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Article

# Microorganisms associated with bacterial wilt disease in *Dendrocalamus asper* (Giant Bamboo) from Ecuador

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## ABSTRACT

*Dendrocalamus asper* (Bamboo) is an essential component of forest ecosystems, whose health and survival are intrinsically linked to complex interactions with its microbiome. This study focuses on the pathogenic dynamics between the fungi *Arthrinium spp*. and *Erwinia spp*. Bacteria and their impact in the shoots wilting and culm rot in bamboo. Through humid chamber induction methods and selective cultivation techniques, pathogenic strains that significantly affect the regeneration and propagation of bamboo were identified and isolated. The investigation revealed a pathogenic synergy resulting in a high prevalence of diseases, 97% of the shoots evaluated showed signs of deterioration. Morphological identification challenged conventional techniques due to the variability of *Arthrinium spp*—conidia, suggesting the need for more specific identification methods. The findings suggest that regulating soil pH and using beneficial biofilms could be promising strategies to mitigate bacterial infection. This study highlights the importance of sustainable management and in-depth knowledge of microbial ecology for integrated disease management in bamboo ecosystems. Understanding these microbial interactions is crucial for developing effective control strategies and long-term conservation of these vital ecosystems.

**Keywords:** *Dendrocalamus asper*, *Arthrinium spp*., *Erwinia spp*., bacterial wilt, disease management.

## INTRODUCTION

Bamboo is vital in forest ecosystems, providing critical structure and support for microorganisms. Microorganisms, in particular, significantly influence the nutrition and health of bamboo. Studies have shown that bacteria such as *Flavobacterium, Bacillus*, and *Stenotrophomonas* are essential in absorbing nitrogen elements in moso bamboo, which could influence its flowering time [1]. Bamboo planting can enrich soil fertility and increase tree species diversity in coastal ecosystems. In the bamboo forests of southeastern China, it has been observed that soil enzymatic and microbial activity is significantly altered [2]. Microorganisms not only improve nutrient availability [3]. Microbial communities also protect against pathogens [4], underscoring the importance of understanding microbial dynamics to sustain bamboo ecosystems.

Bamboo faces various diseases that can vary in prevalence and distribution worldwide, and microorganisms play a central role in these pathologies. Research has revealed that bamboo is an extensive reservoir of microorganisms, including fungi and bacteria, that should be investigated extensively, given its potential role in plant growth and ecosystem functioning [5]. The invasion of native bamboo in subtropical forests can alter soil microbial communities. Disturbance affects litter decomposition and nutrient cycling. The threat of local forest ecosystems is affected due to microbial disturbance [6]. The findings highlight the need to carefully monitor bamboo planting and expansion to prevent adverse impacts on biodiversity and ecosystem health.

Adverse effects negatively correlate with soil pH and the abundance of stress, virulence, and sulfur-cycling genes [7]. Furthermore, specific genera such as *Arthrobacter*, *Pseudomonas*, *Acidobacteria* GP6, and *Pasteuria* may be potential biological control agents for bacterial wilt [8]. The findings underline the importance of understanding soil bacterial community structure for effectively managing bacterial wilt in bamboo.

The interaction between microorganisms and the plant response allows activating the defense mechanisms of *the Dendrocalamus asper* against microbial attacks crucial for survival. Research has shown that biofilm formation in the rhizosphere, observed in organic hydroponic systems, may be responsible for suppressing bacterial wilt [9]. Soil acidification aggravates the occurrence of bacterial wilt, suggesting that the regulation of soil acidification is a prerequisite and fundamental condition for disease control [10]. Previous findings are critical to developing management strategies that improve the physiological and biochemical responses of bamboo to bacterial infection, which could include modulation of soil pH and utilization of beneficial biofilms as part of an integrated approach to control diseases.

Microorganisms, including specific bacteria, play a crucial role in bacterial wilt that affects various plants, including *D. asper*. Studies have identified that strains of bacteria such as *Ralstonia solanacearum* vary considerably in Brazil, suggesting that host resistance to control bacterial wilt should be targeted primarily by region [11]. Researchers have reported relevant findings on endophytic bacteria. Bacteria isolated from live oak trees in Texas showed the ability to inhibit the pathogen that causes oak wilt in vitro. Preinoculation with the bacteria Pseudomonas denitrificans 1-15 resulted in a 50% reduction in the incidence of diseased trees. Preinoculation decreased crown loss in affected trees by 17% [12]. The findings highlight the importance of accurately identifying and classifying wilt-associated bacteria to develop effective control strategies.

