Invitro and *Invivo* screening of Different Botanicals against *Fusarium semitectum* Causing Bamboo Blight Disease

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A B S T R A C T

Bamboos are one of the fastest-growing plants globally and play a significant role in the livelihoods of local communities by serving as raw materials in several industries, including pulp and paper, construction, agriculture, horticulture, basketry, transportation and fishing. Globally, there are approximately 1,575 bamboo species distributed across over 116 genera. However, bamboo productivity is threatened by multiple biotic and abiotic factors, with diseases, especially bamboo blight caused by *Fusarium semitectum*, posing a significant but understudied challenge in Northeast India. This study evaluates the *in vitro* and *in vivo* antifungal efficacy of aqueous leaf extracts from *Mimosa diplotricha*, *Senna alata* and *Wedelia trilobata* against *F. semitectum*. The *in vitro* antifungal activity was assessed using the poison food technique at concentrations of 20%, 25%, 30%, 35% and 40% with three replications. The *in vivo* experiment followed screenhouse pot trials with a completely randomized design and data from both assays were statistically analyzed using ANOVA. Among the treatments, *S. alata* extract at 40% concentration resulted in the highest mycelial inhibition (71.94%) at 10 days after inoculation (DAI) under *in vitro* condition and the lowest Per cent disease incidence (50.00%) after the second spray under *in vivo* condition. Whereas, under *in vitro* condition, *M. diplotricha* and *W. trilobata* showed up to 71.66% and 59.04% mycelial inhibition at 10 DAI, respectively. These findings suggest that *S. alata* is the most effective plant extract against bamboo blight, followed by *M. diplotricha* with *W. trilobata* being the least effective. These findings suggest that potential of plant-based extracts, especially *S. alata* offers promising, environmentally safe alternatives for managing *F. semitectum*, a key pathogen responsible for bamboo blight. Further large-scale field evaluations are recommended to optimize application protocols and integrate these botanicals into sustainable disease management strategies for bamboo cultivation.

Introduction

Bamboo is a group of woody stemmed grass species belonging to Poaceae family and Bambusoideae subfamily. It is most abundantly found in the tropical and subtropical regions between 46°N and 47°S. Bamboos are one of the world's fastest growing plant plays an important role in the economy of the local people

(Emamverdian *et al.,* 2020). Bamboo serves as a raw material in various industries, including pulp and paper, construction, agriculture, horticulture, basketry, transportation, and fishing. It is also used in handicrafts and as an edible shoot, making it a significant non-timber forest product (NTFP) (Rao *et al.,* 1987). There are about 1575 species of bamboos belonging to over 116 genera worldwide, covering over 31 million hectares of forestland. More than 60% of this area is located in China, Brazil and India. Bamboo accounts for over 0.8% of the world's forest area, with the highest abundance in Asia, Africa and Latin America (Sawarkar *et al.,* 2023, FAO, 2010). With an exceptionally fast growth rate of approximately two inches per hour, bamboo can reach a height of 60 feet in just three months (Abdul Khalil *et al.,* 2012). In India, the area occupied by bamboo is found to be around 15.69 million hectares with about 29 genera and 148 species. The most common and economically important bamboo species include *Bambusa bambos, B. nutans, B. tulda, B. vulgaris, Dendrocalamus hamiltonii, D. strictus, Ochlandra travancorica* and *Melocanna baccifera* (Tewari *et al.,* 2019, Sharma and Nirmala, 2015). More than 50% of the recorded bamboo species are found in North eastern India.

The northeastern region alone contains 15 genera and 90 species, covering 5,348.50 thousand hectares, which accounts for approximately 35.79% of the total bamboo-growing area in the country. This region alone contributes around 24,330 thousand culms, accounting for nearly 46% of India's total bamboo culm production (Gogoi *et al.,* 2021).

However, the production potential of bamboo is significantly influenced by various biotic and abiotic factors, such as erratic rainfall, fires, grazing, unscientific harvesting practices and pests and diseases (Remadevi *et al.,* 2017). Among these factors, diseases are one of the most important production potentials limiting factor. The warm and wet climatic conditions of the northeast India are very conducive for development of diseases. Among the other diseases, bamboo blight is the major disease of the bamboo plantations (Bhamra and Borah, 2021).

