Bio-fabrication of Zinc Oxide Nanoparticles from *Picea smithiana* and their potential antimicrobial activities against *Xanthomonas campestris* pv. *vesicatoria* and *Ralstonia solanacearum* causing bacterial leaf spot and bacterial wilt in *Solanum lycopersicum* L.

By

Fazal ur Rehman

Department of Plant Sciences

Faculty of Biological Sciences, Quaid-i-Azam University

Islamabad, Pakistan

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A Thesis Titled

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Dissertation Submitted for

The Fulfillment of the Requirement for the Award of Degree of Master of Philosophy in Plant Sciences

By

Fazal ur Rehman

Department of Plant Sciences

Faculty of Biological Sciences, Quaid-i-Azam University

Islamabad, Pakistan

DEDICATION

To my Research Supervisor Prof. Dr Hassan Javed Chaudhary; I will be failed in my duty if I do not acknowledge the esteemed scholarly guidance, assistance and knowledge, I have received from him towards fruitful and timely completion of this work.

&

To my Parents for their understanding and continuous support to complete this research work.

APPROVAL CERTIFICATE

This is to certify that the dissertation entitled **"Bio-fabrication of Zinc Oxide Nanoparticles from *Picea smithiana* and their potential antimicrobial activities against *Xanthomonas campestris* pv. *vesicatoria* and *Ralstonia solanacearum* causing bacterial leaf spot and bacterial wilt in *Solanum lycopersicum* L."** submitted by **Fazal ur Rehman** is accepted in its present form to the Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan, as satisfying the dissertation requirement for the degree of Master of Philosophy (M.Phil.) in Plant Sciences.

Supervisor: _____

Prof. Dr. Hassan Javed Chaudhary
Professor,
Department of Plant Sciences,
Quaid-i-Azam University,
Islamabad.

External Examiner: _____

Dr. Muhammad Akmal
Associate Professor,
Institute of Soil and Environmental Sciences,
PMAS Arid Agriculture University,
Rawalpindi.

Chairman: _____

Prof. Dr. Mushtaq Ahmad
Professor,
Department of Plant Sciences,
Quaid-i-Azam University,
Islamabad.

PLAGIARISM CERTIFICATE

It is certified that **Fazal ur Rehman (02042113042)** has submitted her M.Phil. dissertation entitled **"Bio-fabrication of Zinc Oxide Nanoparticles from** *Picea smithiana* **and their potential antimicrobial activities against** *Xanthomonas campestris* pv. *vesicatoria* **and** *Ralstonia solanacearum* **causing bacterial leaf spot and bacterial wilt in** *Solanum lycopersicum* L.**"** that has been checked on Turnitin for similarity index (plagiarism).

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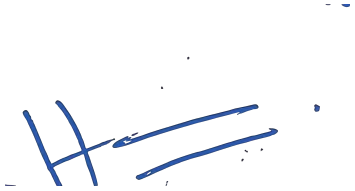

Dr. Hassan Javed Chaudhary
Professor,
Department of Plant Sciences,
Quaid-i-Azam University,
Islamabad.

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I, **Fazal ur Rehman,** student of Master of Philosophy (M.Phil.) in the subject of Plant Sciences session: 2021-2023, hereby declared that the matter printed in this thesis titled **'Bio-fabrication of Zinc Oxide Nanoparticles from** *Picea smithiana* **and their potential antimicrobial activities against** *Xanthomonas campestris* pv. *vesicatoria* **and** *Ralstonia solanacearum* **causing bacterial leaf spot and bacterial wilt in** *Solanum lycopersicum* L**"** is my own work and has not been printed, published, and submitted as research work, thesis or publication in any form in any university, research institution etc. in Pakistan or abroad.

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(Fazal ur Rehman)

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ABSTRACT

Plant diseases and pests are responsible for 20–40% crop losses annually, threatening food security. Agrichemicals are extensively used to manage diseases and pests but their use is raising concerns due to their toxicity to humans and the environment. Nanotechnology has the potency of transforming the pesticide industry by decreasing toxicity, extending durability, and enhancing the dissolution rate of poorly water-soluble pesticides, which can all positively influence the environment. Biogenic-based synthesis of nanoparticles like zinc oxide nanoparticles (ZNPs) are of high interest for various applications such as the management of plant diseases because of their distinctive characteristics compared with engineered nanomaterials synthesized from bulk minerals. The prospect of producing ZNPs with biological activity is of heightened interest for their application against plant pathogens. ZNPs have antimicrobial capabilities and their characteristics depend on their size, shape, and reactivity. As a result, microbe-mediated ZNPs synthesis has dramatically expanded in recent years as an alternative to chemical and physical synthesis methods. These ZNPs have promising targeted antimicrobial properties and low phytotoxicity activities, making them appealing for anti-oxygenic, antiviral, antibacterial, and antimycotic activities against a variety of pathogenic microbes, and for improve agricultural productivity, although potential biosafety issues need to be considered. Due to an inevitable disadvantage of chemical or physical synthesis routes, biosynthesis approach to nanoparticles, especially metallic oxide is attractive nowadays. Metallic oxides nanoparticles (NPs) present a new approach to the control of plant pathogens. ZnO nanoparticles (ZNPs) have very important role in phytopathology. In current study, biosynthesized ZNPs were tested against two devastating bacterial pathogens including *Xanthomonas campestris* pv. *vesicatoria* and *Ralstonia solanacearum* causing bacterial leaf spot and bacterial wilt in tomato. ZNPs were produced using a new extract from the plant *Picea smithiana* using an environmentally friendly, cost-effective and simple procedure. Zinc acetate was added to *P. smithiana* extract, stirred and heated to 200 °C. The white precipitation at the bottom were clear indication of synthesis of nanoparticles, which were further dried by subjecting them at 450 °C. X-ray diffraction pattern (XRD) determined that the ZNPs had a crystallite size of about 26 nm, Fourier transform infrared spectroscopy (FTIR) indicated a peak between 450-550 cm^{-1} and the particle size estimated by Dynamic Light Scattering (DLS) was about 25 nm on average. Scanning electron microscopic (SEM) analysis indicated that the particles were hexagonal in shape 31 nm in diameter. Antibacterial tests showed ZNPs synthesized by *P. smithiana* resulted in clear inhibition zones of 20.1 ± 1.5 and 18.9 ± 1.5 mm and 44.74 and 45.63 % reduction in disease severity and 78.40 and 80.91 % reduction in disease incidence in *Xanthomonas compestris* pv. *vesicatoria* and *Ralstonia solanacearum* respectively at concentration of 100 µg mL^{-1}. Our findings reveal that the concentration of ZNPs was important for their efficient antibacterial activity. Overall, the biosynthesized ZNPs have been found to have effective antimicrobial activities against bacterial wilt and bacterial leaf spot in tomato.

ABSTRACT

Plant growth can be impacted by ZnO nanoparticles (ZNPs), in addition to their effects on photosynthesis and seed germination. The degree to which nanoparticles enhance or inhibit shoot and root growth can vary depending on the specific type of nanoparticles and their concentrations. This research aimed to explore the effects of ZNPs on tomato plants (*Solanum lycopersicum* L.). As nanotechnologies continue to advance rapidly, the likelihood of biological systems being exposed to an excessive number of NPs is increasing. The study measured the growth of the plants, their photosynthetic abilities, chlorophyll, physiology responses, antioxidant activities in 24-day-old plants. The results indicated that ZNPs applications improved germination percentage (30.7%) shoot length (47.6%) and root length (33.1%) of the tomato plants, particularly at 75 µg/mL, and enhanced chlorophyll content including chlorophyll-a (44.4%) and chlorophyll-b (34.1%) and carotenoid content (51.4%) in a manner proportional to the concentration. Moreover, in current research, it has been found that ZNPs applications improved antioxidant response including DPPH (9.7%), TPC (71.7%), TAC (64.1%), TFC (43.0%) and TRP (68.9%). The production of antioxidant enzymes including SOD (27.8%), APX (22.5%), CAT (34.4%) and GOX (6.9%). ZNPs also had a beneficial impact on various root parameters including number of root tips (29.6%), number of branching points (16.6%), average diameter (46.2%), branching frequency (26.6%), total root length (33.1%), network area (26.3%), surface area (24.0%), perimeter (22.0%) and root volume (25.2%). ZNPs had very influential impact these root parameters. According to our best knowledge, this is a novel aspect to study of impact of ZnO nanoparticles on root architecture of *Solanum lycopersicum* L. A significant increase has been found in all root parameter up to 75 µg/mL. Above this concentration, a reduction in all of them have been noticed. The findings collectively indicate that concentrations of ZNPs above 75 µg/mL are harmful to tomato plants. The toxicity is indicated by decreased chlorophyll levels and a damaged photochemical system, hindering photosynthesis and causing a decrease in biomass growth. Moreover, ZNPs appear to stimulate the transcription of various genes which are antioxidant activities, implying that they may bolster the plant's defense mechanism by enhancing antioxidant enzyme activity.

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List of Abbreviations

$AgNO_3$	Silver Nitrate
ATCC	American Type Culture Collection
BHI	Brain Heart Infusion
BSA	Bovine serum Albuimn
CLSI	Clinical & Laboratory Standards Institute (CLSI)
CPS	Counts Per Second
CRA	Congo Red Agar
E. coli	*Escherasia coli*
EDTA	Ethylenediaminetetraacetic acid
EFTEM	Energy-Filtering Transmission Electron Microscoy
ELIZA	Enzyme Linked Immuno-Sorbent Assay
EPS	Extra polymeric Substances
FCC	Face Cubic Centered
FIC	Fractional Inhibitory Concentration
FTIR	Frustrated Total Internal Reflection
FWHM	Full-width at half maximum
LB	Luria Bertani
LPS	Lipopolysacchride
MDR	Multi Drug Resistant
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentrationrs
MPA	Microtittre plate Assay
MRSA	Methicillin Resistant Staphlococcus aureus
MRSE	Methicillin resistant Staphylococcus epidermidis
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NM	Nanao-Materials

NPs	Nanoparticles
OD	Optical Density
PBS	Phosphate Buffer Saline
PEG	Poly Ethylene Glycol
PET	Positron Emission Tomography
PLGA	Poly (lactic-co-glycolic acid
PLH	Poly (l-histidine)
PMS	Phenazine metho-sulfate
RBCs	Red Blood Cells
ROS	Reactive Oxygen Species
S. aureus	*Staphylococcus aureus*
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
TBE	Tris/Borate/EDTA
TEM	Transmission electrons Microscopy
TSC	Tri-Sodium Citrate
UV-Vis	UV-visible spectral analysis
WHO	World Health Organization
XRD	X-Rays Differection
XTT	2, 3-bis [2-Methoxy-4-nitro-5-Sulfophenyl]-2H-tetrazolium-5-carboxanilite inner salt
$ZnSO_4$	Zinc Sulphate

CHAPTER # 1

INTRODUCTION

1. Introduction

1.1. Background

The tomato, a plant in the Solanaceae family, is the second-largest vegetable crop in Pakistan. Tomatoes are advantageous to a balanced and healthy diet. It frequently appears in food and is important to the world's food supply. Given that it is a significant commercial and industrial crop in many parts of the world, it is one of the most significant vegetables (Babalola 2010). The tomato is high yielding, labor-intensive, and has a limited economic life span. Compared to cereal crops, it provides producers with somewhat higher profits and increases the job options for rural workers. In addition to being eaten as a fresh vegetable or salad, tomato is also cooked in sauces and soups, a complement to many vegetable, meat, and fish dishes, and it may be used to make a variety of products, including ketchup, sauces, purées, pulp, and juices (Nachay 2018). Both dry and canned tomatoes are crucial processed foods economically. It has a considerable amount of calcium, iron, vitamin A, and vitamin C (31 mg per 100g) lycopene, a powerful antioxidant found in abundance in tomatoes, helps prevent the spread of certain cancer forms (Borguini & Ferraz da Silva Torres 2009).

On 4.8 million ha, 182 million tons of tomatoes are produced annually, yielding an average of 38 tons per hactre (Ronga *et al.* 2019). The nations that produce the most tomatoes globally are China and India. Among the nations that produce tomatoes, Pakistan comes in at number 33 (Wahid *et al.* 2017). Fresh tomatoes are exported around the world for $8.8 billion, and the value of tomatoes and tomato-related items is now above US$13 billion (Abutalib & Rajeh 2021). Spain is second in the world in terms of exports after Mexico (Pacheco‐López 2005). The USA, Belgium, and Russia are the top importers of fresh tomatoes, respectively (Costa & Heuvelink 2018). Pakistan uses 61 000 hectares of land for tomato farming, yielding an average of 9.5 tonnes per ha and producing 569 000 tonnes of tomatoes annually. After starting from a relatively low foundation in 2001, Pakistan's tomato output increased in the 2000s; nevertheless, the nation is losing its comparative advantage globally as the per ha yield of tomatoes stagnates while the global yield has been steadily increasing during the same time period (Ray *et al.* 2012). Only 25% of the global average yield comes from Pakistan. However, the country's demand for tomatoes and tomato-related products is growing at a pace of 7.3% annually, which is far faster than the country's domestic production growth and is the reason for the growing tomato trade deficit (Johnson 2014). The nation misses out on the opportunity to participate significantly in global export markets and profit from the rapidly rising export of fresh tomatoes and tomato-related products. Pakistan produces only 28% of the average global export price, highlighting the formidable difficulties in improving the tomato value chain. While the global average export-production ratio is 4.7%, the country can only export less than 1% of its total output (Timmer *et al.* 2015). Due to lower farm gate pricing than the global average, Pakistan's export-production ratio has a lot of room for growth.

The Pakistani Planning Commission commissioned this study to identify gaps and potentials, establish a plan with strategies, and put those plans into practice along the tomato value chain in order to increase tomato competitiveness in both domestic and international markets (Louw *et al.* 2008). To account for the regional context, this study's analysis was done at the level of tomato clusters. Numerous stakeholders along the full tomato value chain were involved in order to gather relevant information, Identify opportunities and gaps, then suggest ways to take advantage of those opportunities (Horton *et al.* 2022). Macro data were also analyzed, and related literature was studied. Though grown throughout, there is a greater concentration of tomato farms in Sindh, KP, and Balochistan (Faheem *et al.* 2015). Punjab is dispersed over the province and has a limited overall area. Three main clusters are found in this study and are given in-depth investigation. Which are:

- The Sindh cluster, with Thatta serving as its hub, consists of the districts of Badin, Mirpurkhas, and Badin
- The Baluchistan cluster has Qilla Saifullah as its focal point and includes the districts of Barkhan, Nasirabad, and Barkhan
- The Khyber Pakhtunkhwa cluster has Swat as its focal point and includes Charsadda, Malakand, and Swat (Mehboob & International Studies 2011).

Each cluster's focal point district was built with the intention of concentrating development efforts there. Each cluster was thoroughly characterized, and then a SWOT analysis was performed. This analysis reveals numerous performance gaps in the value chain segments of production, processing, and commerce for each cluster. A few of concerns are inadequate national and international collaboration for the sharing of germplasm, a lack of research, notably on technologies for harvest and post-harvest management and value chain challenges, and the development of scientific ability. Climate change also contributes to water scarcity and rising temperatures (Duhan *et al.* 2017). Lack of improved tomato open pollinated germplasm, commercial cultivars, and local hybrids to produce high quality marketable tomato fruit suitable separately for table consumption and processing. Lack of certified tomato seed, pricey hybrids, healthy tomato nurseries, value chain development for trading in the high-value fresh market.(Kumar *et al.* 2021).

Benchmarks and performance targets are established based on the average global yield, quality, and export, and the interventions are created to meet these targets over a five-year period in order to handle the complex issues in production, product development, and marketing (Noriega *et al.* 2019). For each of the three clusters, particular treatments have been suggested based on these factors while also considering the gaps and potentials. These interventions include the introduction of new varieties and hybrids that have been acquired from other provinces or imported from other countries with a climate similar to Pakistan, in addition to promoting best value chain practices like appropriate picking, handling, transportation, packing, and grading, etc (Smith *et al.* 2021). through building infrastructure for the value chain, such as tomato packing

facilities and improved transportation, among other things. Enhancing institutional and farmer capacities will boost their capacity to create items for the market; one such incentive is to encourage tomato processing, especially the production of tomato puree and tomato powder (Louafi & Welch 2021). These interventions will be started by the government and finished with help from the private sector, including farmers, traders, and their organizations/groups. The development/upgrading plan will reportedly cost US$12.4 million in total for all clusters. The government will cover about 55% of the total investment costs by encouraging tomato-related research, increasing stakeholder capacity, and offering 20% in subsidies and interest-free loans for the construction of infrastructure for the tomato value chain and processing as well as tissue culture labs. These incentives are anticipated to encourage the remaining 45% of the investment to be made in the private sector.

It is suggested that each province should fund the remaining 80% of the public sector investment for the improvement of its tomato cluster, with 20% coming from the PSDP of Pakistan's Planning Commission. Higher operational expenses at various value chain levels will result from these cluster investments, which will be shared by the relevant stakeholders. In comparison to Baluchistan, Sindh, and KP, the upgrade plan's Internal Rate of Return (IRR) is predicted to be 75% over a five-year period when all investment and operational costs, including as those associated with production, processing, and marketing, are taken into account. The total upgrading plan's anticipated Net Present Value (NPV) over a five-year period is $14.4 million USD. The summary sheet that is being provided provides information on the investment, operational costs, benefits, IRR, and NPV by cluster. It should be noted that these costs, benefits, and rates of return are merely approximate for each cluster's focal point (Yang *et al.* 2020). However, we anticipate a significant spillover effect from the investment in each cluster's non-focal areas. Wide-ranging economic and social effects are anticipated from these measures, including increased production of better-quality tomatoes, increased export and income, and the establishment of employment for all tomato industry participants in the three clusters (Samoggia *et al.* 2022). However, the following prerequisites must be satisfied in order to expect these results. To assist them in overcoming the economies of scale issue that small farmers confront, producers are grouped into Farmers' Entrepreneur Groups (FEGs), ii) The operation of infrastructure associated to the value chain, iii) value chain-related reforms are implemented, and iv) relationships between farmers and other stakeholders are improved.

1.2. Tomato Industry of Pakistan

1.2.1. Pakistani Tomato Production

Small farmers in Pakistan are the principal producers of tomatoes. Tomato availability and price varies greatly throughout the year as a result of the production process' extensive seasonality. Pakistan grows two crops each year, the first in the spring and the second in the autumn. However, in Sind, Pakistan's southern region, tomatoes can be cultivated all year round. In Punjab, KP, and Balochistan's cooler regions, tunnel farming is well known (Rana *et al.* 2022). With a tomato-growing area of 27.9 thousand hectares, Sindh has surpassed Khyber Pakhtunkhwa (KP),

Balochistan, and Punjab as the province with the largest tomato production. The Sindh province's climate and soil conditions are ideal for the cultivation of tomatoes. Sindh province has a total tomato area share of 43.6%. Baluchistan, which accounts for 20.8% of tomato-growing land and 24.6% of the nation's production in 2016, is Pakistan's top-producing tomato region and has significant potential for the crop (Table 1.1).

Table 1.1. Tomato yield and area production by Pakistani province (CABI 2017).

Province /District	Area (000 ha)	Production (000 tonnes)	Yield (t/ha)	Share in area (%)	Share in production (%)
Punjab	8.1	105.6	13.0	13.4	18.6
Sindh	26.4	195.8	7.4	43.6	34.4
Khyber Pakhtunkhwa	13.4	127.6	9.5	22.1	22.4
Balochistan	12.6	140.0	11.1	20.8	24.6
Pakistan	60.5	569.0	9.4	100.0	100.0

Source: MNFS&R (2017)

Pakistan's tomato-planting area expanded at a rate of 4.9% per year, from 29.4 thousand ha in 1995 to 61.9 million ha in 2016. The production climbed by 587.1 thousand tonnes in the same year, representing an average yearly growth rate of 4.4%, similar to the preceding case. Without any improvement in yield per hectare, area expansion accounted for the whole rise in tomato production in the nation (Asfaw 2021). Sind has the highest average growth rates for both area and production, at 12.8% and 15.4%, respectively. This may be due to Sindh's tomato producers receiving better prices due to the crop's early season production. The per hectare yield has only slightly improved in Punjab and Sindh (Table 1.1).

In KP, the area and production of tomatoes have decreased; the causes of this need to be looked into. Since 2008, a consistent downward trend has been observed in the Baluchistan tomato region, possibly as a result of the province's water deficit (Ain *et al.* 2020). While the Punjabi government carried out the Australian AID initiative, which saw plastic tunnels delivered to all tomato-growing districts to increase yield. However, because to Sind's faster increase in area and yield, the province's relative share has declined over time. Growing and marketing issues for Pakistani tomato farmers will be covered in the following sections. These limitations lower per ha yield while also lowering yield quality. Up to 40% of the tomato crop is lost each year due to inadequate post-harvest infrastructure, post-harvest illnesses, and short produce shelf lives (Firdous & Research 2021). To fulfil growing local demand and remain competitive in global trade, it is crucial to enhance per-ha output, reduce post-harvest losses, and improve tomato quality.

Table 1.2. Trends by province in tomato production and area during 2001-2016 (CABI 2017).

Year	Punjab		Sindh		KP		Baluchistan		Pakistan	
Year	Area	Prod	Area	Prod	Area	Prod	Area	Prod	Area	Prod
	(000)h	(000)T	(000)h	(000)T	(000)h	(000)T	(000)h	(000)T	(000)h	(000)T
2001-02	4.5	62.2	5.8	32.8	14.1	146.2	5.0	52.9	29.4	294.1
2002-03	4.8	65.2	6.1	35.0	14.6	148.3	5.5	57.8	31.0	306.3
2003-04	5.2	64.0	6.2	35.7	15.1	157.5	12.5	155.6	39.0	412.8
2004-05	5.1	63.7	6.1	34.0	15.8	146.9	14.4	181.6	41.4	426.2
2005-06	5.3	64.6	9.4	48.3	16.1	161.6	15.4	193.6	46.2	468.1
2006-07	5.3	64.8	8.7	60.5	16.1	160.8	17.0	216.2	47.1	502.3
2007-08	5.5	70.1	10.9	91.8	16.1	162.0	20.6	212.3	53.1	536.2
2008-09	5.6	72.5	12.3	100.9	16.5	161.8	19.0	226.7	53.4	561.9
2009-10	6.0	77.9	12.2	100.4	13.1	119.3	18.7	179.2	50.0	476.8
2010-11	6.7	87.8	14.6	114.8	12.6	113.2	18.4	213.8	52.3	529.6
2011-12	6.7	86.0	18.8	141.6	13.7	129.9	18.2	220.4	57.4	577.9
2012-13	6.6	86.3	22.5	174.8	13.6	131.1	15.5	181.9	58.2	574.1
2013-14	7.8	100.1	27.0	200.6	14.0	135.7	14.4	163.3	63.2	599.7
2014-15	7.4	94.6	27.3	202.4	13.3	132.0	12.7	141.6	60.7	570.6
2015-16	7.4	106.2	27.9	206.5	13.7	130.0	12.9	144.4	61.9	587.1
Annual growth (%)	**3.7**	**3.9**	**12.8**	**15.4**	**-1.0**	**-1.5**	**4.7**	**4.5**	**4.9**	**4.4**

Source: MNFS&R (2016)

Note: The difference in the growth rates of production and area can be taken as growth rate in per ha yield in every region.

1.2.2. Pakistan's consumption of tomatoes

One of the most popular and necessary ingredients in Pakistani kitchens, tomatoes are used to prepare practically any vegetable and enhance flavor. Tomato consumption has a high demand elasticity of income. Tomato demand will therefore increase as a result of urbanization, economic growth, and population rise. Pakistan had one of the lowest per capita tomato consumption rates in the world in 2013, at 4.8 kg, according to the FAO Balance Sheet data (Herforth *et al.* 2020). Tomato consumption per person is rising significantly in Pakistan, nevertheless. a 7.5% annual growth rate on average, it has grown from 1.86 kg in 2001. The increased demand for tomatoes is in part due to the urban population's shifting dietary habits, particularly with regard to fast food, which is linked to a large increase in income and urbanization (Cockx *et al.* 2019).

1.2.3. Trade in Pakistani Tomatoes

Being a net importer of fresh tomatoes and tomato paste, Pakistan has a growing tomato trade deficit. Despite the fact that it fell to US$32.4 million in 2017, it climbed from US$0.33 million in 2001 to US$114.9 million in 2016. Between 2001 and 2017, the nation's tomato trade

imbalance grew at an average rate of 49% annually (Morci *et al.* 2020).Despite a substantial rise in domestic tomato production, the growing tomato trade deficit is related to a shift in people's eating habits, particularly with regard to fast food, in the nation. 9 tons in 2001, a very low number, to 24 000 tons in 2016,

Table 1.3. Trade of tomato and tomato products of Pakistan during 2001-2017

Year	Export					Imports					Trade Deficit
	Fresh tomato		Tomato paste		Total export	Fresh tomato		Tomato paste		Total import	
	Q	V	Q	V	V	Q	V	Q	V	V	V
	(tonnes)	(000 US$)	(tonnes)	(000 US$)	(000 US$)	(tonnes)	(000 US$)	(tonnes)	(000 US$)	(000 US$)	(000 US$)
2001	9	1	15	2	3	1585	70	641	260	330	327
2002	234	15	0	0	15	1592	74	622	273	347	332
2003	2413	187	40	20	207	328	29	554	267	296	89
2004	1566	170	34	22	192	512	54	924	375	429	237
2005	224	24	68	59	83	609	70	702	426	496	413
2006	4965	825	40	50	875	1040	198	1883	1078	1276	401
2007	525	94	28	40	134	3089	412	1708	1067	1479	1345
2008	998	179	51	73	252	35860	7213	2721	1518	8731	8479
2009	40907	5051	148	320	5371	104584	34502	1311	1421	35923	30552
2010	5692	903	254	406	1309	76890	22579	1028	1236	23815	22506
2011	45142	18858	304	749	19607	171319	77098	1468	1657	78755	59148
2012	9704	4156	613	1361	5517	247984	115179	1035	1437	116616	111099
2013	5403	2134	275	717	2851	265353	132336	2717	3021	135357	132506
2014	14173	5522	453	1176	6698	287406	126152	3975	5016	131168	124470
2015	9184	3541	720	1757	5298	269285	90851	3944	4928	95779	90481
2016	24792	9465	742	1597	11062	254546	120746	3666	5246	125992	114930
2017	1994	777	740	1733	2510	56855	29430	3596	5470	34900	32390
Growth rate (%)	36.1	48.6	47.2	59.91	41.31	45.49	60.80	11.67	20.81	45.78	48.79

Source: FAOSTAT, Production, Crops http://www.fao.org/faostat/en/#data/QC

Pakistan's tomato exports have increased considerably, representing a 36% yearly growth. During the time, the value of tomato exports has also grown significantly. It is important to note that these growth rates appear spectacular due to the very low base from which they were calculated; Otherwise, the nation still has a negligible export market presence. Pakistan's shipments also kept fluctuating significantly, demonstrating the unreliability of the nation's tomato export market. Tomato export growth rates are substantially lower than import growth rates, on the other hand. In terms of value, Pakistan ranked 14th in the world for tomato imports in 2016. Between 2013 and 2017, The country imported fresh tomatoes and tomato paste for between $135 million and $35 million (Buckle 2018). The fact that Pakistan now sells primarily to the Middle East and

Afghanistan highlights the necessity to find new markets in order to enhance tomato exports during the season of excess supply.

1.3. Bacterial Pathogens of Tomato

1.3.1. Bacterial spot

Bacterial spot caused by *Xanthomonas* spp. is a tomato disease that can pose a significant threat and is challenging to manage under high disease pressure and favorable environmental conditions. The development of the disease is encouraged by warm temperatures and abundant precipitation. The pathogen can spread through different means, including wind-driven rain, handling plants, and seeds. All aboveground parts of the plant can be impacted by the disease. Additionally, the pathogen has shown resistance to copper in various locations. A study conducted in 2017 found copper resistance in *Xanthomonas perforans* populations in Jasper and Smith Counties, Mississippi. It is currently unclear what the prevalence of copper-resistant pathogen populations in Mississippi is. The affected tissues display round, brown lesions that can merge to create dark streaks. Leaves with numerous lesions may turn yellow, and leaves where lesions combine may die off quickly. Dead foliage can stay attached to the plant. Lesions on leaflets can also look like a shot-hole, where the center of the lesion falls out of the leaflet. On fruit, lesions take on a raised blister-like appearance and eventually turn brown and scabby. Symptoms of bacterial spot are quite similar to those of bacterial speck and can be easily mistaken for each other.

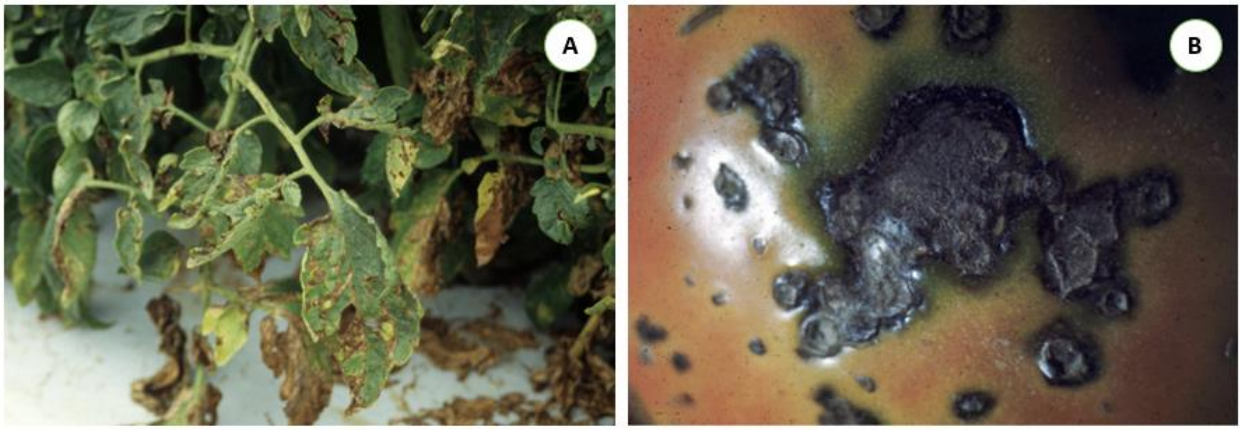

Figure 1.1. (A). Lesions on tomato leaves with bacterial spot. (B). Lesions on a tomato fruit with bacterial spot.

1.3.2. Bacterial wilt

Bacterial wilt caused by the *Ralstonia solanacearum* bacterium can cause extensive harm to both field and greenhouse tomatoes. Favorable conditions for the disease's development include high soil temperatures and moisture levels. This pathogen can infect various crops, and even in the absence of a susceptible crop, it can survive in the soil for extended periods. Once the pathogen is established in a field, it becomes challenging to manage bacterial wilt. The disease can also spread through contaminated water, equipment, tools, and human workers. *R. solanacearum* is classified into five races or subgroups, and not all of these are present in the United States. The tomato-

infecting Race 1 is widespread in the southeastern United States. The pathogen's all strains are classified as select agents by the USDA Animal and Plant Health Inspection Service (APHIS), requiring diagnostic laboratories to follow USDA protocol while handling samples testing positive for this bacterium. Positive diagnoses must also be reported to USDA APHIS. Bacterial wilt causes rapid wilting and death in plants, without displaying symptoms such as yellowing of plant tissue or death of plant tissue. Wilting is generally irreversible, and a brown lesion may appear on the stem's exterior near the plant's base. When cut at the soil/media line, the stem's inside may seem dark and waterlogged. In advanced infections, the stem may be hollow, and cut stems may show abundant bacterial streaming.

Figure 1.2. (A). Brown stem lesion on a tomato with bacterial wilt. (B). Vascular discoloration in a tomato with bacterial wilt. Bacterial streaming from a stem of a tomato plant with bacterial wilt

1.4. Nanotechnology and Plant Pathology

The chemical, pharmaceutical, mechanical, and food processing industries all use nanotechnology extensively. It is currently regarded as a validated cutting-edge technology. Nanotechnology also has applications in computers, electricity production, optical, pharmaceutical delivery, & environmental science (Faisal *et al.* 2021). The green synthesis of ZnO-NPs has environmental benefits as well as numerous biomedical uses. In the synthesis of biogenic ZnO-NPs, metabolites found in the aqueous extract of *Picea smithiana* act as an oxidising, reducing, and capping agent. Plant-based nanoparticle development has many advantages over traditional physicochemical approaches and has numerous applications in medicine and science. The *Picea smithiana* leaf extracts used in this study were used to make zinc oxide (ZnO) nanoparticles (NPs). The technique of biosynthesis of zinc oxide nanoparticles from moringa leaves is examined in this research. Due

to its affordability and lack of harmful environmental effects, this green culinary technique is supported by many academics (Abel et al., 2021). Due to their antibacterial, UV-blocking, and most powerful catalytic and photochemical effects, zinc oxide nanoparticles (ZnO NPs) have received a great deal of interest in practical applications. It is easier to use plant-based leaf extract to create nanoparticles. There is no venom emitted into the environment, nor is it hazardous. Additionally, it needs to be replaced by other nanoparticle preparation techniques (Constantinescu & Sicuia 2020). The goal of the present study was to develop a biomimetic method for the green synthesis of environmentally friendly zinc oxide nanoparticles from *Picea smithiana*. This was accomplished by utilizing UV-visible spectroscopy, scanning electron microscopy, X-ray diffraction characterization, and antimicrobial studies (Alrajhi *et al.* 2021). Chemical reduction, lithography, and beam epitaxy, are only a few of the high-tech techniques used to manufacture nanoparticles. However, the materials utilized in these procedures are both cheap and unfriendly to the environment.

Traditional medicine uses the *Picea smithiana* plant. It is a small tree with thick bark that can be found from northern Pakistan almost to the nothernmost point of India. The plant is also rich in nutrients and contains a variety of phytochemicals. they demonstrate the presence of proteins, carbohydrates, terpenoids, steroids, cardiac glycosides, alkaloids, tannins, and phenols. It is abundant in minerals like calcium, sodium, potassium, and iron as well as vitamins like vitamin C, vitamin E, and vitamin A.

1.4.1. Biological method for the synthesis of zinc oxide nanoparticles

In comparison to conventional chemical and physical methods, biological NP synthesis has a benefit. The "green" approach to NP synthesis has grown in popularity in recent years. When using traditional chemical techniques, it is expensive and necessary to use chemical compounds or organic solvents as reducing agents (Hussain *et al.* 2016). The green synthesis method creates NPs through an environmentally friendly process, as opposed to the conventional chemical reduction method, which uses toxic chemicals that can later result in a variety of health issues due to their toxicity. The green synthesis method is advantageous for biological applications where purity is a concern because it is free of impurities (Ahmed *et al.* 2017). The use of toxic chemicals on the surface of the NPs and non-polar diluents in the chemical synthesis method restricts their use in clinical and biological fields (Mittal *et al.* 2013). The process of coating with biogenic surfactants or capping agents results in NPs synthesized by plants having a higher rate of synthesis, showing variations in shape and size, and being more stable and biocompatible than NPs produced by other organisms (Singh *et al.* 2018). The quicker rate of synthesis is caused by the biological capacity to serve as a catalyst for reactions in aqueous media under usual temperature and pressure conditions.

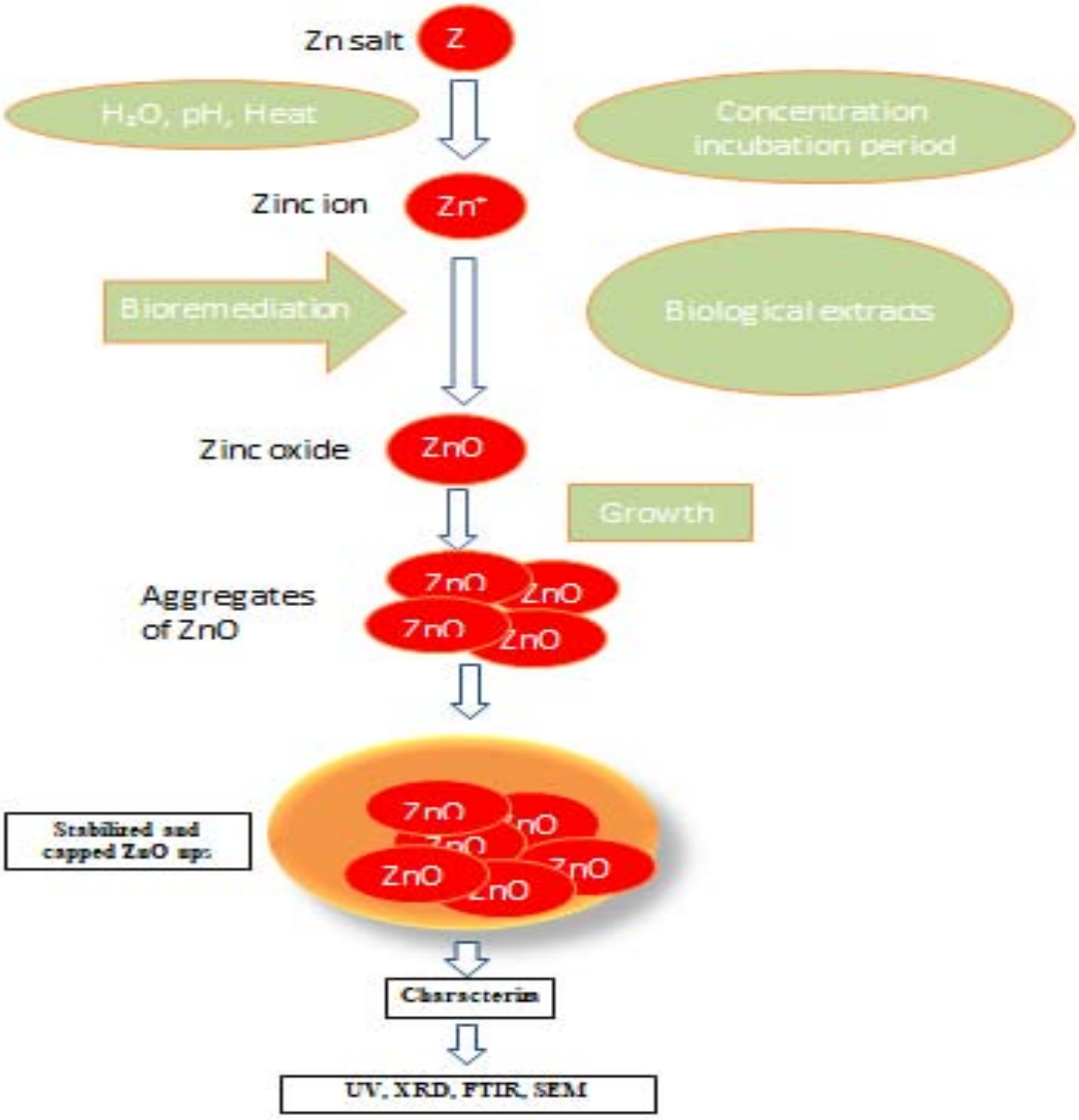

Figure 1.3. Possible process by which zinc oxide nanoparticles are produced biologically.

