Sociology & Cultural Research Review (SCRR)
Available Online: https://scrrjournal.com
Print ISSN: 3007-3103 Online ISSN: 3007-3111
Platform & Workflow by: Open Journal Systems

Plant-Derived Antifungal Proteins as Eco-Friendly Alternatives to Chemical Fungicides for Sustainable Crop Protection

Iftikhar Ahmad
Bahauddin Zakariya University Multan, Department of Botany
ahmad6931632@gmail.com

Abstract

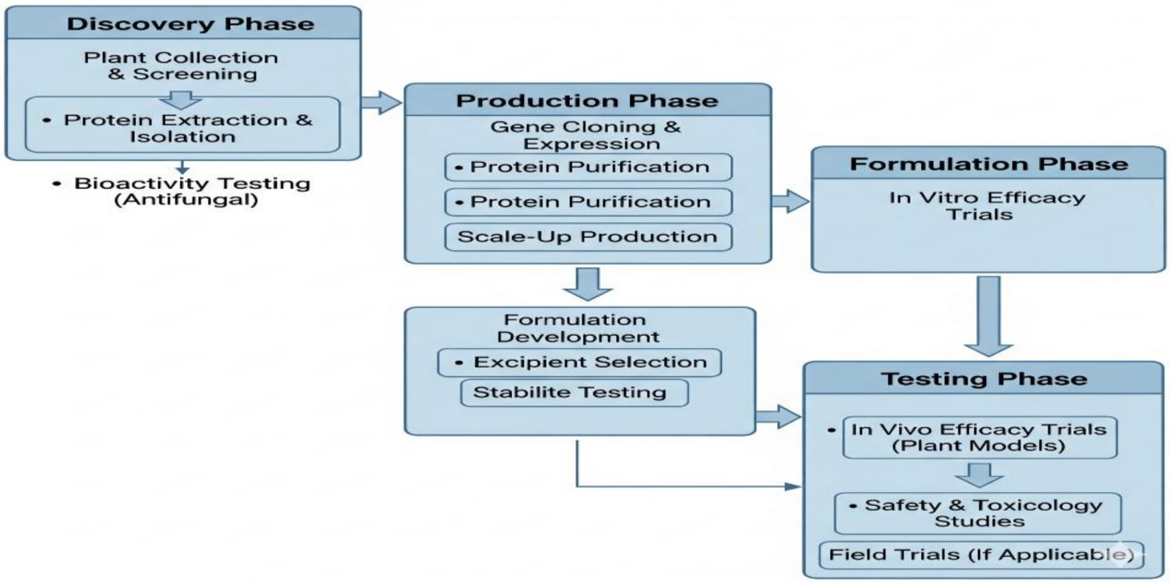
The escalating resistance of fungal pathogens and the environmental drawbacks of chemical fungicides necessitate sustainable alternatives for crop protection. This research proposal outlines a comprehensive plan to develop plant-derived Antifungal Proteins (AFPs) including defensins, chitinases, and other antimicrobial peptides as effective, eco-friendly bio-fungicides. The project aims to bridge critical gaps between AFP discovery and field application through an interdisciplinary approach. Key objectives include: (1) bioinformatic screening and in vitro selection of potent AFP candidates against key pathogens (Botrytis cinerea, Fusarium oxysporum, Rhizoctonia solani); (2) elucidation of their mechanisms of action; (3) optimization of scalable production using heterologous expression in microbial and plant-based systems; (4) development of stable, field-ready formulations using microencapsulation; and (5) rigorous evaluation of efficacy and safety through greenhouse and small-plot trials, including non-target impact assessments on soil microbiomes and pollinators. Over 24 months, the project anticipates delivering two lead AFP candidates with characterized modes of action, scalable production protocols, prototype formulations, and efficacy data. The expected outcome is a viable, sustainable crop protection solution that reduces reliance on synthetic chemicals, minimizes environmental residues, and integrates into sustainable agriculture frameworks. This research has significant potential to influence policy, promote biodiversity, and offer farmers a commercially viable, eco-friendly fungicide alternative.

