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Ecological Safety of RNAi Biofungicides: Assessing the Impact of Spray-Induced Gene Silencing on the Indigenous Phyllosphere Microbiome of Tropical Staples

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Abstract

The recent epidemic of ginger blight disease in Nigeria has resulted in unprecedented yield losses, with economic damages exceeding ₦12 billion in 2023 alone. A complex of soil-borne fungal diseases, mainly Fusarium and Aspergillus species, which have demonstrated a high level of developing resistance to traditional synthetic fungicides, is most likely responsible for this enormous economic loss. Spray-Induced Gene Silencing (SIGS), a non-transgenic application of RNA interference (RNAi), has emerged as a high-precision, eco-friendly alternative for managing such aggressive pathogens by topically applying double-stranded RNA (dsRNA) to silence essential virulence genes. However, the ecological safety of deploying SIGS in the high-biodiversity, high-stress tropical environments of Sub-Saharan Africa remains poorly understood. This review critically evaluates the potential impact of exogenous dsRNA applications on the indigenous phyllosphere microbiome, the diverse community of epiphytic bacteria and fungi that form a vital biological shield on the leaf surfaces of tropical staples like ginger. We examine the stability of SIGS formulations under tropical stressors, such as extreme UV radiation and humidity, and analyze the bioinformatics-based risks of non-target silencing within the local microbial niche. Furthermore, the role of bio-based nanocarriers, such as chitosan, in modulating these microbial interactions is discussed. By synthesizing current molecular findings with tropical ecological imperatives, this paper provides a framework for the safe integration of RNAi-based biopesticides into Nigerian integrated pest management (IPM) systems, ensuring that innovation does not come at the cost of microbial equilibrium.

Keywords: *Spray-Induced Gene Silencing (SIGS), RNA interference (RNAi), Phyllosphere Microbiome, Ecological Risk Assessment, Tropical Agriculture, Ginger Blight, Non-target Effects, dsRNA Stability.*

Introduction

Ginger (*Zingiber officinale*) is a cornerstone of Nigeria's agricultural economy, serving as a vital cash crop that supports rural livelihoods and contributes significantly to foreign exchange earnings. Nigeria has historically maintained a dominant position in the global market; however, the sector is currently facing an unprecedented crisis due to the devastating ginger blight epidemic. Initial outbreaks in 2023 ravaged approximately 90% of farms across the ginger-producing hub of Southern Kaduna, leading to a monumental national loss estimated at over ₦12 billion [1], [2]. Ginger exports fell by 74% to over ₦6.28 billion in just nine months due to the disease's severe economic effects, which led to a six-fold increase in local market prices. [3], [4].

The enduring nature of the blight is attributed to a complex of fungal pathogens, primarily *Fusarium*, *Aspergillus* species, and *Pythium zingiberis*, which have proven difficult to eradicate using conventional and traditional methods [5]. Despite government interventions and the distribution of fungicides, farmers continue to raise concerns as the disease resurfaces in new planting seasons, threatening to permanently displace Nigeria from the $7.5 billion global ginger market [6], [7]. Current management relies heavily on synthetic chemicals, which are not only costly but also raise significant concerns regarding environmental toxicity and the development of pathogen resistance [8].

To safeguard the future of the industry and achieve the government's sector revival targets, there is an urgent need for innovative, sequence-specific, and eco-friendly molecular tools [9]. Spray-Induced Gene Silencing (SIGS) offers a revolutionary, non-transgenic approach by using topically applied double-stranded RNA (dsRNA) to silence essential virulence genes in the target fungi. While global research has validated SIGS as a sustainable alternative to chemical pesticides, its application within the unique tropical agro-ecological niche of Nigeria, specifically regarding its impact on the indigenous leaf microbiome, remains a critical research gap. This review evaluates the ecological safety and technical feasibility of SIGS as a transformative solution for the Nigerian ginger blight crisis.