A diagrammatic representation of the potential biological synthesis mechanism of ZnO NPs has been given in figure. In the biological synthesis of NPs, a metal salt solution is simply combined with a plant/microorganism extract at room temperature. Within hours, the synthesis process is finished. There are many reports on the environmentally friendly synthesis of ZnO NPs. This report summaries a study that used *Picea smithiana* plant leaf extract to biosynthesize ZnO nanoparticles.

1.4.2. Nanoparticles: Alternatives against Drug-Resistant Pathogenic Microbes

Due to the abundance of natural resources and the technique's reputation as being more environmentally friendly, many scientists have recently developed an interest in the green synthesis of nanoparticles (Adam *et al.* 2021). The synthesis and creation of various nanomaterial's are central to the emerging field of nanotechnology. Nanoparticles are objects with sizes ranging from 1 to 100 nm that, due to their size, differ from the bulk material. Copper, zinc, titanium, magnesium, gold, alginate, and silver are now being used to create a variety of metallic nanomaterials (Akhtar *et al.* 2018). Nanoparticles manufactured by nanotechnology range in size from 1-100nm (Yaqoob *et al.* 2020). The human eye cannot perceive such small sizes. Nanoparticles are made up of only a few hundred atoms (Isaac *et al.* 2022). Nanoparticles can operate as an alternative to antibiotics by interacting with bacterial systems and inhibiting or killing them. The many methods in which nanoparticles can kill bacteria are determined by the core size, surface chemistry, and size of the nanoparticles, among other factors (Munir *et al.* 2020).

1.4.3. Types of nanoparticles

There are two major types of nanoparticles including organic and inorganic nanoparticles. Organic nanoparticles are made up of organic molecules or polymeric structures. These can be synthesized by both top down and bottom-up approach. Organic polymeric molecules are in the form of vesicles, micelles, polymerases etc. Conjugation between these systems can result in variation of sizes in organic nanoparticle (Zappi *et al.* 2019). The examples of organic nanoparticles are lipid based and polymeric based nanoparticles. Lipid based nanoparticles possess an internal core that is made up of lipid and an external core that is stabilized by the use of surfactants and emulsifie (Rawat *et al.* 2019). Same is the case with polymeric nanoparticles which contain two cores, the inner core contains a solid mass which on the outer surface various molecules are adsorbed (Rao *et al.* 2000). The other type of nanoparticles includes inorganic nanoparticles. Their major component is the inorganic material like metal, metal oxide, carbon etc. The carbon-based inorganic nanoparticles include carbon based nanotubes that are 1-2 nm in diameters and in the form of rolling graphite sheets. Other carbon based inorganic nanoparticles include fullerenes that have gained wide industrial importance due to their unique electrical conductivity, electron affinity, high strength etc (Ijaz *et al.* 2020). Metal based nanoparticles also come under the type of inorganic nanoparticles. They are purely made up of metals and are known to possess unique electrical properties due to characteristic surface resonance (Kurbanoglu & Ozkan 2018). Although there are many types of nanoparticles but our focus is on zinc nanoparticles.

1.4.4. Methods for Producing ZnO Nanoparticles

For the synthesis of zinc nanoparticles, a metal ion precursor (typically zinc nitrate [$ZnNO_3$]), a reducing agent to change the salt's zinc ions (Zn^+) into metallic zinc (ZnO), then into aggregated clusters, and capping/stabilizing agents to stop the fanned particles from settling and clumping are needed.

1.4.4.1. Chemical Method

The most common procedures include organic and inorganic compound reduction, electrochemical methods, and chemical processes helped by irradiation.

1.4.4.2. Biological Method

Biomolecules that act as chemical stabilizers and reducing agents' substitutes are produced by plants, bacteria, fungi, and algae. Using plants, bacteria, fungi, and algae to produce biocompatible nanostructures is known as "green synthesis" of ZnNPs. As reducing and stabilizing agents in the synthesis of ZnNP, proteins and polysaccharides derived from the natural activity of these organisms are employed.

1.4.4.3. Physical methods

Laser ablation, arc discharge, and evaporation/vapor condensation (the least prevalent). Another method of classification considers the location and timing of metal nanoparticle formation in relation to the production of polymer/metal nanocomposites. Nanoparticles can be produced through a variety of physical, chemical, and biological processes. The use of hazardous substances in biological processes makes them less environmentally friendly than physical and chemical ones, which are frequently preferred. While biological processes are thought to be secure because they don't use risky chemicals and move quickly. Biologically produced nanoparticles are also more stable, which encourages the use of green synthesis.

1.4.5. Characterization of Zn nanoparticles

Following the synthesis of zinc nanoparticles is their characterization. The characterization of the synthesized zinc nanoparticles is very important as their biological nature e.g., antibacterial nature and anti- cancerous nature can be greatly affected by their physiochemical properties. Hence their size, shape, surface area, aggregation, solubility, size distribution is analyzed while characterizing them which ultimately effects their properties (Akbar *et al.* 2019). Various techniques are used in this regard. For example, Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), ultraviolet visible spectroscopy (UV-vis spectroscopy), Dynamic light scattering (DLS), photoelectron Spectroscopy (XPS), scanning electron microscopy (SEM), The properties exhibited by nanoparticles are greatly influenced by the morphology of nanoparticles (Akbar *et al.* 2017).

1.5. Mechanism of action of zinc oxide nanoparticles

Due to the anti-bacterial activity of nanoparticles, they are considered as the substitute of antibiotics in the treatment of diseases. The first step in the mechanism of action of nanoparticles includes nanoparticle adsorption on the cell wall of bacteria. The adsorption of nanoparticles on the cell wall causes disintegration of the cell wall, giving it a porous appearance (Durán *et al.* 2016). This actually occurs due to the de-polymerization of the cell wall that decreases its negative charge and thus makes it permeable. When viewed under laser scanning confocal microscope, the

cell wall of the bacteria appears blurred confirming its disintegration. After the first step which is the entry of nanoparticles into the cell. Second step that is the formation of reactive oxygen species take place (Tayel *et al.* 2011).These reactive oxygen species stop the ATP production and also result in the hindrance of DNA replication. Due to this the respiratory activity of bacteria is disrupted which lowers the chances for cell survival. Moreover, reproduction of the bacteria ultimately stops as the DNA replication is hindered (Yang *et al.* 2009). Furthermore, as the cell wall becomes porous the cellular contents move out of the cell and result in cell death.

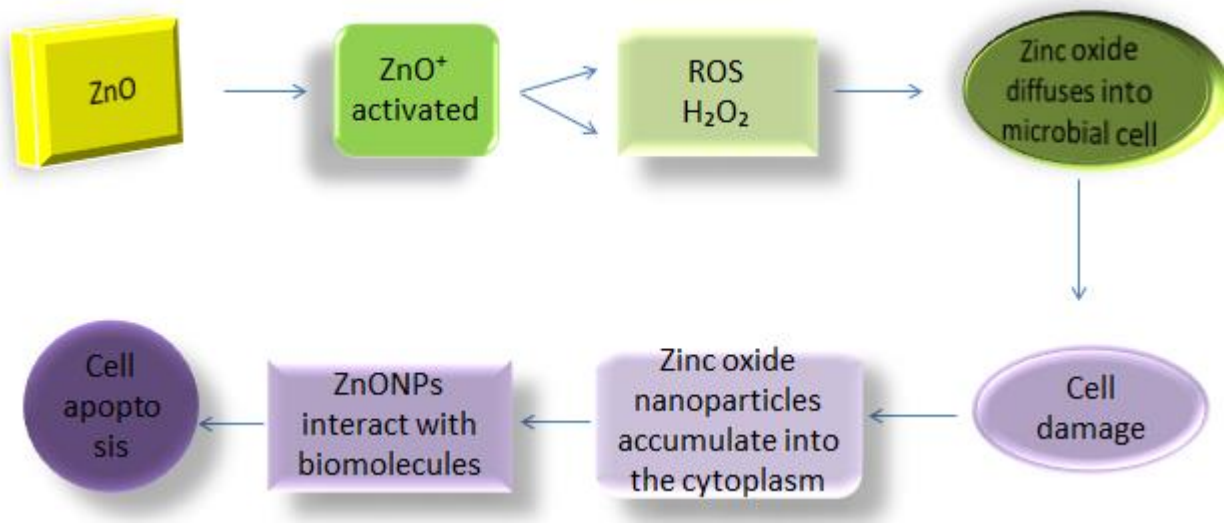

Figure 1.4. Mechanisms of antimicrobial activity of zinc oxide nanoparticles.

1.6. ZnO NPs has more antimicrobial efficacy against bacteria and viruses

The antibacterial properties of zinc oxide nanoparticles and their ability to penetrate cell membranes to stop microbial growth are well known. Stress damages lipids, carbohydrates, proteins, and DNA through the oxidative process. The most significant is certainly lipid peroxidation, which modifies the cell membrane and ultimately impairs essential cellular processes. It has been shown to be supported by an oxidative stress mechanism involving zinc oxide nanoparticles in Escherichia coli. However, bulk zinc oxide suspension is used when H_2O_2 is produced externally. Siddiqi et al. have proposed anti-bacterial properties. Nanoscale. It has also been thought about how toxic nanoparticles that release toxic ions are. Bacterial infectious diseases are serious public health problems that have attracted attention on a global scale as a threat to human health with repercussions for the environment, the economy, and society. People around the world are concerned about increasing pathogenic strain outbreaks and infections, bacterial antibiotic resistance, the appearance of new bacterial mutations, the lack of an efficient vaccine in developing countries, and hospital-associated diseases. For instance, Shigella flexneri infections result in 1.5 million fatalities annually from tainted food and drink (Jones *et al.* 2008). The ZnO-NPs are deemed a promising additive to replace hazardous chemical and physical antibacterial agents because of their highly effective antibacterial action (Zhang *et al.* 2022).

ZnO is described as an inorganic substance with a variety of applications that is useful, wise, hopeful, and adaptable. It is referred to as an II-VI semiconductor because Zn and O are grouped into Groups 2 and Group 6 of the periodic table, respectively. Unusual optical, semiconducting, chemical sensing, electric conductivity, and piezoelectric properties are found in ZnO (Fan & Lu, 2005). This plant's various parts have extraordinary medicinal effects, including being an antiulcer, diuretic, anticancer, antipyretic, anti-inflammatory, antispasmodic, antiepileptic, antihypertensive, anti-diabetic, cholesterol-lowering, and antioxidant. This herb is especially used in South Asian indigenous medical systems (Irfan *et al.* 2021). To decrease dental plaque, prevent calculus from forming, and lessen halitosis, zinc has been added to mouthwashes and toothpaste as an antibacterial (Mahmoudi *et al.* 2011). When ZnO particles are reduced to the nanometer range in size, they demonstrate strong antimicrobial properties. Once within the bacterial cell, they interact with the surface and/or the core of the bacteria and exhibit specific bactericidal mechanisms (Sirelkhatim *et al.* 2015a).

1.7. Scope and significance of zinc nanoparticles

UV protection, antibacterial coatings, and use in skin lotions distinguish ZnO. As a result, covering hospital implants with 4% of these doped ZnO nanostructures will help lower the risk of bacterial infections. However, such doped ZnO can be used in place of undoped ZnO in skin lotions for UV protection (Sirelkhatim et al., 2015). ZnO is described as an inorganic substance with a variety of applications that is useful, wise, hopeful, and adaptable. It is referred to as an II-VI semiconductor because Zn and O are grouped into Groups 2 and Group 6 of the periodic table, respectively. Unusual optical, semiconducting, chemical sensing, electric conductivity, and piezoelectric properties are found in ZnO (Fan & Lu, 2005).

Zinc is known for its anti-microbial activity since very long. The anti-microbial effect is actually possessed by positively charged zinc ion (Zn^{+}). It has potential of killing bacteria, fungi and certain viruses. Microbial growth is retarded through different mode of actions of zinc ions. Zinc ions can bind with the cell membrane and inhibit the transport from the membrane. They can also bind with DNA and hinder the replication of microbe. Furthermore, the respiratory system of bacteria might get blocked by zincs ions which will stop the energy production by cell and hence cause the death of microbe (Busi & Rajkumari 2019). That is among all the metallic Nano particles zinc nanoparticles have gained much importance.

ZnNPs have shown to possess unique properties like thermal, optical and high electrical conductivity. They have gained fame in various industries including food, medical, cosmetic, pharmaceutical industries etc. their unique properties have led to their prominent use in healthcare industry for coating various medical equipment like catheters, vascular grafts, dental materials, stainless stainless steel materials to prevent bacterial colonization. Their role has also been seen in constructing certain household product and food containers. All this is owed to their antibacterial property. They are also used as drug delivery systems, taking drug to a specific site while controlling the rate of drug release. They have a higher densities surface ligand attachment, which improves the surface ligands' stability that plays a major role in drug delivery. Zinc nanoparticles

also act as anticancer agents. These zinc nanoparticles are also known to possess certain other exceptional properties like antiviral, anti- fungal, anti-inflammatory, antiplatelet activity and anti-angiogenesis.

1.8. Current trends

Zinc is known to have significant antibacterial properties in both its metallic and nanoparticle forms, and as a result, it has a wide range of uses. Zinc and other non-antibiotic medicines were abandoned when penicillin and other antibiotics were discovered; however, with the arrival of antibiotic-resistant bacteria and its low risk of resistance development, zinc has regained prominence. Because of their intrinsic therapeutic properties and multi-site action, zinc nanoparticles have a broad-spectrum antibacterial capability against many microorganisms, and they have a huge potential to address emerging issues in the area of microbial resistance in a variety of applications, including therapeutically enhanced healthcare. Zinc nanoparticles (ZnNPs) have shown significant promise in a variety of applications, including detection and diagnostics, drug delivery, biomaterial and device coatings, and so on (Callaghan *et al.* 2016).

CHAPTER # 2

REVIEW OF LITURATURE

2. Review of Literature

2.1. Global Trend of Tomato

In terms of tomato output and export revenue, Pakistan comes in at positions 33 and 52, respectively, in the globe. It provides 1.3% to global tomato area, 0.33% to global production, 0.06% to global tomato export volume, and much less to global tomato export value (Bashir *et al.* 2021). On the global tomato market, Pakistan performs very poorly in terms of per-ha yield, export-production ratio, and export price. Only 25% of the world average is produced there. However, due to the fact that its farm gate prices are far lower than the global average, there is significant potential for global export as well as room for improvement along the value chain (Meemken *et al.* 2021).

Table 2.1. Comparison of world vs. Pakistani Tomato sector, 2016

Parameter	World	Pakistan	Share (%)
Area (000 ha)	4,848	63.2	1.30
Production (Million tonnes)	182.3	0.60	0.33
Yield (tonne/ha)	37.6	9.51	25.29
Value of production (Million US$)	87970	101.7	0.12
Farm gate price (US$/tonne)	483	169	35.06
Volume of trade (000) tonne[1]	7,846	4.5	0.06
Value of international trade (Million US$)	8,472	2.13	0.025
Export quantity as % of production	4.67	0.94	20.12
Export value as % of production value	9.14	2.23	24.4
Average export prices (US$/tonne)	585	166	28.4
Value of export of fresh tomato and tomato products (million US$)	13402	2.51	0.0001

Source: FAOSTAT, Production, Crops http://www.fao.org/faostat/en/#data/QC

Source: FAOSTAT, Trade, Crops and Livestock Products http://www.fao.org/faostat/en/#data/TP

2.2. Global Production Trend

The tomato category is the biggest vegetable category in the world, accounting for 16% of all vegetable land. It has the fastest pace of growth across all categories. In 2017, 182.3 million tonnes of tomatoes were grown on 4.85 million acres worldwide, with an average yield of 37.6 tonnes per hectare. The remaining tomatoes, 42 million tonnes, or 23% of them, are processed into various tomato products before being consumed fresh (Wu *et al.* 2022). Tomato output climbed at a rate of 3.3% annually between 2001 and 2017, which is significantly faster than the 1.19% annual rise in world population. This suggests that tomato consumption per capita is rising internationally. Both the growth in tomato area and its yield are roughly equally responsible for the increase in production. It is important to note that Pakistan's tomato production is increasing faster than the global average, suggesting that Pakistan's contribution to global tomato production is increasing with time (Ahmad *et al.* 2021). Pakistan's share of this particular vegetable is one of

the few to be increasing. But unlike on a worldwide scale, Pakistan's output rise was entirely the result of increasing the land area without increasing the per-ha yield, which made Pakistan less competitive. The top tomato producers, accounting for 70% of global production, are China, India, the United States, and Turkey (Costa & Heuvelink 2018). To raise its per-ha output to at least the level of the global average, Pakistan must learn a lot from China and Turkey, notably in the creation of high-yielding heat- and cold-tolerant hybrids, tomato grafting, and the efficient use of low tunnels.

Table 2.2. Trends in world tomato production during 2001-2017

Year	Area	Production	Yield
	(000 ha)	(000 tonnes)	(t/ha)
2001	3,803	106,715	28.1
2002	3,926	115,762	29.5
2003	3,991	118,226	29.6
2004	4,152	127,032	30.6
2005	4,171	128,363	30.8
2006	4,154	130,452	31.4
2007	4,223	137,155	32.5
2008	4,223	141,648	33.5
2009	4,419	155,309	35.1
2010	4,430	153,305	34.6
2011	4,582	159,516	34.8
2012	4,804	163,181	34.0
2013	4,849	165,296	34.1
2014	4,910	174,862	35.6
2015	4,816	177,501	36.9
2016	4,845	179,508	37.0
2017	4,848	182,301	37.6
Growth (%)	1.63	3.30	1.67

Source: FAOSTAT, Production, Crops http://www.fao.org/faostat/en/#data/QC

From 2001 to 2017, there has been a global increase in tomato production at a rate of 3.3% per year, which is significantly higher than the global population growth of 1.19%. This suggests that there is a rising trend in per capita tomato consumption worldwide. The growth in tomato production is due to an almost equal contribution from the expansion of tomato cultivation area and its yield (as seen in Table 2.2). It is noteworthy that Pakistan's tomato production growth rate is higher than the global average, indicating an improvement in Pakistan's share of international tomato production over time. This is one of the few vegetables where Pakistan's share has improved. However, unlike the global trend, the increase in production in Pakistan is solely due to the expansion of cultivation area without any increase in yield per hectare, which has led to a deterioration of Pakistan's competitive position. According to Table 2.3, the largest tomato producers in the world are China, India, USA, and Turkey, which collectively account for 70%

of global production. To increase its per hectare yield and achieve at least the world average level, Pakistan can benefit from learning techniques such as developing high-yielding heat-resistant and cold-tolerant hybrids, tomato grafting, and economical use of low tunnels from China and Turkey.

Table 2.3. Top Tomato Producing Countries of the World (2016)

Rank	Country	Production (tonnes)	Area (ha)	Yield (t/ha)	Share (%)
1.	China	871,235	41,879,684	48.1	28
2.	India	865,000	16,826,000	19.5	11
3.	USA	159,200	12,902,000	81.0	9
4.	Turkey	304,000	10,052,000	33.1	7
5.	Egypt	216,385	8,544,990	39.5	6
6.	Italy	118,822	6,024,800	50.7	4
7.	Iran	146,985	5,256,110	35.8	3
8.	Spain	58,300	4,312,700	74.0	3
9.	Brazil	60,772	3,691,320	60.7	2
10.	Mexico	98,189	2,997,640	30.5	2
	World Total	**164,493,000**	**112,487,244**		

2.3. International Tomato Consumption

The FAOSTAT Food Balanced Sheet data show that 20.46 kg of tomatoes were consumed annually on average globally in 2013 for per person (Bisangwa 2019). With an improvement of 2.3% annually, it increased from 15.4kg in 2013. Though Pakistan's per capita tomato consumption is only one-fourth of the global average, it is nevertheless increasing at a rate that is more than three times faster than the global average. With a consumption rate of 99 kg annually per person, Turkey came out on top, followed closely by Egypt, Armenia, and Tunisia (Korotayev & Zinkina 2022). Regarding global tomato consumption, Pakistan comes in at position 128.

2.4. International Tomato Trade

It's interesting to note that tomatoes are gradually becoming a global commodity. According to Table 5, the amount and value of tomato exports have grown at rates of 4.6% and 7.1% annually, respectively, while tomato output has expanded just marginally (Ahmed *et al.* 2021b).The gross commerce in tomatoes and products related to tomatoes increased from US$4.4 billion in 2001 to US$13.4 billion in 2017, growing at an average annual growth rate of 6.7%.Fresh tomatoes dominate commerce, accounting for around 67.3% of the overall value of tomato trade (Soethoudt *et al.* 2018). The value of fresh tomatoes has climbed from 64.7% in 2001, indicating that people enjoy them more today. Fresh tomato gross exports reached 8.0 million metric tonnes with a US$9.0 billion value (Cui *et al.* 2022). The 2017 market value of tomato paste was $ 3.0 billion., was the main tomato product sent to other countries, accounting for nearly 22% of the overall export value of tomatoes and tomato products (Løvdal *et al.* 2019). About 10% of the tomato trade is made up of peeled tomatoes, while tomato juice makes up a negligible amount. The top

exporters of tomatoes internationally are Mexico, Netherlands, and Spain. Unless Pakistan improves the value chain for tomatoes, it cannot compete with any of these exporters (Gulati *et al.* 2022). The countries that imported the most tomatoes in 2016 were the United States, Germany, France, the United Kingdom, the Russian Federation, and Poland, making up 76.8% of all imports. Spain, the Netherlands, France, and the United Arab Emirates are those with their nations' tomato markets experiencing the biggest growth since 2013 (Ghonima 2021). Pakistan is not a significant exporter of tomatoes abroad.

Table 2.4. Trend in the global export of fresh tomato and its products during 2001-15

Year	Fresh tomato		Tomato juice		Tomato paste		Peeled tomato		Totale export
	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Value
	(000 t)	(million US$)	(000 t)	(million US$)	(000 t)	(million US$)	(000 t)	(million US$)	(million US$)
2001	4221.3	2880.9	68.3	26.9	1800.9	1072.8	1092.2	472.2	4452.7
2002	4287.9	3371.6	64.3	28.1	1934.4	1281.7	1139.2	560.3	5241.8
2003	4556.8	4286.2	57.1	28.3	2045.0	1475.6	1081.4	707.9	6498.0
2004	4867.4	4455	57.7	29.6	2202.1	1735.4	1063.5	722.1	6942.1
2005	4986.9	5099.5	59.7	32.2	2360.6	1751.4	1129.0	732.4	7615.4
2006	5701.6	5433.2	78.9	42.4	2446.4	1773.5	1213.9	779.6	8028.6
2007	6369.5	6871.5	80.8	48.7	2637.5	2119.3	1283.5	963.8	10003.3
2008	6430.6	7371.5	80.6	55.1	2768.8	3017.6	1300.4	1241.8	11685.9
2009	6854.6	7009.1	71.6	49.8	2542.8	3005.8	1256.3	1269.4	11334.1
2010	7085.9	8251.1	76.8	48.7	2903.1	2945.1	1428.5	1257.6	12502.5
2011	7432.1	8501.6	99.2	68.1	3150.7	3197.6	1500.6	1312.4	13079.7
2012	7263.4	8181.6	97.0	66.9	3093.4	3173.5	1509.1	1306.3	12728.3
2013	7682.6	8803	97.6	68.2	3210.5	3579.4	1562.3	1432.5	13883.1
2014	8287.3	9238.4	85.8	60.2	3217.8	3722.7	1575.7	1523.5	14544.8
2015	7944.2	8349.0	68.8	44.5	3231.3	3283.2	1592.7	1291.6	12968.3

Source: FAOSTAT, Trade, Crops and Livestock Products http://www.fao.org/faostat/en/#data/TP

Table 2.5. Top Tomato Exporting Countries of the World (2017).

Rank	Country	Quantity (000 tonne)	Export (Million US$)	% share in export value
1	Mexico	1535.2	1835.2	21.66
2	Netherlands	1013.5	1697.2	20.03
3	Spain	1004.0	1313.2	15.50
4	Morocco	457.9	429.3	5.07
5	France	229.8	395.5	4.67
6	Turkey	483.0	391.2	4.62
7	Canada	162.2	381.3	4.50
8	USA	211.8	340.6	4.02
9	Jordan	611.5	316.3	3.73
10	Italy	108.3	243.9	2.88

Source: FAOSTAT, Trade, Crops and Livestock Products http://www.fao.org/faostat/en/#data/TP

In 2016, the top tomato importing countries were the USA, Germany, France, UK, Russian Federation, and Poland, collectively accounting for 76.8% of global tomato imports (as indicated in Table 2.6). Spain, Netherlands, France, and United Arab Emirates have experienced the most significant growth in tomato markets among these countries since 2013. However, Pakistan is not a significant participant in the international tomato export market.

Table 2.6. Major tomato importing countries of the world during 2017

	Country	Amount in (US$ Million)	Share in world import (%)
1.	USA	2272.4	24.6
2.	Germany	1493.7	16.1
3.	France	704.8	7.6
4.	United Kingdom	638.3	6.9
5.	Russian Federation	558.7	6.0
6.	Netherlands	339.8	3.7
7.	Canada	332.8	3.6
8.	Poland	222.3	2.4
9.	Belarus	217.5	2.4
10.	Sweden	171.5	1.9

Source: FAOSTAT, Trade, Crops and Livestock Products http://www.fao.org/faostat/en/#data/TP

After analyzing the macro level tomato situation in Pakistan and at a global level, it can be concluded that tomato production in Pakistan has increased significantly from a low starting point during the 2000s. This improvement has led to a higher comparative position among tomato producing countries, moving from 45th in 2001 to 36th in 2017. However, Pakistan's per-hectare yield in tomato has remained stagnant, while international yield continues to improve, resulting in

a loss of comparative advantage globally. Currently, Pakistan is only achieving 25% of the world's average yield. Meanwhile, the demand for tomato and its products in the country is rapidly expanding at a rate of 7.3% per annum, significantly higher than its production increase, resulting in a substantial trade deficit. Pakistan's participation in international export markets is insignificant, despite the fact that the global export of fresh tomatoes and their products has exceeded US$13 billion in 2017. The country's export price is only 28% of the world average, indicating significant challenges in enhancing the tomato value chain. Pakistan's export volume is less than 1% of its production, while the global average export-production ratio is 4.7%. Due to its lower farm gate prices compared to the world average, Pakistan has the potential to increase its export-production ratio. The country can benefit from its good economic and social relationship with neighboring countries, particularly China, to learn about efficient tomato production.

2.5. Description of Tomato Value Chain

Tomatoes are conventionally harvested and handled roughly throughout all three clusters of the value chain. Initially, workers gather the tomatoes in an open field and then pack them directly into various materials, mainly plastic shoppers and bags weighing 15-20 kgs each. Upon reaching the local market, the growers/merchants decide whether to transport the tomatoes to the market destination using wooden crates weighing 20 kgs or in pickups and Mazdas under open conditions. Some growers/merchants prioritize packing materials and opt for plastic or paper crates with a printed brand name, which fetches them better prices in selected markets. The tomato processing phase involves grading, washing, and pre-cooling before packing the tomatoes into paper crates with a brand name. Figure 2.1 depicts the flow of tomatoes through different stakeholders in the value chain.

Tomato production, processing, and trading are major components of Pakistan's business industry, with many farmers, traders, and service providers involved in the tomato value chain. This value chain is integrated with larger input systems in the country, and several well-known companies, such as Shangrila National Foods, Shezan, and Knorr, are engaged in the trade of tomato byproducts. In the Sindh cluster, Mr. Muazum Khoso has established a small dehydrated unit to produce tomato powder, puree, and slices under the brand name "Zamzam Enter Prizes." He is seeking technical and marketing assistance to expand his product line for export. There is enormous potential for small industries to be established in all the tomato clusters to bolster tomato marketing, increase farmer income, enhance GDP, generate foreign exchange, and create job opportunities in rural areas.

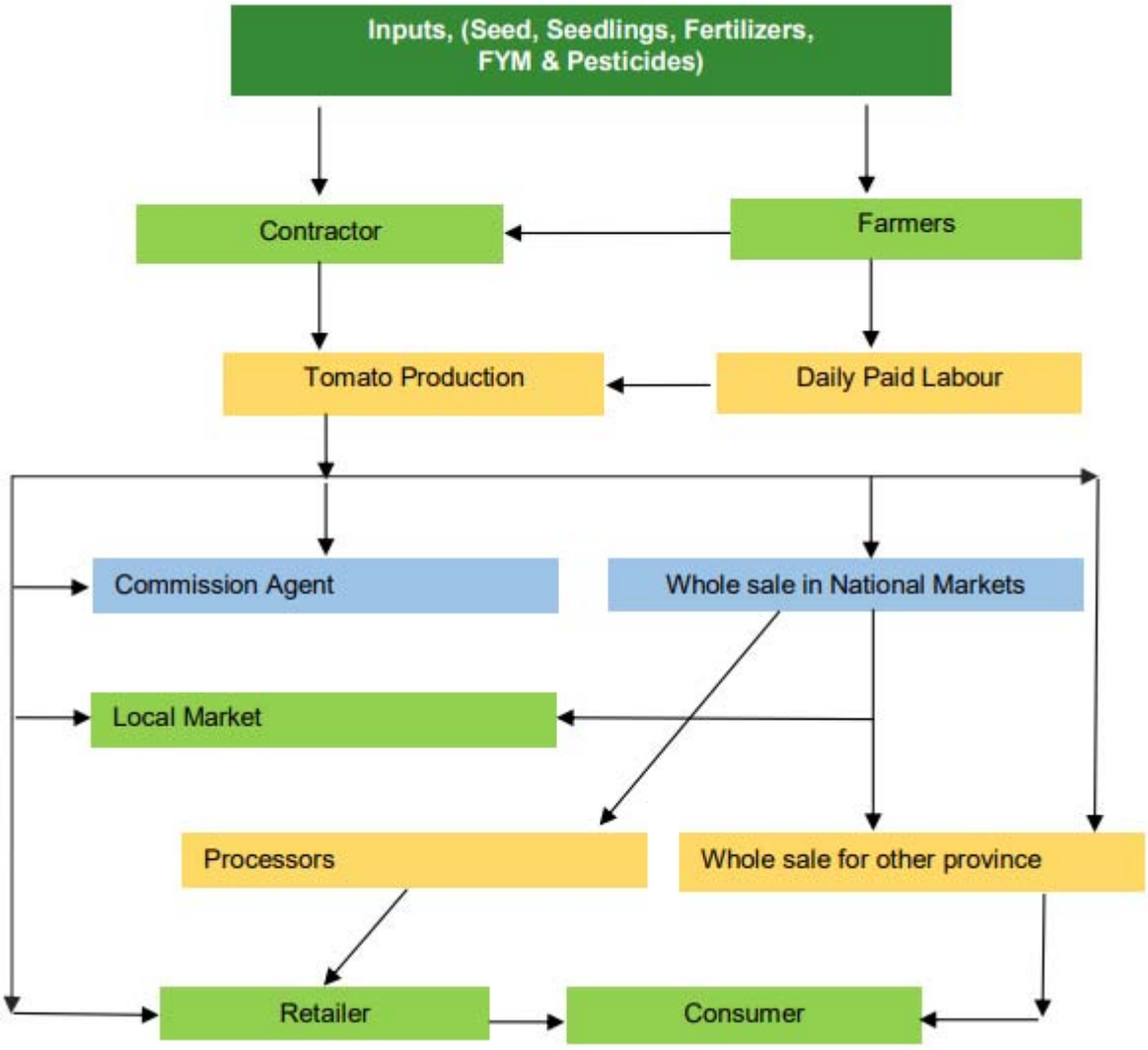

Figure 2.1. Description of Tomato Value Chain in Pakistan

2.6. Constraints at Production Level

Production of tomatoes in different clusters faces various constraints with varying intensity. The primary constraints at the production level include chemical fertilizers and pesticides adulteration, unavailability of certified tomato seeds, high cost of hybrids, and limited access to healthy tomato nurseries. In addition, traditional cultivation techniques, lack of modern production technology, insect and diseases outbreak, and inadequate support from agriculture research and extension workers are also significant issues, although their impact varies across clusters. Electricity load shedding is a common constraint for the processing segment in all the clusters. To expand the processing segment, a diversification strategy is essential to prepare products for the market. Institutes such as the Institute of Food Science and Technology (Agriculture University of Faisalabad) and Post-Harvest Research Center (Ayub Agriculture Research Institute, Faisalabad) have developed models for tomato pulp extraction and paste making. Tomato processing methods

such as pulp, paste, puree, dried slices, and ketchup production are not commonly practiced on a small scale. Despite this, there are some companies in Karachi that engage in tomato processing as indicated in Table 2.7. These companies rely entirely on imported tomato pulp for their processing and manufacturing needs. It is crucial to develop the capacity for puree production at the village level. However, the technologies required for processing, packaging, and storage vary in quality and efficiency.

In tomato cultivation areas, there are no cold stores available, but in larger cities like Karachi, Lahore, and Islamabad, there are some small-scale cold rooms present. Due to the profitability of tomato trading, there are many buyers and sellers, resulting in a competitive market environment. Small-scale trading does not require a license, and there are no significant barriers to entry besides access to finance. Traders aim to maximize their profits by purchasing at the lowest price and selling at the highest price, with little attention paid to product differentiation or quality aspects to achieve price premiums. Communication technologies and internet services are easily accessible, labor is available on both a permanent and seasonal basis, and traders have access to financial services from both formal and informal banking institutions. However, some traders surveyed at fruit and vegetable markets in Karachi, Quetta, and Swat expressed dissatisfaction with the inadequate market facilities provided by the Market Committees/Government.

Table 2.7. Gaps and Constraints at Production Level

S#.	Parameter	Sindh cluster	Balochistan Cluster	Khyber Pakhtunkhwa cluster
2.	New germplasm	Non availability of OPV germplasm& local low-cost hybrids	Non availability of OPV germplasm& very few Local Hybrids	Non availability of OPV germplasm& very few Local Hybrids
		Foreign Hybrids are available but majority of them are un-authenticated and costly	Foreign Hybrids are available but majority of them are un-authenticated	Foreign Hybrids are available but majority of them are un-authenticated
3.	Mother nurseries/seedlings	Small amount	Small amount	Small amount
4.	Orchard size/type	Mix (Small&medium)	Relatively larger	Small
5.	Certified plants/Seed	Less than 50%	Less than 30%	Less than 50%
6.	Extension services	Weak	Weak	Weak
7.	Commercial inputs	Imbalanced use	Imbalanced use	Imbalanced use
8.	Labor input	(Hired+ Family)	(Hired+ Family)	(Hired+ Family)

2.7. Production Potential

In the introduction section, it was mentioned that Pakistan is ranked 33rd in the world for tomato production, with a yield gap of 25.1 tonnes/ha compared to the global average yield of 35 tonnes/ha. The estimated yield for three tomato clusters in Pakistan is 7.69 tonnes/ha for Sindh cluster, 9.78 tonnes/ha for Baluchistan, and 11.64 tonnes/ha for KP cluster. However, there are technologies available, such as the Sandel and Surkhail hybrids released by Ayub Agricultural Research Institute (AARI), as well as Sahel and Anna seeds supplied by the private sector in Punjab and KP, respectively, which have the potential to yield above 180-190 tonnes per ha. In addition, open pollinated varieties are more successful in Sindh and micronutrients supplied by the private sector, such as Esabyan, can increase the size of the tomato significantly. By adopting these technologies and good agricultural practices, Pakistan can increase its tomato production and enhance its quality for high-end domestic and international markets, especially to Iran, Afghanistan, and United Arab Emirates. A 20% increase in average tomato yield can be achieved relatively easily in all clusters, resulting in an additional tomato production of 142.5 thousand tonnes worth Rs3.19 billion (US$23.6 million). This will increase farmers' gross income by about 25% at existing tomato prices and generate employment by about 4.25% in tomato-growing rural areas. In Pakistan, where unemployment is a major problem, adopting the latest production techniques in tomato clusters can not only increase farmers' income but also generate employment opportunities. According to Kalsoom et al. (2008), a ten percentage increase in GDP can lead to the generation of 1.3% additional jobs in the short run and 1.1% more jobs in the long run.

2.8. Demand Potential

Pakistan's population is currently approximately 210 million, and it is increasing at a rapid rate of 2.1% annually, as reported in the 2017 census. The per capita annual availability of tomatoes in the country has improved from 1.5 kg in 2000 to 2.73kg, but it still falls short of the global average of 20.0 kg per capita per year. North America consumes the highest number of tomatoes globally, with 42 kg per year, while Europeans consume around 31 kg per capita per year, according to FAOSTAT-2015. As a result, there is a significant demand gap in the domestic market. Although Pakistan has started exporting tomatoes, it also imports them during the off-season. To bridge this demand gap, Pakistan needs to focus on increasing tomato production, improving quality for export through germplasm diversification, modern production technologies, introduction of high-yielding local tomato hybrids and varieties, good agronomic practices, and improved value chain practices. With these improvements, the country could increase per capita tomato availability by at least 20%.

2.9. Improvement in Production to Export Ratio

Pakistan ranks 33rd in tomato exports, with 0.94% of its production being exported, despite having lower farm gate prices than the world average. There are significant export opportunities in the tomato market, with a growth rate of 4.6% per year, and new opportunities have emerged with the opening of CPEC routes to China, Saudi Arabia, UAE, Iran, and Tajikistan. However, Pakistan's

share in the international tomato market is still quite small. By improving linkages with international markets and adopting appropriate commercial strategies, as outlined in the next section, Pakistan could increase its export-production ratio by 10%. This would allow for an increase in foreign exchange revenue of US$27.2 million, based on the existing average Pakistani export price of tomato, with only half of the enhanced production being exported and the rest going towards improving per capita consumption.

2.9.1. Improvement in Quality

In the tomato sector of Pakistan, a concern is the discrepancy between Pakistani export prices and the global average export prices for tomatoes. Currently, Pakistani tomatoes are exported at a price of US$382, compared to the average international export price of US$1080. Stakeholders have suggested that by improving the tomato value chain, as outlined in the following section, it would be possible to achieve 50% of the international market price. Additionally, improving the value chain could increase the price of locally sold tomatoes by 10%, bringing it in line with the international average export price. This overall improvement to the value chain would not only increase the income of those involved in the value chain but also bring in additional foreign exchange revenue and improve the competitiveness of the entire tomato sector.

2.9.2. Reduction in Post-Harvest Losses

One of the main issues facing agriculture in the country is the significant amount of produce lost after harvest. For instance, tomatoes account for almost 30% of the loss from the point of harvesting to the final market. However, these losses can be curbed by implementing suggested measures such as selecting the appropriate harvest index, proper picking and washing, utilizing modern harvesting methods, packing in small quantities, and ensuring safe transportation with padding in the packing material. Discussions with stakeholders have revealed that if suitable harvest and post-harvest strategies are put in place, these losses can be reduced from 30% to 10%. This reduction will lead to additional income for producers and other stakeholders in the value chain, amounting to Rs. 432 million (equivalent to US$3.2 million).