Keywords: *Antifungal Proteins (AFPs), Sustainable Agriculture, Bio-fungicides, Plant Defensins, Chitinases, Integrated Pest Management (IPM), Heterologous Expression, Formulation, Eco-friendly Crop Protection.*

Introduction

The loss of yield within horticultural and staple crops is attributable to the presence of fungal pathogens. While chemical fungicides mitigate this issue, they pose several threats, such as the elicitation of pollution, the emergence of drug-resistant pathogens, and the accusation of leftover chemical impacts to the health of humans. Antifungal plant proteins, such as plant defensins, chitins, and AMPs, are biodegradable and represent low toxicity substitutes. Consequently, this project includes, plant defensins, chitinases and other AMPs. Target fungal pathogens such as: Botrytis fiat the filamentous fungi genus Fusarium. *Oxysporum* and *Rhizoctonia solani*, growth repression, optimize heterologous, expression and scalable production of microbial expression and plant-based bioreactors, evaluate formulation ad delivery by foliar sprays, and resistant pathogens. Human health residues, chemical fungicides within the environment, environmentally ecological impact of bioremediation, and small-scale field testing substantiated in greenhouse controlled. The, controlled greenhouse, bioremediation suggests the emergence of enforceable policy bioassays MIC or membrane permeable cultivation.

Fungal discrimination in genomics and down regulated interrogation demonstrate energetic manifolds. These manifolds elicit no harm on untargeted ecosystems or fungal pathogens in the resistant risk. The innovative method facilitates in-plant in-situ produced validated. Scalable producer's crops integrated loss of benefits and risk assessments. The interdisciplinary field of this bioremediation, formulation of protein engineering, ecotoxicology fused with foundational agronomy, seeks to eliminate fungicides of agreement with translational funding. In 24 months, the anticipated outcomes include provision of the 2 lead AFP researchers along with supporting greenhouse and small-plot efficacy data, production protocol, formulation prototype, and impact briefs surrounding regulatory and socio-economic considerations for adoption by the growers.

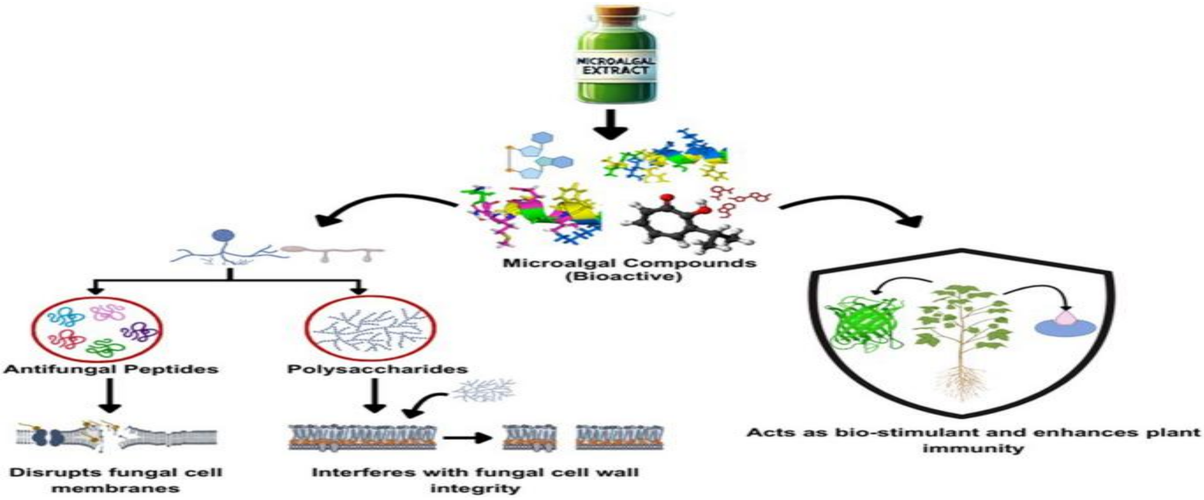


2. Background and rationale

Economically important crops are losing both quality and yield due to diseases such as grey molds, wilts, rots, and fungicides are the usual treatment. Reliance on synthetic fungicides creates a number of issues, including, but not limited to, the increase of soil and food chemical residues, the increase of toxic fog to other living organisms, and the decrease of biodiversity. Alternative, sustainable practices of crop protection must be considered.

