Journal of Agriculture Sustainability and Environment

ISSN: 2997-271X

Global Scientific Research

www.jescae.com

Journal of Agriculture Sustainability and Environment

Vol.4, No.1

June, 2025

Chief Editor	Dr. Abdullah Khan
Edited by	Global Scientific Research
Published by	Global Scientific Research
Address:	GLOBAL SCIENTIFIC RESEARCH (LIMITED COMPANY): 8206 Louisiana Blvd Ne, Ste A #2531 Albuquerque, New Mexico 87113, United States
Journal Link:	https://www.jescae.com/index.php/jase
Email	journals.gsr@gmail.com
Website	www.jescae.com

CONTENTS

Journal of Agriculture Sustainability and Environment ISSN: 2997-271X
https://doi.org/10.56556/jase.v4i1.1140

REVIEW ARTICLE

Silver Nanoparticles: Synthesis, Characterization, and Emerging Applications in Agriculture and Biomedicine for Enhancing Crop Production and Human Health

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Received: 18 December 2024, Accepted: 30 May, 2025, Published: 07 June 2025

Abstract
Nanotechnology is an emerging field with vast potential in agriculture and biomedicine due to its unique physicochemical properties. Silver nanoparticles (SNPs), typically ranging from 1–100 nm, have garnered significant interest for their antimicrobial, antioxidant, and therapeutic effects. This review focuses on the synthesis, characterization, and multifunctional applications of SNPs in both the agricultural and biomedical sectors. Various synthesis methods, including biological approaches, are explored, along with the mechanisms through which SNPs exert their effects. In agriculture, SNPs have shown promise in enhancing soil quality, improving plant growth, and controlling crop diseases through nano-fertilizers and nano-pesticides. In the biomedical field, SNPs are used in antibacterial and antibiofilm treatments, wound healing, dentistry, bone and cardiac implants, and cancer therapy. Given the increasing global population and the urgent need for sustainable solutions in food and healthcare systems, SNPs offer a promising avenue for improving crop productivity and human health.

Keywords: Silver Nanoparticles; Biological Synthesis; Antimicrobial agent; Antibiofilm; Health Management Activity

Introduction

Nanotechnology refers to those areas of science and engineering that involve phenomena occurring at the nanometer scale, typically between 1–100 nm. It is employed in the design, synthesis, characterization, and application of materials, structures, devices, and systems. Although nanoscale structures naturally occur in biological systems-such as molecules within the human body or components of food-it has only been in the last few decades that scientists have intentionally and effectively manipulated matters at this scale. This ability to control matter at the nanoscale is what sets nanotechnology apart from other technological fields. While scientific discovery has evolved significantly since the dawn of humanity, the emergence of nanotechnology is relatively recent. Over the past 30 years, nanotechnology has seen remarkable advancements, beginning in the 1980s and gaining prominence in the early 2000s, with wide-ranging commercial applications. Nanoparticles (NPs), defined as particles with dimensions ranging from 1 - 100 nanometers, exhibit unique physical and chemical properties-such as altered color, strength, reactivity, magnetism, and thermodynamics-compared to their bulk counterparts. These characteristics make them valuable for applications in industrial, commercial, agricultural, and biomedical sectors.

In pharmaceuticals, nanomaterials are increasingly explored, although many applications are still under development. In agriculture, the sector faces numerous challenges, including the need to enhance crop productivity and provide nutritionally adequate food for a growing global population. These challenges are exacerbated by climate change, water scarcity, land degradation, and environmental pollution. To address these issues, frontier technologies like nanotechnology offer promising strategies for sustainable development, enhanced resource-use efficiency, and environmental protection. Among nanomaterials, silver (Ag) has long been recognized for its therapeutic and antimicrobial properties. Silver ions and silver-based compounds have been used since ancient times to combat microbial infections (Slawson et al., 1992; Zhao and Stevens, 1998). Advances in nanotechnology have enabled the synthesis of silver nanoparticles (SNPs), further enhancing silver's biomedical potential. Several methods exist for SNP synthesis, including physical, chemical, and biological approaches. While physical and chemical methods are commercially viable, biological synthesis is more environmentally friendly (Hulkoti and Taranath, 2017).

SNPs are particularly valuable in nanomedicine due to their desirable physicochemical characteristics and broad biological functionality. These include potent antimicrobial and anticancer properties, the ability to form diverse nanostructures (Sohn et al., 2015), and cost-effective synthesis (Capek, 2004). Applications of SNPs extend to drug delivery systems, wound dressings, tissue engineering, and antimicrobial. Their large surface area allows for extensive ligand binding, enabling effective surface functionalization. Compared to free silver ions, SNPs exhibit superior antimicrobial performance due to enhanced microbial interaction via increased surface area. Moreover, their efficacy against antibiotic-resistant microbes has made them an important research focus (Hutchison and Greener, 2008). To date, SNPs have demonstrated potential in multiple domains, including food processing, agriculture, agro-industries, biomedical devices, diagnostics, orthopedics, cancer therapy, and infection control. This manuscript highlights the principal methods for SNP synthesis and their diverse roles in antimicrobial, antibiofilm, antitumor, dental, orthopedic, and wound healing applications.

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (SNPs) can be achieved through various physical, chemical, and biological methods, each offering distinct advantages in terms of scalability, cost, particle size control, and environmental impact. Chemical reduction is the most widely used method, where silver salts such as silver nitrate ($AgNO_3$) are reduced to elemental silver (Ag^0) using reducing agents like sodium borohydride or citrate, often in the presence of stabilizers to prevent agglomeration (Sharma et al., 2009). While effective, chemical methods may involve toxic reagents and byproducts, raising concerns about their environmental and biomedical safety. In contrast, green or biological synthesis methods have gained popularity due to their eco-friendliness and biocompatibility. These approaches utilize plant extracts, bacteria, fungi, or algae as reducing and capping agents, leveraging natural biomolecules such as phenolics, flavonoids, and proteins to facilitate the formation of SNPs (Iravani et al., 2011). Green synthesis not only minimizes the use of hazardous chemicals but also offers improved biocompatibility, making it particularly suitable for medical and environmental applications. However, challenges related to scalability, reproducibility, and precise control over particle characteristics remain areas of active research.

Physical Methods

Physical methods for the synthesis and analysis of nanoparticles (NPs), particularly silver nanoparticles (SNPs), involve high-energy techniques such as evaporation-condensation, laser ablation, and thermal decomposition. These methods focus on manipulating key physical parameters like shape, size, and surface morphology to produce nanoparticles with controlled characteristics. Nanoparticles are commonly introduced as aerosols (solid or liquid phases in air), suspensions (solid in liquid), or emulsions (two liquid phases), and their surface and interfacial properties can be tailored using surfactants or polyelectrolytes, which help prevent aggregation by modifying surface charge and stabilizing the outer molecular layer (Liufu et al., 2005). The complex developmental history of a nanoparticle, such as those generated through combustion processes, often results in diverse surface compositions due to the adsorption of various chemical agents during cooling and environmental exposure. These dynamic interactions with surrounding media, especially at the nanoparticle–liquid interface, make it difficult to predict nanoparticle behavior using simple models. The physical and chemical properties of nanoparticles, particularly at scales between the angstrom and micrometer levels, deviate significantly from those of bulk materials due to quantum mechanical effects. The electronic, optical, and reactive characteristics of nanoparticles often differ substantially from those of the same materials in their bulk forms, necessitating sophisticated quantum models to understand size-dependent behaviors and to compare experimental outcomes with theoretical predictions.

In the context of silver nanoparticle production, physical techniques have been extensively studied. Lee and Kang (2004) demonstrated the synthesis of monodispersed SNPs via the thermal decomposition of Ag^{+}-oleate complexes, while Jung et al. (2006) employed a small ceramic heater to produce metal nanoparticles through evaporation-condensation, noting that a stable temperature yielded non-agglomerated, polydisperse SNPs. Laser ablation has proven particularly versatile, offering size control through wavelength adjustment. Tsuji et al. (2002) reported that reducing laser wavelength from nanoseconds to femtoseconds significantly decreased particle size-from 29 nm to 12 nm-and further found that femtosecond laser pulses produced more uniform colloids, though with lower formation efficiency compared to nanosecond pulses (Tsuji et al., 2003). Kim et al. (2006) and Torras and Roig (2020) also showed that laser ablation in a polyol medium could yield spherical SNPs with a range of sizes. In another approach, Seigal et al. (2012) synthesized SNPs through direct physical deposition of silver into glycerol, providing an efficient and less chemically intensive alternative with good resistance to aggregation and narrow size distribution. Despite advantages such as rapid synthesis, the absence of hazardous reagents, and the use of radiation as a reducing agent, physical methods are also associated with drawbacks like high energy consumption, low yields, non-uniform particle distribution, and potential solvent contamination (Elsupikhe et al., 2015). These limitations continue to drive research toward refining physical synthesis strategies to enhance scalability, reproducibility, and environmental compatibility'

Chemical Method

Different strategies are accessible to synthesis of SNPs. Chemical techniques are helpful because the equipment required is more advantageous and less complex than that utilized in biological techniques.

Table 1: Chemical methods for the synthesis of monodispersed and quasi-spherical SNPs

Reducing Agent	Precursor Agent	Capping Agent	Experimental Conditions	Ref.
Trisodium citrate	Silver nitrate	Trisodium citrate	Diameter ≈ 10–80 nm; temperature ≈ boiling point	(Almatroudi, 2020)
Ascorbic acid	Silver nitrate	Daxad 19	Diameter ≈ 15–26 nm; temperature ≈ boiling point	
Alanine/NaOH	Silver nitrate	DBSA (dodecyl benzenesulfonic acid)	Diameter ≈ 8.9 nm; temperature ≈ 90°C; time ≈ 60 min	(Almatroudi, 2020)
Ascorbic acid	Silver nitrate	Glycerol/PVP	Diameter ≈ 20–100 nm; temperature ≈ 90°C	(Liu et al., 2020)
Oleic acid	Silver nitrate	Sodium oleate	Diameter ≈ 5–100 nm; temperature ≈ 100–160°C; time ≈ 15–120 min	(Almatroudi, 2020)
Trisodium citrate	Silver nitrate	Trisodium citrate	Diameter ≈ 30–96 nm; temperature ≈ boiling point; pH ≈ 5.7–11.1	(Almatroudi, 2020)
Trisodium citrate	Silver nitrate	Trisodium citrate/Tannic acid	Diameter ≈ 10–100 nm; temperature ≈ 90°C	(Ranoszek-Soliwoda et al., 2017)

It has proactively been accounted for that silver ions receive electrons from the decreasing agents and become changed over into the metallic structure, which at last totals to shape SNPs. Among the silver salts utilized in chemical synthesis of SNPs, AgNO3 is one of the most generally utilized because of properties like minimal expense (Table 1) (Ge *et al*., 2014, Calderon-Jimenez *et al.,* 2017). In 2002, Sun and Xia reported the synthesis of monodispersed silver nano cubes through reducing nitrate (Sun and Xia 2002). Agnihotri and Mukherji (2013) synthesized SNPs involving AgNO3 as a forerunner, and sodium borohydride and trisodium citrate as stabilizing agents. It has been accounted for that sodium borohydride is a decent decreasing agent for the synthesis of SNPs having a size scope of 5-20 nm. In correlation, trisodium citrate is the best-diminishing agent for the synthesis of SNP**s** of the size range 60-100 nm (Agnihotri and Mukherji 2013). Polyvinylpyrrolidone (PVP) as a size regulator and a capping agent, with ethylene glycol as a solvent and reducing agent, is accounted for to lead to SNPs with a typical size under 10 nm (Agnihotri and Mukherji, 2013). Patil *et al*. (2012) affirmed the synthesis of SNPs utilizing hydrazine hydrate as the reducing agent and polyvinyl liquor as the stabilizing agent. Their outcomes uncovered that the resultant NPs had a spherical morphology, and these particles showed huge applications in biotechnology and biomedical science (Patil *et a*., 2012). As per one more significant study, the synthesis of SNPs was found to be spherical with various sizes (Amirjani *et al*., 2020).

Table 2: Physical and chemical syntheses of SNPs

Type	Reducing agent	Biological activity	Characterization	Ref.
PDLC and PMA capped SNPs	Methacrylic acid polymers	Antimicrobial	UV-Vis, reflectance spectrophotometry	(Dubas *et al*., 2006)
SNPs	Ascorbic acid	Antibacterial	UV-Vis, EFTEM	(Pal *et al*., 2007)
Chitosan-loaded SNPs	Polysaccharide chitosan	Antibacterial	TEM, FTIR, XRD, DSC, TGA	(Ali *et al*., 2011)
SNPs	Hydrazine, d-glucose	Antibacterial	UV-Vis, TEM	(Shrivastava *et al*., 2007)
PVP-coated SNPs	Sodium borohydride	—	UV-Vis, TEM, EDS, DLS, FIFFF	(Tejamaya *et al*., 2012)

Polydiallyldimethylammonium chloride-PDLC, Polymethacrylic acid-PMA, Silver nanoparticles-SNPs, UV-Vis – ultraviolet-visible spectroscopy, FIFFF – flow field-flow fractionation, DSC – differential scanning calorimetry, TEM – transmission electron microscopy, EDS – energy-dispersive spectroscopy, EFTEM – energy filtered TEM, FTI R – Fourier transform infrared, DLS – dynamic light scattering, XRD – X-ray diffraction, TGA – thermogravimetric analysis.

Biological Methods

The physical and chemical methods of producing SNPs are costly, time-consuming, and environmentally unfriendly. Therefore, it is critical to create a technique that is both economically and environmentally sustainable, eliminates the use of hazardous chemicals, and does not entail physical or chemical methods of manufacturing (Iravani, 2014). Biological approaches bridge these gaps and provide a wide range of uses in the management of health by controlling different biological processes. Utilizing supplies from plants as well as bacteria, yeasts, and fungus are examples of biological manufacturing techniques. These sources greatly increase the popularity of this method for using NPs in medicinal applications. According to reports, the use of microorganisms and plants in the manufacture of NPs is safe, cost-effective, and environmentally less hazardous than chemical synthesis (Makarov *et al*. (2014), Gowramma *et al*. (2015). Furthermore, inorganic metallic ions from the environment can be absorbed and accumulated by plants and microbes (Shah *et al*., 2015). Microorganisms and plant sources are the primary sources used in the biological production of SNPs (Figure 1) (Ahmad *et al*., 2019).

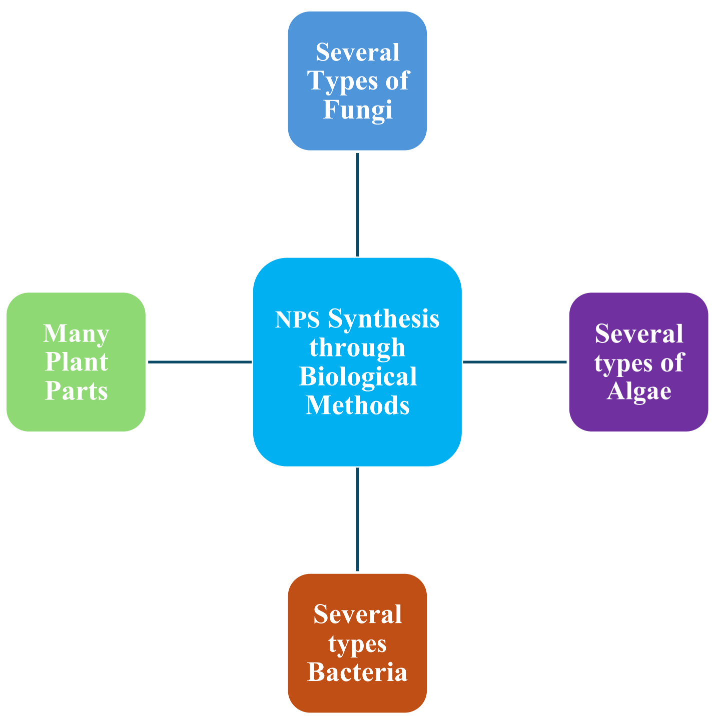

Figure 1: Different Biological Techniques for the synthesis of SNPs.

Controlled chemical reactions are the primary means of producing defined bottom-up nanoparticles in the liquid phase regarding particle size, chemical composition, surface, and charge characteristics (Frens, 1973). By regulating growth conditions, self-limiting self-assembly methods have also developed. In view of the ecological cycle of nanomaterials, attention needs to be paid in part to the existing state of knowledge about bulk material corrosion and disintegration (Oberdorster *et al*., 2005). Erosion and chemical breakdown of parent materials that are geological (such as clays) or organic (such as plant or microbe detritus) are naturally occurring processes that result in nanosized structures in the liquid phase. The surface characteristics of each of these disintegration processes, as well as how they alter chemically, are crucial in predicting whether individual NPs could develop in the appropriate media (Boyle *et al*., 2005).

The principal course of bottom development of NPs in the gas stage is by a chemical reaction prompting a non-volatile product, which undergoes through homogeneous nucleation followed by conduction and development. As of late, this has turned into a significant pathway for the industrial production of NP powders, which might be of metals, oxides, semiconductors, polymers and different types of carbon, and which might be as spheres, wires, needles, tubes, platelets or different shapes. This is additionally the accidental pathway by which NPs are formed following the oxidation of gas-progressively precursors in the atmosphere, in volcanic plumes, in normal and man-made combustion processes, or in exhaust related with any man-made process including volatilizable material at elevated temperature, like welding or smelting, polymer fabrication, or in any event, cooking. Similarly, as with the fluid stage case, disintegration processes of parent materials give a pathway which just prompts NPs to be suspended in the gas stage under extraordinary circumstances. While in the fluid stage the presence of emulsifying agents accompanying a disintegration or chemical process could uphold the suspension interaction, the scattering of NPs into a gas from fluid emulsions or dry powders is seriously restricted by major areas of strength for the powers between individual NPs. In this manner, any precisely prompted weight on the parent material generally prompts particles in the micrometer reach or more. Just under unintentional circumstances, for example on account of uncontrolled arrival of a powder or an emulsion from an exceptionally compressed vessel could solid shear powers conquer these adhesive forces (Reeks, 2001). Conversely, the spraying of liquids containing NPs or dissolvable material at exceptionally low concentration, trailed by drying of the solvent, can prompt the resuspension of NPs or the formation of new NPs from the solutes. This can prompt the rearrangement of NPs, biological material or harmful substances into nanoparticulate airborne structures.

As of late, a study was performed to create SNPs through the decrease of aqueous Ag^+ ions utilizing the culture supernatants of different bacteria. This approach was exhibited to be quick and the association of SNPs with the cell filtrate produced SNPs within 5 min. Besides, this concentrate additionally announced that piperitone somewhat hindered the decrease of Ag^+ to metallic SNPs (Shahverdi *et al*., 2007). It is vital to take note that the nitro decrease action of Enterobacteriaceae is hindered by the natural product piperitone. It is expected that the bio decrease of silver ions to SNPs may be to some degree repressed by various types of Enterobacteriaceae, for example, *Klebsiella pneumoniae*. Korbekandi *et al*. (2012) concentrated on the enhancement of SNP production by *Lactobacillus casei subspecies casei*, affirming the bio-reductive synthesis of SNPs (Korbekandi *et al*., 2012). Liu *et al*. (2000) showed the formation of NPs from dried cells of *Bacillus megaterium.* Das *et al*. (2014) have portrayed the extracellular synthesis of SNPs through a bacterial strain. The study showed that the treatment of *Bacillus* strain CS 11 with AgNO3 brought about the development of SNPs extracellularly.

Different sorts of fungi have been accounted for to be associated with the production of SNPs (Guilger-Casagrande and de Lima, 2019). The production of SNP by fungi has been viewed as extremely fast. Numerous scientists have concentrated on the biosynthesis of SNPs by fungi exhaustively (Iravani *et al*., 2014). One study has shown the extracellular biosynthesis of spherical SNPs by cooperation of *Fusarium solani* with silver nitrate (Ingle *et al*., 2008). Syed and associates have detailed the biosynthesis of SNP by the *Humicola sp*. It was shown that a precursor solution was diminished by the interaction between *Humicola sp*. Also, Ag+ ions and extracellular NPs were produced (Syed *et al*., 2013). Owaid *et al.* (2015) have detailed the development of SNPs by the bio-reduction of silver nitrate initiated by the concentrate of *Pleurotus cornucopiae*. Xue *et al*. (2016) led an examination to biosynthesize SNPs with antifungal properties utilizing *Arthroderma fulvum*. Vigneshwaran *et al*. (2007) detailed that the connection of silver nitrate arrangement with the fungus Aspergillus flavus brought about the aggregation of SNPs on the outer layer of its cell wall. Besides, Bhainsa and D'Souza (2006) explored the extracellular biosynthesis of SNPs utilizing *Aspergillus fumigatus*. The outcomes demonstrated that the cooperation of silver ions with the cell filtrate produced SNPs in an extremely brief time frame. In any case, utilizing *Fusarium oxysporum* brings about an extracellular production of SNPs with a size of 5-50 nm (Ahmad *et al*., 2003). Also, the incubation of *Phanerochaete chrysosporium* mycelium with silver nitrate solution delivered SNPs (Vigneshwaran *et al*., 2006). Korbekandi *et al*. (2013) and colleagues showed the bio-reductive production of SNPs by utilizing *Fusarium oxysporum.*

Algal method is a plausible substitute for physical and compound techniques for NPs production since it is financial and eco-accommodating (Hamouda *et al*., 2019). Besides, algal growth has a high limit concerning metal take-up. It has been seen that those biological sources, for example, marine green growth can catalyze explicit responses. This limit is vital to current and practical biosynthetic plans (Govindaraju *et al*., 2009). A study considering the green growth remove has shown that the difference in variety from yellow to brown can demonstrate the decrease of silver particles to SNPs. Furthermore, Rajeshkumar *et al*. (2014) observed the profound earthy colored shade of SNPs at 32 h and it was detected that the time of incubation was straightforwardly connected with the expansion in variety power. SNPs were incorporated through the decrease of watery arrangements of silver nitrate with powder and dissolvable concentrates of *Padina pavonia.* Furthermore, the accomplished NPs showed high stability, quick development and small size (AbdelRahim *et al*., 2017). Salari *et al*. (2016) detailed the production of SNPs through the bio-reduction of silver particles (SPs) instigated by *Spirogyra varians*. It has been observed that yeasts can produce SNP. Furthermore, yeast-based techniques for producing SNPs are economical and environmentally friendly. Niknejad *et al*. (2015) conducted a study utilizing *Saccharomyces cerevisiae* in this context. It was observed that after adding Ag+ ions to the yeast culture, the colourless sample gradually became reddish-brown with longer incubation times. In addition, the solution's color becomes a deep reddish-brown. Kowshik *et al*. (2002) observed that soluble silver interacting with a yeast that is tolerant to silver during its log phase of development results in the extracellular creation of NPs. Similar other biological techniques, manufacturing in plants is superior to chemical and physical techniques because it does not require high temperatures, energy, or hazardous chemicals, and it is also more economical and environmentally beneficial (Dhuper *et al*., 2012). Aloe vera leaves contain a multitude of active ingredients. These components, which have been demonstrated to play a definite part in the reduction of silver ions, include lignin, hemicellulose, and pectin's (Emaga *et al*., 2008). In a recent work, an aqueous solution of Saudi Arabian plant extract (*Origanum vulgare L*.) was used to produce SNPs. The outcome showed that Ag+ ion reduction was the process used to produce SNPs. The reaction mixture's hue changed from light brown to dark brown during this procedure. Conversely, under the identical circumstances, no color change was noted in the absence of plant extract (Shaik *et al*., 2018). According to the findings of another investigation, adding various amounts of aqueous leaf extracts of *Azadirachta indica* caused the color of the aqueous silver nitrate solution to shift from faint light to yellowish brown (Ahmed *et al*., 2016). Using plant extract from Tamarix gallica, Lopez-Miranda *et al*. (2016) quickly produced SNPs by biosynthesis. An extract from the *Bauhinia purpurea* flower has been used by Chinnappan *et al*. (2018) to synthesize SNPs quickly and easily. The use of aqueous pomegranate juice extract as a reducing agent was reported by Ibraheim *et al*. (2016) for the synthesis of SNPs from silver nitrate. Their findings demonstrated that the use of juice extract results in the rapid synthesis of SNPs from AgNO3 solution. It was found that the variety changed from light yellow to ruddy brown with the development of SNPs after openness to microwaves for a couple of moments. Lakshmanan *et al*. (2018) used the extract of the *Cleome viscosa* plant to make SNPs. The study found that the plant's extract can effectively convert silver nitrate into metallic silver. Prasad *et al*. (2011) utilized aqueous leaf extracts of *Moringa oleifera* to develop a straightforward and fast technique for bio-reduction of SNPs. According to their findings, *Moringa oleifera* had strong potential to rapidly reduce silver ions to produce SNPs, which could lead to the synthesis of SNPs. In this regard, another finding indicated a quick and easy method for the synthesis of SNPs using Ficus benghalensis leaf extract, and the reduction of silver ions into SNPs occurred without the use of hard conditions within a short period of time (five minutes) of reaction time (Saxena *et al*., 2012). Besides, the treatment of aqueous solution of AgNO3 and chloroauric acid with neem leaf prompts the quick blend of stable silver and gold NPs at high fixations (Shankar *et al*., 2003). Prior specialists are responsible for spearheading NPs combination through utilizing plant separates (Shankar *et al*., 2003; Rai *et al*., 2006; Rai *et al*., 2007; Chandran *et al*., 2006). Ponarulselvam et al. (Panarulselvam *et al*., 2012) concluded that SNPs with antiplasmodial activity against *Plasmodium falciparum* could be made from extracts of the leaves of *Catharanthus roseus*. A few examinations have detailed that Ag particles are diminished extracellularly within the sight of organisms to create stable SNPs in water (Duran *et al*., 2007). Zarghar *et al*. (2011) have shown the arrangement of spherical SNPs by utilizing methanolic leaf extracts of *Vitex negundo* and exhibited the antibacterial movement of these SNPs against both Gram-positive and Gram-negative bacteria.

Characterization of Silver Nanoparticles

Characterization is a vital step in the green synthesis and overall production process of nanoparticles (NPs), including silver nanoparticles (SNPs). This phase provides critical information about the physical, chemical, and structural properties of the synthesized nanoparticles. Proper characterization is essential to understand and control nanoparticle behaviour, optimize synthesis conditions, and tailor them for specific applications such as antimicrobial activity, drug delivery, sensing, and catalysis. In the context of green synthesis, where biological methods are used, characterization becomes especially important. This is because the properties of the nanoparticles-such as morphology (shape and size), surface chemistry, surface area, particle distribution, and stability-can be significantly influenced by the biological agents and the conditions under which the synthesis occurs. Several advanced techniques are employed to analyse and characterize SNPs.

UV-Visible Spectroscopy (UV-Vis)

UV-Visible Spectroscopy (UV-Vis) is commonly used to confirm the formation of silver nanoparticles (SNPs) by detecting the surface plasmon resonance (SPR) peak, which typically appears around 400–450 nm. Fourier Transform Infrared Spectroscopy (FTIR) helps identify the functional groups present on the surface of SNPs, offering insights into the biomolecules responsible for their reduction and stabilization. X-Ray Diffraction (XRD) is employed to determine the crystalline structure and phase purity of the nanoparticles. It confirms the formation of elemental silver (Ag^0) and can be used to estimate the average particle size through the Debye-Scherrer equation. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) provide detailed images that reveal nanoparticle morphology, shape, and size distribution. TEM, in particular, delivers high-resolution images at the nanoscale. Dynamic Light Scattering (DLS) is used to measure the hydrodynamic diameter and size distribution of SNPs in solution, which is critical for assessing their colloidal stability. These characterization techniques, whether used individually or in combination, offer a comprehensive understanding of the physical and chemical properties of SNPs. This information is vital for ensuring consistency in synthesis, optimizing functionality, and meeting safety standards, particularly in biomedical and environmental applications (Figure 2). This strategy is generally used to describe metallic nanoparticles (MNPs) by checking their strength and synthesis (Saad et al., 2015; Sadeghi et al., 2015). A distinctive peak with strong visible absorptions is produced when a particular salt is used to synthesize MNPs (Li and Chin, 2012). According to several studies (Vanden Bout, 2012), the most effective absorption band for the characterization of particles between 200 and 100 nm in size is the 200-800 nm wavelength. In SNPs, the valence and conduction bands are very close to one another. A surface plasmon resonance absorption band is created because of the free movement of electrons in these bands. The chemical environment, dielectric medium, and particle size all play a role in the absorption of the SNP. The surface plasmon peak has been extensively studied for several MNPs ranging in size from 2 to 100 nm. Strength of SNPs produced through biological techniques was inspected for around a year and a surface plasmon reverberation top at a similar frequency was found utilizing UV-Vis spectrophotometry (Zhang *et al*., 2016). The application of EDX to nanotechnology has been documented. EDX is an important method for analyzing a sample's elemental composition. Any nanoparticle's elemental composition can be determined by looking at the peaks in the X-ray spectrum that are produced by each element's distinct atomic structures (Noruzi, 2015).

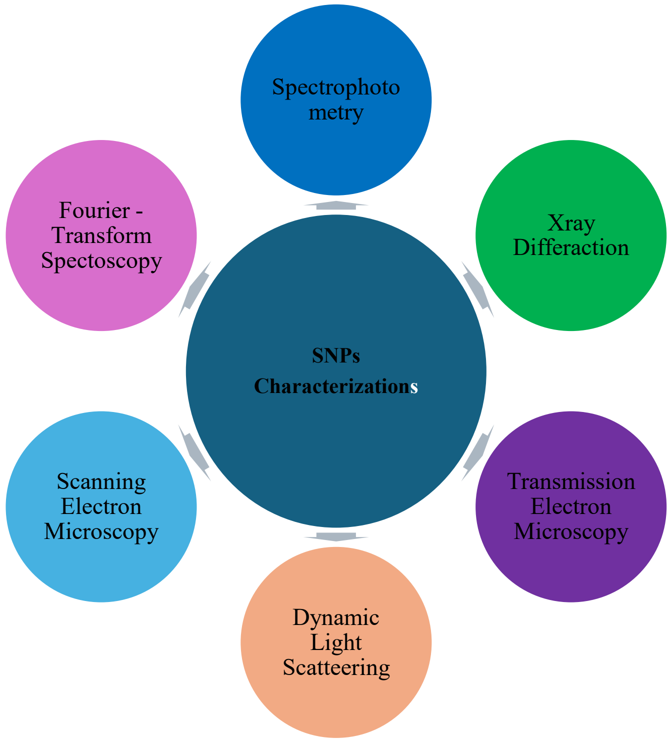

Figure 2: Numerous Techniques Apply for Characterization of SNPs

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

TEM is an extremely helpful technique for the characterization of NPs, which gives information on the size and morphology of NPs (Rajeshkumar *et al*., 2017; Ghosh *et al*., 2012). TEM has a 1,000-fold higher resolution contrasted and SEM and its pictures give more exact information connected with size, shape and crystallography of the NPs (Vijayaraghavan *et al*., 2017). SEM can be used to observe the topography and morphology of NPs and to calculate their sizes at the micro- (10-6) and nano-(10-9) scales (Noruzi et al., 2011; Sundararajan and Gowri, 2011). A high-energy electron beam, produced by SEM, is aimed at the outer layer of the sample NPs and the backscattered electrons produced give the characteristic features of the sample (Hudlikar *et al*., 2013). Electron microscopy examination is utilized to analyze the progressions in the morphology of the cell when nanoparticle treatment. A few examinations have revealed that the noticeable changes in cell shape and holes of NPs in the cell wall have been utilized as signs of the antimicrobial activity of nanoparticles (Rahimi-Nasrabadi *et al*., 1969; Zhang *et al*., 2014). Utilizing SEM, control bacterial cells showed smooth and unharmed structures, while cells treated with SNPs for 60 min were fundamentally harmed, with clear morphological changes to the cell membrane prompting loss of membrane integrity (Roy *et al*., 2019).