Table 1: Comparative Analysis of SIGS vs. Conventional Chemical Fungicides in Nigeria

FEATURE	CONVENTIONAL SYNTHETIC FUNGICIDES	SIGS (RNAI BIOFUNGICIDES)
Specificity	Broad-spectrum (kills beneficial microbes)	High (sequence-specific to target pathogen)
Toxicity	High (residual risk to humans/environment)	None (RNA is a natural, biodegradable molecule)
Persistence	Long-term (soil and water accumulation)	Short-term (rapid environmental degradation)
Resistance	High risk (pathogens adapt quickly)	Low risk (can target multiple essential genes)
Delivery	Knapsack sprayers (common in Nigeria)	Knapsack sprayers / Bio-nanocarriers
Regulatory	NAFDAC Registered (Pesticide)	NAFDAC (Bio-Pesticide) & NBMA (Biosafety)

The Tropical Phyllosphere: A Unique Ecological Niche

The phyllosphere, comprising the total above-ground surfaces of plants, represents one of the most complex and dynamic microbial habitats on Earth. In tropical regions such as Nigeria, the phyllosphere of staple crops like ginger (*Zingiber officinale*) is characterized by high microbial density and diversity, driven by consistent warmth and periodic high humidity [5]. This indigenous microbiome, consisting of bacteria, filamentous fungi, yeasts, and archaea, functions as a vital biological shield, providing the host plant with essential services including nutrient cycling, growth promotion, and natural antagonism against invading pathogens [10].

The tropical phyllosphere is a "high-stress" niche, in contrast to temperate agro-ecosystems where microbial populations may undergo slower turnover.

Epiphytic microbes in the Nigerian ginger belt must withstand intense solar ultraviolet (UV) radiation, fluctuating surface temperatures, and the physical scouring effect of heavy tropical rainfall [2], [8]. These environmental stressors not only dictate the community structure of the microbiome but also influence the residence time and stability of any topically applied biological agents, including double-stranded RNA (dsRNA) molecules used in gene silencing [11].

The introduction of Spray-Induced Gene Silencing (SIGS) into this niche presents a unique ecological question. While SIGS is designed for high target specificity, the sheer diversity of the tropical phyllosphere increases the statistical probability of "off-target" sequences in non-pathogenic, beneficial microbes [12]. Furthermore, the high moisture content typical of tropical ginger farms may facilitate the horizontal transfer or systemic uptake of exogenously applied RNA by a broader range of epiphytes than previously observed in laboratory settings. To ensure that RNAi-based therapies do not unintentionally upset the microbial equilibrium that sustains plant health, it is necessary to comprehend the baseline composition and functional resilience of this indigenous population [13].

Mechanisms of SIGS and Potential Non-Target Interactions

Spray-Induced Gene Silencing (SIGS) operates through the exogenous application of double-stranded RNA (dsRNA) to trigger the highly conserved RNA interference (RNAi) pathway in target pathogens. This mechanism begins with the uptake of dsRNA by the fungal cells, where it is processed by Dicer-like (DCL) enzymes into small interfering RNAs (siRNAs). These siRNAs are subsequently integrated into the RNA-induced silencing complex (RISC), guiding the cleavage of complementary messenger RNA (mRNA) transcripts essential for pathogen virulence or survival [11]. In the context of the Nigerian ginger blight, this allows for the precise silencing of growth-essential genes in pathogens such as *Fusarium* and *Aspergillus* [5].

Despite this precision, the potential for non-target interactions remains a critical safety concern. Non-target effects occur when the applied dsRNA or its resulting siRNAs share sufficient sequence homology with the mRNA of unintended organisms, leading to accidental gene silencing [12]. In the diverse tropical phyllosphere of Nigerian ginger farms, the risk is twofold:

1. Host Plant Interaction: Potential silencing of host genes if the dsRNA sequence overlaps with the *Zingiber officinale* genome.

2. Microbiome Disruption: Impact on beneficial epiphytic microbes that provide natural biocontrol or growth promotion [10].

