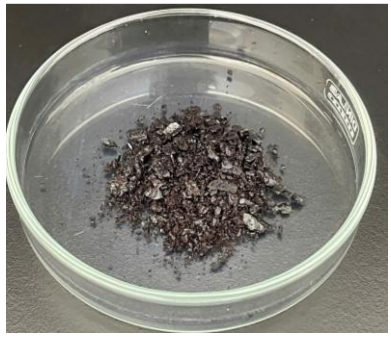
3.1. Extraction yield and physical characteristics of *K. pinnata* methanol extract

The liquid-liquid extraction of *K. pinnata* leaves with methanol resulted in a crude extract yield of 2.20 ± 0.19% (w/w), characterized by a semi-solid consistency, dark brown coloration, and a mild aromatic odor (Figure 1).

Kalanchoe pinnata (Lam.) Pers

Methanol leaf extracts

Ultrasonication of the homogenate



Methanol extraction yield of *K. pinnata*

Figure 1. Methanol liquid-liquid extraction yield of *K. pinnata* leaves

This extraction efficiency is consistent with yields reported for methanol extracts of resistance-inducing plant species (Kagale et al., 2004; Syed-Ab-Rahman et al., 2020; Xa et al., 2023a). Methanol-based liquid-liquid extraction is particularly effective for isolating phenolic compounds, fatty acids, and ester derivatives implicated in plant defense signaling. Although the overall yield was moderate, ultrasonication likely promoted enhanced cell disruption and qualitative enrichment of elicitor compounds, rather than increasing extract mass (Azmir et al., 2013).

3.2. GC–MS profiling of bioactive compounds in *K. pinnata* methanol leaf extract

GC–MS analysis revealed that the methanol leaf extract of *K. pinnata* contained a diverse set of metabolites with potential roles in plant defense induction. A total of 27 compounds were tentatively identified based on NIST library matching, retention times, and mass fragmentation patterns (Figure 2; Table 1). The identified compounds were mainly organic acids and their esters, fatty acids, aromatic dicarboxylic esters, and long-chain hydrocarbons-metabolite classes.

Five compounds were detected at relatively high abundance, including 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (19.08%), dimethyl malate (15.39%), diisooctyl phthalate (10.65%), 5-oxotetrahydrofuran-2,3-dicarboxylic acid (9.43%), and triacontane (3.15%). The dominance of organic acid derivatives and lipid-related metabolites suggests that the extract may influence central metabolic pathways closely linked to plant immunity.

Dimethyl malate and citric acid–related derivatives are intermediates of the tricarboxylic acid (TCA) cycle and have been increasingly recognized as metabolic signals involved in plant defense priming (Mauch-Mani et al., 2017). These metabolites contribute to energy redistribution, redox regulation, and reactive oxygen species (ROS) homeostasis, which are essential for the activation of induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Van Dongen, 2011; El-Ramady et al., 2022). Their presence suggests that *K. pinnata* extract may enhance plants' metabolic readiness to respond rapidly to pathogen attack.

The detection of 5-oxotetrahydrofuran-2,3-dicarboxylic acid further supports the defense-inducing potential of the extract. This compound has been reported in *K. pinnata* and other plant species and is associated with antimicrobial activity and modulation of oxidative stress (Phatak, 2015; Mejia-Mendez et al., 2023). Such organic acids are proposed to act as signaling or priming molecules that strengthen basal defense mechanisms in plants.

