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Original Paper

Optimization of Purple Nutsedge (*Cyperus rotundus* L.) Leachate Against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 Attacking 'Lakatan' Banana

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***Abstract*—Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4), is one of the most destructive diseases affecting banana production worldwide, with 'Lakatan' being highly susceptible. The continuous use of synthetic fungicides for disease management poses environmental and health concerns, prompting the exploration of plant-based alternatives. This study evaluated the efficacy of purple nutsedge (*Cyperus rotundus* L.) leachate derived from different plant parts and concentrations against FocTR4 under in vitro and in vivo conditions. The experiment was arranged in a Completely Randomized Design (CRD). For the in vitro assay, nine treatments were tested with three replications and three culture plates per replicate. Treatments consisted of sterile distilled water as control, purple nutsedge leachates from inflorescence, stem and leaves, and tubers at 25%, 50%, 75%, and 100% concentrations, while fosetyl-Al served as the chemical check. The most effective leachate identified in vitro was further evaluated in vivo using hardened tissue-cultured 'Lakatan' banana meriplants. The in vivo experiment consisted of three treatments replicated three times with five sample plants per replicate. Results showed that the stem and leaves leachate of purple nutsedge exhibited the highest percent growth inhibition against FocTR4 among all treatments in vitro. Moreover, in vivo findings revealed that the efficacy of the stem and leaves leachate in reducing disease incidence and corm discoloration was comparable to the chemical check. The results suggest that purple nutsedge stem and leaves leachate has potential as an eco-friendly alternative for the management of Fusarium wilt in 'Lakatan' banana.**

***Keywords*—antimicrobial, plant-based fungicide, meriplants, weeds**

I. INTRODUCTION

Banana (*Musa* spp.), a monoecious and monocotyledonous plant belonging to the family Musaceae, is considered the largest herbaceous flowering plant in the world [10]. It is one of the most important food crops globally and contributes significantly to the food security and livelihood of millions of people, particularly in tropical and subtropical countries [1]. In the Philippines, banana remains the leading fruit crop in terms of production volume and cultivated area. The country produces several commercially important cultivars, including 'Cavendish' for export, 'Lakatan' as a premium dessert banana, and 'Saba' or 'Cardaba' for processing and staple food consumption [13].

According to the Food and Agriculture Organization, banana is the most traded fruit commodity worldwide, with Ecuador recognized as the leading exporter. In the Philippines, the Davao Region is the top banana-producing area in the country. Among the cultivated varieties, 'Cavendish' accounts for approximately 48% of total production, followed by 'Lakatan' (30%), 'Saba' (11%), and other cultivars (11%). Although 'Cavendish' dominates the export market, 'Lakatan' banana has gained increasing attention because of its superior taste, aroma, and market potential in both local and international trade. Consumer preference is gradually shifting toward 'Lakatan', creating opportunities for expanded exportation and increased production demand [7].

Despite the economic importance of banana, diseases continue to limit productivity and profitability worldwide. Among these diseases, Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4), is regarded as one of the most destructive threats to banana production. The pathogen infects the vascular tissues of the plant, resulting in wilting, yellowing, and eventual plant death. In the Philippines, the occurrence of Fusarium wilt has been reported particularly in Mindanao. Surveys conducted in Region XI revealed the presence of the disease in Davao City and the provinces of Davao del Norte, Davao Oriental, and Compostela Valley affecting cultivars such as 'Lakatan', 'Latundan', 'Abuhon', 'Cavendish', and 'Cardaba' [14]. The spread of the disease poses a serious threat not only to export-oriented cultivars but also to locally consumed varieties, especially 'Lakatan' and 'Latundan' [19].