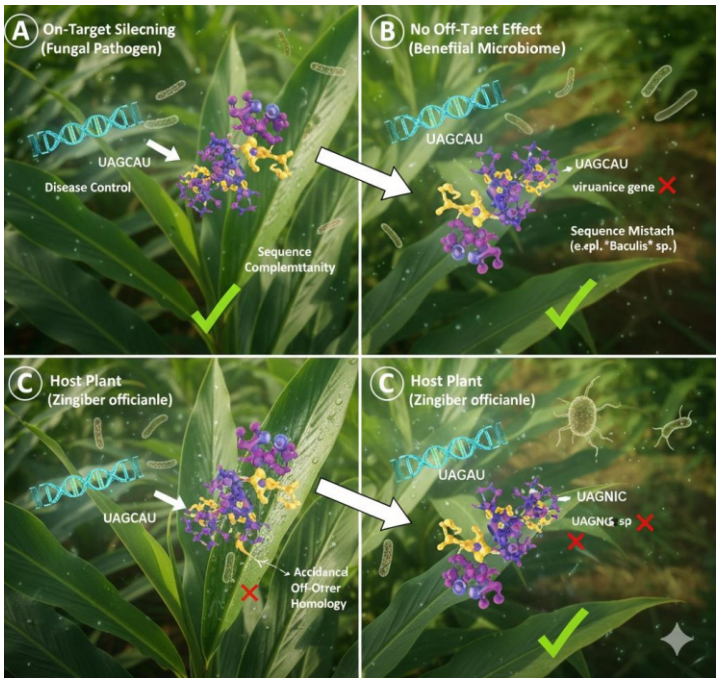

Figure 1: Mechanisms of SIGS and Ecological Non-Target Interactions in the Tropical Phyllosphere

The environmental conditions of the Nigerian ginger belt further complicate these interactions. High humidity and leaf surface moisture may enhance the mobility of dsRNA, increasing the surface contact with a broad range of non-target epiphytes [8]. Additionally, although sequence-specific design might reduce "off-target" dangers, thorough bioinformatics screening is difficult due to the lack of genomic information for many native Nigerian microbial species.[6]. Therefore, assessing the ecological safety of SIGS requires not only bioinformatics prediction but also empirical validation within the local microbial niche to ensure that the suppression of ginger blight does not inadvertently destabilize the phyllosphere's microbial equilibrium.

Environmental Modulation of SIGS in the Tropics

The successful deployment of Spray-Induced Gene Silencing (SIGS) in Nigeria is heavily contingent upon the environmental stability of exogenously applied double-stranded RNA (dsRNA). In tropical agro-ecological zones, such as the ginger-growing belts of Kaduna and Plateau states, climatic factors pose significant challenges to the longevity and efficacy of RNAi-based biopesticides. Unlike controlled laboratory settings, the open-field tropical environment exposes dsRNA to a rapid degradation cycle driven by intense solar ultraviolet (UV) radiation, fluctuating temperatures, and frequent high-intensity rainfall [2], [11].

Solar UV radiation is a primary factor in the abiotic degradation of nucleic acids on leaf surfaces. High levels of isolation can drastically reduce the half-life of "naked" dsRNA, possibly lowering its protective window to less than 48 hours. [14].Furthermore, the physical "wash-off" effect caused by the West African monsoon season presents a major hurdle for adhesion and uptake.

Without adequate stabilization, heavy precipitation can remove the dsRNA from the phyllosphere before it can be internalized by target fungal pathogens like *Fusarium* species [8], [12].

To mitigate these environmental stressors, the integration of advanced bioformulations is essential. Research suggests that utilizing locally-sourced, biodegradable nanocarriers, such as chitosan or clay-based "nanosheets", can provide a protective "scaffold" for dsRNA [10]. These carriers not only shield the RNA from UV-induced photodegradation but also improve rain-fastness and leaf surface adhesion, extending the biopesticide's efficacy from days to weeks [11]. Therefore, in order to maintain resilience under tropical field stresses, the development of SIGS for Nigerian staples must change from a "molecule-only" focus to a "bioformulation-centered" approach. [15], in theirwork Enhancing RNAi efficiency in Phytophthora infestans using cell-penetrating peptide TAT highlighted the use of RNAi in the effective treatment of plant diseases.

Methodological Approaches for Ecological Assessment

The evaluation of Spray-Induced Gene Silencing (SIGS) safety within the Nigerian phyllosphere requires a multi-tiered methodological framework that integrates molecular biology, bioinformatics, and microbial ecology.

Bioinformatics-Based Risk Prediction

The first tier of assessment involves in silico screening to identify potential off-target effects. Using high-throughput sequencing data from Nigerian ginger pathogens, researchers can perform sequence homology searches against the genomes of the host plant (*Zingiber officinale*) and known beneficial epiphytes [11]. This ensures that the designed dsRNA molecules possess minimal complementarity to non-target transcripts [5], [6].