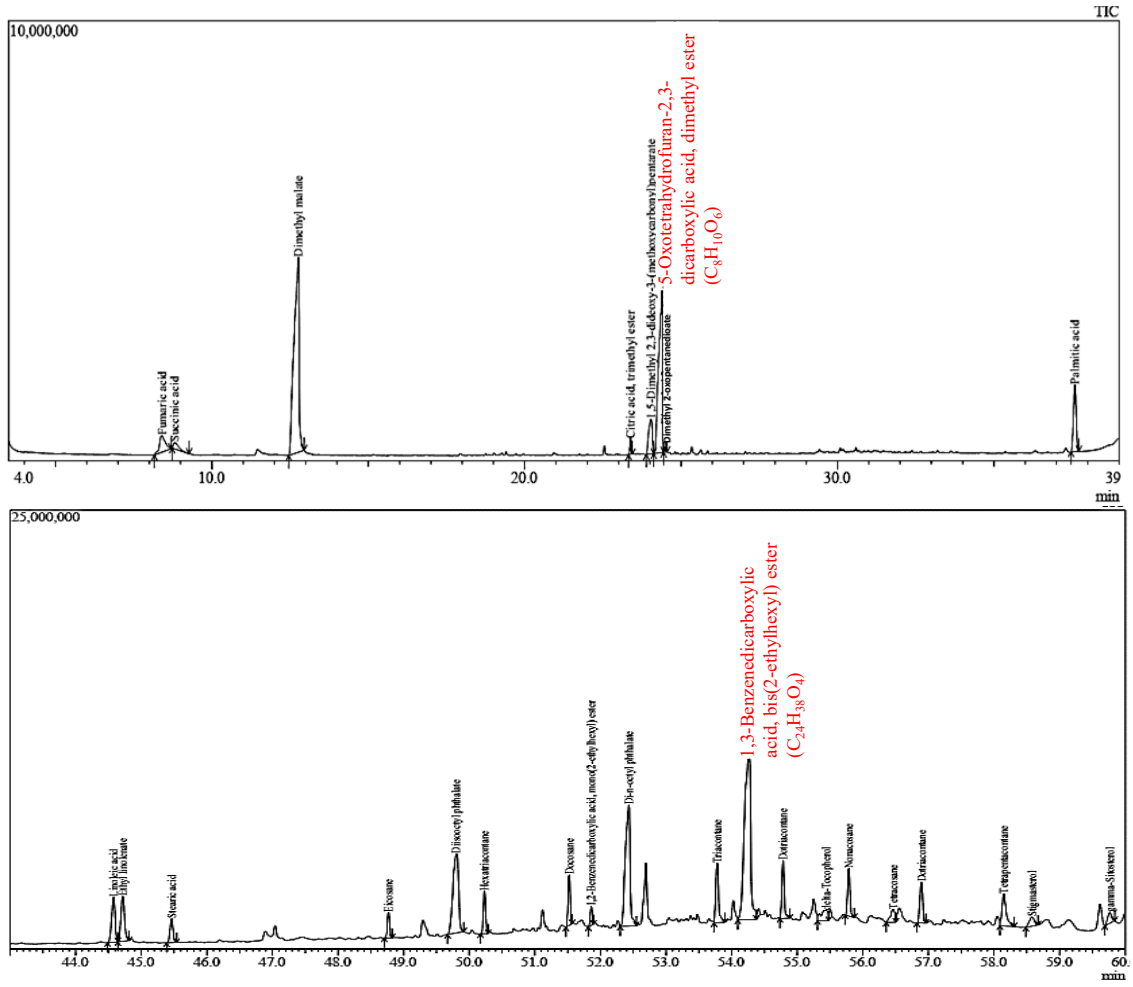


Figure 2. Total ion current GC–MS chromatogram of metabolites in *K. pinnata* methanol leaf extract

Fatty acids, including palmitic acid and linoleic acid, were also identified. Palmitic acid is a key component of membrane phospholipids and plays an important role in maintaining membrane integrity and regulating membrane-associated signaling proteins involved in stress perception (Carta et al., 2017). Linoleic acid serves as a precursor for oxylipins and jasmonic acid–related signaling pathways, which are central to ISR against bacterial and fungal pathogens (Blee, 2002). These findings suggest that lipid-mediated signaling may contribute to the defense-inducing effects of the extract.

Notably, 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester was the most abundant compound detected. Aromatic dicarboxylic esters have been reported to possess antimicrobial properties and may contribute to disease suppression by either limiting pathogen growth or priming host defense responses (Abdul et al., 2022). Its high relative abundance indicates a potential role in both direct and indirect plant protection.