The management of Fusarium wilt commonly relies on synthetic fungicides and other chemical-based approaches. However, the excessive and continuous use of these chemicals has raised concerns regarding environmental pollution, pathogen resistance, and potential risks to human health. Consequently, there is growing interest in exploring environmentally sustainable and plant-based alternatives for disease management. Among the promising biological

strategies is the utilization of weeds with allelopathic and antifungal properties. Purple nutsedge (*Cyperus rotundus* L.), recognized as one of the world's worst weeds, has attracted scientific interest because of its rich content of bioactive compounds such as essential oils, phenolic acids, flavonoids, and ascorbic acids found in its tubers and rhizomes [16]. Previous studies have demonstrated its antimicrobial and antifungal potential against several plant pathogens, suggesting its possible application as a biological control agent.

In the Philippines, particularly in the Davao Region, studies investigating the potential of weeds as biological control agents against fungal pathogens remain limited. Hence, this study was conducted to optimize the effectiveness of purple nutsedge leachate against FocTR4 causing Fusarium wilt in 'Lakatan' banana. Specifically, the study aimed to determine the most effective concentration of purple nutsedge leachate and identify the plant part with the highest antifungal efficacy against FocTR4 under in vitro and in vivo conditions.

II. Materials and Methods

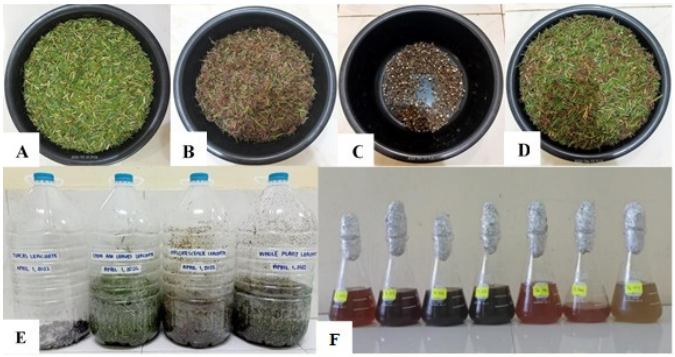
A. Location and Test Plant of the Study

The study was conducted at the University of Southeastern Philippines, Tagum-Mabini Campus, Mabini Unit, Pindasan, Mabini, Davao de Oro. In vitro assessment was done at the Crops Research Laboratory and susceptible 'Lakatan' banana meriplants were used in the experiment from Envirogreen Banana Tissue Culture Laboratory, Davao de Oro.

B. Experimental Treatments and Designs

The in vitro experiment was conducted using a Completely Randomized Design (CRD) consisting of nine treatments replicated three times, with three culture plates serving as sample units per replication, resulting in a total of 81 experimental plates. The treatments included: T1 – control (*Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 [FocTR4] without weed leachate); T2 – FocTR4 + 25% purple nutsedge leachate; T3 – FocTR4 + 50% purple nutsedge leachate; T4 – FocTR4 + 75% purple nutsedge leachate; T5 – FocTR4 + 100% purple nutsedge leachate; T6 – FocTR4 + 100% purple nutsedge inflorescence leachate; T7 – FocTR4 + 100% purple nutsedge tuber leachate; T8 – FocTR4 + 100% purple nutsedge stem and leaves leachate; and T9 – chemical check consisting of FocTR4 treated with fosetyl-Al. The treatments were evaluated to determine the inhibitory effect of purple nutsedge leachates against the growth of FocTR4 under laboratory conditions.

For the in vivo experiment, a Completely Randomized Design (CRD) was likewise employed using hardened tissue-cultured 'Lakatan' banana meriplants. The most effective treatment identified from the in vitro assay was further evaluated under greenhouse conditions. The experiment consisted of three treatments replicated three times, with five sample plants per replication, for a total of 45 sample plants. The treatments were as follows: T1 – control (FocTR4 without weed leachate); T2 – FocTR4 + 100% purple nutsedge stem and leaves leachate; and T3 – chemical check consisting of FocTR4 treated with fosetyl-Al. The experiment aimed to assess the efficacy of the selected purple nutsedge leachate in reducing Fusarium wilt incidence and infection in 'Lakatan' banana meriplants.