High-Throughput Metagenomic Profiling

To capture the real-time impact of SIGS on the indigenous leaf microbiome, metagenomic analysis (16S rRNA and ITS sequencing) is essential. Researchers are able to track changes in variety and guarantee the stability of "helper" microbes by characterising the entire microbial community, including bacteria and fungus, both before and after the administration of bioformulated dsRNA[12].

qRT-PCR Validation of Gene Expression

While metagenomics tracks community structure, quantitative Real-Time PCR (qRT-PCR) is necessary to validate actual gene silencing. This measures the expression levels of the target virulence genes in pathogens versus essential housekeeping genes in non-target epiphytes, confirming that the RNAi effect remains localized [11], [2].

Greenhouse-Based Microcosm Studies

Finally, ecological safety must be validated in controlled environments that mimic the Nigerian climate.

Microcosm studies enable the observation of long-term interactions between the SIGS formulation, the host plant, and the phyllosphere under varying humidity and temperature cycles [10]. This provides the "proof-of-safety" essential for national regulatory approval [7].

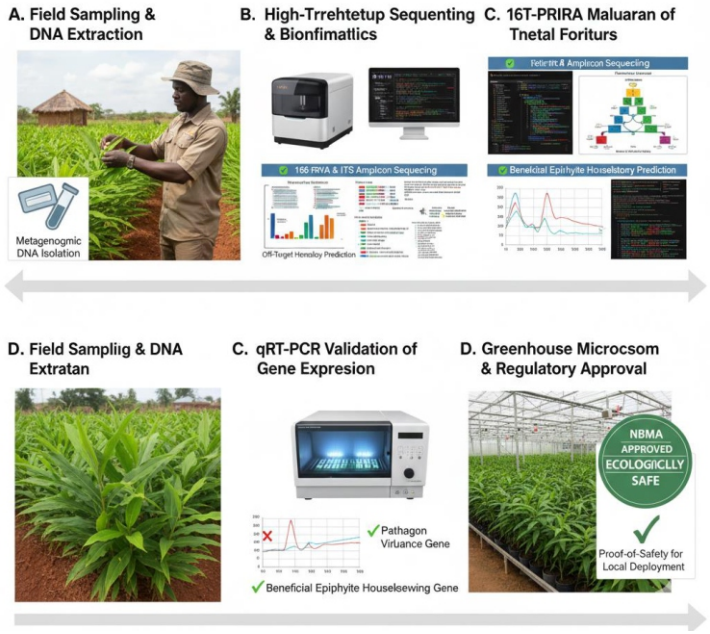

Figure 2: Tiered Methodological Framework for Ecological Risk of SIGS in Nigeria.

Conclusion and Future Perspectives

The emergence of Spray-Induced Gene Silencing (SIGS) represents a paradigm shift in Nigerian plant protection, moving from broad-spectrum chemical interventions to sequence-specific molecular precision. This technology offers a potent and sustainable solution to the ginger blight epidemic that has severely compromised Nigeria's standing in the global spice market. By targeting essential virulence genes in local fungal pathogens, SIGS provides a non-transgenic pathway to suppress disease while eliminating the environmental hazards and health risks associated with synthetic fungicides.

However, the transition from laboratory discovery to field application in the Nigerian ginger belt is contingent upon addressing critical ecological and technical gaps. The high biodiversity and extreme environmental stressors of the tropical phyllosphere, including intense UV radiation and monsoon rainfall, require the development of stabilized bioformulations to ensure dsRNA longevity and efficacy. Furthermore, ensuring the ecological safety of these biopesticides necessitates rigorous metagenomic and bioinformatics screening to safeguard the indigenous microbial communities that underpin plant health and natural biocontrol.

Looking forward, the establishment of a robust regulatory framework by national biosafety authorities is essential to accommodate topically applied RNAi products. Future research must prioritize the scaling of local dsRNA synthesis and the exploration of cost-effective, locally sourced nanocarriers to make SIGS accessible and affordable for smallholder farmers.

By integrating these molecular innovations with ecological foresight, Nigeria can pioneer a sustainable agricultural model that safeguards both crop productivity and environmental integrity for future generations.

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