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Original Article

Fungitoxic potential of *Vernonia amygdalina* and *Cymbopogon citratus* extraction methods and concentrations on *Fusarium proliferatum* of Rice (*Oryza sativa*)

OPEN ACCESS

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A B S T R A C T

This study evaluated the fungitoxic efficacy of Vernonia amygdalina (bitter leaf) and Cymbopogon citratus (lemon grass) extracts against Fusarium proliferatum, a significant pathogen affecting rice production. Treatments comprised of 2 plants (bitter leaf and lemongrass) extracted using 2 methods (Soxhlet and Maceration) at 4 concentrations (0%, 50%, 75%, and 100%) respectively to assess their inhibitory effects on fungal radial growth over a 7-day period. The experiment employed a 2 × 2 × 4 factorial laid in a completely randomized design with three replications. Analysis of variance revealed significant main effects (p<0.05) for plant type, extraction method, concentration, and interactions between all factors in controlling F. proliferatum growth. Results showed that lemongrass extract exhibited superior antifungal activity compared to bitter leaf, with maximum inhibition of 39.88% observed on day 4. Soxhlet extraction proved more effective than maceration, particularly in maintaining consistent inhibition rates during later stages of observation. A clear concentration-dependent response was observed, with 100% concentration showing the highest inhibition rates (50.62 - 63.93%). The interaction between extraction methods and plant types revealed that Soxhlet-extracted lemongrass provided the most stable and sustained antifungal activity. While macerated bitter leaf showed high initial inhibition (69.74% on day 2), its efficacy declined substantially over time. The study suggested that lemongrass extracted via the Soxhlet method represents a promising natural alternative for controlling F. proliferatum in rice production systems, particularly when applied at one hundred percent. These findings contribute to the development of sustainable disease management strategies in rice cultivation.

K E Y W O R D S : *Antifungal, Cymbopogon citratus, Extract, Fusarium proliferatum, Rice,*

INTRODUCTION

Rice (*Oryza sativa*) represents one of the world's most important food crops, with production ranking third globally after sugarcane and maize (Abbade, 2021). However, fungal diseases pose a significant threat to rice production, leading to substantial yield losses and potential contamination with mycotoxins that pose health risks to consumers (Khan *et al.*, 2024). Among these pathogens, *Fusarium proliferatum* has emerged as a significant concern in rice cultivation, affecting

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both yield and grain quality. The rising concerns about *Fusarium proliferatum* in rice production have prompted research into sustainable control methods. *F. proliferatum* has emerged as a significant threat, capable of causing substantial yield losses ranging from 20-50% under favourable conditions while producing harmful mycotoxins, particularly *fumonisins*, that pose serious health risks to consumers (Khan *et al.,* 2024; Peng *et al.,* 2021). The conventional approach to managing fungal diseases in rice has primarily relied on synthetic fungicides. However, their indiscriminate use has led to environmental contamination, development of pathogen resistance, and accumulation of harmful chemical residues in food products (Carlsen *et al.*, 2017). These challenges have spurred interest in exploring natural alternatives that can effectively control plant pathogens while minimizing negative environmental impacts.

Plant-derived fungicides have gained attention as promising alternatives to synthetic chemicals due to their biodegradability and reduced environmental impact (Godlewska *et al.,* 2021). *Vernonia amygdalina* (bitter leaf) and *Cymbopogon citratus* (lemon grass) are two plants known for their antimicrobial properties, though their specific efficacy against *F. proliferatum* in rice cultivation remains understudied. These plants contain various bioactive compounds that may offer natural protection against fungal pathogens without the drawbacks associated with synthetic fungicides (Suleiman *et al.,* 2008) and act as alternatives to synthetic fungicides (Bello and Sisterna, 2010). These botanical solutions offer several advantages, including lower toxicity, biodegradability, and reduced environmental impact (Khursheed *et al.,* 2022). Among the promising plant species studied, lemon grass (*Cymbopogon citratus*) and bitter leaf (*Vernonia amygdalina*) have shown particular potential for fungal control.

