Green Synthesis of Silver Nanoparticles Using Algae and Its Effect on Salt-Stressed Wheat

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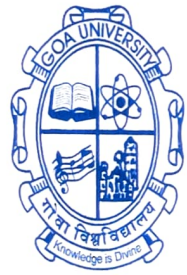

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The synthesis of nanoparticles is currently an interesting area of study due to their wide applications and large surface-to-volume ratio. Cyanobacteria are of ecological importance due to their photosynthetic activity, nitrogen-fixing activity and as they also as nutrient-rich food source. Seaweeds also serve as important food sources and are the potential bio-resources of the marine ecosystem. Physical, chemical and biological methods to synthesise the nanoparticles. However, biological synthesis is crucial as it is eco-friendly, non-toxic and cost-effective. In the present study, the green synthesis of the silver nanoparticles was done by cyanobacteria *Nostoc commune* and seaweed *Enteromorpha intestinalis.* Their characterisation was achieved using an Ultraviolet-Visible Spectroscopy spectrum, revealing that the silver nanoparticle formation increased with the addition of the algal extracts. The spectral peaks were observed at 440 nm and 495 nm in the case of *N. commune* and *E. intestinalis*, respectively. Fourier Transform Infrared Spectroscopy analysis revealed that the primary, secondary, aliphatic amines, amides, carboxyl, alkynes, nitro-compounds and phenols were the biomolecules responsible for the silver ion reduction and Scanning Electron Microscopy images examined the surface morphology and revealed the average size of the silver nanoparticles to be 89.37 nm and 76.42 nm in *N. commune* and *E. intestinalis* respectively. The silver nanoparticles synthesised from both samples showed high antibacterial activity against the gram-positive and gram-negative bacteria, *Bacillus cereus* and *Escherichia coli.* The study was also aimed at analysing the interactive effects of salinity and silver nanoparticles in wheat. On application of nanoparticles, salt-treated plants showed better growth due to increased chlorophyll and carotenoid content. The study thus provides a promising approach to salt tolerance in wheat.

Chapter-1

INTRODUCTION

1. INTRODUCTION

Nanotechnology uses matter on an atomic, molecular, and supramolecular scale for industrial purposes. The associated research and applications are equally diverse, ranging from extensions of conventional device physics to new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale to direct control of matter on the atomic scale.

Nanotechnology may create new materials and devices with many applications, such as nanomedicine, nanoelectronics, biomaterials energy production, and consumer products. On the other hand, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and environmental impact of nanomaterials, their potential effects on global economics, and speculation about various doomsday scenarios.

1.1. Nanoparticles

Nanoparticles are particles ranging between 1 and 100 nanometers (nm). Nanotechnology is one of the new branches of science which is fast growing and shows a high interest amongst researchers due to their wide applications, such as in industries related to medicines, pharmaceuticals, cosmetics, food and agriculture. It deals with the design, synthesis and exploitation of the structure of particles ranging from 1 to 100 nm in size (Shabani *et al.*, 2014).

Nanotechnology examines the relationship between nano-biosynthesis and nanomaterials and expresses how the various parameters like structure, chemical properties and origin can alter the functionality of the nanomachinery of cells (Ahmed *et al.*, 2016). The entire nanoparticle characteristics depend on various parameters such as the nature, size and shape of nanoparticles and their surroundings (Longoria *et al.*, 2011). Nanoparticles have attracted

significant interest from researchers due to their ability to interact efficiently with other particles and their large surface-to-volume ratio (Chugh *et al.*, 2021).

1.1.1. Applications of nanoparticles

There are several examples wherein metal oxide nanoparticles are manufactured and used in households and industries. Zinc nanoparticles are already used in the UV filters in sunscreens (Robert *et al.*, 2007) as well as starting material for UV protection films, electronic appliances, and chemical sensors (Meulenkamp, 1998). The Copper oxide nanoparticles in suspension can be used as a heat transfer fluid in machine tools due to their outstanding thermal conductivity (Gao *et al.*, 2005).

Infections in living organisms, including humans, arise due to several microorganisms like fungi, bacteria, and molds. In order to defend against these infections, various antimicrobial and antibiotic compounds have been discovered (Komolafe, 2004). However, there is a need to develop alternate methods, as over a while, these microorganisms have started exhibiting resistance against most antibiotics. Nanoparticles can be considered an excellent alternative to overcome antibiotic resistance and simultaneously be cost-effective (Kim *et al.*, 2011).

1.1.2. Types of nanoparticles

Inorganic nanoparticles and organic nanoparticles are the two types of nanoparticles. The organic nanoparticles include the quaternary ammonium compounds, chitosan and cationic quaternary polyelectrolytes. The inorganic nanoparticles have metal and metal oxides which include the nanoparticles such as Zinc oxide (ZnO), Silver (Ag), Titanium oxide (TiO_2), Iron oxide (Fe_3O_4) and Copper oxide (CuO). Inorganic nanoparticles are preferred as the organic nanoparticles are less stable at high temperatures (Jain *et al.*, 2014).