Overall, the GC–MS results demonstrate that the methanol leaf extract of *K. pinnata* contains a complex mixture of bioactive metabolites with known antioxidant, antimicrobial, and plant defense–related functions. Although many of these compounds have been extensively studied in medicinal contexts, their application in crop protection remains limited. The present findings provide important chemical evidence supporting the potential use of *K. pinnata* as a natural source of resistance-inducing agents for sustainable plant disease management.

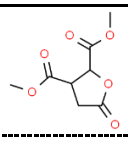
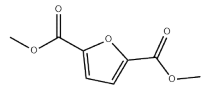
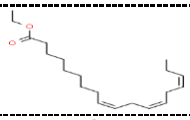
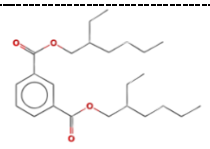
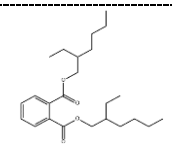
Table 1. Composition of chemical constituents in the methanol extract of fresh leaves of *K. pinnata*

ID	Ret. time	Name of compounds	Molecular formula	Molecular mass (Da)	Composition (%)
1	8.42	Fumaric acid	$C_4H_4O_4$	116.07	1.51
2	8.81	Succinic acid	$C_4H_6O_4$	118.09	0.51
3	12.77	Dimethyl malate	$C_6H_{10}O_5$	162.14	15.39
4	23.38	Citric acid, trimethyl ester	$C_9H_{14}O_7$	234.20	0.30
5	24.04	1,5-dimethyl 2,3-dideoxy-3-(methoxycarbonyl)pentarate	$C_9H_{14}O_7$	234.20	1.92
6	24.39	5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester	$C_8H_{10}O_6$	202.16	9.43
7	24.49	Dimethyl 2-oxopentanedioate	$C_7H_{10}O_5$	174.15	0.45
8	37.59	Palmitic acid	$C_{16}H_{32}O_2$	256.42	2.84
9	44.58	Linoleic acid	$C_{18}H_{32}O_2$	280.45	2.84
10	44.72	Ethyl linolenate	$C_{20}H_{34}O_2$	306.48	3.03
11	45.46	Stearic acid	$C_{18}H_{36}O_2$	284.48	1.31
12	48.77	Eicosane	$C_{20}H_{42}$	282.55	1.09
13	49.80	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.56	8.30
14	50.23	Hexatriacontane	$C_{36}H_{74}$	506.98	1.77
15	51.52	Docosane	$C_{22}H_{46}$	310.60	2.10
16	51.86	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.34	0.72
17	52.34	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.56	10.65
18	53.78	Triacontane	$C_{30}H_{62}$	422.81	3.15
19	54.27	1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.56	19.08
20	54.79	Dotriacontane	$C_{32}H_{66}$	450.87	2.62
21	55.44	Delta-tocopherol	$C_{27}H_{46}O_2$	402.65	1.40
22	55.79	Nonacosane	$C_{29}H_{60}$	408.79	2.16
23	56.46	Tetracosane	$C_{24}H_{50}$	338.65	1.14
24	56.89	Dotriacontane	$C_{32}H_{66}$	450.87	2.18
25	58.15	Tetrapentacontane	$C_{54}H_{110}$	759.45	2.48
26	58.58	Stigmasterol	$C_{29}H_{48}O$	412.69	0.87
27	59.77	Gamma-sitosterol	$C_{29}H_{50}O$	414.71	0.78

3.3. Selection of major bioactive compounds related to plant defense induction

GC–MS analysis of the methanol leaf extract of *K. pinnata* identified 27 bioactive compounds, of which four major constituents (>5%) were selected due to their reported roles in plant defense signaling: 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester; linoleic acid and 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. These compounds represent key metabolic and lipid-related pathways involved in ISR against plant pathogens.