C. Preparation of Weed Leachate

Whole plants of purple nutsedge (*Cyperus rotundus* L.), including the tubers up to the inflorescence, were collected and thoroughly washed with tap water to remove adhering soil and other surface contaminants. The plant materials were air-dried and subsequently separated according to the plant parts used in the study, namely: whole plant, inflorescence, stem and leaves, and tubers (Fig. 1).

Each plant part was then chopped into approximately 3-mm sections, weighed, and exposed to ultraviolet (UV) light inside the Crops Research Laboratory for two hours to minimize microbial contaminants present on the plant tissues. After UV exposure, the prepared plant materials were transferred into sterile containers and soaked in sterilized distilled water (SDW) at a ratio of 1:2 (w/v), wherein one part plant material was combined with two parts SDW. The containers were tightly sealed and maintained at room temperature for 96 hours to allow leachate extraction.

After the soaking period, the leachates from the different plant parts were collected by filtration using sterile filter paper and transferred into sterile containers for storage at 6°C until use. Prior to application, the stored leachates were allowed to equilibrate to room temperature.

Fig. 1. Preparation of purple nutsedge leachates: (A) stem and leaves; (B) inflorescence; (C) tubers; (D) whole plant; (E) different plant parts soaked in sterile distilled water (SDW) for 96 hours; and (F) harvested leachates.

D. Preparation of Culture Medium and Test Pathogen

Potato Dextrose Agar (PDA), one of the most commonly used media for fungal culture and growth, was utilized as the culture medium for the in vitro assay. The medium was prepared following standard laboratory procedures using 250 g potato tubers, 20 g dextrose, 20 g agar, and 1,000 mL distilled water. The potato tubers were boiled, and the extract was collected and combined with the remaining ingredients prior to sterilization. The prepared culture medium was then sterilized in a pressure cooker at 15 psi for 30 minutes to ensure aseptic conditions before use.

The pure culture of the test pathogen used in the study was obtained from the TADECO Plant Pathology Laboratory in Panabo City, where it was previously confirmed as

Fusarium oxysporum f. sp. *cubense* Tropical Race 4 (FocTR4). The pathogen culture was subsequently mass-produced under laboratory conditions for use in both the in vitro and in vivo experiments.

E. Preparation of Meriplants and Pathogen Inoculum

Tissue-cultured 'Lakatan' banana meriplants were obtained from the Envirogreen, and were subsequently transported to the Crops Research Laboratory. The meriplants used in the study were at the hardening stage and were carefully transferred aseptically into sterile glass bottles containing 20 mL sterile distilled water. The plants were then maintained inside a hardening chamber under controlled conditions prior to inoculation and treatment application.

For the preparation of the pathogen inoculum, a one-month-old pure culture of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) grown on Potato Dextrose Agar (PDA) plates was used. The fungal culture was flooded with sterile distilled water, and the mycelial mat was gently scraped using a sterile spatula to detach the fungal structures from the agar surface. The resulting fungal suspension was transferred into a sterile beaker and carefully stirred to fragment the mycelial masses into smaller propagules suitable for uniform inoculation of the meriplants.

F. In vitro Test Evaluation

The efficacy of purple nutsedge leachate against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) was evaluated using the poison food technique. In this method, the test pathogen was cultivated on Potato Dextrose Agar (PDA) medium incorporated with different concentrations and plant-part-derived leachates of purple nutsedge. The prepared leachates were aseptically mixed with molten PDA at approximately 50°C prior to pouring into sterile petri plates at 20 mL per plate. The medium was then allowed to solidify. For the control treatment, sterilized distilled water was used in place of the weed leachate following the same procedure.

After solidification of the culture medium, a 5-mm mycelial disc taken from a seven-day-old pure culture of FocTR4 was aseptically placed at the center of each petri plate. The inoculated plates were incubated at room temperature, and observations were recorded on the 7th day of incubation. Data gathered included colony diameter, percent growth inhibition, and spore count.