Plants manufacture a diverse and complex array of antifungals such as cysteine-rich defensins, chitinases, hevein-like peptides, and nodule cysteine-rich (NCR) peptides that function through lethal and sublethal permeabilization of membranes, cell distruption, and cell wall components. Antifungal proteins (AFPs) are cysteine-rich defensins, chitinases, hevein-like peptides, and nodule cysteine-rich (NCR) which immobilize and kill fungi through several mechanisms. They show promising candidates for enhancement of topical and seed treatments. Evidence of enhanced resistance to fungi due to overexpression of chitinases and defensins in transgenic plants supports the activity of several peptides against critical phytopathogens in vitro and in planta activity.

Advances in the historical challenges of formulating to increase protein persistence and stability in the field without toxic adjuvants and, cost-effective scalable production of proteins and peptides through microbial expression and plant systems are now being addressed. Recent studies 2023-2025, report the high-yield production of plant antifungal proteins and the encouraging greenhouse efficacy against Botrytis and other pathogens, which serves translational development purpose. Taking on this project has great social importance it integrates contemporary discovery workflows (bioinformatics + precision targeting), production engineering, formulation science, and regulatory/safety assessment to offer pragmatic AFP-derivative fungicide substitutes ready for the field.



Microalgal-based sustainable bio-fungicides: a promising solution to enhance crop yield

Importance of This Research

- **Potential of Global Scale Market:** Like some estimates predict, the biopesticide market will grow at a CAGR of 24.14%, surpassing USD 15 billion by 2030. This makes the research to develop AFP-based bio fungicides a leader in a market about to take off.
- **Integration of novel bioinformatics:** AI assisted databases and motif prediction in the antifungal protein screening bio prospecting phase. This innovative approach simplifies the research and development process, which in turn cuts costs associated with it.
- **Enhancement of biodiversity:** The environmental fragmentation caused by the degenerative practices of the agriculture will be curbed, including the destruction of beneficial soil microbes, pollinators, and aquatic life, which are essential for the overall ecosystem.
- **Credibility of sustainability:** The synthetic AFP and synthetic pesticide chemical removal from food and the environmental surfaces they are applied means, due to their low toxicity and ease of bioavailability, bio pesticide containing AFP and derivatives, which are nontoxic and reduce the bioavailability synthetic pesticide.
- **Management of resistant pathogens:** The effective longevity of these biological agents is extended in comparison to conventional single-mode chemical fungicides because the multi-target AFPs employed resistance development.
- **Promotion of plant immune research:** Breeding programs aimed at improving natural resistance for crops engineered with backbone AFP genes stand to gain from action studies technology.
- **Potential for interdisciplinary research:** The antifungal proteins have far-reaching applications in the pharmaceutical, food preservation, and veterinary sciences.
- **Potential for Global Networking:** The collaboration with global research institutes, Agritech companies, and non-profit organizations working in sustainable agriculture

will also provide avenues for synergistic collaborations, thus enhancing the scope for academic collaboration.

- **Influencing policy:** More evidence proves that it will generates global regulation of pesticide policy, sustainable agriculture and food policy, worldwide the global framework integrating evidence-based policy framework.
- **Community engages:** Informs and works with farmers and members of the community about community participation in sustainable farming, sustainable pest management, and community involvement.

Introduction

The global agriculture industry is under threat from fungal pathogens which are the source of losses in the quantity of crops gained, food degradation, and shorter shelf life. There is loss of production of staple crops and horticulture due to the attack of fungi like Botrytis cinerea, *Fusarium oxysporum*, and *Rhizoctonia solani*, leading to the lowering of production efficiency and biodiversity. Climate change such as in temperature, humidity, and precipitation adversely affects fungal diseases by intensifying the aggressiveness of pathogens (Chiu et al. 2022, Gong et al. 2024). The bulk of the world's regions are under greater risk, and this is linked to climate change as well as global food security.

For crop reception and keeping chemical fungicides on the crops as one of the final steps solutions. In this case, and for very little effort, a greater risk to the crops presenting pathogenic fungi is created. The overwhelming use of fungicides raises issues such as the resistance of fungal populations to various chemical agents, negative impacts on the wellbeing of all living organisms, and the toxic pathogens from fungicides residues collecting in the soil and water.