1.2. Silver nanoparticles

The inhibitory action against different microorganisms is exhibited by silver in several modes, and it has been known for its medicinal properties for over the last 2000 years since they possess broad-spectrum antimicrobial activity. The silver-based products are cost-effective and tend to induce antimicrobial resistance (Prabhu and Poulose, 2012). *Caulerpa racemosa* (Kathiraven *et al.,* 2015), *Sargassum muticum* (Azizi *et al.,* 2013), *Navicula atomus, Diadesmis gallica, Sargassum wightii, Fucus vesiculosus* (Asmathunisha and Kathiresan, 2013) are used in the biosynthesis of silver nanoparticles. Silver nanoparticles are used in non-linear optics as intercalation materials for batteries, optical receptors, and catalysts.

1.2.1. Methods of silver nanoparticle synthesis

Several distinct techniques can synthesise silver nanoparticles. The methods are broadly classified as physical, chemical, biological, or green synthesis. Generally, two approaches are concerned with forming silver nanoparticles; the 'bottom to top' approach and the 'top to bottom' approach. In the 'bottom to top' approach, the nanoparticles are formed by assembling smaller atoms of molecules. In the 'top to bottom' approach, the nanoparticles are synthesised by breaking down the bulk material into the minute, fine-sized particles using several lithographic techniques like milling, thermal/laser ablation, grinding and sputtering (Ahmed *et al.,* 2016).

1.2.1.1. Physical method

Numerous techniques involved in the physical method of silver nanoparticle synthesis are thermal evaporation, vapour condensation method, lithographic techniques, ultrathin films, diffusion flame synthesis, ball milling and plasma arching (Praveen *et al.,* 2016).

1.2.1.2. Chemical method

One of the most common methods used to synthesise silver nanoparticles is the chemical method. This method reduces silver ions, stabilising the nanoparticles using various chemicals and reagents (Dawadi *et al.,* 2021). These include methods like the chemical reduction method, catalytic route, microemulsion method, polyol process, sol-gel process, chemical solution deposition, wet chemical method, Tollen's method, Langmuir-Blodgett method (Praveen *et al.,* 2016). The chemical method of silver nanoparticle synthesis is toxic and harmful to living organisms and the environment.

1.2.1.3. Biological / Green Synthesis

Green synthesis of the biological nanoparticle synthesis method is more environmentally friendly than physical and chemical methods. Biological agents such as fungi, bacteria, algae and plant extracts are used to synthesise nanoparticles in the biological methods (Ansari, 2018; Roy *et al.,* 2019). The green synthesis process varies depending on the type and form in which the organism is used. Green synthesis in plants involves using only the plant extracts and the aqueous solution of the silver ions, as the plants have properties of reduction, accumulation and detoxification of metals. The plant extracts also contain polysaccharides, polymers, enzymes, alkaloids, flavonoids and proteins, which are used as reducing agents, and few of them even function as capping agents (Logeswari *et al.,* 2015; Nadaroglu *et al.,* 2017). The size of silver nanoparticles synthesised using plant extracts depends on the Silver nitrate concentration or the plant extract concentration. *Aloe vera, Ocimum sanctum, Coffea arabica, Azadirachta indica, Jatropha curcas, Abutilon indicum, and Emblica officinalis,* plant extracts have been used to synthesise silver nanoparticles (Syafiuddin *et al.,* 2017).

1.2.1.4. Biological synthesis of silver nanoparticles using algae

'Phyconanotechnology' is the term used for the branch of nanoscience, which deals with using algae for nanoparticle synthesis. The most common types of algae used for silver nanoparticle synthesis are *Cyanophyceae, Chlorophyceae, Rhodophyceae* and *Phaeophyceae* (Lewis *et al.,* 2016). Algae are mainly used to synthesise nanoparticles due to their high potential to take in and reduce metal ions and low production costs (Singh and Singh, 2019; Rajkumar *et al.,* 2021). Algae are easy to cultivate and handle and are less toxic to the environment (Negi and Singh, 2018). Algae can also tolerate harsh atmospheric conditions more effectively than other organisms (Khan, 2019). Algae are also known as 'Bionanofactories' since both live and dead, dry algal biomass can be used to synthesise nanoparticles (Omar, 2017). The hydrophilic surface groups such as carboxyl, hydroxyl and sulphate are present in the algal synthesised silver nanoparticles, providing them with unique applicability (Rahman *et al.,* 2020).

Algal silver nanoparticles can be synthesised from algal biomass using intracellular or extracellular mechanisms. Various approaches to synthesising algal nanoparticles include using extracted biomolecules, living cultures, cell-free supernatant, and whole cell. In the case of the intracellular mechanism, the bio-reduction of the metal ion to its nanoparticle occurs through the enzymatic activity inside the cell wall and cell membrane. In contrast, in the extracellular mechanism, bio-reduction occurs on the surface of the algal cell (Negi and Singh, 2018).