Table 2. Commercial compounds replacing bioactive compounds in *Kalanchoe pinnata* leaves

Bioactive compounds			Commercial compounds		
Compound name	**Molecular formula**	**Chemical structure**	**Trade name**	**Molecular formula**	**Chemical structure**
5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester	$C_8H_{10}O_6$		Dimethyl furan-2,5-dicarboxylate (FDME)	$C_8H_{10}O_5$	
Linoleic acid	$C_{18}H_{32}O_2$		Linoleic acid-water soluble (LinA)	$C_{18}H_{32}O_2$	
1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$		Bis(2-ethylhexyl) phthalate (DEHP)	$C_{24}H_{38}O_4$	

To facilitate mechanistic disease assays, commercially available analogs were employed (Table 2), including FDME, LinA, and DEHP, selected based on structural equivalence and availability. Organic acid derivatives such as FDME are implicated in redox regulation and metabolic priming, while ethyl linolenate derivatives activate oxylipin and jasmonate-mediated defense pathways critical for restricting pathogen colonization. Aromatic esters may further enhance disease suppression by priming basal immunity. These findings support the hypothesis that *K. pinnata* derived compounds mitigate disease severity primarily through host defense activation rather than direct antimicrobial toxicity (Walters et al., 2013; Solekha et al., 2020).

3.4. Defense-related effects of selected bioactive compounds on rice responses to bacterial leaf blight

Seed soaking with selected commercial analogs significantly influenced BLB development in rice under net house conditions. Among the tested compounds, FDME and DEHP exhibited disease-reducing effects at specific concentrations, whereas LinA showed no significant suppression compared with the untreated control. At 14 DAI, all treatments differed significantly from the negative control, which exhibited severe lesion development (195.6 ± 8.7 mm). At 21 DAI, FDME at 20 ppm and 25 ppm resulted in significantly shorter lesion lengths (388.9 ± 18.2 mm and 358.2 ± 19.9 mm, respectively) compared with the control (409.0 ± 30.0 mm), indicating sustained disease suppression. In contrast, FDME 15 ppm and DEHP at 20-25 ppm reduced lesion length only at 14 DAI, suggesting a transient protective effect (Figure 3).

The reduction of BLB observed in seed-treated rice is unlikely to be attributed to direct antibacterial effects, as treatment was applied prior to pathogen inoculation, supporting a host-mediated defense priming mechanism. FDME, an analog of organic acid-related metabolites detected in the total methanol extract of *K. pinnata* leaves, may enhance basal resistance by promoting early defense readiness and strengthening constitutive barriers to pathogen invasion. Similarly, DEHP, representing aromatic ester-type metabolites present in the same extract, has been linked to membrane-associated signaling and activation of stress-responsive defense pathways that restrict lesion expansion. The presence of both metabolite types in the methanol extract previously shown to induce resistance in rice further supports their role in activating disease-limiting host responses rather than exerting direct effects on *Xoo*. These findings are consistent with established models of induced resistance in which seed-applied elicitors confer durable protection against bacterial diseases through priming of host defense mechanisms (van Loon et al., 2006; Walters et al., 2013; Khoa et al., 2017).