The present study aimed to evaluate the fungitoxic efficacy of *V. amygdalina* and *C. citratus* extracts on the radial inhibition of *F. proliferatum*. Understanding the antifungal potential of these plant extracts could contribute to the development of sustainable disease management strategies in rice cultivation, potentially reducing reliance on synthetic fungicides while maintaining effective pathogen control (Tsion & Steven, 2019).

Lemon grass essential oil, containing active compounds such as citral, geraniol and myrcene, has demonstrated significant antifungal efficacy (Tyagi and Malik, 2010). Research by Helal Sarhan et al. (2007) revealed that lemon grass oil's mechanism of action involves disrupting fungal cell membranes, leading to cell wall degradation and cytoplasmic disorganization. Studies by Tzortzakis & Economakis (2007) found that lemon grass oil vapours effectively inhibited spore germination and mycelial growth in fungal pathogens, suggesting its potential in disease management. Bitter leaf also contains bioactive compounds including sesquiterpene lactones, flavonoids and steroid glycosides that contribute to its antifungal properties (Yeap et al., 2010). Research by Erasto et al. (2006) demonstrated that both aqueous and ethanolic extracts of *V. amygdalina* effectively inhibited fungal growth, with ethanolic extracts showing superior potency. The antifungal mechanism of bitter leaf is believed to be multifaceted due to its diverse phytochemical composition, potentially involving both membrane disruption and interference with metabolic processes (Ijeh & Ejike, 2011).

The efficacy of plant-based fungicides can be influenced by extraction methods. Maceration, a traditional room-temperature extraction technique, has proven effective for obtaining antifungal compounds (Kalit *et al.,* 2023). Cravotto & Cintas (2007) opined that this extraction technique widely used for extracting bioactive compounds from plant materials. It involves soaking the plant material in a solvent, allowing diffusion to extract soluble components. However, Ncube *et al.* (2008) found that methanol-based maceration yielded higher concentrations of antifungal phenolic compounds compared to aqueous extraction. Alternatively, Soxhlet extraction on the other hand is a widely used sample preparation method for transferring analytes from a solid matrix to a solvent (Sander, 2017). It involves repeated extraction cycles with pure solvent, making it thermodynamically favourable compared to simple soaking (Sander, 2017). Soxhlet extraction, while more efficient in extracting compounds with low solubility (Gopalasatheeskumar, 2018), may risk degrading thermolabile antifungal compounds during the heating process (Azwanida, 2015). While both lemon grass and bitter leaf extracts show promising antifungal properties, their effectiveness can vary depending on extraction methods and environmental conditions. Further research is needed to optimize their formulations and application methods for maximum efficacy against *F. proliferatum* (Ihejirika, 2013). The continued investigation of these natural alternatives represents an important step toward developing sustainable and environmentally conscious approaches to managing fungal diseases in rice production systems

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh leaf samples of *Vernonia amygdalina* and *Cymbopogon citratus* were obtained from Eke-Awka market in Awka and identified by a botanist from the Department of Botany, Nnamdi Azikiwe University, Awka. The leaves were aseptically washed, cut into smaller sizes independently, oven dried for 3 days at 50°C using a laboratory oven and ground into fine powder (250g) with a Qasa electric blender. The powder was preserved in dried plastic containers for extraction.

Preparation of Plant Extracts

Soxhlet Extraction Method

Using cold solvent extraction method, 50g of each plant material was placed in a thimble and inserted into a funnel. 500ml of ethanol was poured into a round bottom flask placed on a heating mantle. The condenser was connected with water inlet and outlet, and the apparatus was operated for 4 hours. The extract was collected in a conical flask and heated in a water bath at 78°C until ethanol evaporation, leaving the pure extract.

Maceration Method

Fifty grams of each plant material was soaked individually in 450ml of sterile distilled water in sterile conical flasks for 48 hours with intermittent manual shaking. The extracts were filtered first with sterilized white cheese cloth and then with sterile filter paper to remove residual debris. The solvent was evaporated using a water bath at 100°C and extracts were stored in airtight containers in a refrigerator.