*D. asper* possesses intrinsic defense mechanisms against microbial attacks, which include complex physiological and biochemical responses. Induction of resistance against diseases such as wilt caused by *Bursaphelenchus xylophilus* using selected pine endophytic bacteria is an example of how microorganisms can manage diseases by inducing systemic resistance in plants [13]. It has been observed that soil acidification can lead to outbreaks of bacterial wilt, underscoring the dynamic interaction between soil conditions and plant susceptibility to diseases [14]. The studies underscore the need to understand better plant responses to bacterial infection to improve disease management strategies.

The Bamboo *Dendrocalamus giganteus* is found in an environment where synergy between microorganisms can play a crucial role in its health and survival. Studies have shown that different species of tree-dwelling insects carry a variety of microorganisms, including pathogens, indicating their potential as vectors of plant diseases [15]. It has been observed that interactions between plant pathogens can lead to disease complexes that are more common than expected. Understanding these mechanisms can have essential implications in the epidemiology and management of plant diseases [16]. Therefore, it is plausible that a synergistic action between microorganisms could be present in pathogenic attacks on *D. giganteus* Munro. However, direct evidence specific to this bamboo species has yet to be established in the scientific literature.

The hypothesis that there is a synergy between different pathogenic microorganisms that contribute to bacterial wilt in *D. asper* in Ecuador is supported by the observation that interactions between plant attackers can be asymmetric, time-dependent and species-specific [17]. These complex interactions can influence the susceptibility of plants to diseases and their ability to resist or succumb to pathogen attacks. It has been reported that plant pathogenic microorganisms can colonize insect tissues, revealing a network of interactions that affects substantial invaders [18]. The findings highlight the need to investigate further the possibility of microbial synergies in bacterial wilt of bamboo, particularly in regions such as Ecuador, where this disease can negatively impact bamboo production. This research aimed to identify and characterize the microorganisms associated with bacterial wilt diseases in *D. asper* (giant bamboo) from Ecuador.

## MATERIAL AND METHODS

### Plant material

The plant material was collected on the premises of the Special Forces Group (SFG) No. 26 CENEPA in Quevedo (Ecuador), which was subjected to a humid chamber to induce the manifestation of the causal agent(s) of the disease. This study area was selected based on the prevalence of disease symptoms in bamboo, which provides a relevant context for pathogen induction for the exact location of the study area (Figure 1). The experimental research was conducted in the "La María" Campus laboratories at the State Technical University of Quevedo, Ecuador.

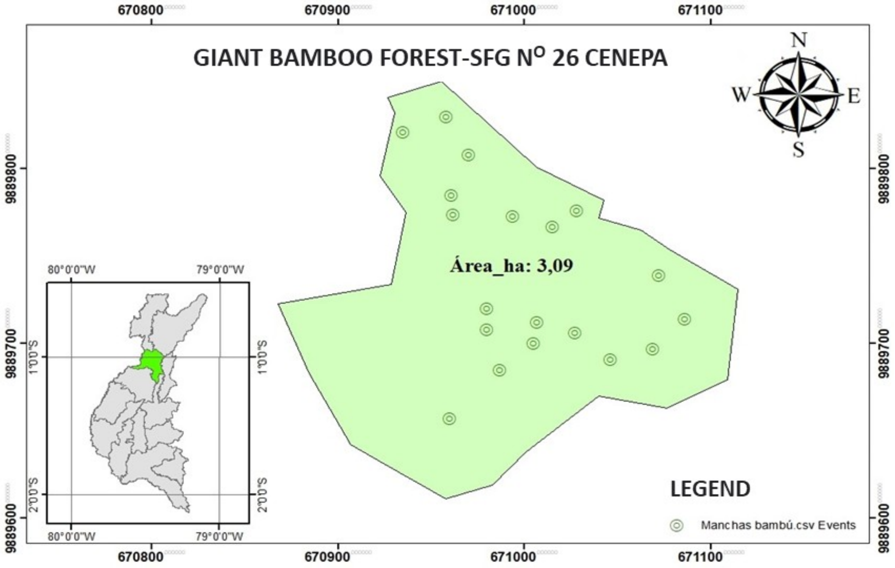

**Figure 1: Location of plant material.**

### Induction in a wet chamber

Plant material with signs of necrosis was placed in 14" x 20" polypropylene covers to induce the manifestation of disease-causing agents in bamboo. A controlled relative humidity was maintained for 72 to 96 hours. The effectiveness of this method was evaluated by observing morphological changes in the bamboo tissue, which were documented photographically. This technique is common in plant pathology to promote the manifestation of pathogens [14].

