

Potential role of Zinc Nanoparticles against Bacterial Leaf Blight of Wheat

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Abstract

Management of plant diseases through conventional agricultural techniques encounters several problems, leading to a serious threat to climate change and toxicity. The development of resistance to biotic stress in plants or by damaging the biochemical potential of pathogens can be possible with the use of Zinc nanoparticles (ZnNPs). This approach is economically feasible and eco-friendly. This chapter reviews the utilization of zinc nanoparticles and their role in plant defense mechanisms and their mechanism of action against the leaf blight pathogen. It was found that ZnNPs play a crucial role in regulating hormonal pathways and also improve the efficiency of various antioxidants to suppress the detrimental effects of bacterial pathogens. Deficiency of zinc makes plants susceptible to various pathogens, while its excess leads to destroying plant defense mechanisms and growth due to toxicity. This chapter discusses the level of zinc necessary for the normal functioning of plants and the role of ZnNPs in boosting the plant defense mechanism against bacterial leaf blight of wheat.

Keywords: Nanotechnology, Bacterial leaf blight, Biotic stress, Wheat, Antibacterial mechanism

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Introduction

On a global level, wheat is regarded as a basic food item and a vital source of food grains for humans. For a large portion of the world's population, particularly in North America, Europe, and Asia, wheat is a staple grain (Erenstein et al., 2022). Wheat is also regarded as a vital source of vitamins and fiber. There might be as much as 11-14% protein in wheat (Iqbal et al., 2022). But it also contains a concentration of lysine and other important amino acids. The production of wheat is reduced by 10 to 40 percent as a result of bacterial infections (Singh et al., 2023). Wheat bacterial infections can cause large yield losses as well as quality issues, such as discoloration, shriveled kernels, decreased grain weight, and poor milling qualities (Mehmood et al., 2023). Significant bacterial infections like *Xanthomonas translucens* pathogens' bacterial leaf streak and black chaff; *Pseudomonas syringae* pathogens' bacterial leaf blight & basal glume rot and diseases of minor importance due to the lack of research on them, like spike blight of wheat, bacterial mosaic of wheat, and gumming disease of spike, caused by genus *Clavibacter* pathogens (Jiang et al., 2022). Multiple aspects of controlling bacterial disease in wheat have been the focus of researchers and scientists, including disease-resistant cultivars, cultural practices, pathogen identification, disease characterization, and biological control techniques (Ayaz et al., 2023).

The management of bacterial disease in wheat has advanced significantly, but there are still a number of issues that need to be resolved, including a lack of integrated management strategies, sustainable disease management practices, extension and farmer education, quick and precise diagnostic tools, and limited disease resistance (Rajendiran & Rethnaraj, 2024). To overcome these obstacles and safeguard the wheat crop against bacterial illnesses, stakeholders must continue their research, work together, and share knowledge. For bacterial illnesses of wheat, this review offers a current, thorough explanation of the symptoms, disease cycle, epidemiology, and management techniques.

Nanotechnology in Agriculture and Managing Bacterial Plant Diseases

Enhancing nutrient utilization for plant development and improving plant disease resistance are the two main goals of nanotechnology in agriculture (Humbal & Pathak, 2023). Nanoparticle technology is crucial for solving plant-pathogen interaction-related agricultural problems and creating novel approaches to product protection. Currently, spraying is used to apply agricultural chemicals to crops; however, in order to be delivered safely, nano agrochemicals need to be designed with certain properties, such as high solubility, stability, efficacy, controlled release mechanisms, improved targeted activities, lower toxicity levels, and effective concentration (Ghorbanpour & Hatami, 2015). Nanoparticles (NPs) have enormous applications in many scientific domains. To combat the increasing risks of crop damage and disease brought on by a

variety of phytopathogens, such as bacteria, fungi, viruses, and some insects, they have significantly improved traditional techniques in the domains of agriculture and food sciences. Food scarcity has become a serious concern as a result of bacterial illnesses that cause widespread agricultural damage by establishing antibiotic resistance (Iqbal et al., 2025; Salam et al., 2023). To solve the problem of antibiotic resistance along with maximizing crop yield and immunity to diseases, several types of nanoparticles with such potential exists in market. NPs made using green synthesis methods have been shown to be more environmentally friendly and effective than those made using chemical processes (Madani et al., 2022). NPs have a variety of important roles in plants, including increased agricultural output, disease prevention, targeted medicine and pesticide delivery within the plant, and pathogen detection biosensors. NPs play important roles in energy transfer, DNA and protein entity modification, reactive oxygen species (ROS) generation, and cellular membrane disruption. They also help plant to cope the biotic and abiotic stress by altering many important mechanisms in plant (Gul et al., 2024).