2.9.3. Improved Processing

The Food Science and Technology Institute in Faisalabad has successfully developed recipes for tomato products such as puree, pulp, paste, and powder, creating ample opportunities for tomato product development. Additionally, there is a large-scale tomato ketchup and paste industry in Karachi, although it currently relies on imports from China and Turkey. If a reliable supply of high-quality tomato paste could be established, there is already a market waiting for it. Tomato farmer enterprise groups (FEGs) could establish small-scale cottage industries in villages to produce tomato puree and supply it to the paste industry under contractual agreements. The paste industry could then supply tomato paste of a higher brix level to the ketchup industry, creating a mutually beneficial relationship. This system could be implemented in the Sindh region, where tomato farmers are close to the paste and ketchup industry. The processing of just 5% of tomatoes from Sindh could generate an additional revenue of RS. 188 million (US$1.39 million). Although

a recipe for tomato powder is also available, it is not well-known among Pakistani consumers, so a campaign would be needed to introduce the product. Small-scale tomato powder making cottage industries could be established in KP and Baluchistan, initially exporting to China and the Middle East and eventually trickling down into the domestic market. If 1% of tomato production in Baluchistan and 2% in KP were converted, processors could earn an additional revenue of RS. 132 million (US$0.9 million).

2.10. Diseases of Tomatoes

2.10.1. Anthracnose

Anthracnose caused by fungi in the *Colletotrichum* species, commonly affects ripe or overripe fruit, although it can also infect leaves, stems, and roots. Even unripe fruit can become infected, but symptoms usually only appear once the fruit starts to ripen. The disease thrives in moist environments and can persist from one crop to the next in plant debris and soil. The pathogens have a wide range of hosts and can be spread through splashing water from rain or overhead irrigation. Ripe fruit infected with Anthracnose typically develop small circular lesions that are slightly depressed, which can enlarge, merge and become more sunken. As the disease progresses, small black spots, called microsclerotia, develop in the center of the lesions. When the conditions are humid, masses of salmon-colored spores can be seen on the surface of the lesions.

2.10.2. Bacterial canker

Tomatoes grown in greenhouses are susceptible to a significant disease caused by the bacterium *Clavibacter michiganensis* subsp. *michiganensis*, known as bacterial canker. This pathogen can survive in infected plant debris, weed hosts, and on production supplies such as stakes and trays. Additionally, the pathogen can be spread from plant to plant through splashing water, pruning, and workers' hands. Infected tomatoes can display a range of symptoms, with wilting being the most noticeable. This typically starts at the lower part of the plant and moves upward, but can also begin at any point where the pathogen enters the plant through wounds. Other symptoms include dark streaks on stems that may split to reveal a brown canker, as well as light yellow to reddish-brown streaks in the vascular tissues, which are more visible at plant nodes. Stems may also develop adventitious roots. Leaflet margins may turn brown and be bordered by yellow margins, and fruits may develop tan to brown spots surrounded by creamy-white halos known as bird's eye spots.

2.10.3. Bacterial speck

Tomatoes can suffer from a serious disease called bacterial speck caused by the *Pseudomonas syringae* pv. tomato bacterium. This disease can be difficult to control, especially when environmental conditions are favorable, such as high humidity and cool temperatures. The pathogen can be transmitted through contaminated tools and equipment, splashing water, and workers, and it can survive from one season to another in crop debris. Although copper resistance has been reported, it is unclear whether resistant pathogen populations are present in Mississippi. Bacterial speck causes round, dark brown to black lesions on leaflets, which may have a yellow halo around them as they grow. When lesions merge, large areas of leaflets may die. Additionally, elongated lesions can form on stems, petioles, peduncles, pedicels, and sepals. Fruit lesions are

also common, surrounded by a dark green halo, and can be easily confused with symptoms of bacterial spot.

2.10.4. Bacterial spot

Bacterial spot, caused by *Xanthomonas* spp. bacteria, is a tomato disease that can pose a significant threat and become challenging to manage under high disease pressure and favorable environmental conditions. Disease development is favored by warm temperatures and abundant precipitation. The pathogen can be spread through wind-driven rain, seed transmission, and plant handling, affecting all aboveground plant parts. Copper resistance in the pathogen has been reported in various regions, including Jasper and Smith Counties in Mississippi in 2017. The extent of copper-resistant pathogen populations in Mississippi remains unknown. Symptoms of bacterial spot include round, brown lesions that may develop into dark streaks on affected tissues. Leaves with numerous lesions may become yellow or blighted, and dead foliage may persist on the plant. Lesions on fruit appear as raised blisters that become scabby and brown. It's important to note that the symptoms of bacterial spot and bacterial speck are similar and can be easily confused.

2.10.5. Bacterial wilt

Bacterial wilt caused by the bacterium *Ralstonia solanacearum* can have devastating effects on both field and greenhouse tomato crops. The development of the disease is favored by high soil temperatures and moisture levels. This pathogen can infect many different crops and has the ability to survive in the soil for extended periods, even without a host crop present. Once established in a field, bacterial wilt is difficult to manage. Spread of the pathogen can occur through contaminated water, tools, equipment, and workers. *R. solanacearum* is categorized into five races, with Race 1 being the most common and found throughout the southeastern United States. However, all races of the pathogen are considered select agents by the USDA APHIS, and positive diagnoses must be reported and handled according to USDA protocol. Plants affected by bacterial wilt rapidly wilt and die without showing signs of yellowing or death of plant tissue. A brown lesion may be visible on the outside of the stem near the plant base. The inside of the stem near the soil/media line may appear dark and water-soaked, and in advanced infections, the stem may be hollow and exhibit profuse bacterial streaming when cut.

2.10.6. Buckeye rot

Tomatoes in the southeastern United States are commonly affected by Buckeye rot, caused by oomycetes such as *Phytophthora nicotianae* var. *parasitica*, *P. capsici*, and *P. drechsleri*. The disease thrives in warm and humid conditions, especially in soils with high moisture levels. The pathogen can spread through water contamination and splashing. Infected fruit develop brown, oily-looking lesions that gradually enlarge and may cover a significant portion of the fruit. Lesions typically show concentric rings, and under moist conditions, a white, cottony fungal growth may appear on the lesion's surface. The disease primarily affects fruit in direct contact with the soil or those situated low on the plant. The foliage is not vulnerable to infection.

2.10.7. Early blight

Tomatoes grown in the field are commonly plagued by early blight, caused by the fungus *Alternaria linariae* (also known as *A. tomatophila*). The disease affects the leaves, stems, and fruits of the plant, with the lower portions being more susceptible to infection. The pathogen thrives in warm, humid conditions and can survive on infected plant debris as well as through seed transmission. Infected plant tissues develop circular or elongated brown lesions with concentric rings that gradually increase in size. In addition, the surrounding leaf tissue may turn yellowish-green. Symptoms of the disease can be observed on all parts of the plant.

2.10.8. Fusarium wilt

Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *lycopersici*, is a disease that is prevalent in warm weather conditions. The pathogen has three races, each capable of causing disease, and can survive in soil for several years. It can spread through contaminated soil, equipment, and infected transplants. The disease is more likely to infect plants with root wounds caused by root-knot nematodes. To manage the disease, resistant cultivars are commonly used. It's worth noting that other species of Fusarium can cause diseases in tomatoes, such as Fusarium crown and root rot. The most common symptoms of Fusarium wilt include wilting and chlorosis of leaves. Initially, the disease may cause wilting during the hottest part of the day, with the plant recovering overnight. Chlorosis may occur on only one side of the plant initially but can spread to the whole plant as the disease progresses. Additionally, the base of infected plants may exhibit a dark red to brown coloration in the vascular tissue.

2.10.9. Gray leaf spot

Tomatoes can be severely affected by Gray leaf spot, a disease caused by the fungi Stemphylium spp. in regions where susceptible varieties are cultivated. The pathogen can persist on plant remnants and vulnerable hosts and can be disseminated by wind. On the upper and lower sides of the leaflets, small, circular to oblong lesions emerge randomly. Lesions may merge to form large areas of necrosis in the leaflets. In the center of the lesions, fissures may arise, and entire leaves may start to turn yellow. Damaged leaves may wither quickly, become brown, and drop off the plant.

2.10.10. Gray mold

Botrytis cinerea, also known as Gray mold, is a widespread disease that affects tomatoes cultivated in enclosed environments and can propagate rapidly. The fungus thrives in damp and cold conditions and requires moisture for its spores to germinate. Airborne spores can spread the disease. The disease can affect all aboveground parts of the plant, but it does not actively infect healthy tissues. The pathogen typically enters through pruning wounds or dead plant tissue. Sclerotia, a type of compact fungal hyphae, allow the pathogen to survive from one season to the next. The disease causes tan or gray lesions to appear at the tips of leaflets, which become covered in brown or gray fungal growth. Infected flowers and calyx tissue also become diseased, and stem cankers may develop due to pathogen invasion at a pruning wound. These large, brown cankers can girdle the stem and cause plant death. Another symptom associated with gray mold is the

production of small, whitish rings or halos on the fruit, called "ghost spots," which result from Botrytis spores germinating but failing to infect the fruit. Infected fruit becomes water-soaked and soft.

2.10.11. Late blight

Late blight, caused by the oomycete *Phytophthora infestans*, thrives in moist weather, cool nighttime temperatures, and warm daytime temperatures, but temperatures above 86ºF are not conducive to the disease's development. The pathogen can be seedborne and survive on vulnerable hosts and weeds, and it infects potatoes, causing late blight symptoms on all aboveground plant parts. On leaves, the disease appears as small water-soaked spots that turn pale-green to brown and rapidly spread to cover large areas. When moist conditions are present, gray to white pathogen growth may form on the lower surface of leaf lesions. Infected foliage eventually browns, withers, and dies. Infected fruits display greasy, brown lesions that can enlarge to cover the entire fruit.

2.10.12. Leaf mold

Leaf mold is a prevalent ailment that affects enclosed tomato crops, but it can also affect those grown in fields. The fungus responsible for this disease was formerly known as *Cladosporium fulvum* and *Passalora fulva*, but it is now called *Fulvia fulva*. The development of the disease is favored by moderate temperatures and high humidity, and the pathogen can survive on residual crop material. The spores of the fungus can be disseminated by rain, wind, insects, clothing, and tools. Leaflets develop pale green or yellow lesions with irregular margins on their upper surfaces. Velvet-like fungal growths that are olive-green in color develop on the underside of leaflets directly beneath the yellow lesions. Infected leaves eventually collapse and wither. Symptoms of leaf mold are only evident on leaflets as it is a foliar disease.

2.10.13. Powdery mildew

While many tomato diseases worsen with wet conditions, powdery mildew caused by *Leveillula taurica* and *Oidium neolycopersici* can develop in dry weather, with high humidity being favorable for disease progression. Wind can quickly spread these pathogens, resulting in powdery white fungal mycelium on the top of leaves infected with *O. neolycopersici.* Leaves infected with this fungus may turn chlorotic and necrotic over time. Meanwhile, *L. taurica* causes irregularly shaped, light green or bright yellow lesions on the upper surfaces of tomato leaflets.

2.10.14. Pythium damping-off and stem rot

Pythium species, which are oomycetes, can lead to the loss of plants and poor establishment of seedlings in both the field and greenhouse due to damping-off and stem rot. Damping-off can occur before or after emergence, and Pythium spp. can survive in the soil for extended periods in the absence of a host, persisting indefinitely on organic matter. The growth of these pathogens is favored by certain conditions, such as moisture. Other pathogens can also cause damping-off, so it is essential to identify the responsible pathogen to select the appropriate fungicide for treatment. Pre-emergence damping-off is characterized by the development of a dark brown to black lesion on germinating seedlings, while post-emergence damping-off results in a water-soaked lesion on

the roots, extending to the stem above the soil line. Infected seedlings typically wilt and may collapse at the point of the stem lesion before dying, while plants with less severe root infections may be stunted.

2.10.14.1. Septoria leaf spot

The fungus known as *Septoria lycopersici*, also known as *Septoria* leaf spot, has the potential to destroy tomato foliage and result in reduced yield. Although the fruit itself is not commonly infected, the damage to foliage can cause the fruit to fail or become susceptible to sunscald. This disease tends to thrive in conditions of long moderate temperature periods, dew, and high humidity. The pathogen can survive on susceptible host plant debris and production equipment during the winter months, and can also infect tomato seeds. Spread of the disease can occur through rain or by workers, insects, and equipment when the plants are wet. Symptoms of the disease can be found on the calyx, leaves, and stems of the tomato plant. Lesions with dark margins and tan to gray centers appear as round spots on the leaves, with black fruiting bodies (pycnidia) developing in the center of lesions about two weeks after infection. Additionally, a thin, yellow halo may develop around lesions on leaflets.

2.10.15. Southern blight

Tomato production may face a significant challenge known as Southern blight, caused by the *Athelia rolfsii* fungus (previously known as *Sclerotium rolfsii*), also called Southern stem rot. This disease is more likely to develop under high temperatures and humid conditions. The pathogen can survive in soil or plant debris for a long time in the form of sclerotia and can spread easily through infected soil or plant material. In the field, it is easy to identify Southern blight by the presence of typical symptoms. The most apparent symptom is a sudden and permanent wilting of the stem due to stem rot, with a brown to black lesion that encircles the stem near the soil line. Under humid conditions, white fungal mycelia appear on the stem lesion, followed by the development of round, tan to brown sclerotia. Moreover, fruits in contact with infested soil may also be affected, and the initial symptoms are sunken and yellow spots on the fruit, which later becomes water-soaked, soft, and collapses. Infected fruits also show white mycelium and sclerotia growth.

2.10.16. Southern blight

The fungus *Corynespora cassiicola*, commonly known as target spot, can pose a significant threat in both field and greenhouse environments. The disease thrives in conditions of extended moisture and moderate temperatures between 68 and 82ºF, and the pathogen tends to produce more spores on the lower surface of leaves than on the upper surface. Symptoms of the disease can appear on various parts of the plant, including leaves, petioles, stems, and fruit. When the disease affects leaflets, it first manifests as small, water-soaked lesions that gradually expand and turn light brown and circular, often exhibiting a target-like appearance. A yellow halo may form around individual lesions, and when they merge, they can cause the collapse of leaflet tissue. Petioles and stems can also develop elongated lesions that may encircle and constrict these structures, leading to the collapse of the affected leaflet or leaf. On young fruit, the disease causes small, sunken brown spots, which can develop into larger craters as the fruit matures. On ripe fruit, circular lesions with brown centers often form, which may crack open over time.

2.10.17. Timber rot

Tomatoes and many other vegetables can be affected by a fungus called *Sclerotinia sclerotiorum*, also known as timber rot, white mold, or *Sclerotinia* stem rot. This disease thrives in cool and moist weather, as well as in conditions with high humidity and free moisture. The fungus feeds on dead or senescent tissue, such as fallen flowers, before infecting healthy tissue. The disease starts as water-soaked areas near leaf axils or in stem joints and progresses to make the stem soft, light gray or tan, and bleached. In favorable conditions, white mycelium often develops on or in infected stems, and black sclerotia, resembling rat droppings, can be found on the fungal mycelium or inside infected stems. The fruit can also be infected, leading to gray coloration and watery rot, along with the characteristic white fungal mycelium and black sclerotia.

2.10.18. Tobacco and tomato mosaic

The Tobacco and Tomato Mosaic viruses, which are caused by similar but distinct viruses (Tobacco Mosaic Virus, TMV, and Tomato Mosaic Virus, ToMV), are the topic of discussion. While TMV is covered in depth in the MSU Extension Information Sheet 1665 The Plant Doctor - Tobacco Mosaic Virus, this article will focus on ToMV. The virus is easily transmitted through sap, and can be spread from plant to plant via contaminated hands, clothes, and tools. Seed contamination is also possible. ToMV is incredibly resilient and can survive for varying periods in plant debris depending on the conditions. Symptoms of ToMV infection in tomatoes vary depending on several factors, including the strain of ToMV, cultivar, timing of infection, and environmental conditions. However, infected plants typically exhibit mottled leaves, stunted growth, smaller and curled or deformed leaves, and fruit that ripens unevenly, is smaller in size, develops yellow rings, or has internal browning. The number of fruit produced on infected plants may also be reduced.

2.10.19. Tomato spotted wilt

The *Tomato Spotted Wilt Virus* (TSWV) is capable of causing significant damage to tomato plants and has a broad range of hosts, including various weed species. TSWV is transmitted by seven different species of thrips, with the virus being acquired by the larvae within 30 minutes of feeding on an infected plant. The virus remains in the thrips through adulthood, and transmission occurs after 3 to 7 days of acquisition, with transmission taking place within 5 minutes of feeding on non-infected tissues. Adult thrips continue to be infective throughout their entire lifespan. Despite the existence of resistant tomato varieties, symptoms may still develop in infected plants, including small, dark spots, a bronze coloration on leaves, and streaks on the stems of infected terminals. Infected plants may also exhibit stunted growth and wilted leaves. If infection occurs prior to fruit set, plants may not produce fruit, whereas infected plants that have already set fruit will display green fruits with raised areas and concentric rings, as well as ripe fruits with chlorotic ringspots featuring concentric rings of red and white or yellow.

2.10.20. Tomato yellow leaf curl

Tomato yellow leaf curl virus (TYLCV) is a highly destructive disease that can cause complete yield loss in tomato plants when whitefly populations are high. TYLCV is transmitted by adult

whiteflies, which can acquire the virus within 15 minutes of feeding on an infected plant and transmit it after 6 hours. The virus can be transmitted to non-infected plants within 15 minutes of feeding, and whiteflies can retain the virus for several weeks. TYLCV can infect a variety of hosts and even resistant tomato varieties can be infected, with greater yield losses in plants infected at an early age. The virus causes severe stunting, flower loss, reduced leaf size, upward leaf curling, mottling, chlorosis, and significant reductions in yield. Fruit production is often decreased due to flower loss.

2.10.21. Verticillium wilt

Verticillium wilt, caused by the fungi *Verticillium albo-atrum* and *V. dahliae*, is a disease that typically occurs in cool weather. The fungi responsible for the disease are present in the soil and can survive for several years even in the absence of a host, by infecting plant debris. Once inside the plant, the pathogen primarily affects the water-conducting tissue (xylem) and can be worsened in the presence of certain nematode species. Symptoms of the disease usually appear in the later stages of infection and include mild to moderate wilting during the hottest part of the day, followed by recovery at night. Leaflets may exhibit marginal and interveinal chlorosis, and characteristic V-shaped lesions may form on them. Additionally, vascular discoloration is visible in stems.

2.11. Nanotechnology

In a 1974 paper, Norio Taniguchi of Tokyo Science University coined the term "nanotechnology," which he defined as "the processing, separation, consolidation, and deformation of materials by one atom or one molecule." In the early 1980s, two significant discoveries propelled nanotechnology and nano science: the rise of cluster science and the introduction of the scanning tunnelling microscope (STM) (Ganguly & Mukhopadhayay 2011). As a result of this achievement, fullerenes were discovered in 1985. Rice University uses three distinct nanotechnologies:

- **Wet nanotechnology** is the study of biological systems that are mostly found in water. The functional nanometer-scale structures of interest here are genetic material, membranes, enzymes, and other biological components. The presence of living entities whose shape, function, and evolution are determined by interactions of nanometer-scale structures demonstrates the efficacy of this nanotechnology (Singh *et al.* 2008).
- **Dry nanotechnology** this branch of chemistry studies the formation of structures in carbon (such as fullerenes and nanotubes), silicon, and other inorganic materials. In contrast to "wet" technologies, "dry" processes allow for the use of metals and semiconductors. These materials are too reactive to operate in a "wet" environment because they contain active conduction electrons, but they also have physical properties that make "dry" nanostructures promising as electrical, magnetic, and optical devices. Another goal is to develop "dry" structures with similar self-assembly properties to wet structures (Singh *et al.* 2008).
- **Computational nanotechnology** Complex nanometer-scale structures can be modelled and simulated. Nature spent hundreds of millions of years developing a workable "wet" nanotechnology; the understanding provided by computation should allow us to reduce the development period of a working "dry" nanotechnology to a few decades, with a significant

impact on the "wet" side as well. These three nanotechnologies are inextricably linked. The use of techniques or the adaptation of material from one has frequently resulted in significant advances for both (Srivastava *et al.* 2001).

2.11.1. Nano technology an introduction

Nano-science and nanotechnology research and applications have grown at an unprecedented rate in recent years. Nanotechnology is manipulation of materials at Nano scale of about 1-100 nm (Hasan, 2015). It has become promising field of science which is capable of providing various novel applications in almost all aspects of life. Nanoparticles are the basic components of nanotechnology. Reducing the size at Nano-scale can modify their chemical biological and physical properties compared with their higher scale particles. These properties are due to their improved mechanical strength, enhanced reactivity and solidity in chemical process and large surface area to volume (Balbus *et al.* 2007). Because of these intrinsic properties, nanoparticles are at the foremost edge of rapidly emerging field of nanotechnology. A nanoparticle had various dimensions depending upon the parameters such as length, breadth and height (Tan *et al.* 2013). They can be of zero dimensional where at single point all the parameters are fixed e.g. Nano dots, one dimensional with one parameter e.g. graphene, two dimensional having two parameters such as breadth and length e.g. carbon nanotubes and three dimensional owning all three parameters for example gold nanoparticles. The nanoparticles can be flat, spherical, conical, cylindrical, tubular, spiral, core and hollow characterized by their size, shape and structure. Moreover, they are made up of three layers (a) The core, which is the central part of nanoparticle; (b) The surface layer, containing metal ions, small molecules, polymers and surfactants; (c) The shell layer (Liu *et al.* 2016).

2.11.2. Nano-particles as a therapeutic agent to avoid the use of antibiotics

Nanoparticles range in size from 1 to 100nm. Their extremely small size makes them useful in a variety of medical procedures. Efforts have been made over the last decade to use nanoparticles in a variety of applications, including drug delivery, imaging, and the rapeuticsn (Kim & Hyeon 2013). Their high surface-to-volume ratio is due to their wide range of applications (Kumari *et al.* 2016). Because of their unique properties, the use of nanoparticles in magnetic resonance imaging (MRI) has grown rapidly in recent years. Nanoparticles have exceptional magnetic moieties and can be subjected to new modification related to targeting ligands due to large surface area. Furthermore, they effectively accumulate at the diseased site due to their anti-bacterial activity (Sirelkhatim *et al.* 2015a).

All of these properties of nanoparticles make them suitable for use in MRI, as they can greatly improve MRI resolution and sensitivity. Cancer drug delivery also includes the use of nanoparticles, which act as carriers and transport the drug to the site of action while having minimal toxic effects on nearby normal cells (Sirelkhatim *et al.* 2015a). Furthermore, these nanoparticles allow for exquisite customization. These changes specifically allow for improved binding to various sites such as cancer cell membranes, nuclear pore sites, and so on. This improves

the effectiveness of anti-cancer therapy (Jesline *et al.* 2015). Traditional drug delivery systems fall short in several areas, including target specificity and controlled drug delivery. The use of nanoparticles in drug therapy is not only target specific, but also a safe method with low toxicity (Aleaghil *et al.* 2016). Furthermore, by modifying or tuning the functional groups on nanoparticles, they can be used effectively as anti-bacterial agents (Li *et al.* 2014).

2.11.3. Antibiotics as conventional anti-bacterial agents

Since their discovery, antibiotics have proven to be effective against a wide range of infectious diseases. The first antibiotic, penicillin, was discovered in 1929, and numerous antibiotics have been introduced to the world since then. The discovery of antibiotics and their role in disease treatment significantly reduced infection mortality. The discovery of penicillin paved the way for the development of other antibiotics, resulting in a significant decrease in infection-related deaths in the United States. The mortality rate fell from 280 deaths per 100,000 people to as low as 60 deaths per 100,000 people (Nielsen *et al.* 2019). However, due to antibiotic resistance, antibiotics have become less effective in providing significant results in recent years. Antibiotic resistance has hindered the treatment of a variety of diseases. Methicillin-resistant S. aureus strains are becoming more common in hospitalized patients (Vysakh & Jeya 2013). Due to increased antibiotic resistance, the world is facing serious problems in treating infections caused by *S. aureus* and *E.coli*. According to WHO, the world will soon enter a post-antibiotic era in which common infections will kill a large number of people.

2.11.4. Inefficacy of antibiotics

Antibiotic resistance in bacteria, whether gram positive or gram negative, is a major cause of the recent increase in the death rate. The misuse or overuse of antibiotics is the leading cause of antibiotic resistance (Davies & Davies 2010). The majority of patients with a history of antibiotic use have developed antibiotic resistance. Antibiotic resistance in bacteria is classified into two types: intrinsic resistance and acquired resistance. Bacterial intrinsic resistance is achieved through normal innate processes found in all bacterial species. Examples of intrinsic resistance include the resistance provided by the bacterial cell wall and the active efflux mechanism (Cox & Wright 2013). Acquired resistance is achieved through either cross resistance caused by mutations in cell genes or horizontal gene transfer between different microbes. Horizontal gene transfer tools include plasmids transferred via conjugation or transformation, transposons transferred via conjugation, and integrins transferred via transduction (Giedraitienė *et al.* 2011). Microbes use a variety of mechanisms that contribute to antibiotic resistance. These mechanisms can include (1) changes to the enzymes that are the antibiotics' actual target. (2) Antibiotic degradation by enzymes, and (3) changes in membrane permeability that impede antibiotic movement within the cell (Read & Woods 2014).

2.12. Nanoparticles as an Antimicrobial Agent

Nanoparticles are highly auspicious particles that are gaining interest in addressing antibiotic resistance concerns because they can be used as better alternative antimicrobial agents.

Antimicrobial nanomaterial has evolved in chemical composition and properties. This is the reason for their various modes of action. Furthermore, bacterial sensitivity to nanoparticles is directly related to many bacterial factors such as their physiological state, genetics, metabolic pathways, and many other components Nath, Banerjee, & pharmacology. Furthermore, environmental factors such as temperature, pH, and aeration have a significant influence on their antimicrobial activity. The physiochemical properties of particles, such as size, shape, and concentration, are also linked to the bacterial lethality of nanoparticles (Read & Woods 2014). Metal nanoparticles have been discovered to be the most effective antimicrobial agent against common pathogens. Silver, zinc oxide, and titanium dioxide have received a lot of attention as therapeutic agents in health care and industry (Loomba & Scarabelli 2013). Several studies have been conducted to investigate the antimicrobial activity of zinc nanoparticles against *Xanthomonas compestris* and *Ralstonia solanacearum*. Zinc ions have the potential to cause similar morphological changes in Gram-positive and Gram-negative organisms. The cytoplasmic membrane separates from the cell wall, revealing a region in the cell's centre containing deoxyribonucleic acid molecules. (S.H. Kim et al., 2011). Antimicrobial activity of zinc nanoparticles against multidrug resistant pathogens is currently being investigated. Methicillin resistant *Staphylococcus aureus* (MRSA) outperformed Methicillin resistant *Staphylococcus epidermidis* (MRSE) and *Streptococcus pyogenes* (Bindu & Thomas 2014). *Klebsiella pneumonia* and *Salmonella typhi* showed little activity against Zn nanoparticles (Dobrucka & Długaszewska 2016). They are also effective against *Salmonella enteritidis*, *Listeria monocytogenes*, and *E. coli* O157:H7. Several other studies have confirmed the catalytic activity against food-borne pathogens like *Salmonella typhimurium* and *Staphylococcus aureus* (Arfat *et al.* 2016).

In recent years, serious contagious contaminations have fundamentally added to the growing ailment and lethality of compromised immune systems patients who require treatment is very extensive, including broad-spectrum antibiotic therapy. There have been few studies on metal nanoparticles' antifungal activity. Zinc nanoparticles have been studied for their static and fungicidal effects on selected pathogenic yeasts that cause incursive life-threatening fungal infection in critical care patients. Zinc NPs have strong antifungal activity against pathogenic Candida spp. at concentrations of around 1 mg/L Zn. Zinc NPs have antifungal properties similar to ionic zinc. The antifungal effects of round zinc nanoparticles on dermatophytes have been investigated. Trichophyton mentagrophytes clinical isolates and ATCC strains were resistant to the Nano Zn. The activity of nano-antifungal Zn is attributed to its effects on mycelia. Nano zinc with antifungal activity has also been reported to be used in the bio stabilization of footwear materials, with a 1% solution inhibiting the growth of the majority of yeast-like parasitic and form strains. Nano-Zn kills parasites by focusing on yeast cell layers and disrupting film potential. The collaboration between Nano-Zn and the layer structure of *C. albicans* cells during Nano-Ag introduction causes changes in the layers of *C. albicans*, which can be seen as "pits" on the film surfaces, according to transmission electron microscopy analysis. Cell passage is thus facilitated by the arrangement of pores. (c) The shell layer of surfactants (Shin *et al.* 2016).

2.13. Nanoparticles-Biomedical Aspect

With the emergence of nanotechnology, nanoparticles have been gaining much interest in biomedical applications with the concern of significant improvement in diagnosis and treatment of diseases. The distinctive properties such as their potential to absorb and carry other compounds, the surface to mass ratio and their quantum properties make them significantly attractive in biomedical applications. Moreover, they have relatively huge surface area to bind and carry other compounds such as proteins, drugs and probes. Due to their small size, nanoparticles can circulate throughout the body and bind with the specific cells as well. This feature has allowed the development of enhanced imaging of organs and diseased tissues in body and brings a revolution in diagnostics. Magnetic nanoparticles are not only used as an alternative to radioactive technetium to detect spread of cancer cells in body along with lymph nodes but also has the ability to kill tumors via hyperthermia. In addition, nanoparticles have the ability to increase the fluorescent imaging along with the enhanced images in ultrasound and positron emission tomography (PET). Their miscellaneous applications in medicine includes neutraceutical, both in vitro and in vivo diagnostic, drug delivery and production of better biocompatible materials (de Jong *et al.* 2005). Recently, with advancement in drug delivery, they are considered as promising treatment of neurological disorders including Alzheimer disease, Parkinson disease and multiple sclerosis. Nanoparticles and Nano fibers have their applications in tissue repair therapies as they have significant role in design and manufacture of innovative framework for bone and tissue repair. Only biocompatible nanostructures such as calcium hydroxide can be used for therapeutic purpose. Also, nanoparticles play their role in the progression of health-related products such as a sunscreen; Optisol was designed with titanium dioxide nanoparticles along with manganese to block the ultraviolet rays on skin. Although, nanoparticles are likely to have improved diagnostic and treatment approaches in medicine but they also have significant impact on human health that needs to be considered regarding their biomedical applications in future. For example, very few studies were conducted about the effect of nanoparticles after they get entered in our body or whether they have any harmful effects on our body. The concerns related to the effectiveness and safety of nanoparticles can be resolved by clinical trials. Nanoparticles required for medical applications should be manufactured under sterile conditions.

2.14. Antibacterial activity of nanoparticles and mechanism of action

Due to the anti-bacterial activity of nanoparticles, they are considered as the substitute of antibiotics in the treatment of diseases. The adsorption of nanoparticles on the cell wall of bacteria is the first step in the mechanism of action of nanoparticles. The adsorption of nanoparticles on the cell wall causes disintegration of the cell wall, giving it a porous appearance (Durán *et al.* 2016). This actually occurs due to the de-polymerization of the cell wall that decreases its negative charge and thus makes it permeable. When viewed under laser scanning confocal microscope, the cell wall of the bacteria appears blurred confirming its disintegration. After the first step this is the entry of nanoparticles into the cell. Second step that is the formation of reactive oxygen species take place (Ahmad *et al.* 2019). These reactive oxygen species stop the ATP production and also

result in the hindrance of DNA replication. Due to this the respiratory activity of bacteria is disrupted which lowers the chances for cell survival. Moreover, reproduction of the bacteria ultimately stops as the DNA replication is hindered (Yang *et al.* 2009). Furthermore, as the cell wall becomes pours the cellular contents move out of the cell and result in cell death.

2.14.1. Comparative Analysis of nanoparticles against Gram negative and Gram-positive bacteria

Difference in the activity of nanoparticles against gram positive and gram negative are quite prominent. This is because gram positive and gram-negative bacteria have different cell wall compositions. Gram negative bacteria have an 8nm thick layer of peptidoglycan and a 1-3um thick layer of lipopolysaccharides. While gram positive bacteria have thick layer of peptidoglycan of about 80nm thick (Pasquina-Lemonche *et al.* 2020). The attachment of nanoparticles with the gram-negative bacteria proves to be more detrimental as they lack thick peptidoglycan layer that acts as a protector like in case of Gram-positive bacteria (Nermina Malanovic, 2016). Another reason for this could be the presence of lipopolysaccharide layer I n gram negative bacteria which carries a negative charge. As the ions released by nanoparticles are positively charged and have higher affinity for negatively charged lipopolysaccharide that results in effective entry of nanoparticles into the gram-negative bacteria.

2.15. Types of nano-particles:

There are many types of nanoparticles. Organic and inorganic nanoparticles are the two main types of nanoparticles. Organic nanoparticles are polymeric structures composed of organic molecules. Both top down and bottom-up approaches can be used to synthesis these. Top-down approach involves the production of organic nanoparticles using various techniques like mechanical milling etc. While bottoms down approach involves the self – assembly if organic molecules. Organic polymeric molecules are in the form of vesicles, micelles, polymersomes etc. Conjugation between these systems can result in variation of sizes in organic nanoparticles (Romero & Moya 2012). The examples of organic nanoparticles can be lipid-based nanoparticles, polymeric based nanoparticles. Lipid based nanoparticles possess an internal core that is made up of lipid and an external core that is stabilized by the use of surfactants and emulsifier (Rawat *et al.* 2011). Same is the case with polymeric nanoparticles which contain two cores, the inner core contains a solid mass which on the outer surface various molecules are adsorbed (Rao & Geckeler 2011).

The other types of nano particles include inorganic nanoparticles. Their major component is the inorganic material like metal, metal oxide, carbon etc. The carbon-based inorganic nanoparticles include carbon based nano tubes are 1-2 nm in diameters and in the form of rolling graphite sheets. Other carbon based inorganic nanoparticles include fullerences that have gained wide industrial importance due to their unique electrical conductivity, electron affinity, high strength etc (Astefanei 2015). Metal based nanoparticles also come under the type of inorganic nanoparticles. They are purely made up of metals and are known to possess unique optoelectrical properties due to characteristic surface resonance. Size controlled synthesis of metal nanoparticles is very

important for various applications in medical (Dreaden *et al.* 2012). Semi-conductor nanoparticles are also an example of inorganic nanoparticles. They find wide applications in literature especially in the processes like photo catalysis and electronic devices (Rizwan *et al.* 2017).

2.16. Applications of nanoparticles

Due to antimicrobial of nano particles, they are frequently being used in coating the medical devices like catheters, surgical instruments etc. Nanoparticles are also being used in agriculture to enhance the growth of the crops for mass production. Major applications of nanoparticles in agriculture are in the form of nano fertilizers, nano pesticides or as the carrier of conventional pesticides. Moreover, they are also used in drug delivery systems in cancer treatment (Barbara Haley, 2008). They are highly appreciated for their use in cancer treatment as they go and bind with the cancer cell membranes, cytoplasmic or nuclear receptor sites. They bind their and allow the drug to act at that specific site while minimizing the effect of drug on normal cells.

Various metallic nano particles have antimicrobial activity and have proved beneficial for human race. These metallic nano particles can be made by varying the composition of the functional groups which play role in binding with the ligands, antibiotics and various other drugs of interest. Due to this property, they play a prominent role in targeted drug delivery, vehicles or carriers for gene and drug delivery. Nano particles are of different types.

The first type is known as metal nano particles. Gold, silver, platinum, copper, titanium, iron, zinc nano particles fall under first type. These nano particles are made up of pure metals hence they are referred as metal nano particles. Among these gold, silver and titanium nano particles are commonly known as noble nano particles. These noble nano particles possess electron resonance oscillation which is known as localized surface plasmon resonance. Various processes have been developed that can integrate these noble nano particles into biological systems without causing any toxic effects. This has paved way for their usage in medicine and biological systems. Gold nano particles are widely used due to their low toxicity and ease of preparation. Moreover, silver nano particles have proved to exhibit exceptional antimicrobial activities due to which they are prominently been used as antimicrobial agents (Jun Sung Kim, 2007). These noble nano particles have wide spread applications in drug delivery, gene delivery, investigation assays etc.

Second type of nano particles is the metallic oxide nano particles. Recent studies have shown their antimicrobial nature. Due to this their role in drug delivery and diagnostic purposes is emerging. Examples of metal oxide nano particles can be TiO_2, ZnO, Al_2O_3, FeO etc. Photo catalysis reactions are preferred to be carried out in the presence of ZnO as it is considered as bio safe material. ZnO nano particles are widely used in photo degradation process as they are prominent photo catalyst candidates (Chin Bon Ong, 2018). Al_2O_3 also possess antimicrobial properties and has various applications in medical systems. Moreover, CuO nano particles have anti cancerous properties and have various biomedical applications. The apoptotic effect of CuO nanoparticles is mainly due to the generation of reactive oxygen species along with the disruption of the

mitochondrial membrane potential (Renu Sankar, 2014). TiO_2 is another nano particle that has antimicrobial properties and is used to inhibit the transmission of various diseases.

The third type of nano particles include doped metal or metal oxide nano particles. These include zinc, copper, TiO_2 etc. Doped nano particles. Proangiogenic properties are possessed by Zn doped Titania nano particles due to which they have multiples of applications. TiO_2 co-doped Ag nano particles and carbonaceous sheets show increased antimicrobial activity (V Poornima Parvathi, 2020).

Forth type of nano particles is the metal sulphide nano particles. Its known example can be of cadmium sulphide nano particle. CdS having surface modified with chitosan can be effectively used for drug delivery and for imaging of cells as it retains their fluorescence within cell while reducing their toxicity (R Harish, 2020). Fifth type of nano particles are the metal organic frame works (MOFs). These consist of metal ions and organic clusters. They possess unique properties like high pore volume, large surface area and informal surface variation. All these properties make MOFs suitable for drug delivery process (Mihad Ibrahim, 2017).

2.17. Approaches for synthesis of nanoparticles

Nanoparticles can be formed by various methods whether physical, chemical or biological but all these methods use one of the two approaches in the nanoparticle synthesis. The two approaches that are being used are the bottom up and top-down approach (Singh,2020).

2.17.1. Bottom-up approach

This approach involves the self-assembly of various smaller components of molecular or atomic size. This self-assembly of nano materials then results in the formation of nano structures. In this approach nano particles are formed atom by atom or molecule by molecule (Jitendra pal singh,2020). Sol gel synthesis, organometallic chemical route, hydrothermal route, electro-disposition etc. are some of the most widely used processes that involve bottom-up approach.

2.17.2. Top-down approach

While in the top-down approach larger initial. Structures are used whose size is then reduced to nano size by various techniques e.g lithography. This technique results in the removal of certain amount of the bulk material to reduce its size to the required one. In other words, it is the miniaturization of the bulk fabrication processes. Bottom-up approach is less expensive and can be used for large scale production while top-down approach is quite expensive and is un-favourable for large scale production. Moreover, imperfection of surface related issues is more common in top-down approach.

Nanoparticles can be synthesized from various physical, chemical and biological methods.

2.17.3. Chemical Methods

Some of the chemical methods for the synthesis of nano particles are as follows:

2.17.3.1. Polyol method

Non aqueous liquids also known as Polyols are used as solvents in this method. Reducing agents are also required in this process. Surface oxidation and agglomeration is minimized by the use of non-aqueous liquids. This method is quite intriguing as it allows to take adjust the size, texture and shape of nanoparticles according to the need. In the synthesis of metal oxide nano particles the most common solvent that is being used is the ethylene glycol (Chieng, 2012). It is suitable for use as it has strong reducing capacity, high boiling point and high dielectric constant. Being a cross linking agent, it links with the metal ion and results in the formation of metal glycolate which is then subjected to oligomerization. When the metal glycolate is calcined in air, it results in the formation of more common metal oxide derivatives. During this process their properties are strictly maintained. Bimetallic alloys and core shell nano particles have also been produces by this method. Yang and colleagues used the Polyol method to create icosahedral and cubic gold particles with diameters ranging from 100 to 300 nm.