The diameter of the fungal colony was determined by obtaining the average of two perpendicular measurements representing the longest and shortest colony diameter (CD) using a ruler and expressed in millimeters (mm). Radial fungal growth was computed using the formula:

$$CD = \frac{d1 + d2 - 2d0}{2} \quad (1)$$

Where d0 represents the diameter of the original mycelial disc, while d1 and d2 refer to the measured diameters of fungal growth on the petri plate. Percent growth inhibition was evaluated after 72 hours of incubation and every other day thereafter until the control plates were fully colonized.

The inhibitory effect of the treatments on fungal growth was calculated using the formula [15]:

$$GI\ (\%) = \frac{CD - TD}{CD} x\ 100 \quad (2)$$

Where *GI* is the percent growth inhibition, *CD* is the average diameter of fungal growth in the control plates, and *TD* is the average diameter of fungal growth in the treated plates. Spore density was likewise determined using a hemocytometer to assess the effect of the different leachate treatments on fungal sporulation.

G. In vivo Test Evaluation

After one week of hardening, each tissue-cultured 'Lakatan' banana meriplant was treated with 15 mL of the assigned purple nutsedge leachate, while the control plants received sterile distilled water only. Twenty-four hours after treatment application, inoculation of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) was carried out by drenching 10 mL of fungal suspension into each glass bottle containing the meriplants. The inoculated plants were maintained under controlled conditions and monitored daily for two weeks for the development of Fusarium wilt symptoms.

Data gathered included the number of days to first symptom appearance, percent disease incidence, and severity of infection. The number of days to symptom appearance was determined by counting the days from inoculation until visible disease symptoms appeared in 50% of the inoculated plants. Daily observations were conducted throughout the two-week monitoring period. Disease incidence was assessed by counting the number of wilted plants per treatment and was expressed as percentage using the formula:

$$DI\ (\%) = \frac{Number\ of\ plants\ showing\ incidence}{Number\ of\ plants\ inoculated} x\ 100 \quad (3)$$

Disease severity was assessed at the termination of the experiment through external examination and cross-sectional evaluation of corm tissues. The severity of infection was rated using the internal symptom evaluation scale [17], where: 1 = no symptoms; 2 = initial rhizome discoloration; 3 = slight rhizome discoloration; 4 = slight rhizome discoloration throughout the vascular system; and 5 = totally necrotic rhizome. The scale was used to evaluate the extent of vascular discoloration and internal tissue damage associated with FocTR4 infection (Fig. 2). To facilitate quantitative comparison among treatments, severity ratings were converted into a Disease Severity Index (DSI) expressed as a percentage using the following formula:

$$DSI\ (\%) = \frac{\sum (n \times v)}{N\ x\ V} x\ 100 \quad (4)$$

where *n* is the number of plants assigned to each severity rating, *v* is the corresponding severity score, *N* is the total



number of plants assessed, and V is the highest severity rating on the scale (5). Higher DSI values indicate greater disease severity and tissue damage caused by FocTR4 infection.

Fig. 2. Degree of severity of internal symptoms progressed from left to right.

H. Statistical Analysis

Data collected were homogenized and checked for normality using the Shapiro-Wilk test through the Statistical Tool for Agricultural Research (STAR) version 1.0. The data were analyzed using Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD). When significant differences among treatment means were detected at the 5% significance level ($p \leq 0.05$), comparisons were further examined using Tukey's Honest Significant Difference (HSD) test.