Synthesis of silver nanoparticles from algal biomass usually involves three steps:

1. Algal extract preparation by in water or an organic solvent at a specific temperature for a specific period.
2. Preparation of the molar solutions of the ionic metallic compounds.

3. Incubation of algal extract mixture with or without continuous stirring (Sharma *et al.*, 2016).

1.2.1.5. Drawbacks of synthesis by physical and chemical methods

Silver nanoparticle synthesis's physical and chemical methods are not environmentally friendly and expensive. There are very high energy requirements in the physical synthesis methods, and the chemical methods involve using hazardous chemicals, which may cause significant environmental risks (Keat *et al.,* 2015). These methods require costly equipment, vacuum conditions and extreme temperatures. Drawbacks of these methods are the structural particle deformation, particle growth inhibition and the low production rate (Natsuki *et al.*, 2015).

1.3. Capping agents

Amphiphilic molecules possessing a non-polar tail and a polar head region are the capping agents. The tail region interacts with the surrounding medium, while the head interacts with the metal atom of the nanocrystals formed by agglomeration (Gulati *et al.,* 2018). Ionic and non-ionic surfactants and polymers are capping agents (Shi *et al.,* 2002). In some cases, capping agents also act as reducing agents.

The experiment, where the algae *Chlamydomonas reinhardtii* was used to synthesise nanoparticles, showed the involvement of the cellular proteins as capping agents (Haider and Kang, 2014). In another study, when *Pithophora oedogonia* was used to synthesise nanoparticles, the secondary amide derivatives, long-chain fatty acids, and terpenoids showed the capping and stabilising of the silver nanoparticles (Sinha *et al.,* 2015).

1.4. Applications of silver nanoparticles

Silver nanoparticles have numerous applications in various fields, such as the food industry, optics, medicines, and water treatment, as they have unique physical, chemical and electrical properties. Recent studies have found that silver nanoparticles can be integrated into electronic components, composite fibres and cryogenic superconducting materials (Xu *et al.,* 2020). Silver nanoparticles are used extensively in pharmaceuticals and medicines due to their low toxicity and stability at different temperatures (Mathur *et al.,* 2018).

1.4.1. Anticancer agents

Recent advances have led to silver nanoparticles' use in cancer diagnosis and treatment. The use of silver nanoparticles as anticancer agents is because the toxicity of the silver nanoparticles is more towards the cancerous cells than the bulk materials. It is inferred from recent studies that the silver nanoparticles suppress tumour cell progression and thus act as antitumour agents. Silver nanoparticles have no lethal effect on normal cells. Silver nanoparticles have inhibitory action in numerous signalling cascades needed for cancer development and pathogenesis (Gomathi *et al.,* 2019). Different human cancer cell lines like the endothelial cells, U251 glioblastoma cells, IMR-90 lung fibroblasts and MDA-MB-231 and MCF-7 breast cancer cells are tested with silver nanoparticles to investigate their anticancer effects (Thapa *et al.,* 2016; Rajeshkumar *et al.,* 2016). Silver nanoparticles have been reported to suppress the growth of the tumour by blocking the process of angiogenesis (El-Naggar *et al.,* 2017).

1.4.2. Drug delivery system

Silver nanoparticles are also used in the drug delivery system as they effectively deliver anticancer drugs to the tumour cells and tissue. The silver nanoparticles can easily penetrate the tissue and bring about effective drug delivery due to their nano-size (Patra *et al.,* 2018).

Using conventional methods in drug delivery may lead to the death of healthy and normal cells and tumour cells (Ivanova *et al.,* 2019).

1.4.3. Antimicrobial agents

Silver nanoparticles have the potential to overcome antibiotic resistance and work effectively against both gram-positive and gram-negative bacteria (Dawadi *et al.,* 2021). The mechanism for bringing about cell death is such that the silver nanoparticles can penetrate the cell wall of the bacteria and cause extreme disturbances in the functioning of the cell (Yan *et al.,* 2018). Colloidal state, shape, size, surface charge and concentration are a few physio-chemical parameters that affect their antimicrobial properties, which depend on the nanoparticle synthesis method (Burdusel *et al.,* 2018).

In *Escherichia coli*, the nanoparticles attach to the bacterial cell wall, forming holes in the membrane responsible for cell death. Larger particles were less potent than smaller particles (Sondi *et al.,* 2004). It has also been reported that 0.06mg/mL or more silver nanoparticle concentration was effective against *E. coli* (Raffi *et al.,* 2008).