Media Preparation and *In-Vitro* Fungitoxicity Test

Sabouraud Dextrose Agar (SDA) was prepared by dissolving 39g in 1000ml of sterile distilled water, autoclaved at 121°C at 15psi for 15 minutes. After cooling, 15ml of molten agar was distributed into 9cm petri dishes with two drops of lactic acid and 10ml of plant extract dilution. Plant extracts were diluted to concentrations of 100%, 75% and 50% using the formula $C_1V_1 = C_2V_2$.

Inoculation and Assessment of Fungal Growth

A 4mm mycelia disc from 5-day old pure culture of *F. proliferatum* was placed at the centre of each prepared plate. The experiment included three replications for each concentration level (100%, 75%, 50% and 0% control). Plates were incubated at 27°C with 12-hour light/dark cycles for 7 days. Radial growth measurements were taken daily using a meter rule and pair of dividers.

Determination of Growth Inhibition

The percentage radial inhibition was calculated using the formula:

$$\%\ \text{growth inhibition} = (dc - dt) / dc \times 100 \qquad (1)$$

Where, dc = diameter of fungal colony in control and dt = diameter of fungal colony in treatment

Experimental Design and Statistical Analysis

The experiment was designed as a 2×2×4 factorial arrangement in a Completely Randomized Design (CRD) with three replications. Factors included two plant extracts (Bitter leaf and Lemon grass), two extraction methods (Soxhlet and maceration), and four concentration levels (0%, 50%, 75%, 100%). Data were analysed using Analysis of Variance (ANOVA) in GENSTAT package 9.2, and means were separated using Least Significant Difference (LSD) at $P<0.05$.

RESULTS

Fungitoxic Efficacy of Plant Extracts against *F. Proliferatum* of rice

The study revealed significant variations in antifungal activity between the two plant extracts. Lemon grass (*Cymbopogon citratus*) recorded consistently superior inhibitory effects compared to bitter leaf (*Vernonia amygdalina*), with the highest inhibition (39.88%) recorded by lemon grass on day 4, while bitter leaf showed lower inhibition rates, reaching its minimum of (28.47%) on day 6 (Table 1). Regarding extraction methods, Soxhlet extraction proved more effective than maceration, particularly in later stages (days 6-7). While maceration initially showed higher inhibition of (39.05%) on day 2, Soxhlet extraction maintained more consistent inhibition rates throughout the study period, suggesting better extraction of stable antifungal compounds.

A clear concentration-dependent response was observed, with 100% concentration consistently showing the highest inhibition rates of (50.62-63.93%), followed by 75% (44.61-52.42%) and 50% (29.02-37.12%). All concentration levels performed significantly better than the control.Interactive Effects of Plant Extracts and Extraction Methods on the Radial inhibition of *Fusarium proliferatum* of rice.

The interaction between plant extracts and extraction methods significantly influenced the radial inhibition of *Fusarium proliferatum* (Table 2). Lemon grass consistently showed higher inhibition rates compared to bitter leaf across both extraction methods. With maceration, lemon grass achieved peak inhibition (40.06%) on day 2, gradually declining to 30.22% by day 7. In contrast, soxhlet-extracted lemon grass demonstrated increasing effectiveness until day 4 (41.53%) before slightly decreasing to 35.18% by day 7.

Bitter leaf recorded lower inhibition rates, with maceration showing highest activity on day 2 (38.05%) followed by a steady decline to 26.70% by day 7. Soxhlet-extracted bitter leaf maintained more stable inhibition rates, ranging from 34.42% to 32.15% throughout the observation period. These differences were statistically significant ($p<0.05$) across all days. The results suggest that lemon grass extracted, using the soxhlet method was more effective and provides more sustained antifungal activity against *Fusarium proliferatum* compared to bitter leaf. Interactive Effects of Plant Extracts and Concentration on Radial inhibition of *Fusarium proliferatum.*

Table 1: Main effects of Plant Extracts, Extraction Methods and Concentration Levels on the Radial Inhibition of *Fusarium proliferatum* in PDA Culture (%).