Very little research has been conducted on bamboo diseases in Northeast India. The management of major bamboo diseases in nurseries and plantations primarily involves cultural and chemical control measures. Although effective to some extent, cultural practices such as crop rotation and sanitation are often labour-intensive, costly and insufficient to eliminate existing infections or manage severe outbreaks. These methods are generally less effective than conventional fungicides. Chemical control strategies, while commonly used, must be applied with caution due to their multiple negative impacts. These include harm to beneficial microorganisms, disruption of plant microbiota, risks to human and animal health, contamination of aquatic environments, increased environmental toxicity and the promotion of pathogen resistance. Given these limitations, conventional approaches must be complemented with cost-effective, eco-friendly alternatives, such as the use of plant-based (botanical) treatments, to achieve sustainable levels of disease control. However, research on botanical disease management methods for bamboo remains limited. Thus, the development of an integrated disease management approach incorporating botanical alternatives is crucial for achieving sustainable and environmentally friendly bamboo cultivation (McLaughlin *et al.,* 2023).

This study, therefore, investigates the antifungal effects of three botanical extracts namely, *Mimosa diplotricha*, *Senna alata* and *Wedelia trilobata* plant extracts at different concentrations against pathogenic *Fusarium* species affecting bamboo, under both *in vitro* and *in vivo* conditions.

Materials and Methods

Source of plant materials

M. diplotricha, *S. alata* and *W. trilobata* were selected for the study based on their antifungal properties and availability in the area. Matured leaves were collected from various location within the Rain Forest Research Institute, Sotai, Assam (Lat 26.782412°N and Long 94.294161°E) and utilized for preparation of plant extracts.

Fungal Strains

Pure culture of *Fusarium semitectum* was acquired from the Division of Forest Protection, ICFRE-Rain Forest Research Instirue (Figure). The pure culture was maintained on PDA plates throughout the experimental period. The stock culture of the fungus was kept in refrigerator at 4°C for future use.

Preparation of the aqueous plant extracts

Freshly collected mature leaves were thoroughly washed multiple times with double-distilled water to remove dust and dirt. The leaves were then roughly chopped into

small pieces measuring about 1–5 cm². A total of 100 g of these chopped leaves was crushed in 100 ml of distilled water using a sterilized mortar and pestle.

The extract was strained through a sieve and further filtered using Whatman No. 1 filter paper to remove any plant debris. The resulting filtrate was used as the stock solution. From the stock solution, plant extracts were prepared at concentrations of 20%, 25%, 30%, 35% and 40%.

In vitro Screening of plant extracts

The screening of aqueous plant extracts from *M. diplotricha, S. alata* and *W. trilobata* was conducted following the method described by Jagani *et al.,* (2023) between September 2024 and January 2025 against *Fusarium semitectum*.

To prepare the treatments, 1 ml of each plant extract at concentrations of 20%, 25%, 30%, 35%, and 40% was added to 15 ml of sterile, molten Potato Dextrose Agar (PDA) in sterile Petri dishes. A 5 mm diameter mycelial disc, cut from a seven-day-old culture of *F. semitectum*, was then placed at the center of each Petri dish. Plates without plant extract served as controls. Each treatment was maintained in triplicate. All plates were incubated at $27 \pm 2°C$ and radial mycelial growth was measured daily from 6 to 10 days of incubation and the results were compared with the control.

Percentage inhibition of plant extracts was calculated using the formula proposed by Vincent (1947)-

$$L = \left(\frac{C - T}{C}\right) \times 100$$

Where,
L=Inhibition rate.
C = Radial growth in control plate.
T= Radial growth in treatment plate.

In vivo efficacy of different botanicals against *Fusarium semitectum*

Based on in vitro evaluation, the plant extracts were selected for further efficacy testing under greenhouse conditions using foliar spray application for the management of *Fusarium semitectum*. For comparison, one treatment included a commercial *Trichoderma* formulation (AAU-Bioveer, containing 2×10^7 viable spores of *Trichoderma viride*/ml), along with an untreated control. For the pot experiment, topsoil collected from the farm area of RFRI, Jorhat, was sterilized using an autoclave. In a completely randomized pot experiment, each pot was filled with sterilized soil and planted with a single *Bambusa tulda* seedling. Before planting, the seedlings were inoculated with 20 ml of *Fusarium semitechtum* spores (3×10^5 spores/ml) for 24 hours. Following this, 20 ml of plant extracts at a concentration of 40% was applied. No treatment was applied in the control pots. Standard management practices, including thinning, watering and weeding were carried out as necessary (Akanmu *et al.,* 2013). Disease incidence was recorded 15 days after the 1st spray, followed by a second spray of the respective treatments. A final assessment of disease incidence was conducted 15 days after the 2nd spray.