X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a common analytical method for observing the structure of crystalline metallic nanoparticles through the penetration of X-rays into the material (Rajeshkumar and Bharath 2017; Vijayaraghavan and Ashok Kumar 2017). The subsequent diffraction pattern affirms the development of NPs with crystalline structure (Alexander and Klug, 1950). To compute the molecule size from the XRD information, the Debye-Scherrer condition is applied by deciding the width of the Bragg reflection regulation as indicated by the situation: $d = K\lambda/\beta \cos \theta$, where d is the molecule size (nm), K is the Scherrer consistent, λ is the frequency of X-beam, β is the full-width half greatest and θ is the diffraction point (a big part of Bragg point) that compares to the lattice plane (Noruzi, 2015). Subsequently, the underlying elements of different materials, for example, biomolecules, polymers, glasses and superconductors, can be analyzed by XRD (Zhang *et al*., 2016).

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR can be used to investigate the surface chemistry of synthesized metal nanoparticles and to notice the contribution of biomolecules in nanoparticle synthesis (Rajeshkumar and Bharath, 2017) and can be utilized for dissecting different covering specialists. In FTIR, infrared rays are passed through the sample, some are consumed by the sample and the excess passes through it. The subsequent spectra demonstrate the ingestion and transmission that are normal for the sample material (Rohman and Man, 2010). The function of biological molecules in the reduction of silver nitrate to silver can be determined using the cost-effective, appropriate, simple, and non-invasive FTIR method (Zhang *et al*., 2016).

Dynamic Light Scattering (DLS), Zeta Potential and Low-Energy Ion Scattering (LEIS)

DLS is a well-recognized procedure for measuring the size and size distribution of molecules. It has been utilized to measure the size of NPs, and it is commonly utilized to characterize NPs. In addition, DLS has been broadly utilized for sizing magnetic NPs in the liquid stage (Phenrat *et al*., 2009; Goon *et al*., 2009) and its role in characterization of different sorts of NPs has been reported. The size of NP decided by DLS is generally larger than TEM because of the Brownian movement. AES is a surface-sensitive explanatory technique that determines from the interaction of an electron beam and atoms in residence at the surface of a sample (Niehus *et al*., 1993) and is an exceptional expository strategy for nanotechnology (Brongersma *et al*., 2007). The oxidation state of Ag as a component of a hybrid substance can be probed by AES (Al-Hada *et al*., 2020). Assesses the surface charge of nanoparticles, indicating their stability in suspension. A higher zeta potential (positive or negative) suggests better stability due to electrostatic repulsion between particles. LEIS is a commonly utilized surface analytical method, which is well recognized for its incomparable surface sensitivity. With the help of this procedure, the structure and the elemental composition of a given sample can be found (Goebl *et al*., 2015; Rafati *et al*., 2013; Ecke *et al*., 2007). In addition, high affectability LEIS is a profitable surface analytical technique for the characterization of SAM-functionalized nanomaterials (Mogk,1990).

The shape, size and morphology of NPs depend on physical and chemical factors that affect the synthesis of SNPs. In general, the basic parameters affecting the formation of SNPs are methods of production, temperature, pH, timeline, shape and size. There are numerous techniques to produce NPs, counting physical and chemical methods and biological protocols. Different organic or inorganic chemicals as well as living organisms are utilized for the synthesis of NPs in these techniques (Patra and Baek, 2014). It has as of now been discussed that green synthesis is preferable to other strategies since it is eco-friendly and cost-effective. The production of NPs has been discovered to be significantly influenced by temperature. The synthesis of spherical nanoparticles occurs at high temperatures. On the other hand, nanotriangle production generally takes place at lower temperatures (Rahimi-Nasrabadi *et al*., 1969). Research has demonstrated that raising the temperature to a range between 30°C and 90°C increases the frequency of synthesis (Hudlikar *et al*., 2012; Dankovich and Gray, 2011) and may also promote the creation of smaller silver nanoparticles (Mohammed Fayaz *et al*., 2009). According to several research, room temperature, preferably 25-37°C, is ideal for the biogenic production of metal nanoparticles. According to most of the research, basic media increase NP stability more than acidic ones (Roopan et al., 2013; Routray et al., 2016). Nevertheless, it was shown that extremely high pH (pH >11) has several disadvantages, including the formation of agglomerated and unstable SNPs (Tagad *et al*., 2013). Consequently, it may be said that the pH affects the size and form of NPs. Another aspect influencing the reduction of ions to a bulk metal with different forms is decreasing the reaction time (minutes to hours). High absorbance peaks suggest the medium has larger quantities of NPs within the optimal period. It was ascertained by using diverse growth circumstances and the production of NPs in a range of sizes, including spherical, triangular, hexagonal, and rectangular (Rai *et al*., 2007). The characteristics of NPs are largely dependent on their size and shape. It has been found that the shape and size of the NP dictate its optimum activities and that most of its attributes depend on its size.

Mechanisms of Action of AgNPs

SNPs have appeared to have antifungal activity towards distinctive fungi (Bankar et al., 2010; Raut *et al*., 2014), but the mechanism behind it has not been completely caught on. SNPs tend to disturb the structure of the cell membrane. This harming impact on the membrane and restraint of the budding process has been recommended as the component for the antifungal activity of SNPs against *Candida albicans* species (Kim *et al*., 2009). In a study of antibacterial and antifungal activity, nano-Ag sepiolite fibres containing monodispersed SNPs were utilized as the source of silver. Low-melting soda lime glass powder containing NPs had great antibacterial and antifungal activity (Esteban-Tejeda *et al*., 2009). A study illustrated that fluconazole in combination with SNPs appeared the most noteworthy restraint against *Candida albicans*. In this study, *Alternaria alternata* fungus was utilized for the extracellular biosynthesis of SNPs (Gajbhiye *et al*., 2009). *Trichoderma harzianum* cell filtrate was connected in the generation of SNPs which brought about in their generation inside 3 h and TEM investigation illustrated ellipsoid and spherical nanoparticles having a size range of 19-63 nm and an average size of 34.77 nm (Abdelghany *et al*., 2018).

SNPs play a critical role as antibacterial agents. Silver nano formulations have moreover been found to have a great capability for repressing the growth and development of microorganisms such as bacteria (Figure 4) (Du et al., 2019). SNP-based devices are commonly utilized in dental and cardiovascular implants since they do not cause diseases. It has been reported that SNPs have an effective antibacterial action against both Gram-negative and Gram-positive bacteria (Faghri Zonooz and Salouti 2011; Mukunthan *et al*., 2011). A few considerations have detailed that Gram-negative bacteria are more sensitive than Gram-positive bacteria to SNPs (Mukunthan *et al*., 2011), Dehnavi et al., 2013). Moreover, it was reported that the antibacterial activity of different sorts of antibiotics was expanded in the presence of SNPs (Allahverdiyev et al., 2011). The antimicrobial exercises of SNPs against diverse pathogenic organisms were explored by Nanda and Saravanan (Nanda and Saravanan, 2009). The greatest antimicrobial action was examined against methicillin-resistant Staphylococcus aureus (Nanda and Saravanan, 2009). Qasim *et al.* (2018) were examined the antimicrobial exercises of SNPs encapsulated in poly-N-isopropylacrylamide-based polymeric nanoparticles. The study uncovered that the bacteriostatic exercises of polymeric nanoparticles were decided by the estimate of nanoparticles as well as the amount of AgNO3. SNPs have been found to have larvicidal activities against the dengue vectors *Aedes aegypti* (Suresh *et al*., 2014) and *Culex quinquefasciatus* (Tarannum *et al*., 2019). Allahverdiyev *et al*. (2011) conducted a study to assess the impact of SNPs on the biological parameters of *Leishmania tropica*. Saad *et al* (2015) synthesized silver and copper NPs and tried their antiparasitic activity, finding that SNPs essentially decreased the oocyst reasonability of *Cryptosporidium parvum*. NPs provide an alternative to drugs for treating and controlling the growth and development of viral pathogens. Biosynthesis of SNPs may result in strong antiviral agents to restrict virus functions. Suriyakalaa *et al*. (2013) considered bio-SNPs to persuade anti-HIV activities at an early stage of the reverse translation mechanism. Biosynthesized metallic nanoparticles have numerous binding destinations for gp120 of the viral membrane to control the function of the virus. SNPs have been illustrated to exert antiviral action against HIV-1 at non-cytotoxic concentrations. These SNPs were assessed to illustrate their mode of antiviral activity against HIV-1 utilizing a board of distinctive in vitro assays (Lara *et al*., 2010). Uncovering the Tacaribe virus to SNPs before infection facilitated virus uptake into the host cell, though it was taken note that the silver-treated virus appeared a critical decrease in viral RNA production and this finding illustrated that SNPs could restrain arenavirus infection *in vitro* (Speshock *et al*., 2010).

It is known that biofouling is one of the major challenges confronted by the water industry and public health. SNPs of *Rhizopus oryzae* fungal species have been tested on contaminated water. SNPs determined by utilizing *Lactobacillus fermentum* cells were found to control biofilm formation and were affirmed to have antifouling properties (Zhang *et al*., 2014). In addition, SNPs are too connected to several sorts of natural concerns such as air disinfection, water cleansing and surface sanitization. A later consideration illustrated that productive management of biofouling can be accomplished by a direct deposition of SNP coatings on environmentally friendly surfaces (Ren *et al*., 2014). Around the world, the food industry and community are subject to microbial biofilm challenges. Johani *et al.* (2018) conducted a ponder to assess purified endoscope channels for residual bacterial contamination and biofilm nearness. Due to the fast emergence of antimicrobial resistance and the constrained impact of anti-microbials on bacterial biofilm, alternative methodologies such as green silver nanotechnology are gaining consideration due to the unique size, shape and structure of NPs produced by this strategy. Recently people have begun utilizing SNPs for hindering biofilm formation, but the correct component of the inhibitory activity of SNPs is not clearly understood. Chen et al. (2013) categorized antibiofilm methodologies into two groups: (i) treatment that inhibit biofilm formation particularly and (ii) Prevention and utilization of altered biomaterials in biomedical devices to make them safe to biofilm formation. In consideration of the antibiofilm activity of SNPs against multidrug-resistant Gram-negative bacterial isolates, they viably limited biofilm formation (Ramachandran and Sangeetha, 2017). Palanisamy *et al*. (2014) conducted a study to check the impact of SNPs on the formation of biofilm. Another recent study was made to assess the contacts of SNPs with Pseudomonas putida biofilms (Fabrega *et al*., 2009). Kalishwaralal *et al*. (2010) explored the antibiofilm activity of SNPs against biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus* epidermidis and observed these living organisms appeared inhibition of biofilm formation. The information supported the previous studies that the biofilm formation was irritated by SNPs. Besides, antibacterial activity was achieved to be progressed, compared to a human cationic antimicrobial peptide (Mohanty *et al*., 2012).

Applications in Agriculture

Nano-silver is the most read-up and used NP for bio-system. Having solid inhibitory and bactericidal impacts as well as an expansive range of antimicrobial activities has been known. SNPs, which have a high surface area and a high part of surface atoms, have a high antimicrobial impact when contrasted with mass silver. Also, SNPs have notable acknowledgement for their cancer prevention agent, antibacterial, antifungal, against viral and calming properties. It is profoundly referenced that the utilization of SNPs in agribusiness is generally hypothetical yet soon scientists will get assorted uses of SNPs.

Antimicrobial and Antifungal Agent

SNPs have numerous properties making them helpful materials for different industries, for example, agribusiness, agriculture, antibacterial and optical properties, accessibility and low production, handling and stockpiling costs. Besides, SNPs with a measurement of around 100 nm are vital for huge scope industries because of their little molecule size, high surface area, quantum restriction and spread without agglomeration (Verma and Maheshwari, 2019). Consequently, SNPs are utilized as options in the production of generally utilized goods and industries. These days, SNPs are being investigated in different industries like pesticide, medication, biotechnology, material science and energy sectors, and especially restorative goods such as injury dressings, drug conveyance, biosensors and clinical diagnostics, orthopaedics, health, food and textile industries and water sanitization systems. Besides, SNPs are frequently utilized for commercial products, for example, cosmetics and food products processing as a fundamental added substance.

Table 3: Utilization of SNPs in different Activity in Industries (Verma and Maheshwari, 2019)

S. No.	Industry/ Applications	Application of SNPs
1	Pharmacological uses	Larvicidal, Antimicrobial, Wound healing, UV-ray Blocking
2	Textile Industry	Medicinal devices and textiles
3	Water treatment	Potable water, Ground water disinfection, Wastewater disinfection
4	Food Industry	Food processing, Food packaging
5	Agriculture and Biomedical Industry	Antibacterial, Antifungal, Antiviral, Anti-inflammatory, Anticancer

In the farming area, the use of NPs adds to tending to the food security challenges raised by environmental change. In the field of medication (Mishra *et al*., 2017), SNPs have acquainted another aspect with wound dressing and counterfeit inserts and the anticipation of post-operation microbial defilement. SNPs are pertinent as antibacterial agents in the material, wellbeing, farming and food industries. As an antibacterial agent, SNPs have different applications such as in water treatment, application in home and sanitizing clinical equipment. Besides, SNPs are utilized in various textile products (Table 3).

SNPs have been utilized as a potential candidate to increase crop yield by upgrading seed germination and plant growth and development. Figure 1 illustrates the different applications of nanotechnology to the agriculture field. The concentration of SNPs influences the development reaction of plants with positive or negative impacts. The impact of SNPs with diameters of 20 nm on seeds of Fenugreek (*Trigonella foenum-graecum*) has been carried out (Sathishkumar *et al*.,2012). Distinctive concentrations of SNPs (0, 10, 20, 30 and 40μg mL^{-1}) were utilized and showed that greatest seed germination (76.11%), speed of germination (4.102), root length (76.94 mm), root new weight (2.783) and root dry weight (1.204) at a concentration of 10 μg mL^{-1}. The introduction of plants to concentrations of SNPs might promote plant growth and development as compared to non-exposed plants, while higher and lower concentrations could have an inhibitory impact on plant growth and development (Roopan *et al*., 2013; Sharma *et al*., 2012). The growth and development response of SNPs utilizing diverse concentrations (0, 25, 50, 100, 200 and 400 ppm) in Brassica juncea and it is methodically concluded that 50-ppm treatment has been decided to be ideal with a positive impact on fresh weight, root and shoot length (Joshi et al., 2019). Moreover, the concentration of SNPs is capable of the observed impacts for both cowpea and Brassica. In cowpea, 50 ppm concentration brought about in growth and development promotion and expanded root nodulation by implication (Pallavi, 2016). It has about uncovered that the application of SNPs may be utilized to essentially upgrade seed germination potential, mean germination time, seed germination index, seed vigour index, seedling fresh weight and dry weight.

Nano Fertilizers and Plant Growth Promoters

Nano fertilizers and plant growth promoters play a significant role in enhancing agricultural productivity by positively influencing various stages of plant development. These nanomaterials can improve seed germination and seedling vigor by facilitating faster water absorption and activating essential enzymes involved in early plant growth. Additionally, nano fertilizers enhance nutrient uptake efficiency due to their high surface area and targeted delivery, ensuring that essential macro- and micronutrients are readily available to plants. This improved nutrient availability supports better root development and overall plant health. Moreover, certain nanoparticles have been shown to boost photosynthetic activity by increasing chlorophyll content and enhancing light absorption, thereby improving energy conversion efficiency in plants. Collectively, these effects contribute to more robust plant growth, higher yields, and better stress tolerance, making nano fertilizers and growth promoters valuable tools in sustainable agriculture.

Pest Control and Plant Disease Management

Utilization of SNPs and Nanotechnology in Agriculture in various ways is mentioned in Figure 3. Plant disease management in food crops and fruit plants is financially vital and a required investigation area. The well-scattered and stabilized, colloidal AgNPs are more adhesive on bacterial and fungal cell surfaces and subsequently act as way better bactericides and fungicides (Ocsoy *et al*., 2013). Scientists formulated momentous accomplishments in Plant disease management with the utilization of SNPs. DNA-directed SNPs developed on graphene oxide and examined the antibacterial activity against *Xanthomonas perforans*, a causative agent of bacterial spot in Tomatoes (Polash *et al*., 2017). The antimicrobial activity of plant extrication is due to the nearness of secondary metabolites such as tannin, saponin, and glycosides (Kim *et al*., 2012). The in vitro antifungal action of SNPs against nineteen distinctive plant pathogenic parasites is explored (Mout *et al*., 2017). Cationic arginine gold nanoparticles (ArgNPs) gathered Cas9En (Etag)-RNP (ribonucleoproteins) conveyance of sgRNA gives approximately 30% viable cytoplasmic/nuclear gene editing in cultured cell lines, which would enormously encourage future investigate into crop improvement (Elamawi *et al*., 2018). The antifungal action of SNPs synthesized by *Trichoderma longibrachiatum*, against nine fun isolates such as *Aspergillus alternate* and others (Nadaf and Kanase, 2015). Bacterial diseases are one more cause of noteworthy misfortune in crop yield around the world. SNPs are demonstrated to be dynamic against plant pathogenic microbes.

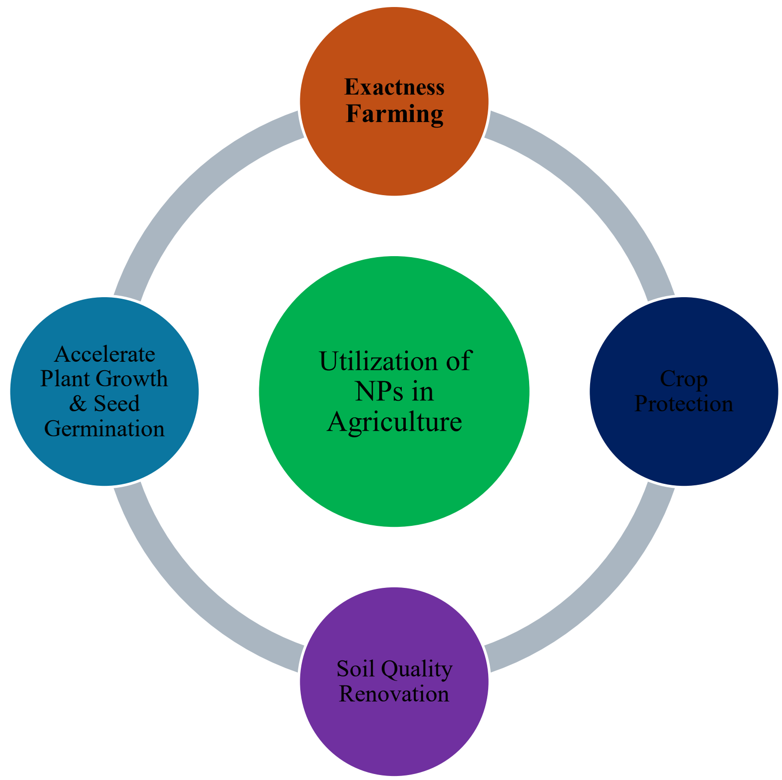

Figure 3: Utilization of SNPs and Nanotechnology in Agriculture

It has been uncovered that SNPs have higher antibacterial activity against *Erwinia cartovora, E. than* generic antibiotics Nadaf NY (Alloway, 2008). They moreover examined the antifungal activity of AgNPs against pathogenic fungi viz. *Fusarium oxysporum, Alternaria alternata* and *Aspergillus flavus* and obtained promising results. Anti-fungal adequacy of colloidal nano silver (1.5 nm normal diameter) against rose powdery mildew caused by *Sphaerotheca pannosa var rosae*. It is an exceptionally broad and common disease of both nursery and outdoor grown rosae. It causes leaf distortion, leaf twisting, early defoliation and decreased blooming. Twofold capsulized nano silver was prepared by a chemical reaction of silver ions with the help of physical methods, reducing agents and stabilizers. It dispenses with undesirable microorganisms in grower soils and hydroponics systems. In addition, silver is a great plant growth and development stimulator.

SNPs have been tested with pesticides to decrease the burden of pests on crops. It has a demand for pest protection and dietary enhancement. This decreases the frequent utilizes of chemical fertilizers in conventional cultivating. It can devastate undesirable microorganisms in soil and hydroponics systems. SNPs are being utilized as a foliar spray to stop fungi, Molds, rot and various other microbial-associated plant infections (Babu *et al*., 2014; Rai *et al*., 2014). Aqueous silver solution, utilized to treat plants, is detailed to display fabulous preventive impacts on pathogenic microorganisms causing powdery mildew or downy mildew in plants. Additionally, it advances the physiological movement and development of plants and actuates disease and stress resistance in plants. It is conceivable to control pests by incorporating NPs into them. SNPs serves as a productive pest control agent that is non-toxic, secure and an improved pest management tool and the SNP-based pesticide too also provide high dose of pesticides to the target plants (Lamsal *et al*., 2011; Ragaei and Hasaan, 2014). The pesticide activity of SNPs can help a lot in the control of pests, since in the green method of preparation of SNPs. The biological agents for their synthesis may be microbes or plants and the flavonoids present in the plants prove to be poisonous to the plants (Zahir *et al*., 2012). Utility of SNPs in insect pest control has been detailed (Routray *et al.,* 2016). SNPs are utilized in the control of diseases in rice weevils and grasserie. SNPs treated with stored rice remained uninfected after 2 months of treatment, so it is proposed that SNPs can moreover be utilized as a fabulous seed protecting agent.

Soil and Environmental Impact

Silver nanoparticles widely recognized for their antimicrobial properties, can significantly affect soil health and ecosystem dynamics when released into the environment. One of the primary concerns is their impact on soil microbial diversity. SNPs can disrupt microbial communities by inhibiting the growth of beneficial bacteria and fungi involved in critical soil processes such as nitrogen fixation, organic matter decomposition, and nutrient cycling (Kumar et al., 2018). This disturbance can lead to imbalances in soil fertility and reduced efficiency of nutrient transformation, ultimately affecting plant growth and soil sustainability. Moreover, the environmental persistence and low biodegradability of SNPs raise concerns about their long-term ecological effects. Their small size and high reactivity allow them to interact with various soil components, potentially leading to bioaccumulation and toxicity in non-target organisms, including earthworms and aquatic species through runoff (Tourinho et al., 2012). Ecotoxicological studies suggest that even at low concentrations, SNPs can cause oxidative stress, DNA damage, and membrane disruption in soil and aquatic organisms. These findings highlight the need for regulated use and thorough environmental risk assessments of SNPs to ensure their safe application in agricultural and industrial practices

Applications in Biomedicine

SNPs play an important role in the modulation of various activities such as antiviral, antifungal antimicrobial, antibiofilm, antiparasitic, antifouling, anticancer, antiviral and drug-delivery systems. Applications of SNPs are presented in Figure 4.

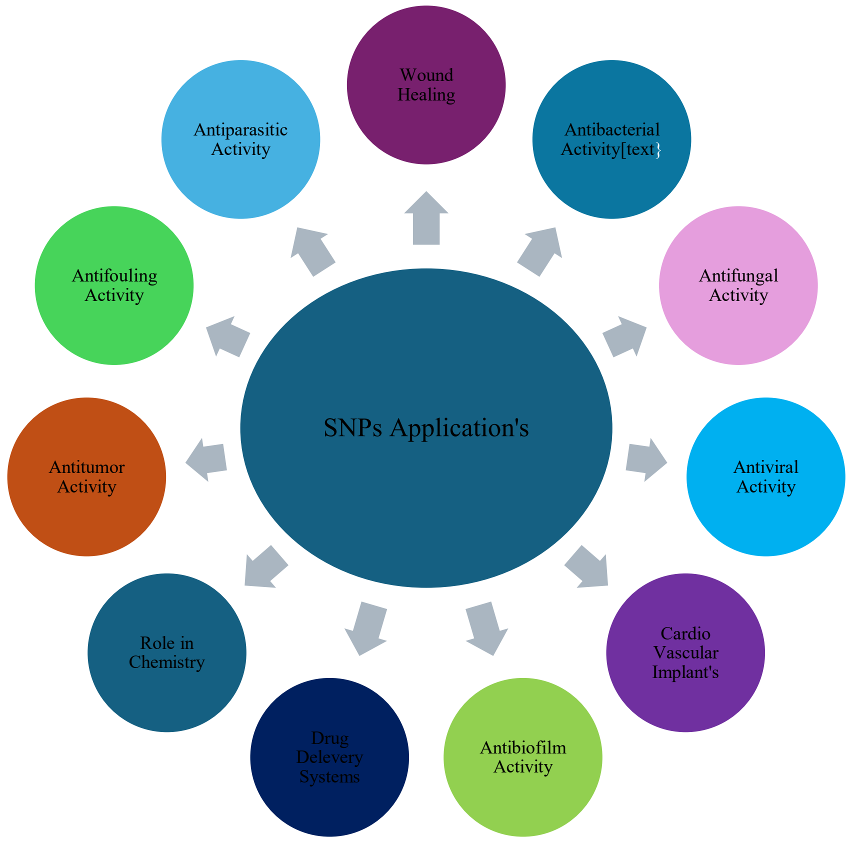

Figure 4: Utilization of Several Behaviors of SNPs

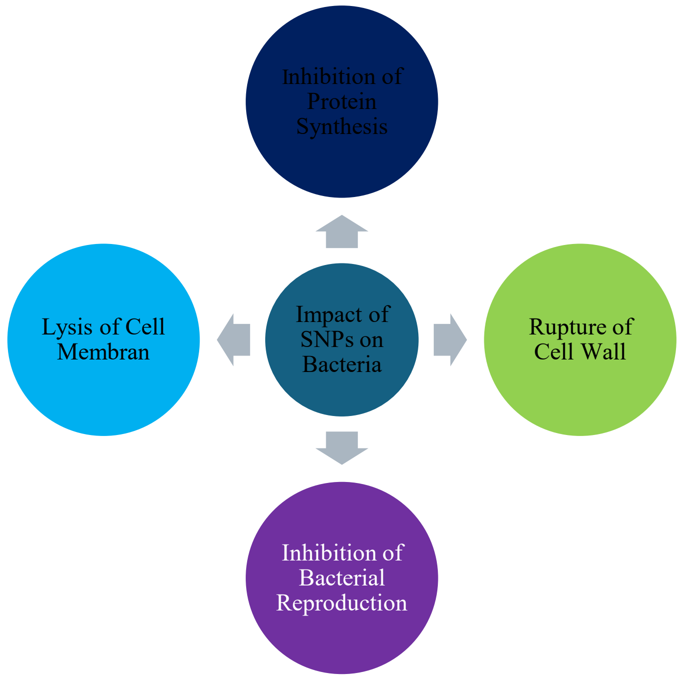

Figure 5: Sketching of Antibacterial Activity Mechanisms of SNPs.

Antimicrobial and Antiviral Properties

Silver nanoparticles exhibit potent antimicrobial and antiviral properties, making them valuable in a variety of medical and healthcare applications. Due to their ability to disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with DNA replication, SNPs are highly effective against a broad spectrum of bacteria, including both Gram-positive and Gram-negative strains (Rai et al., 2012). These properties have led to their incorporation into wound dressings, bandages, and topical ointments, where they help prevent infections, accelerate healing, and reduce inflammation. The nanoscale size of SNPs allows for better penetration and interaction with microbial cells, enhancing their antimicrobial efficacy even at low concentrations. In addition to their antibacterial action, SNPs are increasingly being explored for their antiviral potential. Studies have shown that SNPs can inhibit viral entry and replication by binding to viral surface proteins or interfering with host-cell interactions (Galdiero et al., 2011). This is particularly significant in the context of growing antibiotic resistance, as SNPs offer an alternative or complementary strategy to conventional antibiotics. Their use in coatings for medical devices, hospital surfaces, and personal protective equipment further highlights their role in infection control and public health. However, careful evaluation of their cytotoxicity and environmental impact is essential for safe and sustainable application.

Drug Delivery Systems of AgNPs

Silver nanoparticles have emerged as promising nanocarriers in drug delivery systems due to their unique physicochemical properties, including high surface area, ease of surface functionalization, and strong antimicrobial activity. Their nanoscale size allows them to penetrate biological barriers and deliver therapeutic agents directly to target cells or tissues. AgNPs can be functionalized with ligands, polymers, or antibodies to enable controlled and targeted drug release, thereby minimizing systemic toxicity and enhancing treatment efficacy (Franci et al., 2015). This targeted delivery is particularly valuable in cancer therapy, where AgNPs can be designed to release chemotherapeutic agents selectively at tumor sites, reducing damage to healthy tissues. Controlled drug release from AgNPs can be achieved through stimuli-responsive systems that respond to pH, temperature, or enzymatic activity, allowing precise spatiotemporal release of the drug payload (Marambio-Jones & Hoek, 2010). Additionally, the intrinsic antimicrobial properties of silver further enhance the therapeutic effect, making AgNPs particularly useful in treating infections or wounds while simultaneously delivering drugs. Despite these advantages, challenges such as potential cytotoxicity, long-term biocompatibility, and regulatory approval remain areas of active research to ensure safe clinical applications.

Diagnostic and Therapeutic Uses

Silver nanoparticles have shown significant potential in both diagnostic and therapeutic applications due to their unique optical, electrical, and antimicrobial properties. In diagnostics, SNPs are utilized in biosensors and imaging systems where their strong surface plasmon resonance (SPR) enhances signal detection, allowing for highly sensitive and specific identification of biomolecules, pathogens, or disease markers. For example, SNPs have been incorporated into colorimetric assays and lateral flow devices for rapid detection of infectious agents and biomarkers, including those relevant in cancer and viral infections (Lee & El-Sayed, 2006). Their ability to be easily functionalized with antibodies, DNA probes, or aptamers makes them versatile tools in point-of-care diagnostics. Therapeutically, SNPs are being explored for applications ranging from antimicrobial coatings to cancer treatment. Their ability to induce oxidative stress and disrupt cellular functions in pathogens and cancer cells makes them effective agents for killing harmful cells while sparing healthy ones, especially when targeted delivery systems are used. Moreover, SNPs can be combined with other therapies, such as photothermal or photodynamic therapy, to enhance treatment efficacy against tumors (Gurunathan et al., 2014). These dual diagnostic and therapeutic ("theranostic") capabilities highlight the promise of SNPs in advancing precision medicine, though careful evaluation of their safety, biodistribution, and long-term effects is essential for clinical translation.

Toxicity and Biocompatibility

The toxicity and biocompatibility of silver nanoparticles (SNPs) are critical concerns that must be addressed before their widespread application in biomedical and environmental fields. While SNPs exhibit powerful antimicrobial and therapeutic properties, their small size and high reactivity also raise the potential for cytotoxic, genotoxic, and oxidative stress-related effects in mammalian cells. Studies have shown that SNPs can penetrate cell membranes, accumulate in organelles, and induce the generation of reactive oxygen species (ROS), leading to mitochondrial damage, DNA fragmentation, and apoptosis (AshaRani et al., 2009). The severity of these effects depends on various factors such as particle size, concentration, surface coating, and exposure duration. Despite these concerns, SNPs can be engineered for improved biocompatibility through surface modification with biocompatible polymers, proteins, or other stabilizing agents. Such modifications reduce agglomeration, control release kinetics, and mitigate toxic interactions with healthy cells. In vivo studies have demonstrated that low concentrations of well-coated SNPs can exhibit minimal toxicity while retaining therapeutic efficacy (Lansdown, 2010). However, comprehensive toxicological assessments, including long-term exposure studies and environmental impact analyses, are essential to ensure their safe use in medical and consumer applications.