1.4.4. Wound healing

Since various cell lineages are involved in wound healing, it is a time-consuming and complicated process, and the rate of wound healing rate is affected by various intrinsic and extrinsic factors like the size, depth and age of the wound, the nutritional status, and the medication. An ideal wound dressing should protect against microorganisms, remove excess fluids or dead cells, and maintain a moist, humid environment surrounding the wound (Konop *et al.,* 2016). Antiseptics, enzyme treatment, herbal therapeutics, granulation tissue suppressing agents, tropical antibiotics, and other tropical agents are conventional drugs used in wound healing. These drugs have limitations like allergies, skin irritation, cytotoxic effect on body cells and narrow antimicrobial spectrum. Due to properties like low toxicity

to the system and effectiveness against multidrug-resistant pathogens, silver nanoparticles can be used as an alternative to conventional methods (Gunasekaran *et al.,* 2012).

1.5. Algae

Algae are a group of autotrophic organisms having ecological and economic importance. They are simple chlorophyll-bearing organisms and can exist as single cells or multicellular organisms. Algae vary in size from giant kelps up to 70 meters, and certain unicellular algae are 3-10 microns. Algae are ubiquitous and widely found in habitats such as freshwater, marine water or damp rock surfaces. The algal cell walls are generally composed of cellulose and pectin, which is responsible for the slimy nature of the algae (Lewis *et al.,* 2016).

Algae can be generally categorised into two kingdoms; the eukaryotic unicellular algae belonging to the kingdom Protista and the Eukaryotic, multicellular algae belonging to the kingdom Plantae. The algae are not differentiated into distinct stems, roots and leaves, i.e., thalloid. Algae are found to exist in different shapes and sizes and may occur as single cells, filaments or colonies. Algae need sunlight and moisture for their growth and sustenance as they are photosynthetic (Michael, 2001).

They may be microscopic (microalgae) or macroscopic (macroalgae). The microalgae are usually found in the littoral and benthic habitats and throughout ocean waters as phytoplanktons, including diatoms, dinoflagellates, green and yellow flagellates and blue-green algae. The macroalgae usually found in the littoral zones are classified into three classes based on the structure of the cell and the pigments. They include Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae). Algae provide almost half of the oxygen needed for the survival of living organisms (Chapman, 2013). Algae are used in paints, cosmetics, pharmaceuticals, and food sources (Wang *et al.,* 2015).

1.5.1. Cyanobacteria

Cyanobacteria, or blue-green algae, are found in various habitats ranging from the Artic regions to the hot springs. They are called blue-green algae as they contain the green chlorophyll pigment and a unique blue pigment called phycocyanin. Phycocyanin is a water-soluble pigment that facilitates light harvesting for photosynthesis. They can exist as single-celled or in colonies; in some cases, the colonies may form filaments, sheets or hollow balls. Few species grow as single cells enclosed in a mucilage sheath, e.g. *Chrococcus.* Other aggregates of the cells in colonies are rounded (*Nostoc*), coiled (*Spirulina*), rod-shaped (*Oscillatoria*) or elongated filaments (*Phormidium*) (Chakraborty *et al.,* 2009).

Cyanobacteria are of great ecological importance as they perform over 90% of the total photosynthetic activity, serve as food sources for herbivores and detritivores, and several species are capable of fixing the atmospheric nitrogen in symbiosis with other plants (e.g. *Nostoc* with *Cycus*, *Anaebana* with *Azolla*) or as free-living organisms (*Nostoc, Anaebana, Oscillatoria*). Some blue-green algae that produce gold nanoparticles are *Spirulina subsalsa, Plectonema boryanum, Lyngbya majuscule* (Lengke *et al.,* 2006). *Nostoc, Spirulina platensis* synthesises silver nanoparticles (Hassan and Hosny, 2018). *Nostoc* is used to increase the nutrient value of soil and is essential for its nitrogen-fixing ability. They are used as a food source as they are rich in proteins and vitamin C. They can produce the hydrogen required for biofuel production. Various species also exhibit antibacterial and antiviral activity (Vanlalveni *et al.,* 2018).

1.5.2. Seaweeds

Seaweeds or marine macroalgae are plant-like organisms. They are generally attached to solid structures or the bottom of the sea by holdfasts (Govindan *et al.,* 2019). They form dense growth on the rocky shores and are well established along the margins of the seas. They include green algae, red algae and brown algae. They belong to the group of

multicellular algae. The size of marine algae can vary from large to very tiny (Elechiguerra *et al.,* 2005).

Seaweeds are renewable and natural living resources in the marine ecosystem. Seaweed extracts contain more than 60 elements, micro and macronutrients, carbohydrates, proteins, amino acids, cytokinins, auxins and abscisic acids (Jensen, 1993). Industrial products such as carrageenan, agar agar and alginates are the sources obtained from the seaweeds (Shelar *et al.,* 2012). Seaweeds are crucial in adsorbing heavy metals such as manganese, copper, zinc and lead (Vijayaraghavan *et al.,* 2009).