Disease incidence

$$\text{Per cent Disease Incidence} = \frac{\text{Number of plant showing disease symptoms}}{\text{Total number of sample used}} \times 100 \%$$

Statistical analysis

The data so generated were subjected to analysis of variance (ANOVA) at values of P=0.05% following Completely Randomized Design (CRD) after suitable angular transformation. Whenever variance ratio was found significant, critical difference (CD) was calculated at 5% level of significance, otherwise only Standard Error of Difference (S. Ed ±) was mentioned.

Results and Discussion

Invitro Evaluation of Plant Extracts against *Fusarium semitechtum* Using the Poison Food Method

Extracts from three different plants (*Senna alata*, *Mimosa diplotricha*, and *Wedelia trilobata*) were tested for antifungal activity at concentrations of 20%, 25%, 30%, 35% and 40%. The mycelial growth inhibition of *F. semitectum* was measured and per cent inhibition was calculated for each extract.

Effect of *Senna alata* leaf extract

The data on mycelial growth inhibition of *Fusarium semitectum* at various concentrations of *S. alata* aqueous extract are presented in Table 1. Different concentrations

significantly affected the radial mycelial growth at 6, 7, 8, 9 and 10 days after inoculation (DAI). The highest inhibition was recorded at 40% concentration, with 83.15% at 6 DAI and 71.94% at 10 DAI. In contrast, the lowest inhibition was observed at 20% concentration, with 36.20% at 6 DAI and 29.37% at 10 DAI. At 35% concentration, inhibition reached 80.82% at 6 DAI and 69.87% at 10 DAI. The control plates showed the highest fungal growth with no inhibition (Figure 1).

Effect of *Mimosa diplotricha* Extract

The antifungal effects of *M. pudica* aqueous extract on the radial growth of *Fusarium semitectum* are shown in Table 2. The 40% extract showed the highest inhibition, with 79.47% at 6 DAI and 71.66% at 10 DAI. The 35% extract followed with 64.42% inhibition at 6 DAI and 54.72% at 10 DAI. The lowest inhibition was recorded at 20% concentration, with 18.23% at 6 DAI and 14.33% at 10 DAI. No radial mycelial growth inhibition was observed at the control plate.

Effect of *Wedelia trilobata* Extract

W. trilobata extract showed the least antifungal activity among the three extracts tested (Table 3). At 40% concentration, inhibition was 65.95% at 6 DAI and 59.04% at 10 DAI. The lowest inhibition was observed at 20% concentration, with only 7.93% at 6 DAI and 4.44% at 10 DAI.

Invivo evaluation of aqueous plant extracts against *Fusarium*

The in vivo efficacy of the aqueous plant extracts was evaluated based on per cent disease incidence (PDI) in bamboo seedlings following two treatment sprays. The results, summarized in Table 4, showed that the untreated control group exhibited 100% disease incidence after both the first and second spray, indicating complete susceptibility to *Fusarium semitectum* infection. Among the plant extracts tested, *Senna alata* showed the highest level of disease suppression. Seedlings treated with *S. alata* extract showed a PDI of only 25% after the first spray and 50% after the second, indicating strong antifungal effects. Treatment with *Mimosa diplotricha* extract resulted in moderate disease control, with a PDI of 50% following the first spray and 75% after the second. Whereas, *Wedelia trilobata* extract showed the least effectiveness, with a consistent PDI of 75% after both sprays. The performance of the commercial biocontrol agent, *Trichoderma viride*, was comparable to

that of *M. pudica*, resulting in 50% PDI after the first spray and 75% after the second. Overall, these results indicate that the aqueous extract of *S. alata* provided the most effective protection against *F. semitectum in vivo*, followed by *M. diplotricha* and *W. trilobata*.

The increasing concerns over environmental safety and resistance development associated with synthetic fungicides have prompted researchers to explore eco-friendly alternatives for disease management in plants. Plant-derived extracts are increasingly recognized for their potential as safe, ecofriendly, effective, selective and cost-effective alternative in the control of plant diseases (Deresa and Diriba, 2023). Many conventional chemical approaches have been tried to control bamboo diseases and to reduce economic losses, but not many eco-friendly measures have been investigated. In the present study, the antifungal activitieas of plant extracts of *M. diplotricha, S. alata* and *W. trilobata* were evaluated under *in vitro* and *in vivo* conditions against *Fusarium semitectum*, a causal agent of bamboo blight. Among the tested plant extracts, *S. alata* exhibited the highest antifungal activity against *F. semitectum*, followed by *M. diplotricha* and *W. trilobata*. These findings are correlate with earlier reports that highlight the antifungal efficacy of *S. alata* against various *Fusarium* species.