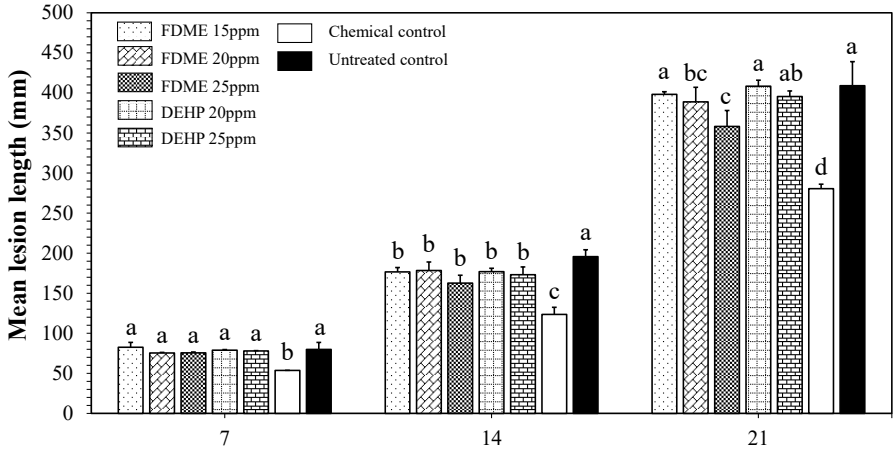


Figure 3. Effects of seed soaking with commercial compounds on bacterial leaf blight lesion length in rice cv. Jasmine 85 under net house conditions

FDME: dimethyl furan-2,5-dicarboxylate compound (used as a substitute for the bioactive compound 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester); DEHP: bis(2-ethylhexyl) phthalate compound (used as a substitute for the bioactive compound 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester). At each assessment time point, bars with the same letters are not significantly different at $p \leq 0.05$ by Duncan's multiple range test. Each value is the mean ± standard deviation of three replications.

3.5. Combined bioactive compounds prime rice defense responses against BLB

Seed soaking with selected commercial analogs revealed that FDME and DEHP, but not LinA, significantly reduced BLB severity in rice under net house conditions. FDME showed a sustained disease-suppressive effect at higher concentrations, maintaining shorter lesion lengths up to 21 DAI, whereas DEHP exhibited a more transient effect, with significant suppression mainly at 14 DAI (Figure 4). These results indicate that FDME primarily contributes to prolonged defense activation, while DEHP may be involved in early-stage defense responses. Because seed treatment preceded pathogen inoculation, the observed BLB reduction is unlikely due to direct antibacterial activity, supporting a host-mediated defense priming mechanism.

Based on these complementary temporal effects, FDME and DEHP were combined to enhance both the magnitude and durability of induced resistance. FDME, a structural analog of an organic acid derivative detected in the total methanol extract of *K. pinnata*, is likely associated with metabolic priming and redox homeostasis, reinforcing basal immunity. In contrast, DEHP, representing aromatic ester-type metabolites present in the same extract, may modulate membrane-associated signaling and stress-responsive defense pathways. Under net house conditions, seed soaking with the FDME+DEHP combination reduced lesion length to levels comparable to the chemical control Starner 20WP (Figure 4), whereas treatments with either compound alone produced a smaller reduction in lesion length and did not reach the efficacy observed for Starner 20WP (Figure 3). Their co-occurrence in the methanol extract previously shown to induce resistance in rice supports their joint contribution to host defense activation. This rationale aligns with previous studies demonstrating that combining elicitor molecules with distinct but complementary modes of action can strengthen and prolong systemic resistance without imposing selective pressure on pathogens, offering a sustainable approach for BLB management (Walters et al., 2013; Khoa et al., 2017; Xa et al., 2023a).

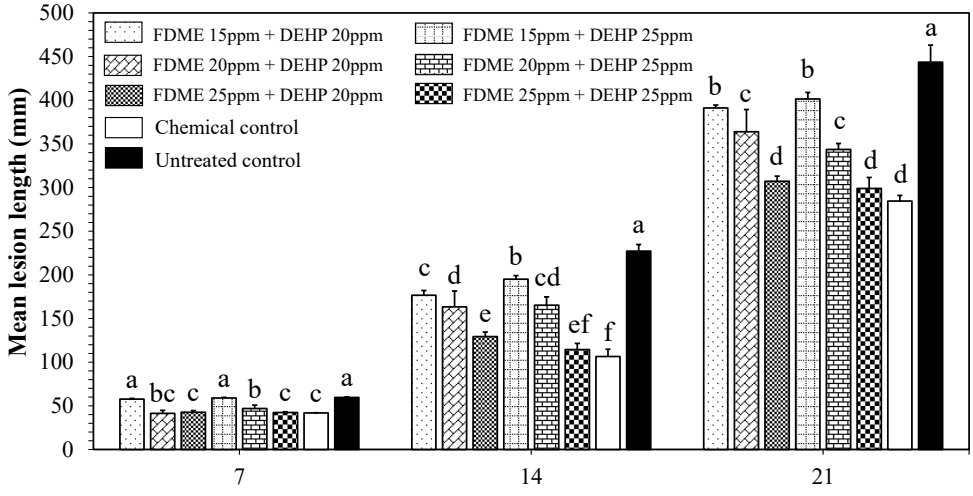


Figure 4. Effects of seed soaking with commercial compound mixture on bacterial leaf blight lesion length in rice cv. Jasmine 85 under net house conditions