### Preparation of culture medium

Two modified culture media were prepared to promote the selective growth of fungi and bacteria. The PDA medium was enriched with Streptomycin (1mg $L^{-1}$) to inhibit unwanted bacterial growth, while nutrient agar was used for bacterial cultivation. Both media were diluted in distilled water and autoclaved at 121°C for 25 minutes. These media were selected based on previous literature indicating their effectiveness in isolating pathogens from bamboo [19].

### Sowing in Petri plates

Aseptic samples of the induced plant material were seeded in Petri plates containing the prepared culture media [20]. Pieces of bamboo culm were cut with a length and width of 2.5 cm and a thickness of 0.5 to 1 mm and incubated at 28 ± 2 °C for 8 days. Incubation was performed in a calibrated incubator to ensure consistency of growth conditions.

### Sowing in carrot sandwiches

The carrot sandwich technique was used as a complementary method for pathogen induction. Carrots were washed, peeled, and disinfected with 80% ethanol before exposure to UV light for 15 minutes. The culm pieces were placed between two slices of carrot and secured with paper tape. This method allows a detailed observation of the pathogen-host interaction.

### Isolation of microbial strains

Colonies developed in the Petri dishes were isolated using a sterile seed loop. They were transferred to new Petri containing the modified culture media [PDA (Streptomycin, 1mg $L^{-1}$) and nutrient agar (1mg $L^{-1}$)] to obtain pure cultures [21]. This step was critical for the subsequent identification of the pathogens through biochemical and molecular tests

## RESULTS

### Description of the symptoms of the disease

The disease was observed to affect shoots in the early stages of development, with symptoms of internal rot evident in roots and young tissues at growth sections. This condition seriously compromises the production of new shoots, resulting in diminished natural regeneration, stunted growth and, ultimately, plant death. Affected shoots present dark brown to black exudates in the apical area, and the leaves are dark brown with black spots (Figure 2).

**Figure 2: Comparison of healthy shoots and those affected by the disease. A) Healthy shoots from *D. asper*; B) *D. asper* shoots with exudations in the apical area.**

The enzymatic action of *E. sinocalami* and *Arthrinium spp*. It resulted in spraying the internal part of the shoots, stopping the disease when reaching the culm area, which presents resistance due to its high cellulose content (Figure 3).

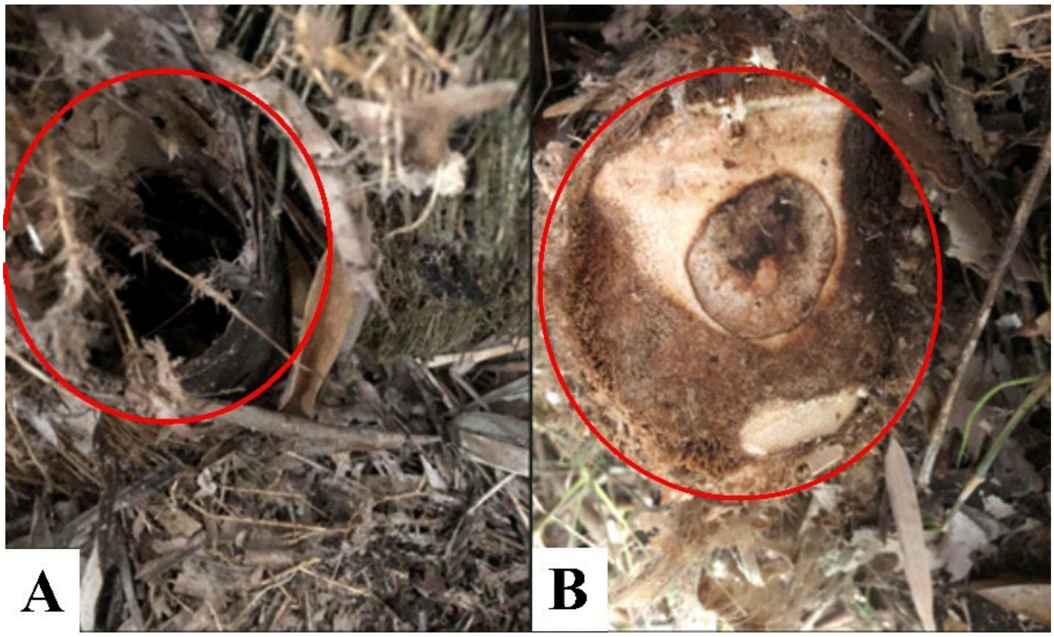

**Figure 3: Evidence of enzymatic action on shoots and culm. The enzymatic action of *E. sinocalami* and *Arthrinium spp*. A) Internal part of the shoot pulverized by bacterial action; B) Attack by *E. sinocalami* and *Arthrinium spp*. in the culm internode.**