III. RESULTS

A. Colony Diameter and Growth Inhibition

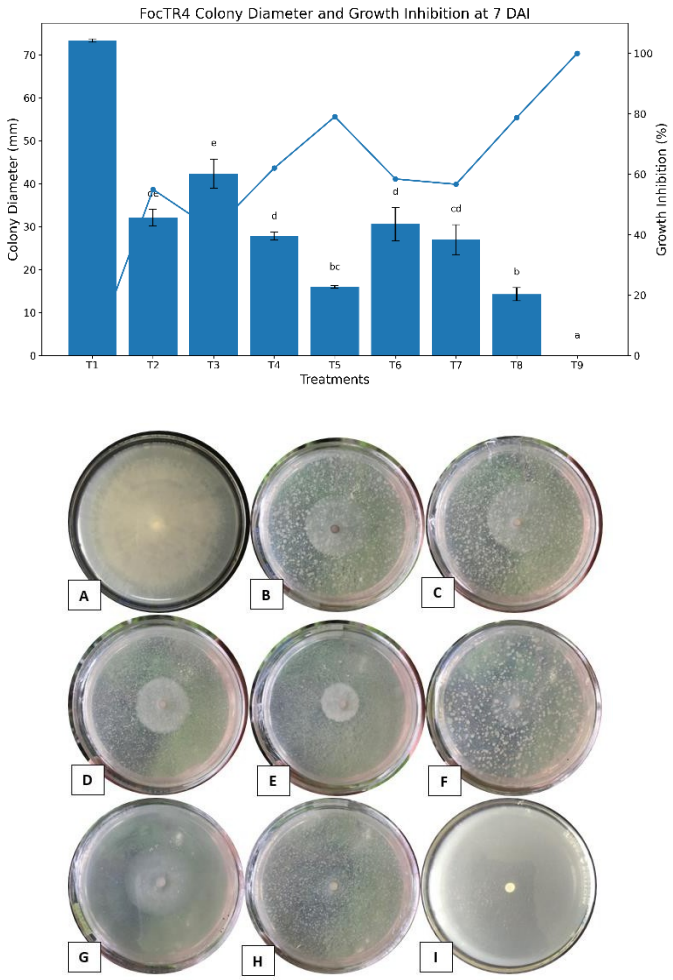
The mean colony diameter of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) demonstrated that all purple nutsedge leachate treatments reduced fungal growth compared with the untreated control across three, five, and seven days of incubation. The untreated control consistently recorded the highest colony diameter, indicating unrestricted fungal growth in the absence of any antifungal treatment.

Among the different treatments, the chemical check (fosetyl-Al) completely inhibited fungal growth throughout the observation period, resulting in zero colony diameter at day 7. Among the purple nutsedge treatments, the 100% stem and leaves leachate (T8) and the 100% whole plant leachate (T5) showed the strongest suppression of fungal growth. These treatments consistently produced the smallest colony diameters across all incubation periods, indicating high antifungal activity against FocTR4.

The 75% whole plant leachate (T4) and 100% tuber leachate (T7) also reduced fungal growth substantially, although their effects were less pronounced than those of T5 and T8. In contrast, lower concentrations of whole plant leachate, particularly 25% (T2) and 50% (T3), exhibited comparatively larger colony diameters, suggesting that antifungal efficacy increased with increasing leachate concentration. The 100% inflorescence leachate (T6) showed moderate inhibition but was less effective than the stem and leaves and tuber treatments.

Fig. 3. Mean colony diameter and growth inhibition under different purple nutsedge leachate treatments at 7 days of incubation.

The percent growth inhibition further confirmed the antifungal potential of purple nutsedge leachates against FocTR4. The chemical check consistently exhibited 100%



growth inhibition throughout the incubation period, serving as the highest standard of fungal suppression. Among the botanical treatments, the 100% stem and leaves leachate (T8) and the 100% whole plant leachate (T5) achieved the highest inhibitory activity, with inhibition values approaching those of the chemical fungicide by the seventh day of incubation.

The inhibitory effect of the treatments generally increased over time, indicating that prolonged exposure to the leachates enhanced suppression of fungal growth. The 100% stem and leaves leachate maintained high inhibition values from day 3 onward, demonstrating strong and stable antifungal activity. Similarly, the 100% whole plant leachate showed substantial inhibition throughout the incubation period. These findings suggest that the antifungal compounds in purple nutsedge may have cumulative or sustained effects on fungal development.

Moderate inhibition was observed in the 75% whole plant leachate (T4), 100% tuber leachate (T7), and 100% inflorescence leachate (T6), while the lower concentration treatments, particularly 25% and 50% whole plant leachates, recorded the lowest inhibition percentages among the treated groups. This trend indicates a concentration-dependent response, wherein increasing leachate concentration enhanced antifungal efficacy.