Antibacterial Role of NPs

Several nanoparticles possess remarkable antibacterial properties, both bactericidal and bacteriostatic. Usability of NPs based on several factors such as their synthesis techniques, their structure and shape, concentration used, and to obtain the best one against bacteria many analyses are underway by the researchers (Slavin et al., 2017). While gram-negative bacteria with an extra outer layer of polysaccharides prevent NPs from entering bacterial cells, gram-positive bacteria with thick peptidoglycan layers create pores and negative charges on the cellular membranes, allowing NPs to enter the bacterial cells efficiently (Fazal et al., 2022). Research has suggested that biosynthesized metals (e.g., AgNPs, AuNPs, CuNPs), metal oxides (e.g., ZnONPs, TiONPs, CuONPs), carbon (e.g., GONPs), and magnetic (e.g., FeONPs) NPs present efficient antibacterial activity against plant bacterial pathogens (Gul et al., 2024). These properties are discussed in Table 1.

Table 1: Utility of nanoparticles in controlling various plant diseases spread by bacterial infection.

Nanoparticles	Bacteria	Crop	Diseases	Reference
ZnNPs	<i>Xanthomonas translucens</i>	<i>Triticum aestivum</i>	Bacterial leaf spot	(Hangamaisho, 2022)
MgONPs				
CuONPs				
ZnONPs	<i>Pseudomonas syringae</i>	<i>Triticum aestivum</i>	Bacterial leaf blight	(Ayisigi et al., 2020)
Nanosized Ag-Silica hybrid Complex (NSSC)	<i>Pseudomonas syringae</i>	<i>Arabidopsis thaliana</i>	Blight diseases	(Chu et al., 2012)
SiO ₂ NPs	<i>Pseudomonas syringae</i>	<i>Arabidopsis thaliana</i>	Blight diseases	(El-Shetehy et al., 2021)
AgNPs	<i>Pseudomonas syringae</i>	<i>Nicotiana benthamiana</i>	Tobacco wildfire	(Jiang et al., 2022)
ZnONPs	<i>Fusarium oxysporum f.sp. lentis</i>	<i>Lens culinaris</i> Medick	Gall formation	(Siddiqui et al., 2018)
	<i>Pseudomonas syringae</i> pv. <i>syringae</i>		Nematode multiplication	
			Bacterial wilt	
			Leaf spot	
ZnONPs	<i>Pantoea ananatis</i>	<i>Zea mays</i>	Maize white spot	(Mamede et al., 2021)
ZnONPs	<i>Xanthomonas translucens</i>	<i>Triticum aestivum</i>	Bacterial leaf streak	(Ocoy et al., 2013)
Calcium Nanocrystals	carbonate <i>Xylella fastidiosa</i>	<i>Olea europaea</i>	Olive quick decline syndrome	(Baldassarre et al., 2020)

Understanding Bacterial Blight in Wheat

Bacterial leaf blight, caused by *Pseudomonas syringae* pv. *Syringae* Van Hall was first identified as an important wheat disease in the early 1970s. The bacterium is found in soil in large quantities and inhabits wheat leaves as a member of the resident bacterial population. Additionally, it is seedborne (Nadeem et al., 2025). The pathogenicity of the strains to wheat varies greatly among the population. According to some theories, the rise in bacterial leaf blight cases could be brought on by a shift in *P. syringae* virulence linked to increased vulnerability of more recent cultivars (Lamichhane et al., 2015). Certain environmental factors can cause leaf blight. These are times of rainy weather, particularly driving rain, which could help the virus enter the leaf, and temperatures ranging from 15 to 25°C. Initially appearing as tiny, wet patches, symptoms gradually get bigger (Nadeem & Batool). Over a few days to develop bleached white necrotic patches and uneven tanning as well as streaks. Even though none were noticeable previously, symptoms frequently start to show up abruptly around the onset stage. Due to the fact that virulent strains live on the leaf surface, symptoms may appear quickly on every leaf when the environment supports the bacterium's growth and spread (Rajput et al., 2024).