2.17.3.2. Micro emulsion

This method involves the mixing of water and oil. As this happens two phases are formed as water and oil are immiscible. The water-water/oil-oil contentions that are present must be replaced with the water-oil connection. About 30-50 dynes/cm of interfacial surface tension can be present between the oil and the water phases. This can be removed by the use of surfactants as they contain lipophilic and hydrophilic groups. When metal nanoparticles are to be prepared with micro emulsion method, the major process that takes is the mixing of two micro emulsions contains metal salt and reducing agent. After the mixing of two micro emulsions Brownian motion is formed due to proper collision. Proper collision further results in the good fusion and mixing of reactants hence good quality of nanoparticles.

2.17.3.3. Thermal decomposition

This is another technique being used for the chemical synthesis of nano particles. It is also known as thermolysis. In this process the chemical bonds in the compound undergoing thermal decomposition are broken down by applying heat. This process is usually endothermic. A thermal runaway is produced by this process is the process is sufficiently exothermic. Partial et al. investigated the infrared spectra and thermal decomposition of metal acetates and dicarboxylates. This investigation yielded positive results.

2.17.3.4. Chemical vapor decomposition

In this process the substrate is exposed to vapor phase precursors which then deposit on the substrate and form a thin layer. Then there is a CDV reactor in which the previously vaporized precursors are placed. These vaporized precursors then adsorb on the surface of the substance that has already been placed in the CDV reactor at an elevated temperature (Ajit Behera,2020). The adsorbed precursors then react with other molecules in the surface and result in the formation of crystals. During this process various by products are produced during the gas phase. These by

products should be removed. The nano particles are formed as a result of a chemical reaction that occurs in the gaseous phase. By carefully controlling the chemical reaction taking place in the gaseous phase, ultrafine nano particles with sizes less than 1um can be formed.

2.17.4. Physical methods

Some of the methods used for the synthesis of nano particles are as follows:

2.17.4.1. Plasma

It is one of the physical methods used for the production of nano particles. In this process the evacuated chamber contains a pestle in which initial metal is enclosed. This evacuated chamber is wrapped around with high radio frequency (RF) coils which heat the metal above their evaporating point (Ahmed, 2021). Helium gas is allowed to flow through the system. As it passes through the system, high-temperature plasma forms in the region where the coils are present. This results in the generation of metal vapors that nucleate on the helium gas atoms. These then diffuse on cold collector rod from where the nanoparticles can be collected. Then the nanoparticles that have been collected are passivated with treating them with oxygen gas.

2.17.4.2. Microwave irradiation

This is another physical method used for nano particle synthesis. This is a rapid process and size of the nano particles can be effectively controlled by this process. It is quite efficient and economic process. This process requires a reducing agent, a stabilizer and a liquid medium in which the process will take place (Saifuddin, 2009). Then this whole setup is acted upon by microwave irradiation. The product result after this is monitored for its properties using various techniques. Various nano particles have been produced by this technique. Ag nanoparticles have been produced by micro irradiation using an aqueous solution of silver nitrate and trisodium citrate. In this process formaldehyde was used as the reducing agent.

2.17.4.3. Pulsed laser method

This method is used to create zinc nanoparticles. It entails using a blender-like device to mix zinc nitrate solution and a reducing agent. This blender device contains a disc which is irradiated or Pulsed with the laser beam. This results in the formation of hot spot in the disc (Mohammad Zamakhsari, 2019). Formation of zinc nano particles actually takes place in these discs by the reaction of silver nitrate with the reducing agent. The zinc nano particles that are being formed are separated by centrifugation. Angular velocity of the disc and the energy if the laser striking the disc are the two factors that play an important role in determining the size of silver nanoparticles.

These are some of the physical and chemical techniques that are being used for the production of nano particles. There are number of other techniques that are being used as well. Chemical techniques use both bottom up and top-down approaches while physical techniques involve the use of top down approach only.

2.17.5. Green Synthesis of nanoparticles

Micro-organisms, plants and their by-products like lipids, proteins etc. can be used in the green synthesis. Green synthesis does not involve the use of expensive chemicals, uses less energy and produces products or by products that are eco-friendly (Pal *et al.* 2019). Metallic nanoparticles are being made from the plants. During this synthesis of nano particles from plants, plants are used as bio-reducing agents while metallic salts are used as precursors. This results in the formation of biocompatible nanoparticles. Green synthesis used the bottom-up approach. Using bottom-up approach nanoparticles are synthesized using reducing agents and stabilizing agents.

Green synthesis is preferred over physical or chemical synthesis of nano particles. The main reason for this is the use of reagents that are non-toxic, eco-friendly and safer to use. Green synthesis is a very simple and easy process as it is a one step process. The nanoparticles synthesized from this process are quite stable and of diverse nature. High radiation along with highly concentrated reductants are being used in the physical and chemical synthesis of nanoparticles which are detrimental for the environment that is very they are not much appreciated (Thakkar *et al.* 2010). During the chemical synthesis, the size of the synthesized zinc nanoparticle is stabilized by using certain chemical agents. As these toxic chemicals get absorbed on the surface, they prove to be hazardous for medical uses (Ramya & Subapriya 2012). These chemical agents being used either for synthesis or for the stabilization are non- eco-friendly. The synthesis of zinc nanoparticles from plant extract is ecofriendly as it does not produce any toxic chemicals and acts as a natural source of capping agents. Moreover, this process is also very cost effective as this process is free from the processes like culture isolation, media preparation etc.

The needles of the *Picea smithiana* tree is exceptionally sturdy and coated in a thick covering of exceedingly unique. The tips of the petals and sepals of the flowers feature prominent green spots. From southeast Pakistan nearly all the way to the southernmost point of India, *Picea smithiana* can be found in tropical dry forests. It was just discovered in western Bangladesh. Despite its large range, *Picea smithiana* habitat is severely threatened by urbanization and agriculture. (Slender Forms: Moringa Concanensis, M. Oleifera, M. Peregrina, n.d.). Antiulcer, anti-helminthic and anti-dysentric properties are possessed by this plant (Indra, 2013).

2.18. Nanotechnology dominating world

Nano technology is an emerging field due to its numerous benefits that it offers. It is a technology or engineering that is strictly conducted at nano scale level. By nano scale it means that it deals with things that are between the sizes of 1-100 nano meters. This technology majorly deals with the manipulation that is being implemented at atomic or molecular level (Bandyopadhyay *et al.* 2012). High precision and accuracy is required. Physicist Richard Feynman is known as the father of nano technology. In 1959, at American physical society meeting held in California institute of technology a talk was held that actually introduced the concept of nanotechnology even before the term was coined. This talk was entitled “There’s Plenty of Room at the Bottom”. Richard Feynman used this platform and explained how various processes can be used to control the manipulation at

atomic or molecular level (Sandhu 2006). Nanotechnology has gained much fame in medicine, electronics and construction. Its contribution in medicine is revolutionizing as it plays an evident role in gene therapy, drug delivery and diagnostics (Khan *et al.* 2019). High surface area along with extremely small size of nanoparticles they are known to possess unique physical and chemical properties. The optical properties and reactivity of nanoparticles are all size dependent. Depending upon the size they impart different colors due to the absorption of light in visible region .Various properties of the synthesized silver nanoparticle effects their biological characteristics e.g., size distribution, particle composition, efficiency of iron release, particle reactivity in solution, dissolution rates, chemical sensing, bio sensing, catalysis etc. which in turn greatly affect their medical nature (Chandra Hembram *et al.* 2016). One very crucial factor in determining the cytotoxicity is the type of reducing agent used while synthesizing it.

CHAPTER # 3

Objectives of Research

3. Objectives

1. The aim of current study was the synthesis of ZnO NPs from needles extract of *Picea smithiana* via green synthesis approach.
2. It was also aimed to do the characterization of biogenically synthesized ZNPs through Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) analysis, Dynamic light scattering (DLS) and UV-visible spectroscopy.
3. Mainly current study was aimed to investigated the antimicrobial activity against *Xanthomonas compestris* pv. *vesicotoria* (FCBP Acc. No.; FCBP-PB-0003) and *Ralstonia solanacearum* (FCBP Acc. No.; FCBP-PB-0407) obtained from First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, Punjab University, Lahore, Pakistan.
4. It was also aimed to investigate the impact of ZNPs on tomato plants morphology, particularly on their roots and physiology to determine its optimal dosage for tomato growth promotion.

CHAPTER # 4

Material and Methods

SECTION # 1

ZnO Nanoparticles Synthesis and Characterization and Antibacterial Activity

4. Material and methods

4.1. Nanoparticles Synthesis and Characterization

4.1.1. Plant Sampling and Extract Preparation

Healthy plants of *Picea smithiana* L. were selected for biogenesis of ZnO nanoparticles from forest plants in Kafar-Khn (Talah) (34◦ 19′ 0″ N and 73◦ 44′ 0″ E), Muzaffarabad, Azad and Jamu Kashmir, Pakistan at the altitude of 2756 m (Fig. 3.1). Plant needles samples were collected randomly using sterilized polythene bags during August 2022 and then brought to Environmental and Microbial Botany Lab. In order to remove the surface impurities, the needles were washed thoroughly in 70% ethanol followed by washing with deionized water. Fifty g of were macerated in a blender with 150 ml of deionized water, shade dried and ground to a fine powder for further studies. Ten grams of powdered needles were added to 100 mL of distilled water in a conical flask and heated on a hot plate at 70 degrees Celsius for 2 hours. The resulting light brown extract was cooled, filtered twice using Whatman filter paper for removal of any residue, and stored for future use (Bhagat *et al.* 2017).

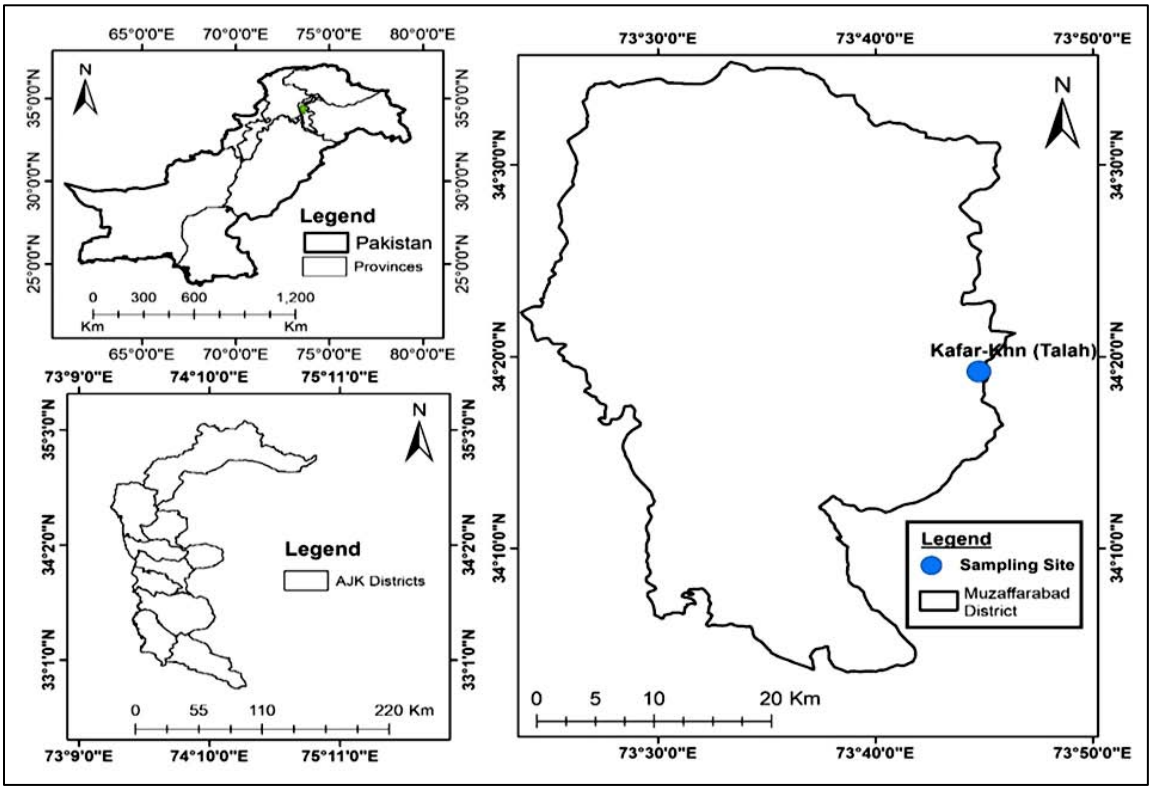

Figure 4.1. Map of sampling site

4.1.2. Biosynthesis of ZnO nanoparticles

ZNPs synthesis was carried out using the needles extracts of *Picea smithiana*. The aqueous solution of 200 ml of 0.04 M zinc acetate ($Zn(CH_3CO_2)_2$) was prepared in double distilled water. This zinc acetate solution was added in the 200 ml of plant extract by drop wise separately. The mixture was stirred completely by using magnetic stirrer for 15 min. The basic pH of solution was maintained by adding NaOH. The solution was heated with microwaves at a frequency up to 300 GHz to disruption of weak hydrogen bonds. The solution was allowed to rest for 12 hours at ambient temperature and then centrifuged at 6000 rpm to obtain a pellet. The pellet was dried at 70 °C for 3 hours (Karam & Abdulrahman 2022).

Figure 4.2. Biosynthesis of ZnO NPs from extract of *Picea smithiana* by using Zinc acetate.

4.1.3. Characterization of biosynthesized ZnO NPs

4.1.3.1. UV–Vis spectroscopy

To monitor the reduction of zinc ions, the optical density (OD) of the reaction mixture containing the extract and zinc acetate was measured before drying the ZNPs. This was achieved by diluting a small portion of the reaction mixture with Milli-Q water and analyzing it using UV-Vis spectrophotometry. The analysis was performed with a UV-Vis spectrophotometer (Multiskan GO, ESW Version 1.01.12 with Serial Number 1510-05040C).

4.1.3.2. Dynamic light scattering (DLS) analysis

The particle size distribution and average size of ZNPs were analyzed using a particle size analyzer that employed dynamic light scattering (Malvern Zetasizer, Nano Z500 UK). The sample was

prepared by diluting it with Milli-Q water, centrifuging, and transferring it to a cuvette. The sample holder temperature was maintained at 25 degrees Celsius during the analysis. The results are mainly affected by various factors such as the surface structure, core size, type of ion present in the mixture, and concentration of particles.

4.1.3.3. X-ray diffraction

The size of ZNPs was studied using X-ray diffraction. Firstly, the centrifugation of biogenically synthesized was done ZNPs at 10 °C for 12 minutes at 3000 rpm. Afterwards, the resulting pellet was washed with ethanol and sterile Milli-Q water, and the procedure was repeated two to three times. The purified ZNPs were dried in oven at 70 °C. Then precipitates of purified ZNPs were ground with a ceramic pestle–mortar. Finely ground sample of ZNPs was analyzed using X-ray diffractometer (Model Theta-Theta S/N 65022; STOE Germany). The scanning of sample was done at 20 to 80 of 2θ (degree).

4.1.3.4. Scanning electron microscopy

The morphological analysis was carried out using JEOL JSM-5910. By using small amount of ZNPs powder, thin film was prepared on carbon-coated tape of grid. The excess powder was delicately wiped away with blotting paper. The mercury lamp of SEM was used to dry the film for 5 minutes, which helped to determine the surface structure of ZNPs.

4.1.3.5. FTIR analysis

FTIR analysis was carried out to determine the functional groups that played a role in the reduction of zinc ion and the capping of reduced ZNPs. FTIR spectrum was recorded using FT/IR-Model Spectrum 100; Perkin Elmer, USA infrared (IR) double-beam spectrophotometer. Potassium bromide (KBr) pellet method was used for FTIR analysis of dried ZNPs in 30:1 ratio (KBr: NPs) and in transmittance mode, spectrum was obtained at a resolution of 4 cm^{-1}. The wave number was plotted on X-axis and the peaks obtained in the form of stretching were plotted on Y-axis as transmittance. The recorded and analyzed spectrum had a wave number range of 450-4000 cm^{-1}.

4.1.4. Antimicrobial assay

4.1.4.1. Laboratory study

The lima bean (LB) broth medium was prepared having four different ZNPs concentrations including 25, 50, 75 and 100µg/mL. The bacterial cultures including *Ralstonia solanacearum* and *Xanthomonas compestris* pv. *vesicotoria* were inoculated on broths media having ZNPs different concentrations. The optical density (OD) was calculated at every next day.

The inoculation of fresh colonies of *Ralstonia solanacearum* and *Xanthomonas compestris* pv. *vesicotoria* was done into 250 ml of lima bean (LB) broth medium. The bacterial growth was observed using a UV-Visible spectrophotometer at 600 nm with intervals of 7 hours, until the optical density (OD) of 0.9 was obtained. OD of 0.92 corresponded to about 6.72 x 10^8 CFU mL^{-1} of bacterial strains. The ability of biogenically synthesized ZNPs to inhibit the growth of bacterial

pathogens was assessed through an *in vitro* agar well diffusion assay. The LB agar media was sterilized and then cooled and approximately 20 ml of cooled sterilized media was poured in petri dishes.

Already prepared isolates of *Ralstonia solanacearum* and *Xanthomonas compestris* pv. *vesicotoria* in LB broth medium were poured (15 mL) on agar plates. On agar plates, the bacterial cultures were spread uniformly. After pouring and solidifying agar media on Petri dishes, the wells of 6 mm diameter were made by using cork borer. In this study, four concentrations of the ZNPs suspension (25, 50, 75 and 100µg/mL) used. Each concentration was prepared by dispersing 50 µL of each concentration into separate wells. The positive control was maintained by adding Streptomycin (10 µg/ml). After incubation overnight at 35 °C, the zones of inhibition were measured using a Vernier caliper.

4.1.4.2. Greenhouse Study

Tomato seeds of variety Naqeeb obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan were used for In vitro screening under greenhouse conditions. The seeds were treated with surface sterilization prior to planting by being soaked in 0.5% NaOCl for 1.5 minutes and rinsed three times with double distilled water. The sterilized seeds were germinated in plastic germinator trays filled with sterilized sand and sterilized farmyard manure with the ratio of 1:1 as potting mixture. After 20 days, the tomato seedlings were carefully uprooted, dipped into the inoculum of each bacteria for about 10 min, and then sown in plastic pots having sterilized clay:sand:farm yard manure with 1:1:1 as potting mixture. The untreated seedlings dipped in double-distilled water and set as control. 60 pots were used to grow the tomato plants, with five sets of replications. The tomato plants were grown in 60 pots with five replications. The experiment was conducted in the greenhouse and pots were watered regularly. The experimental design was a completely randomized design (CRD).

The disease scoring was recorded three days after inoculation and continued every three days for up to four ratings. The disease parameters including disease severity and disease incidence were recorded. At each rating time, disease incidence (%) and disease severity were recorded. Disease severity rated with a 0-9 scale was used to calculate the disease severity index percentage as proposed by Bayaa et al. (1995) with minor modification. The plants with score of 0 were considered as immune (with 0% infection), those with rating scale values 1-3 were considered as 1-25% infected, with 4-6 as 25-50% infected and with score of 7-9 were considered as susceptible (50% or more) to both pathogens. The formula of Kranz (1988) was used to determine the severity index percentage

$$Disease\ Severity\ Index = \frac{\Sigma(a \times b)}{N \times Z} \times 100$$

where, N = Total number of plants per pot

Z = Highest scale value

(a x b) = Sum of the symptomatic plants and their corresponding severity rating value

After observing the disease symptoms, the four different concentrations of ZNPs were used against both disease as treatment. ZNPs suspensions of concentration 25, 50, 75 and 100 µg/ml were prepared in double-distilled water. For application, suspensions of 40 ml properly mixed using vortex mixture were applied. After ZNPs applications, the disease severity index of treated plants was again recorded after the interval of 7 days as mentioned above.

4.1.5. Statistical study

Five replicates were conducted for all of the tests, and the results were presented as the mean ± standard errors. A one-way variant analysis was used to analyze the results.

SECTION # 2

ZnO Nanoparticles and Plant Growth Promotion

4.2. Nanoparticles and Plant Growth Promotion

4.2.1. Plant materials and Source of ZnO nanoparticles

The tomato seeds (*Lycopersicon esculentum* Mill. cv. Naqeeb) were procured from the National Agriculture Research Centre, Islamabad, Pakistan. The ZNPs were sourced from the Environmental and Microbial Botany Lab at the Department of Plant Sciences at Quaid-I-Azam University in Islamabad, Pakistan. The required concentration of ZNPs (25, 50, 75 or 100 µg/mL) was suspended in deionized water (10mL) in a volumetric flask of 100 mL.

4.2.2. Seed Germination Assay and Seedling Assay

The seeds were subjected to surface sterilization by first washing them with 70% ethanol for 2 minutes and then with distilled water three times. After washing for 5 minutes with 1.5% sodium hypochlorite solution and rinsing with distilled water four times, the specimens were subjected to liquid solutions with ZNPs of concentrations including 0, 25, 50, 75 or 100 µg/mL to evaluate their effect. At the onset of the investigation, 20 seeds that had been sterilized were placed in every petri dish having 10 mL of the nanoparticle solutions. The seeds were left for germination for six days in dark condition. Daily counts of the number of germinated seeds were made, and the percentage of germination (GP%) was calculated by the equation given below:

$$Germination\ Percentage = \frac{Germinated\ Seeds}{Total\ Seeds} \times 100$$

In the next stage of the experiment, the impact of ZNPs on shoot and root length was evaluated by transferring the seedlings from pots and exposing them to various concentrations of ZNPs in a greenhouse setting (25° C) for 8 days. Then the tomato plants were carefully removed from the soil and their root and shoot lengths were measured using a meter scale. An electronic balance was used to measure the fresh and dry masses, while a portable leaf area meter was utilized to measure the leaf area. The plant biomasses were recorded and the vigor index was obtained using the following equation:

$$Vigor\ Index = [Average\ Root\ Lenght + Average\ Shoot\ Lenght] \times Germinatin\ Percentage$$

Three plants of equal size were included in each replicate, and the treatment was repeated three times.

4.2.3. Photosynthetic Pigments

The treated and untreated leaves (100 mg) of plants samples were taken for extraction used for assessment of photosynthetic pigments and chilled acetone:ethanol (1:1, v/v). After centrifugation for 12 minutes at 8000 rpm, the supernatant was obtained. The total chlorophyll content including chlorophyll a and b as well as carotenoid content was determined by measuring the absorbance at 663, 645, 480, and 510 nm (Hashemi *et al.* 2019).

$$Total\ Chlorophyll\ (mg/g) = \frac{[\{20.2(A645) + 8.02(\mathrm{A663})\} \times \mathrm{V}]}{(1000 \times \mathrm{W})}$$

$$Chlorophyll\ a\ (mg/g) = \frac{[\{12.7(\mathrm{A663}) - 2.63(\mathrm{A645})\} \times \mathrm{V}]}{(1000 \times \mathrm{W})}$$

$$Chlorophyll\ b\ (mg/g) = \frac{[\{22.9(\mathrm{A645}) - 4.68(\mathrm{A663})\} \times \mathrm{V}]}{(1000 \times \mathrm{W})}$$

$$Carotenoid\ (mg/g) = \frac{[\{7.6\ (\mathrm{A480} - 2.63(\mathrm{A645})\} \times \mathrm{V}]}{(1000 \times \mathrm{W})}$$

4.2.4. Proline content analysis

For extract preparation, 3 mL of 3% sulfosalicyclic acid dihydrate and 0.1 g of freeze-dried powder was used and the mixture was shaken at 200 rpm for 25 min (Ramzan *et al.* 2022). After centrifuging the supernatant at 3000 rpm for 8 minutes, it was passed through a syringe filter (0.2-µm) using a syringe of 1-mL and which was stored. Further dilutions of supernatant were prepared with 3% sulfosalicyclic acid dehydrate, and spectrophotometer was used for measuring the proline content (Bates *et al.* 1973). In a 15-mL tube, a mixture of the supernatant (500 µL), acid ninhydrin (500 µL) and acetic acid (500 µL) was prepared and then subjected to vortexing for 25 seconds and shaking at 120 rpm in a water bath kept at 95 °C for 1 hour. After rapid cooling of mixture on ice for 10 minutes, toluene (1 mL) was added to the supernatant, which was further vortexed and then centrifuged at 4000 rpm for 8 minutes. After centrifugation, the supernatant was transferred to a 96-well plate. The sample was analyzed at 520 nm using a microplate reader. A commercial L-proline standard which gives linear range from 0–100 µg mL^{-1} was used for measuring the proline content.

4.2.5. Indole acetic acid

IAA was measured by extracting 100 mg of leaves from both treated and controlled tomato plants in 10 mL of chilled acetone and ethanol used with 1:1, v/v. The analysis of indole acetic acid content was conducted following the procedure outlined by Larsen and his colleagues (Miller *et al.* 1987).

4.2.6. Antioxidant Enzymatic Assay

The harvested plants were used for enzyme analysis by following (Heath & Packer 1968). The control and treated seedlings were mixed in a solution of sodium acetate buffer (0.1 M) (pH 4.8) with NaCl (10 mM) and subsequently subjected to centrifugation. The supernatant was utilized to evaluate the activities of ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (GPX) (Singh *et al.* 2020). To calculate the SOD activity, nitrobluetetrazolium (NBT) was photochemically reduced. For APX activity, the reaction mixture comprised of enzyme extract, H_2O_2 (1.0 mM), EDTA (0.1 mM), phosphate buffer (25 mM) (pH

7.0), APX, and the absorbance was recorded at 290 nm for 60 seconds. The monitoring of the oxidation of guaiacol to tetraguaiacol was used to determine the GPX activity, by recording of absorbance changes at 470 nm. On the bases dissociation of H_2O_2, CAT activity was evaluated and the decrease in absorbance at 240 nm was monitored. The enzymatic activity was quantified as U/gram of fresh plant weight for all enzymes.

4.2.7. Extracts Preparation and Antioxidative Potential

The tomato plant material was dried ground using a mortar and pestle and a 100 mg/ml suspension was made in Dimethyl Sulfoxide. It was placed in Eppendorf tubes and stored for 48 hours at normal temperature before centrifugation for 3 minutes at 5500 rpm. The supernatant was then used to determine the antioxidant activities (total reducing power, DPPH-based free radical scavenging activity and total antioxidant potential) and non-enzymatic antioxidants including flavonoids and phenolics.

4.2.7.1. Free Radical Scavenging

DPPH reagent was utilized to assess the free radical scavenging activity (Rehman *et al.* 2014). 10 µL extract was mixed with 0.004% DPPH in methanol (190 µL) and left to incubate in the dark for 1 hour. The OD was recorded by using a microplate reader at 515 nm. The positive standard was ascorbic acid while the negative control was DMSO. The inhibition percentage was calculated using the following formula:

$$\%\ Scavenging\ Activity = Percent\ Inhibition = (1 - \frac{Ab_S}{Ab_C}) \times 100$$

Ab_c is absorbance of the negative control having reagent and solvent; Ab_s is absorbance of the solution of DPPH with sample

4.2.7.2. Total Antioxidant Capacity (TAC)

TAC of the extracts was measured using a modified method from Fatima and her colleagues (Fatima *et al.* 2015). 100 µL from each extract's stock solution was combined with 900 µL of a reagent mixture consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. This solution was further incubated for 90 minutes at 95°C. After cooling, OD was measured by using spectrophotometer at 695 nm with a microplate reader. Dimethyl Sulfoxide was used as a control instead of the test samples. Ascorbic acid was used for calibration curve as a positive control. The TAC was determined by measuring the amount of ascorbic acid equivalent per unit of fresh weight which was expressed as µg AAE/mg FW.

4.2.8. Total Reducing Power (TRP)

The estimation of reduction potential was done by using the method (Ul-Haq *et al.* 2012). 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (250 µL) were added to each sample which was incubated at 50◦C for 20 minutes. After that, 10% trichloroacetic acid (200 µL) was used to acidify the reaction mixture. After centrifugation for 10 minutes, a supernatant layer of

150 µL was obtained, which was combined with 0.1% ferric chloride solution (50 µL). Ascorbic acid was used as positive control. The OD was then measured at 630 nm, and the results were reported as µg ascorbic acid equivalent per mg of fresh weight (µg AAE/mg FW).

4.2.8.1. Total Phenolic Contents (TPC)

TPC was measured by previously described method (Ul-Haq *et al.* 2012). To summarize, 20 µl of DMSO-extracted sample was added to each well of a 96 well plate. 7.5% Na_2CO_3 (90 µl) and Folin–Ciocalteu reagent (90 µl) were added after five minutes. After one hour incubation, absorbance was measured by using a microplate reader at 650 nm. Standard (gallic acid in DMSO) and Blank (DMSO) were also included as controls. A calibration curve was generated in parallel using gallic acid (6.25–50 µg/mL) under the same conditions, resulting in a TPC value reported as µg gallic acid equivalent per mg FW (µg GAE/mg FW).

4.2.8.2. Total Flavonoid Content (TFC)

The estimation of TFC was carried out by using the method previously outlined (Ul-Haq *et al.* 2012). 20 µL of extract was mixed with 1 M potassium acetate solution (10 µL) and 10% aluminum chloride solution. To final volume of 200 µL, distilled water was added. The mixture was leftover for 30 minutes, and the absorbance was measured at a wavelength of 415 nm by using a microplate reader (Bioteck, USA). The quercetin was used as standard to make calibration curve ($y = 0.0301x + 0.00854$, $R_2 = 0.989$), ranging from 0 to 40 µg/mL.

4.2.9. Statistical Analysis

The research aimed to examine the impact of nanoparticles on the germination and plant characteristics of tomato seeds. Four seeds were placed in each of the five flasks containing different concentrations. The data has been given as the average with a standard error. The antioxidant tests were conducted three times, and the results were analyzed using ANOVA and LSD.

CHAPTER # 5

Results_Section: 1

5. Results

5.1. XRD analysis

The biogenically synthesized ZNPs were subjected to the powder XRD analysis annealed at 450 °C under an air atmosphere for 2.15 h and has been illustrated in Fig. 5.1. The analysis was performed within an angular range of 2θ, ranging from 20 to 80 degrees. Nine diffraction peaks were observed in XRD pattern of the sample scattering from the (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2) and (2 0 1) planes. These diffraction peaks were properly assigned by using JCPDS Card No. 01-080-0074. These diffractions peaks were consistent with ZnO hexagonal structure (P63mc). The corresponding space group of ZNPs was P63mc with space group number of 186. The nanoscale nature of biogenically synthesized sample was confirmed by observing relatively broad Bragg peaks (Yogamalar *et al.* 2009).

The broadening of the X-ray diffraction line was probably caused by both instrumental and sample broadening. The standard samples were used for the assessment of instrumental broadening and the instrumental corrected broadening (β_{hkl}) was derived by using the following equation to deconvolute these two contributions.

$$\beta_{hkl}^2=[\beta_{measured}^2-\beta_{instrument}^2]$$

The Scherrer method was employed to estimate the average crystallite size using the XRD pattern for peak width analysis.

$$D = \frac{k\lambda}{\beta_{hkl}\cos\theta}$$

Where λ is the incident wavelength, in correspondence with diffraction angle θ, β_{hkl} is the full width at half maximum of the XRD Bragg peak and k is the shape factor. By using Scherrer method, the calculated average crystallite size was found to be D = 26.43 nm (Table 1.1). In addition to crystallite size and instrumental broadening, lattice strain (ε) also contributes to XRD peak broadening (Venkateswarlu *et al.* 2010). In nanoparticles, the primary source of lattice strain is the stress field produced by the extra volume of grain boundaries. The diffraction line broadening caused by lattice strain (ε) changes with tan θ, whereas the broadening due to crystallite size (D) changes with 1/cos θ.

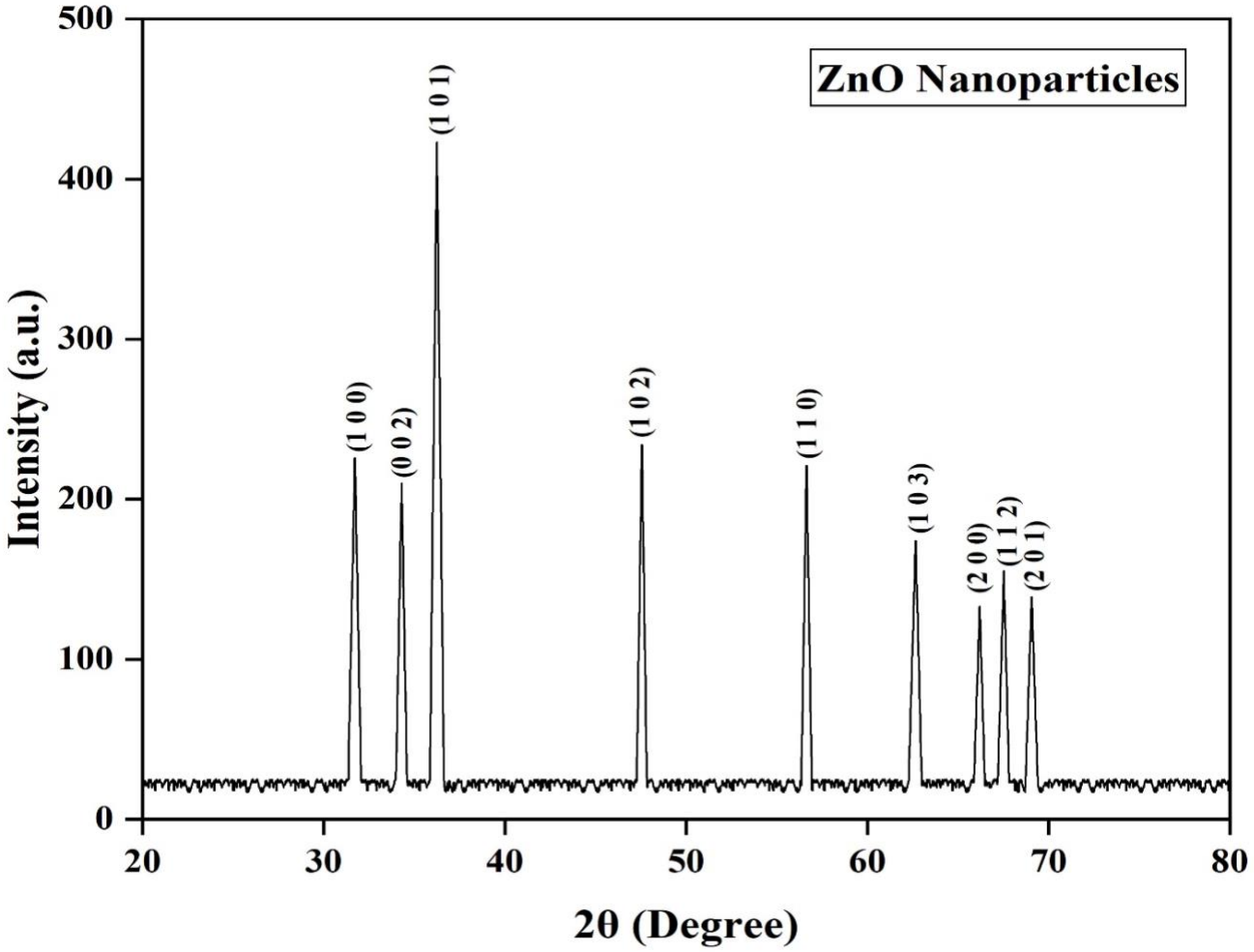

Figure 5.1. XRD pattern for green synthesized ZnO nanoparticles of *P. smithiana.*

Table 5.1. Peak Position (2θ), FWHM (β) along with size (nm) obtained from XRD analysis.

Sr. No.	Peak Position (2θ)	FWHM (β)	Size (nm)	Average Size (nm)
1	31.71795	0.37958	21.74882559	
2	34.2975	0.32132	25.86414361	
3	36.24739	0.40596	20.58268629	
4	47.55732	0.2918	29.73906557	**26.42695265**
5	56.64612	0.30172	29.89907376	
6	62.65059	0.38175	24.35186952	
7	66.19069	0.31771	29.83553019	
8	67.52139	0.33583	28.44291933	
9	69.06544	0.35209	27.37846001	

5.2. Potential mechanism of the reaction and chemical bonding

FTIR spectroscopy analysis confirmed the formation of ZNPs. The bioactive compounds involved in the formation of ZNPs were also recognized by Fourier transform IR analysis. FTIR spectrum was recorded to monitor the interaction between zinc precursor salt and biologically active compounds in *Picea smthiana* extract during the formation of ZNPs. The capping agent of bio-reduced ZNPs and possible bioactive molecules responsible for ZnO reduction were identified in the FTIR spectrum by defined wave numbers. Fourier transform IR spectrum obtained after reaction of $Zn(CH_3CO_2)_2$ and leaf extract are shown in Fig. 5.2. The band of absorption were observed at 3447, 3340, 1580, 1404, 1270, 1161, 1031, 910, 831, 763, 660 and 450-500 cm^{-1} when zinc acetate was added in needles extract *Picea smithiana* (Table 5.2) FTIR spectrum G-ZNPs confirmed alcohol and phenol of -OH stretching are around 3447 cm^{-1}. These results have also been reported in encapsulation of curcumin using fucoidan stabilized zein NPs (Zhang *et al.* 2021). The presence of bonds due to OH bond of carbohydrates proteins and polyphenols and O–H stretching had the wavelength of range 3340 cm^{-1} (Ezati *et al.* 2020). The formation of peak at 1580 cm^{-1} showed the presence of 1° Amines. Similar outcomes have been documented in the creation of NH^{2-}graphene through the reduction of graphene oxide in situ (Lai *et al.* 2011). The maximum peak found at 1404 cm^{-1} in the spectrum has been attributed to the stretching of carboxyl side groups in amino acid residues that make up protein molecules (Ramesh *et al.* 2015). These results also coincide with already reported results of synthesis of ZNPs from the leaf extract of *Solanum nigrum* (Ramesh *et al.* 2015). The absorption band obtained at 1270 cm^{-1} corresponds to C-H waging of Alkyl halides (Arivazhagan & Kavitha 2012). The peaks at 1161, 1031, 910, 831, 763 and 660 cm^{-1} showed the presence of C-O-C Symmetric Stretching, -CH = CH_2, Aromatic Compound, C-N Stretching, C-Cl Stretching of Alkyl halides and Halogens (Cl-Br). The absorption bands in the range of 1161 to 660 cm^{-1} revealed the crystal structure, the strengths of the IR bands, and the chemical bonds of various compounds. Meanwhile, the stretching of ZNPs was observed in the range of 450-500 cm^{-1} (Sohail *et al.* 2017). Overall, the presence of peaks in the data suggests that biogenically synthesized ZNPs were surrounded by functional groups present in proteins and other metabolites, such as terpenoids.