Treatments	Mean
T1- Untreated Control	2.5125×10^6
T2- 25% whole plant	0.00
T3- 50% whole plant	3.37×10^5
T4- 75% whole plant	0.00
T5- 100% whole plant	5×10^4
T6- 100% inflorescence	0.00
T7- 100% tubers	2.5×10^5
T8- 100% stem and leaves	2.5×10^4
T9- Fosetyl-Al at 0.1g/50ml of water	0.00

Fig. 4. Mycelial growth of FocTR4 on PDA after seven days of incubation as affected by different concentration and different plant parts of purple nutsedge weed leachate: (A) Control (no leachate); (B) 25% whole plant purple nutsedge leachate; (C) 50% whole plant purple nutsedge leachate; (D) 75% whole plant purple nutsedge leachate; (E) 100% whole plant leachate; (F) 100% inflorescence leachate; (G) 100% tubers leachate; (H) 100% stem and leaves leachate; and (I) Fosetyl-Al at recommended rate.

B. Spore Count

The results presented in Table I revealed that purple nutsedge leachates significantly reduced the spore production of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) compared with the untreated control. The control treatment (T1) recorded the highest mean spore count at 2.5125×10^6, indicating prolific sporulation of the pathogen in the absence of inhibitory substances. This finding confirms the aggressive reproductive capacity of FocTR4 under favorable conditions.

Among the treatments evaluated, complete suppression of spore production was observed in T2 (25% whole plant leachate), T4 (75% whole plant leachate), T6 (100% inflorescence leachate), and T9 (chemical check using fosetyl-Al), all of which recorded zero spore count. The absence of spores suggests that these treatments were highly effective in inhibiting fungal sporulation, which is an important factor in reducing pathogen spread and disease development. The result obtained from the chemical check validates the effectiveness of fosetyl-Al as a standard fungicide against FocTR4. Interestingly, some purple nutsedge leachate treatments exhibited comparable suppressive effects, indicating their potential as natural antifungal agents.

The 100% stem and leaves leachate (T8) recorded one of the lowest spore counts among the botanical treatments at 2.5×10^4, followed by the 100% whole plant leachate (T5) with 5×10^4. These findings indicate that higher concentrations of purple nutsedge leachate, particularly from the stem and leaves, substantially reduced fungal sporulation. Similarly, the 100% tuber leachate (T7) and 50% whole plant leachate (T3) also suppressed spore production compared with the control, although their effects were less pronounced.

TABLE I. Mean Spore Count Of Foctr4 Under Different Purple Nutsedge Leachate Treatments

C. Incubation Period, Disease Incidence, and Disease Severity Index

The results presented in the Table II showed that the application of 100% stem and leaves purple nutsedge leachate influenced the incubation period, disease incidence, and severity of infection of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) in tissue-cultured 'Lakatan' banana meriplants (Fig. 5). Significant differences among treatments were observed at the 1% level based on Tukey's HSD test.

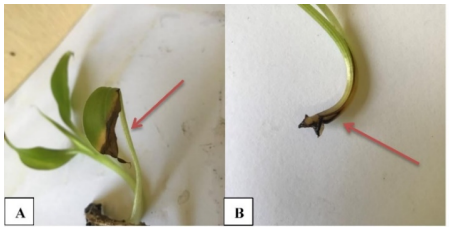


Fig. 5. (A) Sample plant showing yellowing symptom of FocTR4 and (B) pseudostem splitting.

In terms of incubation period, the fosetyl-Al treatment recorded the longest incubation period (9.00 days), indicating delayed symptom development of Fusarium wilt. In contrast, symptom appearance was observed after 7.40 days in the untreated control and 6.60 days in plants treated with the 100% stem and leaves leachate. Based on Tukey's HSD test, the fosetyl-Al treatment had a significantly longer incubation period than the stem and leaves leachate treatment, while the untreated control was statistically comparable to both treatments. These results suggest that fosetyl-Al tended to delay the onset of disease symptoms, whereas the stem and leaves leachate appeared to exert its protective effect primarily through reducing disease incidence and severity rather than extending the incubation period.

