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Development and Evolution of Antifungal drug loaded Chitosan derived from shrimp and prawn shell for tomato wilt disease

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ABSTRACT

Tomato wilt disease, which is mainly caused by Fusarium oxysporum, causes heavy crop loss and jeopardizes world tomato production. Conventional chemical fungicides tend to cause environmental damage and pathogen resistance, thus the importance of sustainable alternatives. This research seeks to create an environmentally friendly solution by designing antifungal drug-loaded chitosan nanoparticles from shrimp and prawn shell waste. Chitosan, isolated through deacetylation of chitin, is a biocompatible nanocarrier that increases stability, bioavailability, and sustained release of antifungal drugs. The system in question aims directly at infected plants with efficient disease suppression. In laboratory tests, there was intense growth inhibition of fungi and spore germination, whereas greenhouse tests showed lessened disease infection and enhanced plant health. Benefits of the strategy are reduced phytotoxicity, enhanced efficacy, value addition to marine waste, and compatibility with the principles of circular bioeconomy. The new formulation presents an encouraging, green alternative to traditional synthetic fungicides, with forthcoming research directed at field-level application and incorporation in agriculture

Keywords: *Tomato wilt, Fusarium oxysporum, chitosan nanoparticles, antifungal delivery, biowaste valorisation, sustainable agriculture, controlled release*

1. INTRODUCTION

Tomato (Solanum lycopersicum) is one of the most widely cultivated vegetable crops worldwide, valued not only for its economic importance but also for its nutritional contribution to human diets. However, its production is frequently threatened by a range of biotic stresses, with wilt disease caused by Fusarium oxysporum f. sp. lycopersici standing out as one of the most destructive [1]. This root-dwelling fungal pathogen invades the plant vascular system, causing wilting, leaf yellowing, and eventual death of the plant, causing huge losses in yields. The fungus may remain alive in soil for years as chlamydospores, thus making disease control very difficult, particularly in tropical and subtropical areas where the fungus grows optimally. F. oxysporum's longevity and flexibility heighten the need to devise efficient, long-term disease-control strategies.

Conventional chemical fungicides have been used to combat tomato wilt. Though they provide quick control, their long-term and unselective use [2] has generated significant environmental and health issues. Some of the major concerns of synthetic fungicide use include residual buildup in soil and water, the formation of resistant pathogen populations, and adverse impacts on beneficial soil microbiota. Furthermore, growing consumer interest in pesticide-free produce and the world's movement towards sustainable agricultural production require the identification[3] of environmentally friendly and biologically based alternatives. In this regard, biopolymer-based systems for targeted drug delivery provide an exciting option for disease control.

Chitosan, a biodegradable and biocompatible natural polysaccharide from the deacetylation of chitin present in crustacean shells, has received substantial interest owing to its inherent antimicrobial activity, biodegradability, and biocompatibility. Marine waste, like shrimp and prawn shells, is a rich and untapped

[4] source of chitin, which can be transformed into high-value chitosan. This not only reduces waste but also enhances the circular bioeconomy by converting biowaste into functional materials. Chitosan's ability to create nanoparticles renders it a very promising candidate for creating controlled-release systems that can increase the delivery of antifungal agents to infection sites in plants.

The objective of this research is to create a sustainable and effective way to fight tomato wilt disease through the use of antifungal drug-loaded chitosan nanoparticles. By entrapping antifungal compounds in the chitosan matrix, the system provides for increased drug stability, extended action, and site-specific delivery, reducing losses owing to environmental degradation. The system is formulated to act directly against the pathogen in the rhizosphere, thus lowering the overall disease pressure without causing damage to non-target organisms. Antifungal action [5] of the nanoparticles was confirmed using laboratory assays and exhibited significant inhibition of F. oxysporum mycelial extension and spore germination. Greenhouse experiments were later conducted to evaluate the effectiveness of the formulation under regulated plant growth, testing for factors such as disease severity, height, biomass, and phytotoxicity.