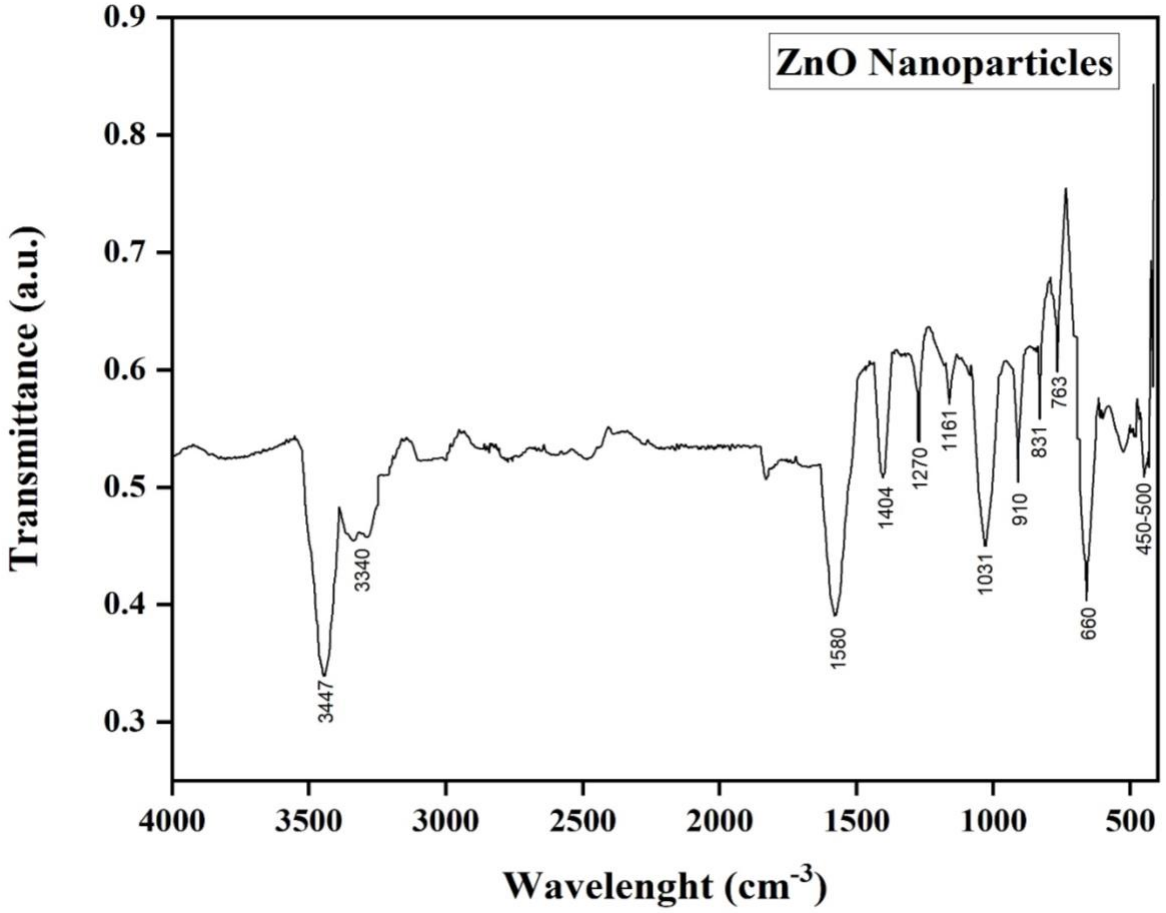

Figure 5.2. FTIR spectra of biologically synthesized ZNPs using *Picea smithiana*.

Table 5.2. FTIR absorption peak values (cm-1) along with functional groups.

Sr. No.	Absorption Peak (cm^{-3})	Functional Group
1	450-500	ZnO Stretching
2	660	Halogens (Cl-Br)
3	763	C-Cl Stretching of Alkyl halides
4	831	Aromatic Compound
5	910	$-CH = CH_2$
6	1031	Ethers / C-O-C Symmetric Stretching
7	1161	C-N Stretching / Vibration of Protein
8	1270	C-H Waging of Alkyl halides
9	1404	Alkanes
10	1618	Diketones
11	1580	1° Amines
12	3340	OH Carbohydrates Proteins and Polyphenols
13	3447	Alcohol and Phenol of -OH Stretching

5.3. Scanning Electron Microscopy

To investigate the dimensions and morphology of ZNPs synthesized through biogenic means, various magnification levels were used (Fig. 5.3). The formation of agglomerated form was observed in SEM images. Various studies report that the antimicrobial activities of ZNPs majorly depend upon surface morphology (Nguyen *et al.* 2022; Thirumoorthy *et al.* 2021; Vasantharaj *et al.* 2021). The particles were mainly hexagonal in shape with size of 25 nm, which was also confirmed by XRD analysis.

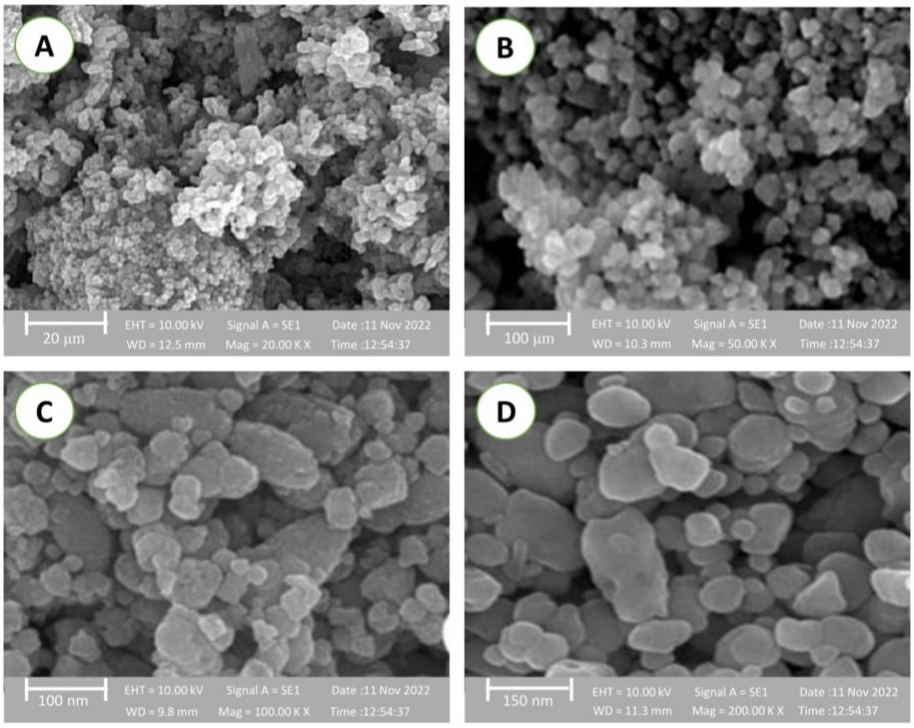

Figure 5.3. SEM images of ZnO nanoparticle of *P. smithiana*.

5.4. Ultraviolet–visible spectroscopy

The optical properties of ZNPs were analyzed through UV–visible absorbance spectrum by the strong and clear absorption band in spectrum obtained at 380 nm (Fig. 5.4.). The capability of crystalline structure to absorb electrons at specific wavelength depend on the arrangement of atoms in that structures (Ramesh *et al.* 2015). It suggests excitation of ZNPs by ultra violet light. The majority of the spectrum absorbed by bulk ZnO is in the vicinity of 385 nm. In comparison with bulk ZnO, the blue shift absorption for the biosynthesized ZNPs may be attributed to their decreased particle size (Lee *et al.* 2019). It is observed that the absorption capability of the G-ZNPs is more prominent in the ultra violet than in the visible regions of the spectrum, which may be advantageous for optical applications (Mydeen *et al.* 2020b). We noted that the G-ZNPs exhibit a UV range of 320-390 nm, with a peak at 365 nm (Jamdagni *et al.* 2018). Calculating the optical band gap energy requires exciting valence band electrons into the conduction band. The optical band energy was computed using the Tauc Equation, (Makuła *et al.* 2018):

$$\alpha h\nu = (h\nu - E_g)^n$$

Where Eg is optical band gap, λ is the wavelength of absorption peak, α is the absorption coefficient, h is Plank's constant (6.626×10^{-34} Joules/s), c is velocity of light (2.99×10^{-8} m/s) and *ν* is the incident photon frequency. The absorption process involved in sample is characterized by value of n i.e. n = ½ and n = 2 are for direct and indirect band gap transitions respectively. The absorption band was obtained at 380 nm and the band gap, calculated using the Tauc equation, was found to be 3.262 eV. The biosynthesized nanoparticles' small particle size is mainly responsible for the wide band gap.

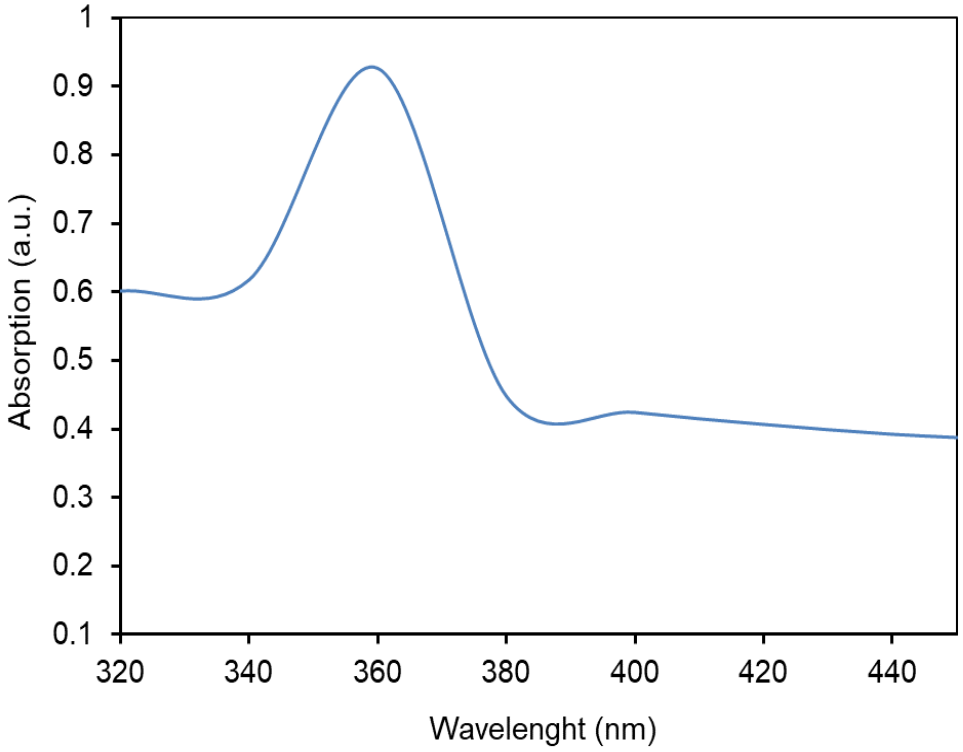

Figure 5.4. UV-Vis absorption spectrum of ZnO nanoparticles of *P. smithiana*

5.5. Dynamic light scattering analysis

DLS analysis is influenced by several factors such as surface structure size, particle core size, type of ions presents, and concentration of particles. On the base fluctuations in scattered light intensity, the diffusion coefficient of diffusing particles can be measured. The stability of the particles can also be determined by DLS. Through studying the increase in hydrodynamic radius by using DLS analysis, it can also be determined whether the particles aggregate over time. If the radii of huge number of particles are comparatively larger, the particles will aggregate. The results of Dynamic Light Scattering (DLS) analysis on biogenically synthesized ZNPs showed a mean size of 25 nm, represented by a single peak at about 100%, with a Polydispersity Index (PDI) of 0.223 (Fig. 5.5).

Figure 5.5. DLS graph of ZnO nanoparticles of *P. smithiana.*

5.6. Antimicrobial Activity Analysis

5.6.1. Optical Density

The antimicrobial activities of biologically synthesized G-ZNPs were determined against *Ralstonia solanacearum* and *Xanthomonas compestris* pv. *vesicotoria* at four different concentrations. The antimicrobial efficacy of biogenically synthesized ZNPs depends upon on type of phytopathogen (Abdallah *et al.* 2020). The OD of ZNPs treated broths with various concentrations having bacterial cultures were measured. The highest OD was recorded in case of control and it was lowest in case of 100 µg/mL. At concentration of 100 µg/mL, the OD values for *Xanthomonas compestris* pv. *vesicotoria* at 3rd, 4th and 5th day were 0.50, 0.55 and 0.54 while at 25 µg/mL were 0.83, 0.88 and 0.85 respectively. At concentration of 100 µg/mL, the OD values for *Ralstonia solanacearum* at 3rd, 4th and 5th were 0.52, 0.57 and 0.55 while at 25 µg/mL were 0.83, 0.90 and 0.87 respectively (Fig. 5.6).

Figure 5.6. The OD of ZNPs treated broths with various concentrations having bacterial cultures at 3rd, 4th and 5th day (A). *Xanthomonas compestris* pv. *vesicotoria* (B). *Ralstonia solanacearum*

5.6.2. Inhibition Zone

The presence of a distinct inhibition zone demonstrated the biocidal effect of ZNPs. Studies have revealed the various mechanisms behind this biocidal action, including membrane disruption, a high rate of surface oxygen species formation, and ultimately, the death of pathogens. ZNPs inhibited growth of both phytopathogens as evidenced by ZOI's (Fig. 5.7). The ZOI grew larger as the concentration of ZNP increased, and the zones were slightly bigger for *X. campestris* pv. *vesicatoria* compared to *R. solanacearum*. The maximum inhibition zones against *Ralstonia solanacearum* and *Xanthomonas campestris pv. vesicatoria* have been observed at 100 µg/ml with the size of 18.9 ± 1.5 and 20.1 ± 1.5 mm, respectively (Fig. 5.8).

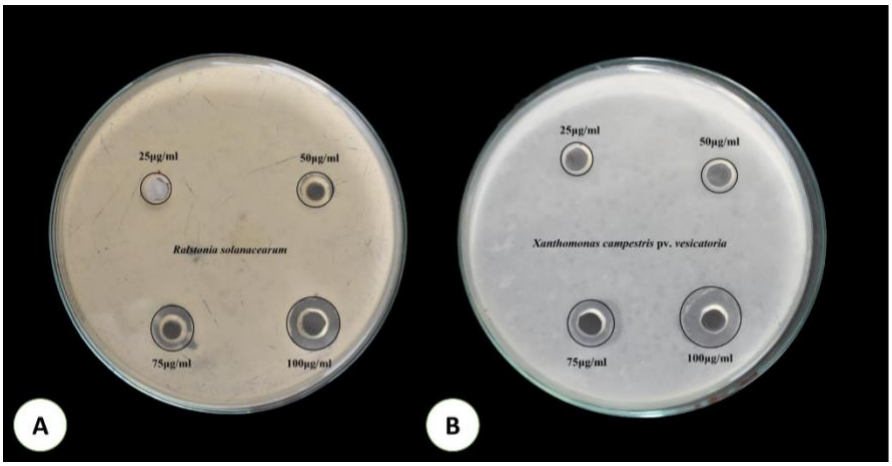

Figure 5.7. Testing pathogenic organism's susceptibility to various concentrations of ZNPs (A). *Ralstonia solanacearum* (B). *Xanthomonas campestris* pv. *vesicatoria* with concentrations of 1). 25 µg/ml, 2). 50 µg/ml, 3). 75 µg/ml and 4). 100 µg/ml.

Figure 5.8. Impact of various concentrations of ZNPs on the zones of inhibitions shown by *Xanthomonas campestris* pv. *vesicatoria* and *Ralstonia solanacearum*.

5.7. Disease severity

The disease severity was measured 7 DAI in five replicates (Fig. 5.9). The area under disease progressive curve of disease severity has been given in Fig. 5.10 and Fig. 5.11. It was reduced to 12.5 ± 1.2 % in *Xanthomonas campestris pv. vesicatoria*, whereas, 13.66 ± 1.1 % in *Ralstonia solanacearum* after the ZNPs applications at 100 µg/ml. In control, the disease severity was recorded as 68.06 ± 1.2 and 63.98 ± 1.1 % in *Ralstonia solanacearum* and *Xanthomonas*

campestris pv. vesicatoria, respectively. The lesser disease reduction was recorded at 25 µg/ml against both bacteria with the means value of disease severity 20.21 ± 1.1 and 20. ± 1.3 %. From the obtained results, it has been demonstrated that the applications of ZNPs at concentrations of 100, 75, 50, 25 µg/ml and streptomycin treatment gave about 52.56 ± 1.23, 46.45 ± 1.27, 44.78 ± 1.98, 43.87 ± 1.89 and 60.12 ± 1.41 % biocontrol efficacy against *Xanthomonas campestris pv. vesicatoria,* whereas, 54.47 ± 1.56, 50.9 ± 1.34, 48.38 ± 1.21, 47.15 ± 1.99 and 63.45 ± 1.89 % against *Ralstonia solanacearum* respectively (Table. 5.3).

Table 5.3. Tomato bacterial leaf spot and tomato bacterial wilting severity 7 days after inoculation with *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria* respectively along with ZnO NPs treatments at various concentrations including 100, 75, 50 and 25.

Treatments	*Xanthomonas campestris*	*Ralstonia solanacearum*
Control	62.99 ± 1.98^{d}	65.97 ± 1.34^{d}
ZnO NPs 100 µg/ml	12.22 ± 1.65^{a}	14.78 ± 1.67^{a}
ZnO NPs 75 µg/ml	16.78 ± 1.67^{b}	17.45 ± 1.56^{b}
ZnO NPs 50 µg/ml	19.02 ± 1.54^{c}	20.89 ± 1.87^{c}
ZnO NPs 25 µg/ml	20.21 ± 1.12^{c}	21.19 ± 1.34^{c}
Streptomycin 10 µg/ml	13.45 ± 1.77^{a}	14.54 ± 1.71^{a}

Values are the means of 5 replicated plots treatment.

5.8. Disease Incidence

The disease incidence was measured 4 DAI in five replicates. It was recorded as 5.16 ± 1.3 % in *Xanthomonas campestris pv. vesicatoria*, whereas, 6.74 ± 1.1 % in *Ralstonia solanacearum* after the ZNPs applications at 100 µg/ml. In control, the disease incidence was recorded as 85.06 ± 1.45 and 83.59 ± 1.56 % in *Ralstonia solanacearum* and *Xanthomonas campestris pv. vesicatoria*, respectively. The lesser reduction in disease incidence was recorded at the concentration of 25 µg/ml against both bacteria with the means value of disease incidence 40.04 ± 1.23 and 37.50 ± 1.45 % (Table 5.4).

Table 5.4. Tomato bacterial leaf spot and tomato bacterial wilting incidence 4 days after inoculation with *Xanthomonas compestris* pv. *vesicatoria* and *Ralstonia solanacearum* respectively along with ZnO NPs treatments at various concentrations including 100, 75, 50 and 25 µg/ml. Control treatment is represented by distilled water.

Treatments	*Xanthomonas campestris*	*Ralstonia solanacearum*
Control	86.43 ± 1.98^{e}	89.56 ± 2.02^{e}
ZnO NPs 100 µg/ml	5.66 ± 0.78^{a}	7.96 ± 0.87^{a}
ZnO NPs 75 µg/ml	12.56 ± 1.09^{b}	14.56 ± 1.17^{b}
ZnO NPs 50 µg/ml	28.96 ± 1.32^{c}	26.44 ± 1.43^{c}
ZnO NPs 25 µg/ml	40.04 ± 1.45^{d}	37.50 ± 1.55^{d}
Streptomycin 10 µg/ml	9.78 ± 0.99^{a}	8.32 ± 0.89^{a}

Figure 5.9. (A). *Xanthomonas campestris* pv. *vesicatoria* causing tomato wilt disease symptoms (B). *Ralstonia solanacearum* causing tomato leaf spot disease symptom (C). Treated with ZnO nanoparticles. (D). Control.

5.9. Area under disease progressive curve

Extracts of *Picea smithiana* needles contain sterols, terpenoids, alkaloids, tennis, phenols, ethyl acetate, n-hexane, acetone, saponins, methanolic, quinones, chloroform, glycosides, flavonoids, anthraquinones, quinones, and many and isoquinoline alkaloids (Thapa-Magar *et al.* 2020). These alkaloids include indoles, quinolizidines, pyrrolizidines, piperidines, tropanes, imidazoles and isoquinoline purines (Sharma *et al.* 2004). The functional groups present in all these compounds play a vital role in the reduction of ZNPs. Among the piperidine compounds, 1,6-dehydropinidine had the greatest antimicrobial effect (Virjamo *et al.* 2020). Stilbenes, with heterologous bridges connecting two aromatic rings (phenolic compounds), are also produced by various conifers, including *Picea* spp. (Bhardwaj *et al.* 2021; García-Pérez *et al.* 2012). Excellent antimicrobial activity of stilbenes has been reported (Metsämuuronen & Sirén 2019). The barks for conifers contain the cis-stilbene, trans-pinosylvin, piceatannol and resveratrol. Tannins cause microbial inhibition with pro-oxidative enzymes, transition metal chelation and free radical scavenging (Koleckar *et al.* 2008). These antioxidant properties, lead to complexes of proteins and other compounds by causing binding through making these bound molecules unavailable for microbial growth inhibiting extracellular microbial enzymes and by acting directly on microbial metabolisms by altering the proteins in the cell membrane, causing denaturation (Koleckar *et al.* 2008). Other than tannins, polyphenols are also present in in *Picea smithiana* extract which suppress microbial activity through lipid peroxidation inhibition, and direct capillary constrictive action (Willför *et al.* 2007).

It seems that the way ZNPs exert their antimicrobial effect is by releasing oxygen species on their surface, causing harm to the phytopathogenic bacteria. The various studies reveal that the dissociation of Zn^{2+} plays a vital role in antimicrobial activities of ZNPs. The ZNPs have great potential to cause the lysis of bacterial cells through cell membrane disintegration followed by leakage of all cytoplasmic material (Kaushik et al., 2019). The Nanoparticles can also diffuse through porins that are beta-barrel proteins and this passive internalization has been seen in Gram-negative bacteria (Kalia *et al.* 2020). The Gram-positive bacteria have a dense and thick cell wall which makes difficulty and causes hinder in passive internalization of ZNPs. They result in dissolving Zn^{2+} that is present in the vicinity of the cell surface and released by ZNPs and chelated by lipoteichoic acid (Agarwal *et al.* 2018). Once they are within the bacterial cell, the incorporated nanoparticles (NPs) may have a substantial impact on the cell membrane, internal biomolecules, and other catalytic and soluble molecules through production of reactive oxygen species (ROS) using Fenton reaction or other non-Fenton processes (Patra *et al.* 2015). The released O species than react with H^+ ion and form H_2O_2 which gives antimicrobial activities by penetrating the bacterial cell membranes (Padmavathy & Vijayaraghavan 2008; Xie *et al.* 2011). The disintegration of ZNPs simultaneously or eventually releases ions, resulting in metal/nonmetal ion toxicity and cell death (Liu *et al.* 2020). Another intriguing method is to prevent the formation of biofilms by reducing the expression of the genetic makeup or functions controlled by quorum-sensing in bacteria (Al-Shabib *et al.* 2016).

The absorption of Zn^{2+} ions by bacterial cells through intercellular forces occurs when ZNPs interact with the negatively charged bacterial membrane (Murali *et al.* 2021; Sirelkhatim *et al.* 2015b). This changes the permeability of cell membrane and leads to mitochondrial distortion in bacterial cell (Ahmadi *et al.* 2019). ZNPs generate nicks and breaches in bacterial cell membrane, causing fragmentation and disunity in the membrane, ultimately leading to the death of the cell. Additionally, ZNPs interact with the bacterial cell wall and produce reactive oxygen species (ROS) that result in oxidation of unsaturated fatty acids, such as oleic acid, in the walls of bacterial cells, resulting in the destruction of the cells (Wang *et al.* 2017)

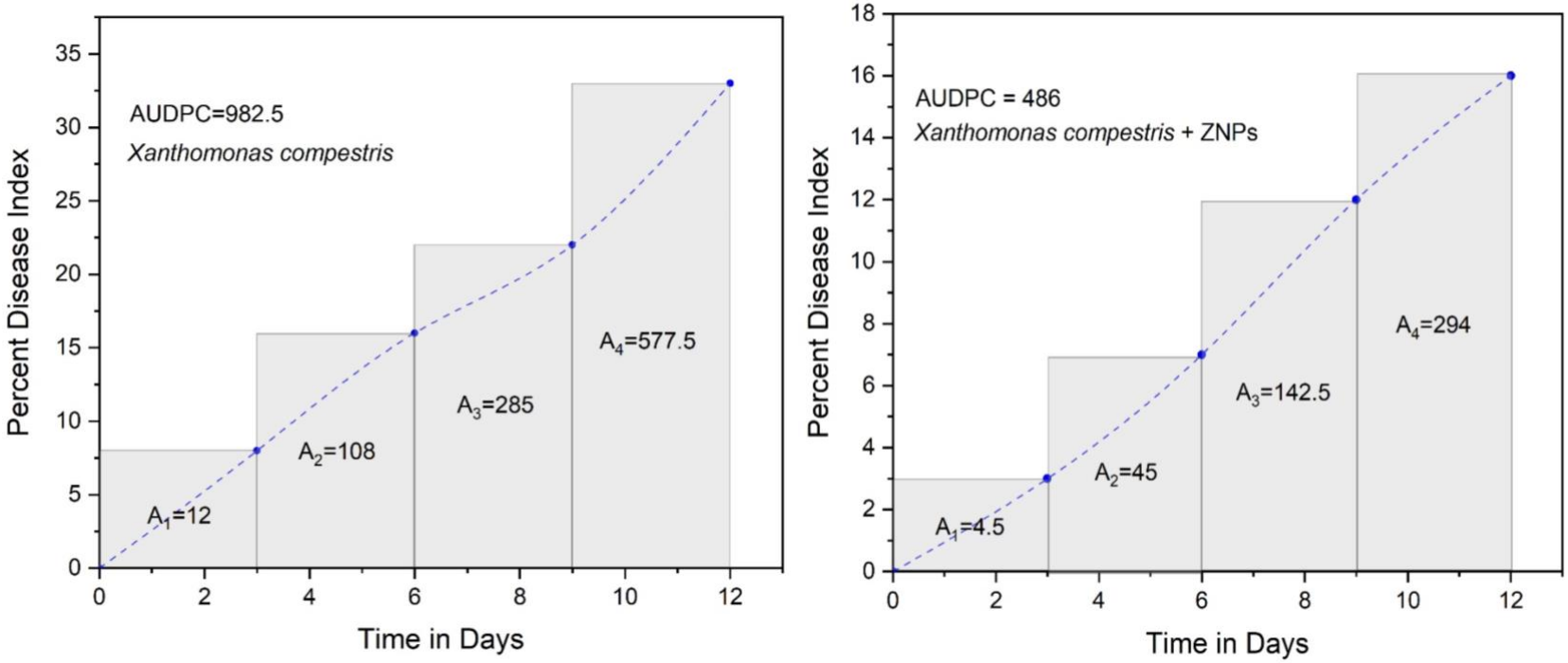

Figure 5.10. Area under disease progressive curve. AUDPC of tomato plant infected with *Xanthomonas compestris* pv. *vesicatoria* (Left) and infected plant treated with ZnO nanoparticles (Right).

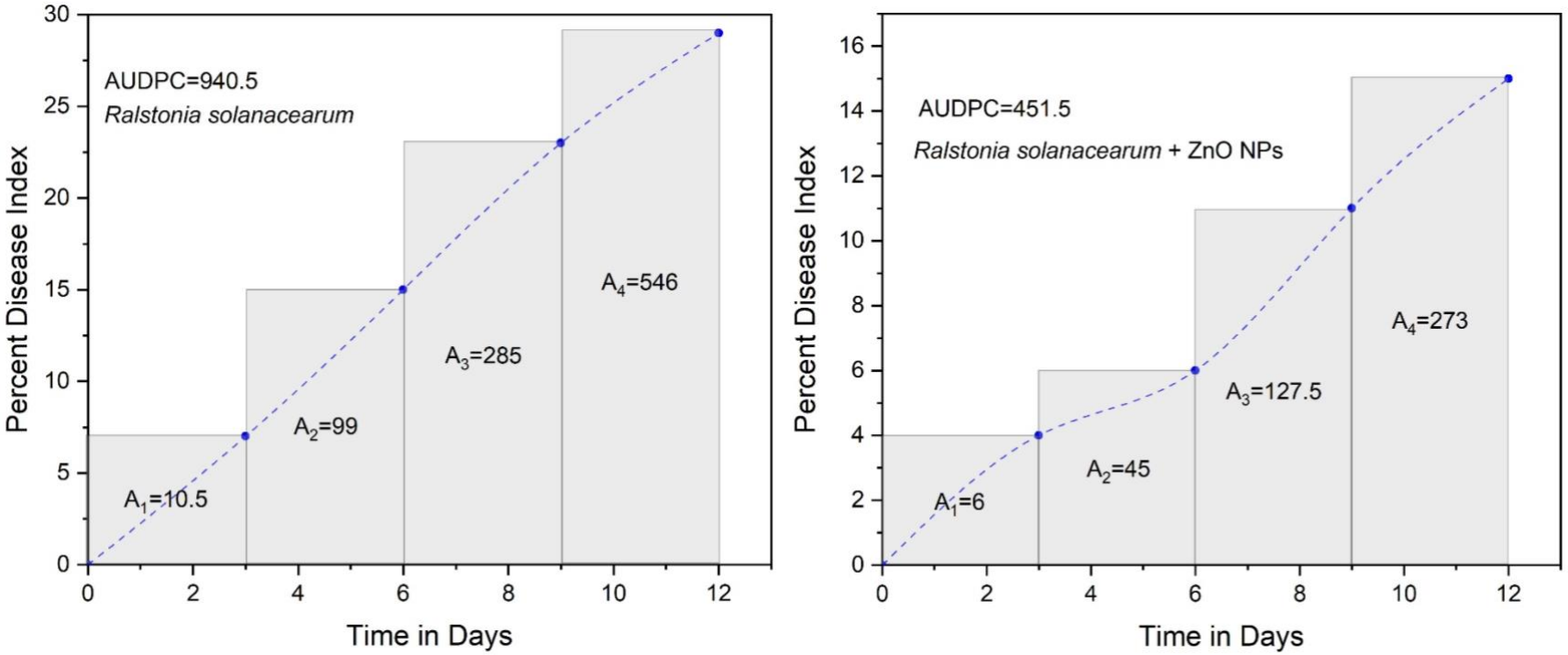

Figure 5.11. Area under disease progressive curve. AUDPC of tomato plant infected with *Ralstonia solanacearum* (Left) and infected plant treated with ZnO nanoparticles (Right).

CHAPTER # 5

Results_Section:2

5.10. Seed Germination and Seedling Assay.

The results obtained from current research demonstrated that the application of ZNPs enhanced the germination rate of tomato seed. In germination assay, it was found that the rate of germination of seeds in petri dish was markedly high in case of 75 µg/mL. There was a bit reduction in gemmation at concentration of 100 µg/mL. The germination time was mainly affected ZNPs application and it was noticed in each treatment of ZNPs, the germination was started earlier. At control, germination was started at 7th day but at concentration of 70 µg/mL, the germination was noticed at 6th day. But the germination at 25, 50 and 1000 µg/mL was at 7th day. According to the data presented in Table 5.5, tomato seedling shoot length demonstrated a favorable response until 75 µg/mL of ZNPs was reached. The highest positive impact was seen in seed treated with 75 µg/mL, as indicated in Table 5.5. The study showed that tomato treated with 75 µg/mL of ZNPs had a 20% increase in germination percentages compared to the control group. However, an increased amount of ZNPs negatively impacted the germination of the seeds, with 100 µg/mL reducing seed germination by 4% compared to the T3, as demonstrated in Table 5.5.

The study showed that tomato treated with 75 µg/mL of ZNPs had also increase in shoot and root length with respect to control group as in Fig. 5.12 (A-D). However, a higher ZNPs concentration had a negative impact on rootlet growth, with 100 µg/mL resulted in reduction of rootlet growth by 13% comparison with control, as demonstrated in Fig. 5.12 (D). The results of current study reveal that a significant correlation exists between the length of the roots and the ZNPs concentrations. The seedling vigor index, which is a combination of factors such as growth percentage, root length, and shoot length, is an effective tool for determining the impact of ZNPs on germination quality and quantity. As seen in Fig. 5.12 (B and C), the best concentration of ZNPs for promoting maximum seedling vigor in tomato is between 50-75 µg/mL. However, this index also indicates that concentrations higher than 75 µg/mL have a detrimental effect on the seedlings.

Figure 5.12. Impact of various concentrations of ZnO Nanoparticles on growth and morphology of tomato; (A). Control (B). 50 µg/mL (C). 75 µg/mL (D). 100 µg/mL

Table 5.5. The effect of various ZNPs concentrations on the seed germination time and germination rate percentages of tomato.

Sr. No.	Treatments	8-Day	10-Day
1.	Control	60 ± 1.5^{a}	65 ± 1.5^{a}
2.	T1	64 ± 1.5^{b}	68 ± 1.5^{b}
3.	T2	74 ± 1.5^{c}	79 ± 1.5^{c}
4.	T3	79 ± 1.5^{d}	86 ± 1.5^{d}
5.	T4	76 ± 1.5^{cd}	81 ± 1.5^{cd}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

Studies indicate that the impact of ZNPs on different plant species varies, and even the method of foliar application and root feed solution can result in distinct effects on germination and growth parameters (Keerthana *et al.* 2021). Effect of various concentration of ZNPs also varies greatly between species. For instance, the treatment of Black gram seeds with 600 mg/L of ZNPs resulted in significant effects on germination parameters, including the highest growth percentage, highest seedling vigor, maximum germination length, and maximum root length (Raja *et al.* 2019). Various researches have been conducted on the impact of ZNPs on plants belonging to the Brassicaceae family. The study on *Brassica juncea* found that seedling growth was positively affected by a concentration of 25 ppm, but a concentration of 100 ppm had a toxic effect (Nayan *et al.* 2016). This study examines the effect of ZNPs on tomato which yielded similar results to those found in *B. juncea* (Nayan *et al.* 2016). Other investigations into the long-term impact of ZNPs on *Brassica napus* support these findings (Sarkhosh *et al.* 2022). Another study found that the use of ZnO-nanorods had a synergistic effect on the growth of *Brassica oleracea* and the biomass of *P. indica* when both were present in a symbiotic relationship (Singhal *et al.* 2017). Research has shown that applying ZNPs (1000 ppm) to *Arachis hypogaea* seeds enhances germination rate, enhances seedling vitality, promotes earlier blooming, and increases chlorophyll content in leaves (Iqbal *et al.* 2021). The impact of these particles on root and stem growth has also been established. The treated samples showed a 34% increase in pod production with respect to control group (Iqbal *et al.* 2021). In another study of *Capsicum annuum* L., it was found that treating seeds with 750 ppm ZNPs improves germination rate, increases root and stem length

(García-López *et al.* 2018). Even low concentrations of ZNPs have a significant impact in some species. Another research on pearl millet showed that a mere 10 ppm of ZNPs (applied via foliar) led to a significant increase in buds, root growth, pigment and protein content (Tarafdar *et al.* 2014). Studies have shown that low concentrations of ZNPs have positive impacts on germination of seeds and seedling growth in various species. For instance, in *Triticum aestivum*, a concentration of 50 ppm ZNPs was found to improve germination, root development, and overall plant growth (Munir *et al.* 2018). However, a concentration of 400 ppm showed toxic effects, inhibiting root growth and seedling germination (Munir *et al.* 2018). Studies on *Cicer arietinum* have shown that seedlings treated with 1.5 ppm ZNPs in a foliar treatment saw the highest growth, while a concentration of 10 ppm had an adverse effect on root growth (Burman *et al.* 2013). Pandey et al. also found that ZNPs increase root phytohormones, such as IAA, which improve root growth. Another study on corn showed that germination percentage is enhanced by a 10 ppm concentration of ZNPs, but the impact on root growth varies (Pandey *et al.* 2010). Research on maize revealed that concentrations above 800 ppm ZNPs have toxic and inhibitory effects and reduce the seeds germination and seedling growth (Liu *et al.* 2015), with similar toxic effects at concentrations above 400 ppm (Yang *et al.* 2015). However, there have been conflicting findings in some studies. For example, Lin and Jing conducted research to demonstrate the impact of ZNPs on *Lactuca sativa* and various other species. They discovered that high doses of this NPs (2000 mg/L) did not hinder seed germination. Contrarily, it was discovered that ryegrass and corn's germination rate was reduced by up to 50% with low levels of ZNPs, ranged from 20 to 50 mg/L (Lin & Xing 2007).

5.11. Morphological parameter

The application of ZNPs significantly germination of tomato. The concentration of ZNPs was found to have a direct impact on the germination efficiency of seeds, as demonstrated by the data presented in Table 5.6 for the 8th and 10th day of seed inoculation. The stimulating effect of presence of ZNPs in the culture media was noticed on both root and shoot growth, up to a certain concentration limit. Specifically, the maximum increase in shoot length (47.67% compared to control) was observed at 75 µg/mL ZNPs, while an increase in root length (41.30%) was observed up 100 µg/mL (Table 5.6.). However, it should be noted that while there was a positive effect on shoot lengths up to 75 µg/mL, the fresh weight of shoots decreased with increasing ZNP concentration above 50 µg/mL, likely due to a reduction in stem diameter. It was maximum at 50 µg/mL with the value of 2.59% compared to control. At 100 mg/L ZNPs, 1.86% reduction in shoot FW was observed with respect to 50 µg/mL. Comparatively root FW significantly increased for 25 to 100 µg/mL; 2.60% at 25 µg/mL and 10.81% at concentration of 100 µg/mL. There was no notable difference observed in the shoot of DW at concentrations ranging from 25 to 100 µg/mL but root dry weight was significantly high at 75 µg/mL with 21.42% compared to control.

Table 5.6. The effect of various ZNPs concentrations on root and shoot length, fresh weight and dry weight of shoot and root.