1.5.3. Application of algae in the generation of pharmaceuticals and nutraceuticals

The secondary metabolites extracted from plants were initially utilised for medicinal and health purposes. Low yield combined with the seasonal availability of plants is the primary reason for the researchers to look for alternatives such as microalgae. The bioactive components encompassed by algae are considered similar in composition to alternative counterparts of natural plants (Chew *et al.,* 2021). Marine algae are considered an excellent source of pharmaceuticals. Algae contain many functional components, such as carotenoids, chlorophyll, docosahexaenoic acid, eicosapentaenoic acid, and astaxanthin. These components possess numerous benefits for value-added food applications and are widely sought after in the current market. As the expenditure is high for nutraceutical items, researchers are looking towards enhancing the yield with nanotechnology.

1.5.4. Application of nanotechnology in algae

The following pathway biosynthesises the nanoparticles in algae-(i) algae extract preparation in water or organic solvent by heating for a particular interval, (ii) ionic metal compounds are prepared in molar solutions, (iii) incubation of both these solutions with/without stirring at particular controlled atmosphere (Dahoumane *et al.,* 2014). Algal cells can either

synthesise nanoparticles intracellularly or extracellularly depending on the species and dose of the metal extracts.

1.5.5. Developments of algal nanotechnology for pigments and antioxidant compounds extraction

Algal nanotechnology includes the introduction of nanoparticles as a source of oxidative stress on the algal species, as well as the synthesis of metal nanoparticles from the original metal. However, the former route is sometimes harmful and is applied to tackle algal blooms in water bodies (Chen *et al.,* 2022). A high concentration of nanoparticles in water affects algae growth and damages the cell (Koh *et al.,* 2018).

1.5.6. Challenges hindering algal nanotechnology in food science and technology

Nanoparticles (NPs) have a wide range of applications due to their unique and distinguished properties (Chang *et al.,* 2019). Thus, NPs can be helpful in food packaging materials, disinfectants, optical product formulations, fabric cleaners, cosmetics, biology and medicine, textile, composites and energy, biosensor and diagnostics, electronics, and agricultural products (Sedaghat *et al.,* 2018).

1.5.7. Sustainability and Prospects

The utilisation of nanotechnology for algae cultivation for the enhanced accumulation of secondary metabolites and biomolecules is an attractive option for industries, which look into increasing the production of pharmaceuticals currently. Due to the capability of magnetic nanoparticles to clog, NPs could reduce the expensive harvesting cost of algae.

1.6. Interactive effect of silver nanoparticles and salinity on growth and photosynthetic parameters of wheat

The agronomic food security of the world is under serious threat due to the exponential increase in human population and the drastic climate changes (Francini *et al.,* 2019). Salinity or salt stress is a significant abiotic stress that affects crop productivity and yield and reduces the land usage area for agricultural practices, especially in the arid and semi-arid regions of the world (Rehman *et al.,* 2016). The spread of salinity stress is mainly due to anthropogenic activities (Hossain, 2019). From previous research studies, it is known that salt stress alters the physiological responses in plants, such as the reduction in the photosynthetic efficiency, decrease in the stomata aperture size, generation of excessive reactive oxygen species (ROS), disruption of the integrity of the cell membrane and insufficient accessibility of the antioxidant enzymes (Muchate *et al.,* 2016). There is also a disturbance in the uptake and distribution as well as the availability of the essential in the plants due to the ionic stress, which is caused by the higher accumulation of the sodium (Na^+) and chloride (Cl^-) ions (Thor, 2019). Wheat is a staple food in most countries, with a global annual yield of about 650 million tons, according to the FAO 2013. To ensure food security, wheat production is needed to double by the year 2050 (Ray *et al.,* 2013). In order to ensure sustainable crop production in salinised soils (Rizwan *et al.,* 2015) as the salt stress causes a decrease in the grain yield, growth, gas exchange characteristics, mineral nutrients and chlorophyll content in wheat (Shafi *et al.,* 2010).

Studies have reported the effects of silver nanoparticles in the discipline of agriculture, focusing mainly on plant growth (Kim *et al.,* 2018), photosynthesis rate (Wang *et al.,* 2020) and seed germination (Singh *et al.,* 2016). Since silver nanoparticles have unique physio-chemical properties (Yousaf *et al.,* 2020), which impart antioxidant and antimicrobial attributes and therefore have an advantage over the other existing nanoparticles (Chouhan,

2018). The silver nanoparticles are also referred to as ‘biocompatible precursors’ for inducing the traits responsible for the overall growth of the plants due to their non-toxicity and chemical stability (Gonzalez *et al.*, 2019).

OBJECTIVES

The present study was aimed at the green synthesis of silver nanoparticles using algae and its effect on salt-stressed wheat plants.