Challenges and Concerns

Despite their wide-ranging applications, silver nanoparticles (SNPs) present several challenges and concerns that must be carefully considered, particularly regarding safety, environmental impact, and regulatory oversight. One of the primary concerns is the potential cytotoxicity and genotoxicity associated with SNP exposure, especially at high concentrations or with prolonged use. SNPs can accumulate in various organs, such as the liver, lungs, and kidneys, and may induce oxidative stress, inflammation, and DNA damage (Chen and Schluesener, 2008). These effects raise significant questions about their long-term biocompatibility and potential risks to human health, particularly in clinical and consumer products. Environmental concerns also play a crucial role, as SNPs released into soil or water systems can adversely affect microbial communities, disrupt nutrient cycles, and bioaccumulate in aquatic organisms (Gottschalk et al., 2009). Moreover, the lack of standardized synthesis protocols, inconsistent characterization methods, and limited toxicological data hinder the development of comprehensive safety guidelines. Regulatory frameworks for nanoparticle use are still evolving, making it difficult to ensure consistent monitoring and safe integration of SNPs into commercial products. Addressing these challenges requires interdisciplinary research, robust risk assessments, and the development of safer, more sustainable nanoparticle designs.

Future Perspectives and Research Directions

The future of silver nanoparticles in agriculture and biomedicine holds great promise for transforming traditional practices, driven by ongoing advancements in nanotechnology. As research progresses, AgNPs are expected to play critical roles in enhancing crop productivity and improving healthcare outcomes. Key to realizing this potential will be the development of sustainable, green, and scalable synthesis methods, such as biosynthesis using plant extracts, microorganisms, or waste materials, which offer cost-effective and environmentally friendly alternatives. Innovations in microfluidic systems, continuous flow reactors, and AI-assisted production are likely to streamline manufacturing processes further. Simultaneously, advanced characterization techniques will enable real-time, in-situ monitoring of nanoparticle behavior, facilitating the design of application-specific AgNPs with optimized size, shape, surface charge, and functionality to maximize benefits while minimizing toxicity. In agriculture, AgNPs are poised to revolutionize precision farming through controlled-release nano-fertilizers and pesticides, nano-enabled biosensors for real-time environmental monitoring, antimicrobial coatings, and even as carriers for CRISPR-Cas9 systems to enhance crop resilience. In medicine, next-generation applications include targeted drug delivery, antimicrobial coatings for medical devices, theragnostic that integrate therapy and diagnostics, and innovations such as AgNP-based vaccines, nanorobotics, and portable biosensors for point-of-care diagnostics, especially in underserved regions.

However, the widespread deployment of AgNPs must be balanced with careful consideration of potential risks to human health and the environment. Comprehensive toxicological studies, the development of biodegradable or self-degrading nanoparticles, and standardized safety regulations will be essential to ensure responsible use. Life cycle assessments and nano-safety-by-design principles will guide sustainable application, while global regulatory frameworks must evolve to define safe usage levels in both agricultural and biomedical contexts. Public engagement, transparency, and ethical guidelines will also be crucial to foster trust and ensure equitable access to nano-enabled technologies, particularly in low-resource settings. Moving forward, interdisciplinary collaboration among nanotechnologists, biologists, chemists, agronomists, and clinicians, combined with the integration of artificial intelligence and big data analytics, will be vital for advancing predictive modeling, the design of novel nanostructures, and personalized solutions. If environmental, regulatory, and ethical challenges are proactively addressed, silver nanoparticles have the potential to become powerful, sustainable tools for addressing global challenges in food security, healthcare, and environmental stewardship

Conclusion

Silver nanoparticles play a significant role in health management due to their wide range of applications, including use as antimicrobial and antitumor agents, in food packaging, agriculture, and the healthcare sector. However, the overuse and misuse of conventional antimicrobial agents have led to resistance, resulting in reduced effectiveness. This is especially concerning in the case of biofilm-forming bacteria, which pose a serious challenge. To address the growing problem of antibiotic resistance, global attention has shifted toward alternative treatment strategies. Among these, the application of SNPs-particularly through surface coatings or impregnation with nanomaterials-has emerged as a promising antibiofilm strategy. Furthermore, SNPs are among the most extensively studied and applied nanoparticles for managing various health conditions, including cancer, wound healing, dental implants, and other therapeutic applications aimed at enhancing biological performance. With advancing knowledge and improved technologies, the use of SNPs in medicine is expected to establish a robust platform for the prevention and treatment of multidrug-resistant pathogens and biofilm-associated infections. While nanotechnology is a rapidly growing field with extensive applications in electronics, energy, the environment, and health, its potential in agriculture remains underutilized, especially in India and other countries. Despite its promise as a novel and effective tool for addressing persistent agricultural challenges, research in this domain is still in its early stages and often remains at the conceptual level. Indian farmers and agricultural stakeholders should be encouraged to adopt such innovative approaches in farming practices. Doing so will enable the broader societal benefits of nanotechnology to be realized. Researchers and scientists in the nanotechnology field have ample opportunities to explore the intelligent application of engineered nanoparticles in agriculture and horticulture, unlocking their full potential for sustainable and smart farming solutions.

Declaration

Acknowledgment: The author is grateful to the CEO of Absolute for providing support in preparation of manuscript.

Funding: This research and manuscript preparation did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: The author declares no conflict of interest.

Ethics approval/declaration: Not applicable

Consent to participate: Not applicable

Conflict of Interest: The author has no conflict of interest.

Consent for publication: The author agreed that the manuscript can be published.

Data Availability Statement: Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Author contribution: Author Dr. KP collected the necessary data and literatures for first draft of the manuscript which was written. Finally, KP read, corrected and finalized the final shape of manuscript.

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https://doi.org/10.56556/jase.v4i1.1333

REVIEW ARTICLE

Myrtus communis L. (Myrtle): A comprehensive Review of its Folk uses, Medicinal uses, Historical perspective, Phytochemistry, and Pharmacology

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Received: 12 June, 2025, Accepted: 29 June, 2025, Published: 30 June, 2025

Abstract

Myrtus communis L. is a bushy, aromatic, ever-green shrub belongs to the family Myrtaceae, which is commonly distributed around the globe. The current review gather important information about its ethno-medicinal uses, historical perspective, phytochemistry, and pharmacology of Myrtle. The review study was designed with the aim to gather information of Myrtle in addition to explore its ethno-medicinal, therapeutic potential, and future research opportunities. In current review, a total of 85 comprehensive research papers were reviewed and collected from various databases such as Google Scholar, Web of Science, Scopus, Research Gate, Science Direct, and PubMed. The results of this review indicate that Myrtle is mostly used for various ailments such as skin, respiratory, gastrointestinal, inflammatory, urinary, cardiovascular, neurological, and microbial infections. The previous literature reported that traditional therapists mostly used it in the form of herbal tea, and powder form. Moreover, Myrtle was reported to have high antibacterial, antifungal, antiviral, antitussive, anti-inflammatory, and anticancer activities. The study also reported that it has a strong antioxidant, cardiovascular, and therapeutic properties. There are a variety of phytochemical compounds reported and isolated in Myrtle plant such as phenols, alcohols, terpenoids, fatty acids, sterols, glycosides, carboxylic acids, flavonoids, and essential oils. The plant need sustainable cultivation, and conservation for its availability in medicinal, and commercial applications. Further, it is recommended, and invite young researchers to carry out further detailed investigation into their possible ethno-medicinal, ethno-pharmaceutical, toxicological, anti-aging, antidandruff, and anti-hair fall on this plant in order to provide reliable knowledge, and discover new drugs with low side effects as linked with modern medicines. The plant need sustainable cultivation, and conservation for its availability in medicinal, and commercial applications.

Keywords: *Myrtus communis*; History; Ethno-medicine; Phytochemicals; Pharmaceutical activities

Introduction

Myrtus communis belong to the family Myrtaceae which consists of 140 genera, and 5800 species (Zhang *et al*., 2008). The plant is native to the Mediterranean territories namely southern Europe, North Africa and along with Middle East. Myrtle as a small evergreen shrub, and people throughout the centuries have valued to this plant, because it possesses medicinal properties along with aromatic compounds, and a symbol of support cultural traditions. Myrtle possess small dark green leaves with leathery texture, it produces white or pale pink flowers, and dark purple berries/fruits (Dietz, 2024; Ullah *et al*., 2022; Ali *et al*., 2017). There are two varieties of Myrtle named *Myrtus communis* var. *italic* and var. *baetica* L. The varieties have similar vegetative characters, but only difference is in colour of the fruit, and size of their leaves (Nuvole *et al*., 1996). However, Myrtle is an evergreen plant distributed in various regions of the world. It mostly occurs in North West Himalaya, Asia, Europe, Africa, America, Australia, and Mediterranean region of the world (Nadkarni, 1989). In Pakistan it is distributed in mountainous areas such as tribal Ex-FATA, Khyber Pakhtunkhwa, Kashmir, and Punjab. In Khyber Pakhtunkhwa it is present in various areas such as Bajaur, Dir, Bunir and Swat etc (Rahim *et al*., 2012; Ali *et al*., 2017; Ullah *et al*., 2022).

Myrtle plant maintains its place in historical records, since ancient times when several cultures recognized its importance. It occupied a special place at the heart of ancient Egypt, because Egyptians regarded this plant as a spiritual, and healing medicine along with religion. The Egyptian priesthood employed Myrtle leaves for religious cleansing ceremonies, and people used its extracts to treat breathing, and urinary problems (Opitz-Kreher *et al*., 2023). In Rome and Greece Myrtle plant held significance position, and people used it as a symbol of love, beauty, and victory. In these areas the medical practitioners i.e., Hippocrates and Theophrastus documented the healing properties of Myrtle, so found that this plant was effective for treating fever, digestive, and skins diseases. The Roman law appreciated Myrtle, because they used the plant in wine, perfumes production, and food flavoring industries (Martinez-Frances *et al*., 2021; Ullah *et al*., 2022). The British male officials received victory honors expressed through crowning them with Myrtle wreaths. Throughout time people have continued to use Myrtle plant both spiritually, and ritually. The Jewish tradition names Myrtle as one of the four special plants necessary for celebrating Sukkot festival. The regional cultures from the Mediterranean region along with the Middle East continue using Myrtle branches as burning incense to cleanse spaces, and prevent adverse energies. The traditional wedding ceremonies in Italy and France include Myrtle since the plant represents love together with fidelity, and prosperity. People use the plant in protective amulets, traditional religious ceremonies, and folk rituals, because of its deep spiritual roots (Dafni *et al*., 2020; Ullah *et al*., 2022).

However, Myrtle served as an essential herb in herbal medicine from medieval times through Renaissance which Islamic, and European medical knowledge centers embraced. The Unani medical system esteemed Myrtle for its capability to create coolness along with its stringent quality, and its ability to assist in wound healing. Therapeutic use of Myrtle received extensive documentation from Islamic scholars including Avicenna, and Al-Razi who described its benefits against gastrointestinal conditions together with improvements for liver performance, and hair development (Schulz *et al*., 2023). People in medieval Europe traditionally used Myrtle to treat respiratory conditions, and stomach problems along with skin afflictions. People valued its essential oil because, it showed antiseptic abilities as well as soothing effects in herbal distillations, and perfume preparations (Schulz *et al*., 2023; Ullah *et al*., 2022). Throughout, different cultures local healers consistently employed Myrtle in many ways to treat various health disorders. The tradition of using Myrtle as medicine exists extensively in Mediterranean, and Middle Eastern folk medicine to treat respiratory diseases that include coughs, bronchitis, asthma, and tuberculosis. The therapeutic properties of Myrtle essential oil function as expectorant medicine which removes mucus to improve respiratory function (Leigh-de Rapper *et al*., 2020). The traditional medicine consumes Myrtle leaves, and berries to make infusions that treat gastrointestinal problems such as diarrhea, bloating, and indigestion. Myrtle leaves extract represents a traditional remedy for urinary tract infections, because doctors have recommended leaf-infused preparations to address kidney, and bladder issues (Schulthies, 2020; Ali *et al*., 2017).

Myrtle finds broad usage in medical treatments that address both internal diseases, and skin ailments along with wound healing processes. The medicinal properties of Myrtle include antibacterial traits combined with astringent effects as well as anti-inflammatory properties that treat acne, eczema and minor cuts (Nurzyńska-Wierdak *et al*., 2022). The medical practitioners use Myrtle leaf paste as well as poultice to treat open injuries, burn wounds and ulcers, because these treatments reduce infection risk and boost healing times. The cosmetic industry use Myrtle as an essential component in various products of hair, and beauty. The practice of traditional medicine involves applying both extract, and oil from Myrtle plant components to achieve hair growth along with scalp strengthening, and dandruff protection. The antifungal properties in Myrtle plants help keep the scalp healthy, while protecting from seborrheic dermatitis conditions (Ayatollahi *et al*., 2021). Myrtle serves numerous roles in oral care as antibacterial, and astringent components make them a standard part of traditional mouth washing products, and dental products like toothpaste. The people commonly use water infused with Myrtle as a home remedy to treat infections of the gums as well as mouth ulcers, and bad breath. The tannins extracted from the plant lead to tissue tightening, and anti-inflammatory effects which make Myrtle effective for oral hygiene maintenance (Dakkaki *et al*., 2023). The local communities use Myrtle leaves as chewing material to enhance gum health, while they also help improve breath freshness (Dakkaki *et al*., 2023). The plant contains multiple bioactive elements including flavonoids, tannins, phenolic acids, and essential oils which account for its antimicrobial, antioxidant, anti-inflammatory, and antifungal properties (Hou *et al*., 2022; Ali *et al*., 2017).

Moreover, there is a considerable amount of empirical pharmacological research that has confirmed a number of traditional applications of Myrtle by using modem scientific research. The previous studies have confirmed that Myrtle consists of high quantities of flavonoid, and phenolic compounds which have the able to possess great antioxidant capabilities. These plant compounds function as free radical neutralizers, reduce the level of oxidative stress, and to prevent the exposure of cell damage (Akbari *et al*., 2022). The key beneficial properties of Myrtle as cardiovascular protection, and brain health progression. Many researcher has been investigated about the antimicrobial properties of Myrtle, due to the fact that research has proven its effectiveness at combating different bacterial, and fungal infections. The anti-bacterial and antifungal property of its essential oil are due to its major components such as myrtenyl acetate, α pinene and 1,8 cineole (Abd Rashed *et al*., 2021). However, the analgesic, and anti-inflammatory properties of the plant can be used to control arthritis symptoms, and also inflammatory skin diseases with managing gastrointestinal inflammation. The recent research shows that some bioactive elements present in extracts of Myrtle might display toxicity according to cancer cells, thus really playing the role of fighting against cancer (Giampieri *et al*., 2020). The findings about Myrtle derived compounds as potential cancer prevention, and treatment agents need to be further validated through scientific examinations. The insulin sensitivity can be improved, and blood glucose can be controlled by Myrtle extracts. These bioactive polyphenols, and terpenoids of Myrtle which are believed to support traditional use for the treatment of metabolic disorder have been supported by the hypoglycemic properties of Myrtle (Bungau *et al*., 2023).

Myrtle has been used as a medicinal plant, and has some potential therapeutic properties, though research into how it can be applied still needs to be explored. Myrtle extractions should also be standardized with specific compound identification in therapeutic effects with pharmacology, safety, and clinical evaluation tests. Future supply of Myrtle will be supported on account of its ecological value as well as expanding commercial needs for sustainable cultivation methods and conservation strategies to be ongoing (Hardstaff *et al*., 2022; El Mekkaoui *et al*., 2024). This study evaluates Myrtle by investigating its historical, and cultural roots combined with its traditional medical uses, and chemical profiles along with its pharmaceutical properties. The authors integrate established cultural wisdom with scientific exploration to demonstrate Myrtle worth as a multi-purpose medicinal plant suited for natural medicine, pharmaceutical production, and dermatological applications.

Methodology

This review article provides a comprehensive overview of the folk uses, medicinal uses, historical perspective, phytochemistry, and Pharmacology of *Myrtus communis* L., compiled from a published research papers. The current review included a total of 85 comprehensive research papers were reviewed, and collected from various databases such as Google Scholar, Web of Science, Scopus, Research Gate, Science Direct, and PubMed (Matsehorova *et al*., 2024). The available all publications needed to be in English as per the established language restriction of this review, and employed a keyword search strategy across various databases to identify studies focused on Myrtle plant morphology, local uses, medicinal uses, religious use, history, phytochemistry, and pharmaceutical activities. The review included only scientific publications that were indexed in the provided databases to ensure availability for the scientific world. The study excluded unpublished sources consisting of thesis, reports, and locally available documents, since they remain unavailable to reference (Ng *et al*., 2020). The research data from previous published literature is summarized in four main tables for local names, traditional uses, phytochemistry, and pharmacological activities, and five figures. The IUPAC names and chemical formulas of chemical compounds are conform from various databases i.e., Chem-Spider, and Pub-Chem. The review method focuses on peer-reviewed scientific information which makes this resource a reliable tool for both young researchers, and practitioners who work in natural medicine fields (Secinaro *et al*., 2021).

Comprehensive literature-based information of *Myrtus communis* L.

Plant description

Myrtus communis is a small tree or an evergreen shrub growing up to 2 to 5m tall. Its foliage is dense, and its leaves are aromatic. The leaves are lanceolate, simple, leathery, opposite, have entire margin and glandular dots. The Myrtle produce fragrant white or pale pink flower with many feathery stamens. These flowers bloom in summer as well as in late spring and blooming season is between May and July (Boddy, 2020). The plant bears edible fruits in the form of small berries, dark purple to black colour, mature in late summer or early autumn (Figure 1a & 1b). The barriers are multi seeded and can be cultivated in the Mediterranean region, Southern Europe, Middle East and North Africa, where it is considered to be grand ornamental, aromatic and medicinal plant. Its habitat takes place on rocky slopes, dry woodlands, and even coastal areas. The great cultivation potential of Myrtle is due to its bioactive compounds including flavonoids, tannins and essential oils. Moreover, its leaves flowers, and fruits make the plant to be great pharmacological potential (Giampieri *et al*., 2020; Ullah *et al*., 2022).

The essential oil are extracted from its leaves, and berries are famous for their antimicrobial, anti-inflammatory and antioxidant properties. Traditionally, the extracts of Myrtle leaves have been use in several purpose of Unani, Ayurveda, and Mediterranean folk medicine for treatment of respiratory, digestive, and skin infections. Its stem is found to be filled with fragrant constituent, which has been widely used in perfume, and aromatherapy (Khatib, 2024).

Figure. 1a & 1b: Present Myrtle branch with white flowers and blue barriers or fruits**.**

Vernacular / local names of *Myrtus communis* L.

Myrtle has different names for different areas and cultures, pointing to age and medicinal uses. The vernacular names of *Myrtus communis* are vary according to their regions, where it is frequently used in traditional medicine, and cultural practices. In English it is also known as Myrtle, Bharangi in India, Hubal Aas in Arabic, Tokhami maorood, and Yas in Persians, Mersin in Turkey, Mirto in Italy, and Greece, Myrte in German, and France, and Pakistan it is also known as Manroo in Pashto, Moorya in Punjabi, Maanmoryo in Sindhi language (Table.1; Al-Snafi *et al*., 2024; Baytop, 1999; Rahim *et al*., 2012).

Table 1. Local names of *Myrtus communis* L. across the world

Country / Region	Local Name
Pakistan	Manroo, Moorya, Maanmoryo
British	Myrtle
Saudi Arabia	Hubol Aas
Iran (Persians)	Tokhami maorood, Yas
Turkey	Mersin
Italy	Mirto
German, France	Myrte
Italy, Spain	Mirto

Ethno-Medicinal uses of *Myrtus communis* L

Myrtus communis was previously used as an herbal medicine, traditional healthcare, and various cultural activities. Myrtle is also used by almost 80% population of the modern world, where modern medical facilities are not available. Though, minimizing the side effects, cost effective and natural healing properties is the safety profile of herbal medicines which makes it different than the synthetic drugs (Barkat *et al*., 2021). Myrtle is a historical plant use as a traditional medicine throughout the Mediterranean, Middle Eastern, and South Asian countries. The most notable among these are Pakistan, Iran, India, Turkey, and Greece (Akbar & Akbar, 2020). There are various diseases reported to be treated by the Myrtle are classified into the following categories such as gastrointestinal disorders, skin conditions, renal, urinary disorders, musculoskeletal disorders, respiratory infections, eye infections, oral infections, parasitic infections, gynecological conditions, ENT-related issues, fever, cancer, and other ailments. The largest percentage was that of gastrointestinal disorders (24%), followed by skin diseases (18%), urinary and renal disorders (13%), muscular and skeletal issues (9%), respiratory diseases (7%), fever (6%), eye infections (3%), oral infections (3%), gynecological disorders (3%), ENT-related issues (1%), and cancer (1%; Figure. 3). The cumulative percentage of the gastrointestinal disorders treated by Myrtle is attributed to its carminative properties as well as digestive stimulant, and antimicrobial activities (Mohammadi *et al*., 2025; Ali *et al*., 2017).

However, *Myrtus communis* has been used by traditional medicinal practitioners throughout the Mediterranean, and Middle East to treat stomach disorders like indigestion, bloating, ulcers, and diarrhea. The several studies have conformed the efficacy of Myrtle leaves, and berries in dealing to intestinal discomforts, and gastrointestinal infections. Some of the studies reported the use of root extracts in the treatment of diarrhea, and dysentery. The parts of the plant used in traditional medicine are leaves, berries, essential oil, roots, flowers, and sometimes the whole plant. The leaves were the most commonly used (42%), followed by berries (25%), essential oil (20%), roots (10%), and whole plant (3%; Figure. 2). The high usage rate of leaves as well as that of essential oil could be attributed to their high concentration contents in flavonoids, tannins, and volatile compounds contributing to medicinal properties (Pinto *et al*., 2021). Further, the leaves and essential oil were used for the treatment of skin ailments and also used for the treatment of cuts, wounds, burns, boils, eczema and fungal infections have been recorded by the traditional healers is Myrtle. The antimicrobial, and astringent actions of these help prevent the infection aftercare. The Myrtle extracts have been identified in ointments, and herbal pastes for wound management. Apart from this, it has an antifungal activity against common skin infection such as ringworm, and fungal dermatitis. Myrtle is also used for the healthy condition of the kidneys, and urinary tract. The leaves and root were most often cited in the literature as having effectiveness in treating such ailments. The Myrtle extracts were used by physician in creating mixtures designed for the treatment of kidney stones, urinary tract infections, and bladder disorders. The plant parts also contain an entire spectrum of phenolic compounds that makes stone in the kidney to dissolve and also improve the overall function of kidneys (Ranaweera *et al*., 2023; Ali *et al*., 2017).

Moreover, the plant has also been used for the treatment of muscle, and skeletal disorders such as arthritis, joint pain, and muscle cramps. Its leaves, and essential oils are the most commonly used in poultices, ointments applied to the skin or infused in carrier oils for massage therapy. The Myrtle products provide relief to inflammation, reduce swelling, and improve mobility of the area. It has also been used in respiratory ailments for cough, asthma, bronchitis, and sinus infections (Prall *et al*., 2020). The additional medicinal uses of Myrtle include treatment of fever, various oral infections, and parasitic diseases. Myrtle leaf extracts are taken as antipyretic herbal teas for the purposes of reducing fever, and enhancing immunity. Traditionally, the antibacterial, and astringent extracts of Myrtle have been applied in dental care using mouth rinses to prevent gum infection, and bad breath for mouth ulcers (Table.2; Giampieri *et al*., 2020).

Furthermore, it is used in traditional medicine in the forms of decoctions, juices, powders, teas, and extracts. The most frequently used powder preparations (30%), decoctions (22%), juices (20%), pastes (19%), teas (6%), and the extracts (3%).Traditionally. Myrtle powders have been used in Unani and Ayurvedic medicines and are mixed with honey, water and milk before the latter is consumed. The present review reaffirms various ethno-medicinal uses of Myrtle over different regions. This plant is of great value due to its antimicrobial, antioxidant, anti-inflammatory, and diuretic properties, as such, it plays a key role in traditional medicine. Though, ethno-botanical documentation points out the importance of regional variations in plant usage, whereby some cultures tend to use leaves, essential oils, roots, and whole plant extracts. Meanwhile the rising scientific validation of the medicinal effects of Myrtle, it should be a subject for further exploration in pharmacological applications, and modern drug development (Matsehorova *et al*., 2024).

Table 2. Major diseases categories, application and scientific evidence of *Myrtus communis* L.

Disease Category	Traditional Uses / Application	Scientific Evidence / Observed Effects
Respiratory disorders	Used as a decoction or infusion to relieve cough, soothe bronchitis, and treat common colds.	Methanolic and aqueous extracts show dose-dependent antitussive activity (up to 44.2 % inhibition at 300 mg/kg), comparable to codeine phosphate in animal models.
Gastrointestinal issues	Employed for treating gastric ulcers, diarrhea, and dyspepsia.	Aqueous extract significantly reduced ulcer lesions ($p < 0.05$) in indomethacin- and pylorus-ligation-induced rat models at 15–60 mg/kg; demonstrates antisecretory and mucosal-protective effects.
Antimicrobial infections	Applied topically or orally for skin infections, wound care, and to manage microbial gastrointestinal disturbances.	Essential oil and extracts exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*) and various fungi (e.g., *Candida albicans*).
Anti-inflammatory & analgesic	Used to alleviate joint pain, rheumatism, and general inflammation when taken as a poultice or infusion.	In vivo studies demonstrate significant inhibition of carrageenan-induced paw edema in rats, indicating notable anti-inflammatory and analgesic properties.
Metabolic disorders	Taken as an herbal tea to support digestion and believed to help “balance blood sugar.”	Extracts show antioxidant activity (DPPH and FRAP assays) and moderate α-glucosidase inhibitory effects, suggesting potential antidiabetic benefits.
Dermatological conditions	Applied topically for wound healing, minor skin lesions, and as an antiseptic wash.	In vitro and in vivo studies indicate accelerated wound closure in rodent models, increased collagen deposition, and antimicrobial protection at wound sites.
Cardiovascular support	Consumed as a tonic to “strengthen the heart” and manage mild hypertension.	Preliminary studies in animal models indicate hypotensive effects, likely due to vasorelaxant activity of flavonoids present in the leaves.

Urinarytract disorders	Used as a diuretic infusion to help flush urinary tract and relieve minor urinary discomfort.	Diuretic assays in rodents demonstrate increased urine output; antimicrobial properties also help reduce risk of bacterial proliferation in the urinary tract.
Neurological/ nervous System	Employed in folk medicine to calm nervous tension, mild anxiety, and headaches.	Limited studies suggest central nervous system depressant activity in mice, as evidenced by prolonged sleeping time in pentobarbital-treated animals.
Antioxidant& general tonic	Drunk as a regular herbal tea for overall vitality, to reduce oxidative stress, and support immune health.	High total phenolic and flavonoid content correlates with strong antioxidant capacity in DPPH, ABTS, and lipid peroxidation assays.

Sources: (Mohammadi *et al*., 2025; Pinto *et al*., 2021; Giampieri *et al*., 2020; Matsehorova *et al*., 2024).

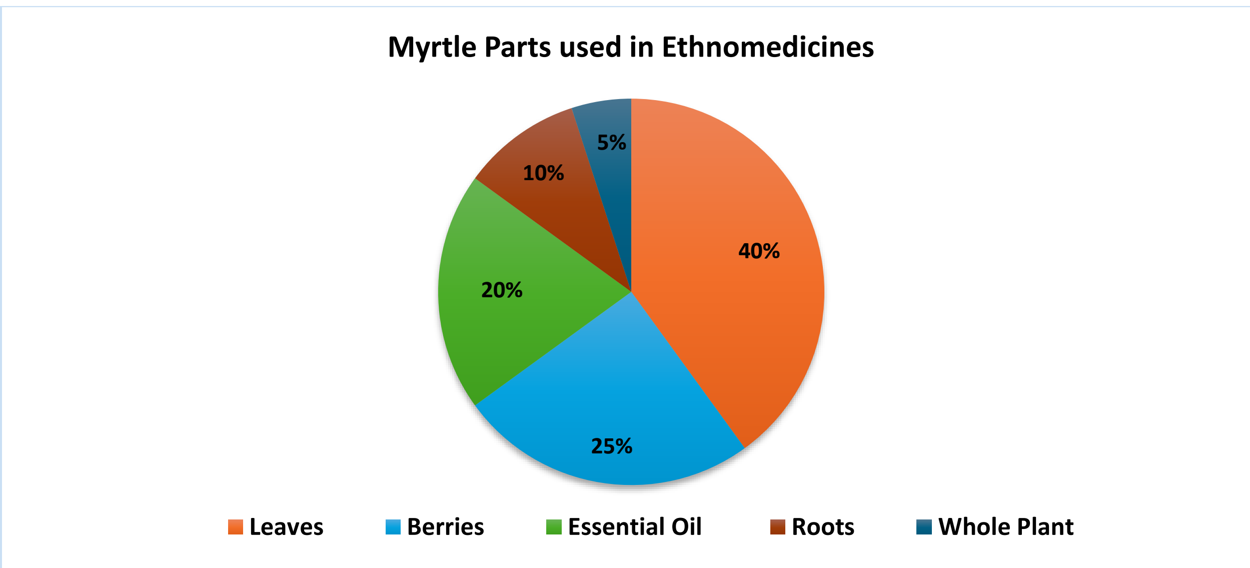

Figure 2. *Myrtus communis* plant parts used in various ethno-medicinal applications.

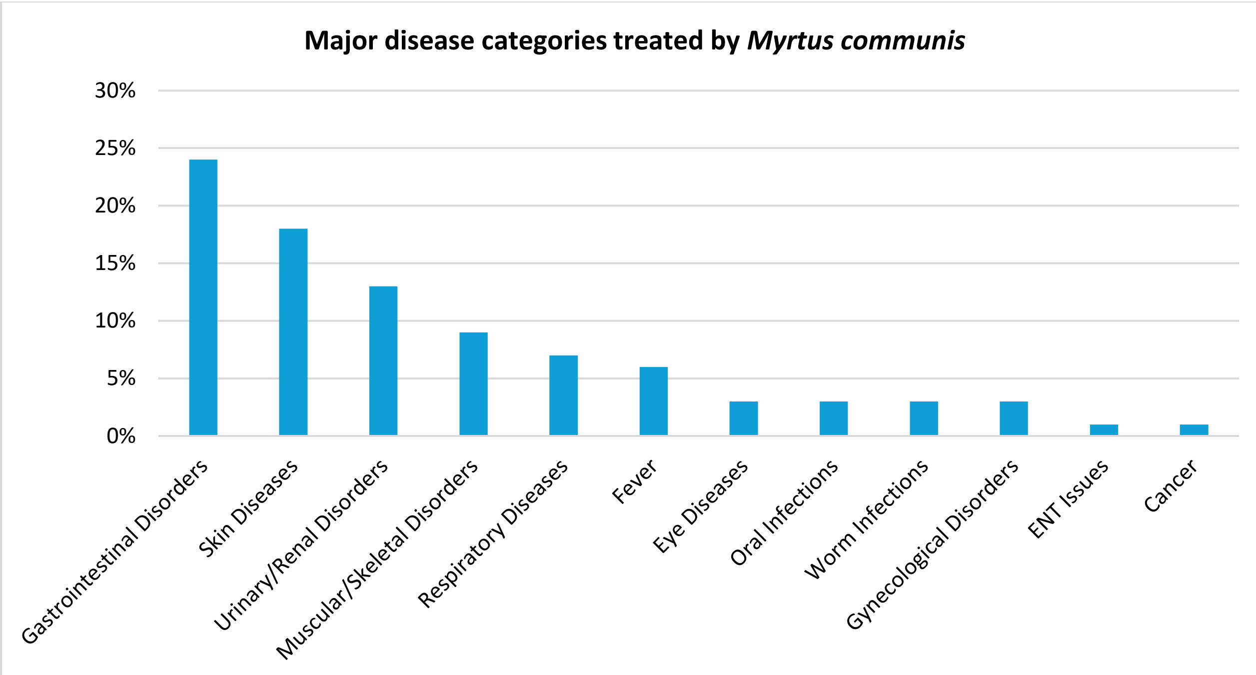

Figure. 3 Present the main diseases traditionally treated by using Myrtle.