Akanmu *et al.,* (2013, 2014) reported significant antifungal activity of *S. alata* against *F. anthophilum*, *F. scirpi*, *F. verticillioides* and *F. oxysporum* in millet, both *in vitro* and *in vivo*. Similarly, Rathnamalala *et al.,* (2025) studied the antifungal activity of three aqueous plant extracts of *Mikania micrantha*, *Senna alata* and *Datura metel* at various concentrations of 25%, 50%, 75% and 100% against *F. oxysporum*, the causal agent responsible for Panama wilt in banana.

In the present study, *M. diplotricha* followed *S. alata* in antifungal activity, with a 79.47% mycelial inhibition of *F. semitectum* after six days, aligning with results by Neela *et al.,* (2014), who reported that ethanol and acetone extracts of *Mimosa diplotricha* at a 25% concentration were effective in inhibiting the radial mycelial growth of *Fusarium oxysporum*, the pathogen responsible for tomato wilt, achieving 100% inhibition at 12 days after inoculation (DAI) under in vitro conditions. Durgadevi and Karthika (2018) also studied antifungal activity of *Mimosa* leaf extract, reporting a 15 mm zone of inhibition against *Fusarium* sp. at 100 mg concentration.

Table.1 Screening of *Senna alata* plant extract against *Fusarium semitechtum*

Treatment	Mycelial growth inhibition (%)				
	6 DAI	**7 DAI**	**8 DAI**	**9 DAI**	**10 DAI**
T$_1$= 20 % aqueous extract	36.20 (36.99)^d	35.65 (36.66)^e	34.44 (35.94)^e	31.60 (34.20)^e	29.37 (32.81)^e
T$_2$= 25 % aqueous extract	42.83 (40.88)^c	41.32 (40.00)^d	39.17 (38.74)^d	38.62 (38.42)^d	37.55 (37.79)^d
T$_3$= 30 % aqueous extract	63.44 (52.80)^b	53.79 (47.17)^c	50.56 (45.32)^c	49.52 (44.72)^c	48.03 (43.87)^c
T$_4$= 35 % aqueous extract	80.82 (64.03)^a	76.34 (60.90)^b	73.75 (59.18)^b	72.40 (58.31)^b	69.87 (56.71)^b
T$_5$= 40 % aqueous extract	83.15 (65.77)^a	79.97 (63.41)^a	76.39 (60.93)^a	74.46 (59.64)^a	71.94 (58.02)^a
T$_6$= Control	0.00 (0.08)^e	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f
SE.d (±)	1.01	0.43	0.40	0.34	0.43
C.D. (P=0.05%)	2.22	0.95	0.89	0.75	0.95
Figures in the parenthesis are the angular transformed values, DAI- Days after Inoculation					

Table.2 Screening of *Mimosa diplotricha* plant extract against *Fusarium semitechtum*

Treatment	Mycelial growth inhibition (%)				
	6 DAI	**7 DAI**	**8 DAI**	**9 DAI**	**10 DAI**
T$_1$= 20 % aqueous extract	18.23 (25.28)^e	17.99 (25.10)^e	16.86 (24.25)^e	15.79 (23.41)^e	14.33 (22.25)^e
T$_2$= 25 % aqueous extract	23.36 (28.90)^d	21.59 (27.69)^d	20.82 (27.15)^d	19.74 (26.38)^d	19.11 (25.92)^d
T$_3$= 30 % aqueous extract	41.42 (40.06)^c	39.58 (38.99)^c	37.29 (37.63)^c	35.65 (36.66)^c	34.31 (35.86)^c
T$_4$= 35 % aqueous extract	64.42 (53.38)^b	62.97 (52.52)^b	60.08 (50.81)^b	57.89 (49.54)^b	54.72 (47.71)^b
T$_5$= 40 % aqueous extract	79.47 (63.06)^a	76.61 (61.08)^a	74.84 (59.89)^a	72.85 (58.59)^a	71.66 (57.84)^a
T$_6$= Control	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f
SE.d (±)	0.77	0.59	0.62	0.62	0.39
C.D. (P=0.05%)	1.70	1.30	1.37	1.36	0.87

Figures in the parenthesis are the angular transformed values, DAI- Days after Inoculation

Table.3 Screening of *Wedelia trilobata* plant extract against *Fusarium semitechtum*