The objectives of the present study are as follows:

1. To biosynthesise silver nanoparticles from the cyanobacteria, *Nostoc commune* and the seaweed, *Enteromorpha intestinalis* using 0.01 M Silver nitrate solution.
2. Characterising the synthesised silver nanoparticles using Ultraviolet-Visible Spectrophotometry, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy imaging.
3. To evaluate the antibacterial activity of the synthesised nanoparticles from the algal species against the gram-positive bacteria, *Bacillus cereus* and the gram-negative bacteria, *Escherichia coli*.
4. To analyse the interactive effect of the synthesised silver nanoparticles and salt stress on the wheat plants.

Chapter-2

MATERIALS & METHODS

2. MATERIALS AND METHODS

2.1. Algal species used

2.1.1. *Nostoc commune*

Nostoc is a genus of blue-green algae and is prokaryotic and photosynthetic. Various species of *Nostoc* are found mainly in freshwater as colonies or at the bottom of lakes, or attached to rocks. They are unbranched and filamentous and found in a gelatinous colony. The filament has a chain of cells appearing like beads on a string. The cells are oval or spherical, and there are specific specialised cells called heterocysts which are the sites of nitrogen fixation. The colonies are of different colours, shapes and sizes and are called *Nostoc* balls when spherical (Vanlalveni *et al.*, 2018).

Table 1. Classification of *Nostoc*

Class	Cyanophyceae
Order	Nostocales
Family	Nostocaceae
Genus	*Nostoc*
Species	*commune*

2.1.2. *Enteromorpha intestinalis*

The *Enteromorpha intestinalis,* known as the *Ulva intestinalis*, is a green alga which belongs to the division Chlorophyta. It is also known as sea lettuce or gut weed (Silva *et al.,* 2009). Furanone a, furanone b and hexanol are the volatile compounds in the marine *Enteromorpha intestinalis.* This algal extract is rich in bioactive compounds such as polyphenols and thus has antioxidant activities (Horincar *et al.*, 2011).

Table 2. Classification of *Enteromorpha*

Class	Ulvophyceae
Order	Ulvales
Family	Ulvaceae
Genus	*Enteromorpha*
Species	*Intestinalis*

2.2. Collection of algal samples

2.2.1. *Nostoc commune*

Nostoc commune was collected as balls from the rocks, Goa University, Taleigao plateau. The entire collection was carried out in June 2022. The collected algae were washed using tap water to remove extraneous materials (Kumar *et al.,* 2013). Washing was followed by repeated surface sterilisation using sterile distilled water. The sample was stored at -20^{0}C until further use.

2.2.2. *Enteromorpha intestinalis*

The lithophytic marine algae, *Enteromorpha intestinalis*, were collected from Vagator Beach due to the abundance and easy accessibility. The collection of the marine algae was carried out in August 2022. The collected algae were thoroughly washed using tap water to remove extraneous materials (Kumar *et al.,* 2013) and subjected to surface sterilisation using sterile distilled water. The sample was stored at -20^{0}C until further use.

2.3. Preparation of aqueous algal extract

The *Nostoc commune* and *Enteromorpha intestinalis* algal extract were prepared with slight modifications by the intracellular extraction methodology (Kannan *et al.,* 2013; Kumar *et al.,* 2013).

2.3.1. *Nostoc commune* aqueous extract

The stored *Nostoc commune* balls were cleaned again to remove any remaining contaminants (Senthilkumar and Sudha, 2012). The *Nostoc commune* balls were weighed using an analytical balance, and a weight of 2.7 kg was recorded. Further, the specimen was shade-dried for three weeks to eradicate the moisture content. In order to prevent fungal contamination, the sample was oven-dried at 50^0C for 25-30 minutes. The dried specimen was ground to a fine powder with a mortar and pestle using liquid nitrogen. A constant weight of 31.3 grams of the powdered sample was recorded. The powdered sample was stored in an air-tight container until further use. The aqueous algal extract was prepared by dissolving 30 g of the powdered *Nostoc commune* in 300 mL of sterile distilled water in a ratio of 1:10. The extract was heated for 20 minutes in a water bath at 60℃ (Kumar *et al.,* 2013). The extract was filtered using a muslin cloth, followed by Whatman no.1 filter paper and the collected filtrate was stored at 4℃ and used for further analysis.

2.3.2. *Enteromorpha intestinalis* aqueous algal extract

The stored samples of the marine algae *Enteromorpha intestinalis* were rewashed using sterile distilled water (Senthilkumar and Sudha, 2012). A weight of 1.6 kg was recorded on weighing the cleaned and stored marine algae. The marine algae were shade-dried for two weeks to eradicate the moisture content. Furthermore, the sample was oven-dried at 50 ℃ for 20 minutes to prevent fungal contamination. The oven-dried sample was weighed, and a dry weight of 21.9 g was recorded. The wholly dried sample was ground to powder with a

mortar and pestle using liquid nitrogen, and the resultant weight of the obtained powder was 20.03 g. The powdered *Enteromorpha intestinalis* (20 g) was dissolved in 200 mL of distilled water in a 1:10 ratio. The algal extract was heated in a water bath at 60℃ for 20 minutes (Kumar *et al.,* 2013). The extract was filtered using a muslin cloth followed by Whatman no.1 filter paper. The collected filtrate of *Enteromorpha intestinalis* was refrigerated at 4℃ and used for further analysis.