FDME: dimethyl furan-2,5-dicarboxylate compound (used as a substitute for the bioactive compound 5-oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester); DEHP: bis(2-ethylhexyl) phthalate compound (used as a substitute for the bioactive compound 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester). At each assessment time point, bars with the same letters are not significantly different at $p \leq 0.05$ by Duncan's multiple range test. Each value is the mean ± standard deviation of three replications.

Methanol extraction of *K. pinnata* leaves yielded a complex mixture of defense-related metabolites, as confirmed by GC-MS profiling, which identified 27 compounds dominated by organic acid derivatives, fatty acids, and aromatic esters. Several of these metabolites, including dimethyl malate, 5-oxotetrahydrofuran-2,3-dicarboxylic acid derivatives, linoleic acid, and 1,3-

benzenedicarboxylic acid esters, are closely associated with central carbon metabolism, redox regulation, and lipid-mediated signaling pathways that underpin induced resistance in plants. Such metabolites are increasingly recognized as metabolic and signaling cues that prime salicylic acid and jasmonic acid-dependent defense pathways, thereby enhancing the plant's capacity to respond rapidly to pathogen attack rather than exerting direct antimicrobial activity (Bolton, 2009; Walters et al., 2013; El-Ramady et al., 2022).

Bioassays using commercial analogs demonstrated that FDME and DEHP significantly reduced bacterial leaf blight severity when applied via seed soaking, whereas LinA showed no consistent protective effect. Disease suppression was concentration- and time-dependent, with FDME conferring more sustained protection and DEHP contributing mainly to early-stage disease reduction. DEHP is subject to regulatory restrictions due to concerns over persistence and potential toxicity; however, its application at low concentrations via seed treatment in this study likely minimizes environmental exposure and associated risks while providing early-stage disease suppression. Future studies should prioritize phthalate-free or naturally derived alternatives with comparable efficacy and evaluate their environmental fate and biosafety under field conditions.

Because seed treatment was applied well before pathogen inoculation, the observed effects are best explained by host-mediated defense priming rather than direct inhibition of *Xoo*. The enhanced efficacy observed when FDME and DEHP were combined suggests complementary modes of action, whereby organic acid–related metabolites support metabolic readiness and redox homeostasis, while aromatic esters modulate membrane-associated signaling and stress-responsive defenses. This synergistic priming effect is consistent with previous reports showing that combinations of elicitors targeting distinct defense layers can strengthen and prolong induced resistance against bacterial diseases without imposing selective pressure on pathogens (van Loon et al., 2006; Walters et al., 2013; Khoa et al., 2017).

4. CONCLUSIONS

This study demonstrates that methanol extracts of *K. pinnata* leaves are a rich source of bioactive metabolites that induce rice resistance against bacterial leaf blight. GC–MS-guided selection and biological validation identified FDME and DEHP-related metabolites are among the compounds associated with disease suppression, possibly through host defense priming. Seed soaking with these compounds, particularly in combination, provided sustained protection comparable to chemical control under net house conditions. These findings support the potential of *K. pinnata* derived metabolites as natural resistance inducers and provide a scientific basis for developing sustainable, plant-based strategies for BLB management. The results of this study should be confirmed under field conditions. Furthermore, molecular mechanisms underlying FDME- and DEHP-mediated defense priming should be investigated, considering that these commercial analogs may not fully show identical activities of their natural compounds in the extract.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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