Many plant diseases, notably wheat leaf blight, are caused by *P. syringae*. *P. syringae* is a very diverse group that is a member of *Pseudomonas*' RNA homology group I, which is a subclass g. from the Proteobacteria class. This species exhibits genetic diversity and the DNA homology of its pathogens ranges from 40 to 100%, while that of strains of one specific *P. syringae* strains were characterized using a variety of molecular approaches as mentioned in Table 2, which revealed that there was significant genetic variability in the various strains, such as those that were separated from the identical host (Khezri & Mohammadi, 2018).

Classification and Features

Gammaproteobacteria is the class to which *P. syringae* belongs. There is still much disagreement regarding the species' precise categorization. According to Young and associates, all fluorescent and plant-pathogenic oxidase-negative *Pseudomonas* isolates should be grouped into a single species, *P. syringae*, which will then be further subdivided into pathovars (Young et al., 1978). Large genetic variation

among the groupings has been revealed by several DNA hybridization investigations; nonetheless, biochemical traits, with a few exceptions, prevented those groups from being elevated into different species (Dudnik & Dudler, 2013).

Table 2: *Pseudomonas syringae* pv. *Syringae* strains studied in wheat.

Strain	Host	Stage of isolation	Location	References
PW1	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Dariush et al., 2012)
PW2	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Dariush et al., 2012)
PW3	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Khezri & Mohammadi, 2018)
PW4	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Siahkal	(Khezri & Mohammadi, 2018)
PW5	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Siahkal	(Gul et al., 2024)
PW6	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roobar	(Gul et al., 2024)
PW7	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Amlash	(Gul et al., 2024)
PW8	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Amlash	(Gul et al., 2024)
PW9	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Talesh	(Gul et al., 2024)
PW10	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Talesh	(Dariush et al., 2012)
PW11	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Astara	(Dariush et al., 2012)
PW12	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Astara	(Dariush et al., 2012)
PW14	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Astara	(Dariush et al., 2012)
PW15	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Dariush et al., 2012)
PW16	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Ayisigi et al., 2020)
PW17	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Siahkal	(Ayisigi et al., 2020)
PW18	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Siahkal	(Ayisigi et al., 2020)
PW19	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Amlash	(Ayisigi et al., 2020)
PW20	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Ayisigi et al., 2020)
PW21	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Ayisigi et al., 2020)
PW22	WHEAT (<i>Triticum aestivum</i>)	<i>In vivo</i>	Roodbar	(Ayisigi et al., 2020)

PW=*Pseudomonas* wheat

Challenges in Controlling Bacterial Leaf Blight

Certain meteorological variables determine the disease's significance for wheat. Several days with high humidity, low temperatures (15–25°C), and a lot of rain are when the disease is most frequently seen (Von Kietzell & Rudolph, 1997), and plants are more susceptible at the boot stage. Otta (1974) revealed significant output losses in South Dakota, where areas with 75% or more necrotic leaves experienced extremely high infection severity due to an epidemic outbreak. Additionally, growing sensitive cultivars like Chris, Era, Scout 66, and Winoka resulted in large yield losses in North America. Since resistant wheat varieties have supplanted these susceptible ones, the disease is now sometimes found in North America. For the bacterial leaf blight disease brought on by *P. syringae* pv. *Syringae*, there are no conventional control techniques. However, planting very susceptible varieties is discouraged.