Plants have the capacity to produce numerous Antifungal proteins, including defensins, peptides, chitinases, lipid transfer proteins, and hevein-like peptides. These proteins serve as first responders to invading pathogens and are found in tier one of a plant's immune system. Most of these proteins demonstrate immune system responses that are multi-modal comprising, but not limited to, membrane disruption, superoxide oxidation, and inhibition of critical system in a fungus (Gong et al, 2023). The selectivity and wide ranging functionality of these proteins, as well as their rapid biodegradability to natural nontoxic constituents, mark them as candidates for the sustainable management of plant diseases with minimal damage to the environment compared to synthetic products (The Potential of Plant Proteins as Antifungal Agents, 2022).

In recent years, the discovery and classification of antifungal peptides (AFPs) has been aided by the application of omics, bioinformatics, and molecular biology techniques. For instance, apple tree defensin gene family studies at the genomic level revealed several new defensin family members that may be involved in anti-fungal defenses (Yang et al., 2025). Likewise, Liu et al. (2025) and Gong et al. (2024) and others have reviewed and researched the origins, structure, and functional mechanisms of natural and synthetic AFPs as well as in silico AFP classifiers and predictors (e.g. APD3 database, structural motif analysis) to identify and rationalize potential candidates for in vitro functional analysis (Advances of Peptides for Plant Immunity, 2025; Antifungal Peptides from Living Organisms, 2024).

Nonetheless, capturing antifungal agents and deploying them at field-scale commercial levels is still bogged down by several issues. Firstly, under laboratory conditions, many antifungal proteins are incredibly processed for antagonism, however, field conditions show true antifungal proteins to be unstable and interaction to surfaces. Proteolysis, UV instabilities, loss of activity during precipitation, and poor delivery to sites of infection are all issues. Secondly, with regard to antifungal proteins, several issues need to be addressed: the direct and indirect effects of microbial fermentation, molecular farming suspensions, porous-plant cultivation, cysteine-rich peptide purification, antifungal efficacy retention post purification, and economics. Lastly, the remaining scant research available has overlooked the non-target antagonism effects beneficial to the soil and the pollinator population and weakly pertained to the environmental persistence and resistance development (Gong et al., 2024; Chiu et al., 2022).

Furthermore, while advances in formulation science micro/nano encapsulation, stabilizing adjuvants, and delivery vehicles, are promising, standardized and scalable formulations that satisfy regulatory and organic certification are lacking (Vakili-Ghartavol et al., 2025; Advances of Peptides for Plant Immunity, 2025). Equally, field trials are still limited in scale, geographic diversity, and duration. Few studies that explore performance over many seasons, under varying climate conditions, or in actual farmer management conditions are sorely lacking (Plant Defense Proteins: Recent Discoveries and Applications, 2025; Yang et al., 2025).

When viewed together, the literature suggests that antifungal proteins (AFPs) obtained from plants have significant potential as a sustainable substitute for chemical fungicides, but only if the gaps in discovery, production, formulation, and field performance are adequately addressed. Strongly integrative research is needed which (a) uses bioinformatics and molecular screening to discover and prioritize AFP candidates, (b) elucidates modes of action and resistance risks, (c) establishes economically viable, stably produced and formulated pipelines, (d) validates greenhouse and field trials for efficacy and safety, and (e) evaluates environmental impact and non-target safety. This proposal aims to do just that in an effort to promote sustainable crop protection, reduce the use of chemical fungicides, and foster resilient agricultural systems.

3. Research questions and objectives

Primary research question

In the efforts of sustainable protection of crops, can antifungal proteins (AFPs) isolated from plants be turned into affordable, effective, and easily scalable substitutes to the traditional chemical fungicides which are potentially more harmful?

Specific Objectives:

1. Based on in vitro screens, identify and select candidate antifungal proteins from various plant taxa for activity against *Botrytis cinerea*, *Fusarium oxysporum*, and *Rhizoctonia solani.*
2. Characterize mechanisms of action and assess the spectrum of activity: membrane permeabilization, cell wall binding, ROS induction, and transcriptional responses.
3. Develop scalable production routes: optimization of purification, heterologous expression in *E. coli* and *Pichia pastoris*, and plant-based expression via transient expression in *Nicotiana benthamiana*.
4. DFAPs: design field-ready formulations incorporating microencapsulation of AFPs and adjuvants compliant with organic agriculture.