For disease incidence, the untreated control recorded the highest incidence at 100%, indicating that all inoculated plants developed symptoms of Fusarium wilt. In contrast, treatment with 100% stem and leaves leachate reduced disease incidence to 66.67%, which was lower than the chemical check at 86.60%. The reduced disease incidence observed in the leachate-treated plants indicates that purple nutsedge stem and leaves possess antifungal compounds capable of suppressing pathogen infection and reducing disease establishment in 'Lakatan' banana meriplants.

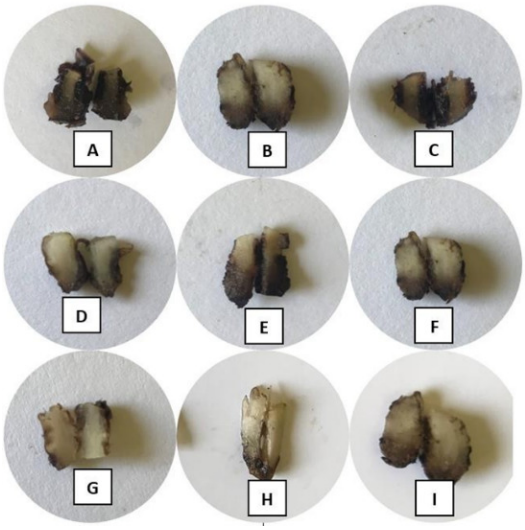
Similarly, severity of infection was highest in the untreated control with a mean value of 57.33, indicating extensive rhizome discoloration and tissue damage caused by FocTR4 infection. Application of the 100% stem and leaves leachate reduced disease severity to 34.66, which was lower than the severity observed in plants treated with fosetyl-Al (37.33). These findings suggest that the purple nutsedge stem and leaves leachate was effective in minimizing internal disease symptoms and restricting pathogen development within the plant tissues (Fig. 6).

TABLE II. INCUBATION PERIOD, DISEASE INCIDENCE, AND DISEASE SEVERITY INDEX OF Foctr4

Fig. 6. Corm discoloration on 'Lakatan' meriplants: (A-C) Untreated control; (D-F) 100% stem and leaves purple nutsedge leachate; (G-I) and Fosetyl-Al.

Treatment	Incubation Period (days)**	Disease Incidence (%)**	Severity of Infection (%)**
T1- Untreated: Inoculated Control	7.40[ab]	100.00[b]	57.33[b]
T2- 100% stem and leaves leachate	6.60[b]	66.67[a]	34.66[a]
T3- Fosetyl-Al at 0.1g/50ml of water	9.00[a]	86.60[a]	37.33[a]
CV%	**11.27**	**11.79**	**19.31**

**Significant; Means in column having the same letter superscripts are not significantly different at 1% level, Tukey's HSD.



IV. Discussion

In the present study, in vitro results revealed that all purple nutsedge leachate treatments suppressed the mycelial growth of FocTR4 compared with the untreated control. The greatest reduction in colony diameter and highest percent growth inhibition were observed in the 100% stem and leaves leachate and the 100% whole plant leachate treatments. These findings indicate that the antifungal efficacy of purple nutsedge increased with increasing leachate concentration. Although the present study did not include phytochemical characterization of the purple nutsedge leachates, previous studies have reported that *Cyperus rotundus* contains various secondary metabolites, including phenolic compounds, flavonoids, terpenoids, and essential oils with antimicrobial properties [11, 16]. Therefore, it is plausible that one or more of these compounds contributed to the antifungal activity observed in the study. However, this remains a hypothesis and requires confirmation through future phytochemical analyses and bioactivity-guided studies.