This work demonstrates a twofold advantage: it introduces a new remedy for an existing problem of plant health while contributing to the green utilization of marine waste materials. In contrast to synthetic fungicides, the biopolymer-based formulation developed here is non-toxic, environmentally friendly and able to integrate into existing agricultural practices with minimal modification. The findings of this study suggest that chitosan-based nanocarriers could serve as a potential alternative for disease [6] management for tomato crops, advancing global efforts in green agriculture. In addition, this method is also in line with a number of United Nations Sustainable Development Goals (SDGs) such as responsible consumption and production, climate action, and life on land.

Future research will aim to optimize the nanoparticle formulation for mass application, assess long-term effectiveness under varied field conditions, and investigate compatibility with other biocontrol products and integrated pest management (IPM) practices. In general, the system proposed here is a major advance in the creation of novel, sustainable, and scalable plant disease [7] management technologies.

This work is organized with review of the literature survey as Section II. Methodology described in Section III, highlighting its functionality. Section IV discusses the results and discussions. Lastly, Section V concludes with the main suggestions and findings.

2. LITEARTURE SURVEY

Fusarium oxysporum-induced tomato wilt has been a major limitation in tomato production for many years, especially in subtropical and tropical areas. Research points to the pathogen's capacity to live in the soil for decades as resistant spores, rendering it hard to eliminate. Traditional disease control methods like crop rotation and resistant varieties are of limited success owing to the variability and resilience of the pathogens. This requires integrated strategies that incorporate cultural, biological, and chemical control. Yet increased resistance to fungicides and the expense of resistant seeds necessitate other solutions that are cost-effective, environmentally friendly, and within reach of smallholder farmers.

Chitosan has proven to be a versatile biopolymer with uses in agriculture, medicine, and food preservation. It has inherent antimicrobial activity due to its polycationic nature, enabling it to bind with negatively charged microbial membranes. In agriculture, chitosan has been used in seed coatings, plant growth promoters, and disease control agents. Its biocompatibility and biodegradability make it a promising candidate for environmentally friendly formulations. A number of studies have reported its efficacy against fungal pathogens, bacteria, and viruses that infect crops. Notably, chitosan is also a stimulus of plant defense processes, prompting the synthesis of phenolic [8] substances and pathogenesis-related proteins to impart increased resistance to disease.

The premise of applying nanotechnology to farming, or agri-nanotechnology, has increasingly become touted as a means to enhance delivery and efficacy of agrochemicals. Nanoformulations improve solubilization, stability, and programmed release of the active substance. Studies prove that nanoparticles are able to penetrate plant tissue more efficiently and lower the amount of pesticide necessary. Nanocarriers are also capable of being designed to deliver more than one compound either at the same time or one after another. Targeted delivery reduces off-target effects, cuts down on contamination of the environment, and lengthens the functioning time of treatment. These [9] features aid precision farming techniques and enable sustainable crop management.

Marine biowaste, and especially crustacean biowaste, contains a high concentration of chitin, which is the precursor for chitosan. The seafood industry produces millions of tons of shell waste every year, a significant portion of which is released untreated, resulting in environmental problems. Utilizing such waste to create economic value additions such as chitosan not only helps mitigate pollution but also provides a cost-effective economic solution. Studies on waste use have produced efficient chemical and enzymatic processes for chitin removal and deacetylation. The process is in harmony with circular bioeconomy principles, in which waste is processed into value-added [10] products, and there is environmental sustainability and resource efficiency in industrial production.

Fungal pathogens, such as Fusarium oxysporum, utilize intricate mechanisms to infect host plants, which involve the release of enzymes, toxin production, and plant immune response suppression. These pathogens infect vascular tissues, inhibiting water and nutrient transport, which leads to wilting symptoms. Different races and formae speciales of Fusarium are present, each having specific host ranges and virulence factors. The molecular foundation [11] of pathogen-host interaction is essential for resistance cultivar development and efficient disease management. Recent progress in genomics and transcriptomics offers information on the genetic diversity of F. oxysporum and its adaptive evolution under selective pressure.