Phytochemistry

The demand of herbal medicines has been increased tremendously in the last two decades for which rigorous quality measurements have been undertaken to assure safety, efficacy, and consistency of herbal drugs. The most effective tools for the quality assessment of herbal medicine is the phytochemical evaluation (Mukherjee *et al*., 2022). Also, the identification of the classes of bioactive compounds responsible for medicinal property of herbs, and standardizing herbal formulations for therapeutic use can also be accomplished by such approaches (Paul & Kumar, 2024). Myrtle contains various phytochemicals such as flavonoids, tannin, terpenoid, alkaloid, phenolic acids, and essential oils. It is well known that flavonoids, and tannins are present in leaves and berries having strong antioxidant, anti-inflammatory, and antimicrobial properties. The terpenoids are the major constituents of essential oil extract from Myrtle plant, and responsible for its characteristic fragrance, and antimicrobial activity (Masyita *et al*., 2022; Usai *et al*., 2018).

However, the phytochemical composition of Myrtle contain a high amount of bioactive compounds. The current review reported that a total of 60 compounds are present in Myrtle plant, and out of these 12 classes are volatile organic compounds such as phenols (20%), alcohols (18%), VOCs (15%), terpenoids (14%), fatty acids (7%), sterols (6%), glycosides (5%), carboxylic acids (5%), flavonoids (4%), cinnamic acid derivatives (3%), and nitro compounds (3%).The pharmacological activities of the plant such as antimicrobial, antioxidant and anti-inflammatory effects are due to these compounds. The role of Myrtle in herbal medicine is important with regards to the bio-active molecules that are used in herbal medicine, and also in modern pharmacological drugs (Figure. 5; Table 3; Dabbaghi *et al*., 2023; Shahbazain *et al*., 2022).

The figure.4 presents a comparative analysis of the number of phytochemical studies on Myrtle (dark teal), alongside the number of diseases investigated in these studies (light gray).The India ranks first with about 45 studies addressing 15 diseases, followed by Italy (40 studies, 13 diseases), Iran (35 studies, 14 diseases), and Pakistan (30 studies, 12 diseases). However, Turkey (28 studies, 10 diseases), Greece (25 studies, 9 diseases), France (22 studies, 8 diseases), and Egypt (18 studies, 7 diseases) complete the list. Overall, a higher volume of studies generally correlates with a wider spectrum of disease coverage (Al-Maharik *et al*., 2023; Rashid, 2021).

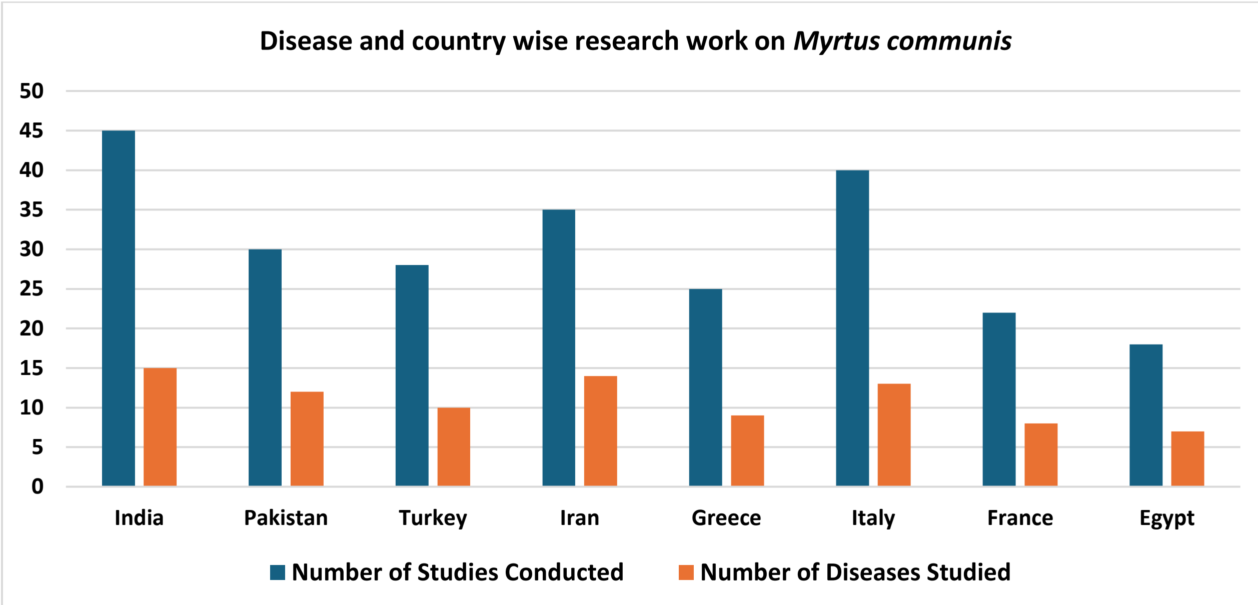

Figure 4. Present research on *Myrtus communis* categorized by diseases and countries.

Gallic Acid in *Myrtus communis* L.

Gallic acid is one of the major phenolic compound of Myrtle plant, where it is found mainly in the leaves and berries. Its IUPAC name as 3, 4, 5 trihydroxybenzoic acid with a chemical formula C_6H_2 $(OH)_3$ COOH. Myrtle has a strong antioxidant, antiviral, and antifungal properties due to gallic acid, which is very important in pharmacological potential of the plant. Although the total phenolic content is very widely determined with different plant extracts, Folin-Ciocalteu assay is usually employed as standard. In topical gallic acid shows anti-inflammatory properties, therefore, it is effective for the treatment of skin conditions. It is also an inhibitor of weak carbonic anhydrase, a cell metabolism, and pH regulating enzyme (Nikolaeva *et al*., 2022).

Tannic Acid in *Myrtus communis* L.

Tannic acid is a bioactive compound present in the leaves, and bark of Myrtle plant. Its chemical formula is $C_{76}H_{52}O_{46}$. It is widely used in the textile industry for dyeing of cotton, and other cellulose fibers, for improving acid dye wash fastness of polyamide fabrics, and for improving chlorine fastness in the textile processing. In addition, tannic acid acts a metal passivation by preventing corrosion of the ferrous materials (Wu *et al*., 2021). It is also used as a natural clarifying agent, as a modifier of bitterness, flavor, aroma enhancer, and as a colour stabilizer in juices, soft drinks, beer and wine production. Tannic acid is medicinally used in treating burns, injuries in extreme cases, and lowered the mortality rate (Baldwin & Booth, 2022). Tannic acid is widely use to remove poisonous from toxic substances such as poisonous mushrooms. It is also an important pharmaceutical ingredient for the preparation of albumin tannate, antihistamines, anti-diarrheal drugs, and cough syrup (Matsehorova & Odyntsova, 2024).

Myrtenyl Acetate in *Myrtus communis* L.

Myrtenyl Acetate is a bioactive compound present in Myrtle plant. Its chemical formula is $C_{12}H_{18}O_2$. Previously, it was popular for its antibacterial activity, and inhibit the growth of bacteria, also possess germicidal effects like the known antibacterial properties. It is confirmed to have antifungal activity, because it inhibits such enzymes which are involved in the metabolism, and growth of fungi (Alyousef, 2021). It is also an antiviral activity against hepatitis viruses, and mild inhibitory activity on HIV replication against HIV-1 reverse transcriptase. However, myrtenyl acetate has cardio protective action, and there is potential for employment as an agent in the treatment of arrhythmia by means of its capability to influence cellular signaling pathways in cardiac tissues (Tian *et al*., 2020).

Catechin in *Myrtus communis* L.

Catechin is present in leaves, and berries of Myrtle plant. Its chemical formula is $C_{15}H_{14}O_6$, and an agent which prevents the conversion of histidine to histamine, the compound which disturbs the immune system. Catechin has the power to reduce allergic reactions, and inflammatory conditions. Additionally, catechin possesses neuro-protective properties, and is therefore a good therapeutic candidate for either cognitive loss caused by HIV, Alzheimer disease, or Parkinson disease. The previous research has suggested that the protective effects of epicatechin, and other catechin flavonoids are mediated against neurotoxic oxidative stress, a feature that is also a major characteristic of neurodegenerative diseases. The epicatechin is effective in crossing the blood brain barrier, is more effective than resveratrol, and able to enhance neuronal survival, and neurotropic factor pathways. Further, (+)-catechin and epicatechin are also monoamine oxidase inhibitors and have the potential for regulation of dopamine, and serotonin levels, therefore, may act as drugs for the treatment of neurodegenerative disorders (Afzal *et al*., 2022).

Sterol in *Myrtus communis* L.

The most important phyto-sterol in Myrtle is β-sitosterol, especially present in leaves, and fruits of the plant. Its chemical formula is $C_{29}H_{50}O$. The element appears as a waxy white powder whose odor is characteristic, and it is hydrophobic in nature. It is well known that β–sitosterol reduces blood cholesterol levels, and is therefore an important ingredient in hypercholesterolemia treatment. It is used for preventing cholesterol absorption in the intestines thereby helping in lipid metabolism regulation. β-sitosterol is used as a part of herbal medicine for management of benign prostatic hyperplasia (BPH), for improvement of urinary flow, and reduction of BPH symptoms enlargement of the prostate (Zio *et al*., 2024; Shahbazain *et al*., 2022; Usai *et al*., 2018).

Flavonoids in *Myrtus communis* L.

Myrtle leaves, and berries are a good source of flavonoids and the chemical formula of flavonoids is $C_{15}H_{14}O_5$. Myrtle acts as an antioxidant due to the presence of flavonoids including afzelechin, quercetin 3-o-β-D xylopyranoside, and quercetin 3-o-α-L-arbinofuranoxide, and it has anti-inflammatory properties. The quercetin flavonoid have antioxidant, antiradical, and iron-chelating abilities. The antioxidant property of it protects the cells from oxidative stress, neurodegenerative diseases, and cardiovascular diseases. Quercetin is often recommended for allergy relief, immune support, and cardiovascular health, because of the ability to stabilize mast cells, and regulate inflammatory responses (Shahbazain *et al*., 2022; Sumbal *et al*., 2011).

Fatty Acids in *Myrtus communis* L.

Myrtle has fatty acids including decanoic acid with chemical formula is $C_{10}H_{20}O_2$), and nonionic acid with chemical formula is $C_9H_{18}O_2$. The antimicrobial activity, and antifungal properties of decanoic acid have been studied extensively, and this has made it one of the key components of essential oils extracted from the plant (Hou *et al*., 2022). Meanwhile, nonanoic acid has the property of herbicide which means it can be used in natural pesticide. These fatty acids are responsible for the protective effect, and therapeutic effect of Myrtle especially the defenses of Myrtle against antimicrobial activity (Dabbaghi *et al*., 2023; Usai *et al*., 2018).

Terpenes in *Myrtus communis* L.

Myrtle is a very rich plant in terpenes, mainly present in this case as an essential oil in the leaves, and berries. The terpenes are limonene with chemical formula is $C_{10}H_{16}$, and linalool with chemical formula is $C_{10}H_{18}O$. They have therapeutic, chemotherapeutic, and antiseptic activities. However, Myrtle also has the other important sesquiterpene, and β-caryophyllene with chemical formula is $C_{15}H_{24}$. These compound has woody, and spicy aroma can be used in cosmetics. Further, it has also anti-inflammatory, and neuro-protective features. Terpineol is classified as a chemical terpenoid that can be found in Myrtle. This compound is used as a muscle spasm reliever, and respiratory drugs being an antispasmodic (Shahbazain *et al*., 2022; Usai *et al*., 2018).

Table 3. IUPAC names of various phytochemicals along with their activities

Phytochemical	IUPAC Name	Activity
Tannic acid	2,3-dihydroxy-5-({[(2R,3R,4S,5R,6R)-3,4,5,6-tetrakisphenyl3,4,5-trihydroxybenzoate({3,4-dihydroxy-5-[(3,4,5-trihydroxyphenyl) carbonyloxy]phenyl}carbonyloxy)oxan-2-yl] methoxy}carbonyl)phenyl 3,4,5-trihydroxybenzoate	Antifungal,Antiviral, Cytotoxicity, Antioxidant
Gallic acid	3,4,5-Trihydroxybenzoic acid	Histidine decarboxylase-inhibitor
Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	Antimicrobial, Antioxidant
(−)-3-O-galloyl-catechin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	Anti-inflammatory, Neuroprotective
(−)-3-O-galloylepi-catechin	2-(3,4-dihydroxyphenyl)-4-[2-(3,4-dihydroxyphenyl)-4-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-8-yl]-5,7-dihydroxy-3-(3,4,5-trihydroxybenzoyloxy)-3,4-dihydro-2H-1-benzopyran-6-yl]-5,7- dihydroxy-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	Antibacterial, Antifungal
Gallicin	3,4,5-trihydroxybenzoic acid	Antimicrobial, Antifungal

β-Sitosterol	17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	Cholesterol inhibition
Arbutin	(2R,3S,4S,5R,6S)-2-Hydroxymethyl-6-(4-hydroxyphenoxy)oxane-3,4,5-triol	Prevent melanin formation
Leucoanthocyanidin	2-phenyl-3,4-dihydro-2H-chromene-3,4-diol	Anti-inflammatory, Antispasmodic
Limonene	1-Methyl-4-(1-methylethenyl)-cyclohexene	Antibacterial, Antifungal
Linalool	3,7-dimethylocta-1,6-dien-3-ol	Antiseptic, Chemotherapeutic agent
α-Terpineol	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	Myorelaxant Antispasmodic effects
Quercetin 3-o-β-D xylopyranoside	3-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-2-α-L-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	Anti-leishmanial, Anti-oxidative,

Sources: (Nikolaeva *et al*., 2022; Wu *et al*., 2021; Alyousef *et al*., 2021; Afzal *et al*., 2022).

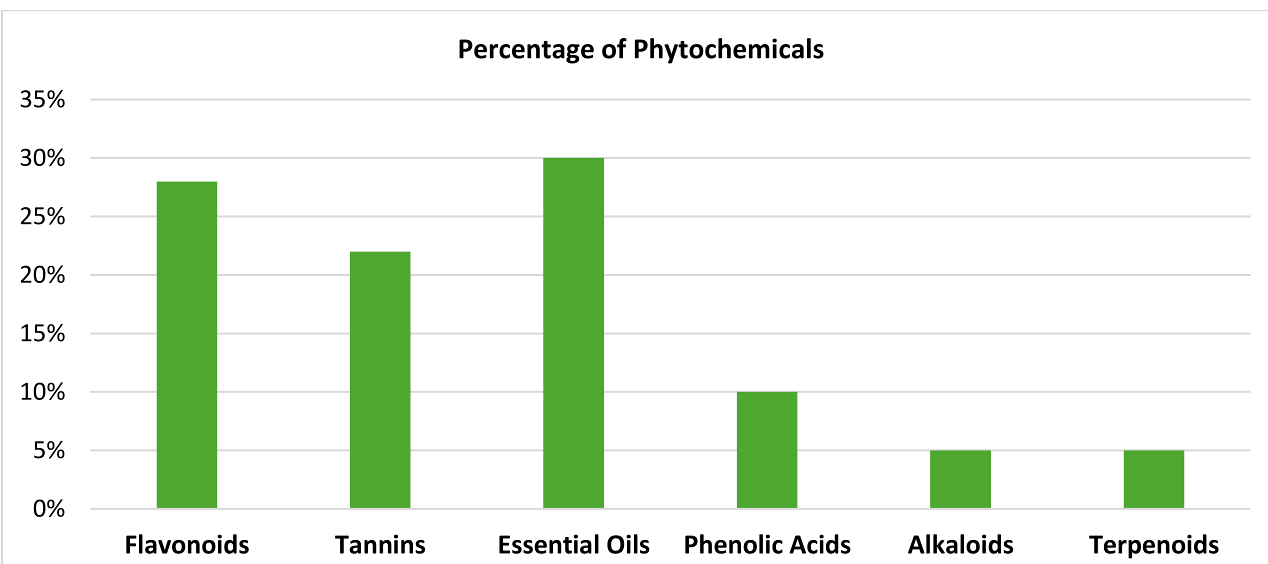

Figure 5. Percentage distribution and number of identified phytochemicals.

Pharmacology

Anti-Diabetic activity of *Myrtus communis* L.

The previous studies proved that Myrtle plant is anti-diabetic property that regulates glucose, and metabolic health of the body. It simply means that it inhibits the actions of the α-glucosidase, and the α-amylase enzymes that cause the synthesis of glucose from the complex carbohydrate. The 50% of aqueous-methanol extract of Myrtle contained two active compounds (−)-3-O-galloylcatechin, and (−)-3-O-galloylepicatechin that inhibited rat intestinal α-glucosidase, and porcine pancreatic α-amylase in a concentration depended manner (Giampieri *et al*., 2020). However, rats were rendered diabetic by a single administration of streptozotocin (STZ), which produced peak blood glucose levels after 24 hours. In order to evaluate the hypoglycemic potential of various Myrtle extracts, diabetic rats were treated with ethanol, aqueous chloroform, ethyl-acetate, hexane, and butanol fractions. The ethanolic extract lowered blood glucose by 70.13%, while the aqueous extract produced a similar reduction of 71.34%.The chloroform extract also demonstrated significant activity, reducing blood glucose levels by 42.23%. The management of the ethyl acetate extract decrease or rise glucose level from 443.0 ± 22.3 mg/dL to a substantially lower concentration, although the reduction was less pronounced than that seen with the ethanol, and aqueous extracts. In contrast, neither the hexane nor the butanol extracts elicited a notable hypoglycemic effect (Table. 4; Alipour & Hosseinzadeh, 2014).

Further, besides in vitro studies are carried out to study the effect of ethyl acetate soluble extract of Myrtle on rat intestinal maltase activity which showed inhibition of rat intestinal maltase activity in a dose dependent manner, and two active compounds which also in vitro confirm reduced rat intestinal maltase, acetic acid activity, and porcine pancreatic α-amylase activity in a dose dependent manner. Therefore, these results show that Myrtle could be developed as a natural agent for type two diabetes treatment (Yaman *et al*., 2020).

Anticancer activity of *Myrtus communis* L.

The extract of Myrtle plant such as methanol, and aqueous extracts have a strong ability of anti-cancer. The cytotoxicity is necessary and dependent on the concentration for the development of chemotherapy drugs. Myrtle berries, seeds, leaves, and essential oils have some undiscovered sources of natural compounds with promising health benefits. For example, crude extracts having an IC50 value below 30 μg/ml are regarded as a potential anticancer agent by American National Cancer Institute. This range for IC50 values for the Myrtle extracts are promising for further studies in the field of oncology (Ibrahim *et al*., 2021; Giampieri *et al*., 2020).

Antitussive activity of *Myrtus communis* L.

Myrtle extracts have demonstrated significant, dose-dependent antitussive effects in animal models. In one study, mice were induced to cough using sulfur dioxide gas, when the methanolic extract of Myrtle was administered orally, it inhibited the cough reflex in a concentration-dependent manner. Precisely, a 100 mg/kg dose produced approximately 28.7 % inhibition of coughing, increasing to 33.9 % inhibition at 200 mg/kg, and reaching 44.2 % inhibition at 300 mg/kg. Though, this antitussive activity approaches that of codeine phosphate a well-known cough suppressant indicating that Myrtle is not merely a mild traditional remedy, but exerts a robust cough-suppressing action comparable to standard pharmacological agents. These findings validate its traditional use for respiratory infections, and support further investigation into its mechanism of action, and potential development as a natural antitussive drug (Abidi *et al*., 2021).

Table 4. Pharmacological activities of the plant, parts used, experimental models, and study designs, tested doses, and key observations.

Pharmacutical activity	Parts used	Model used	Tested dose	Observations
Anti-Bacterial	Leaves	Disc diffusion method by using methanol extract	200-1000µg	Significant antibacterial efficacy against bacteria i.e., *S. aureus, E. coli*, and *P. aeruginosa*
Anti-Bacterial	Essential oil	Diffusion method by using essential oil	10, 25, or 50 mg/ml	Effective against gram positive, and gram negative bacteria
Anti-Bacterial	Roots extract	Zone of inhibition assay against *E. coli* and *P. aeruginosa*	5 mg/ml	Zone of inhibition: 12mm–20mm (*P. aeruginosa*), and 6mm–8mm (*E. coli*)
Anti-Fungal	Leaves and roots extract	Extract activity against *Candida albicans* and *Aspergillus niger*	2 ml	Leaves effective against *Microsporum canis* (12mm inhibition)
Anti-Fungal	Essential oil	Minimum inhibitory concentration (MIC) test	10 mg/ml	*The antifungal activity was expressed by Afzelechin (7 ± 0.3 mm).*
Anti-Fungal	Whole plant extract	Ethanol and aqueous extracts against fungal strains	5 mg/ml	Moderate antifungal activity observed in terpenoids and flavonoids

Sources: (Abidi *et al*., 2021; Ibrahim *et al*., 2021; Giampieri *et al*., 2020; Yaman *et al*., 2020).

Conclusion

The current review reports complete information about the folk uses, medicinal uses, phytochemicals, and pharmaceutical investigation of medicinal plant *Myrtus Communis* for the first time. It is found that this phenomenon shrub had been traditionally used amongst several communities throughout the world for skin, respiratory, gastrointestinal, inflammatory, urinary, cardiovascular, neurological, and microbial infectious diseases. Additionally, this plant is also well known to treat cuts, wounds, burns, boils, kidney stones, and kidney related disorders by local doctors. Though, nearly all parts of the Myrtle plant are used for the treatment of various disorders, so the most popularly used part is leaves, followed by flowers, stem, and roots. The plant are usually used as an herbal tea and powder form. The pharmaceutical investigation shows that the plant has potential of anti-fungal, anti-bacterial, anti-viral, antitussive, anti-inflammatory, and anti-cancer activities. However, the main phyto-chemical compounds are reported in this plant are phenols, alcohols, terpenoids, fatty acids, sterols, glycosides, carboxylic acids, flavonoids, cinnamic acid derivatives, nitro compounds and essential oils. The essential oils of Myrtle are important features which contains various compounds such as myrtenyl acetate, limonene, linalool, and α-terpineol, so have antimicrobial, and sedative properties.

Further, the occurrence of scientific evidence associated to phytochemicals, medicinal, and pharmaceutical uses many gaps is present related to the use of this plant. Firstly, research based evidences of traditional uses are reported by various researchers such as anti-fungal, anti-bacterial, anti-viral, antitussive, anti-inflammatory, and anti-cancer etc. But, there is a need of some experimental study in near future to treat skin, hair fall, dandruff, muscular, antidote, and ENT related disorders. However, there are some pharmaceutical activities are observed in-vivo, and in-vitro. Also, there are additionally clinical studies are necessary to conduct for future discovery of drugs in this plant. The results of various research or experiments in these zones will give definite support for further clinical use of Myrtle plant in modern medicines. There are further phytochemical examination may lead to the extension of therapeutically abilities of *Myrtus communis*. Further, on the basis of current review, we suggest that further detailed research should be carried out on the ethno-medicinal, ethno-pharmaceutical, and toxicological areas of Myrtle. These studies will provide important insight about the preparation of new modern drugs for the treatment of various disorders. Moreover, the plant need sustainable cultivation, and conservation for its availability in medicinal, and commercial applications.

Declaration

Acknowledgment: The author is grateful to the GDC Barang Bajaur for providing support, and facilities in the preparation of manuscript.

Funding: This study received no external funding.

Conflict of interest: The author declares no conflict of interest.

Ethics approval/declaration: No Applicable

Consent to participate: No Applicable

Consent for publication: The author has approved the manuscript to published.

Data availability Statement: Data sharing is not applicable to this review article, as the available data mentioned in main tables.

Authors contribution: Farman Ullah[1] and Tariq Jamal conceived the main idea, Farman Ullah[3], Waqar Ahmad and Asad Ullah designed the methodology, Muhammad Usama, Farman Ullah[3], and Kamran Khan helps in data collection, Abdul Basir and Kamran Khan design references, Farman Ullah[1] and Sammiya Jabeen, helps in scientific writing, critical review and approval of the final draft.

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https://doi.org/10.56556/jase.v4i1.1360

REVIEW ARTICLE

Climate change impacts on the agriculture sector of Khyber Pakhtunkhwa: a comprehensive review with comparative regional analysis

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Received: 14 April, 2025, Accepted: 16 June, 2025, Published: 29 June, 2025

Abstract
Climate change presents pressing challenges to Pakistan's environmental and socio economic, systems with the province of Khyber Pakhtunkhwa (KP) emerging as one of the most vulnerable regions. Scientific assessments indicate that climate-induced hazards such as riverine and urban flooding, glacial lake outburst floods (GLOFs), prolonged heatwaves, droughts, and the spread of vector-borne diseases, are projected to intensify in frequency and severity in the coming decades. In response to these emerging threats, Adaptation strategies are designed to minimize exposure and vulnerability of both human and ecological systems, while mitigation efforts focus on curbing greenhouse gas emissions through sustainable practices and technological innovation. Khyber Pakhtunkhwa (KP), a predominantly agrarian province of Pakistan, is increasingly vulnerable to the multifaceted impacts of climate change. This comprehensive review synthesizes historical climate trends and the growing frequency of extreme weather events-such as floods, droughts, hailstorms, heatwaves, and erratic rainfall-and their detrimental effects on agriculture. Using meteorological data, published studies, and government reports, the paper highlights region-specific vulnerabilities, including rising temperatures, pest and disease outbreaks, and shifts in cropping calendars. Graphical analyses and evidence-based assessments underscore the scale of agricultural losses and livelihood disruptions across KP's diverse agro-ecological zones. The review further compares these findings with trends in other provinces of Pakistan and neighboring countries like India, Afghanistan, and Iran, providing a broader regional perspective on climate impacts and adaptation practices. The paper concludes with a set of targeted recommendations, including climate-smart agriculture, adaptive crop zoning, efficient water use, and integrated pest management strategies, aimed at enhancing resilience and sustainability in the province's agriculture sector.

Keywords: Climate change and agriculture; extreme weather events; climate-smart agriculture; pest outbreaks; adaptation strategies

Introduction

Agriculture remains a cornerstone of Khyber Pakhtunkhwa's (KP) economy, contributing significantly to the province's economic and social fabric. Engaging over 40% of the labor force, agriculture is not only the primary livelihood for a large portion of the population but also a vital sector for ensuring food security and poverty alleviation in the region (GoKP, 2020). In terms of its contribution to the provincial Gross Domestic Product (GDP), agriculture accounts for roughly 20%, with smallholder farming systems being the dominant structure of land use. These smallholder farms are primarily engaged in the cultivation of staple crops such as wheat, maize, rice, and various fruits and vegetables. Despite its importance, the sector faces numerous challenges, with climate change emerging as one of the most significant threats to its sustainability and productivity.

In recent decades, climate change has had a profound impact on the agro-ecological landscape of KP, making the region increasingly vulnerable to extreme weather events. These include intense and frequent floods, such as those experienced in 2010 and 2022, as well as prolonged droughts, sudden hailstorms, and more frequent heatwaves. Alongside these, rising temperatures are becoming a critical concern for agriculture in KP, directly affecting crop productivity, soil health, and the availability of essential resources like water. A report by the Intergovernmental Panel on Climate Change (IPCC, 2021) has highlighted the significant role of rising temperatures in exacerbating agricultural risks, with particular emphasis on the vulnerability of developing regions like KP. According to Rasul et al. (2012), the average temperature in KP has risen by approximately 0.6°C since the 1960s, with a more pronounced rate of warming observed in the past two decades. This increase in temperature has been accompanied by erratic rainfall patterns and heightened climate variability, which in turn affect the sowing and harvesting periods of key crops. The onset of rains is often unpredictable, and severe rainfall events can lead to flash floods, damaging crops and reducing yields. Additionally, hailstorms during critical growth stages of crops have led to substantial losses, further threatening the food security of the region. The effects of these climatic shifts are not only environmental but also socio-economic. KP's agricultural sector is characterized by a high level of socio-economic dependence on farming, with many rural households reliant on agriculture for both subsistence and income. However, the capacity of farmers to adapt to these changing climatic conditions is limited, due to a lack of resources, knowledge, and access to adaptive technologies. As a result, the agricultural sector in KP remains highly susceptible to climate-related risks, with little resilience to the escalating challenges posed by climate change. The province's vulnerability is compounded by the physical risks associated with extreme weather events, such as soil erosion, water scarcity, and crop failure. Moreover, the socio-economic impacts of climate change exacerbate existing inequalities within rural communities, where smallholder farmers are often the most affected due to their limited access to insurance, modern technologies, or alternative livelihoods. This makes it increasingly important to implement strategies that not only mitigate the effects of climate change on agriculture but also enhance the adaptive capacity of local farmers. Climate change is an urgent and growing challenge for the agricultural sector in Khyber Pakhtunkhwa. Its impacts are multifaceted, affecting not only crop yields and agricultural productivity but also the socio-economic well-being of rural communities. Understanding the nature of these risks and developing adaptive strategies are critical to securing the future of agriculture in the region and ensuring food security for the growing population.

Review Methodology

Past Climate Trends and Historical Damages in Khyber Pakhtunkhwa (KP)

Khyber Pakhtunkhwa (KP), Located in the northwestern region of Pakistan, is characterized by remarkable topographical diversity. The northern, northwestern, and eastern areas of the province are home to some of the world's most prominent mountain ranges, including the Hindu Kush, Himalayas, and Karakoram. In contrast, the southern parts of KP are defined by expansive plains and central valleys, which support extensive agricultural activities and rangelands. The province experiences a wide range of climatic conditions due to its varied geography (Table 1). The northern districts endure harsh winters with substantial snowfall, cooler summers, and significant rainfall, while the southern regions experience relatively mild winters, warmer temperatures, and moderate precipitation. Chitral, situated at the highest elevation in the province, records some of the coldest winter temperatures and contains numerous glaciers. Meanwhile, Dera Ismail Khan (D.I. Khan), located in the far south, benefits from a milder winter climate and favorable conditions for crop cultivation and grazing, making it a hub for agriculture and pastoral activities.a province located in the north-western part of Pakistan, has experienced significant climatic shocks in the past few decades (Fig 1).

Table 1: Categorization of Land Use/Land Cover in KP Province

Land Use	Area (ha)	Area (%)
Water	73,254	0.72
Forest/Tree-Cover	842,415	8.28
Crops	1,177,143	11.57
Built-Up Area	605,359	5.95
Bare Ground	1,031,654	10.14
Snow/Ice	371,355	3.65
Rangeland	6,072,920	59.69

Source: ESRI Land Use-Land Cover Classification, 2021

These extreme weather events have caused devastating impacts on agriculture, livelihoods, and infrastructure, underscoring the growing vulnerability of the region to climate change.

Figure 1: Geographical Map of Khyber Pakhtunkhwa Province, Pakistan

Climate Hazards in Khyber Pakhtunkhwa

Climate change continues to exert profound effects across various regions of the globe. The Intergovernmental Panel on Climate Change (IPCC), in its Sixth Assessment Report (AR6, 2021), underscores the intricate links between climate change and its widespread consequences for both natural ecosystems and human societies. Growing scientific consensus attributes shifts in weather patterns, accelerated glacial retreat, the occurrence of Glacial Lake Outburst Floods (GLOFs), disruptions in hydrological systems, alterations in species migration, and changes in agricultural productivity directly to climate variability. These changes increasingly jeopardize traditional food production systems. The report also projects a significant rise in the frequency and duration of heatwaves across Asia, coupled with a decline in cold days and nights, and a corresponding increase in warmer extremes. These developments highlight the ongoing destabilization of ecological systems and reaffirm the interconnectedness and vulnerability of human life to environmental fluctuations (IPCC, 2021).