Zinc Nanoparticles (ZnNPs) Synthesis

Zinc nanoparticle (Zn NP) eco-friendly synthesis techniques have drawn interest because they have less adverse effect on the environment than conventional chemical synthesis techniques. Biological resources like plant extracts, microbes, and biopolymers are frequently used as reducing and stabilizing agents in these "green synthesis" techniques (Alsaiani et al., 2023). Because of its simplicity, affordability, and capacity to generate zinc nanoparticles without producing any toxic byproducts, plant-based synthesis is especially preferred (Ehsan et al., 2022). Commonly utilized plant extracts include those from *Azadirachta indica* (neem) and *Cassia fistula* (golden shower); these extracts contain bioactive substances such as flavonoids, tannins, and alkaloids that help reduce zinc ions into nanoparticles (Agarwal et al., 2017). The most widely used technique for the straightforward synthesis of ZnONPs from leaves or flowers involves thoroughly washing the plant portion under running water and sterilizing it with double-distilled water (some people disinfect it with Tween 20) (Dejene & Geletaw, 2024). The plant portion is then allowed to dry at room temperature before being weighed and crushed with a crusher and pestle. Using a magnetic stirrer, the mixture is continuously stirred while Milli-Q H₂O is introduced to the plant portion in accordance with the desired concentration (Rajeshkumar et al., 2016). Whatman filter paper is used to filter the solution, and the clear solution that is produced is used as a plant extract (sample). To ensure effective mixing, a certain volume of the extract is combined with 0.5mm of hydrated zinc nitrate, oxide, or sulfate, and the combination is cooked at the appropriate temperature and duration (Ochieng et al., 2015).

At this stage, some people optimize by varying the temperature, pH, extract concentration, and time. The mixture turns yellow during the incubation phase, providing visual proof of the produced NPs (Rajeshkumar et al., 2016). The synthesis of NPs is next verified by UV-Vis spectrophotometry, then the mixture is centrifuged, and the pellet is dried in a hot air oven to obtain the crystal NPS (Yasmin et al., 2014). Moreover, X-ray diffractometers (XRD), energy dispersion analysis of X-rays (EDAX), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), *in vivo* emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM), thermal-gravimetric differential thermal analysis (TG-DTA), photoluminescence analysis (PL), X-ray photoelectron microscopy (XPS), Raman spectroscopy, attenuated total reflection (ATR), UV-visible diffuse reflectance spectroscopy (UV-DRS), and dynamic light scattering (DLS) are used to further characterize synthesized nanoparticles (Ali et al., 2014).

Physicochemical Properties

Nanoscale size increases their specific contact area, which, in turn, increases reactivity and allows for better interactions with bacterial membranes (KhokharVoytas et al., 2023). Some of the unique physicochemical characteristics of ZnNPs include high surface area, individual size of particles, and structural stability that make them more effective antibacterials (Gohar et al., 2024). In addition, their shape, which is often spherical or rod-shaped, breaks bacterial cells by affecting particle stability and promoting interactions through membrane adsorption (Sirelkhatim et al., 2015). ZnNPs' antimicrobial properties comprise the capacity to penetrate biofilms and bear environmental conditions, making them more resilient and ideal to apply in antibacterial coatings as well as in medical devices. They also have the ability in them to produce reactive oxygen species (ROS) when exposed to light. Even in the presence of different environmental stressors, a persistent and effective antimicrobial action against diverse pathogenic microorganisms is ensured by the retention of high reactivity coupled with structural integrity (Regiel-Futyra et al., 2017).

Antibacterial Mechanism of Zn NPs

Production of Reactive Oxygen Species (ROS)

Upon UV or visible light exposure, zinc nanoparticles (Zn NPs) can produce reactive oxygen species such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($\text{OH}\cdot$), and superoxide anions (O_2^-). Oxidative stress induced by these highly reactive ROS degrades critical cellular components such as proteins, lipids, and DNA in bacterial cells, as indicated by Figure 1. Bacterial viability is disrupted by this oxidative stress, which disrupts biological activity. Zn NPs are versatile for applications over a spectrum because they can generate ROS even without light, which means they should also be efficient in dark environments (Ali et al., 2018). As earlier stated, internalization of ZnONPs within the bacterial cell is not always required for their toxicity. Environmental alterations close to the bacterium, like the release of ROS or enhancement of ZnONP solubility, can lead to cell damage (Hajipour et al., 2012).

Disruption of Cell Membrane

Nanoparticles also have great usefulness due to their specific interaction with the bacterial cell membrane. ZnNPs have positive charge on them that allows them to bind with the negatively charged bacterial cell membrane, leading to membrane instability (AlQurashi et al., 2025). Increased membrane permeability due to this interaction can lead to the release of ions, proteins, and DNA, which ends up in cell lysis and death. Zinc nanoparticles (ZnNPs) are shown by studies to be excellent at entering and lysing bacterial membranes due to their small size and positive surface charge (Lallo da Silva et al., 2019).