		Length (cm)		Fresh Weight (g)		Dry Weight (g)	
Sr. No.	Treatments	Shoot	Root	Shoot	Root	Shoot	Root
1	Control	15.61 ± 0.12^{a}	6.31 ± 0.14^{a}	6.76 ± 0.12^{a}	3.38 ± 0.11^{a}	0.76 ± 0.12^{a}	0.11 ± 0.11^{a}
2	T1	19.98 ± 0.12^{b}	7.42 ± 0.13^{ab}	6.84 ± 0.13^{ab}	3.47 ± 0.12^{b}	0.77 ± 0.11^{a}	0.12 ± 0.12^{ab}
3	T2	24.46 ± 0.11^{c}	8.97 ± 0.12^{b}	6.94 ± 0.12^{c}	3.58 ± 0.13^{c}	0.77 ± 0.11^{a}	0.13 ± 0.13^{b}
4	T3	29.83 ± 0.13^{d}	9.44 ± 0.11^{c}	6.82 ± 0.11^{ab}	3.68 ± 0.12^{cd}	0.76 ± 0.12^{a}	0.14 ± 0.11^{b}
5	T4	24.68 ± 0.11^{c}	10.75 ± 0.11^{d}	6.81 ± 0.12^{ab}	3.79 ± 0.11^{d}	0.76 ± 0.14^{a}	0.13 ± 0.14^{b}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same lowercase letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

The growth of shoots in response to NPs may be a result of the nutritional behavior of nanoparticles, but at non-lethal concentrations. On the other hand, the direct contact of roots with ZNPs leads to shorter root length due to accumulation on the root surface or root tissue. The presence of nanoparticles in media has not much good impact on elongation of roots as the agar media is non-porous and has water logging and less dissolved oxygen due to ZNPs. Nanoparticles are thought to hinder cell division by obstructing prophase initiation. Even though NPs caused a rise in shoot length, the shoots were slim and had greater distances between nodes. The decline in shoot fresh weight (FW) that was observed could be attributed to the presence of NPs. However, there was no significant impact on the water holding capacity of the shoots, resulting in no significant change in dry weight (DW). This outcome is similar to previous studies on ryegrass and broad bean, which found comparable effects at higher concentrations (2000 mg/L) (Feng *et al.* 2015). Another has research found no negative effects on zucchini, while the root dry weight and fresh weight declined as the concentration of NPs increased (Stampoulis *et al.* 2009). The presence of zinc NPs or dissolved ions had an impact on root biochemistry and physiology, resulting in changes in length and weight. Previous study also showed inhibition of root growth in various plants including cucumber, radish, rape, ryegrass, Arabidopsis and lettuce (Lee *et al.* 2010; Lin & Xing 2007, 2008). It has been observed that the inhibitory impact of NPs may vary depending on factors such as NPs size and shape, plant species, adhesion to the root surface, release of metallic ions in the surrounding environment and ability to dissolve and translocate from root to shoot (Bhattacharjee *et al.* 2022). In field conditions, wheat biomass reduction has been reported due to ZNPs. The detrimental effects of heavy metals on seed germination, growth and development of plant, biochemical and physiological processes have been extensively researched (Du *et al.* 2011).

5.12. Photosynthetic pigments

The chlorophyll content of leaves was used as a metric to evaluate the plant's photosynthetic ability. The results, shown in Table 5.7, revealed that the chlorophyll content in 24-day-old tomato plant leaves significantly increased with the application of ZNPs by foliar spray and root dip. A significant increase in chlorophyll content (a+b) as well as carotenoid ranging from 15.17% to 45.33% and 10.89% and 54.24% for ZNPs concentrations of 25-100 µg/mL, respectively (Fig. 5.13). Our findings align with previous researches, showing an increase in chlorophyll content in various plant species upon exposure to biologically produced ZNPs. However, there have also been conflicting reports where no change in chlorophyll was observed. These divergent results may be due to the varying properties of nanoparticles, such as dosage, exposure concentration, and delivery method. The utilization of ZNPs exhibited a beneficial impact on the growth of cotton, resulting in a significant increase in both growth (130.6%) and biomass (131%).

Table 5.7. The effect of various ZNPs concentrations on photosynthetic pigment of tomato.

Sr. No.	Treatments	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total Chlorophyll	Carotenoid (mg/g)
1.	Control	6.52±0.24^{a}	7.01±0.23^{a}	13.53±0.54^{a}	5.07±0.34^{a}
2.	T1	8.43±0.41^{b}	7.52±0.42^{ab}	15.95±0.52^{b}	5.69±0.45^{ab}
3.	T2	10.66±0.19^{c}	8.27±0.12^{b}	18.93±0.29^{c}	7.39±0.52^{b}
4.	T3	11.73±0.31^{cd}	10.64±0.20^{c}	22.37±0.42^{d}	10.45±0.32^{c}
5.	T4	12.97±0.50^{d}	11.78±0.42^{d}	24.75±0.28^{e}	11.08±0.53^{d}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same lowercase letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

ZNPs elevated soluble protein (179.4%), reduced malondialdehyde (MDA) and effectively boosted the levels of chlorophyll a, b, and carotenoids (141.6%, 134.7%, and 138.6%, respectively) in plant leaves (Venkatachalam *et al.* 2017). In addition, the enzymatic activities of catalase, superoxide dismutase (264.2%), and peroxidase (182.8%) were enhanced, thereby leading to further growth improvement in cotton plants (Venkatachalam *et al.* 2017). The underlying mechanism behind the impact of nanoparticles on photosynthetic pigments remains unclear. The increase in chlorophyll content in our study was further supported by the rise in photosynthetically active radiation absorption in tomato plant leaves with increased ZNPs concentration in foliar application. This suggests that nanoparticles may enhance the rate of plant photosynthesis.

5.13. Endogenous IAA

The application of various concentrations of ZNPs on tomato plants showed a significant increase in IAA levels. Among the treatments, the 75 µg/mL of ZNPs exhibited the greatest impact on IAA content, outperforming the corresponding control plants. The results, shown in Table 5.8 and figure 5.13, revealed that IAA in 24-day-old tomato plant leaves significantly increased with the application of ZNPs by foliar spray and root dip. The results demonstrated that there is a significant increase in IAA ranging from 17.08% to 46.54% for ZNPs concentrations of 25-75 µg/mL, respectively as compared to control. At 100 µg/mL, the reduction in indole acetic acid was noticed (Table 5.8; Fig 5.13). Our results were also in accordance with a study in which ZNPs inhibited IAA production above a specific concentrations of ZNPs (Dimkpa *et al.* 2019). It has been found that the combination of CuO and ZNPs led to a moderate increase in IAA production compared to when each was used alone. The same effect on IAA levels produced by CuO NPs could be replicated by the ion concentration released from the NPs (Dimkpa *et al.* 2019).

5.14. Proline content

The application of ZNPs to the roots of tomato plants resulted in an enhancing in the proline content in the leaves, compared to control. Specifically, at 75 µg/mL, the roots treated with ZNPs exhibited the highest proline content, which was 30.41% higher than the control. However, with increase in ZNPs concentration up to 100 µg/mL, the proline content decreased to 24.77 (mg/g). The trend of proline accumulation in plants following treatment with varying concentrations of ZNPs was as follows: 75>100>50>25>0 µg/mL (ZNPs) as given in Table 5.8 and Fig 5.13. Proline acts as a non-enzymatic antioxidant and stabilizes subcellular structures, including cell membranes and proteins. It scavenges free radicals, buffers redox potential during stress, and acts as a molecular chaperone to enhance enzyme activity and protect protein integrity. Proline also provides protection against free radical-induced and singlet oxygen damage caused by excess ROS, making it a unique and effective compatible solute for plant protection. The results of current study are consistent with previous reports, which showed that due to application of ZNPs, accumulation of proline was enhanced (Parveen & Siddiqui 2021; Siddiqui *et al.* 2019).

Table 5.8. Different concentrations of ZNPs were applied to observe the effect of Indole Acetic Acid and proline content on growth of tomato.

Sr. No.	Treatments	IAA (mg/100 g)	Proline Content (mg/g)
1.	Control	12.76 ± 0.78^{a}	19.78 ± 0.98^{a}
2.	T1	13.39 ± 0.67^{ab}	21.98 ± 0.76^{b}
3.	T2	17.86 ± 0.56^{b}	23.22 ± 0.58^{bc}
4.	T3	23.87 ± 0.88^{c}	28.45 ± 0.98^{d}
5.	T4	22.76 ± 0.71^{cd}	24.77 ± 0.87^{c}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same lowercase letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

In banana plants, the addition of ZNPs to the MS media induced proline synthesis and enhanced tolerance to biotic stress, as well as the activity of CAT, SOD and POX (Faizan *et al.* 2018). Similarly, in basil plants, SiO_2-NPs led to increased proline accumulation and chlorophyll content (Kalteh *et al.* 2018; Siddiqui *et al.* 2014). This suggests that the elevated proline content induced by ZNPs can be beneficial for plant growth and development. The complete understanding of the mechanisms which is responsible enhanced proline content and improved antioxidant defenses in plants exposed to ZNPs remains largely unexplored. Further investigation of these mechanisms is needed for sustainable agriculture practices.

Figure 5.13. Heat maps exhibiting the trend of fold-change in chlorophyll content, proline and indole acetic acid in tomato plant in response to application of biogenically synthesized nanoparticles. Colored blocks define a fold-change in these content for various concentrations of ZnO nanoparticles compared to untreated control plants.

5.15. Antioxidant Enzymes

The study found that the response of antioxidant enzymes differed at different concentrations of ZNPs. ZNPs were observed to have the ability to trigger the generation of ROS within the system. When the cell's antioxidative capacity increases, it eventually leads to the death of the cell. The activity of SOD in tomato increased from 25 µg/mL (12.37 Unit/g) to 50 µg/mL (15.68 Unit/g) ZNPs with the percentages of 19.07 and 36.16% respectively but decreased up to 21.11% at 100 µg/mL afterwards; however, it was higher than the control (10.01 Unit/g) at all ZNPs treated treatments. The activity of APX in tomato was higher than the control up to 100 µg/mL ZNPs but it started declined at 75 µg/mL. A similar response was also recorded on case of CAT activity and there was a gradual increase up to the applied 100 µg/mL concentration of ZNPs from 0.25 to 0.39

Unit/g. A decline in GPX activity was noticed at 50 µg/mL concentrations in shoot from 44.99 to 41.85 Unit/g up to the concentration of 100 µg/mL (Table 5.9; Fig. 5.14).

Zinc is recognized for its function as a SOD cofactor, which act as an antioxidant by aiding plants in ROS suppression (Lee 2018) In this study, the introduction of ZNPs led to a rise in zinc levels, which in turn resulted in an increase in antioxidant enzymes activity at specific ZNPs concentrations. This likely facilitated the regulation of ROS and promoted better plant growth. Higher levels of ZNPs led to increased Zn accumulation, but it also caused plants to become overburdened and disrupted Zn homeostasis. Therefore, lower concentrations of ZNPs were found to have a positive effect on growth of plant. ZNPs were discovered to act as a natural plant growth regulator, promoting growth and development by modulating essential physiological parameters under both stressed and non-stressed conditions (Singh *et al.* 2021). ZNPs' small size enables them to penetrate plant cells, facilitating seed germination and growth (Ali *et al.* 2021).

Figure 5.14. 4Ys-Y-YYY plot exhibiting the trend of fold-change in Antioxidant Enzymes content in tomato plant in response to application of various concentrations of ZnO nanoparticles. Colored lines define a fold-change in Antioxidant Enzymes content for various concentrations of ZnO nanoparticles compared to untreated control plants

Table 5.9. The effect of various ZNPs concentrations on antioxidant enzymes of tomato.

Sr. No.	Treatments	SOD (Unit/g)	APX (Unit/g)	CAT (Unit/g)	GPX (Unit/g)
1.	Control	10.01 ± 0.32^{a}	40.91 ± 0.52^{a}	0.25 ± 0.39^{a}	39.97 ± 0.43^{a}
2.	T1	12.37 ± 0.22^{b}	43.67 ± 0.33^{b}	0.32 ± 0.42^{ab}	45.69 ± 0.23^{c}
3.	T2	15.68 ± 0.39^{d}	47.78 ± 0.62^{c}	0.35 ± 0.22^{b}	44.99 ± 0.52^{b}
4.	T3	13.87 ± 0.13^{c}	52.84 ± 0.34^{e}	0.37 ± 0.23^{bc}	42.95 ± 0.42^{ab}
5.	T4	12.69 ± 0.42^{bc}	49.91 ± 0.17^{d}	0.39 ± 0.19^{c}	41.85 ± 0.65^{ab}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same lowercase letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

5.16. Antioxidative Response

Total antioxidant potential, reducing power potential, DPPH radical scavenging activity, flavonoid contents and total phenolic content significantly varied in tomato shoots on ZNPs exposure. DPPH increased up to 72.46% in shoot at 50 µg/mL and afterward, there was a gradual decrease in free radical scavenging activity up to the concentration of 75 µg/mL and again an increase was recorded at 100 µg/mL. A steady increase in Total Antioxidant Capacity (TPC) activity from 3.01 to 12.78 µg GAE/mg was observed in shoots parts at concentration of 25 to 100 µg/mL. No decline was recorded up to the applied 100 µg/mL, over all there was a huge increase in TPC percentages which was recorded as 38.82% at 25 µg/mL and 76.44% at 100 µg/mL with respect to control. Tomato shoots also exhibited a noteworthy shift in Total Antioxidant Capacity (TAC). A steady rise in TAC was documented as the concentration of ZNPs increased up to 50 µg/mL, with the highest value noted as 2.86 µg AAE/mg, which equated to a 73.42% increase compared to the control. Likewise, an unvarying enhancement in Total Flavonoid Content (TFC) was recorded as the ZNPs concentration rose to 100 µg/mL, with an increase from 24.48% to 52.56% compared to the control. Total Reducing Power (TRP) was also consistently increased from 3.06 to 11.22 µg AAE/mg with enhanced ZNPs concentration from 25 to 100 µg/mL. The maximum obtained value of TRP was recorded 72.72% higher with respect to control (Table 3.10; Fig. 3.15).

Tomato shoots experienced oxidative stress due to the existence of ZNPs in the medium (Faizan *et al.* 2018). Additionally, direct contact with ZNPs in the roots resulted in oxidative stress during root dip inoculations. The levels of non-enzymatic antioxidants, specifically flavonoids and phenolics, were associated with the reducing power potential, free radical scavenging capacity and total antioxidant response, all of which varied. The concentration flavonoid and phenolic content in tomato shoots exhibited noteworthy changes. Numerous investigations have centered on the induction of oxidative stress in living beings due to exposure to NPs, as noted by a previous study (López-Moreno *et al.* 2016). The capacity of ZnO to catalyze light has been associated with a response in organisms to reactive oxygen species (ROS) owing to its band gap energy of 3.37 eV, according to (Ma *et al.* 2013), despite the fact that band gap energy is not much important element for production of ROS, as stated by (Navarro *et al.* 2008). Zinc plays a crucial role in many metabolic processes, but excessive concentrations may disrupt photosynthesis and lead to oxidative stress. It is worth noting that some nanoparticles (NP) may also have a bio-protective effect against oxidative damage (Gondal *et al.* 2021). It has been observed that *Allium cepa* plants exposed to ZNPs experienced a rise in ROS levels (Kumari *et al.* 2011).

Table 5.10. The effect of various ZNPs concentrations on antioxidant responses in tomato.

Sr. No.	Treatments	DPPH (%)	TPC (µg GAE/mg)	TAC (µg AAE/mg)	TFC (µg QE/mg)	TRP (µg AAE/mg)
1	Control	61.23±0.78^{a}	3.01±0.78^{a}	0.76±0.73^{a}	0.37±0.67^{a}	3.06±0.55^{a}
2	T1	68.37±0.99^{c}	4.92±0.45^{b}	1.57±0.48^{b}	0.49±0.58^{b}	5.15±0.68^{b}
3	T2	72.46±0.88^{d}	6.27±0.76^{c}	2.86±0.76^{d}	0.57±0.76^{c}	7.17±0.67^{c}
4	T3	67.83±0.97^{c}	10.64±0.72^{d}	2.12±0.36^{c}	0.65±0.88^{d}	9.87±0.54^{d}
5	T4	64.67±0.67^{b}	12.78±0.54^{e}	1.78±0.56^{bc}	0.78±0.56^{e}	11.22±0.72^{e}

Note: T1-25 µg/mL, T2-50 µg/mL, T3-75 µg/mL, T4-100 µg/mL. the standard error is represented by ±. The letter a-d in superscripts show significant differences between application of various ZNPs concentrations at $p<0.005$. If the means in the same column have the same lowercase letter, according to Duncan's multiple range test at a 95% confidence interval, they are not significantly different.

Fig. 3.15 Chord Diagram exhibiting the trend of fold-change in antioxidant responses in tomato plant in response to application of various concentrations of ZnO nanoparticles. Colored chords define a fold-change in antioxidant responses for various concentrations of ZnO nanoparticles compared to untreated control plants.

5.17. Root Growth and Development

Through application of ZNPs at different concentrations, impacts were found in various roots parameters. Overall, the major increase in percentages of various parameter was found from concentration of 25 to 75 µg/mL (Fig. 5.17-5.18). By application of ZNPs, an increase in number of root tips was found with the increase in concentration from 25 to 75 µg/mL but by increasing ZNPs concentration above 75 µg/mL, reduction in number of roots tips was found (Fig. 5.16 C). Up to 75 µg/mL, 29.68% increase in roots tips was found with respect to control. Moreover, the number of branching points was also increase with increase in ZNPs concentration. At concentration of 100 µg/mL, the maximum branching point was found with 22.22 % as compared to control (Fig. 5.16 D). Various ZNPs concentrations had also major impact roots diameter. About 46.23 % increase in roots diameter was observed with respect to control at 75 µg/mL and after that 13.88 % reduction in root diameter was found by further increasing concentration up to 100 µg/mL. The branching frequency was also majorly influenced by the various concentration of ZNPs. By

increasing ZNPs concentrations up to 100 µg/mL, the branching frequency was increased up to 14.76 % in comparison with control. The root network area was also influenced by ZNPs applications which was increased up to 26.39 % at 75 µg/mL with respect to control and after that concentration, 6.38 % reduction in root network area was record at 100 µg/mL. Similar trends were recorded in surface area, perimeter and volume. The maximum increase in surface area, perimeter and volume was noticed up to 75 µg/mL with 24.08, 22.09, and 25.27 % respectively as compared to control. At 100 µg/mL, 5.51, 14.09 and 5.74 % reduction were recorded in surface area, perimeter and volume with respect to 75 µg/mL.

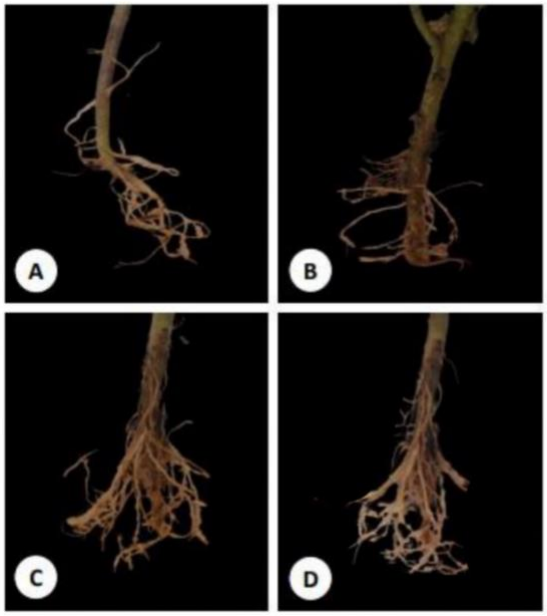

Figure 5.15. Impact of various concentrations of ZnO Nanoparticles on tomato root morphology; (A). Control (B). 50 µg/mL (C). 75 µg/mL (D). 100 µg/mL

Root growth can also be impacted by nanoparticles, in addition to their effects on photosynthesis and seed germination. The degree to which nanoparticles enhance or inhibit root length can vary depending on the specific type of nanoparticle. For example, ZNPs concentrations of 0.40 and 0.80 mL^{-1} had a negative impact on germination of seed and were therefore excluded from further study (Parveen & Siddiqui 2021). Additionally, previous research has shown that high concentrations of ZNPs can lead to inhibited tomato shoot and root growth (Wang *et al.* 2018), as well as reduced photosynthetic efficiency due to decreased chlorophyll content (Malea *et al.* 2019). Although Zn^{+2} was present in small amounts, ZNPs suspensions did not hinder tomato growth, leading researchers to conclude that the toxic effects observed were due to the ZNPs themselves rather than the release of Zn^{+2} (Wang D. *et al.* 2016). ZNPs are widely utilized and it is crucial to evaluate their potential negative impacts on environment as well as on human health. Different metals can be accumulated in different cellular compartments due to these NPs and interfere with various physiological and biochemical processes in a concentration-dependent manner. The presence of metal oxide

nanoparticles can stimulate the production of ROS, which serve as an indicator of NP phytotoxicity. ROS are known to be a major contributor to DNA damage, as well as being signaling molecules for both abiotic and biotic stresses, and play a role in programmed cell death.

The absorption of ZNPs by the roots of *Cicer arietinum* and *Vigna radiata* seedlings resulted in increased root length and biomass (Gahoi *et al.* 2021; Shekhawat *et al.* 2021). However, the effects of NPs on root elongation vary from plant-to-plant species. In barley, root length was increased (Doğaroğlu & Köleli 2017) while in *Lactuca sativa*, it was inhibited (Xu *et al.* 2018). In *Crocus sativus*, the enhancement of root growth was found to be caused by the blocking of ethylene signaling (Rikabad *et al.* 2019). AgNPs were found to increase the length of roots in barley, cabbage and maize, compared to AgNO3, as noted in (Siddiqui *et al.* 2015). The morphology of NPs is also important in regulation of root growth, as demonstrated by (Lee *et al.* 2013). Among the three different morphologies of Ag nanoparticles tested on Arabidopsis seedlings, the decahedral morphology was the most effective in promoting root growth, while the spherical morphology had no effect on root growth and instead induced high levels of anthocyanin accumulation (Syu *et al.* 2014). In addition, Ag nanoparticles inhibited root elongation by reducing the expression of ACC oxidase 7 and ACC oxidase 2 and activating ACC in Arabidopsis seedlings, indicating that Ag NPs impeded the perception and synthesis of ethylene (Syu *et al.* 2014). The utilization of metal-based NPs, specifically silicon, high copper levels, palladium, low gold levels, and a blend of gold and copper, showed promising outcomes in enhancing seedling growth and shoot-to-root ratio (Agrahari & Dubey 2020). However, the use of cerium oxide NPs had only influenced root growth of *Lactuca sativa* at 2000 mg/L (Cui *et al.* 2014). Meanwhile, the treatment of parsley seeds with nano-anatase stimulated germination, roots and shoots length, and chlorophyll content (Dehkourdi & Mosavi 2013). Similarly, pumpkin root elongation was observed when exposed to iron oxide NPs (Zhu *et al.* 2008). Soybean roots experience elongation when exposed to ZNPs (Xiao *et al.* 2022). In the case of *Cyamopsis tetragonoloba*, exposure to ZnO nanoparticles led to an improvement in various growth parameters such as plant biomass, protein and chlorophyll synthesis, and more (Bazzi *et al.* 2021). Conversely, rape and radish plant roots experienced a decrease in growth when incubated in a suspension of ZNPs (Ma *et al.* 2010), while ZNPs did not show such inhibition due to the selective permeability of the seed coat (Zhang R. *et al.* 2015). NPs tend to aggregate on the soil surface, allowing for uptake by plants. Soil colloids can act as carriers for strongly adsorbed nanoparticles, such as soil clay material. For instance, in corn plants treated with ZNPs grown in soil media, aggregated ZNPs were found on the root epidermis and then transported to the endodermis through the symplastic pathway.

The growth of plants such as carrot, cucumber, cabbage, and corn were found to decrease as a result of pure alumina NPs (13 nm), which reduced root elongation without any modifications (Zhang R. *et al.* 2015). On the other hand, the presence of Cu nanoparticles resulted in an increased shoot to root ratio during seed germination in lettuce when compared to plants without nanoparticles (Shah & Belozerova 2009). When *Tetrahymena pyriformis* was cultured in a nutrient medium supplemented with nanotubes and protein, there was an unexpected growth simulation (Agrahari & Dubey 2020). This growth stimulation was believed to be due to the binding between

nanotube supplements and protein, resulting in increased protein penetration into cells and enhanced growth. Carbon-based NPs such as graphene and carbon nanotubes have displayed differing effects on plant shoot and roots (Lahiani *et al.* 2016). The carbon nanotubes were found to induce root elongation in onion and cucumber plants and form nanotube sheets on the cucumber roots surface upon interaction with CNTs and fCNTs, but were not able to penetrate the roots (Cañas *et al.* 2008). Conversely, these nanotubes had no impact on carrot and cabbage plants (Cañas *et al.* 2008). fCNTs were found to inhibit root elongation in lettuce, while CNTs had the same effect on tomato plants, which were also highly sensitive to SWCNTs (De & Chakrabarti 2013). Scientists found that SWCNTs enhanced the growth of cucumber roots and onion at concentrations of 0.5, 0.9, and 0.16 gL^{-1} (Cañas *et al.* 2008). Of all the carbon-based nanoparticles, graphene displayed the most notable impacts on seed germination (Lahiani *et al.* 2016). Tomato seedlings that were exposed to graphene in a study had longer roots and stems compared to the control group on the 19th day, with increases of up to 12.5% and 17% respectively (Zhang M. *et al.* 2015).

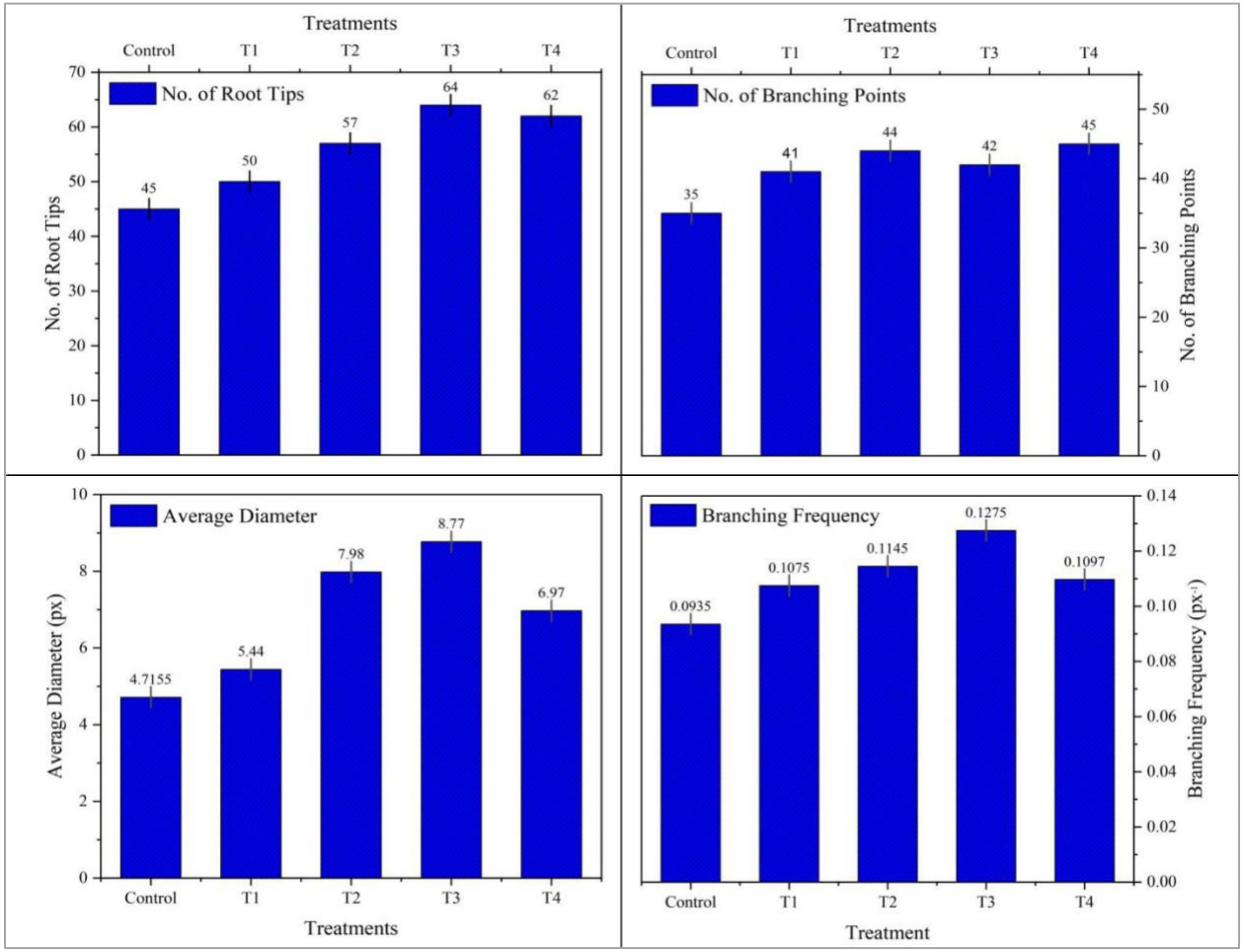

Figure 5.16. Impact of various concentrations of ZnO Nanoparticles on different root parameters of tomato including number of root tips, number of root branching, average root diameter, branching frequency.

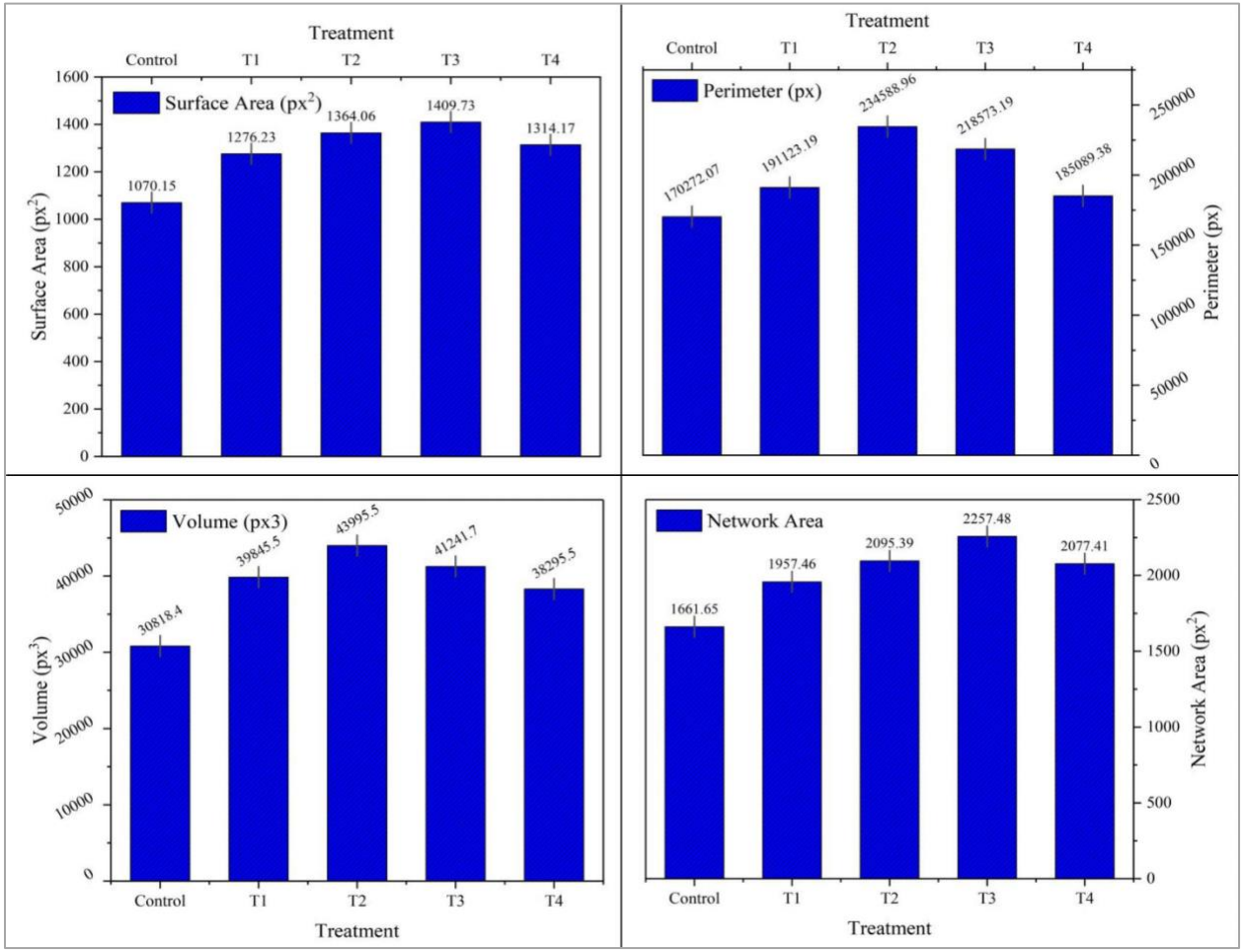

Figure 5.17. Impact of various concentrations of ZnO Nanoparticles on different root parameters of tomato including root surface area, root perimeter, volume and total root network area

CHAPTER # 6

Discussion

6. Discussion

Plant diseases and pests are posing increasing the food security challenges to sustainable agriculture productivity (Balaure *et al.* 2017). Currently, the common practice in agriculture sector for pest and disease management is use of pesticides, but the heavy usage of synthetic pesticides can be environmentally damaging and increase the risk of fungicide resistant in phytopathogen populations (Rehman et al. 2021a; Rehman et al. 2021b; Rehman et al. 2021c). Health effects due to high antioxidant and nutritional values of green vegetables have increased their consumption immensely. Various important vegetable crops of the genus *Solanum* are highly susceptible to various phytopathogens including nematodes, bacterial, fungal pathogens (Seid *et al.* 2015). These pathogens are causing huge losses in production. Two diseases, bacterial wilt of tomato (BWT) and bacterial leaf spot (BLS) caused by Gram-negative bacteria including *Ralstonia solanacearum* (Nion & Toyota 2015) and *Xanthomonas campestris* pv. *vesicatoria* (XCV) (Obradovic *et al.* 2004) respectively cause significant losses to of all nightshade crops worldwide. Various synthetic pesticides including copper substances, benzimidazole derivatives, dithiocarbamate, antibiotics and quinolones are commonly used to control BLS and BWT diseases in tomato crops, but their huge applications have resulted in the emergence of fungicide resistant pathogens. Various chemical-based products i.e. Actigard induces immunity in plants and are used to minimize the impacts of bacterial infections, but there effectiveness varies among various nightshade crops (Stroud *et al.* 2022). There is a great need for biocontrol agents to control these diseases (Bragante *et al.* 2022; Matich *et al.* 2021; Türkmenoğlu & Özmen 2021; van Bruggen *et al.* 2016). There are many scientific efforts in developing eco-friendly pesticides by identifying new antimicrobial agents (Bhardwaj *et al.* 2014). One new hotspot in this field of research is nanotechnology.

Nanotechnology is being used in many science fields, including chemical, biological, physical, material and pharmaceutical sciences (Dulta *et al.* 2022; Halamoda-Kenzaoui *et al.* 2022). As well as in agriculture (Saranya *et al.* 2019). Due to increased surface to volume ratio along with relatively small size and characteristics optical properties of NPs, they have many applications such as with fertilizers, nutrition and plant protection (Qasim *et al.* 2022). The efficiency and effect of NPs on plant metabolic and growth activities vary among plants (Rastogi *et al.* 2019). The available amount of nanoparticles highly influences the process of seed germination along with plant growth (Waani *et al.* 2021). Among various nanoparticles, ZNPs have very promising role in agriculture sector (Elshayb *et al.* 2021).

Nanotechnology is a research hotspot in the field of materials science. This technology offers a wide range of novel applications, including fabric compounds, agriculture, food processing, and medicine. Nanotechnology is defined as the production, exploration and characterization of nanometer-scale materials (1–100 nm). The unique physicochemical properties of nanocrystalline particles (NCPs) make nanotechnology the most strenuous and dynamic research field based on electronic, optical catalysis, and magnetism (Sharma *et al.* 2009). NCPs are used for diagnosis, molecular detections, therapeutic, and antimicrobial activities. For example, zinc oxide

nanoparticles (ZNPs) are applied in various fields such as optics, biomedical and electronics, environment, and catalysis. The synthesis of ZNPs is achieved by various methods including electrochemical (Liu *et al.* 2020), chemical (Hasnidawani *et al.* 2016), photochemical (Gbur *et al.* 2011), and biological (Vidya *et al.* 2013) syntheses. Most synthesis methods require harsh reaction conditions and the use of toxic chemicals. The most commonly method of ZNPs synthesis is the chemical method due to its short reaction time and ease in handling.

The synthesis of ZNPs can involve microbes, i.e. fungi or bacteria, as well as algae and plant extracts (Bayrami *et al.* 2019). The synthesis known as green synthesis has a lower toxicity level than physicochemical methods, therefore, it is more recommended than chemical synthesis (Jeevanandam *et al.* 2016). Green synthesis of ZNPs is a cost-effective, safe, eco-friendly, biocompatible approach (Bose & Chatterjee 2016) that is scalable for large-scale production of ZNPs (Yuvakkumar *et al.* 2014). ZNPs synthesized from biological synthesis have reduce the use of toxic and expensive chemicals and more enzymatic activity. The surface modification of green synthesized ZNPs allows direct application in living systems (Singh *et al.* 2016). Various plant extracts and microbes are being exponentially used because of the benefits of green synthesis (Alavi & Nokhodchi 2021; Gunalan *et al.* 2012). Research has been done for the biosynthesis of ZNPs by applying extracts obtained from various plants that can work as reducing as well as capping agents (Sundrarajan *et al.* 2015).

Plant pathogens and insect pests cause huge yield losses in many agricultural commodities, jeopardizing food security at the household, national, and international levels (Rehman *et al.* 2021; ur Rehman[1] *et al.* 2020; ur Rehman 2020). Pathogens and pests are thought to cause an annual 20-40% reduction in global agriculture production (Savary *et al.* 2019). These are extensively controlled by pesticides in the form of fungicides, insecticides, or herbicides (Kalsoom *et al.* 2020; ur Rehman 2020). Unfortunately, the over-application of chemicals, non-targeted insects can be impacted and resistance to various fungicides and pesticides have been documented in pathogens and insects, respectively (Ghormade *et al.* 2011). Therefore, there is a dire need to replace these chemicals with some other products and technologies which have less or no impact on the environment (Ur Rehman *et al.* 2021).

Zinc, a micronutrient, is vital in protein and sugar production (Bhantana *et al.* 2021). It promotes stem growth along with the synthesis of chlorophyll which imparts green color to the plant (Akhtar *et al.* 2022). Alkaline soils especially in dry climates results in Zn deficiencies which can also happen in acidic soils. Needed in small amounts, Zn is essential for plant growth hormones, proteins synthesis, and cell membranes (Umair Hassan *et al.* 2020). Zinc sulphate and zinc oxide are commonly applied to compensate for Zn deficiency, but these fertilizers are in in effective because Zn is unavailable to the plants. Additionally, the chemical fertilizers can adversely affect beneficial soil microorganisms reducing soil fertility. To deal with these issues, a product is needed that can control plant pathogens as well as to reduce Zn deficiency. ZNPs are a more bio-available form of Zn that can increase plant development and plant immunity against phytopathogens (Salih *et al.* 2021).

While several physical and chemical methods exist for the production of ZNPs, but their major constituents are toxic to plants (Ismail *et al.* 2019; Mahamuni *et al.* 2019; Mohan *et al.* 2020). An eco-friendly and environmentally benign method for biogenesis of ZNPs is needed (Karthika *et al.* 2021). One approach is biological synthesis of ZNPs using various plant parts which can be low cost, energy-efficient, and eco-friendly (El Shafey 2020; Veerakumar *et al.* 2014). Biogenic synthesis of NPs has been reported by using plant extracts of *Punica granatum*, *Parthenium hysterophorus*, *Olea ferruginea*, *Pelargonium zonale*, *Aegle marmelos*, *Berberis vulgaris*, and *Aesculus hippocastanum* (Anzabi 2018; Asadi *et al.* 2018; Çolak & Karaköse 2017; Fowsiya *et al.* 2019; Murali *et al.* 2021; Umavathi *et al.* 2021; Vahidi *et al.* 2019). The mechanisms of action of ZNPs involves the formation of reactive oxygen species (ROS) which have significant antibacterial properties (Sirelkhatim *et al.* 2015b). ZNPs application induces the free radicals formation which reduces the level of glutathione and increases malondialdehyde (Gordon *et al.* 2011). ZNPs exhibit antibacterial properties due to reduced size of particles leading to enhanced surface reactivity. Zinc oxide is a biologically safe material that possesses photocatalysis and photo-oxidizing impacts on biological and chemical species (Sirelkhatim *et al.* 2015b).