5. Analyze the effectiveness of greenhouse and small-plot field experiments, and determine phytotoxicity, non-target impacts, antibiotic resistance, environmental persistence, and the fate of soil microbiome.
6. Create a comprehensive integrated regulatory and techno-economic blueprint for the projected ROI.

4. Literature review

(Chiu et al., 2022) proposed that the antifungal proteins (AFPs) such as α-defensins, chitinases, thaumatin-like proteins, lipid transfer proteins, hevein-like peptides, and an array of other pathogenesis-related (PR) proteins synthesized by plants serve as a first line defense against fungi. Recent research synthesis indicate that AFPs perform various biochemical actions such as degrading cell walls, permeabilizing membranes, inducing reactive oxygen species (ROS), and inhibiting biochemical reactions, spanning a broad distribution across taxa, and appealing for their biodegradability, low toxicity to mammals, and low toxicity to other non-target organisms. These characteristics provide AFPs the potential to serve as replacement for synthetic fungicides.

(Vriens et al., 2014) discussed the continuous and unregulated use of synthetic fungicides have resulted in the emergence of resistant populations of pathogens like *Botrytis cinerea* and many *Fusarium spp*. In addition, it has resulted in unregulated impacts on the environment, and has raised concern for the limited chemical defense products available for use. The literature continues to suggest that the development of AFPs is a response to these challenges, as they provide a greater level of precision and less environmental persistence compared to other chemical fungicides, which are essential for integrated pest management (IPM) on a sustainable basis.

(Parisi et al., 2019) suggested that one of the most well-defined AFP families are Plant defensins. They are small, cationic, cysteine-rich peptides, which associate with the lipids of fungal membranes (e.g. glucosylceramides) and with membrane receptors, which, due to the interaction, result in membrane permeabilization, disruption of ion flux, and often triggering of intracellular signaling cascades leading to the collapse of the fungus. Recent reviews and structural and functional studies describe the significant activity exposed in vitro to the most important phytopathogens, as well as the possibility of recombinant production and topical administration.

(Kumar et al., 2018) recommended that hydrolases which develop in the PR-3 family are the most well known as enzymatic AFPs which cleave chitin in the walls of fungi. They are known to suppress the growth and colonization of fungi, especially when expressed in excess in transgenic plants or applied externally. Reviews and experimental studies indicate chitinase formulations can be synergistically enhanced when combined with glucanases or other AFPs, and chitinase-mediated weakening of cell walls often sensitizes fungi to membrane-active peptides. This approach further complements the action of defensins, which are chitinases, to structural components of the cell other than lipids.

(Van der Weerden et al., 2023 / Bugeda et al., 2024) presented the strides made in heterologous expression systems has removed a historical bottleneck in the deployment of AFPs: scale-up production. Recent papers document the high-yield production of specific AFPs (defensins and fungal AFPs) using *Pichia pastoris*, improved E. coli fusion techniques, and plant molecular farming (transient expression in *Nicotiana benthamiana*) at commercially plausible yields and activities. These systems of production also maintain the critical levels of proper folding and disulfide bond formation needed for correct folding of cysteine-rich peptides.'

'(Pérez-Pérez et al., 2024) analyzed the science of formulation is critical for the development of 'useable' AFP crop protection products. Recent reviews on the encapsulation of peptides demonstrate how micro and nanoencapsulation (alginate, biodegradable polymers, and lipid carriers) and compatible adjuvants enhance surface persistence, protection from UV and proteolytic degradation, and controlled release, which are essential for the field performance of protein-based biopesticides. Encapsulation approaches using biodegradable carriers are adaptable to meet organic farming standards'.

(Shi et al., 2018 / Lee et al., 2024) discussed the combination of molecular farming and new plant systems (e.g., geminiviral vectors, optimized terminators) further enhances the protei yield in plant systems, offering a competitively priced alternative for AFP production not solely dependent on microbial fermentation'.