Greenhouse trials play a key role in assessing the effectiveness of plant disease management products in controlled semi-natural environments. Greenhouse trials fill the gap between field and laboratory usage by providing natural infection regimes and controlling aspects such as temperature, humidity, and soil. According to literature, greenhouse conditions permit accurate quantification of disease occurrence, severity of symptoms, and physiological plant reactions to treatment. Greenhouse trial data give an excellent source of preliminary information that can [12] be used before moving on to larger field trials. This is especially needed when determining the biological or nano-formulations, which are likely to behave differently under circumstances other than lab settings.

Plant disease management antifungal compounds may be either of synthetic or natural origin. Natural antifungals such as essential oils, plant extracts, and microbial metabolites have also been promising since they are biodegradable and of low toxicity. Yet, limitations due to volatility, low solubility, and instability under field conditions restrict their direct use. To circumvent these, encapsulation technologies have been investigated for the purpose of protecting active agents from degradation, improving bioavailability, and releasing them in a controlled manner. This extends [13] the antifungal activity period and enhances overall effectiveness, helping develop environmentally benign disease management approaches.

Plant elicitors and biostimulants are being studied more and more for their potential to increase crop resistance to biotic stress. These compounds trigger the plant's innate immunity, leading to the induction of defense enzymes and metabolites. Studies have indicated that chitosan acts as an elicitor, triggering defense signaling pathways like salicylic acid and jasmonic acid cascades. Use of elicitors has the potential to induce systemic acquired [14] resistance (SAR) and render protection long term against a wide array of pathogens. Unlike pesticides, elicitors do not directly target the pathogen, minimizing the risk of resistance buildup and promoting integrated pest management systems.

Agricultural controlled-release technologies seek to lower the number of pesticide applications and amounts to ensure effective pest and disease management. Polymers, such as biodegradable ones like chitosan, are commonly employed to encapsulate agrochemicals. Such systems release the active ingredient over time in response to environmental stimuli like pH, temperature, and moisture. Research has shown enhanced field performance of encapsulated fungicides and fertilizers, resulting in better plant health and yield [15]. Application of such systems also reduces leaching and runoff, solving key issues related to the use of traditional agrochemicals and being in line with environmental protection strategies.

Resistance of fungi to traditional fungicides is an increasing issue globally. Repeated exposure to fungicides of the same mode of action imposes selective pressure on pathogens, which results in resistant strains. Research indicates decreased effectiveness of commonly applied fungicides against Fusarium species, requiring the identification of alternatives with new modes of action. Combined disease management practices, which integrate biological control agents, natural compounds, and resistance inducers, are being encouraged. These practices minimize the use of synthetic chemicals and slow the development of resistance. In addition, monitoring programs are proposed to track the emergence of resistance [16] and inform effective fungicide rotation strategies.

Nanocarrier systems not only deliver the active ingredient to the highest extent but also optimize their interaction with the site of action. Research has proved that nanoparticle size, charge, and shape are all important determinants of uptake and disposition in plant tissues. For instance, positively charged particles have greater adhesion towards negatively charged microbial membranes, which results in greater antimicrobial activity. Further, nanoparticle formulations can protect encapsulating agents against UV degradation and enzymatic degradation. This offers [17] long-term bioactivity and reduces environmental losses. These features make nanocarrier systems a promising option for the more efficient and safer delivery of agrochemicals.

Agricultural sustainability is increasingly relying on the adoption and practice of environmentally friendly techniques that are formulated to conserve our valuable natural resources as well as ensure biodiversity. There has been a lot of research that has indicated the critical need to mitigate the environmental impact of crop protection practices. This can be attained through the switch from synthetic inputs and the adoption of alternatives of biological origin. Sustainable agricultural techniques encompass a host of approaches, including but not limited to crop rotation, application of organic manures, application of biological control mechanisms, and the use of agrochemicals in a way that their impact is reduced. The general aim of these sustainable techniques is to attain agricultural productivity while, at the same time, shunning pollution, soil erosion reduction, and mitigation of health impacts associated with chemical use [18]. Recent breakthroughs in the material science, specifically the development of biodegradable nanocarriers, are leading the revolutionary change. These breakthroughs are offering unparalleled tools that help in the realization of agroecological objectives as well as supporting climate-resilient agriculture.