Treatments	Days					
	D2	**D3**	**D4**	**D5**	**D6**	**D7**
Plant extract						
Bitter leaf	36.23	34.97	33.43	32.85	28.47	29.43
Lemon grass	39.12	39.78	39.88	37.19	34.58	32.70
$LSD_{0.05}$	Ns	1.981	1.642	1.780	1.772	1.173
Extraction Method						
Maceration	39.05	37.16	35.91	34.21	29.39	28.46
Soxhlet	36.30	37.59	37.40	35.83	33.66	33.67
$LSD_{0.05}$	Ns	Ns	Ns	Ns	1.772	1.173
Concentration level (%)						
0	0.00	0.00	0.00	0.00	0.00	0.00
50	36.02	37.12	35.79	31.99	29.46	29.02
75	50.75	50.26	52.42	50.59	44.59	44.61
100	63.93	62.13	58.40	57.48	52.04	50.62
$LSD_{0.05}$	4.563	2.801	2.322	2.517	2.506	1.659
Interactions						
P.E * E.M	*	*	*	*	*	*
E.M *CL	*	*	*	*	*	*
P.E*CL	*	*	*	*	*	*
P.E*E.M*CL	*	*	*	*	*	*

xx = highly significance @0.01; NS = not significant; x= significant @0.05 PE = plant extract; EM = extraction method; CL = concentration level; D2=day 2; D3=day3; D4= day4; D5=day5; D6=day6; D7=day7

Table 2: The Interaction Effect of Plant Extracts and Extraction Methods on the Radial Inhibition of *Fusarium proliferatum* in PDA Culture. (%)

Plant Extract	Extraction Method	Days					
		D2	**D3**	**D4**	**D5**	**D6**	**D7**
Bitter Leaf	Maceration	38.05	34.72	33.59	32.61	25.78	26.70
	Soxhlet	34.42	35.22	33.26	33.08	31.16	32.15
Lemon grass	Maceration	40.06	39.60	38.22	35.80	32.99	30.22
	Soxhlet	38.18	39.97	41.53	38.57	36.17	35.18
$LSD_{0.05}$		4.56	2.80	2.32	2.52	2.51	1.66

D2 =day 2; D3 =day3; D4 = day4; D5 =day5; D6 =day6; D7 =day7

The interaction between plant extracts and concentrations significantly affected the radial inhibition of *Fusarium proliferatum* (Table 3). At 100% concentration, bitter leaf showed maximum inhibition (67.23%) on day 2, though this decreased to 49.67% by day 7. Lemon grass at 100% exhibited more consistent inhibition, ranging from 60.63% on day 2 to 51.57% on day 7. Both extracts demonstrated concentration-dependent activity, with 75% concentrations maintaining moderate inhibition (bitter leaf: 47.65 - 42.61%; lemon grass: 53.85 - 46.62%) throughout the period. The lowest inhibition was observed at 50% concentration for both extracts, while control (0%) showed no inhibition. All differences were statistically significant ($p<0.05$). These results suggested that while bitter leaf shows higher initial antifungal activity at maximum concentration, lemon grass provides more stable and sustained inhibition over time, indicating it may be more reliable for long-term fungal control.