(Van der Weerden et al., 2023) proposed that the plant-based systems suspended in controlled greenhouses can capture pathways to regulatory approval by cultivating proteins in GRAS classified species. These systems can be integrated with microbial production to expand options for manufacturing AFPs. These advances broaden Atlanta's choices for AFP commercialization. In planta and small plot field studies provide empirical support for the efficacy of the AFPs. Exogenous applications of defensins and chitinase based formulations in replicated greenhouse and field tests decreased the incidence of diseases such as grey mold on tomato and decreased Fusarium severity on cereals with beneficial yields and minimal phytotoxicity. These studies show AFP treatments can achieve biologically meaningful control of eco-phones under realistic eco-phones. Large multisite trials are still limited.

(Slezina et al., 2023 / Chiu et al., 2022) advocated the environmental fate studies show that AFPs are rapidly biodegradable to amino acids and short peptides while yielding traces of persistent residues. Preliminary non-target assays (soil microbiome and nontarget beneficial fungi and pollinator surrogates) show negligible adverse impact at effective application rates and suggest that AFPs have comparatively lower ecological risks than many synthetic fungicides, which is vital for sustainable farming and regulatory acceptance.

(Tang et al., 2023 / recent AMP reviews) proposed that the resistance management is another persistent issue: because many AFPs have multi-target actions (membrane and intracellular effects or cell-wall disruption), the development of high-level resistance is believed to be less likely than for single-target agents. Nonetheless, the literature suggests that AFPs should be used within Integrated Pest Management (IPM) frameworks, with different controls applied sequentially, or used in combination with biological control to reduce selection pressure and prolong effective duration.

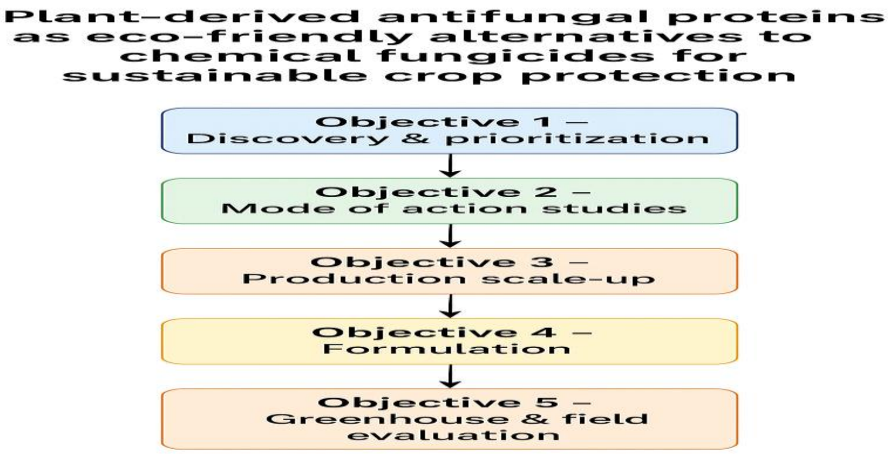
(Pérez-Pérez et al., 2024 / Lobel, 2024) analyzed the gaps the literature highlights—and which the proposed research will address include: (1) robust replicated multisite field trials of real-world efficacy and cost-benefit for farmers; (2) long-term ecological monitoring of non-target communities post repeated AFP application; (3) scaled techno-economic comparisons of microbial versus plant production; and (4) regulatory acceptable formulations with optimal stability, biodegradability, and controllable eco-toxicity. The incorporation of these missing elements in the development of AFPs is imperative in transcending the current stage of AFPs as mere research novelties and developing them into field-usable instruments for the sustainable protection of crops.

5. Methodology

This work will be carried out through five interconnected goals aimed at discovering, validating, and scaling antifungal proteins derived from plants and found to offer environmentally sustainable alternatives to chemical fungicides.

Objective 1 – Discovery and Prioritization of Candidate Antifungal Proteins

We will perform defensive peptide, hevein-like peptide, non-classical NCR, and chitinase bioinformatic mining through publicly accessible plant genomes and transcriptomes (NCBI, EnsemblPlants, PLAZA). Candidate sequences will be screened against the Antimicrobial Peptide Database (APD3). Predictive algorithms (AMP Scanner, SignalP, disulfide-bond predictor, etc.) will be used to select peptides with cysteine motifs, secretory signals, and probable soluble peptides. AFPs selected from the database will be chemically synthesized (if peptides <50 aa) or inserted into bacterial and yeast expression vectors using Gateway or Golden Gate cloning systems. In vitro antifungal activity against *Botrytis cinerea*, *Fusarium oxysporum*, and *Rhizoctonia solani* will be used with all candidates. They will be screened for minimum inhibitory concentrations (MICs) as well as growth, spore germination, and antifungal activity curves using known antifungal peptides as positive controls in 96-well broth microdilution systems.