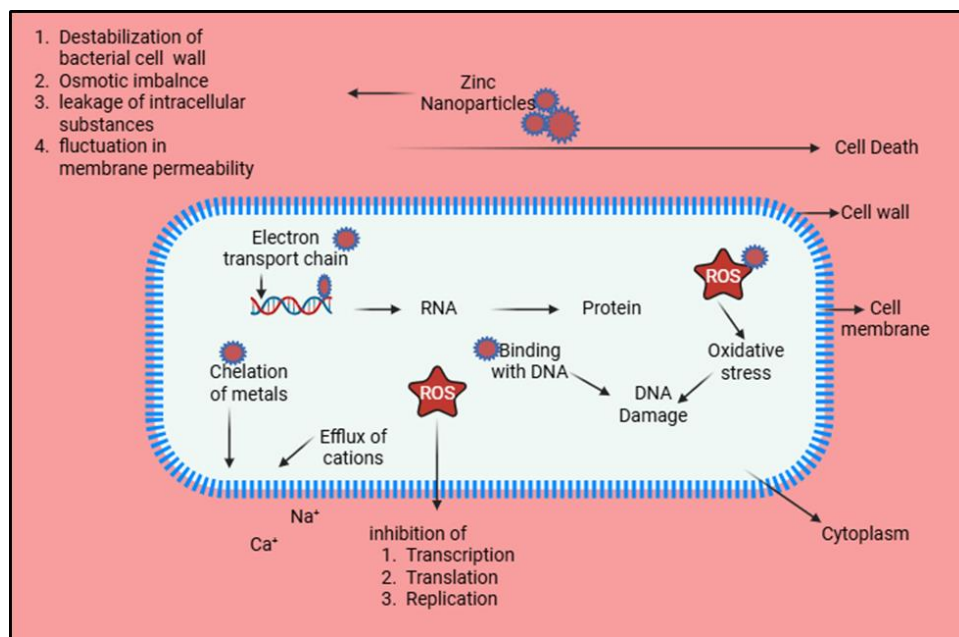


Fig. 1: Mechanism of action of Zn nanoparticles in generating ROS and cell membrane disruption, which leads to bacterial cell death.

Nutrient Deprivation

ZnNPs bind with the bacterial membrane more strongly as compared to other required nutrients for growth and development of bacteria, and when these nutrients are blocked to get entry into the cell, cell may damage and death can occur of such cells (Sirelkhatim et al., 2015). Research indicates that this inhibition can lead to a significant reduction in the uptake of essential nutrients, thereby affecting the growth and viability of bacteria (Mustafa et al., 2024). In addition, ZnNPs can induce conformational alterations in transport proteins, which further inhibits their activity. These conformational changes can be caused by contact of ZnNPs with the lipid bilayer of the membrane, potentially destabilizing the membrane-anchored transport proteins (Altunbek et al., 2018).

Consequently, the metabolic activities on which bacteria rely for energy and reproduction are disrupted, and nutrient uptake efficiency decreases. Production of ROS by ZnNPs may also be responsible for interference with nutrient transport. Membrane lipids and transport proteins are some of the cellular structures which ROS can damage, further interfering with the mechanisms through which nutrients are absorbed (Ozougwu, 2016). This oxidative stress might overwhelm the protective mechanisms of the bacteria and further inhibit their capacity

to sustain homeostasis as well as absorb vital nutrients. Furthermore, it has been proved through research that Zn NPs can affect the proton motive force (PMF), necessary for most bacterial species' active uptake of nutrients. A number of transport processes rely on the PMF to operate, and ZnNPs can interfere with it, resulting in a domino effect on nutrient uptake (Wang et al., 2024).

Application Methods and *In vivo* Efficacy of ZnNPs

ZnNPs for Seed Nano-Priming

Seed priming when done with ZnNPs they boost the growth, nutrient uptake and also able plant to be tolerant against stresses. Due to small size of NPs they can easily move inside the plant tissues through cell wall and promotes the activities of various important pathways of cell. For instance, research on rice has shown that ZnNP priming results in taller plants, stronger root systems, and higher yields. In drought situations, where nano-primed seeds exhibit improved root development, water uptake, and enzyme activity, this technique has been proven to be very successful in helping the plant adapt to stress (Nile et al., 2022).