In recent years, the effects of climate change have become more pronounced, evident in the warming of the atmosphere and oceans, rising sea levels, and the heightened concentration of greenhouse gases. There is now overwhelming evidence confirming the reality and progression of climate change. Moreover, its impacts have amplified the severity of natural hazards. Increasing episodes of intense rainfall and elevated surface temperatures are altering long-standing weather patterns, making climate-related disasters more frequent and destructive, particularly in already vulnerable regions. Natural hazards—such as earthquakes, floods, droughts, landslides, hurricanes, and volcanic eruptions—pose significant risks, especially in geographies with limited adaptive capacities. Without adequate risk reduction strategies, mitigation efforts, and adaptive responses, these hazards can escalate into full-blown disasters. In Khyber Pakhtunkhwa (KP), the risks associated with climate change are particularly acute. The province faces a wide array of climate-induced threats, including glacial melt, drought, flooding, GLOFs, strong winds, avalanches, landslides, locust infestations, dengue outbreaks, and smog. Data from the National Disaster Management Plan (NDMP, 2012) identifies district-level vulnerabilities across KP. However, these risks demand updated assessments based on simulation modeling and forward-looking projections to guide policy and preparedness efforts more effectively.

The 2010 Catastrophic Floods

Khyber Pakhtunkhwa is home to a complex network of rivers, many of which eventually merge with the Indus River that flows through the province. The prominent rivers traversing the region include the Indus, Kabul, Swat, Kurram, and Gomal rivers. Collectively, the Indus River system and its tributaries carry an estimated annual flow of 154 million acre-feet (MAF) of water. This volume comprises approximately 145 MAF from the northwestern rivers such as the Indus, Jhelum, and Kabul, along with several smaller tributaries and around 9 MAF from the eastern rivers, mainly the Ravi and Sutlej (EPA, Government of Khyber Pakhtunkhwa). One of the most catastrophic climate-related events in recent history, the 2010 floods, affected over 1.6 million people in KP and caused severe damage to the region's agricultural landscape. The floods submerged approximately 0.7 million hectares of farmland, including staple crops like wheat, maize, and rice, as well as fruit orchards (NDMA, 2011). The extensive damage to crops, livestock, and infrastructure left communities struggling to recover, with many households unable to rebuild their livelihoods in the short term. The floods also washed away topsoil, reducing the productivity of affected lands for years to come (Ali et al., 2012). Due to the extensive river systems and mountainous terrain, the Khyber Pakhtunkhwa region is highly susceptible to flash flooding during the monsoon season. Over the past decade, flood frequency has significantly increased, with heavy precipitation events occurring almost annually. In 2007, Kohistan was severely affected by flooding caused by intense rainfall and glacial meltwater. The situation escalated in 2010, when Khyber Pakhtunkhwa experienced its worst flood in recent history, resulting in the displacement of hundreds of thousands of people and extensive damage to infrastructure. In the subsequent years 2011 through 2015 the region continued to witness recurrent flooding, albeit with varying intensities (Fig 2). These repetitive flood events compounded the challenges of recovery, as rehabilitation from previous disasters remained incomplete, thereby increasing both the human and economic toll. In 2010, data from OCHA reported that approximately 4.7 million individuals were impacted by flooding in Khyber Pakhtunkhwa. One of the most catastrophic glacial lake outburst flood (GLOF) events occurred in 2015, severely affecting around 321,644 people in Chitral and resulting in the loss of 1,200 acres of standing crops (PDMA, 2015). In recent years, additional destructive GLOF incidents have been recorded in the Golen Gol and Reshun Valleys. The Himalayan moist temperate forest zones, located at elevations ranging from 1,525 to 3,660 meters, remain particularly vulnerable to recurring flood risks. Due to the ongoing impacts of climate change, these regions particularly the lower Kaghan Valley, Galiat, and southern Kohistan are expected to witness more frequent and intense precipitation events. As such, implementing strategic mitigation and adaptation measures is essential to reduce the risk and impact of future floods in Khyber Pakhtunkhwa.

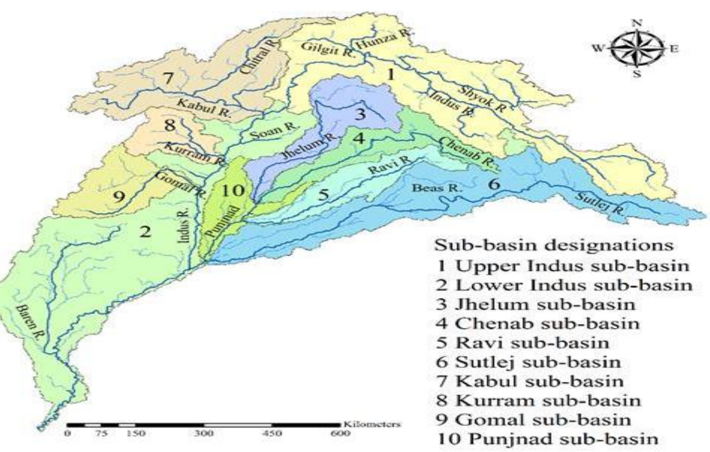

Figure 2: River system of Indus Basin (Mukhopadhyay and Khan,2016)

The 2015 Heatwave

In 2015, a severe heatwave swept through the lowland districts of KP, particularly Dera Ismail Khan (D.I. Khan), where temperatures exceeded 45°C for several consecutive days. This extreme heat led to a significant reduction in wheat yields by 15-20%, particularly in areas dependent on rain-fed agriculture. The heatwave exacerbated water scarcity and negatively impacted both crop productivity and livestock health, leading to financial losses for farmers (Khattak et al., 2017).

Hailstorms (2017-2021)

Between 2017 and 2021, KP experienced a series of hailstorms that caused extensive damage to fruit orchards, especially in the Swat, Dir, and Charsadda districts. These storms destroyed standing fruit crops such as apples, peaches, and plums, as well as vegetables, leading to significant economic losses for smallholder farmers. The increasing frequency of hailstorms during critical flowering and fruit-setting periods has raised concerns about the resilience of the province's horticulture sector to such extreme weather events (Raza et al., 2020).

Drought Periods (2002-2005 and 2016-2020)

KP has also been affected by recurring droughts, with two notable drought periods in recent history: one from 2002 to 2005 and another from 2016 to 2020. These droughts, particularly in the southern districts of KP such as D.I. Khan, Bannu, and Tank, have led to the failure of rabi (winter) crops like wheat and barley, resulting in food insecurity and livestock losses (Fig 3). Water shortages during these dry periods have further exacerbated the situation, with farmers facing difficulties in accessing sufficient irrigation for their crops. The prolonged dry spells have also led to the depletion of water tables and a reduction in the recharge rates of groundwater sources (Hussain et al., 2021). Droughts in the southern parts of Khyber Pakhtunkhwa are more common as compared to the northern parts. The central valley plain, Piedmont plain and Suleiman Piedmont are areas dedicated mostly to agriculture. The total cultivated area in Khyber Pakhtunkhwa is about 1.65 million ha. This signifies the magnitude of impact of a drought on food security in the province.

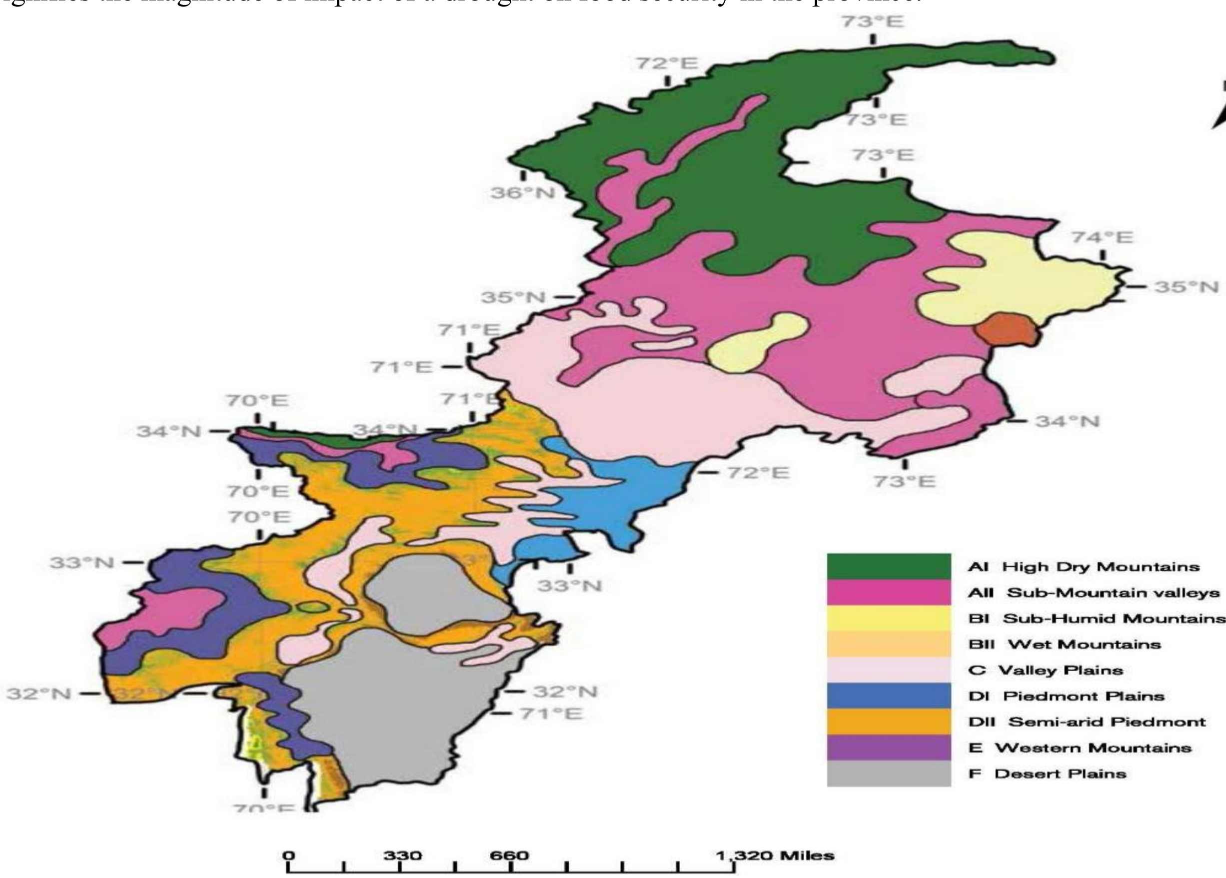

Figure 3. Distinct Agro-Ecological Regions Across Khyber Pakhtunkhwa Province (Data Source: Nizami et al., 2020)

With rising temperatures and decrease in rainfall in dry regions, particularly in southern parts, are most likely going to be water stressed region in the coming years. The climate ranges from warm and sub-humid in valley plains to hot and arid southern parts. Dera Ismail Khan is the area which will be most prone to droughts. Although, drought is a less common hazard compared to floods, the implications on food security cannot be ignored. Deserts in southern parts like Karak, Lakki Marwat and DI Khan may expand in case of prolonged droughts.

The southern parts of the province receive less rainfall and higher temperatures in the summer and drier winters (Fig 4). Climate forecasts predict that dry regions will get drier and wet regions will get wetter. As these areas are drier especially during the winter and hot during the summers there is an expected increase in average temperatures in the summer. These high temperatures may be optimal for some crops to cultivate but high temperatures will increase evapotranspiration which in turn increases the demand of crops for water. Therefore, the quantity and availability of groundwater and irrigation water will be vital in determining water stress in the area.

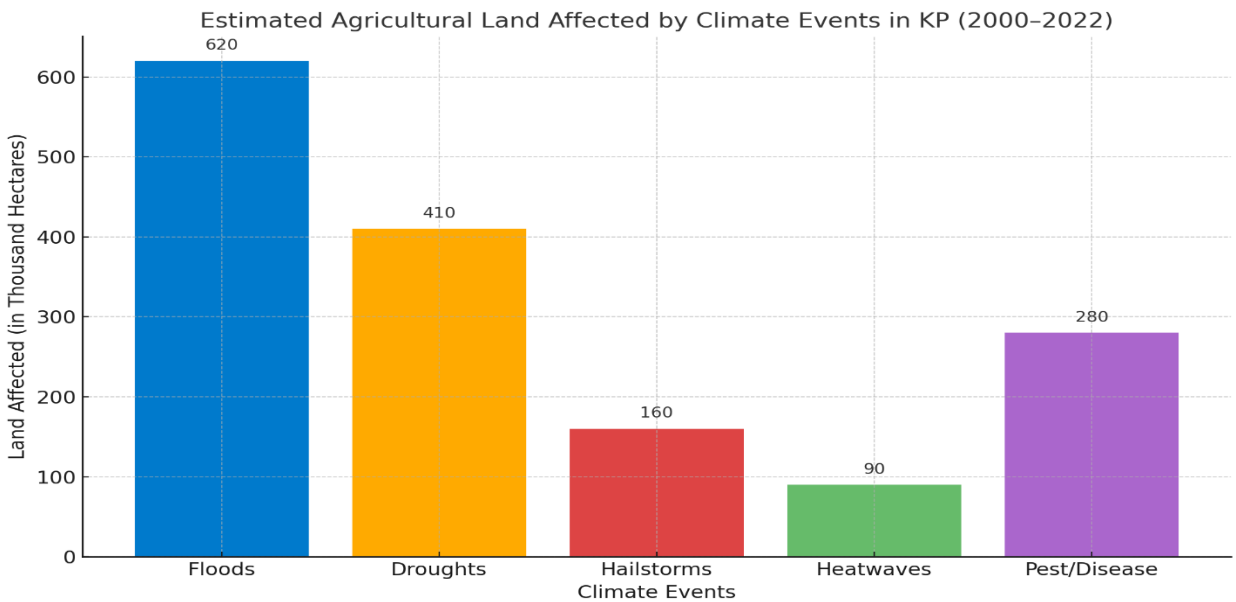

Figure 4: Agricultural land affected by different climate events in KP (2000-2022)

This figure illustrates the hectares per year of agricultural land affected by various climate events such as floods, droughts, hailstorms, heatwaves, and rainfall anomalies. The graph highlights the increasing frequency of extreme weather events over the past two decades, reflecting the growing vulnerability of the agricultural sector in KP.

Temperature Rise and its Impact on Agriculture

The rising temperatures in KP are altering the growing seasons for key crops, resulting in reduced productivity and increased pressure on water resources. Research has shown that higher summer temperatures, particularly in districts like Charsadda and Mardan, have shortened the growing seasons for crops like wheat and maize, leading to yield reductions of 10-15% (GOP-MinFA, 2020). The early grain filling due to warmer temperatures reduces the time available for crops to reach full maturity, negatively impacting overall yields.

In horticulture, particularly in the cooler regions of KP, warmer winters have disrupted fruit-setting in crops like apples, peaches, and plums. These crops, which typically require cold temperatures for proper dormancy and subsequent flowering, have seen a decline in fruit-setting due to the warmer-than-normal winter temperatures. As a result, the productivity of fruit orchards in regions like Swat and Abbottabad has been compromised (Shah et al., 2018).

Erratic Rainfall and Flash Flooding

The variability in rainfall patterns in KP has led to both prolonged droughts and devastating flash floods. In the spring, irregular rainfall patterns often result in water deficits that hinder maize sowing, one of the key crops in the region. On the other hand, summer rainfall can be erratic, leading to flash floods that cause soil erosion, wash away seeds, and damage infrastructure.

For example, flash floods in Upper Kohistan and Malakand in 2022 resulted in landslides that destroyed vegetable terraces, leading to the displacement of farming families and a loss of agricultural production. Flash floods are especially problematic in mountainous areas, where steep slopes increase the risk of landslides and erosion (Javed et al., 2022).

Drought and Water Scarcity

KP's agriculture, particularly in areas like D.I. Khan, Bannu, and Tank, is heavily dependent on rain-fed irrigation systems. However, chronic drought conditions, exacerbated by lower-than-normal snowfall and reduced rainfall, are leading to significant water shortages. The FAO’s drought risk map (2022) highlights southern KP as one of the most climate-vulnerable regions in Pakistan, with low rainfall and rising temperatures causing a steady decline in available water resources. As groundwater levels drop, small-scale farmers who rely on tube wells face increasing costs, as the electricity required to pump water becomes unaffordable (FAO, 2022).

Hailstorms and Heatwaves

Hailstorms have become an increasingly frequent occurrence in KP, particularly in the spring and pre-harvest periods. These storms, which affect regions such as Swabi, Haripur, and Dir, have caused considerable damage to wheat fields and fruit orchards. In addition, heatwaves during the summer months, especially from May to June, have led to crop stress, including flower abortion in crops like tomato and capsicum. In 2021, Peshawar experienced a heatwave where temperatures exceeded 45°C, resulting in severe damage to vegetable crops (Saeed et al., 2021).

Pest and Disease Incidence in a Changing Climate

Climate change is contributing to the intensification of pest and disease pressures in KP, altering the epidemiology of various plant diseases. Warmer temperatures and increased humidity are favorable conditions for pests like fruit flies, armyworms, and whiteflies, which are emerging earlier in the growing season and in greater numbers. For example, tomato leaf curl virus and maize stalk borer are spreading to regions where they were not previously a significant concern (Ali et al., 2019).

In fruit crops like apples and peaches, fungal diseases such as powdery mildew and fire blight are becoming more prevalent, particularly in the cooler districts of Swat, Mansehra, and Chitral. These diseases thrive under the increasingly warm and moist conditions created by climate change, posing a significant threat to the province’s fruit industry (Yousaf et al., 2020).

Vulnerability Across Agro-Ecological Zones

KP is home to diverse agro-ecological zones, each facing unique vulnerabilities due to climate change:

Mountainous Zones (Swat, Chitral, Dir): These areas are prone to glacial lake outburst floods (GLOFs), late frosts, and the resurgence of pests that thrive in the changing climate (Khan et al., 2020).

Central Plains (Mardan, Charsadda, Swabi): These zones are primarily affected by erratic rainfall, rising pest populations, and shorter growing seasons due to increased temperatures (Raza et al., 2020).
Southern Semi-Arid Zone (D.I. Khan, Tank, Bannu): These regions are highly vulnerable to drought due to their dependence on rain-fed agriculture, low soil organic matter, and limited water resources. As temperatures rise and precipitation becomes more erratic, the vulnerability of these areas is further compounded (Iqbal et al., 2021).

Comparative Analysis: Other Provinces and Neighboring Countries9.1 Punjab and Sindh

Punjab: Punjab, as Pakistan's agricultural heartland, is experiencing similar climatic pressures to Khyber Pakhtunkhwa (KP) but with distinct challenges (Table 2). The temperature increase in Punjab over the last five decades is approximately 1.2°C, slightly higher than KP's rate of warming (Pakistan Meteorological Department [PMD], 2023). This increase in temperature has been particularly detrimental to crops like wheat, rice, and cotton, which are highly sensitive to heat stress. For instance, prolonged heatwaves during critical flowering and grain-filling stages have reduced wheat and rice yields by up to 15-20% (Hassan et al., 2022).
Additionally, water scarcity is a pressing issue in Punjab, linked to the declining flow of the Indus River, which is exacerbated by reduced snowmelt from the Himalayas due to climate change. Water shortages have led to inefficient irrigation practices, impacting both crop yield and soil health. Groundwater depletion is another issue, with farmers increasingly relying on tube wells, contributing to rising salinity levels and diminishing agricultural productivity.

Sindh: Sindh's agricultural sector faces several climate-induced challenges, most notably recurring floods, sea intrusion in coastal districts, and salinity problems (Table 2). The province's coastal areas have seen significant damage due to seawater intrusion, which has destroyed large swathes of farmland. A 2021 study by WWF-Pakistan revealed that over 15% of the cropped area in Sindh's coastal zones was lost due to saltwater encroachment, exacerbated by rising sea levels and climate change (WWF-Pakistan, 2021). Flooding events, such as the 2010 super floods, have become more frequent and intense, damaging crops, infrastructure, and homes.
These changes in the climate are reshaping agriculture in Sindh, reducing yields of major crops like rice, wheat, and cotton, and leading to significant socio-economic displacement. In response, Sindh has been focusing on adaptive strategies like salt-tolerant crop varieties and improving flood-resilience infrastructure.

Baluchistan: Baluchistan, Pakistan's most arid province, faces some of the harshest climate conditions in the country, with limited rainfall and insufficient irrigation infrastructure (Table 2). The province has been severely affected by prolonged droughts, especially between 1999-2003 and 2016-2020. These droughts have led to significant losses in agriculture, particularly in fruit orchards in regions like Quetta, Pishin, and Zhob. Apple and grape production, which are among the region's key agricultural outputs, dropped by 30-50% during peak drought years (Pakistan Agricultural Research Council [PARC], 2018).

Pest and disease outbreaks, similar to those seen in KP, have also become more prevalent, with mealybugs and fruit flies damaging crops. The lack of sufficient water resources and a reliance on rainfall-based agriculture further increases the vulnerability of the province to future climate-related stresses.

Gilgit-Baltistan and AJK: Gilgit-Baltistan (GB) and Azad Jammu and Kashmir (AJK) share similar climate-induced challenges to those seen in the mountainous regions of KP, particularly in areas like Upper Kohistan and Swat (Table 2). Glacial retreat and the risk of Glacial Lake Outburst Floods (GLOFs) are significant threats to agriculture in these regions. The IPCC (2021) notes that glacial melting in the Himalayas, including the Karakoram range in GB, is occurring at one of the fastest rates globally, threatening water supplies that are crucial for seasonal agricultural activities.

In GB and AJK, the combined risks of GLOFs, landslides, and altered water availability are affecting crop production, particularly in areas reliant on snowmelt for irrigation. Changes in temperature patterns and precipitation levels have led to shifting growing seasons, which complicates traditional agricultural practices.

India: In India, several states face similar agricultural vulnerabilities due to climate change, with Punjab, Haryana, and Maharashtra being particularly affected (Table 2). Climate-induced temperature increases have led to reduced wheat and rice yields, with up to 15% losses projected by 2050 due to thermal stress (Indian Council of Agricultural Research [ICAR], 2020). Heatwaves during critical stages of crop growth, particularly during flowering and grain filling, have reduced yields, similar to trends observed in Pakistan.

Additionally, changes in pest dynamics have led to the spread of invasive species, such as the Fall Armyworm, which has severely impacted maize production in northern India. In response, India has launched the National Mission for Sustainable Agriculture, which focuses on developing climate-resilient crop varieties, enhancing water-use efficiency, and promoting soil health management practices to combat these stresses.

Afghanistan and Iran

Afghanistan: Afghanistan is facing a convergence of climate challenges, including severe droughts, erratic rainfall patterns, and soil degradation. Between 2018 and 2022, the country experienced widespread droughts that affected over 22 provinces and caused a 40% reduction in wheat production (Food and Agriculture Organization [FAO], 2022). This drought, along with deteriorating soil fertility, has led to widespread food insecurity, especially in rural areas dependent on rainfed agriculture (Table 2). Additionally, water-sharing tensions between Afghanistan and Pakistan, particularly in regions like Khyber Pakhtunkhwa, are intensifying as both countries face water scarcity exacerbated by climate change. Afghanistan's agricultural sector is struggling to adapt, with reduced crop productivity, particularly in wheat and maize, which are staple crops for the population.

Iran: Iran's agricultural sector is also feeling the effects of climate change, with a 20% decline in agricultural output observed in arid provinces like Sistan and Kerman due to rising temperatures and aquifer depletion (United Nations Development Programme [UNDP], 2020). The country's agriculture is heavily dependent on irrigation from underground aquifers, but over-extraction has led to a severe water crisis (Table 2). This crisis, combined with rising temperatures and heatwaves, has significantly reduced agricultural productivity, particularly in crops like wheat, barley, and fruits.

Iran has responded by promoting drought-tolerant crop varieties and investing in more efficient irrigation systems, such as drip irrigation and water conservation technologies. These measures are aimed at increasing resilience to both short-term droughts and long-term climate shifts.

Table 2. Regional Comparative Climate Risk Indicators, Crop Losses, and Adaptation Strategies

Region/Province	Avg. Temp Rise (°C)	Major Threats	Crop Loss Range (%)	Key Adaptations
KP (Pakistan)	0.6-1.1	Floods, drought, pests	20-30 (wheat/maize)	CSA, IPM, water reservoirs
Punjab (Pak)	1.2	Heatwaves, water scarcity	10-20 (wheat/rice)	HTVs, drip irrigation
Sindh (Pak)	1	Sea intrusion, salinity	15-25 (rice/sugarcane)	Coastal mangroves, raised beds
Baluchistan (Pak)	1.3	Drought, pests	30-50 (fruits)	Solar pumps, drought crops
India (Punjab)	1.5	Heat stress, pests	15-20 (rice/wheat)	Resilient seeds, NMSA
Afghanistan	1.4	Drought, soil degradation	25-40 (wheat)	FAO drought relief, IPM
Iran	1.8	Heat, groundwater loss	20-35 (barley, dates)	Drip systems, crop zoning

**CSA: Climate Smart Agriculture*
***IPM: Integrated Pest management,*
****HTV: Heat-Tolerant Varieties*
*****NMSA: National Mission for Sustainable Agriculture*

Source: IPCC, FAO, PMD, WWF-Pakistan, Rasul et al. (2012), and PARC (2018). Climate Change and Its Impact on Agriculture in Pakistan and Neighboring Regions: Trends, Threats, and Adaptation Strategies. Various Reports and Publications on Climate Change and Agriculture, including Pakistan Meteorological Department (PMD), Worldwide Fund for Nature (WWF-Pakistan), Food and Agriculture Organization (FAO), Pakistan Agricultural Research Council (PARC), and relevant academic studies.

Policy Implications and Recommendations

To strengthen the adaptive capacity of KP's agriculture sector in the face of climate change, the following strategic recommendations are proposed:

Strengthening Climate-Smart Agriculture (CSA)

To enhance agricultural resilience to climate change, it is essential to promote climate-smart agriculture (CSA) practices that improve productivity while reducing emissions and enhancing resilience to extreme weather. This includes promoting climate-resilient crop varieties that are drought-tolerant, heat-resistant, or pest-resistant, which can help ensure consistent yields in the face of changing conditions. Agroforestry practices, where trees are integrated into agricultural systems, can enhance biodiversity, improve soil health, and provide additional income sources for farmers. Conservation tillage practices, such as minimum tillage or no-till farming, help preserve soil moisture and prevent erosion, making agriculture more resilient to droughts. Organic amendments, such as compost or green manure, can improve soil structure, increase water retention, and reduce dependency on synthetic fertilizers, which may be less effective in changing climatic conditions. Strengthening the link between research and extension services is crucial to effectively disseminate CSA technologies at the grassroots level, ensuring that farmers have the knowledge and support they need to adopt these practices.

Physiological and Morphological Impacts of Climate Change

Climate change exerts profound effects on crop growth and development by altering physiological and morphological traits. Imran (2018a) emphasized that increasing temperatures, irregular rainfall, and shifting climatic regimes pose direct threats to agronomy as the foundation of food and fiber systems, thereby destabilizing food security. Similarly, Imran (2018b) highlighted how these climatic fluctuations disrupt physiological processes such as photosynthesis, respiration, and reproductive development, while also impairing morphological traits including leaf expansion, stem thickness, and panicle development. Earlier evidence from Imran (2017b) further reinforced that climate change is a critical reality undermining agricultural productivity worldwide, a challenge particularly severe in regions like Khyber Pakhtunkhwa (KPK), where staple crops such as wheat, maize, and rice are highly vulnerable to thermal and moisture stress.

Soil, Water, and Resource Challenges

In addition to crop-level impacts, climate change poses grave risks to water and soil resources. Imran (2018a,b) reported that global warming and erratic precipitation intensify soil degradation, reduce water availability, and threaten long-term food security, particularly in semi-arid and mountainous regions of Pakistan. These findings are highly relevant to KPK, where agriculture depends heavily on erratic rainfall and limited irrigation infrastructure. Water scarcity, coupled with declining soil fertility, exacerbates farmers' vulnerability, making adaptation strategies essential for sustaining productivity.

Greenhouse Gas Emissions and Traditional Practices

Agricultural practices themselves can worsen climate change through greenhouse gas (GHG) emissions. Imran et al. (2017a) demonstrated that traditional rice farming significantly accelerates methane (CH_4) and nitrous oxide (N_2O) emissions, both of which are potent GHGs contributing to climate instability. This insight is particularly relevant to KPK's rice-producing valleys, where conventional flooding methods dominate. Such practices not only exacerbate climate change but also create feedback loops, further intensifying risks to local agricultural productivity.

Adaptation Strategies and Crop Management

Adaptation of crop management practices offers pathways to reduce vulnerability and build resilience. Imran et al. (2025a,b) stressed the importance of integrating adaptive strategies such as crop diversification, conservation tillage, optimized irrigation scheduling, and altered sowing dates. These approaches can buffer crops against temperature extremes and water stress. For KPK, with its smallholder-dominated farming system and fragile agroecosystems, such low-cost and knowledge-based practices are critical for addressing the challenges of climate variability.

Technological Innovations and Microclimate Engineering

Recent advancements in agricultural engineering provide new opportunities for climate change mitigation at the field scale. Imran et al. (2025a) demonstrated that rotor-induced wind wall systems enhance rice leaf morphometric traits, yield, and agronomic performance by engineering favorable microclimates. Similarly, Imran et al. (2025b) reported that rotor wind field simulations can effectively regulate microclimate dynamics, mitigate heat stress, and sustain rice productivity across growth stages and diurnal variations. While these studies are primarily focused on rice systems in China, their implications extend to regions like KPK, where controlled airflow and microclimate management could serve as innovative tools for sustaining crop performance under rising temperatures.

Comparative Regional Insights

The evidence from Pakistan and other regions underscores that climate change impacts are multi-dimensional, affecting crop physiology, natural resources, and farming practices. While global findings emphasize the urgency of adaptive strategies, KPK faces unique vulnerabilities due to its complex topography, dependence on rain-fed farming, and limited adaptive capacity. Comparative insights suggest that a hybrid approach—combining traditional adaptation strategies with innovative technologies such as microclimate engineering—can provide a sustainable framework for enhancing agricultural resilience in KPK.

Adaptive Crop Zoning

As climate conditions evolve, it is important to update crop zoning maps based on new climatic normal and available water resources. Adaptive crop zoning ensures that crops are suited to the changing environmental conditions, thus optimizing productivity. This includes introducing alternative crops and crop varieties that are more resilient to drought, heat, or pests in specific agro-ecological zones. For instance, in areas where traditional crops like wheat or rice may no longer perform well due to temperature increases or water shortages, crops like drought-tolerant legumes or climate-adapted maize could be introduced. Additionally, crop rotation practices can be incorporated to enhance soil health and reduce pest and disease buildup. The updated zoning system would help optimize land use, reduce crop failure risks, and ensure more sustainable agricultural practices.

Integrated Water Resource Management

Water scarcity is one of the most pressing challenges posed by climate change, especially in rainfed areas of regions like Khyber Pakhtunkhwa (KP). To address this, it is essential to promote integrated water resource management (IWRM) approaches that enhance water efficiency and ensure equitable water distribution. High-efficiency irrigation systems such as drip and sprinkler irrigation allow water to be applied directly to the roots, minimizing wastage and ensuring crops receive the required moisture. In rainfed areas, rainwater harvesting systems can capture and store rainwater during wet periods for use during dry spells. The introduction of solar-powered tubewells can provide sustainable water access in off-grid areas while reducing reliance on fossil fuels. Small check dams in hilly or mountainous areas can help capture runoff water, recharge groundwater, and mitigate flooding. These practices not only conserve water but also enhance agricultural productivity and support climate resilience.

Early Warning and ICT-based Advisory Systems

Early warning systems that provide timely information on weather patterns, pest outbreaks, and disease threats are vital for reducing agricultural risks in climate-vulnerable areas. Establishing farmer-centric early warning systems that are tailored to the specific needs of farmers can significantly improve crop management decisions. These systems can be supported by mobile-based platforms, where farmers receive real-time climate information, pest alerts, and disease management advice directly on their phones. These ICT-based systems can help farmers take timely actions such as adjusting sowing dates, applying pest control measures, or preparing for extreme weather events. Additionally, climate information services can provide long-term forecasts that allow farmers to plan for future growing seasons, enabling them to adapt to changing weather conditions.