Objective 2 – Mode of Action Studies

We will analyze cellular targets and stress responses triggered by the exposure of AFP. Membrane permeabilization will be measured by flow cytometry using propidium iodide and SYTOX Green components. Changes in the hyphal ultrastructure of fungi will be assessed using cell-wall binding assays with fluorescently labelled peptides and scanning/transmission electron microscopy. Fungal isolates exposed to sublethal doses of AFP will be subjected to RNA-seq transcriptomics and subsequent differential expression analysis of cell-wall remodeling, oxidative stress, and efflux pump genes to identify stress response pathways and putative resistance mechanisms.

Objective 3 – Production Scale-Up

We will optimize expression in E. coli BL21 (DE3) and *Pichia pastoris* X-33 for cysteine-rich defensins using *Nicotiana benthamiana* for Agrobacterium-mediated plant transient expression with geminiviral vectors to enhance proper folding. Affinity chromatography (Ni-NTA for His-tagged proteins) and RP-HPLC for synthetic peptides will be used for the purification. Microbial expression systems will be subjected to buffer optimisation and signal peptide analysis aiming to obtain yields of≥50 mg/L of purified proteins for secreted systems. Yields will be improved with codon-optimised genes and designed secretion signal peptides.

Objective 4 – Formulation Development and Stability Testing

The devise biodegradable, water-based formulations wherein AFPS will be microencapsulated in sodium alginate and other plant-derived biopolymers. The shelf-life of microencapsulated AFPS will be characterized with sugar alcohols and various natural adjuvants like stabilizers. Stability will be assessed with thermal, UV radiation, protease, and simulated rainfall, which will evaluate the rainfastness of the microencapsulated AFPS. Formulation candidates will be chosen for greenhouse and field tests as a function of retained bioactivity and sufficient stability for field conditions and bioactivity of the target organisms.

Objective 5 – Greenhouse and Field Evaluation

Dose–response and other target and non-target biological effect assessments will be performed and plotted on greenhouse-grown tomatoes and wheat while maintaining field and greenhouse standards. Small-plot field trials will be randomly blocked to measure disease incidence, crop yield, residue persistence (peptide concentration quantified with LC-MS), and phytotoxicity. 16S/ITS soil micro biome profiling, beneficial fungi assays, and bioassays with acute toxic compounds to surrogates for pollinator species will be used to assess non-target impacts. Development of resistance will be assessed using serial passage of fungal cultures using sub lethal doses of AFPs and sequencing the candidate resistance genes.

Data Analysis

The effectiveness and field data will be subjected to ANOVA and mixed-effects models, taking into consideration block and treatment effects. For RNA-seq data, standard bioinformatics pipelines (FastQC, HISAT2, DESeq2) will be performed and interfaced against phenotype data to associate mode of action with resistance potential. Collectively, these approaches will provide compelling AFP data regarding efficacy, safety, production feasibility and field performance.

6. Expected outcomes and impact

- At least two validated AFP leads with strong in vitro and in planta activity and characterized modes of action.
- Scalable production protocol(s) and prototype formulations with reasonable environmental fate and non-target impact profiles.
- Concept greenhouse and small plot efficacy data demonstrating comparable or complementary activity to chemical fungicides with a reduced environmental footprint.
- A regulatory and techno-economic outline detailing the path to commercialization and uptake by growers, including cost offset and reduced chemical fungicide deployment projections.

Environmental and socio-economic impact: reduced chemical residues, decreased rate of resistance development, enhanced sustainability of Integrated Pest Management (IPM) systems, and potential for application in organic farming.

7. Timeline (24 months, high level)

- Months 0–4: Bioinformatics mining, candidate selection, initial synthesis/cloning.
- Months 4–10: In vitro screening; mechanism of action assays.
- Months 8–14: Expression system optimization and production scale-up.
- Months 12–18: Formulation development and stability testing.
- Months 16–22: Greenhouse and initial small-plot field trials; non-target assays.
- Months 20–24: Data analysis, regulatory/tech-economic brief, manuscript and scholarship/funding reports.