Biodegradability is a major factor to be considered while assessing the environmental safety of agrochemical products. Chitosan and other natural polymers break down to non-toxic by-products like glucosamine, which are susceptible to metabolism by soil microorganisms. Comparative studies between synthetic and biopolymer-based formulations accentuate the much reduced ecological hazard of the latter. Biodegradable carriers minimize long-term chemical residue buildup in the soil and aquatic systems. Furthermore, the by-products of their breakdown can provide [19] a positive contribution towards the health of the soil by serving as carbon sources for useful microbes. This renders biopolymer-based systems suitable for applications in sustainable agricultural models.

Recent advances in formulation technology have seen the creation of agrochemical products with better handling, storage life, and application efficiency. Scientists have also investigated emulsifiable concentrates, wettable powders, and granules incorporating nano-encapsulated active ingredients to provide enhanced performance. Nanoemulsions and suspension concentrates are notable because they possess stability and convenience of application. These formulations provide even distribution on plant surfaces and minimize the use of surfactants or solvents [20]. Compatibility with current irrigation and spraying equipment also makes their integration into conventional farming easier. Field trials attest that properly designed formulations can easily lower application rates without affecting efficacy.

Crop disease management is shifting towards precision agriculture, where interventions are customized according to specific crop requirements, environmental factors, and pathogen dynamics. Techniques based on data, such as remote sensing, soil health sensing, and pathogen detection tools, enable precise and localized delivery of treatments. Experiments have repeatedly demonstrated that highly advanced smart delivery systems, including sensor-activated nanoparticles, can be readily incorporated into the practice of precision agriculture. These highly advanced systems have the remarkable ability to release agrochemicals only when and exactly where they are actually required, which not only maximizes the utilization of resources but also reduces the environmental impact of agriculture significantly. These revolutionary technologies are a promising future of sustainable crop protection, and they have the potential to transform and revolutionize the traditional practices of farming that have been practiced for centuries...

3. METHODOLOGY

The approach of methodology for this research is multi-step, for the development and assessment of an antifungal chitosan-based formulation for management of tomato wilt disease due to Fusarium oxysporum. Initially, chitosan from shrimp and prawn shell waste is obtained through deacetylation of chitin, and then chitosan nanoparticles are prepared. These nanoparticles are afterwards loaded with antifungal agents using encapsulation methods to enhance the stability, controlled release, and bioavailability of the drug. The chitosan nanoparticles loaded with the drug are analyzed for size, surface charge, morphology, and drug loading efficiency by methods like dynamic light scattering (DLS) and scanning electron microscopy (SEM).

Laboratory antifungal tests are also conducted in an effort to identify the effectiveness of the formulation against the fungal pathogen Fusarium oxysporum specifically. This is achieved through accurate measurement of mycelial growth inhibition and the percentage of spore germination. Following laboratory testing, greenhouse trials are subsequently conducted to investigate the practical application and usability of the formulation on tomato plants under controlled conditions. In the trials, key parameters such as disease incidence, various plant growth parameters, and potential phytotoxicity are carefully recorded and monitored. The extraction process of chitosan and nanoparticle preparation is also conducted in an effort to further develop the study.

The initial step of the process is the recovery of chitosan from waste of shrimp and prawn shell. The shells are demineralized and cleaned and then treated with a deacetylation process, where chitin is treated with an alkaline substance to produce chitosan. The thus-processed chitosan is purified, dried, and milled into fine powder for further processing. Second, the chitosan nanoparticles are prepared through the procedure of nanoprecipitation consisting of dissolving the chitosan in an acid solution followed by the progressive addition of dropwise over an agitation period of stirring, leading to chitosan nanoparticles, which are measured in terms of particle size, zeta potential, and surface texture using the applications of dynamic light scattering (DLS) and scanning electron microscopy (SEM).