### Disease severity

The severity of the pathogen attack was reflected in a meager natural regeneration rate. The phenological evaluation showed that 97% of the shoots were affected and showed signs of spraying by phytopathogens (Table 1).

| ITEM | Cane average | DCH | Live shoots (%) | Dead shoots (%) |
|---|---|---|---|---|
| **Scale 1** | 0,2 | 8,80 | 3% | 97% |
| **Scale 2** | 10,0 | 12,50 | | |
| **Scale 3** | 45,0 | 10,68 | | |
| **Scale 4** | 3,0 | 14,16 | | |
| **Scale 5** | 6,0 | 17,46 | | |

*Note: The phenological scale was arbitrarily based on vigor, diameter and hue.*

**Table 1. Phenological scale and percentage of affected shoots**

### Morphological recognition of microorganisms associated with bacterial wilt in D. asper

A strain of filamentous fungi and a bacterial strain of the genus Erwinia, closely related to bacterial wilt, were isolated and identified. Bacterial strains grew optimally on modified nutrient agar at room temperature, while fungal colonies preferred a PDA culture at 28°C. Identification was based on Gram staining and trypan blue pigmentation techniques, using microscopy with close-ups of 10, 40 and 100x and taxonomic keys to determine the genus of the strains.

Arthrinium species can produce varied fruiting bodies depending on growing conditions. It was observed that the aerial hyphae took on a white tone in the incubator and brown at room temperature. The identification of Arthrinium was confirmed by the observation of conidiogenous structures and spores with a germinal cleft (Figure 4).

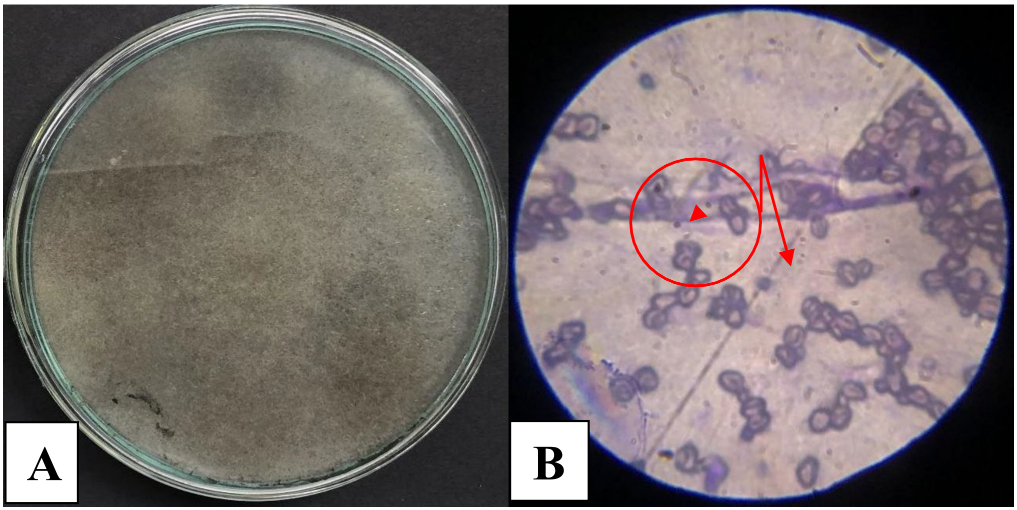

**Figure 4: Morphology of *Arthrinium spp*. Observed in culture. A) Macroscopic view of *Arthrinium spp.*; B) Spores with germinal cleft.**

## DISCUSSION

This study provides a detailed insight into the complex interaction between pathogenic microorganisms and *D. asper*, revealing the synergy between *E. sinocalami* and *Arthrinium spp*. in the induction of bacterial wilt and culm degradation The complex interplay between microorganisms and bamboo, particularly in the context of disease management and control strategies, is a critical area of focus. Recent studies have shed light on various aspects of this interaction, offering insights that could be instrumental in developing more effective disease management strategies for *D. asper*. Darma et al. [22] explored the potential of *Bacillus subtilis* BMB26, a bacterium isolated from bamboo powder, as a biocontrol agent against phytopathogenic fungi. This finding is particularly relevant to our study as it suggests the possibility of utilizing bamboo-associated microorganisms for biologically controlling pathogens in *D. asper*. The use of such biocontrol agents could provide a sustainable alternative to chemical control methods, aligning with the need for eco-friendly disease management practices in bamboo ecosystems [22]. The impact of forest management practices on the soil microbial community was highlighted by Yang et al. [23]. Their study indicated that converting secondary broadleaf forests to Moso bamboo plantations alters the soil bacterial community structure. This alteration could have significant implications for disease dynamics in bamboo plantations, underscoring the importance of considering forest management practices in disease management strategies.