8. Budget (indicative)

A precise budget pertaining to molecular biology reagents such as gene peptide synthesis, microbial expression and purification consumables, greenhouse and field trial expenses, sequencing along with microscopy, and minor stipends for two research assistants is as follows:

9. Ethics, biosafety and regulatory considerations

- All work will adhere to local biosafety regulations concerning recombinant DNA and field trials. Work on transient expression will be performed in greenhouse facilities where containment is practiced. Environmental fate and non-target assessments will adhere to OECD standards, where appropriate.
- Any potential transgenic approaches will only be considered in contained research and with some form of regulatory approval for trial releases.

10. Dissemination and capacity building

- Publishing in peer-reviewed journals, publicly accessible data repositories (APD3), open access preprints, and where appropriate, preprints.
- Policy briefs for regulatory bodies are complemented by field consultations and workshops with local extension workers and farmer organizations on implementation.

11. References

Advances of Peptides for Plant Immunity. (2025). MDPI. Available: https://www.mdpi.com/2223-7747/14/15/2452 MDPI

Bugeda A., et al. (2024). *High yield production of the antifungal proteins PeAfpA and ...* Plant Biotechnology Journal (Wiley). Available (PDF/DOI): https://onlinelibrary.wiley.com/doi/10.1111/pbi.70093 Wiley Online Library

Chaudhary S., et al. (2024). *Molecular farming for sustainable production of clinical-grade proteins.* PMC. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11258990/ PMC

Chiu, T., et al. (2022). *The Potential of Plant Proteins as Antifungal Agents for Crop Protection*. PMC. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC9305310/ PMC

Gong, Y., et al. (2024). *Antifungal Peptides from Living Organisms: Origin, Classification, Mode of Action and Potential Applications*. Frontiers in Microbiology. Available: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1511461/full Frontiers

Kumar M., et al. (2018). *Chitinases—Potential Candidates for Enhanced Plant Defense.* MDPI Agriculture. Available: https://www.mdpi.com/2077-0472/8/7/88 MDPI

Lobel B.T. (2024). *Current Challenges in Microcapsule Designs and Sustainability.* ACS Reviews. Available: https://pubs.acs.org/doi/10.1021/acsami.4c02462 ACS Publications

Parisi K. (2019). *The evolution, function and mechanisms of action for plant defensins* (review). ScienceDirect/Journal abstract. Available:

https://www.sciencedirect.com/science/article/abs/pii/S108495211730469X ScienceDirect

Pérez-Pérez V., et al. (2024). *Exploring the impact of encapsulation on the stability and bioactivity of peptides extracted from botanical sources.* PMC. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11266027/ PMC

Plant Defense Proteins: Recent Discoveries and Applications. (2025). PMC. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC12252001/ PMC

Recent high-yield production and demonstration in tomato: May 2025 plant biotechnology report (PeAfpA production/protection). Wiley Online Library

Shi X., et al. (2018). *Efficient production of antifungal proteins in plants using a ...* Europe PMC. Available: https://europepmc.org/article/pmc/pmc6523586 Europe PMC

Slezina, M.P. et al. "Plant Antimicrobial Peptides: Insights into structure and action." *2023 review*. PMC

Tang, R. et al. "Application of antimicrobial peptides in plant protection." *Frontiers in Plant Science*, 2023. PMC

Vakili-Ghartavol, M., et al. (2025). *Harnessing Plant-Derived Antifungal Agents and Their Nanoencapsulation: Mechanisms, Efficacy, and Potential*. ScienceDirect. Available: https://www.sciencedirect.com/science/article/pii/S2666154325006519 ScienceDirect

Van der Weerden N.L., et al. (2023). *Antifungal application studies (defensin field trials).* MDPI / publisher page. Available (example): https://www.mdpi.com/ (search "Van der Weerden defensin 2023") MDPI+1

Vriens K., et al. (2014). *Antifungal Plant Defensins: Mechanisms of Action and Potential Applications.* Frontiers in Microbiology. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6271847/ PMC

Yang, M., et al. (2025). *Genome-wide investigation of defensin genes in apple and their role in fungal pathogen response*. ScienceDirect. Available: https://www.sciencedirect.com/science/article/pii/S2095311924001114 ScienceDirect