Comprehensive Interactive Effects of plant extracts, extraction methods and concentrations on radial inhibition of *Fusarium proliferatum.*

Table 3: Interaction effect of plant extract and concentration on Radial Inhibition of *Fusarium proliferatum* in PDA Culture. (%)

Plant extract	Conc.	Days					
		D2	D3	D4	D5	D6	D7
Bitter Leaf	0	0.0	0.0	0.00	0.00	0.00	0.00
	50	30.05	29.00	26.37	26.27	23.12	25.43
	75	47.65	48.64	50.51	48.62	41.30	42.61
	100	67.23	62.24	56.82	56.50	49.47	49.67
Lemon grass	0	0.0	0.0	0.00	0.00	0.00	0.00
	50	41.99	45.24	45.21	37.72	35.81	32.61
	75	53.85	51.88	54.33	52.57	47.88	46.62
	100	60.63	62.01	59.97	58.46	54.61	51.57
$LSD_{0.05}$		6.453	3.961	3.283	3.560	3.545	2.346

D2 = day 2; D3 = day3; D4 = day4; D5 = day5; D6 = day6; D7 = day7

The three-way interaction between plant extracts, extraction methods, and concentrations revealed complex patterns in inhibiting *Fusarium proliferatum* (Table 4). Macerated bitter leaf at 100% concentration showed the highest initial inhibition (69.74% on day 2) but declined substantially to 43.38% by day 7. In contrast, Soxhlet-extracted bitter leaf at 100% maintained more stable inhibition (64.72% to 55.95%). For lemon grass, Soxhlet extraction at 100% demonstrated the most consistent inhibition (62.28% to 55.23%), while maceration showed slightly lower but steady inhibition (58.99% to 47.92%). At 75% concentration, both plants maintained moderate inhibition across both extraction methods (40-55% range). The 50% concentration showed variable effectiveness, with lemon grass generally performing better than bitter leaf. Control treatments (0%) showed no inhibition. All differences were statistically significant ($p<0.05$). These findings suggest that Soxhlet extraction generally provides more stable antifungal activity over time compared to maceration, particularly at higher concentrations. While bitter leaf shows higher initial inhibition, lemon grass maintains more consistent activity across both extraction methods, making it potentially more reliable for sustained fungal control.

Table 4: Interaction effect of plant extracts, extraction methods and concentrations on radial inhibition of *Fusarium proliferatum* on SDA in culture (%)

Plant Extract	Extraction Method	Conc. (%)	Days					
			D2	D3	D4	D5	D6	D7
B. Leaf	Maceration	0	0.00	0.00	0.00	0.00	0.00	0.00
		50	31.21	21.73	22.64	22.70	19.25	22.83
		75	51.24	51.73	53.42	51.15	39.52	40.59
		100	69.74	65.43	58.30	56.60	44.37	43.38
	Soxhlet	0	0.00	0.00	0.00	0.00	0.00	0.00
		50	28.90	36.27	30.11	29.84	26.98	28.03
		75	44.07	45.56	47.59	46.09	43.07	44.63
		100	64.72	59.05	55.35	56.39	54.58	55.95
L.Grass	Maceration	0	0.00	0.00	0.00	0.00	0.00	0.00
		50	45.44	46.05	43.25	34.74	32.83	27.06
		75	55.81	52.80	53.87	52.59	48.06	45.90
		100	58.99	59.54	55.77	55.88	51.06	47.92
	Soxhlet	0	0.00	0.00	0.00	0.00	0.00	0.00
		50	38.55	44.42	47.17	40.70	38.80	38.15
		75	51.90	50.97	54.79	52.54	47.69	47.33
		100	62.28	64.48	64.16	61.05	58.17	55.23
$LSD_{0.05}$			9.126	5.602	4.643	5.034	5.013	3.318

B. Leaf = Bitter Leaf; L. Grass = Lemon Grass; D2 = day 2; D3 = day3; D4 = day4; D5 = day5; D6 = day6; D7 = day7

DISCUSSION

The current study demonstrated significant variations in antifungal activity between lemon grass and bitter leaf extracts against *Fusarium proliferatum.* Lemon grass exhibited superior and more consistent inhibitory effects throughout the observation period, particularly when extracted using the Soxhlet method. This finding aligns with previous research by Iwuagwu *et al*. (2020), Tchinda *et al.,* (2009), who reported strong antifungal properties of *C. citratus* essential oils against various *Fusarium* species, attributing this to its high *citratus* content, a compound known for its antimicrobial properties.