Treatment	Mycelial growth inhibition (%)				
	6 DAI	**7 DAI**	**8 DAI**	**9 DAI**	**10 DAI**
T$_1$= 20 % aqueous extract	7.93 (16.35)^e	5.46 (13.51)^e	5.21 (13.19)^e	4.71 (12.54)^e	4.44 (12.16)^e
T$_2$= 25 % aqueous extract	18.74 (25.65)^d	12.32 (20.55)^d	11.51 (19.83)^d	6.95 (15.28)^d	5.92 (14.08)^d
T$_3$= 30 % aqueous extract	31.89 (34.38)^c	29.33 (32.79)^c	27.67 (31.74)^c	25.81 (30.53)^c	24.57 (29.72)^c
T$_4$= 35 % aqueous extract	49.73 (44.85)^b	48.05 (43.88)^b	46.71 (43.11)^b	45.91 (42.65)^b	44.48 (41.83)^b
T$_5$= 40 % aqueous extract	65.95 (54.30)^a	63.81 (53.01)^a	61.37 (51.57)^a	60.42 (51.02)^a	59.04 (50.21)^a
T$_6$= Control	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f	0.00 (0.08)^f
SE.d (±)	0.62	0.51	0.44	0.39	0.36
C.D. (P=0.05%)	1.36	1.12	0.96	0.85	0.80
Figures in the parenthesis are the angular transformed values, DAI- Days after Inoculation					

Table.4 Percentage disease incidence of *Fusarium* s*emitechtum* after treatment with plant extracts on bamboo seedlings.

Treatment No.	Treatments	Per Cent Disease Incidence (%)	
		After 1st Spray	**After 2nd Spray**
T$_1$	*Mimosa diplotricha* 40% aqueous extract	50.00 (45.00)	75.00 (60.00)
T$_2$	*Senna alata* 40% aqueous extract	25.00 (30.00)	50.00 (45.00)
T$_3$	*Wedelia trilobata* 40% aqueous extract	75.00 (60.00)	75.00 (60.00)
T$_4$	Commercial *Trichoderma viride* formulation	50.00 (45.00)	75.00 (60.00)
T$_5$	Control (Untreated)	100.00 (90.00)	100.00 (90.00)

W. trilobata showed the lowest antifungal activity, with a 65.95% inhibition of mycelial growth, making it the least effective among the three plant extracts. This aligns with Govindappa *et al.,* (2011), who reported that aqueous extracts from different parts of *W. trilobata* showed low antifungal efficacy against several *Fusarium* species, including *F. solani*, *F. oxysporum*, and *F. verticillioides*. Although the fresh leaf water extract of *W. trilobata* showed strong antibacterial effects against a range of bacterial pathogens viz., *Pseudomonas fluorescens*, *Xanthomonas oryzae* and *Staphylococcus*

aureus, it had no significant effect on fungal isolates. phytochemical investigation by Wei *et al.,* (2009) reported that the antimicrobial activity of *W. trilobata* varies with the type of solvent used. Ethyl acetate extracts, particularly from the leaf and stem, exhibited the most potent antimicrobial activity, while water extracts, especially from the flower were the least effective.

The antifungal activity observed across all tested plant extracts is likely due to the presence of bioactive

compounds such as alkaloids, flavonoids, terpenoids, phenols, glycosides, saponins, and tannins, which have been widely documented for their antimicrobial properties (Gandhiraja *et al.,* 2009). These compounds disrupt fungal cell membranes, inhibit cell wall and DNA, RNA and protein synthesis, mitochondrial dysfunction, thereby suppressing pathogen growth and infection (Hosee *et al.,* 2025).

In conclusion, bamboo is a vital plant resource for the rural people of Northeast India, playing a significant role in agriculture, cottage industries, arts, culture, and daily life. However, the economic loss of this valuable resource due to biotic and abiotic factors, such as diseases and pests, has become a major concern. Therefore, proper Integrated Pest and Disease Management (IPDM) strategies need to be developed through further research. Botanicals such as *M. diplotricha* and *S. alata* extract, extract have shown positive effects under both in vitro and in- conditions. However, further large-scale field studies are necessary to determine their efficacy, economic feasibility and optimal application dosage. Implementing these eco-friendly management strategies, alongside cultural and chemical control measures, can help safeguard bamboo trees until comprehensive studies on the complex pest and disease issues affecting different bamboo species are completed and effective remedies are identified. The present study has allowed identify the best antifungal plant to develop and implement Phyto fungicide to control crop diseases, with the ultimate goal of developing a green alternative to synthetic fungicides.

Author Contributions

Rajib Kumar Borah: Investigation, formal analysis, writing—original draft. Asma Begum: Validation, methodology, writing—reviewing. Gurpreet Kaur:—Formal analysis, writing—review and editing. Kalyani Morang: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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