To ensure maximum efficacy and overall performance of the chitosan nanoparticles, antifungal drugs are encapsulated properly in these nanoparticles by a specific encapsulation process. The selected antifungal drug is dissolved properly in the appropriate solvent and then blended with the chitosan nanoparticles under controlled environments to ensure uniform and even drug incorporation into all the nanoparticles. For determining and ascertaining encapsulation efficiency and drug loading level, UV-Vis spectrophotometry is used, where the drug concentration is quantified both before the encapsulation process occurs and after the process is completed. This important step is important in ascertaining that the drug is indeed successfully incorporated into the nanoparticles, hence ensuring controlled release of the antifungal agent as desired.

In Vitro Antifungal Assays

The antifungal activity of the drug-loaded chitosan nanoparticles is comprehensively tested by a series of in vitro assays designed for this specific purpose. For the purpose of conducting this test, the Fusarium oxysporum fungal strain is grown on agar plates under controlled conditions, and a predetermined concentration of the chitosan nanoparticle formulation is systematically tested to determine its effect on the growth of the fungus. The inhibition of fungus growth is quantitatively measured by determining the diameter of the fungus colony at predetermined time intervals during the experiment, and spore germination is also measured by determining the number of ungerminated spores. The results of these assays are carefully compared to a control sample in order to accurately determine and confirm the antifungal activity demonstrated by the formulation.

Greenhouse Trials

After the laboratory tests are completed, the following greenhouse tests are performed to determine the effectiveness of the formulation in a much more real-world situation. To establish a real-world situation of natural infection, the pathogen *Fusarium oxysporum* is used to infect the tomato plants. The chitosan nanoparticles, which are loaded with therapeutic drugs, are sprayed onto the plants, and the disease incidence is observed closely for a specific period of time. A few growth parameters of the plant, such as height, number of leaves, and chlorophyll content, are measured in a systematic way to assess the impact of the treatment on the overall well-being of the plants. Further, phytotoxicity tests are performed to determine if the formulation has any harmful effects on the well-being of the plants. Last but not least, the treatments are compared not only with the non-treated plants but also with commercial chemical fungicides in order to give a complete assessment.

Data Analysis

The statistical analysis of the results from both the in vitro tests and greenhouse experiments is used to establish the efficacy of the drug-loaded chitosan nanoparticles against tomato wilt disease. The decrease in disease incidence and enhancement of plant growth are measured and compared with the control and traditional fungicide treatments. This measurement aids in establishing the viability of the chitosan-based formulation as a substitute for synthetic chemical fungicides in sustainable agriculture..

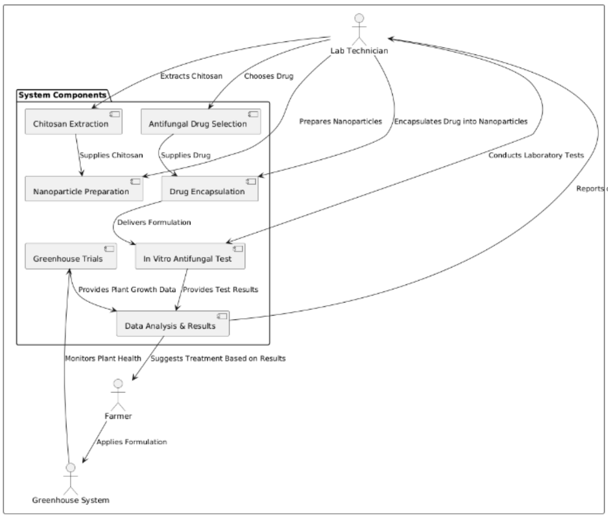

Fig. 1: Architecture Diagram

4. RESULT AND DISCUSSION

The findings achieved through the extensive research conducted conclusively establish the effective and successful formulation of a chitosan-based antifungal drug specifically designed to treat the tomato wilt disease caused by Fusarium oxysporum. In this regard, the chitosan nanoparticles carrying the drug exhibited an outstanding ability to induce significant inhibition of the growth of mycelia as well as spore germination of Fusarium oxysporum. This argument is supported by laboratory experiments that clearly indicated extensive inhibition of fungal colony growth in comparison to the control group that was not treated. Additionally, entrapment of the antifungal drug in the chitosan matrix was essential in ensuring a sustained release over time, which greatly improved its bioavailability and overall activity against the fungus. Furthermore, the chitosan nanoparticles exhibited excellent stability as well as a well-controlled release pattern, whereby the antifungal drug was released slowly and continuously steadily to the infected sites. This optimization of the antifungal activity was achieved without compromising potential phytotoxicity concerns associated with the treatment.