2.4. Synthesis of silver nanoparticles (AgNP)

Aqueous Silver nitrate ($AgNO_3$) (0.01 M) was used to synthesise silver nanoparticles from algae. The synthesis medium for both the samples – *Nostoc commune* and *Enteromorpha intestinalis* was prepared by adding 20 mL of the respective algal extract to 180 mL of 0.01M silver nitrate. The synthesis medium was stirred using a magnetic stirrer and was gradually heated up to 60℃ to ensure the complete reduction of the metal ion (Kumar *et al.,* 2013; Rajesekaran, 2012). The synthesis medium was maintained in triplicate and under dark conditions to minimise silver nitrate photoactivation. An absolute control setup was maintained in distilled water (Kannan *et al.,* 2013), whereas, the 0.01M silver nitrate solution served as a negative control.

The colour change from pale greenish-brown to reddish-brown solution in the case of *Nostoc commune* (Vanlalveni *et al.,* 2018) and the colour change from greenish-yellow to reddish-brown in the case of *Enteromorpha intestinalis* (Haglan *et al.,* 2020) served as a visible confirmation for the silver nanoparticle formation before undergoing the characterisation process. The synthesis medium was centrifuged at 8000 rpm at 28℃ for 30 minutes. The silver nanoparticles were deposited at the bottom upon centrifugation, forming a pellet. The pellet of silver nanoparticles was oven-dried at 50℃ till the moisture content was removed. Using a pestle, the silver nanoparticle pellet was pressed into powdered form (Bhuyar *et al.,* 2020).

2.5. Characterisation of the synthesised silver nanoparticles

Characterisation of the nanoparticles is crucial to understanding and controlling nanoparticle synthesis and their applications. The morphology of the synthesised silver nanoparticles was observed and confirmed by using various techniques of characterisation such as UV-Vis Spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) imaging (Gomathy *et al.,* 2021).

2.5.1. UV-Vis Spectrophotometric Analysis

UV-Vis spectrophotometry confirms the silver nanoparticle formation by showing the surface plasmon resonance (Kholoud *et al.,* 2010). The formation of silver nanoparticles and the reduction of Ag^{+} ions were monitored using a UV-Vis Spectrophotometer. The UV-Vis spectrum of the synthesis medium comprising the algal extract and 0.01M silver nitrate was taken at regular intervals in the wavelength range between 300nm and 800nm. The UV-Vis readings were recorded. Microsoft Excel analysis tools were used to analyse the data.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The Fourier transform infrared spectroscopy (FTIR) of the synthesised silver nanoparticles from *Nostoc commune* and *Enteromorpha intestinalis* were analysed by performing the powder analysis using the software 'LabSolutions' in the range between 4000 cm^{-1} and 400 cm^{-1}. The Potassium Bromide (KBr) pellets were oven-dried and powdered (Sahayaraj *et al.,* 2012). 2 mg of the synthesised silver nanoparticles from each sample were mixed with 200 mg of purified FTIR grade powdered KBr respectively. This mixture was placed on the mount and used for FTIR analysis of each sample.

2.5.3. Scanning Electron Microscopy (SEM) Analysis

The morphology and size of the synthesised nanoparticles were characterised by using Scanning Electron Microscopy (SEM). The silver nanoparticles in the powdered form were cast onto the carbon-coated stubs and placed on the microscope stage. The high-resolution images of the silver nanoparticles were recorded at 300X and 50,000X magnifications.

2.6. Antibacterial activity of silver nanoparticles

The antibacterial activity of the synthesised nanoparticles was evaluated against the gram-negative bacteria, *Escherichia coli* and the gram-positive bacteria, *Bacillus cereus*, with four different concentrations of the synthesised silver nanoparticles using the disc diffusion method (Vijayan *et al.,* 2014). Aqueous dispersions of the desired concentrations of the silver nanoparticles (0.25 mg/mL, 0.50 mg/mL, 0.75 mg/mL, and 1.00 mg/mL) of both – *Nostoc commune* and *Enteromorpha intestinalis* were made by initially using a Sonicator to make a stock solution of the synthesised silver nanoparticles (Hassaan and Hosny, 2018). 5 mm discs were made from the Whatman no.1 filter paper and placed in the four concentrations of the synthesised silver nanoparticles. The 0.01M silver nitrate solution was used as a negative control, and ampicillin (1mg/mL) was used as a positive control. The bacterial suspension was prepared by a single colony sub-culture overnight in distilled water (saline). Mueller-Hinton agar (MHA) medium was prepared; 15-20 mL of the media was poured into sterilised Petri plates and solidified at room temperature. Using the spread plate method, the plates were streaked with 0.1 mL of the bacterial suspension (Nabikhan *et al.,* 2010). The discs immersed in the four silver nanoparticle concentrations, water and ampicillin, were placed in the respective Petri plates. The inoculated and streaked plates were then incubated at room temperature in the incubator for 24 h. The experiment was

performed in triplicates to measure the zone of inhibition. The zone of inhibition was measured after incubation and was expressed as millimetres (mm) in diameter (Annamalai and Nallamuthu, 2016).