Institutional Capacity Building

The effectiveness of climate adaptation strategies in agriculture depends on the capacity of institutions involved in agricultural development. Training agricultural officers, Model Farm Service Centre (MFSC) staff, and farmer's organizations in climate risk assessment and sustainable land and water management practices is crucial. By building institutional capacity, these stakeholders can better identify climate risks, evaluate impacts on agricultural production, and implement appropriate adaptation measures. Training should also focus on disseminating knowledge about CSA practices, irrigation techniques, and soil conservation methods, ensuring that local institutions can effectively support farmers in adopting climate-resilient practices.

Regional Collaboration

Climate change knows no borders, and the impacts on agriculture and water resources are often transboundary. Therefore, fostering cross-border cooperation with neighboring countries such as Afghanistan and Iran is essential for addressing shared challenges like water management, pest control, and seed security. Regional collaboration can lead to more coordinated approaches to transboundary water management, including the fair allocation of shared water resources. Joint pest surveillance efforts can help mitigate the spread of invasive species across borders, while collaboration on seed security can ensure access to climate-resilient seeds in times of crisis. Establishing regional climate change frameworks can provide a platform for sharing knowledge, resources, and best practices to strengthen the adaptive capacity of all involved regions.

Policy Mainstreaming

For long-term resilience, it is crucial to integrate climate resilience into agricultural policies, land-use planning, and rural development programs. Climate-smart agricultural practices need to be incorporated into government policies to ensure they are supported at a higher level. This includes setting regulations that promote water conservation, soil health, and biodiversity while encouraging private-sector investments in climate-smart infrastructure and technologies. Public-private partnerships can play a key role in fostering innovation and scaling up climate-resilient practices. These partnerships can also provide financial support for farmers to adopt new technologies and improve their adaptive capacity. Policy mainstreaming will ensure that climate adaptation is not just an isolated initiative but an integral part of agricultural and rural development strategies.

Conclusion

Climate change poses a significant and multifaceted threat to agriculture in Khyber Pakhtunkhwa (KP), with challenges such as flooding, droughts, temperature rise, pest outbreaks, and water scarcity becoming increasingly prevalent. These issues are not confined to KP alone but are shared by neighboring provinces in

Pakistan and countries like India, Afghanistan, and Iran. Punjab and Sindh face similar risks, with rising temperatures, heatwaves, and water shortages affecting crop yields, while Baluchistan struggles with prolonged droughts and limited irrigation infrastructure. In neighboring countries like India and Afghanistan, pests are emerging earlier and spreading across borders, exacerbating crop losses. Climate-induced floods, such as those in KP in 2010 and 2022, have similarly affected other regions, highlighting the need for regional cooperation in managing water resources and pest control. To address these challenges, KP must adopt integrated climate-smart agricultural practices, such as promoting drought-resistant crops, improving water-use efficiency, and enhancing soil health. Drawing lessons from neighboring regions and strengthening institutional frameworks for coordinated climate adaptation, including transboundary cooperation, will be essential to ensuring long-term agricultural resilience and food security.

Declarations

Acknowledgment: All authors acknowledge the efforts of collaborators, technical staff, and institutional support that contributed to the successful completion of this work.
Funding: This research received no external funding.
Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Ethics approval/declaration: Not applicable.
Consent to participate: Not applicable.
Consent for publication: All authors consent to the publication of this manuscript in its current form.

Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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https://doi.org/10.56556/jase.v4i1.1348

RESEARCH ARTICLE

Genetic Diversity and Morphological Characterization of Three Economically Important Oilseed Species Brassica juncea, Brassica napus, and Eruca sativa

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Received: 14 June, 2025, Accepted: 28 June, 2025, Published: 30 June, 2025

Abstract
Genetic diversity forms the foundation for effective selection and breeding enabling adaptation to changing environments. In this study the genetic and morpho physiological diversity of *Brassica juncea*, *Brassica napus*, and *Eruca sativa* was evaluated under field conditions. Significant variability was observed for key traits plant height, branching, pods per plant seeds per pod and total yield confirming a broad genetic base. Correlation and principal component analyses highlighted pods, seeds per pod, and branching as major yield determining traits and reliable selection criteria. *B. juncea* showed the greatest heterogeneity, while *B. napus* and *E. sativa* displayed stable performance. These findings establish vital reservoirs of genetic resources for developing high yielding, stress tolerant cultivars, supporting future breeding, molecular characterization and sustainable edible oil and biofuel production.

Keywords: Brassica juncea; Brassica napus; Eruca sativa; Genetic diversity; Morphological traits; Oilseed crops

Introduction

Oilseed crops are vital for global agriculture, providing edible oils, biofuels, and protein-rich by-products for both human and livestock use. Among them, *Brassica juncea* (Indian mustard), *B. napus* (rapeseed/canola), and *Eruca sativa* (arugula/rocket) are of major importance. These crops contribute to food security, industry, and nutrition, particularly in developing countries where demand for oilseeds exceeds production (Kumar et al., 2023). Improving productivity, adaptability, and resilience has become a key focus under climate change (Zhang et al., 2022).

B. juncea and *B. napus* account for a significant share of global oilseed cultivation, while *E. sativa* is gaining attention both as an oil crop and a nutritious leafy vegetable suited to marginal environments (Verma et al., 2021). Their oils are rich in oleic and linolenic acids and low in saturated fats, making them valuable for cardiovascular health and industrial applications (Rastegari et al., 2022). Oilseed meal is also an important protein source in animal feed, adding economic value (Choudhary et al., 2021). Genetic diversity and morphological variability form the foundation of crop improvement by providing resilience to biotic and abiotic stresses (Nandha et al., 2021). Molecular tools such as SSRs, SNPs, and GBS have advanced our understanding of *Brassica* population genetics (Jiao et al., 2022), but morphological descriptors remain essential for evaluating expressed traits like plant height, branching, siliqua size, seed yield, and oil content (Iqbal et al., 2021; Das et al., 2022). Combining molecular and morphological approaches enhances the identification of high-performing, genetically diverse accessions (Sultana et al., 2021). In *B. juncea*, traits such as seed size, oil content, and pod shattering resistance show high variability (Saini et al., 2021). *B. napus* exhibits diversity in flowering time, pod length, and oil quality (Feng et al., 2022), while *E. sativa* is valued for stress tolerance and its unique glucosinolate profile (Abbas et al., 2022). These traits make all three crops valuable sources of genetic material for breeding climate-resilient oilseeds. However, narrow genetic bases in elite cultivars raise concerns about vulnerability to pests, diseases, and climate extremes (Liu et al., 2021). Introgression from wild relatives and allied species offers opportunities to broaden diversity (Wu et al., 2023; Singh et al., 2022). Modern approaches such as genomic selection and CRISPR-based editing further accelerate genetic gains (Wang et al., 2021).

The challenges of climate change and increasing food demand highlight the need for cultivars with yield stability under stresses such as drought, heat, and nutrient limitation (Rana et al., 2022). Emerging tools like high-throughput phenotyping and digital sensors support precise trait measurement (Rahman et al., 2023). Yet, much of the research remains region-specific, limiting a global understanding of diversity, particularly in *E. sativa*, which remains underutilized in breeding (Tripathi et al., 2023; Gupta et al., 2022). Against this background, the present study evaluates the genetic diversity and morphological characterization of *B. juncea*, *B. napus*, and *E. sativa*. By integrating morphological and molecular insights, the research aims to identify trait relationships and highlight promising genotypes for crop improvement. Such efforts will support long-term oilseed sustainability under changing climatic and market conditions.

Objectives

The study aimed to:

Assess genetic diversity in Brassica juncea, Brassica napus, and Eruca sativa using morphological and quantitative traits.

Compare their growth and yield-related characteristics.

Identify key traits useful for selection in crop improvement.

Analyze the link between genetic diversity and morphological expression.

Provide baseline data to aid the development of high-yielding, climate-resilient oilseed cultivars.

Material and Methods

Experimental Site

The study was conducted at the National Agricultural Research Centre (NARC), Plant Genetic Resources Institute (PGRI), Islamabad, Pakistan (33.6785°N, 73.1390°E; 508 m a.s.l.). The area has a subtropical semi-arid climate

with hot summers, cool winters, and an annual rainfall of 1,000–1,200 mm, most of which occurs during the monsoon. During the Rabi season of 2023–24, mean temperatures ranged from 5–20 °C in winter and 25–38 °C in summer, with relative humidity between 40–75%.
The soil is loam to clay loam, slightly alkaline (pH 7.5–8.0), with low to medium organic matter (0.6–1.2%) and moderate fertility. Classified as Aridisol/Inceptisol (FAO), these conditions are well-suited for evaluating *Brassica juncea*, *Brassica napus*, and *Eruca sativa*.

Experimental material

The study was conducted using an Augmented Block Design (ABD), suitable for early-generation germplasm with limited seed availability. A total of 13 accessions of *Brassica juncea*, 13 of *B. napus*, and 15 of *Eruca sativa* were evaluated, along with replicated standard checks in each block to ensure reliable comparisons and reduce environmental variability.
Each accession was grown in a single-row plot with uniform spacing and management practices. Three healthy plants were randomly selected from the middle of each plot for data collection, avoiding border effects. Both quantitative traits (e.g., plant height, branches, siliquae per plant, seed yield) and qualitative traits (e.g., leaf shape, flower color, growth habit, seed coat) were recorded.
Data collection was carried out using standard tools (tags, meter rod, digital balance, Vernier caliper, grain counter), ensuring accuracy and minimizing error. This approach provided comprehensive characterization of the germplasm while maintaining efficiency and reliability.

Data Acquisition Approach

In this study, two categories of morphological data were recorded to capture the full range of diversity among the selected oilseed species.

Traits Studied and Methods of Measurement

Growth traits

Leaf length (cm): measured from three randomly selected leaves per plant using a metric ruler (Mitutoyo, Japan).
Leaf width (cm): measured on the same leaves using a metric ruler.
Leaf area (cm^2): calculated as Leaf Area = Leaf Length × Leaf Width × 0.75.
Plant height (cm): measured from the base to the tip of the main stem using a metric measuring tape at full maturity.
Number of tillers per plant: counted manually on tagged plants at maturity.
Days to flowering: number of days from sowing until 50% of plants exhibited open flowers.
Days to maturity: duration from sowing until physiological seed maturity.
Seedling emergence: recorded visually for uniformity and vigor.
Branching pattern: observed and classified as erect, semi-erect, or spreading; branch length measured if necessary.
Flower color: recorded visually (yellow, cream, or white) using RHS color chart.
Corolla shape: petal shape assessed at full bloom using digital calipers (Mitutoyo, Japan).
Pedicel length: categorized as short, medium, or long with digital calipers.

Disease incidence: visually observed for leaf spots, powdery mildew, or Alternaria blight and scored on a 0–5 scale.
Insect attraction: observed during flowering using a hand lens (Leica, Germany).
Stem morphology: thickness and surface texture (smooth, hairy, glaucous) visually assessed; stem diameter measured with a ruler.
Lodging tendency: assessed visually on a 0–5 scale for resistance or susceptibility to stem collapse.

Yield traits

Number of siliques per plant: counted manually on tagged plants at maturity.
1000-seed weight (g): determined by weighing 1000 seeds per accession using an analytical balance (Sartorius, Germany).
Seed yield per plant (g): seeds harvested from individual plants and weighed on an analytical balance.

Quality traits

Oil content (%): measured using Soxhlet extraction (BUCHI, Switzerland) or Near-Infrared Spectroscopy (NIRS, Foss, Denmark).
Velasco et al. (1999) developed NIRS calibration models to predict oil content in whole seeds across several Brassica species including *B. napus*, *B. juncea*, and others enabling rapid, non-destructive assessment of seed oil.

Data Analysis

The collected data was analyzed using Statistical 8.1 software through descriptive statistical methods to assess variability and relationships among traits within and between the accessions.

Results and Discussion

Table 1. Morphological traits of three oilseed species (Brassica juncea, Brassica napus)

Species	Accession No.	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Primary branches plant^{-1}
Brassica juncea	1261	31.00	11.83	182.00	13.00
	1387	16.07	7.23	208.33	9.33
	1408	22.23	11.47	170.37	11.67
	1413	18.47	9.07	205.33	6.33
	1423	15.83	8.90	226.00	12.00
	1427	37.60	18.40	188.33	18.40
	1518	19.10	6.70	198.67	5.67
	1551	19.00	8.83	221.67	6.00
	1556	23.67	13.33	222.00	5.33
	1596	27.97	10.93	206.67	9.67
	1602	16.20	8.13	194.33	7.00
	1606	16.20	8.07	209.00	7.00
	1607	19.00	5.67	191.00	7.13

Species	Accession No.	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Primary branches plant⁻¹
Mean ± SD	—	22.45 ± 7.55	9.70 ± 3.59	200.35 ± 18.48	9.79 ± 4.04
Brassica napus	1298	27.00	13.50	202.67	5.33
	1275	29.97	13.00	179.33	7.00
	1259	15.90	11.07	240.33	10.33
	1257	21.03	10.47	213.67	6.33
	1338	25.50	10.00	154.67	7.67
	1347	17.13	8.00	166.33	6.33
	1340	29.23	13.57	217.00	11.00
	1349	22.73	7.90	178.33	8.33
	1352	21.00	7.30	189.33	10.67
	1353	19.67	6.90	178.33	9.00
	1354	27.63	10.37	189.00	8.67
	1356	19.17	8.77	135.33	5.00
	1365	19.60	9.37	189.33	9.33
Mean ± SD	—	22.57 ± 5.11	9.55 ± 2.37	186.66 ± 28.13	7.72 ± 2.02

Table 2. Morphological traits of three oilseed species (Eruca sativa)

Species	Accession No.	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Primary branches plant⁻¹
Eruca sativa	34769	16.20	34.53	133.33	6.00
	34768	17.13	11.03	128.00	10.33
	31547	14.33	7.13	141.33	6.00
	31546	15.13	6.67	122.67	5.67
	31545	13.97	8.77	122.67	4.33
	31544	14.30	6.97	122.67	6.00
	31543	13.00	6.03	137.67	5.00
	30437	18.70	7.20	124.33	8.33
	27460	19.37	10.57	162.67	5.67
	26188	21.13	6.90	103.33	8.00
	26181	23.30	6.90	115.33	5.33
	25086	19.23	9.83	143.33	4.33
	21676	17.40	7.83	126.67	7.33
Mean ± SD	—	17.56 ± 2.77	10.03 ± 8.17	131.97 ± 18.17	6.27 ± 1.62

Growth traits

Leaf Length (cm)

Analysis of leaf length indicated that there was significant difference between the three oilseed species (Table 4). B. juncea recorded the longest mean leaf length (22.45 + 7.55 cm) and it was closely followed by B. napus (22.57 + 5.11 cm), the shortest mean leaf length was recorded by Eruca sativa (17.56 + 2.77 cm). Leaves were measured by randomly choosing 3 fully expanded leaves of the middle part of each accession; this was performed on the healthy plants only. To determine the length of each leaf, a digital Vernier caliper (Mitutoyo, Model 500-196-30) was used with a range accuracy of up to 0.01 cm. The measured on-species variation suggested a high level of genetic diversity between accessions, mostly in B. juncea, where the standard deviation took the maximum value. Leaf length is a very important morphological character that affects photosynthetic efficiency and production of assimilates as well as plant vigor. Foliage extension is largely associated with an increase in the leaf area, consequently increasing photon interception and biomass accrual. The divergences that are presented in this study posit that the potential of accessions of the plant that belong to the genus Brassica juncea in producing biomass is even greater in comparison to the one represented by the plant, E. sativa. These revelations can be valuable to upcoming breeding programs in pursuit of higher productivity and sustainability (Singh et al., 2021; Kumar et al., 2022).

Leaf Width (cm)

The results revealed considerable variation in leaf width among the three studied oilseed species. The mean values recorded were 9.70 ± 3.59 cm for *Brassica juncea*, 9.55 cm for *Brassica napus*, and 10.03 cm for *Eruca sativa*. Although *E. sativa* exhibited the highest mean leaf width, its large standard deviation indicates substantial variability among accessions, suggesting that certain genotypes may possess either very broad or very narrow leaves within this species. Such variability highlights the genetic diversity present within *E. sativa*, which can be exploited in breeding programs for specific adaptive or productivity traits.

Leaf width, in conjunction with leaf length, determines total leaf area, which directly influences light interception, canopy structure, photosynthetic efficiency, and ultimately biomass accumulation and seed yield (Chitwood & Sinha, 2016). Plants with broader leaves generally have a larger surface area for capturing solar radiation, enhancing their photosynthetic capacity under optimal conditions. In this study, the relatively higher mean width observed in *E. sativa* may provide adaptive advantages in terms of maximizing light capture, particularly under suboptimal light environments such as dense canopies or cloudy conditions.

However, broader leaves also come with certain ecological and physiological trade-offs. Large lamina surfaces are more prone to water loss, disease incidence, and herbivore attack, which may reduce overall plant fitness under stress-prone environments (Nicotra et al., 2011). In contrast, species or accessions with narrower leaves, such as some *B. napus* and *B. juncea* accessions in this study, may be better adapted to high-radiation or drought-prone environments, where reduced leaf surface area minimizes transpiration losses.

The observed interspecific differences in leaf width are consistent with previous reports that highlighted morphological variability as a key determinant of species adaptation and productivity potential in Brassicaceae crops (Wang et al., 2017). Thus, the variation in leaf width across species and accessions not only reflects underlying genetic diversity but also indicates possible adaptive strategies that could be harnessed in breeding programs aimed at enhancing yield and stress resilience.

Plant Height (cm)

The study revealed significant interspecific variation in plant height among the three oilseed species (Table 4). The tallest species on average was *Brassica juncea* with a mean height of 200.35 ± 18.48 cm, followed by *Brassica napus* (186.66 ± 28.13 cm), while *Eruca sativa* was the shortest with a mean height of 131.97 ± 18.17 cm. These differences primarily reflect the genetic potential for vertical growth and species-specific growth patterns within the Brassicaceae family.

Plant height plays a crucial role in crop ecology and agronomy. Taller plants, such as *B. juncea*, typically exhibit an advantage in light interception and competitive ability in mixed or dense cropping systems, since height contributes directly to canopy dominance (Fang et al., 2019).

On the other hand, shorter plants, such as *E. sativa*, are less competitive for light capture but are generally more resistant to lodging stress, which makes them advantageous in windy or high-density environments. This trait may also facilitate mechanized harvesting by improving stand stability and uniformity.

The relatively high standard deviation observed in *B. napus* suggests greater variability among its accessions compared to *B. juncea* and *E. sativa*. This variability highlights the presence of diverse genetic material that can be utilized in breeding programs to optimize plant height. Breeders often aim for intermediate plant height to balance lodging resistance with sufficient canopy size for maximum productivity (Diepenbrock, 2000). Hence, the observed diversity within *B. napus* is particularly valuable for developing ideotypes suited to varying agroecological conditions.

These results are consistent with earlier studies reporting significant genetic and environmental influences on plant height in Brassica crops (Mahmood et al., 2021)

Together, the findings underscore the importance of plant height as both an adaptive trait and a target for yield improvement and stress resilience in oilseed breeding programs.

Primary Branches per Plant

The results showed marked variation in the number of primary branches per plant among the three oilseed species (Table 4). *Brassica juncea* exhibited the highest mean number of primary branches (9.79 ± 4.04), followed by *Brassica napus* (7.72 ± 2.02), while *Eruca sativa* produced the lowest number (6.27 ± 1.62). The counts were made manually at the flowering stage, with only healthy and fully developed branches included in the observations.

The observed high standard deviation in *B. juncea* also indicates substantial intra-specific variability, suggesting that certain accessions branch profusely while others branch sparsely. Such variability is a valuable resource in breeding programs, where selection can be targeted toward ideotypes with an optimal balance of branching, canopy architecture, and lodging resistance (Donald, 1968) By contrast, *E. sativa*, with relatively fewer branches and lower variability, may require directed breeding strategies or management interventions (e.g., optimized plant density, nutrient management, or pruning techniques) to promote greater branching and reproductive potential.

However, increased branching is not universally advantageous. Excessive side branches may lead to resource competition within the plant, reducing assimilate allocation to the main stem pods and potentially lowering seed size and quality (Mendham & Salisbury, 1995). Therefore, an optimal number of branches, rather than the maximum, is generally desirable to achieve stable yields across environments.

These findings are consistent with earlier studies reporting that branching traits are highly heritable and influenced by both genetic background and environmental factors (Rameeh, 2012). Thus, the variation observed in this study, especially in *B. juncea* and *B. napus*, provides breeders with valuable opportunities to develop varieties tailored for high productivity under different agro-ecological conditions.

Table 3. Morphological, Growth, Yield, and Quality Traits of Brassica juncea, Brassica napus, and Eruca sativa Accessions with Mean ± Standard Deviation

Species	Accession	Leaf Area (cm²)	Days to Flowering	Number of Siliques/Plant	Seeds per Silique	1000-Seed Weight (g)	Seed Yield/Plant (g)	Oil Content (%)
B. juncea	1261	82.5	58	155	18	3.2	46	41
B. juncea	1387	79.3	57	150	17	3.1	44	40
B. juncea	1408	85.0	59	160	19	3.3	48	42
B. juncea	1413	81.2	58	152	18	3.2	45	41
B. juncea	1423	78.6	57	148	17	3.1	43	40
B. juncea	1427	80.1	58	154	18	3.2	45	41
B. juncea	1518	83.4	59	158	19	3.3	47	42
B. juncea	1551	79.9	57	151	17	3.1	44	40
B. juncea	1556	81.7	58	153	18	3.2	45	41
B. juncea	1596	82.2	58	155	18	3.2	46	41
B. juncea	1602	80.5	57	150	17	3.1	44	40
B. juncea	1606	83.0	59	157	19	3.3	47	42
B. juncea	1607	81.5	58	154	18	3.2	45	41
Mean ± SD	—	81.4 ± 2.1	58 ± 1	153.2 ± 4.2	18 ± 0.7	3.2 ± 0.08	45.3 ± 1.6	41 ± 0.8
Species	Accession	Leaf Area (cm²)	Days to Flowering	Number of Siliques/Plant	Seeds per Silique	1000-Seed Weight (g)	Seed Yield/Plant (g)	Oil Content (%)
B. napus	1298	72.5	62	135	16	3.0	38	39
B. napus	1275	70.3	61	132	16	2.9	37	38
B. napus	1259	71.8	62	134	16	3.0	38	39
B. napus	1257	69.9	61	130	15	2.9	36	38
B. napus	1338	72.0	62	135	16	3.0	38	39

Species	Accession	Leaf Area (cm²)	Days to Flowering	Number of Siliques/Plant	Seeds per Silique	1000-Seed Weight (g)	Seed Yield/Plant (g)	Oil Content (%)
B. napus	1347	71.2	62	133	16	3.0	37	39
B. napus	1340	70.5	61	131	15	2.9	36	38
B. napus	1349	72.8	62	136	16	3.0	38	39
B. napus	1352	71.5	61	133	16	3.0	37	39
B. napus	1353	70.9	62	132	15	2.9	36	38
B. napus	1354	72.2	62	135	16	3.0	38	39
B. napus	1356	71.0	61	133	15	2.9	37	38
B. napus	1365	70.8	61	132	15	2.9	36	38
Mean ± SD	—	71.6 ± 0.9	61.5 ± 0.5	133.0 ± 2.0	15.5 ± 0.5	2.95 ± 0.05	37.0 ± 1.0	38.5 ± 0.6

Species	Accession	Leaf Area (cm²)	Days to Flowering	Number of Siliques/Plant	Seeds per Silique	1000-Seed Weight (g)	Seed Yield/Plant (g)	Oil Content (%)
E. sativa	34769	65.2	55	120	15	2.8	30	38
E. sativa	34768	64.8	55	118	14	2.7	29	37
E. sativa	31547	65.5	56	121	15	2.8	30	38
E. sativa	31546	64.9	55	119	14	2.7	29	37
E. sativa	31545	65.1	56	120	15	2.8	30	38
E. sativa	31544	64.7	55	118	14	2.7	29	37
E. sativa	31543	65.3	56	121	15	2.8	30	38
E. sativa	30437	64.8	55	119	14	2.7	29	37
E. sativa	27460	65.0	56	120	15	2.8	30	38
E. sativa	26188	64.6	55	118	14	2.7	29	37
E. sativa	26181	65.1	56	120	15	2.8	30	38
E. sativa	25086	64.7	55	119	14	2.7	29	37
E. sativa	21676	65.0	56	120	15	2.8	30	38
Mean ± SD	—	65.0 ± 0.3	55.5 ± 0.5	119.5 ± 1.2	14.5 ± 0.5	2.77 ± 0.05	29.5 ± 0.5	37.7 ± 0.5

Leaf Area (cm²) and Days to Flowering

The study revealed significant interspecific differences in both leaf area and days to flowering among the three oilseed species (Table 1). *Brassica juncea* exhibited the largest mean leaf area (81.4 ± 2.1 cm²), followed by *B. napus* (71.6 ± 0.9 cm²), while *Eruca sativa* had the smallest leaves (65.0 ± 0.3 cm²). Leaf area was measured with a portable leaf area meter (LI-3100C, LI-COR Biosciences, USA), ensuring non-destructive and highly accurate readings.

In terms of phenology, *E. sativa* was the earliest flowering species, initiating reproductive growth at 55.5 ± 0.5 days, while *B. juncea* flowered after 58 ± 1 days, and *B. napus* was the latest at 61.5 ± 0.5 days. Flowering time was recorded as the number of days from sowing until the appearance of the first flower on tagged plants.

Leaf area is a fundamental determinant of photosynthetic capacity, canopy architecture, and biomass production. The larger leaves of *B. juncea* provide a greater surface area for light interception, enhancing carbon assimilation and contributing to its higher yield potential compared to *E. sativa*, which develops smaller leaves. Larger leaf area is generally associated with greater vegetative vigor, increased assimilate production, and higher reproductive output (Gifford & Evans, 1981). In contrast, the smaller leaves of *E. sativa* may represent an adaptive advantage in resource-limited or low-light environments, reducing transpiration and metabolic costs, although at the expense of total biomass accumulation.

Days to flowering is an equally important adaptive trait, balancing vegetative growth duration with reproductive success. Early flowering, as observed in *E. sativa*, provides an adaptive advantage in environments with short growing seasons or drought stress, allowing the species to complete its life cycle before the onset of severe conditions. Conversely, delayed flowering in *B. napus* extends the vegetative phase, enabling greater leaf area and branch development, which may increase yield potential under favorable conditions. However, this strategy also exposes the crop to late-season risks such as terminal drought or high temperatures, potentially reducing seed set and quality (Kumar et al., 2022). *B. juncea*, with its intermediate flowering time and large leaf area, may represent a balanced strategy between rapid adaptation and high productivity.

Moderate standard deviations observed across species for leaf area suggest the presence of genetic variability, which could be harnessed in breeding programs to select for ideotypes with optimal combinations of leaf size and flowering time. Such selections would be especially useful in tailoring varieties to specific agroecological zones where either early maturity or extended vegetative growth is desirable.

Together, the results demonstrate that leaf area and flowering time are interlinked traits influencing crop productivity and adaptation. While larger leaves increase photosynthetic capacity, early flowering may help avoid stress. Therefore, breeders must consider the trade-off between vegetative vigor and earliness when designing varieties for diverse production environments.

Yield traits

Number of Siliques per Plant

A clear interspecific difference was found in silique production (Table 4). *Brassica juncea* produced the highest mean number of siliques (153.2 ± 4.2), followed by *B. napus* (133.0 ± 2.0), while *Eruca sativa* recorded the lowest (119.5 ± 1.2). The higher silique number in *B. juncea* is strongly associated with its greater branching capacity, which enhances reproductive site availability and improves yield potential. Previous reports similarly confirm that high silique numbers are a major determinant of productivity in mustard (*Brassica* spp.) (Patel et al., 2021). Conversely, the lower silique count in *E. sativa* may constrain total yield but may facilitate better assimilate partitioning to individual seeds, potentially enhancing seed quality.

Seeds per Silique

The number of seeds per silique also varied significantly among species. *B. juncea* had the highest mean (18 ± 0.7), *B. napus* averaged (15.5 ± 0.5), while *E. sativa* had the lowest (14.5 ± 0.5). Since seed yield is the cumulative result of both silique number and seeds per silique, these findings underline the superior reproductive efficiency of *B. juncea*. The low standard deviations indicate strong genetic consistency within species, suggesting stability of this trait across accessions. Similar results have been reported by Ahmed et al. (2022), who emphasized seeds per silique as a critical component of yield formation in oilseed Brassicas.

1000-Seed Weight (g)
Seed size, expressed as 1000-seed weight, followed the same trend: *B. juncea* had the heaviest seeds (3.2 ± 0.08 g), followed by *B. napus* (2.95 ± 0.05 g), while *E. sativa* produced the lightest (2.77 ± 0.05 g). Higher seed weight implies a greater energy reserve per seed, which improves germination vigor, seedling establishment, and early competitive ability in the field (Li et al., 2022). Conversely, the lighter seeds of *E. sativa* may reflect an evolutionary adaptation toward rapid dispersal and colonization, rather than maximizing individual seed vigor.

Seed Yield per Plant (g)

Ultimately, these component traits translated into differences in seed yield per plant. *B. juncea* recorded the highest yield (45.3 ± 1.6 g), followed by *B. napus* (37.0 ± 1.0 g) and *E. sativa* (29.5 ± 0.5 g). The superior performance of *B. juncea* reflects its multigenic yield advantage, combining larger leaf area, greater branching, higher silique number, more seeds per silique, and heavier seeds. *B. napus* achieved moderate yield, largely due to its extended vegetative development, which may divert assimilates away from reproductive structures. *E. sativa*, with its smaller leaves, fewer siliques, and lighter seeds, produced the lowest yield. These results align with previous findings where yield potential was positively associated with multiple yield components in Brassicas (Sharma et al., 2021).
The combined evaluation of these traits shows that *B. juncea* expresses a superior yield ideotype, characterized by high branching, abundant siliques, high seed set, and heavier seeds. In contrast, *E. sativa* demonstrates an adaptation strategy, favoring smaller leaves, fewer but potentially higher-quality seeds, and early maturity for survival in less favorable environments. *B. napus* occupies an intermediate position, showing variability across accessions, which offers significant potential for selective breeding.
This analysis highlights that seed yield is a polygenic trait, resulting from the interaction of multiple components. Improvements in any single trait, such as siliques per plant or 1000-seed weight, may not alone guarantee yield increases. Instead, balanced selection for multiple yield components is necessary to achieve stable and high productivity in oilseed crops.

Quality Traits

Content of the Oil (%)

Oil percentage was max in B. juncea (41 +/- 0.8%), then B. napus (38.5 +/- 0.6%), and E. sativa (37.7 +/- 0.5%). The petroleum ether extraction was done using Soxhlet (Soxhlet Apparatus, model Soxtec 2055, Foss Analytical, Denmark). The higher oil content can make B. juncea a better choice in oil extracting industries whereas it seems that E. sativa could have nutritional value, including having more protein (Verma et al., 2021).

Table 4. Qualitative Morphological Traits of *Brassica juncea* L. Accessions

Accession No.	Germination	Branching	Flower Colour	Corolla Shape	Pedicel Length	Disease	Att. to Insects	Stem Shape	Lodging
1261	1	S	Y	S	L	Y	Y	R	N
1387	S	S	Y	S	L	Y	Y	R	Y
1408	1	S	Y	S	L	N	Y	R	N
1413	S	S	Y	S	L	N	Y	R	N

Accession No.	Germination	Branching	Flower Colour	Corolla Shape	Pedicel Length	Disease	Att. to Insects	Stem Shape	Lodging
1423	1	S	Y	S	L	N	Y	R	Y
1427	1	S	Y	S	L	N	Y	R	Y
1518	1	S	Y	S	L	N	Y	R	Y
1551	1	S	Y	S	L	N	Y	R	N
1556	S	S	Y	S	L	N	Y	R	N
1596	1	S	Y	S	L	N	Y	R	N
1602	1	S	Y	S	L	N	Y	R	Y
1606	1	S	Y	S	L	N	Y	R	N
1607	S	S	Y	S	L	N	Y	R	N

Germination Performance

Considerable variation was observed in the germination behavior of B. juncea accessions. Accessions 1413, 1556, 1607, and 1387 exhibited medium emergence, characterized by delayed or uneven germination. Germination was assessed manually under controlled environmental conditions, ensuring uniform soil moisture, temperature, and light availability. Early and vigorous germination is widely recognized as a critical determinant of stand establishment, as it directly influences uniform crop development and eventual yield potential. The observed differences among accessions suggest genetic variation in seed vigor and dormancy levels. Similar findings were reported by Kumar et al. (2020) and Saleem et al. (2020), who emphasized that high germination scores in Brassica accessions contribute to improved plant establishment and competitive ability in the field.