The extraction method significantly influenced the stability and efficacy of the plant extracts' antifungal activity. While maceration initially showed higher inhibition rates, Soxhlet extraction maintained more consistent inhibition throughout the study period, particularly evident in the later stages (days 6-7). This observation supports the findings of Yu*et al.,* (2023), who demonstrated that Soxhlet extraction more effectively isolates stable bioactive compounds from medicinal plants compared to traditional maceration methods. The superior performance of Soxhlet extraction could be attributed to its continuous cycling process, which enables better extraction of heat-stable antifungal compounds.

A clear concentration-dependent response was observed in both plant extracts, with 100% concentration consistently showing the highest inhibition rates. However, an interesting pattern emerged in the interaction between concentration and plant type. Bitter leaf showed higher initial inhibition at 100% concentration but experienced a substantial decline over time, while lemon grass maintained more stable inhibition rates. This differential response pattern suggested distinct mechanisms of action and stability profiles between the two plant extracts. Similar concentration-dependent effects were reported by Iwuagwu *et al* (2022) Muthomi *et al.,* (2017) in their study of plant extracts against fungal pathogens.

The three-way interaction between plant extracts, extraction methods, and concentrations revealed complex patterns that could have practical implications for antifungal applications. Notably, Soxhlet-extracted lemon grass at 100% concentration provided the most consistent long-term inhibition, while macerated bitter leaf showed high initial activity but poor persistence. This finding is particularly relevant for developing sustainable agricultural fungicide alternatives, as highlighted by Iwuagwu *et al* (2018) Iwuagwu *et al* (2024), Al-Samarrai *et al.,* (2012) and Oribhabor and Iyekekpolor, (2023) in their review of botanical fungicides.

The moderate but stable inhibition observed at 75% concentration across both plants and extraction methods suggested a potential optimal concentration for practical applications, balancing efficacy with resource efficiency. Furthermore, the superior performance of lemon grass at lower concentrations (50%) compared to bitter leaf indicated its potential economic advantage in agricultural applications.

The findings suggested that while both plants possess significant antifungal properties, their application strategy should consider the temporal aspects of their efficacy. Lemon grass, particularly when Soxhlet-extracted, emerges as a more reliable option for sustained fungal control, while bitter leaf might be more suitable for situations requiring immediate but shorter-term fungal suppression. These results contribute to the growing body of evidence supporting the use of plant-based alternatives to synthetic fungicides.

CONCLUSION AND RECOMMENDATION

The investigation of *Vernonia amygdalina* and *Cymbopogon citratus* extracts against *Fusarium proliferatum* has revealed significant insights into their potential as natural fungicides, and that both extraction method and plant source substantially influence antifungal efficacy. Lemon grass consistently performed better than bitter leaf in terms of sustained antifungal activity, particularly when extracted using the Soxhlet method. This superior performance can be attributed to the stability of its bioactive compounds and their persistent antifungal properties over time. The Soxhlet extraction method proved more effective than maceration in maintaining consistent inhibition rates, suggesting its superiority in isolating stable antifungal compounds from plant materials.

The concentration-dependent response observed in both extracts provide valuable guidance for practical applications. While 100% concentration showed maximum inhibition, the moderate but stable inhibition achieved at 75% concentration suggested a potentially optimal concentration for field applications, balancing efficacy with resource efficiency. It could therefore be recommended that farmers should adopt the use of biopesticides (*Vernonia amygdalina* (bitter leaf) and *Cymbopogon citratus* (lemon grass)) that were used in this research for control of diseases of rice since they were very effective in *in-vitro* inhibition of the test organism instead of synthetic fungicides.

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Authors contribution

VCN provided the fund, involved in field and Laboratory data collections as well in the write-up of results of the research. CCI conceptualized the research, provided guidance in relation to facts, principles and procedures in conducting the research as well as in editing the write-up. GOI was involved in providing guidance in relation to facts, principles and procedures in conducting the research as well as in editing the write-up.

Ethical Statement

Not applicable.

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