The colony diameter and growth inhibition exhibited a concentration-dependent response, the sporulation data did not consistently follow the same trend. No spores were detected in the 25% and 75% whole plant leachate treatments, whereas a small number of spores were recorded in the 100% whole plant leachate treatment. This inconsistency may be attributed to biological variability in fungal sporulation, the heterogeneous distribution of spores within fungal cultures, or limitations associated with hemocytometer-based spore quantification at very low spore densities [3, 12]. Furthermore, sporulation and mycelial growth are regulated by different physiological and genetic mechanisms; therefore, treatments that strongly suppress vegetative growth may not necessarily produce a proportional reduction in spore production [5, 12]. Similar discrepancies between mycelial growth inhibition and sporulation inhibition have been reported in studies evaluating antifungal compounds against phytopathogenic fungi, suggesting that fungal reproduction may respond differently to inhibitory substances than radial colony growth.

Nevertheless, the in vivo assay further confirmed the antifungal potential of purple nutsedge stem and leaves leachate against Fusarium wilt in tissue-cultured 'Lakatan' banana meriplants. Application of the leachate significantly reduced disease incidence and severity of infection compared with the untreated control, indicating its ability to suppress pathogen establishment and disease progression within plant tissues. The reduced corm discoloration observed in treated plants further suggests that the leachate may have restricted vascular colonization by FocTR4, utilizing plant-derived extracts rich in secondary metabolites with antifungal properties [4, 5]. Similar observations have been reported in plant-pathogen interactions where treatments do not necessarily prevent initial infection but can suppress pathogen growth, reduce vascular colonization, or limit tissue damage after infection has occurred [2, 18].

Interestingly, the 100% stem and leaves leachate resulted in lower disease incidence and severity than the fosetyl-Al treatment. While this finding suggests promising antifungal activity of the leachate under the conditions of the study, it should be interpreted with caution. Fosetyl-Al is a systemic fungicide whose efficacy is influenced by several factors, including application rate, timing of application, host growth stage, and pathogen pressure [6, 8]. Moreover, phosphonate-based fungicides such as fosetyl-Al often require sufficient time for systemic translocation and induction of host defense responses before maximum protection is achieved [9]. Therefore, the relatively short interval between treatment application and pathogen inoculation, together with the use of tissue-cultured meriplants under controlled laboratory conditions, may not have fully optimized the performance of the fungicide. Further studies under greenhouse and field conditions, using different application schedules, inoculum levels, and fungicide rates, are necessary to validate these findings and establish a more robust comparison between purple nutsedge leachate and conventional fungicides.

V. Conclusion and Recommendation

The study demonstrated that purple nutsedge (*Cyperus rotundus* L.) leachate possesses antifungal activity against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4), the causal agent of Fusarium wilt in 'Lakatan' banana. Among the treatments evaluated, the 100% stem and leaves leachate consistently exhibited the greatest antifungal activity under in vitro conditions, as evidenced by the lowest colony diameter, highest percent growth inhibition, and reduced spore production. Likewise, under hardening-chamber conditions, the same treatment resulted in lower disease incidence and severity of infection, as indicated by reduced corm discoloration in tissue-cultured 'Lakatan' banana meriplants. These findings suggest that purple nutsedge stem and leaves leachate has

promising potential for suppressing FocTR4 and warrants further investigation as a plant-based approach for Fusarium wilt management.

However, the results should be interpreted within the limitations of the study. Only the most effective treatment identified during the in vitro screening was evaluated in the in vivo assay, preventing the assessment of a dose-response relationship under plant conditions and limiting conclusions regarding the effectiveness of lower concentrations. In addition, the leachates were not subjected to physicochemical or phytochemical characterization; therefore, the specific compounds responsible for the observed antifungal activity remain unknown. Since the study was conducted under laboratory and hardening-chamber conditions, further research is recommended to evaluate different leachate concentrations under greenhouse and field conditions, determine optimum application rates, identify the bioactive compounds involved, and elucidate their mode of action, including possible endophytic effects. Such studies will help validate the practical applicability of purple nutsedge leachate for the sustainable management of Fusarium wilt in banana production systems.

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