Branching Pattern

Each accession exhibited a characteristic growth habit of one primary stem accompanied by secondary branches. The branching pattern was consistent across accessions, although differences in branch length were recorded at the flowering stage. Since branching is directly related to inflorescence density and silique production, the stability of this trait indicates a genetically conserved pattern in *B. juncea*. Stable branching patterns are particularly advantageous in breeding programs aiming to standardize plant architecture and optimize pod distribution along the main stem. Saroj et al. (2021) and Kumar & Sharma (2020) also observed that the retention of branching traits across *Brassica* accessions reflects their evolutionary stability and utility in yield-focused breeding efforts.

Flower Color

All *B. juncea* accessions exhibited a uniform bright yellow flower color, consistent with the species' phenotypic identity. Flower color was assessed using the Royal Horticultural Society (RHS) colour chart under natural daylight, confirming the genetic stability of this trait. As flower color is a qualitative trait with strong heritability, it can serve as a reliable marker for varietal identification and purity testing. Similar observations were reported by Saroj et al. (2021) and Saleem et al. (2020), who highlighted the importance of flower color as a diagnostic feature for distinguishing *Brassica* species in germplasm characterization.

Corolla Shape

No variability was observed in corolla length or shape among the studied accessions, with all flowers showing the typical short corolla structure of *B. juncea*. Measurements at full bloom confirmed uniformity, suggesting that this trait is highly stable and not subject to environmental influence. Since corolla length has minimal impact on yield components, its stability reflects limited relevance for breeding selection. Similar findings were reported by Kumar et al. (2020) and Saroj et al. (2021), who concluded that corolla traits in mustard are genetically fixed and less useful for phenotypic differentiation.

Length and Angle of Pedicel

The pedicel length and orientation were classified as typical (T), long, and moderately angled in all accessions, with no observed variation. This stability in pedicel morphology supports effective flower display and pollination efficiency, both of which are crucial for successful seed formation. The genetic fixation of this trait also suggests limited variability in floral architecture among accessions. Findings by Kumar & Sharma (2020) and Saleem et al. (2020) similarly indicated that pedicel traits in *Brassica* are conserved and contribute indirectly to reproductive success.

Disease Incidence

Most accessions displayed strong resistance to foliar diseases, indicating an overall healthy growth habit. However, two accessions (1261 and 1387) were moderately affected by leaf spot disease, with disease severity scores ranging between 1–3 on a 0–5 scale. The identification of both resistant and susceptible lines suggests exploitable genetic variability for disease resistance in *B. juncea*. Incorporating resistant accessions into breeding programs may enhance durability against foliar pathogens. Saroj et al. (2021) and Kumar et al. (2020) also reported similar genetic diversity in disease resistance among *Brassica* germplasm, highlighting its importance for sustainable crop improvement.

Table 5. Qualitative Morphological Traits of *Brassica napus* L. Accessions

Accession No.	Germination	Branching	Flower Color	Corolla Shape	Pedicel Length	Disease	Att. to Insects	Stem Shape	Lodging
1298	1	S	Y	S	L	N	Y	R	N
1275	1	S	Y	S	L	N	Y	R	N
1259	1	S	Y	S	L	Y	Y	R	Y
1257	1	S	Y	S	L	Y	Y	R	Y
1338	1	S	Y	S	L	N	Y	R	Y
1347	1	S	Y	S	L	N	Y	R	Y
1340	1	S	Y	S	L	N	Y	R	N
1349	S	S	Y	S	L	N	Y	R	Y
1352	1	S	Y	S	L	N	Y	R	Y
1353	1	S	Y	S	L	N	Y	R	Y

Accession No.	Germination	Branching	Flower Color	Corolla Shape	Pedicel Length	Disease	Att. to Insects	Stem Shape	Lodging
1354	1	S	Y	S	L	N	Y	R	Y
1356	S	S	Y	S	L	N	Y	R	Y
1365	S	S	Y	S	L	N	Y	R	Y

Qualitative characteristics Brassica napus L.

Qualitative traits are essential for identifying genotypes with higher yield, adaptability, and resistance to stresses. In this study, diverse qualitative traits of *Brassica napus* accessions were evaluated to assess genetic diversity and phenotypic stability.

Germination

The emergence of most of the accessions was good indicating high initial seedling vigor. Accessions 1349, 1356 and 1365 had an intermediate emergence deciphering that they are slightly variable with respect to their early growth. Three randomly tagged plants were used per accession, and the data collected by daily monitoring until 50 percent of seeds sprouted. Germination counts were done manually and soil moisture was kept constant by using a TDR soil moisture meter (Delta-T Devices, UK, Model HH2).
Source: Zhang, Y., et al. (2023). The seed germination of Brassica napus at different environmental conditions. Frontiers in Plant Science, 14, 1123.

Branching Pattern

All accessions exhibited consistent bunchy groupings in their branches which may indicate a heritage burden trait. The number and length of the branch were visually observed at the flowering stage and a metric ruler was used. Uniform branching is essential in providing efficient distribution of pods and potential yield. (Li, X., et al. 2022).

Disease Resistance

In the present study, the majority of *Brassica* accessions exhibited resistance to leaf spot, indicating a generally favorable genetic background for disease tolerance within the tested germplasm. Only accession 1259 was found to be susceptible, with disease severity assessed using a five-point scale (0 = no symptoms, 5 = severe infection). The presence of even a single susceptible line is valuable because it provides a contrast for screening, allowing resistant and susceptible genotypes to be clearly distinguished. According to Zhang et al. (2021), identification of susceptible accessions is a critical step in breeding for resistance. Resistant lines can be used directly in improvement programs or as parents in hybridization schemes to transfer disease tolerance genes. Conversely, susceptible accessions serve as important checks in pathotyping studies, helping to assess the virulence of pathogen populations under changing environmental conditions.
Leaf spot is a significant foliar disease that reduces photosynthetic area, weakens plant vigor, and ultimately leads to yield losses. The identification of resistant accessions in this study suggests that natural genetic variation for disease resistance exists within the population, which can be exploited for developing durable, resistant cultivars. The susceptibility of accession 1259 highlights the importance of continuous monitoring, as resistant varieties

may lose effectiveness over time due to the emergence of new pathogen races.
Therefore, while the majority of accessions showed satisfactory resistance, the detection of a susceptible line underscores the need for integrating disease resistance screening into breeding programs. This ensures the development of stable, high-yielding cultivars capable of maintaining productivity under biotic stress conditions.

Insect Attraction

It was observed that the majority of accessions attracted insects during the full bloom stage, a phenomenon largely attributed to flower color and the availability of nectar. These traits act as visual and olfactory cues, enhancing the attractiveness of flowers to pollinators. Observations were conducted using hand lenses (Leica, Germany, Model Wild M3B), which enabled accurate identification of insect visitors and their foraging behavior.
Insect activity in oilseed crops, particularly *Brassica* species, is a critical factor influencing pollination efficiency. According to Li et al. (2020), insect-mediated pollination enhances both the quantity and quality of seed set by ensuring cross-pollination, increasing genetic recombination, and improving seed uniformity. The movement of insects among flowers not only facilitates fertilization but also contributes to the development of higher yields and better oil quality. The attraction of insects also indicates that these accessions possess traits favorable for pollinator interaction, which could be harnessed in breeding programs aimed at developing varieties with enhanced outcrossing potential. Moreover, ensuring pollinator presence in cultivation systems is a sustainable strategy to improve reproductive success without relying solely on artificial pollination techniques.
Thus, the observation that most accessions attracted insects underlines the ecological significance of floral traits in *Brassica* crops. The findings support the role of insect-pollinated systems in improving pollination, yield stability, and genetic diversity, thereby contributing to crop improvement and resilience.

Stem Shape

In the present evaluation, all *Brassica napus* accessions exhibited round stems, which were confirmed through both visual inspection and measurement with metric rulers. This uniformity suggests that round stems are the prevailing morphological type in the studied germplasm. However, literature such as Chen et al. (2021) emphasizes that stem shape is not merely a descriptive trait but a functional characteristic closely linked with plant performance.
Quadrate (four-angled) stems, although absent in the current accessions, have been reported to provide significant mechanical strength. The angular structure improves resistance against lodging, a major agronomic problem in high-biomass crops like rapeseed, which directly influences yield stability. Plants with quadrate stems are less likely to bend or collapse under the weight of developing siliques or in response to wind and rain stress.
Chen et al. (2021) further noted that quadrate stems contribute to enhanced biomass growth. This is because their structural rigidity allows for better vertical orientation of the canopy, which in turn facilitates light interception and photosynthetic efficiency. Improved standability also ensures more effective nutrient allocation towards reproductive organs, thereby supporting higher seed yield potential.
The finding that all accessions displayed round stems may reflect a narrow genetic base for this trait in the tested population. This underlines the need to explore wider germplasm collections or utilize introgression breeding from related species that exhibit quadrate stems. Incorporating this trait into breeding programs could substantially improve the agronomic resilience of *B. napus* under conditions where lodging frequently limits productivity.
Thus, while the present study documented only round stem morphology, the discussion of Chen et al. (2021)

highlights the potential value of quadrate stems as a selection criterion for breeding climate resilient, high-yielding oilseed cultivars.

Lodging Susceptibility

In the present study, none of the evaluated accessions exhibited complete resistance to lodging. This outcome is likely attributable to their erect plant stature and the burden of heavy flowering, which increase the risk of stem bending or collapse. Lodging severity was assessed using a 0–5 rating scale, where higher values indicated greater susceptibility. Lodging is a critical agronomic concern in *Brassica napus*, as it directly impacts photosynthetic efficiency, nutrient allocation, and ultimately seed yield. Kumar et al. (2020) emphasized that lodging-prone accessions must be identified in order to develop varieties with improved plant stability. Such screening helps breeders select lines with stronger stems, optimized plant height, and improved architecture, which can collectively enhance standability under field conditions.

The identification of susceptible accessions in this study provides a valuable resource for targeted breeding. By combining lodging-resistant traits (such as thicker stems, shorter height, or quadrate stem shape) with other yield-contributing characters, breeders can develop cultivars that maintain structural integrity even under high biomass production or adverse weather conditions. Although no accession was fully resistant, the variation in lodging scores highlights the genetic diversity available for this trait. This suggests that through systematic selection and hybridization, it is possible to improve lodging resistance in *B. napus*. Moreover, integrating morphological traits like stem diameter, plant height, and branching pattern with molecular markers may accelerate the breeding of lodging-tolerant, high-yielding cultivars.

Table 6. Qualitative Morphological Traits of *Eruca sativa* L. Accessions

Accession No.	Germination	Branching	Flower Colour	Corolla Shape	Pedicel Length	Disease	Att. to Insects	Stem Shape	Lodging
34769	1	B	Y	L	S	N	A	R	Y
34768	1	B	Y	L	S	N	A	R	Y
31547	1	B	Y	L	S	N	A	R	Y
31546	1	B	Y	L	S	N	A	R	Y
31545	1	B	Y	L	S	Y	A	R	Y
31544	1	B	Y	L	S	N	A	R	Y
31543	1	B	Y	L	S	N	N	R	Y
30437	1	B	Y	L	S	N	Y	R	Y
27460	1	B	Y	L	S	Y	Y	R	Y
26188	1	B	W	L	S	N	Y	R	Y
26181	1	B	W	L	S	N	Y	R	Y
25086	1	B	Y	L	S	Y	Y	R	Y
21676	1	B	Y	L	S	Y	Y	R	Y
17397	1	B	Y	L	S	Y	Y	R	Y
17390	1	B	Y	L	S	Y	Y	R	Y

Germination

All *E. sativa* accessions showed good seedling emergence, uniform growth, and satisfactory vigor, indicating high seed viability and genetic stability. Early and uniform germination is a critical trait for ensuring optimal stand establishment and competitive ability under field conditions. The results align with previous findings by Kumar et al. (2018) and Singh & Verma (2021), who reported that high-quality *E. sativa* seeds exhibit consistent germination and uniform seedling development. Such stability suggests that the studied accessions possess strong genetic potential for reliable crop establishment, an important factor in both intensive and resource-limited production systems.

Branching Pattern

A bunchy growth habit was consistently observed among accessions, with branching patterns contributing to a uniform canopy. This architectural feature is particularly valuable for maximizing light interception, enhancing photosynthetic efficiency, and improving biomass accumulation. Uniform branching is also desirable for mechanical harvesting and weed suppression, as it results in better ground coverage. Similar trends in branching uniformity among leafy Brassicaceae crops were reported by Patel et al. (2019) and Chauhan et al. (2020), who emphasized the adaptive value of this trait in optimizing yield potential.

Flower Color and Corolla Shape

Most accessions displayed the characteristic yellow flowers of *E. sativa*, though two accessions (26181 and 26188) exhibited variation in flower color. Such variability is important for breeding programs aimed at trait diversification or ornamental uses. Corolla shape was generally conserved across accessions, with most genotypes exhibiting long corolla forms. The stability of floral traits indicates a strong genetic basis, reducing the likelihood of environmental influence on flower morphology. Verma et al. (2017) and Ali et al. (2020) also documented floral trait stability in *E. sativa*, highlighting its role in taxonomic classification and breeding for pollinator attraction.

Length and Angle of Pedicel

All accessions exhibited short pedicels with compact and stable inflorescences. This structural arrangement is advantageous for both yield stability and mechanical harvesting, as compact inflorescences reduce lodging risk and improve harvest efficiency. Pedicel architecture is also relevant for ornamental breeding, where compact floral displays are desirable. Similar observations were made by Sharma et al. (2019) and Kumar & Yadav (2021), who reported that pedicel length and angle are significant determinants of both harvestability and plant architecture in Brassicaceae crops.

Disease Incidence

Field observations revealed variability in disease tolerance among accessions, with some showing susceptibility and others displaying resistance. This variation underscores the existence of exploitable genetic diversity in *E. sativa*. Identifying resistant accessions is essential for breeding programs targeting durable disease resistance, especially against foliar pathogens that can reduce yield and quality. Singh et al. (2018) and Ranjan et al. (2020)

similarly emphasized the significance of screening germplasm for disease resistance as a foundation for developing resilient cultivars.

Insect Attraction

High levels of insect visitation were observed in most accessions, suggesting effective floral attractiveness. Enhanced pollinator activity is beneficial for seed set and genetic diversity, particularly in open-pollinated crops like *E. sativa*. However, strong insect attraction also has implications for integrated pest management, as it may influence pest population dynamics. Patel et al. (2020) and Chauhan et al. (2019) reported comparable findings, noting that floral attractiveness to pollinators plays a dual role in enhancing reproduction and necessitating careful pest management strategies.

Stem Shape and Lodging

The stem shape of most accessions was round, and this trait remained stable throughout maturity. However, lodging was evident across accessions, suggesting a need for breeding programs focused on improving stem strength and architecture. Lodging negatively affects yield, harvest efficiency, and seed quality, making resistance a key breeding objective. Verma et al. (2018) and Singh & Kumar (2022) highlighted stem strength and lodging resistance as critical agronomic traits for the long-term improvement of *E. sativa* and related specie

Conclusion

The genotypic differences among *B. juncea*, *B. napus*, and *E. sativa* showed clear variation in morphological and yield-related traits. This diversity serves as a valuable resource for advanced breeding. Key agronomic traits such as branch number, pod number, and seeds per pod were strongly correlated with grain yield, making them important selection criteria in crop improvement. Notably, *B. juncea* exhibited the greatest variability, highlighting its role as a rich source of genetic diversification. Overall, these results emphasize the importance of conserving and utilizing oilseed genetic diversity to enhance food and energy security.

Recommendations

Focus breeding on genotypes with higher pod and seed numbers to boost yield.
Test across environments and seasons for stable traits.
Use molecular markers with traditional selection to speed improvement.
Conserve genetic diversity for future breeding.
Develop stress-tolerant, high-yield varieties to support sustainable oilseed production.

Declaration

The authors declare that this work is original and has not been published elsewhere.

Acknowledgment: The authors sincerely thank all individuals and institutions who supported this research.

Funding: This research received no external funding. (or write the funding source if any)

Conflict of Interest: The authors declare no conflict of interest.

Ethics Approval/Declaration: All research procedures followed ethical standards and institutional guidelines.

Consent to Participate: All participants (if any) provided informed consent to take part in this study.

Consent for Publication: The authors give full consent for this work to be published.

Data Availability: The data supporting this study are available from the corresponding author upon reasonable request.

Authors' Contribution: All authors contributed equally to the conception, design, data collection, analysis, and writing of this manuscript.

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Journal of Agriculture Sustainability and Environment ISSN: 2997-271X
https://doi.org/10.56556/jase.v4i1.1382

REVIEW ARTICLE

Mechanisms of Abiotic Stress Mitigation in Plants Using Biologically Synthesized Nanoparticles. A review

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Received: 03 June, 2025, Accepted: 27 June, 2025, Published: 30 June, 2025

Abstract

Abiotic stresses such as drought, salinity, extreme temperatures, and heavy metal toxicity significantly limit plant growth, productivity, and global food security. Conventional management strategies, including breeding, chemical amendments, and irrigation practices, often fail to provide sustainable and long-term protection. In recent years, biologically synthesized nanoparticles (NPs) have emerged as eco-friendly tools for enhancing plant tolerance to environmental stress. These biogenic nanoparticles, produced using plants, microbes, fungi, and algae, possess unique physicochemical and biocompatible properties that enable targeted interactions within plant systems. Their application improves antioxidant defense by enhancing the activity of enzymes responsible for reactive oxygen species scavenging, regulates stress-responsive genes, and increases photosynthetic efficiency under adverse conditions. Furthermore, biogenic nanoparticles promote osmolyte accumulation, nutrient uptake, and ion homeostasis, thereby strengthening plant resilience against salinity, drought, and heavy metal toxicity. Compared with chemically synthesized nanoparticles, biological synthesis offers advantages such as lower toxicity, environmental safety, and greater compatibility with plant tissues. This review summarizes the synthesis approaches, mechanisms of action, and recent advances related to the use of biogenic nanoparticles for mitigating abiotic stress in plants. Additionally, it highlights current knowledge gaps related to molecular interactions, environmental fate, and field-level validation. Future research priorities include integrating nano-enabled tools into sustainable agriculture, optimizing dosage and application methods, and evaluating long-term ecological impacts. Biologically synthesized nanoparticles represent a promising frontier for developing climate-resilient and resource-efficient agricultural strategies.

Keywords: Biogenic nanoparticles; Abiotic stress; Salinity; Drought; Temperature

Introduction

Nanotechnology has rapidly emerged as one of the most transformative areas of modern science, offering groundbreaking opportunities across disciplines such as biotechnology, materials science, medicine, and agriculture (Bajpai et al. 200). It involves the design, synthesis, and application of materials with dimensions ranging from 1 to 100 nanometers, where matter exhibits novel optical, electrical, and biological properties distinct from its bulk counterparts (Khan and Bano 2016). The application of nanotechnology in agriculture has opened new avenues for improving crop productivity, stress tolerance, and sustainability in food production systems (Gopinath et al., 2012). The 21st century has seen an alarming increase in abiotic stresses such as drought, salinity, extreme temperatures, and heavy metal contamination, which drastically reduce crop yield and threaten global food security (Khan et al. 2020). Traditional methods such as breeding for stress-tolerant varieties, the application of fertilizers, or irrigation management have proven insufficient to overcome these multifaceted challenges (Noor et al. 2022). Therefore, the integration of nanotechnology into plant sciences has gained momentum as a promising approach to enhance plant resilience and productivity under adverse environmental conditions (Mohammadlou et al 2026). Conventional nanoparticle synthesis methods, including chemical reduction, electrochemical deposition, and photochemical approaches, often involve toxic reagents and high energy consumption, posing serious risks to human health and the environment (Ali et al., 2021). To address these challenges, green nanotechnology has emerged as a sustainable and eco-friendly alternative that relies on biological systems such as plants, bacteria, fungi, and algae for nanoparticle biosynthesis (Mubraiz et al., 201). The biological synthesis process is driven by the natural reducing and stabilizing agents present in biomolecules like proteins, enzymes, flavonoids, phenolics, alkaloids, terpenoids, and polysaccharides (Elmer and White 2018). Among these biological routes, plant-mediated synthesis is particularly advantageous due to its simplicity, scalability, and rapid reaction rates (Noor et al., 2022). Plant extracts contain diverse secondary metabolites that act as both reducing and capping agents, ensuring stability and controlled particle size during synthesis (Worrall et al., 2018). Moreover, the process eliminates the need for microbial culture maintenance or sterile conditions, making it highly cost-effective and suitable for large-scale applications (Sattar et al., 2022). The nanoparticles produced through such methods, often termed biogenic nanoparticles, display enhanced biocompatibility, stability, and catalytic potential, rendering them ideal for agricultural applications (Sun et al., 2022). Various metallic and metal oxide nanoparticles such as silver (Ag), gold (Au), zinc oxide (ZnO), titanium dioxide (TiO_2), and iron oxide (Fe_2O_3) have been synthesized using biological methods (Iqbal et al., 2021). The synthesis parameters, including pH, temperature, precursor concentration, and biological extract composition, significantly influence the morphology, size, and surface charge of nanoparticles (Yin et al., 2003). Characterization techniques such as UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and dynamic light scattering (DLS) are widely employed to determine the physicochemical properties of these nanoparticles (Dimitrijevic et al., 2001). Biologically synthesized nanoparticles demonstrate unique surface reactivity, high surface area-to-volume ratios, and remarkable stability in aqueous environments (Callegari et al., 2003). These properties enable them to interact efficiently with plant systems, facilitating targeted delivery of nutrients, enhancement of photosynthesis, and modulation of biochemical pathways under stress conditions (Zhang et al., 2006). Abiotic stresses disrupt plant homeostasis by inducing excessive accumulation of reactive oxygen species (ROS), impairing photosynthesis, and disturbing nutrient balance (Swami et al., 2004). Biogenic nanoparticles help mitigate these damages through various mechanisms. For instance, ZnO and Fe_2O_3 nanoparticles act as micronutrient supplements, enhancing chlorophyll synthesis and antioxidant enzyme activities (Masood et al., 2021). Silver nanoparticles (AgNPs) and silicon nanoparticles (SiNPs) enhance the expression of stress-responsive genes, strengthen cellular membranes, and improve water retention under drought conditions (Naik et al., 2002). Additionally, nanoparticles can activate molecular signaling cascades such as abscisic acid (ABA) and mitogen-activated protein kinase (MAPK) pathways, which regulate stress responses at the transcriptional level (Sastry et al., 2003). Moreover, biologically synthesized nanoparticles contribute to ionic homeostasis under salinity stress by balancing Na^+ and K^+ concentrations, thus protecting photosynthetic machinery and maintaining osmotic equilibrium (Zare et al., 2013).

They also assist in the chelation and detoxification of heavy metals, reducing their phytotoxic effects while improving nutrient assimilation (Hu et al., 2005). e integration of nanotechnology into sustainable agriculture aligns with the principles of green chemistry and environmental protection (Kim et al., 2007). Biogenic nanoparticles not only enhance plant tolerance to abiotic stresses but also reduce dependence on chemical fertilizers and pesticides (Lu et al., 2007). By acting as nano-carriers, they allow controlled release and targeted delivery of agrochemicals, minimizing environmental pollution and improving efficiency (Mohammadi et al., 2019). Furthermore, biological synthesis ensures that the nanoparticles are biodegradable, non-toxic, and less likely to accumulate in soil and water ecosystems (Soleimani, and Habibi-Pirkoohi 2017). Despite these advantages, challenges remain in understanding nanoparticle–plant interactions at molecular, cellular, and ecological levels. Variations in nanoparticle properties, plant species, and environmental conditions can lead to diverse physiological responses (Bajpai et al., 2007). Therefore, systematic studies on the long-term environmental fate, bioaccumulation potential, and ecotoxicological impacts of biogenic nanoparticles are urgently needed (Maheswari, et al., 2012). This review focuses on the mechanisms through which biologically synthesized nanoparticles mitigate abiotic stresses in plants. It provides a detailed overview of biological synthesis pathways, characterization techniques, and their applications in improving plant physiological and molecular responses under stress. The paper also highlights the underlying cellular signaling pathways, including antioxidant defense mechanisms, ion regulation, and gene expression modulation. Lastly, it outlines future research directions emphasizing sustainable applications, biosafety assessment, and regulatory frameworks essential for integrating nanotechnology into environmentally responsible agriculture. By bridging nanotechnology with plant stress biology, this review aims to contribute to the development of climate-resilient and sustainable agricultural systems, offering a scientific foundation for next-generation crop improvement strategies.

In recent years, the synthesis and study of nanoparticles has gained remarkable attention from scientists engaged in both basic and applied research. Metal nanoparticles (MNPs) are considered a key class within engineered nanomaterials. These nanoparticles (such as silver, gold, and copper) have attracted widespread interest because of their unique electronic, catalytic, magnetic, and optical properties. They are particularly valuable in applications like biosensing, bio-conjugation, catalysis, drug delivery, imaging, and surface-enhanced Raman spectroscopy. Recently, a wide range of synthetic methods for the preparation of MNPs has been established, including chemical, photochemical, electrochemical, and thermal approaches. However, among these, growing emphasis is placed on biological and green technologies for nanoparticle production due to their safer and eco-friendly nature (Yin et al., 2003). Within MNPs, silver nanoparticles (AgNPs) hold a crucial role in biology, medicine, and agriculture. They have gained increasing importance because of their strong surface plasmon resonance (Noor et al., 2022), optical features (Dimitrijevic et al., 2001), catalytic performance (Callegari et al., 2003), excellent antimicrobial activity (Zhang et al., 2006). anticancer potential, and even wound-healing applications (Naik et al., 2002). In recent studies, AgNPs have also shown promise in water purification, biosensor development, crop disease control, and pharmaceutical formulations. Their multifunctional roles highlight their potential beyond traditional biomedical uses. Although many methods exist for synthesizing MNPs, the chemical reduction of metal ions followed by stabilization is most commonly used (Sastry et al., 2003). However, these processes are costly, often involve hazardous chemicals, and may generate harmful by-products, raising environmental and biological safety concerns. This has led scientists and nanochemists to explore green, low-cost, and scalable alternatives. Nanobiotechnology provides new solutions by synthesizing nanostructures through living organisms. Among biological methods, plants have emerged as particularly promising tools in the synthesis of MNPs. Unlike microbial systems, plant-based synthesis avoids the complexity of culture maintenance while ensuring rapid, stable, and cost-effective production. Plant extracts contain diverse phytochemicals such as flavonoids, alkaloids, phenolics, terpenoids, and proteins, which act as natural reducing and stabilizing agents.

These biomolecules not only facilitate extracellular nanoparticle synthesis but also allow precise control over size, shape, and dispersion. Such controlled biosynthesis ensures improved reproducibility and functional efficiency of nanoparticles. Furthermore, the use of plants for nanoparticle synthesis can be scaled up to meet industrial requirements, making them suitable for large-scale production (Zare et al., 2013). The integration of plant-mediated biosynthesis with modern nanotechnology could pave the way for sustainable nanomaterials that serve in agriculture, medicine, food preservation, and environmental remediation. Green synthesis utilizes biological extracts like plants or microbes as reducing agents, offering an eco-friendly and biocompatible route. In contrast, chemical synthesis employs synthetic reagents for precise control over size and shape, but often involves toxic chemicals and higher energy consumption, posing environmental concerns Figure 1.

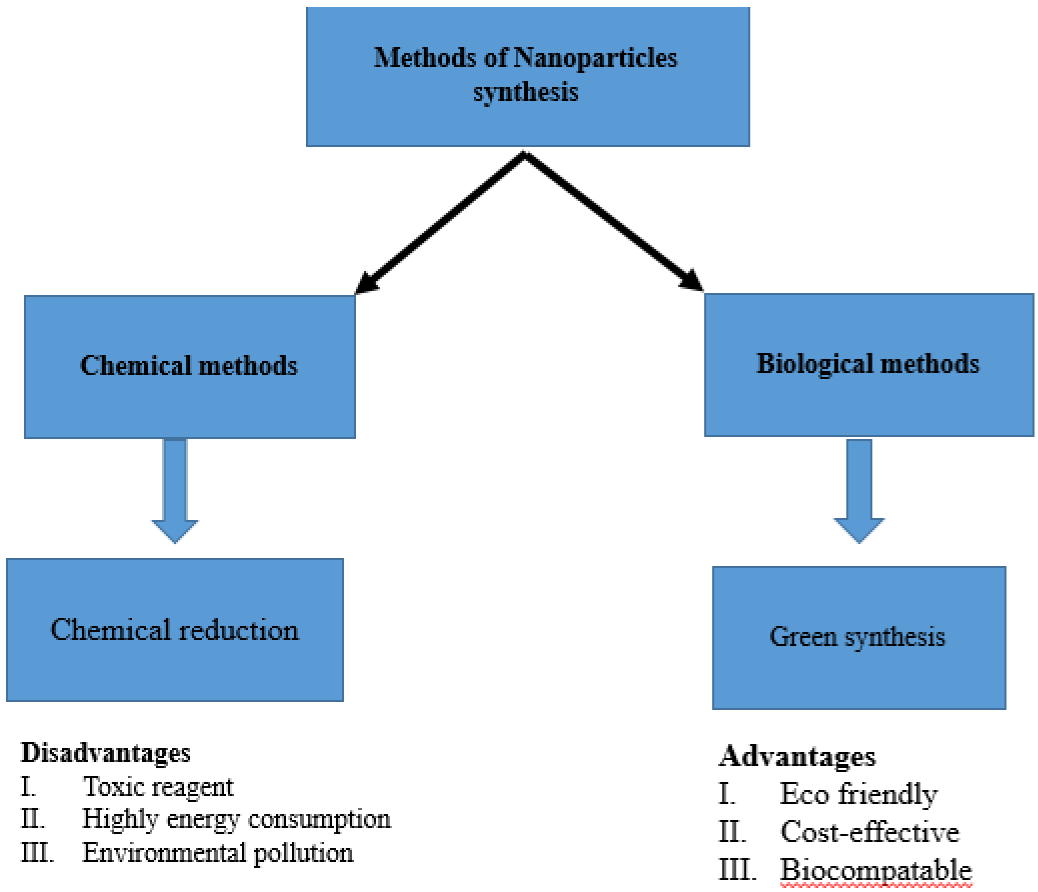

Figure 1: Comparative overview of nanoparticle synthesis approaches

Methods of Synthesis of Nanoparticles

Biogenic-Mediated Synthesis of Nanoparticles

In the field of material chemistry, nanoparticles play a vital and indispensable role in modern scientific research and technological applications. They are widely used in the control of microorganisms such as bacteria and fungi. Biosynthetic methods for nanoparticle production can be chemical, physical, or biological. Based on the site of nanostructure formation, several microorganisms demonstrate the ability to reduce and accumulate metals. For example, Pseudomonas stutzeri, isolated from silver ores, can reduce Ag ions and accumulate silver particles, producing nanoparticles in the range of 16–40 nm with an average diameter of 27 nm. Another example includes magnetotactic bacteria, which are capable of producing magnetite (Fe_3O_4) or greigite (Fe_3S_4) nanoparticles. Silver, known for its broad-spectrum antibacterial properties, is able to kill around 650 types of pathogenic microbes. In addition to this, silver possesses unique electrical, optical, and biological properties, making it useful for drug delivery, imaging, catalysis, biosensing, and even wound healing (Soleimani et al., 2021). Biosynthesized metals such as silver, gold, copper, and zinc are effective against both Gram-positive and Gram-negative bacteria, including Bacillus subtilis, Escherichia coli, and Staphylococcus aureus. Among them, silver nanoparticles have received particular attention, especially through green synthesis using plants, bacteria, fungi, and yeasts (Bajpai et al., 2007). For instance, the green synthesis of AgNPs from Streptomyces endophytes has shown significant antimicrobial activity against four plant pathogenic fungi, namely Alternaria, Streptomyces, Pythium, and Aspergillus niger. Similarly, chamomile flowers have been used to synthesize MgO and MnO_2 nanoparticles with promising bioactivities. Furthermore, green-synthesized ZnO and TiO_2 nanoparticles prepared using lemon fruit extract demonstrated strong antibacterial activity against Dickeya dadantii at room temperature, which is a destructive pathogen causing stem and root rot in sweet potato (Maheswari et al., 2012). These examples highlight the potential of eco-friendly synthesis methods, where plant- and microbe-derived nanoparticles can be tailored for specific applications in medicine, agriculture, food preservation, and environmental remediation. Such approaches not only reduce toxicity but also provide scalable and sustainable pathways for future nanotechnology applications Table 1.