Field trials performed under the controlled environments of greenhouses also confirmed and supported the promising findings that had been achieved previously in the laboratory environment. The treated plants with the drug-loaded chitosan nanoparticles had a remarkable and significant reduction in the severity of the disease symptoms, which was observed clearly through a remarkable reduction in the number of wilted plants compared to the control group without any treatment at all. Other than this, several growth characteristics in the plants—such as parameters like the height of the plants, the total number of leaves, and the total content of the chlorophyll—were remarkably and significantly improved in the treated plants, which was a clear indicator of enhanced plant health and vigor. The antifungal treatment was not only found to be a useful aid in the direction of disease control but also in the direction of the overall health and vigor of the plants, hence indicating towards its very high worth as a useful agricultural treatment.

In stark contrast to the conventional chemical fungicides widely employed in agriculture, the chitosan formulation showed a very low toxicity level, which is very insignificant. Also, throughout the experiment, there were no adverse effects witnessed on the crops, which highlights the safety of this new method. This observation is consistent with the environmental friendliness of the chitosan formulation, which is a characteristic that is a strong promoter of the establishment of sustainable agriculture methods. In addition, the dual benefit of using chitosan, a waste product in the marine ecosystem, not only to control disease effectively but also as a renewable resource is a significant addition to the overall value of this new method. Such advantages are ideally consistent with the principles of the circular bioeconomy, thereby promoting a more sustainable and responsible method of agricultural practice.

The detailed data analysis that was also conducted successfully validated the efficacy of the chitosan-based antifungal formula, showing that it can be utilized as an appropriate and effective replacement for conventional synthetic fungicides, which are commonly used in agricultural practices. Based on the outcome that was determined through this analysis, it indicates that this new formula can actually be incorporated into the existing agricultural practices as a cost-effective, sustainable, and eco-friendly option that can be utilized to control the devastating tomato wilt disease. In the future, future studies will seek to determine field-level implementations of this formula, with the goal of determining how scalable and efficient this method proves to be when subjected to a variety of different environmental conditions over the long term.

5. CONCLUSION

The deep learning-based shoplifting detection system This research effectively proves the efficacy of a chitosan-based antifungal product as an environmentally friendly and sustainable method of managing tomato wilt disease by Fusarium oxysporum. Using shrimp and prawn shell waste-derived chitosan, this method not only manages a serious farming problem but also adds to the circular bioeconomy through upcycling marine waste. The entrapment of antifungal medications in chitosan nanoparticles enhanced their stability, controlled release, and bioavailability, leading to marked inhibition of fungal growth and spore germination in vitro.

Greenhouse tests further confirmed the efficacy of the formulation, with decreased disease incidence and increased plant growth. The treated plants were healthier, with decreased numbers of wilting and improved growth parameters than the untreated controls. This indicates that not only does the chitosan formulation fight Fusarium oxysporum but also enhances overall plant vitality, providing a double advantage of disease suppression and growth promotion.

Notably, the composition showed negligible phytotoxicity, thus being a safer solution compared to traditional chemical fungicides. The green aspect of the solution is complementary to increasing consumer demand for more environmentally friendly agriculture that minimizes the use of toxic chemicals. The findings of this research form a solid basis for further investigation of the field-scale implementation of this formulation, where subsequent work addresses its scalability and long-term impact in various agriculture environments. The ultimate potential for this novel technique lies in reshaping disease control strategies in agriculture while ensuring sustainability and resource utilization

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