Picea smithiana L., belongs to the family *Pinaceae*, an evergreen tree native to the western Himalaya and adjacent mountains and most commonly found in northern areas of Pakistan, northeast Afghanistan, India and central Nepal (Wani *et al.* 2022). Its common names are West Himalayan spruce and morinda spruce (Quamar & Stivrins 2021). It grows in forests together with blue pine, pindrow fir and deodar cedar at altitudes of 2,400-3,600 m (Semwal *et al.* 2014).

Medically, *Picea smithiana* has very importance in treating asthma, diabetes, tuberculosis and diarrhea (Jan *et al.* 2009). Resin obtained from this plant is used for the treatment of sores and cuts (Malik *et al.* 2015), stomach and joints pain (Kayani *et al.* 2015) and cracked heels and wounds (Gairola *et al.* 2014). Various other diseases including kidney stone, rheumatism and heat problem are also treated by the plant extract obtained from *Picea smithiana* (Khan & Khatoon 2007; Sher & Al_yemeni 2011).The powder of grounded cones mixed in hot water are used for the treatment of chest pain.

Phytochemicals present in extract obtained from the needles of *Picea smithiana* have antimicrobial, antifungal, antibacterial, cytotoxic and antiproliferative properties (Thapa-Magar *et al.* 2020). For example, ethanol, various essentials oils, acetone and ethyl acetate are highly active against various pathogenic bacterial strains including *Pseudomonas alcaligenes*, *Bacillus subtilis*, *Micrococcus luteus* as well as against various fungal pathogens including *Curvularia lunata* and *Bipolaris spicifera* (Ali *et al.* 2020; Sati & Joshi 2013; Verma *et al.* 2018; Verma & Nailwal 2019). Extracts from *Picea smithiana* have been shown to have antimicrobial properties against both gram-positive and gram-negative bacteria. It is likely that the extracts from the needles of *P. smithiana* have a significant impact on the formation of ZNPs. These phytochemicals are sterols, chloroform, alkaloids, carbohydrates, flavonoids, saponins, terpenoids, resins, n-hexane, glycosides, tannis, acetone, methanolic, quinones, phenolsethyl acetate, and anthraquinone (Ahmadi *et al.* 2019; Ali *et al.* 2020; Bhagat *et al.* 2017; Rahman *et al.* 2016; Sati & Joshi 2013).

Many other essential oils are also present as the major components in *Picea smithiana* extract including beta pinene, P-cymene, delta-3-carene, limonene, camphene, beta phellandrene, L-bornyl acetate, a-bisabolol, α-pinene, α-salinene, monoterpene compounds and Alpha-terpinolene (Gupta *et al.* 2017; Shah *et al.* 2014).

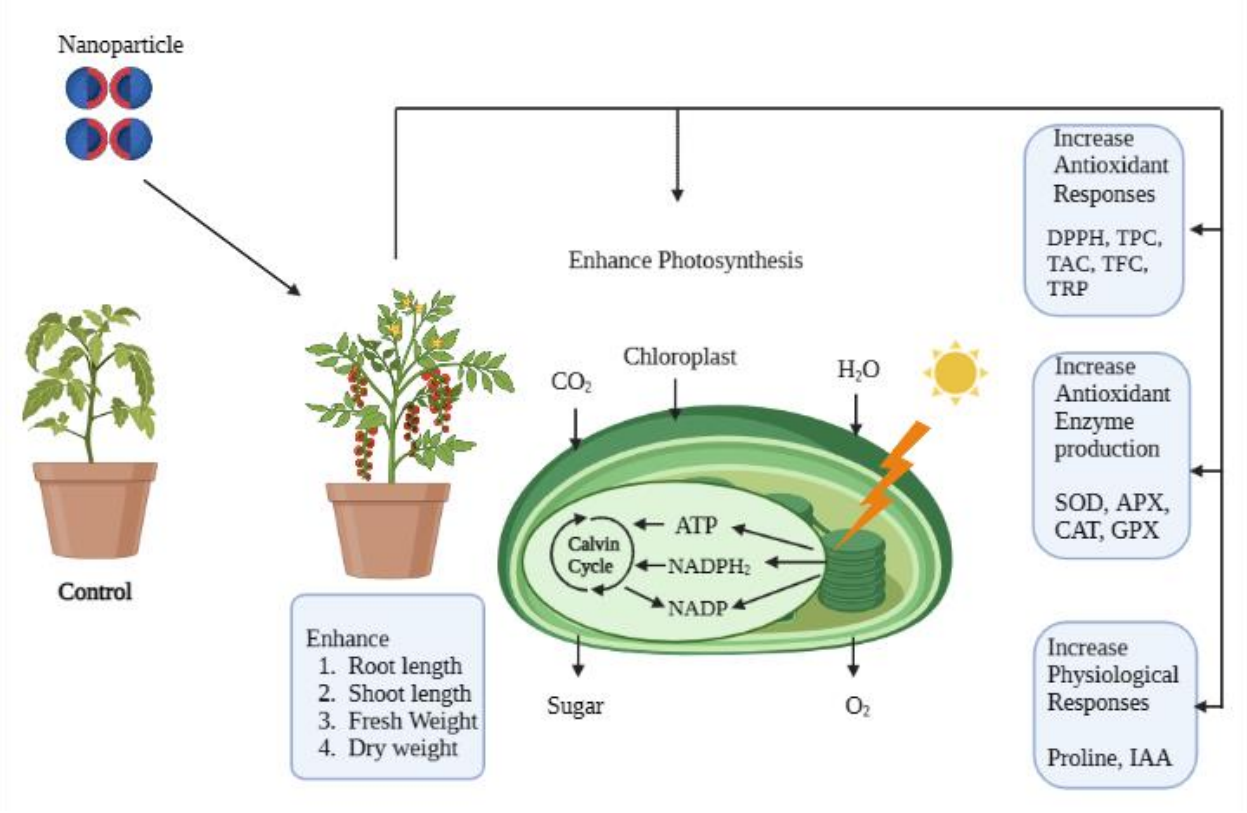

Figure 6.1. Plant growth promoting activities of ZnO NPs in Tomato plant

Nanotechnology is one of the rapidly growing branches of science that holds great promise across a wide range of areas in our daily lives. It encompasses a variety of disciplines, such as biology, engineering, physics and chemistry, and involves the creation and use of engineered nanoparticles (NPs). Nanomaterials that are smaller than 100 nm are utilized in diverse fields such as medicine, agriculture and the environment (Hernández‐Díaz *et al.* 2021). These materials have unique physical and chemical properties, which differ from those of bulk materials. As a result, their impact on living cells is yet to be fully understood. The rapid growth of nanotechnology is largely due to its vast potential for revolutionizing various industries, including electronics, medicine, and agriculture (Mazari *et al.* 2021). On the other hand, the consequences of the decay, deposition, and effects of nanomaterials in the environment are not well understood. The production and utilization of nanoproducts has led to their buildup and dissemination in the environment. With the growing use of NPs in various industries, such as agriculture where they are used as fertilizers and anti-stress agents, there is an increased likelihood of accidental leaks into undesirable locations like ponds or groundwater (Rehman et al. 2021a; Rehman et al. 2021b; Rehman et al. 2021c). A nanoparticle that has been engineered is described as being less than 100 nm in size, and having a

dimension that is less than 100 nm, examples of which include titanium dioxide and zinc oxide. Zinc (Zn) is a vital micronutrient for animals and plants, as it performs crucial functions in metabolic processes and enzyme reactions. If plants are deficient in zinc, it results in low yields, while in humans, it can cause malnutrition and various health issues (Praharaj *et al.* 2021). However, excessive amounts of zinc can also harm the soil and water ecosystem. The ideal soil concentration of zinc should be between 70 to 400 mg/kg (Wang F. *et al.* 2016). Zinc enters the environment from sources such as mining, smelting, and agriculture and can cause an increase in the soil and water levels. When plants absorb an excess amount of zinc, it can accumulate in the food chain and ultimately result in humans having levels that are higher than what is considered optimal (Butt *et al.* 2018).

The negative impacts of presence of zinc in a system have been recorded on plant growth, resulting in physiological and biochemical changes (Wang Y. *et al.* 2016). ZNPs are commonly used in lots of products such as sunscreens, cosmetics, biosensors, solar cells, paints, coatings, medical supplies, personal care items, and even in the food and agriculture industry as fertilizers, pesticides and fungicides (Kolenčík *et al.* 2020; Srivastava *et al.* 2016; Srivastava *et al.* 2015). Being integral part of Pakistan GDP, agriculture need much improvements (Ur Rehman et al. 2021a; Ur Rehman et al. 2021b; Ur Rehman et al. 2020). Despite the potential benefits, such as increased germination, shoot growth, root growth, dry weight, fresh weight and pod yield, observed in plant species like *Cicer arietinum*, *Vigna radiata* and *Solanum lycopersicum* L. (Burman *et al.* 2013; Kareem *et al.* 2022), the toxic effects of ZNPs have been also been reported in several other plant species, including *Brassica napus*, *Lactuca sativa*, *Raphanus sativus*, *Lolium multiflorum*, *Cucumis sativus*, *Oryza sativa* and *Zea mays* by hindering seed germination and root growth (Alcantara-Cobos *et al.* 2020; Chen J. *et al.* 2018; Chen X. *et al.* 2018; Deng *et al.* 2020; Singh & Kumar 2019; Zhang R. *et al.* 2015).

According to the study, ZnO nanoparticles can enter the cells of ryegrass by traveling through the apoplastic pathway, crossing both the epidermis and cortex (Shukla *et al.* 2016). The use of capped ZnO and CuO nanoparticles has been shown to increase the production of sweeteners in *Stevia* rebaudiana by boosting shoot growth (Ahmad *et al.* 2020). The disturbance of the ROS antioxidant machinery in *Allium cepa and Lathyrus sativus* L. as a result of exposure to ZNPs leads to DNA damage, cell-cycle arrest, and cell death, causing genotoxicity and cytotoxicity (Panda *et al.* 2017; Sun *et al.* 2019). Currently, various types of metal oxide nanoparticles are used in agriculture for purposes of fertilization and protection from both abiotic and biotic stress (Zhao *et al.* 2020). In order to sustain agricultural productivity, recent studies have extensively investigated how nanoparticles (NPs) affect plant germination, growth, and biochemical reactions. NPs are introduced into the environment both intentionally and unintentionally through their enhanced applications in consumer products and other industries, which leads to their presence in both terrestrial and aquatic as well as atmospheric environments. The distinctive features of NPs and their unintentional occurrence may give rise to unforeseen health or environmental hazards (Boey & Ho 2020). Living organisms like algae, fungi and plants are likely to experience an impact from their contact with NPs. Zinc oxide NPs become less available to plants as the soil pH rises due to

a decrease in their solubility (Ali *et al.* 2019). The transport of these nanoparticles into various parts of plants may be hindered by the presence of root barriers. Nevertheless, an increase in the production and release of ZNPs may have negative effects on both terrestrial and aquatic ecosystems.

Growing vegetables is an effective way to support people's well-being and nutritional requirements, with tomatoes being a highly prevalent, lucrative, and popular vegetable worldwide, following only potatoes in terms of consumption. Despite its long lifespan, commercial farming generally treats it as an annual crop that self-fertilizes. *Lycopersicon esculentum* Mill., commonly known as tomatoes and belonging to the Solanaceae family, are a crucial vegetable crop in Pakistan, second only to potatoes. They play a crucial role in providing a balanced and nutritious diet, being widely consumed as a staple food item. With high yields and short growth duration, Tomatoes are economically viable and provide employment opportunities in rural areas. They are often consumed fresh or used in dishes such as sauces, soups, and meat dishes. Research demonstrates that Zinc is crucial in enhancing the yield and quality of tomatoes (Ahmed *et al.* 2021a). All living organisms require zinc as a crucial nutrient.

Zinc plays an important role in various plant functions, such as boosting enzyme activity, increasing chlorophyll levels, and acting as a key component in various proteins (Brown *et al.* 1993). Applying zinc through foliar application has been shown to improve tomato yield by promoting photosynthesis in green plants and enhancing fruit set and yield (Ahmed *et al.* 2021a). The impact of NPs on plants can result in various morphological and physiological changes, which are dependent on the chemical, physical, size, shape, surface, and effective dose of the NPs. The efficacy of NPs is measured by these factors. It has been found that the proper application of ZNPs to the shoots, roots and grains of wheat can control plant growth and alleviate Zn deficiency (Munir *et al.* 2018). However, when B and Zn are applied at 250 ppm and 150 ppm respectively through foliar application, the dry weight of leaves, stem, and root increases more effectively (Munir *et al.* 2018). The most significant tomato yield was achieved when Zn was applied through foliar application at a concentration of 250 ppm (Ahmed *et al.* 2021a). Similarly, chili plants exhibited better outcomes in terms of branching points and branching number per plant, stem diameter, and plant spread when treated with a 1.0% foliar spray of Zn alongside Fe and B (Munir *et al.* 2018). Nawaz and his colleagues discovered that the application of 10 ppm Zn in combination with 150 kg/ha of nitrogen and phosphorus via foliar spray increased the total yield by 100% and maximized the number of tomato fruits per plant (Nawaz *et al.* 2012). As a result, it is advisable to utilize a mix of macronutrients in the soil and foliar spray the plants to improve the quality and productivity of tomatoes. The most significant results were seen in growth, flowering, yield and quality when a foliar spray consisting of 0.2% calcium nitrate, ferrous sulfate, zinc sulfate and 0.1% B was applied (Zahed *et al.* 2021). The use of ZNPs (20 ppm) showed a significant increase in root and shoot biomass in mungbean seedlings, with a 40.9% increase in root biomass and 76.0% increase in shoot biomass (Ahmed *et al.* 2021a; Dixit *et al.* 2018). On the other hand, the application of 1 ppm of ZNPs in chickpea seedlings resulted in 26.6% and 37.1% growth in shoot and root biomass (Mahajan *et al.* 2011). The main aim of this study was to investigate the impact of ZNPs on tomato

plants, particularly on their roots, and determine its optimal dosage. It was hypothesized that lower doses of ZNPs would have a beneficial effect, but higher doses could lead to reduced growth and plant stress due to increased accumulation of zinc.

It has been demonstrated by many laboratories that ZNPs have high antimicrobial activities against a vast range of fungal and bacterial plant pathogens (Chen *et al.* 2021), as well as against viral diseases (Rodelo *et al.* 2022). ZNPs have been used to control *Sclerotia sclerotiorum* blight in sugar beet (Derbalah *et al.* 2013), causal organisms of causing bacterial leaf spot in rose including *Pseudomonas syringe* and *Xanthomonas campestris* (Paret *et al.* 2013), *Fusarium graminearum* or *Fusarium moniliforme* causing head blight in sorghum (Dimkpa *et al.* 2013b), *Xanthomonas citri* subsp. citri. causing canker in citrus (Graham *et al.* 2016), *Elsinoe fawcetti* causing scab in citrus, and *Diaporthe citri* causing melanose in citrus (Graham *et al.* 2016). Field experiments with ZNPs applied for disease management are still limited. More research work is required to improve the effectiveness of nanoparticles against phytopathogens. Nonetheless, Zinkicide® has been registered against various phytopathogens. It has been reported that the disease management through the application of ZNPs was much better than the application of ZnO bactericides. Moreover, the registrations of Nano-Zn-based products for plant disease management are being more encouraged as a reliable alternative to traditional disease management strategies (Sharma & Bhandari 2020). In this review, the mechanisms of biological synthesis of ZNPs from plant extracts as well as microbes, and algae will be discussed along with their mode of action as antimicrobial products.

The cubic zincblende and hexagonal wurtzite are the two major forms of ZNPs. The most stable and common structure is the wurtzite structure. ZNPs have a hexagonal lattice and belong to the P63mc space group further composed of two sublattices O^{2+} and Zn^{2+} in such a way that that Zn^{2+} is interconnected with a tetrahedra of O^{2-}. In this way, the tetrahedral coordination forms a polar symmetry. This polar symmetry has a hexagonal axis (Sabir *et al.* 2014) that provides spontaneous polarization and piezoelectricity in ZNPs. Moreover, the polar symmetry is essential in defect generation, etching, and crystal growth. It has two polar termination faces, including a Zn-terminated face and an O-terminated face, which is c-axis oriented. The non-polar faces are interconnected with equal numbers of O and Zn atoms. The polar termination faces have different physical and chemical properties. The polar O-terminated face has a comparatively different electronic structure than the other three O faces (Dulub *et al.* 2002).

Plants produce exclusive chemicals. Various plant parts, such as fruit, leaf, stem, seed, or root can be used for the synthesis of ZNPs (Table 6.1). The plant-mediated biosynthesis gives a large quantity of homogenous product with limited impurities (Resmi *et al.* 2021). The reduction of metallic ions is done to 0 valence nanoparticles which are also regarded as bio-reduction and are aided by phytochemicals available in plant extracts such as polysaccharides, polyphenols, vitamins, terpenoids, alkaloids, and amino acids (Heinlaan *et al.* 2008). Nanoparticles are synthesized using fresh leaf extract and characterized by Fourier transform infrared spectroscopy (FTIR), UV–visible spectrophotometry, X-ray diffractometer (XRD), scanning electron

microscopy (SEM), energy dispersion analysis of X-ray (EDAX), dynamic light scattering (DLS), atomic force microscopy (AFM), and thermal-gravimetric differential thermal analysis (TG-DTA) (Santhoshkumar *et al.* 2017). ZNPs have been synthesized by using plant extract of red tomato fruit (*Lycopersicon esculentum* M.), olive leaves (*Olea europaea*) and chamomile flower (*Matricaria chamomilla* L.). The synthesized ZNPs were characterized by UV-Vis, FTIR, X-ray diffractometer (XRD), transmission electron microscopy (TEM), Energy dispersive X-ray spectroscopy (EDS) profile and scanning electron microscopy (SEM) (Ogunyemi *et al.* 2019).

The process of synthesis of ZNPs from plant extracts involves first the washing of plant samples in tap water and then double distilled water (Figure 6.2). Nonionic surfactants can be used for sterilization. Next samples are dried at room temperature, weighted and crushed in a fine powder. A desired volume of milli-Q water is added and the mixture is boiled along with continuous stirring (Acharya *et al.* 2019; Qu *et al.* 2011) followed by a filtration to get clear solution of plant extract through Whatman filter paper. Then 0.5 mM of hydrated Zn $(NO_3)_2$, $ZnSO_4$, or $Zn(C_2H_3O_2)_2$ is added, and boiled for effective mixing (Rajeshkumar *et al.* 2016) (Figure 6.2). Specific pH, temperature, and extract concentration are usually required for optimal performance. A color change to yellow is a visual confirmation of ZNPs synthesis (Rajeshkumar *et al.* 2016). The confirmation of ZNPs synthesis is also done by UV-visible spectrophotometry. Finally, the mixture is centrifuged and the pellet is dried to obtain NCPs of ZnO (Nezamzadeh-Ejhieh & Bahrami 2015). Moreover, the characterization of ZNPs is done by SEM, DLS, FTIR, TG-DTA, XRD, TEM, FESEM, AFM, UV-DRS, EDAX, Roman spectroscopy, XPS, PL, and ATR (Amjadi *et al.* 2019). Bacteria have the innate ability to be mobilizers and immobilizers of metals, and the ability of metal reduction at a nanometer scale (Qiao *et al.* 2018). Various plant extracts have been used for the biosynthesis of ZNPs from bacteria (Table 6.2). In the biogenic synthesis of ZNPs (Figure 6.3), bacteria are first grown in nutrient broth media. Then, about 25 mL of culture is diluted, and again grown. Next, a precursor salt Zn acetate is added, and the culture is incubated under constant shaking which yields white-colored precipitations at the bottom of the braker, indicating the transformation process (Kirthi *et al.* 2011; Prasad & Jha 2009).

The production of crystallized nature ZNPs has been done by using *Aeromonas hydrophila* and confirmed by XRD and an AFM study gave evidence of spherical morphology with an average size of 57.72 nm (Jayaseelan *et al.* 2012). The ZNPs synthesized from *Rhodococcus pyridinivorans*, a bacterium involved in biodegradation by metabolizing hydrophobic compounds that can survive in harsh conditions of environment (Otari *et al.* 2012), were 100–130 nm in size with physical morphology, as shown by XRD and FESEM analysis. These ZNPS had β-lactone, phosphorus compound, amine salt, hydroxy aryl ketone, mononuclear benzeneresence secondary sulphonamide, enol of 1-3-di ketone and alkane, as shown by FTIS (Tripathi *et al.* 2014). *Bacillus licheniformis* has also been used for the green synthesis of ZnO nanoflowers that have potential photocatalytic activities (Tripathi *et al.* 2014). They enhance the photocatalytic activities of any substance by light absorption and cause degradation of organic waste matter.

NPs biogenically synthesized by fungi are solid colloidal metal particles with a 1-100 nm range in size. Various plants have been used for the biosynthesis of ZNPs (Table 6.3). The fungi are first cultured as a culture suspension for 24 hours and the fungal culture is then centrifuged for 30 minutes (Figure 6.4). Around 0.1 g of ZnO is added to the supernatant in Erlenmeyer flasks (Kirthi *et al.* 2011)and stirred at 200 rpm for another 24–48 hours (Figure 6.4). The myconanoparticles differ from nanoparticles synthesized by plants or bacteria in terms of optical, electrical, and catalytic properties (Elsoud *et al.* 2018). Due to metal bioaccumulation characteristics and better tolerance in harsh environment, fungi are more commonly used and more preferred for the biosynthesis of ZNPs than bacteria and (Haque *et al.* 2020).

ZNPs have been synthesized from *Aspergillus fumigatus* with a size ranging from 1.2-6.8 nm with an average of 3.8 nm, as shown by DLS, and an average height of 8.56 nm, as shown by Atomic Force Microscopy (AFM) (Pavani *et al.* 2012). *Aspergillus terreus* has also been used to synthesize NPs with 54.8–82.6 nm in size, as shown by SEM and XRD (Agarwal *et al.* 2018). FTIR studies revealed the presence of aromatic nitro compounds, amide, primary alcohol, and amines. ZNPs synthesized by using *Aspergillus* species were mostly spherical shaped. *Aspergillus niger* has also been evaluated for the extracellular synthesis of ZNPs. The presence of aromatic rings and carboxylic acid has been confirmed by FTIR (Kalpana *et al.* 2018). *Candida albicans* have also been used for nano-synthesis and NPs of 15-25 nm is size have been synthesized, as shown by TEM, SEM, and XRD (Mashrai *et al.* 2017). *Pichia fermentans* have also been used for the biogenic synthesis of ZNPs (Chauhan *et al.* 2015).

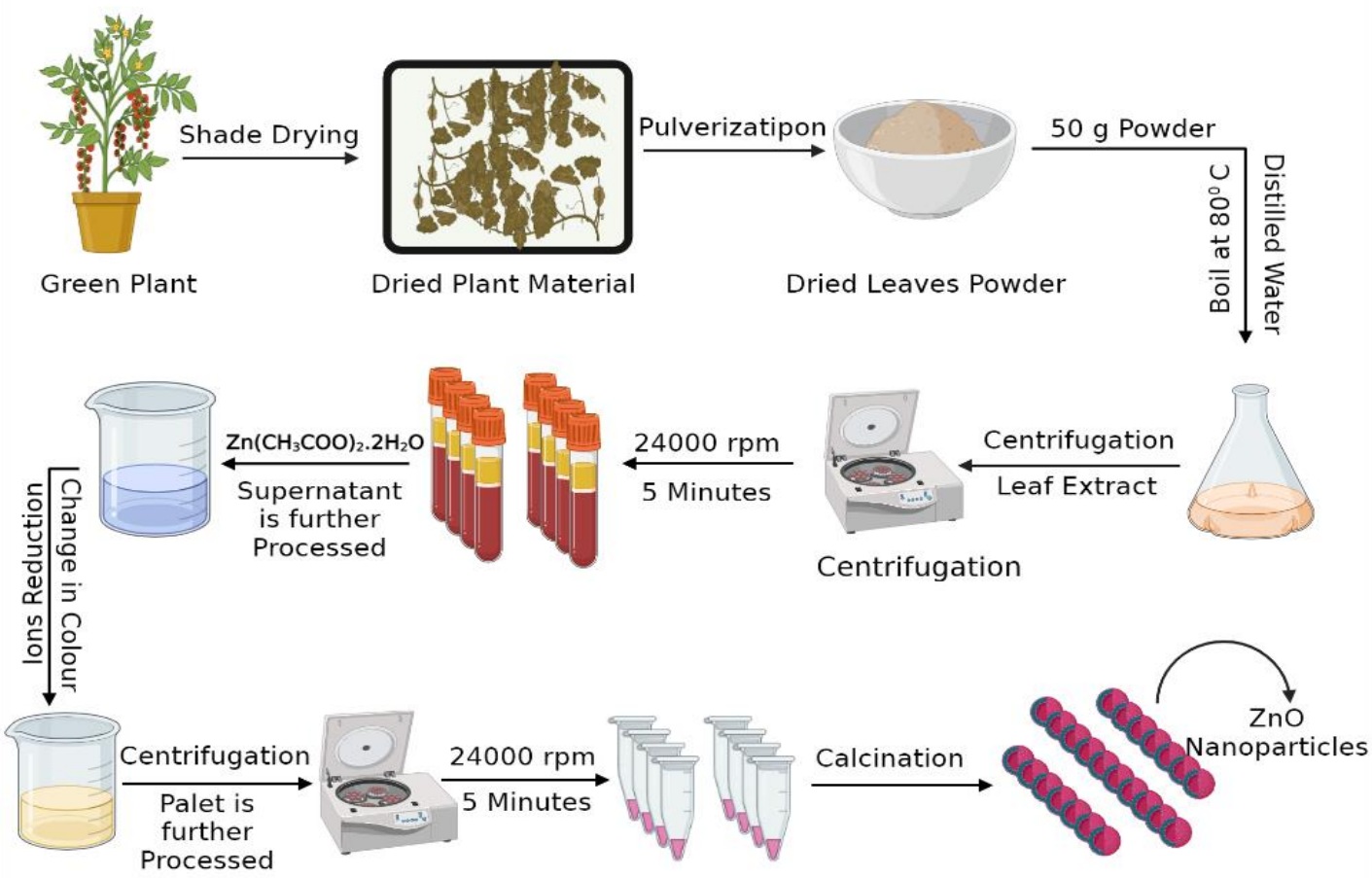

Figure 6.2. A systematic and diagrammatic description of biosynthesis of ZnO nanoparticles from plant extracts. As plant extracts have various biomolecules including protein, carbohydrates etc, which act as a reducing agent to promote the formation of ZnO nanoparticles. More commonly, proteins with functionalized amino groups ($–NH_2$) present in plant extracts can actively participate in the reduction of Zn ions.

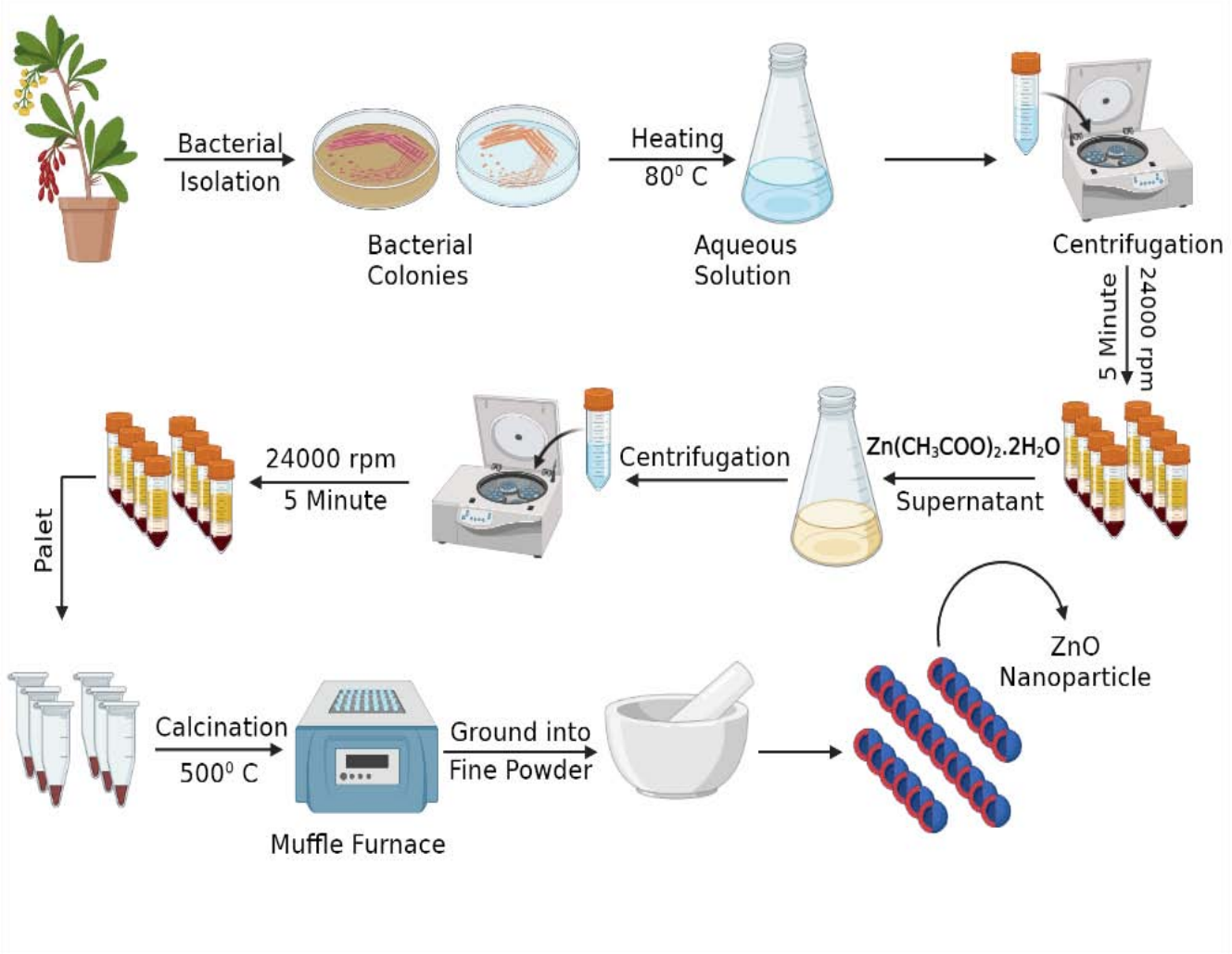

Figure 6.3. A systematic and diagrammatic description of biosynthesis of ZnO nanoparticles from bacterial suspensions. The formation of ZnO nanoparticles by bacteria involves Zn metal capture, enzymatic reduction, and capping. Firstly, Zn ions are trapped on the surface or inside of the microbial cells and then reduced to ZnO nanoparticles in the presence of enzymes.

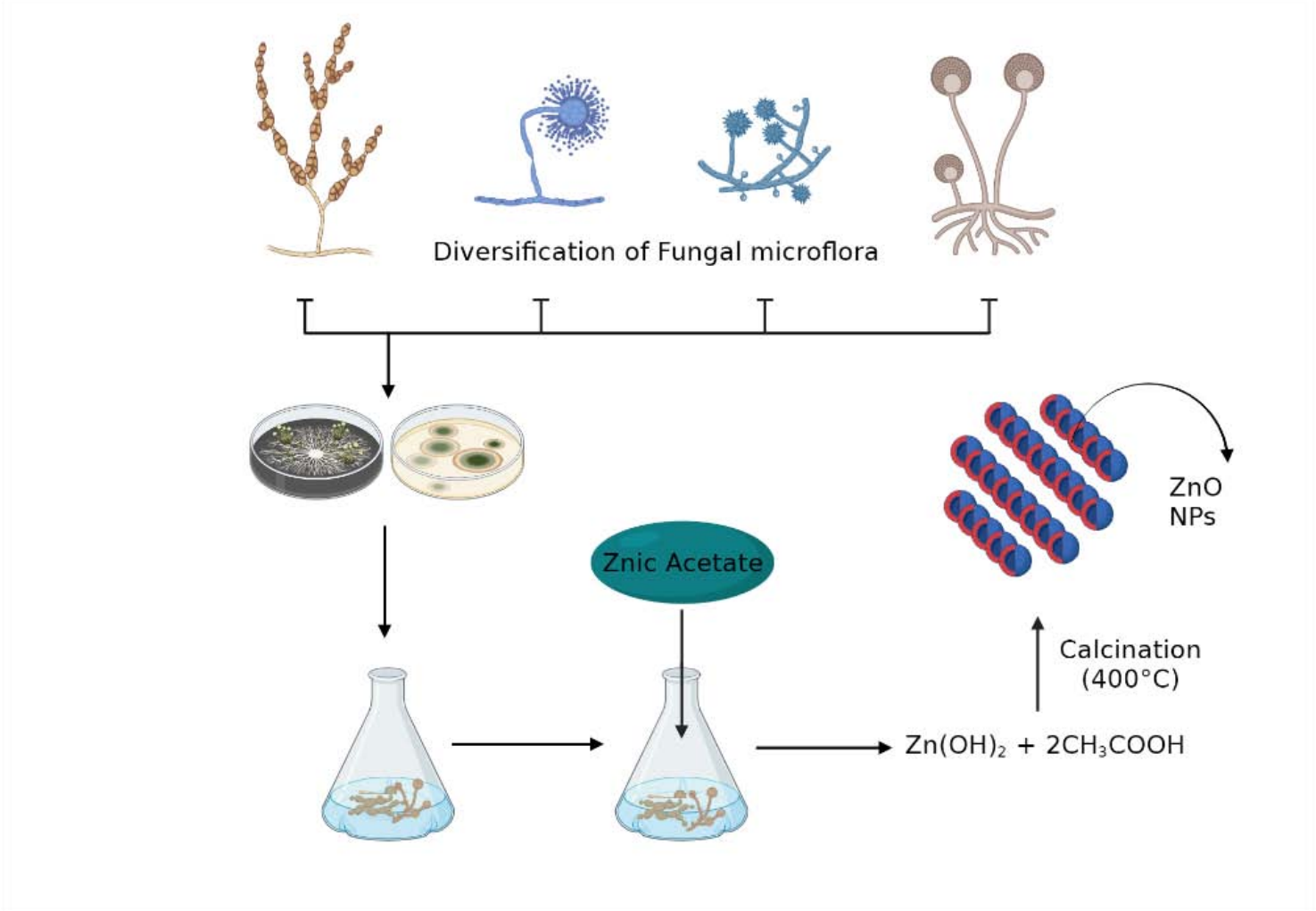

Figure 6.4. A systematic and diagrammatic description of biosynthesis of ZnO nanoparticles from fungi. The synthesis of ZnO nanoparticles involves the trapping of the Zn ions on the surface and inside the cells and reducing them in the presence of enzymes which are secreted by fungi extra and intracellularly.