Foliar Application

ZnNPs can also be applied to plants through foliar spray of it after making their liquid solution of certain concentrations. Foliar application facilitates rapid absorption of zinc nanoparticles (ZnNPs) through leaf pores, making them instantly available for physiological activities. ZnNPs promote improved plant growth and biomass accumulation by increasing chlorophyll production and photosynthetic efficiency. Because Zn NPs may break down bacterial cell walls and stop infections like bacterial leaf blight, foliar sprays are also very helpful in reducing pathogen attacks and enhancing plant health (Hong et al., 2021).

Environmental and Health Considerations

Numerous creatures, especially aquatic ones, have been demonstrated to be toxicologically affected by zinc nanoparticles. For example, research shows that zinc nanoparticles (ZnNPs) can build up in aquatic creatures, which can result in bioaccumulation and even biomagnification in food webs. Significant acute toxicity was noted in tests employing models such as *Artemia salina* and zebrafish, with deadly dosages resulting in physical deformities and impairing general health. This emphasizes how crucial it is to assess the amounts at which these nanoparticles endanger aquatic environments (Sibiya et al., 2022). Since biological/green methods are made from plants, beneficial bacteria, algae, and other sources, they are safe, economical, biocompatible, and environmentally friendly, making them less harmful to the ecosystem than physical and chemical approaches. As plant extracts consist of large amount of useful phytochemicals and also easily available and environmentally friendly approach, has gained attention for the manufacturing and synthesis of NPs. Beyond plants, microorganisms can also be used to synthesize NPs as their extract also enriched with many important nutrients and vitamins (Rajeshkumar, 2016). Microorganisms that are the beauty of nature and also play crucial role in the cycling of nutrients and the health of ecosystems, can be impacted by these nanoparticles and change the chemistry of the soil. Research has shown that ZnNPs might alter soil microbial activity, which could upset the equilibrium of soil ecosystems (Chan & Dudeney, 2008).

Challenges in *In Vivo* Application

In Vivo applications of NPs are not yet gained much attention by the small scale farmers as their resources are much limited and they are reluctant to use or implement new technologies rather they preferred traditional practices more to gain benefit immediately (Vamuloh et al., 2020). Another problem that reduce their *In Vivo* application is scalability issues as they can be produced in laboratory settings but scaling up these NPs to meet the agricultural requirements need much attention. Homogeneity in their size, quality and manufacturing procedures is necessary to make nanoparticles more effective. The methods employed must also be economically viable and sustainable in order to promote widespread adoption in agricultural operations (Ur Rahim et al., 2021). Regulatory limitations are a significant barrier to the application of ZnNPs in agriculture. The long-term effects on ecosystems and the potential toxicity of zinc nanoparticles on non-target animals need to be thoroughly examined. Due to no strict check and balance and regulations of NPs farmers also hesitate to employ these NPs in agriculture settings (Mishra et al., 2022).

Future Prospects and Challenges

Green synthesis of NPs is the demand of the time due to reason that they are synthesized from renewable sources and research institutions are trying their best to shift their synthesis to green method in place of metallic synthesis of NPs. Along with their synthesis methods researchers are also focus to maximize their efficacy and their potential to tackle the problems for which they are used. In addition, novel methods for surface modification are being investigated to enhance ZnNPs' antibacterial activities. Such modification increases the antibacterial activity of nanoparticles by increasing their interaction with microbial cells. As ZnNPs and other therapeutic agents can be employed synergistically in order to overcome microbial resistance, nanocarrier systems comprising them are very interesting as well. Not only does this holistic approach enhance overall treatment effectiveness but also reduce the chances of infection resistance. Exploring new applications, including the addition of zinc nanoparticles to agricultural applications, is also becoming more popular. To enhance plant health and disease resistance, they can be incorporated in foliar sprays and seed treatments. This can increase agricultural output and lower the demand for chemical pesticides.

Conclusion

Traditional farming techniques of disease management have several setbacks, which present a significant hazard to toxicity and climate change. One of the possible, sustainable, and economically viable ways of controlling bacterial leaf blight of wheat through a multitude of ways

is employing specific nanoparticles, particularly zinc nanoparticles. They modify signal transduction pathways, enhance antioxidant activity, and destroy the infections' several crucial metabolic processes. ZnNPs' environmental safety is also aided by green synthesis methods, making them suitable for sustainable agriculture. A delicate balance between zinc's effectiveness and toxicity, though, must be achieved to optimize plant health and production for optimal outcomes.

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