Zhang et al. [24] found that chicken farming under Moso bamboo forests significantly increased bacterial and fungal diversity. This increase in microbial diversity could influence the prevalence and severity of diseases such as bacterial wilt in bamboo, suggesting that agricultural practices in bamboo forests must be carefully managed to maintain ecological balance and prevent disease outbreaks [24].

Fuke et al. 25 emphasized rhizospheric microbial communities' role in bamboo health. These communities play a significant role in nutrient immobilization and phytoremediation, which could enhance the overall health and disease resistance of *the Dendrocalamus asper*. Understanding and harnessing these microbial interactions could improve bamboo resilience against pathogens. The study by Shen et al.[26] on endophytic fungi isolated from Moso bamboo seeds revealed their broad-spectrum antimicrobial activity. These fungi could be a valuable source of bioactive compounds for developing plant defense activators, offering a novel approach to disease management in bamboo.

The interaction of fungi of the genus *Arthrinium* and bacteria *Erwinia spp*. with bamboo is complex and multifaceted. These microorganisms can exist in symbiosis with bamboo or become disease-causing pathogens [27]. In particular, *E. sinocalami* and *Arthrinium spp*. have been identified as causing bacterial wilt and rot. of shoots and culms, respectively [28]. This study reveals a coordinated attack of these pathogens on bamboo shoots, suggesting more complex disease dynamics than previously recognized. The persistence of bacterial wilt in bamboo, associated with E. sinocalami, has been documented for over two decades [29]. The limitation of this bacteria to attack only young shoots has significant implications for the regeneration and propagation of bamboo, as observed in the bamboo patches evaluated, where most shoots were affected or destroyed by pathogens. Identifying species of the genus Arthrinium is challenging due to the similarity of their conidia between different species and the morphological variability depending on the incubation environment [30,31]. In this study, identification was achieved by observing conidiogenous structures and spores with a germinal cleft, which aligns with the existing literature's descriptions. Furthermore, it is essential to consider the role of natural products in crop protection, as discussed in the work of Dayan et al. [32], which highlights the need to discover and develop new pesticides based on natural products to replace compounds lost due to stricter regulations. This could be relevant for the development of biological control strategies against pathogens in bamboo.

The diversity of microorganisms in fermented foods and beverages globally, as reviewed in the study by Tamang et al. [33], also provides a context for understanding the complexity of microbial communities and their potential in biological control applications. Finally, the review by Bhardwaj et al. [34] on xylanases highlights the importance of these enzymes in the cleavage of complex polysaccharides and their application in various industrial and biotechnological sectors. Since Arthrinium spp. and Erwinia spp. are involved in the revision of plant tissues, understanding the enzymes involved could offer ways to mitigate their pathogenic impact.

These findings and comparisons with existing literature underscore the need for a deeper understanding of interactions between pathogens and host plants. They could inform the development of integrated disease management strategies for the conservation and sustainable use of bamboo ecosystems.

## CONCLUSIONS

This study provides a detailed insight into the complex interaction between pathogenic microorganisms and *D. asper*, revealing the synergy between *E. sinocalami* and *Arthrinium spp*. in the induction of bacterial wilt and culm degradation in bamboo. The findings demonstrate that *E. sinocalami* targets young shoots, while Arthrinium spp. is associated with the degradation of culm structure, suggesting a coordinated attack mechanism that could have significant implications on the health and viability of bamboo plantations. Identifying *Arthrinium spp*. was particularly challenging due to the morphological similarity of its conidia to other fungal species. However, accurate identification was achieved through detailed analysis of hyphae, conidiogenous structures and spores, highlighting the importance of meticulous diagnostic techniques in plant pathology. The results of this study emphasize the need to develop integrated disease management strategies that consider the interaction between different pathogens. Furthermore, it is crucial to continue research to understand better the epidemiology of bacterial wilt and pathogen-host interactions in bamboo to develop effective and sustainable control measures that ensure the protection of this important plant species in ecosystems: forestry and agricultural production. Collaboration between plant pathologists, microbiologists and agronomists will be essential to address the challenges presented by bacterial wilt in *D. asper* and other bamboo genera, ensuring the sustainability of these valuable resources worldwide. Finally, these studies collectively highlight the need for a holistic approach to disease management in bamboo ecosystems. By considering both beneficial and pathogenic microorganisms and the impact of environmental and management practices, we can develop more effective and sustainable strategies for the conservation and health of *D. asper*.

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**Conflicts of Interest:** There is no conflict of interest.

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