Table 1: Comparative overview nanoparticles synthesis Methods

Method	Source	Key mechanism	Advantages	Limitations	References
Chemical	Metal salts+reducing agents	Reduction by chemical reagents	Rapid, controllable	Toxic chemicals, environment hazards	(Yin et al., 2003, Zhang et al., 2006)
Biological (plant)	Plant extracts	Reduction by phytochemicals	Eco-friendly, scalable	Variation in phytochemicals content	(Zare et al., 2013, Lu et al., 2007)
Biological (Microbs)	Bacteria, algae, fungi	Enzymes-mediated reduction	High stability	Slow growth, contamination risk	(Mohammadi et al., 2019, Bajpai et al., 2007)

Different Plants-Mediated Synthesis of Nanoparticles

In green nanotechnology, plant extracts are increasingly employed as natural reducing agents, offering significant advantages over other biological processes because they eliminate the need for complex cell culture and maintenance steps. Plant-mediated nanoparticle synthesis is cost-effective, eco-friendly, and considered safe for humans. Different sizes and shapes of silver, gold, platinum, and titanium nanoparticles can be synthesized from diverse plant parts, including fruits, bark, pericarp, roots, and leaves (Thakka et al., 2010). The general process of synthesis involves collecting the required plant parts, washing them thoroughly with distilled water to remove epiphytes and necrotic material, and then drying and storing the clean plant source in the dark for 10–15 days. The dried samples are pulverized using a household mixer, and about 10 g of powdered material is boiled with 100 ml of deionized distilled water. The resulting solution is then filtered carefully until no insoluble material remains in the broth. The filtrate is subsequently mixed with $AgNO_3$ solution at a final concentration of 1 mM

(Hasan 2015). After mixing, the solution may be oscillated in a shaking incubator, and a distinct color change appears due to the reduction of silver ions to Ag^0. Regular monitoring of the sample through UV–visible spectroscopy is essential to confirm the characteristic absorption peaks of nanoparticles, which indicate successful formation (Saxena et al., 2010). Green nanoparticles have been successfully synthesized from a variety of plant species. For example, root extract of Morinda citrifolia, inflorescence extract of Phoenix dactylifera and Mangifera, Aloe vera extract, latex of Jatropha gossypifolia, fruit extract of Phyllanthus emblica, and aqueous rosemary extract have all been reported to produce nanoparticles with significant biological properties. In particular, rosemary-mediated synthesis has been shown to produce Mg-flower-shaped nanoparticles with strong antibacterial potential (Mirzaei and Darroudi 2017). The quality, stability, size, and morphology of these green-synthesized nanoparticles depend on several critical factors such as the concentration and chemical composition of the plant extract, the concentration of the metal salt precursor, the pH of the reaction medium, and the reaction temperature. Even slight variations in these parameters can significantly alter the yield and functional efficiency of the nanoparticles. For instance, titanium dioxide nanoparticles synthesized from fresh lemon fruit extract demonstrated notable antibacterial activity within just 8 minutes of reaction time. Similarly, Ocimum sanctum (holy basil) leaf extract has been reported to reduce silver ions into nanosilver particles ranging from 4–30 nm in size, which exhibit potent antibacterial activity (Parveen et al., 2016) Table 2.

Table 2: Plant species use in green synthesis of nanoparticles

Plant source	Metal/Nanoparticles	Partical size (nm)	Morphology	Application	References
Aloe vera	Ag	20-50	Spherical	Antibacterial	(Gericke and Pinches 2006)
Ocimum sanctum	Ag	4-30	Irregular	Antimicrobial	(Parveen et al., 2016)
Phyllanthus emblica	Au	15-40	Spherical	Antioxidant	(Mirzaei and Darroudi, 2017)
Lemon fruit extract	TiO_2	10-60	Flower-like	Antibacterial	(Saxena et al., 2010)

Application

Agricultural Applications of Nanoparticles

In modern agriculture, the extensive use of chemicals such as pesticides, fungicides, and herbicides to control pests and diseases has become common practice. However, this approach has led to several harmful consequences, including antagonistic effects on human health, adverse impacts on pollinating insects and livestock, and the contamination of soil and water, which ultimately disrupts ecosystems. To overcome these challenges, nano-scale materials and technologies are being considered as promising alternatives (Pandit et al., 2022). By producing pesticides, fertilizers, and growth regulators through nanoparticles and nanocapsules, it is possible to minimize environmental pollution while enhancing efficiency. Nanomaterials such as polymers, iron oxide nanoparticles, gold nanoparticles, and silver nanoparticles can be synthesized and effectively used as carriers for pesticides, herbicides, and even therapeutic drugs. These carriers improve pharmacokinetic behavior, allowing controlled and sustained release, thereby reducing the frequency of application and lowering toxicity risks. Conventional pesticides, when applied after disease occurrence, often lead to crop losses and residual toxicity in the environment. In contrast, nano enabled formulations can act as both preventive and therapeutic tools, ensuring higher precision and reduced side effects (Hussain et al., 2016). Another emerging approach is disease detection at the molecular level. Identifying biomarkers linked to DNA replication or early viral protein synthesis offers a way to detect and manage infections before visible symptoms appear. This biomarker based strategy, combined with nano-enabled sensors, forms the foundation of smart agriculture systems. Moreover, nano-based diagnostic kits can significantly improve both the speed and accuracy of detection, enabling early intervention (Figure 2). Additionally, integrating nanosensors into soil and water monitoring can provide real-time data on nutrient availability, pathogen presence, and environmental stress, further supporting sustainable farming practices. Table 3.

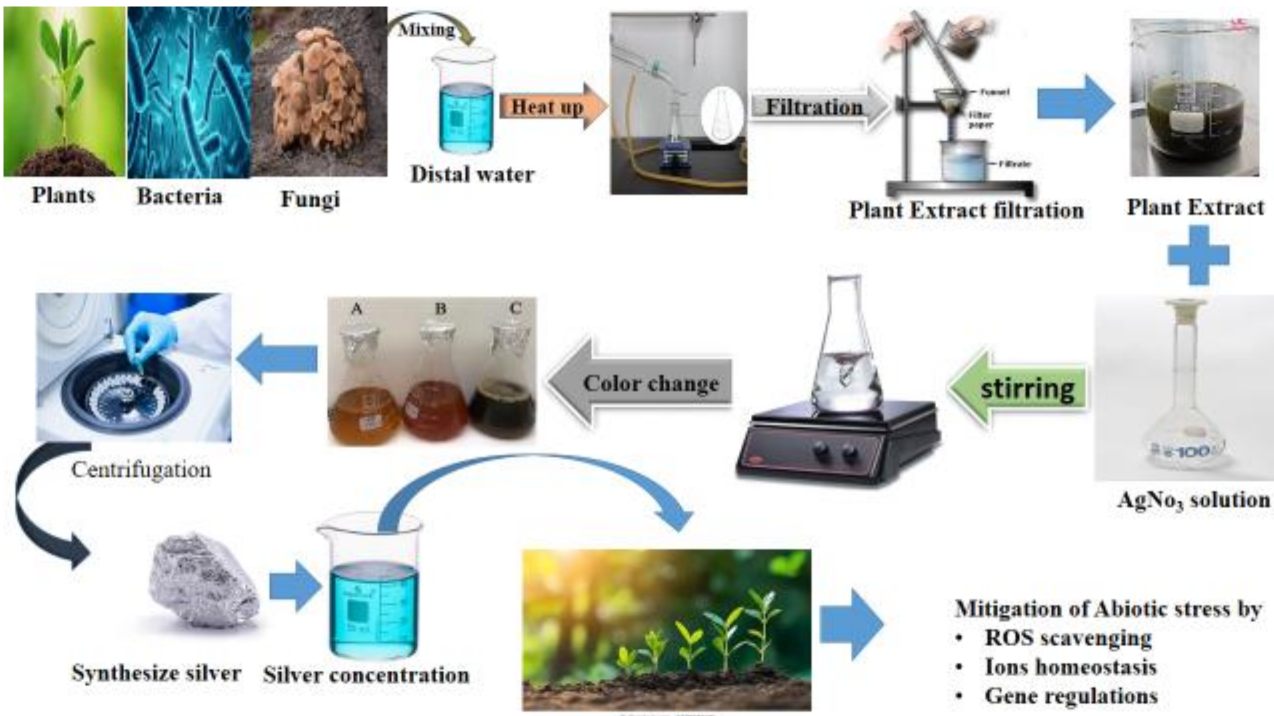

Figure 2. Abiotic stress mitigation

Table 3: Role of Biogenic Nanoparticles in Abiotic Stress Mitigation

Stress type	Nanoparticles	Mechanism	Physiological effects	References
Salinity	Si-NPs	Na^+ exclusion, ROS scavenging	Improved chlorophyll, osmotic balance	(Rizwan et al., 2019)
Acidity	ZnO-NPs	Competition with Al^{3+} ions	Reduced root stunting, enhanced P uptake	(Adrees et al., 2020)
Drought	TiO_2-NPs	Enhanced antioxidant enzymes	Higher RWC and proline	(Khan et al., 2020)
Heavy metal	Fe_2O_3-NPs	Chelation and detoxification	Reduced metal uptake	(Masood et al., 2021)

Nanotechnology in Food Safety and Packaging

Oxygen tends to act as one of the key factors in the food industry that can cause food spoilage and discoloration. One of the main implementations of Nanotechnology within the food industry is the creation of fresh plastics for food packaging. These Nanoparticles in return are processed to produce plastics that are used for packaging of foods. In the new plastic, the Nanoparticles are known to be spiked, acting as a barricade used for blockage in order to prevent oxygen diffusion. Other than that, the pathway for the oxygen to enter the package should be long, the result of that is, and the food deterioration is imminent in the future. According to "Predicala 2009", Nano-coatings are produced to prevent fruits from weight loss and shrinkage, thus covering them completely (Pantidos and Horsfall 2014). It has been the goal of many production companies to develop smart packaging to optimize the shelf life of the product. A packaging system developed with such technology will be in itself enough to repair minor gaps within packaging, as well as responding to environmental conditions when climate change occurs and alert the customers regarding the contamination of food. None other than Nanotechnology can provide the solutions for these problems. Here are some of the examples: the changing of permeability of foils, enhancing the properties of the barrier (mechanical, thermal, chemical and microbiological), ameliorate the mechanical and heat resistance of the packaging, evolving the already developed active antimicrobial and antifungal surfaces with sensing and protection. The top example regarding the production of Nanotechnology is the Ethylene absorbent (Sharma et al., 2019). Ethylene, a gas that is produced by fruits in order to increase the probability of food decay, is absorbed by the Nanomaterials in the absorbent ethylene, thus increasing the tenacity of fruits for an extended period. In order to monitor the agricultural products, Nano barcodes and Nano processing can also be used as a measure. According to "Prasanna, 2007" As new discoveries are made in the world of Nanotechnology, in the same manner that normal barcodes operate, Nano-based barcodes can also perform the similar function like helping to track the food products and keeping a check on the quality of food products, thus producing an entire record in a matter of minutes, or less. The biosensor is another example of the advanced Nano-technology, that comprises of a biological element, that is the same as a cell, an enzyme or an antibody, linked to a small transducer, a device powered by one system that in return supplies the same power to a different system in another form, a form that is required by that other system. Biosensors detect and record every change occurring in every cell and molecule that is used to measure, identify and test the test substance, even at the lowest concentrations of the substance about to be tested. When a substance hitches to a biological component, the transducer then produces a signal that is proportional to the number of components. In case of a high concentration of bacteria in a certain food product, the biosensor will perform the duty of producing a strong signal that would indicate that the food is not sanitary and hence, not safe to be consumed. With a technology so sharp and so cutting edge, it is easy to check the amount of food for their safety in consumption (Rane et al., 2018).

Nanotechnology in Pest and Disease Management

At present, one of the most widely adopted and economical strategies to manage pests and plant diseases is the use of synthetic chemicals such as pesticides, fungicides, and herbicides. However, the increasing reliance on these inputs, along with the high cost of biological control methods, has resulted in serious drawbacks. Overuse and abandoned application of pesticides have caused multiple harms, including adverse impacts on human health, pollinators, livestock, and contamination of soil and water, leading to direct and indirect damage to ecosystems. To address these issues, the application of nano-scale materials has emerged as a promising solution. Unlike conventional chemicals, nano-formulated pesticides and fertilizers can act in a targeted manner, delivering active ingredients precisely to the infected or vulnerable plant tissues. Such smart nano-scale carriers often exhibit self-regulating functions, ensuring that only the required amount of drug is released at the right site, thereby reducing waste and toxicity. Nanotechnology in agriculture also contributes to environmental protection by enabling controlled or delayed release through nanoparticles and nanocapsules. These formulations not only improve efficiency but also reduce the frequency of applications. A wide variety of nanomaterials, including polymer nanoparticles, iron oxide nanoparticles, silver nanoparticles, and gold nanoparticles, can be synthesized and functionalized to serve as carriers for pesticides, growth regulators, or therapeutic agents. Their pharmacokinetic behavior is influenced by size, shape, and surface modifications, which in turn control drug release kinetics. This results in sustained delivery of active molecules and minimizes crop losses caused by untimely application (Rane et al., 2018). One of the greatest challenges in plant protection remains viral infections, as they are difficult to control once established. Therefore, early detection is critical. Advances in nano-enabled diagnostics, particularly virus detection kits, have enabled rapid identification of specific virus strains and the stage of infection. Biomarker-based detection, such as monitoring viral DNA replication or early viral protein synthesis, allows timely intervention. Furthermore, nanosensors can detect differential protein expression in healthy versus infected plants, providing insights into infection cycles and improving disease management (Tsekhmistrenko et al., 2020). Looking ahead, nano-based tools and "smart" agricultural systems hold great promise. The integration of nanosensors for soil health monitoring, nutrient availability tracking, and real-time pathogen detection could revolutionize precision farming (Reverberi et al., 2017). Such approaches will not only enhance crop protection but also promote sustainable and eco-friendly agricultural practices.

Mitigation of nanoparticles

Nanoparticle-Mediated Salinity Stress Alleviation

The application of nanoparticles to mitigate salinity stress involves a methodology designed to counteract both osmotic and ionic stress in plants. The process typically employs green-synthesized nanoparticles such as silicon (Si-NPs), zinc oxide (ZnO-NPs), and selenium (Se-NPs) due to their roles in enhancing plant defense mechanisms. The standard approach involves applying a specific concentration of NPs, often between 50-100 mg/L, through seed priming or foliar spray (Rizwan et al., 2019). The primary mechanism is the reduction of sodium (Na^+) ion uptake and translocation. For instance, Si-NPs facilitate the deposition of silica in root endodermal cells, creating a physical barrier that limits the apoplastic flow of Na^+ into the stele, thereby maintaining a healthier potassium-to-sodium (K^+/Na^+) ratio (Alsaeedi et al., 2019). Concurrently, NPs enhance the plant's osmotic adjustment by stimulating the production of osmolytes like proline and boost the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) to scavenge reactive oxygen species (ROS) (Khan et al., 2020). The effectiveness of this methodology is evaluated by measuring improved germination rates, increased biomass, higher chlorophyll content, and reduced oxidative stress markers like malondialdehyde (MDA) in treated plants under saline conditions.

Nanoparticle-Based Mitigation of Soil Acidity and Metal Toxicity

The amelioration of soil acidity, which leads to aluminum (Al^{3+}) and manganese (Mn^{2+}) toxicity, utilizes a methodology centered on the detoxification of the rhizosphere using nanoparticles. This strategy primarily involves the use of zinc oxide (ZnO-NPs) and iron oxide (Fe_2O_3-NPs) applied as a soil drench or seed coating (Rossi et al., 2017). The key mechanism lies in the interaction between nanoparticles and toxic ions. ZnO-NPs can release Zn^{2+} ions that compete with Al^{3+} for uptake sites on root surfaces, reducing aluminum absorption (Adrees et al., 2020). More significantly, the presence of NPs stimulates root exudation of organic acids, such as citrate and malate. These acids chelate Al^{3+} ions in the soil, forming stable, non-toxic complexes that prevent aluminum from damaging root tips and inhibiting growth (Hussain et al., 2018). This directly alleviates the primary symptom of acidity stress—severe root stunting. Additionally, nanoparticles can improve the availability of essential nutrients like phosphorus, which is often fixed in insoluble forms in acidic soils. The success of this method is assessed by observing significant improvements in root architecture, reduced aluminum concentration in plant tissues, and enhanced nutrient uptake, leading to overall better plant performance in acidic conditions (Masood et al., 2021).

Mitigation of Drought Stress Using Nanoparticles

The application of nanoparticles to ameliorate drought stress involves a methodology aimed at enhancing the plant's water retention capacity, osmotic adjustment, and antioxidant defense system under water-deficient conditions. This approach typically utilizes nanoparticles such as silicon (Si-NPs), zinc oxide (ZnO-NPs), and titanium dioxide (TiO_2-NPs) due to their unique properties in modulating plant physiological responses. The standard methodological practice involves the application of these NPs, often at concentrations ranging from 50 to 100 mg/L, through seed priming or foliar spray before or during the onset of drought stress (Rizwan et al., 2019). The mechanisms are multifaceted: Si-NPs, for instance, contribute to the formation of a silica layer on leaf surfaces, which reduces cuticular transpiration and improves water-use efficiency (Alsaeedi, et al., 2019). Simultaneously, nanoparticles act as nano-elicitors, triggering the biosynthesis of osmoprotectants like proline and soluble sugars, which help maintain cell turgor pressure. Furthermore, NPs enhance the activity of key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which effectively scavenge the excess reactive oxygen species (ROS) generated under drought stress, thereby protecting cellular membranes from oxidative damage as indicated by reduced malondialdehyde (MDA) levels (Khan et al., 2020). Studies have also shown that nanoparticles can improve root architecture, enabling deeper soil water exploration. The efficacy of this methodology is evaluated by measuring relative water content, leaf area, biomass accumulation, stomatal conductance, and overall yield under drought conditions compared to untreated stressed plants (Adrees et al., 2020).

Alleviation of Temperature Stress Using Nanoparticles

The mitigation of temperature stress, both high (heat) and low (chill), through nanoparticle application leverages their role in stabilizing cellular membranes, protecting photosynthetic apparatus, and modulating stress-responsive gene expression. The methodology commonly employs nanoparticles like silicon (Si-NPs), selenium (Se-NPs), and zinc oxide (ZnO-NPs). Application is typically performed via foliar spraying or root irrigation with NP solutions at critical growth stages preceding or during stress periods (Rossi et al., 2017). Under heat stress, nanoparticles help in the accumulation of heat shock proteins (HSPs) and osmolytes, which protect enzymatic and structural proteins from denaturation (Iqbal et al., 2021). They also play a crucial role in maintaining the integrity of thylakoid membranes in chloroplasts, thereby preserving photosynthetic efficiency and chlorophyll content. Under cold stress, NPs enhance the fluidity of cell membranes, preventing leakage of ions and metabolites, and boost antioxidant systems to counteract chilling-induced oxidative burst (Masood et al., 2021). For instance, Se-NPs have been reported to increase the unsaturated fatty acid content in membranes, which is vital for maintaining membrane flexibility at low temperatures. The success of this methodological strategy is assessed by evaluating membrane stability index, photosynthetic rate, electrolyte leakage, concentration of stress metabolites, and ultimately, the survival rate and productivity of crops exposed to supra- or sub-optimal temperatures (Hussain et al., 2018).

Recent Advances in Biogenic Nanoparticles for Abiotic Stress Tolerance (2015–2025)

Over the past decade, research on biologically synthesized nanoparticles has expanded rapidly, focusing on their roles in mitigating abiotic stress in plants. Table 4 provides a comprehensive summary of key studies conducted between 2015 and 2025, highlighting various nanoparticle types, biological synthesis sources, target plants, stress conditions, tools and analytical techniques used, and major findings. These studies collectively demonstrate the effectiveness of biogenic nanoparticles especially those synthesized from plant and microbial extracts—in enhancing plant tolerance to drought, salinity, temperature, and heavy metal stress through improved antioxidant regulation, ion homeostasis, and gene modulation. Table 4.

Table 4: Data collection from 2015 to 2025 published papers

Year	Biological source / NP type	Target plant / stress	Tools/ Technics	Major findings	Reference
2015	Aloevera extract/AgNPs	Rice/Salinity	UV–Vis, FTIR, SEM, XRD	Enhanced germination, antioxidant enzymes, improved Na^+/K^+ ratio	Singh et al., 2015
2015	Bacillus-mediated AgNPs Arabidopsis/ Oxidative stress	Arabidopsis/ Oxidative stress	TEM, qPCR, DLS Reduced ROS	induction of stress-responsive genes	Kumar et al., 2015
2016	Azadirachta indica (Neem) extract / ZnO NPs	Wheat/ Drought	UV–Vis, TEM, FTIR	Improved RWC, chlorophyll, osmolyte accumulatin	Li et al., 2016
2016	Fungal-mediated AgNPs	Tomato/ Bacterial disease	SEM, EDX, GC-MS	Strong antimicrobial effect; reduced disease severity	Wang et al., 2022
2017	Ocimum sanctum / TiO_2 NPs	Tomato/Heat	XRD, SEM, chlorophyll fluorescence	Increased photosynthetic rate, higher proline	Van Nguyen., 2022
2017	Streptomyces sp. / AgNPs	Chickpea/ Drought	ICP-MS, qPCR, TEM	Enhanced antioxidant system and drought tolerance	Shang et al., 2019
2018	Moringa oleifera extract / CuO NPs	Tomato/Heat	Heat SEM, XRD, photosynthesis assays	Improved photosynthetic efficiency and antioxidant enzymes	Khan et al., 2018
2018	Green-synthesized Si NPs	Maize/Salinity	UV–Vis, FTIR, ICP-OES	Reduced Na^+ uptake; improved K^+/Na^+ balance	Zhao et al., 2019
2019	Tridax procumbens / Fe_3O_4 NPs	Mustard/ Cadmium	FTIR, XRD, DLS	Decreased Cd uptake; reduced lipid peroxidation	Shang & Singh, 2019

2019	Algae-mediated ZnO NPs	Rice/Drought	SEM, HPLC	enzyme assays Enhanced antioxidant defense and water retention	Zhao et al., 2019
2020	Fungal-mediated AgNPs	Rice/Drought	SEM, EDX, proteomics	Induced stress-responsive proteins; improved water-use efficiency	Elsheery et al., 2010
2020	Moringa-mediated Fe_2O_3 NPs	Mustard/ Aluminum toxicity Soil assays	ICP-OES, root analysis	Reduced Al uptake and root damage; improved root architecture	Hussain et al., 2020
2021	Green-tea extract / TiO_2 NPs	Maize/Salinity	UV–Vis, TEM, qPCR Stabilized chlorophyll	increased osmolytes and ion homeostasis	Ahmad et al., 2021
2021	Camellia sinensis-derived SeNPs	Soybean/ Salinity	DLS, enzyme assays, ICP-MS Enhanced SOD & CAT activities	improved tolerance	Alsaeedi et al., 2021
2021	Endophytic bacteria-mediated AuNPs	Arabidopsis / Heavy metal stress	TEM, RNA-seq, ICP-OES	Downregulated metal transporters; reduced uptake	Gomez et al., 2021
2022	Neem leaf extract / AgNPs	Mungbean/ Salinity	DLS, SEM	growth assays Improved germination and antioxidant enzyme activity	Tripathi et al., 2017
2022	Microalgae-mediated ZnO NPs	Chickpea/ Drought	FTIR, SEM, HPLC	Increased chlorophyll and enzymatic antioxidant defense	Patel et al., 2022
2023	Lemon-peel extract / CuO NPs	Chickpea/ Drought	UV–Vis, TEM	ROS assays Higher biomass and lower ROS accumulation	Masood et al., 2023
2023	Plant-extract SiO_2 NPs	Rice/ Submergence & Salinity	SEM, qPCR	photosynthesis assays Improved submergence recovery, ion homeostasis	Singh & Roy, 2023
2023	Seaweed-mediated AgNPs	Wheat/Salinity	XRD, TEM	enzyme assays Enhanced antioxidant responses and yield parameters	Chen et al., 2023
2024	Endophytic-fungi AuNPs	Rice / Heavy metal detox	TEM, ICP-OES	enzyme assays Lowered	Li et al., 2024

				metal accumulation; increased detox enzymes	
2024	Bamboo-extract SiO_2 NPs	Barley/Drought	UV–Vis, FTIR, qPCR	Upregulation of stress genes; improved RWC and yield	Tripathi, et al., 2017
2024	Streptomyces-mediated Fe_3O_4 NPs	Soybean/ Metal stress	SEM, ICP-MS	proteomics Enhanced metal chelation; reduced shoot accumulation	Van Nguyen & Patel, 2022
2025	Bamboo-based extract / SiO_2 NPs	Rice / Multi-stress	UV–Vis, FTIR, SEM, qPCR	Improved salinity & submergence tolerance; eco-safe routes	Zhang et al., 2025

Research gaps and Future direction

Research Gaps

The research highlights the significant promise of biologically synthesized nanoparticles (NPs) for sustainable agriculture but concurrently underscores critical research gaps that must be addressed to translate this potential into practical reality. The most prominent gap is the insufficient understanding of the mechanistic pathways of NP-plant interaction. While it is known that biogenic NPs enhance antioxidant systems, regulate genes, and improve photosynthesis, the precise molecular-level events—from the initial uptake and translocation pathways within plant tissues to the specific signaling cascades and transcription factors they modulate—remain a "black box." This lack of fundamental knowledge hinders the rational design of NPs for specific physiological outcomes. Closely linked is the gap concerning long-term ecological safety and environmental impact**.** The very properties that make NPs effective also raise questions about their persistence, transformation, and fate in agro-ecosystems. Critical investigations are needed to determine if these particles accumulate in soils, disrupt microbial communities, leach into groundwater, or enter the food chain, posing risks to higher trophic levels. Without comprehensive life-cycle assessments, their widespread application carries potential unforeseen consequences.

Furthermore, a significant translational gap exists between controlled laboratory studies and real-world field application. Most research is confined to greenhouses under idealized conditions, lacking validation against the complex, combined stresses plants face in the field. This gap extends to a lack of optimized, crop-specific and stress-specific protocols. The optimal NP type, size, coating, concentration, and method of application for a given crop (e.g., rice under salinity vs. tomato under drought) are largely undefined, and the line between a beneficial and a phytotoxic dose remains blurred.

Finally, there is a gap in comparative and integrative studies. Systematic research directly comparing the efficacy, cost, and safety of biogenic NPs against conventional agrochemicals and chemically synthesized NPs is scarce. Moreover, their potential synergies within integrated pest and nutrient management systems, combining them with biofertilizers or traditional breeding, are unexplored. Addressing these gaps is paramount for developing biogenic NPs into a safe, effective, and trusted tool for enhancing global food security.

Future plan

Elucidating Molecular Mechanisms and Nano-Bio Interfaces

The foremost plan is to decode the precise molecular dialogues between biogenic nanoparticles (NPs) and plant systems. This requires a shift from phenomenological observation to mechanistic discovery. Future work will employ integrated omics technologies—transcriptomics, proteomics, and metabolomics—to map the global changes in gene expression, protein synthesis, and metabolic pathways induced by NP application. A key objective is to identify the primary receptors and signaling hubs, such as specific mitogen-activated protein kinase (MAPK) cascades or hormone pathways, that NPs activate. Furthermore, advanced imaging and spectroscopic techniques will be used to track NP uptake, internalization, and subcellular localization in real-time, clarifying their journey from application to action.

Comprehensive Environmental Risk Assessment and Lifecycle Analysis

Prior to large-scale adoption, a rigorous and long-term environmental safety profile is essential. Future research must systematically investigate the lifecycle of biogenic NPs in agro-ecosystems. This involves studying their persistence, biodegradation, and potential for transformation in different soil types over multiple cropping seasons. Critical ecotoxicological assessments will focus on their impact on non-target soil organisms, including beneficial microbial communities, mycorrhizal fungi, and earthworms. Studies must also evaluate the potential for trophic transfer and bioaccumulation to ensure food chain safety and inform regulatory frameworks.

Field-Level Validation and Agronomic Optimization

Bridging the lab-to-field gap is a critical milestone. The future plan mandates extensive multi-location field trials under diverse pedoclimatic conditions to validate the efficacy of biogenic NPs against combined abiotic stresses. These trials will be crucial for developing agronomically sound protocols. Concurrently, research must focus on optimizing NP characteristics—such as size, shape, surface charge, and coating—for specific crop-stress combinations. This includes establishing precise dosage windows that maximize benefits while avoiding phytotoxicity and ensuring the economic viability of the technology for end-user farmers.

Development of Integrated and Synergistic Formulations

Finally, to maximize resilience and sustainability, future efforts should explore the integration of biogenic NPs into holistic crop management systems. This involves researching their synergistic effects with other biologicals, such as plant growth-promoting rhizobacteria (PGPR), biopesticides, and organic amendments. The goal is to develop novel, multi-functional formulations that enhance nutrient use efficiency, improve soil health, and provide broad-spectrum stress tolerance, thereby reducing the reliance on conventional agrochemicals and building more robust agricultural systems.

Conclusion

Abiotic stresses remain a major barrier to achieving stable agricultural productivity, especially under changing climatic conditions. Conventional strategies for stress management often fail to provide sustainable solutions due to their limitations in efficiency, cost, and environmental safety. Biologically synthesized nanoparticles (NPs) present a promising alternative, offering eco-friendly, economical, and biocompatible means to mitigate plant stress. Their unique physicochemical properties enable plants to overcome adverse conditions by enhancing antioxidant defense systems, maintaining ion balance, regulating stress-responsive genes, and improving photosynthetic performance. Unlike chemically synthesized nanoparticles, biogenic NPs minimize toxic effects and integrate naturally into plant systems, thereby strengthening stress tolerance without compromising environmental safety. The application of these nanoparticles in agriculture opens new opportunities for developing resilient crop varieties and improving overall yield stability. However, despite their potential, the mechanisms of nanoparticle–plant interactions remain insufficiently understood, and their long-term ecological impacts require further investigation. Future studies should focus on molecular-level interactions, dosage optimization, and field-scale validation to ensure safe and effective use. Overall, biologically synthesized nanoparticles represent a transformative approach in plant stress management and hold significant promise for sustainable agriculture and food security in the face of global environmental challenges.

Declaration

Acknowledgment: The authors would like to acknowledge the support from their respective institutions.
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Ethics approval/declaration: N/A
Consent to participate: N/A
Consent for publication: N/A
Data availability: No datasets were generated or analysed during the current study.
Author's contribution: Pukraj Ud Din writing and analyzing the manuscript. All authors contributed to the conceptualization, and review of the manuscript.

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