Table 6.1. Various plant extracts used for the biogenic synthesis of ZnO nanoparticles

Sr. No.	Plant	Family	Plant Part	Size (nm)	Functional Group	Reference
1.	*Azadirachta indica*	Meliaceae	Leaves	18	Amine, ketone, carboxylic acid	(Elumalai & Velmurugan 2015)
2.	*Cocus nucifera*	Arecaceae	Water	20–80	O-H of alcohol C = O of ketones	(Krukiewicz & Zak 2016)
3.	*Ocimum basilicum*	Lamiaceae	Leaf extract	50	___	(Salam *et al.* 2014)
4.	*Calatropis Gigantea*	Legumes	Flowers	60-70	Hydroxyl, -C-O	(Dobrucka & Długaszewska 2016)
5.	*Aloe vera*	Liliaceae	Leaf peel	25–65	___	(Qian *et al.* 2015)
6.	*Vitex negundo*	Lamiaceae	Flowers	10–130	___	(Ambika & Sundrarajan 2015)
7.	*Solanum nigrum*	Solanaceae	Leaf extract	20–30	O-H, aldehydic C-H	(Ramesh *et al.* 2015)
8.	*Agathosma betulina*	Rutaceae	Dry leaves	12–26	O-H of hydroxyl group	(Thema *et al.* 2015)
9.	*S. album*	Santalaceae	Leaves	100	O-H of alcohol	(Yuvakkumar *et al.* 2014)
10.	*P. amboinicus*	Lamiaceae	Leaves	50–180	Zn-O, C-O of C-O-SO3	(Fu & Fu 2015)

	Ixora coccinea	Rubiaceae	Leaves	145.1	OH stretching vibrations, The C-H stretch in alkanes, C=C stretch in aromatic ring and C=O stretch in polyphenols, O-H stretch in carboxylic acid	(Yedurkar *et al.* 2016)
11.	*Prosopis juliflora*	Fabaceae	Fresh leaves	65	C–H stretching, symmetric COO– stretching, O–H stretching, hydroxyl group	(Mydeen *et al.* 2020a)
12.	*Mangifera indica*	Anacardiaceae	Leaves	60	_	(Rajeshkumar *et al.* 2018)
13.	*Hibiscus sabdariffa*	Malvaceae	Leaves	9	C–H bond in alkene group, C=C stretching, primary amines, amides I and II, O–H groups	(Mahendiran *et al.* 2017)
14.	*Aloe vera*	Liliaceae	Leaf gel	18	C–H bond in alkene group, C=C stretching, primary amines, amides I and II, O–H groups	(Mahendiran *et al.* 2017)
15.	*Calotropis gigantea*	Apocynaceae	Leaves	10	Stretching of zinc carboxylates, –CH stretching, –OH stretching	(Chaudhuri & Malodia 2017)
16.	*Plectranthus amboinicus*	Lamiaceae	Leaves	88	Stretch band of zinc and oxygen, C-O−SO3, Symmetric C-O vibration	(Fu & Fu 2015)
17.	*Phoenix dactylifera*		Leaves	16 to 35	O–H stretching, (N–H) bending, (C=C) stretch, (C–Cl) bond	(Salih *et al.* 2021)

18.	*Plectranthus amboinicus*	Lamiaceae	Leaves	88	C-N vibrations, C-O belonging	(Zheng *et al.* 2019)
19.	*Nephelium lappaceum*	Sapindaceae	Leaves	25–40	C–O and –C–O–C stretching modes, O-H stretching vibration, H-O-H bending vibration,	(Karnan & Selvakumar 2016)
20.	*Albizia lebbeck*	Fabaceae	Leaves	66.25	C–O of primary saturated alcohol, C=O amide band, O–H bending, C=CH stretching	(Umar *et al.* 2019)

Table 6.2. Various bacterial suspensions used for the biogenic synthesis of ZnO nanoparticles

Sr. No.	Bacteria	Family	Size (nm)	Functional Group	Reference
1.	*E. coli*	Enterobacteriaceae	13	Hydroxyl radicals (˙OH)	(Reddy *et al.* 2007)
2.	*Salmonella typhimurium*	Enterobacteriaceae	≤50	___	(Tayel *et al.* 2011)
3.	*P. aeruginosa*	Pseudomonadaceae	57.72	Free OH groups, H-H bonds	(Jayaseelan *et al.* 2012)
4.	*Candida albicans*	Saccharomycetaceae	15–32	C–OH stretching	(Palanikumar *et al.* 2014)
5.	*Salmonella choleraesuis*	Enterobacteriaceae	100	Carboxylic groups	(Espitia *et al.* 2012)
6.	*E. faecalis*	Enterococcaceae	41.60–167.61	___	(Narayanan *et al.* 2012)
7.	*Staphylococcus aureus*	Staphylococcaceae	19.8	___	(Baek & An 2011)
8.	*Streptococcus agalactiae*	Streptococcaceae	60–100	C-O bonds and-OH groups	(Huang *et al.* 2008)
9.	*Lactobacillus plantarum*	Lactobacillaceae	13.09	symmetric stretching mode of water molecules,	(Selvarajan & Mohanasrinivasan 2013)

				symmetric stretching mode of SO42−	
10.	*Aeromonas hydrophila*	Enterobacteriaceae	42–64	Enol of 1,3 diketone, alkanes, monosubstitued alkyne	(Jayaseelan *et al.* 2012)
11.	*Lactobacillus Spp.*	Lactobacillaceae	18.6	Asymmetric stretch of –C-C=C and C=C, C=C–C stretching	(Suba *et al.* 2021)
12.	*Lactococcus lactis*	Streptococcaceae	55–60.5	C-H stretching vibration, O–H stretching, C=O stretching	(Mahdi *et al.* 2021)
13.	*Bacillus sp*	___	99	C-H stretching vibration, O–H stretching, C=O stretching	(Mahdi *et al.* 2021)
14.	*Bacillus licheniformis*	___	200	N-H bond stretching, carbonyl (-C-O-) stretching, peaks of –N-H and COO-	(Tripathi *et al.* 2014)

Table 6.3. Various fungal solutions used for the biogenic synthesis of ZnO nanoparticles

Sr. No.	Fungi	Shape	Size	Activities	References
1.	*Alternaria tenuissima*	Spherical	15–45 nm	Antimicrobial, antioxidant	(Abdelhakim *et al.* 2020)
2.	*Aspergillus strain*	Spherical	50–120	___	(Pavani *et al.* 2012)
3.	*Aspergillus fumigatus*	Oblate spherical	1.2–6.8	P-mobilizing enzyme secretion	(Raliya & Tarafdar 2013)
4.	*Aspergillus terreus*	Spherical	54.8–82.6	Antibacterial	(Chandrasekaran *et al.* 2016)
5.	*Candida albicans*	Quasi-spherical	15–25	Catalytic Properties	(Mashrai *et al.* 2017)
6.	*Xylaria acuta*	Cylindrical, rod	30–50	Antifungal	(Sumanth *et al.* 2020)
7.	*Fusarium* spp.	Triangular	>100	___	(Velmurugan *et al.* 2010)
8.	*Aspergillus fumigatus*	spherical	60-80	Antibacterial	(Rajan *et al.* 2016)

ZNPs have antimicrobial activities. For bacteria, a retarded growth has been documented in the presence of ZNPs (Graham *et al.* 2016; Indhumathy & Mala 2013). ZNPs inhibit the growth of gram-positive bacteria by 90% but less that of gram-negative bacteria (Adams *et al.* 2006). Moreover, bacterial pathogens can be controlled by ZNPs (Derbalah *et al.* 2013; Paret *et al.* 2013). For example, bacterial leaf spot (*Xanthomonas campestris*) was managed in roses by light-activated ZNPs synthesized by photocatalyst technology. Disease management via ZNPs was equivalent to conventional bactericide applications and became the ornamental industry standard for control of *Xanthomonas campestris* (Paret *et al.* 2013). ZNPs are also widely used for the management of various bacteria diseases in citrus including canker (Young *et al.* 2017). ZNPs formulations such as the particulate Zinkicide^{TM} GS6 and the plate-like Zinkicide^{TM} SG4 showed the same suppression activities of citrus canker (*Xanthomonas citri* pv. citri) (Graham *et al.* 2016). Two foliar applications of Zinkicide^{TM} GS6 showed a 38% reduction of canker lesion formation in sweet orange while injection resulted in a 42% reduction of canker lesions (Graham *et al.* 2016). In full-scale field studies, Zinkicide^{TM} GS6 reduced more efficiently disease incidence than conventional bactericides including zinc oxide and cuprous oxide. ZNPs from *Aeromonas hydrophila* had a spherical morphology with an average size of 57.72 nm, as shown by AFM (Jayaseelan *et al.* 2012). Minimal inhibitory concentration and well diffusion methods were used to check the antibacterial and antifungal activities of ZNPs. The inhibition zone due to ZNPs (25 µg/mL) against *Aspergillus flavus* and *Pseudomonas aeruginosa* was ~19±1 mm and ~22±1.8 mm respectively (Jayaseelan *et al.* 2012). Zinkicide^{TM} applications suppress citrus scab (*Elsinoe fawcetti*) and citrus melanose (*Diaporthe citri*) (Graham *et al.* 2016). Comparative foliar applications of ZNPs, tetraconazole, diatomaceous earth nano-silica, and bacterial biocontrol demonstrated that ZNPs was second only to tetraconazole in reducing the severity of sugar beet leaf blight (*Cercospora beticola*). ZNPs improved the biocontrol efficacy of *Pseudomonas chlororaphis* against *Fusarium graminearum in vitro* (Dimkpa *et al.* 2013b).

Similarly, ZNPs have antifungal activities against *B. cinerea*, *Rhizoctonia solani*, *A. alternata, Mucor plumbeus, F. oxysporum, Penicillium expansum, Sclerotinia sclerotiorum* and *Rhizopus stolonifera* (He *et al.* 2011; Sardella *et al.* 2017). A comparative effectiveness between ZNPs at the rate of 500 to 800 µg/mL and conventional bactericides indicated 26% more effectiveness of ZNPs against *F. graminearum* (Dimkpa *et al.* 2013b). ZNPs act also against nematodes such as *Meloidogyne inconita* (Kaushik & Dutta 2017).

Table 6.4. ZnO nanoparticles against various plant pathogens

Sr. No.	Size of Nanoparticles	Dose of Application	Disease	Pathogen	Reduction rate (%)	Reference
1.	~30 ± 10nm	0.5ml	Black spot	*Alternaria alternate*	78.56	(Wani & Shah 2012)
2.	~30 ± 10nm	0.5ml	Fusarium wilt	*Fusarium oxysporum*	60.41	(Wani & Shah 2012)
3.	~30 ± 10nm	0.5ml	Soft rot	*Rhizopus stolonifer*	71.63	(Wani & Shah 2012)
4.	<100 nm	≈0.7 ± 0.05	Fusarium head blight	*Fusarium graminearum*	26%	(Dimkpa *et al.* 2013a)
5.	<100 nm	0.1 mg	Bacterial Blight	*Xanthomonas axonopodis pv. phaseoli*	___	(Siddiqui *et al.* 2018)
6.	20-25 nm	100 µg	Corn Ear Rot	*Aspergillus flavus*	75%	(Shinde 2015)
7.	20-25 nm	100 µg	Mold	*Aspergillus fumigatus*	75%	(Shinde 2015)
8.	405 nm	---	Grey mould	*Botrytis cinerea*	80%	(Luksiene *et al.* 2020)

9.	42-64 nm	25 µg/mL	____	*Pseudomonas aeruginosa*	____	(Jayaseelan *et al.* 2012)
10.	42-64 nm	25 µg/mL	____	*Aspergillus flavus*	____	(Jayaseelan *et al.* 2012)
11.	____	0.10 mg/mL	Leaf Blight	*Alternaria dauci*	52.5	(Siddiqui *et al.* 2019)
12.	____	0.10 mg/mL	Dry Rot	*Fusarium solani*	47.4	(Siddiqui *et al.* 2019)
13.	____	0.05 mg/mL	Leaf Blight	*Alternaria dauci*	24.7	(Siddiqui *et al.* 2019)
14.	____	05.0 mg/mL	Dry Rot	*Fusarium solani*	22.4	(Siddiqui *et al.* 2019)
15.	<100	500 mg/mL	Root rot	*Fusarium graminearum*	75	(Dimkpa *et al.* 2013a)
16.	27	25 lg/mL	Black mold	*Aspergillus niger*	25	(Rajiv *et al.* 2013)
17.	27	25 lg/mL	Rot disease	*Aspergillus flavus*	25	(Rajiv *et al.* 2013)
18.	13.07-22.25	20 µg/mL	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	___	(Cheema *et al.* 2022)
19.	13.07-22.25	50 µg/mL	Brown spot	*Bipolaris oryzae*	72.68	(Cheema *et al.* 2022)

20.	13.07-22.25	50 µg/mL	Narrow brown spot	*Sphaerulina oryzina*	95.78	(Cheema *et al.* 2022)
21.	48.2	4.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	47.5	(Ogunyemi *et al.* 2019)
22.	65.4	4.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	38.9	(Ogunyemi *et al.* 2019)
23.	61.6	4.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	34.2	(Ogunyemi *et al.* 2019)
24.	48.2	8.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	59.4	(Ogunyemi *et al.* 2019)
25.	65.4	8.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	50.2	(Ogunyemi *et al.* 2019)
26.	61.6	8.0 µg/ml	Bacterial blight	*Xanthomonas oryzae pv. Oryzae*	46.3	(Ogunyemi *et al.* 2019)
27.	60.8	50 µg/ml	Root rot	*Dickeya dadantii*	60	(Hossain *et al.* 2019)

ZNPs have also been used to manage different virus diseases in plants (El-Megharbel *et al.* 2021; Sharmin *et al.* 2021). Biogenically synthesized ZNPs have viral-inhibition and viral-neutralizing properties (Kumar *et al.* 2018). Tomato plants pre-treated with low concentration NPs for one week reduced disease incidence and triggered defense responses to tomato mosaic virus (Sofy *et al.* 2021). Foliar application of ZNPs in tomato conferred control of tomato spotted wilt virus (TSWV) even when ZNPs was applied one day after TSWV inoculation (Vargas-Hernandez *et al.* 2020). Two foliar applications of ZNPs 24 hours before and after inoculation reduced symptom severity of tobacco mosaic virus (TMV) by 90%, concomitantly with the upregulation of phenylalanine ammonia-lyase, pathogenesis-related-1, chalcone synthase, and plant peroxidase genes (Abdelkhalek & Al-Askar 2020). ZNPs controlled TMV infection under laboratory and field conditions (Cai *et al.* 2019). After 2 hours of pretreatment with ZNPs, TMV particles displayed significant aggregation and breaking *in vitro* and virus accumulation was reduced in ZNPs-treated tobacco plants compared with control plants two days later. However, the inactivation effects were insufficient to prevent viral multiplication and accumulation at seven days post-inoculation. Daily foliar spraying of NPs onto tobacco leaves for 12 days, on the other hand, significantly suppressed TMV replication due to the activation of the plant defenses. In NP-treated plants, the accumulation of reactive oxygen species, peroxidase activity, catalase activity, and pathogenesis-related proteins were all increased. Further research revealed that ZNPs caused increases in SA and ABA phytohormone levels of 162% and 517 %, respectively (Cai *et al.* 2019).

Disease suppression activities of ZNPs has been reported by laboratory studies as well as by field studies (Derbalah *et al.* 2013; Graham *et al.* 2016). Antimicrobial ZNPs can be used in the management of plant diseases and improvement of plant health. The applications of ZNPs are a very encouraging for the management of diseases and can be reliable alternatives to traditional strategies for the control of phytopathogens (Table 6.4).

ZNPs have great potential to cause the lysis of bacterial cells through cell membrane disintegration followed by leakage of the cytoplasmic material (Kaushik *et al.* 2019). Nanoparticles can also diffuse through porins that are beta-barrel proteins and this passive internalization has been seen in Gram-negative bacteria (Kalia *et al.* 2020). The Gram-positive bacteria have a thick cell wall which makes difficult the passive internalization of ZNPs, resulting in dissolving Zn^{2+} released by ZNPs in the vicinity of the cell surface and chelated by lipoteichoic acid (Agarwal *et al.* 2018). Once inside the cell, incorporated NPs may cause significant damage to the cell membrane, internal biomolecules, and other catalytic and soluble molecules via Fenton- or non-Fenton-based reactive oxygen species (ROS) (Patra *et al.* 2015). The disintegration of ZNPs releases ions, resulting in metal/nonmetal ion toxicity and cell death (Liu *et al.* 2020). Another intriguing method is the prevention of biofilm formation by reduced expression of quorum-sensing controlled genetic makeup or functionalities in bacteria (Al-Shabib *et al.* 2016). ZNPs can also aid the inhibition of plant-pathogen generated biofilms, resulting in the development of resistance to bacterial pathogens which major crop losses. The mechanisms of action of ZNPs against bacteria have been illustrated in Figure 6.5.

There is a huge amount of information on the fungicidal aspects of ZNPs (Sun *et al.* 2018). Nanoparticulate fungicide formulations are applied at lower treatment dosages than conventional fungicides, potentially addressing the toxicity concerns caused by metal cations (Elmer *et al.* 2018; Elmer & White 2018). Internalization of ZNPs occurs in the fungal cell via three processes: (a) direct entry of nonspecific, mostly spherical and small NPs, (b) a specific receptor-based adsorption of NPs, and (c) the membrane-spanning ion transport proteins. Some NPs, especially metal and nonmetal oxides, can inhibit mycelial growth by (i) antifungal activities resulting from the genesis of reactive oxygen species and dissolution of NPs in the vicinity of the cell to release specific ions for metal/nonmetal ion toxicity, and (ii) reducing or suppressing the expression they can also regulate the mycotoxin-producing genes (Figure 6.6).

ZNPs have antiviral activities. Interference with viral attachment, binding to the plasma membrane, interaction as well as competition for binding with host cell between the NPs and virus, viral particles may become inactive before entering, binding of NPs with viral particles, interaction with double-stranded DNA and inhibition of binding between host cell and virus-cell and penetration are the main mechanisms of nanoparticle antiviral activity. Mechanisms could involve a full or partial denaturation of the virus particle and inhibition of virus replication, and may vary depending on the size and nature of nanoparticles utilized. Nanoparticles frequently modify the structure of the viral nucleocapsid, resulting in a reduction in infectivity. Moreover, the indirect mechanism of antiviral activity involves a shift in membrane permeability that prevents virus entrance into the cell. The mechanisms of action of ZNPs against viral have been demonstrated in Figure 6.7.

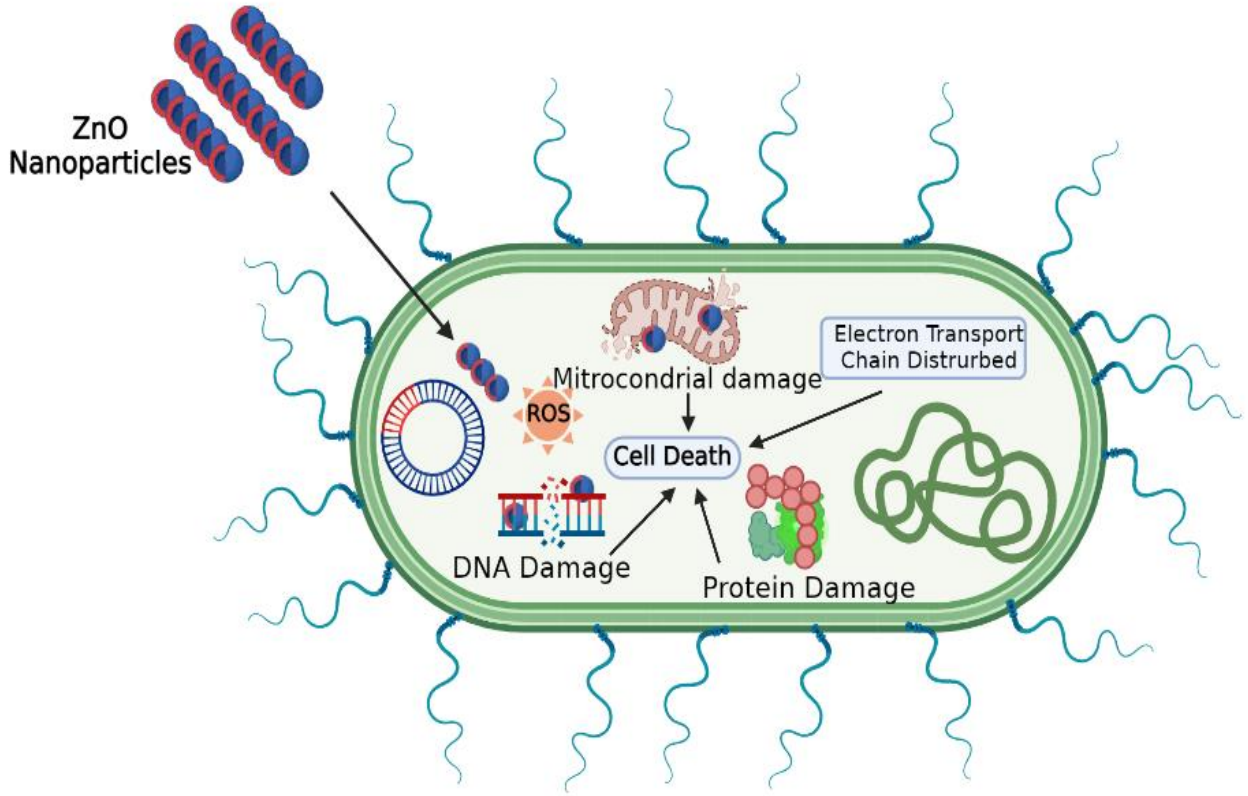

Fig. 4 A systematic and diagrammatic description of mechanisms of action of ZnO nanoparticles against bacteria. ZnO nanoparticles exhibit antimicrobial potential through multifaceted mechanisms. Firstly, the ZnO nanoparticles adhere to bacterial cell surface which results in production of reactive oxygen species. ROS penetration inside the bacterial cells have been recognized as the most prominent mode of antimicrobial action. Various other activities of ZnO nanoparticles inside bacterial cells are inhibition of cell wall/membrane synthesis, photocatalysis, enzyme inhibition, disruption of energy transduction, and reduced DNA production.

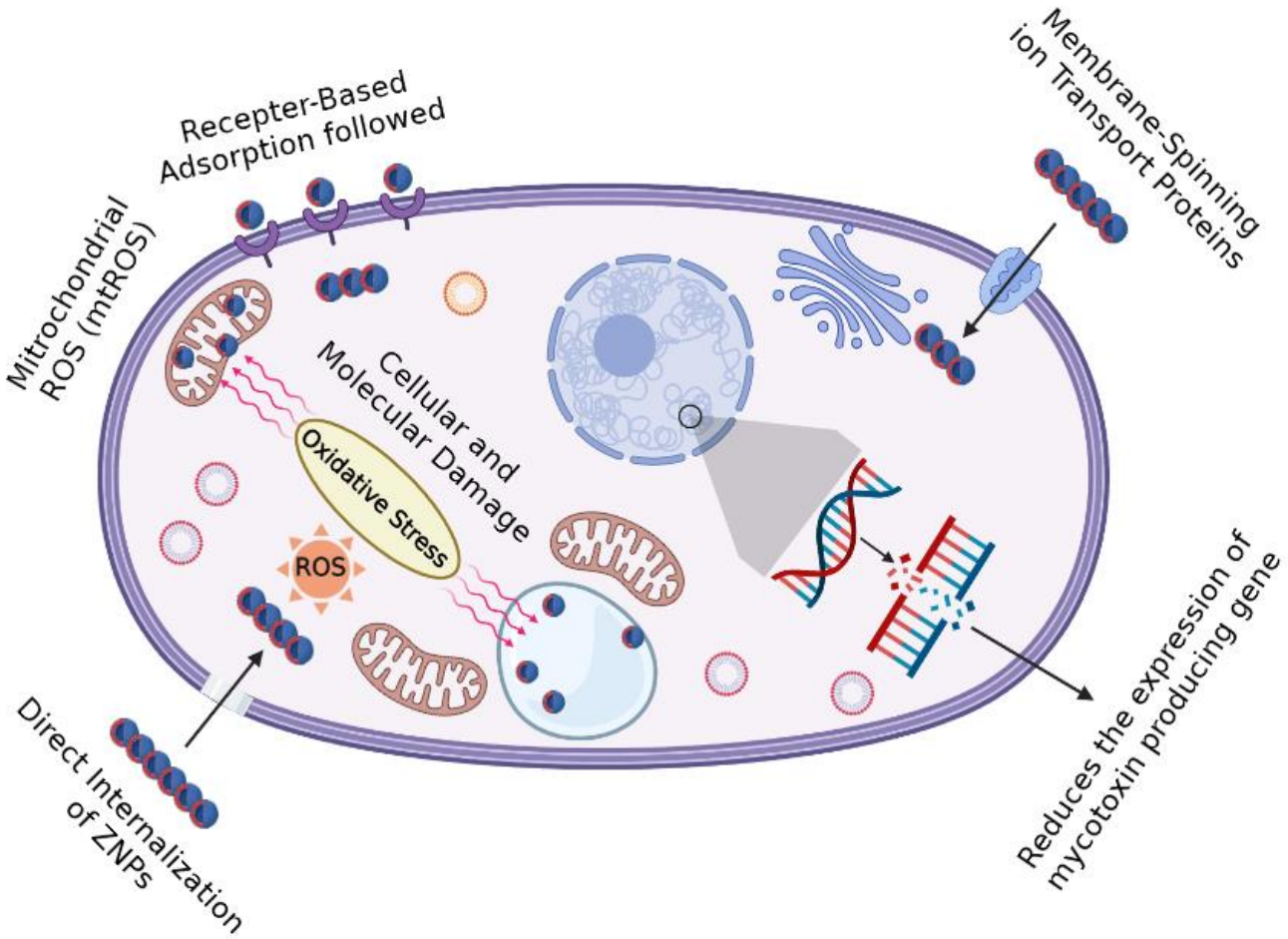

Fig 5 A systematic and diagrammatic description of mechanisms of action of ZnO nanoparticles against fungi. Firstly, ZnO nanoparticles disturb fungal cell membrane integrity through their fungicidal activity. Damaged cell membrane results in leaching of nucleic acid and other ions into cell free filtrate.

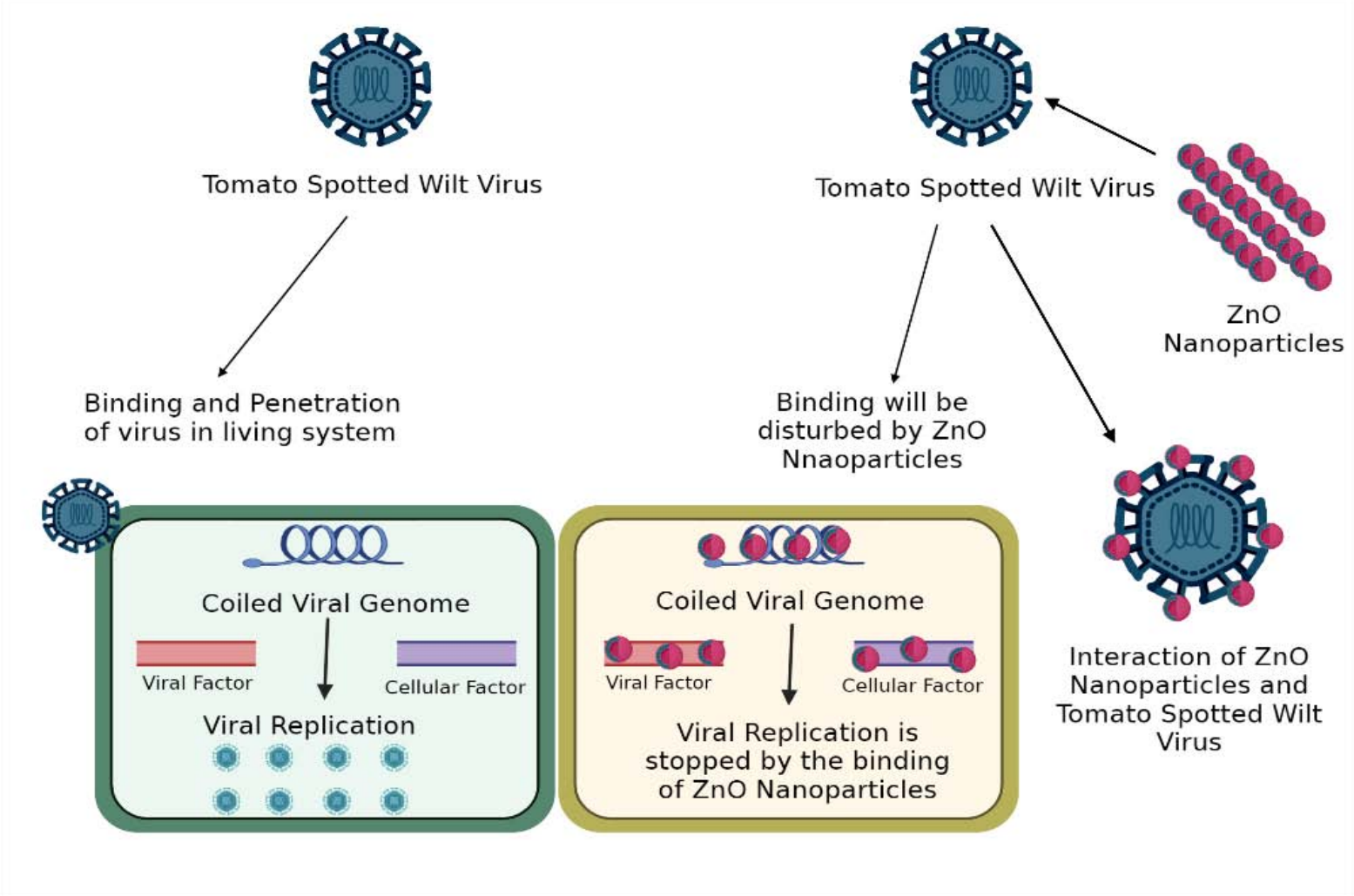

Fig 6 A systematic and diagrammatic description of mechanisms of action of ZnO nanoparticles against viruses in which the binding of viral particles with host cell is disturbed by ZnO nanoparticles.

ZNPs have activities against soil-borne pathogens. Some of the nanoscale material target soil pathogens in agriculture for more effective nutrient delivery as well as improved soil and plant health through microbiome improvement (Lowry *et al.* 2019). Several studies document NPs boosting plant growth, and photosynthetic efficiency under abiotic stress (Wu *et al.* 2017), biomass/yield, and fruit quality (Kole *et al.* 2013).

Due to wide interactions and the overall absence of instrumentations for suitable particle detection for environmental matrices, it is very difficult to understand the mode of action of NPs in soil. As a result, research is lacking in this area, while recent studies have explored the effect of ZNPs on chemical and physical features in surroundings. ZNPs characteristics will be altered as a result of interactions with abiotic and biotic components of soil that will likely impact ZNPs durability, accumulation, transport, and availability. Silver NPs have a higher mobility in negatively charged soils with a substantial effect on the mobility potential (Tolaymat *et al.* 2010). The inclusion of stabilizing substances including amines, amides, sodium citrate, polyvinyl pyrrolidone, and sugars changed the interaction with soil particles and drastically modified the ensuing mobility. Sulfidation of silver nanoparticles was achieved under both aerobic and anaerobic conditions (Zhang *et al.* 2018), and the altered NPs particles changed mobility and activity. A link between silver nanoparticles transport and clay content in the soil was documented, while no precise mechanisms were revealed (Cornelis *et al.* 2012). The quick dissolution of ZNPs was investigated in cowpeas' rhizosphere before ionic Zn absorption into plant tissues (Rajput *et al.* 2020). Much more work is needed, but the existing research clearly shows that surface charge, size, and particle type, along with physical and chemical properties of soil, influence the transport and bioavailability of NPs in soil.

Limited element bioavailability in neutral or slightly alkaline soils severely limits micronutrient-based disease reduction techniques. When the pH of soil increases, nutrients including copper, magnesium, iron, and zinc become progressively less accessible, resulting in inadequate uptake by the roots of the crop. Few studies investigated the influence of soil pH and other factors on nanoparticles effects in the soil. Because variations in pH of soil might reduce the availability of nutrients over time, a successful soil-based NPs supplement for pathogen management must consider soil physical and chemical parameters like pH. Soluble Zn content was about 200-fold higher in acidic soil with ZNPs along with a 10-fold higher amount in shoots of wheat in comparison to basic soil (Watson *et al.* 2015). The wheat plants grown in acidic soil amended with ZNPs produced more lateral roots than in basic soil. After 50 days of exposure to ZNPs, the accumulation of Zn in soybean leaves was 344.07 mg/kg (Priester *et al.* 2012) although the organic matter in the soil would had a major effect on particle aggregation and stability. Adsorption of humic acid on nanoparticles of Al_2O_3, TiO_2, and ZnO was pH-dependent, but electrostatic interactions inhibited the adsorption of SiO_2 nanoparticles on the oxide surface (Yang *et al.* 2009). Silver showed higher mobility when humic acid was available (Tian *et al.* 2010). Another issue to consider is the availability of organic carbon, which is increasingly being used as a soil amendment in the form of biochar. Biochar can be made from different feedstock materials and a variety of processes. These differences can have a big impact on the char's sorption properties. Biochar can

adsorb and hold other compounds in soil (Elmer & Pignatello 2011). However, another research found that there is no effect on the availability of CeO_2 nanoparticles to plant or worm species due to biochar amendment (Servin *et al.* 2017). These results illustrate the foreseeably substantial impact that soil properties have on the availability of nanoparticles. A comprehensive evaluation of how nanoparticles interact with soil elements is required before nanomaterials can be successfully used for crop nutrition and pathogen management as a soil amendment.

Ryegrass biomass drastically decreased, root tips shortened, and root epidermal and cortical cells were extensively vacuolated or collapsed in the presence of ZNPs. Zn levels in shoots under ZNPs treatments were much lower than those under Zn^{2+} treatments, and Zn^{2+} ion levels in bulk nutrient solutions containing ZNPs were lower and below the toxicity threshold of Zn^{2+} to ryegrass (Paschke *et al.* 2006). As a result, ZNPs' poor solubility in the nutrient solution or rhizosphere did not directly cause phytotoxicity. However, the mechanism of toxicity is still unknown, and there is little evidence available about the possible uptake of nanoparticles by plants and their outcome in food webs (Lin & Xing 2008). Long-term research is needed to accurately predict the consequences of the widespread usage of ZNP-based products in agriculture. ZNPs produced from neem leaf extracts were found to have increased antibacterial efficacy and photocatalytic activity Although the average size of the ZNPs differed somewhat from 25.97 nm for biosynthesized ZNPs to 33.20 nm of sol-gel ZNPs (Haque *et al.* 2020), a higher efficiency of ZNPs that have been synthesized by using neem extract was attributable to increased dispersion stability due to interface biocompatibility by the leaf phenolics or terpenoids. Size-dependent processes govern the stability of ZNPs compositions. Furthermore, the ionic strength and surface charge direct ZNPs aggregation, flocculation, and sedimentation (Hou *et al.* 2017). ZNPs are most likely stabilized via changing the steric hindrance (sterically stabilized dispersions) or charge (charge-stabilized dispersions).

CHAPTER # 7

FUTURE RESEARCH

7. Future Research

The ZNPs have an exponential potential to reduce the application of conventional pesticides. ZNPs can be used to control pathogens both in closed greenhouse/screenhouse environments and open fields, and they can be administered to agricultural plants in a variety of ways. Plant diseases diminish average production output by 10–20%, costing the US agriculture industry billions of dollars (Luck *et al.* 2011). Although pathogen control techniques exist for a variety of crops, all of them have substantial limitations. This, combined with the growing demand for expanded agricultural production and the possible problems posed by varying climatic conditions, requires improved pathogens control strategies. Plant micronutrients including copper, iron, nickel, magnesium, and zinc are recognized to play a key role in pathogens resistance by activating enzymes that produce defense barriers (Elmer & White 2018). However, the efficacy of amendment techniques is limited by low soil micronutrient bioavailability and poor intra-plant translocation. Nowadays, there is a great interest in applications of nanotechnology for improvement in the agriculture sector, with a major emphasis on better or more focused fertilizer and pesticide delivery, nano-sensing for improving the performance, and revolutionary treatment strategies based on nano-sized particles to reduce output losses. Early work has shown that ZNPs have high efficacy against phytopathogens due to a variety of antimicrobial mechanisms of action, including photo-oxidation, which produces ROS, cell membrane destabilization, toxicity due to zinc ion release, and disintegration of organelles and cellular macromolecules. To gain a better knowledge of the underlying interplay in a complicated bio-nano system, multidisciplinary work with complementing skills from diverse domains is needed. Thorough knowledge of the ZNPs' structural features, such as shape, size, molecular structure, and active biosorption potential, can serve as a valuable preliminary step for selecting ZNPs for a particular plant pathogen. It is also critical to use a dependable and repeatable method for assessing biocompatibility and effectiveness at the plant cell, pathogen, and ecosystem levels. Research and development on ZNPs are attracting considerable interest, since processes for manufacturing effective products have been intensively studied.

Agriculture is a vital economic force that drives the livelihoods of most countries. Agrichemical products are used to improve physiological quality (fertilizers) and control pest and diseases (pesticides). Before the use of zinc nanomaterial-based agriproducts can be envisioned in the open, environmental toxicity issues need to be addressed. ZNPs are being synthesized for their amenability for simple and low-cost production procedures, and ecotoxicity issues result from the oxidative stress-inducing nature and heavy metal nature of zinc nanomaterials (Ivask *et al.* 2010). Many biogenic metallic nanoparticles have been shown to present improved biological effects, as well as lower (or no) phytotoxicity, compared to metallic nanoparticles synthesized using chemical methods (Itroutwar *et al.* 2020). Primed seeds increased amounts of the superoxide dismutase (SOD) enzyme, which protects plant tissue from ROS damage and counteracts phytotoxic consequences (Acharya *et al.* 2019). Zinc is a crucial part of living organisms, but excessive amounts make it hazardous. Numerous earlier investigations demonstrated Zn^{2+} phytotoxicity (El-

Ghamery *et al.* 2003). According to a study it has been reported that the IC50 of Zn^{2+} ions for several plant species ranged from 43 to 996 Zn mg/L (Paschke *et al.* 2006). Plants restricted in growth are a common indication of Zn^{2+} phytotoxicity, as seen in ryegrass under both ZNPs and Zn^{2+} applications. The lower IC50 and yellow and withered branches at elevated Zn^{2+} levels suggest that Zn^{2+} is more harmful to ryegrass than ZNPs, raising the question of the ZNPs phytotoxicity resulting from their complete dissolution in nutritional solutions. ZnO is frequently categorized as virtually insoluble in water (Han *et al.* 2010), yet significant dissolution of ZnO in water was recorded (from 1 mg/L to several thousand mg/L), which was size- and pH-dependent. Just under 8 mg/L of available Zn was present in the ZNPs-treated nutritional mixtures, which was below the lethal concentration of Zn^{2+} for ryegrass. Therefore, ZNPs toxicities could not be a direct outcome of their disintegration in the bulk nutrient solution. Investigations were also conducted into the disintegration of ZNPs and how that affected the toxicity of ryegrass. The root absorption and phytotoxicity of ZNPs in a hydroponic growth system were compared and verified using Zn^{2+} ions. Light, scanning electron, and transmission electron microscopies were used to see the root uptake and phytotoxicity.

The cyto and ecotoxicity of ZNPs are determined by the surface charges. About 33-82% of soil aggregation was improved and enhanced by green fabricated nanoparticles along with 10-14% increased water retention ability while fertilizer requirements was reduced by 80%, nutrient mobilization was increased by by 30% (Chhipa 2019). Because of these properties, ZNPs can be a viable alternative to synthetic fertilizers as well as innovative agents for agricultural production .The application of NPs for plant disease reduction, on the other hand, has not been well investigated. Materials of nano-sized gain physical and chemical properties not seen in analogous bulk materials. As a result, biogenically synthesized ZNPs may be useful in pathogen management by suppressing disease-causing pathogens or altering host resistance mechanisms, i.e., systemic-acquired resistance. Various research suggests that nanoscale micronutrients applied on leaves or roots have a significant potential to reduce disease and boost crop outputs. Future studies should focus on determining the exact mechanism of these benefits, as well as measures to improve treatment efficacy. Additionally, more studies into the activity of these nanonutrients in various soil types is needed. Nanomaterials can be exploited for growth and yield practices in addition to fertilization since they can promote water diffusion and permeation into the seed. Herbicides could benefit from targeted herbicide-encapsulated nanoparticle on weed plants. However, before developing nanoparticles for use in agriculture, their influence on each level of the ecosystem needs to be investigated, and their toxicity must be tested.

CHAPTER # 8

CONCLUSION

8. Conclusion

Nanotechnology is the fabrication and application of materials with nanoscale dimensions. Nanoparticles have specialized features due to the huge volume fraction of the nanoscale dimension. Due to their wide bandwidth and high exciton binding energy, ZNPs have been the subject of extensive recent research. The nanoformulations of ZNPs are used to suppress various bacterial, fungal, and viral diseases in plants. The ZNPs formulations can manage diseases in the greenhouse and field. Green approaches using plants, fungi, bacteria, and algae have been implemented due to the presence of harmful chemicals employed in the physical and chemical processes for manufacturing ZNPs. The inhibition of phytopathogens by ZNPs is due to various mechanisms including the formation of ROS from the photo-oxidation process, destabilization of various macromolecules as well as the cell membrane and other cell structures, i.e., organelles. The successful biosynthesis of ZNPs was achieved in this study using an extract from *Picea smithiana* needles. The resultant ZNPs were characterized using XRD, SEM, DLS, FTIR and UV – Visible spectroscopy. The average size of ZNPs was 26.42 nm. These ZNPs inhibited growth of *Xanthomonas compestris pv. vesicatoria* and *Ralstonia solanacearum* in broth and agar media and that inhibition increased with increasing concentrations of ZNP. On tomato roots, ZNPs gave 44.74 and 45.63 % reduction in severity and 78.405 and 80.915 % reduction in incidence of disease on tomato roots inoculated with *Xanthomonas compestris pv. vesicatoria* and *Ralstonia solanacearum,* respectively. Our results show that a high concentration of ZNPs is crucial for effective antibacterial activity. The ZNPs produced through biosynthesis were found to be effective against the bacteria causing bacterial leaf spot and bacterial wilt in tomato. The potential of ZNPs to improve plant growth and development through increased uptake of nutrient and water and enhanced photosynthetic activity is promising. Further advancements are required to optimize the usage of ZNPs in agriculture, which includes the creation of nanoparticles tailored to specific targets that can effectively promote plant growth and enhance its physiological parameters. Specifically, attention should be given to utilizing nanoparticles that can enhance photosynthesis, an area that has received minimal research. Biosynthesized nanoparticles can be utilized by controlling their concentration and size to determine their mechanism and process of toxicity in plants. To minimize the impact on the ecosystem in terms of toxicity, transportation, and bio-availability, adjustments can be made to these factors through modulation. The potential influence of nanoparticles in the soil beneath plant roots requires further investigation.

CHAPTER # 9

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9. References

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