2.7. Effect of silver nanoparticles on salt-stressed wheat

2.7.1. Plant growth condition and treatment

Wheat seeds were obtained from Goa Bagayatdar Bazaar Porvorim, Bardez, Goa. The seeds were surface sterilised using 0.5 % Sodium hypochlorite solution for 2-3 minutes, then rinsed with distilled water thrice to remove traces of the Sodium hypochlorite solution. The seeds were soaked in water for 24 h. Five seeds were sown in each plastic pot containing vermiculite. The experiment was set as:

Control,
Control+*Nostoc* AgNP,
Control+ *Enteromorpha* AgNP,
200 mM NaCl,
200 mM NaCl+*Nostoc* AgNP
200 mM NaCl+*Enteromorpha* AgNP.

The control pots were watered with the Hoagland solution (Hoagland *et al.*, 1998) at pH 6.4. The *Nostoc* AgNP and *Enteromorpha* AgNP with 0.25 mg/mL concentration were used for the treatment, whereas 200 mM NaCl was used to induce salt stress. The plants were allowed to grow for ten days in a controlled chamber under a 16h photoperiod, 25±2°C temperature with 65-70% Relative humidity.

2.7.2. Determination of Biomass and Shoot/Root length

The plants were harvested on the 10th day of treatment, washed with distilled water to remove the extraneous vermiculite, and recorded for shoot/root length and biomass (shoot and root), on obtaining the fresh weight. The samples were dried at 80°C for 48 h and weighed to note their dry weight (Wang *et al.,* 2014).

2.7.3. Pigment analysis by spectrophotometry

The contents of Chlorophyll a, Chlorophyll b and Carotenoids were measured spectrophotometrically (Yang *et al.,* 1998). Fresh leaf tissue (0.2 g) was homogenised with 2 mL of 100 % acetone containing a few crystals of Butylated hydroxytoluene (BHT). The extracts were incubated at 4°C overnight. After 24 h, the extracts were centrifuged at 7000 rpm for 10 minutes. Using a UV-Vis Spectrophotometer, the supernatant was used to measure the absorbance at 663, 645 and 470 nm. The Chlorophyll a, Chlorophyll b and the carotenoid content were calculated using the formula

$$\text{Chlorophyll a (µg/mL)} = 12.25\ A_{663} - 2.25\ A_{645}$$

$$\text{Chlorophyll b (µg/mL)} = 20.31\ A_{645} - 4.91\ A_{663}$$

$$\text{Total Carotenoids (µg/mL)} = \{1000\ A_{470} - 2.27\ (\text{Chl a}) - 81.4\ (\text{Chl b})\}/227$$

Chapter-3

RESULTS

3. RESULTS

3.1. Silver Nanoparticle Synthesis

The green synthesis of the silver nanoparticles (AgNPs) was carried out with 0.01M silver nitrate solution with the respective algal extracts in a ratio of 1:10 respectively. The negative control solution was 0.01M silver nitrate solution without any algal extract, and absolute control was maintained with the same ratio in distilled water and the respective algal extracts. The experimental synthesis medium with 0.01 M silver nitrate and the cyanobacteria *Nostoc* sp. extract showed a slightly turbid light brown solution with the addition of the silver nitrate solution indicating the initiation of the reaction (**Fig. 1e**).

A similar turbid change was also observed in the case of the synthesis medium containing seaweed *Enteromorpha intestinalis* extract (**Fig. 2e**). The reduction of the silver ions to silver nanoparticles was thus visually identified by the colour change from pale yellowish-green to dark reddish-brown and dark brown in the *Nostoc commune* and *Enteromorpha intestinalis* reaction mixture (**Fig. 1h and 2h**), respectively. The formation of brown colour in the reaction mixture was due to the oscillation of free electrons. The deep reddish-brown colour in the reaction mixture of *Nostoc commune* was attained at 192 h, and the dark brown colour in the *Enteromorpha intestinalis* was attained at 288 h, indicating the increase in colour density is directly proportional to the time of incubation. On the other hand, the negative control with just the 0.01M silver nitrate remained colourless on stirring and gradual heating for 192 h and 288 h (**Fig. 1f and 2f**). In contrast, the colour of the absolute control solutions having the distilled water and the algal extracts showed a pale yellow to greenish colour due to the presence of the algal extracts (**Fig. 1g and 2g**).

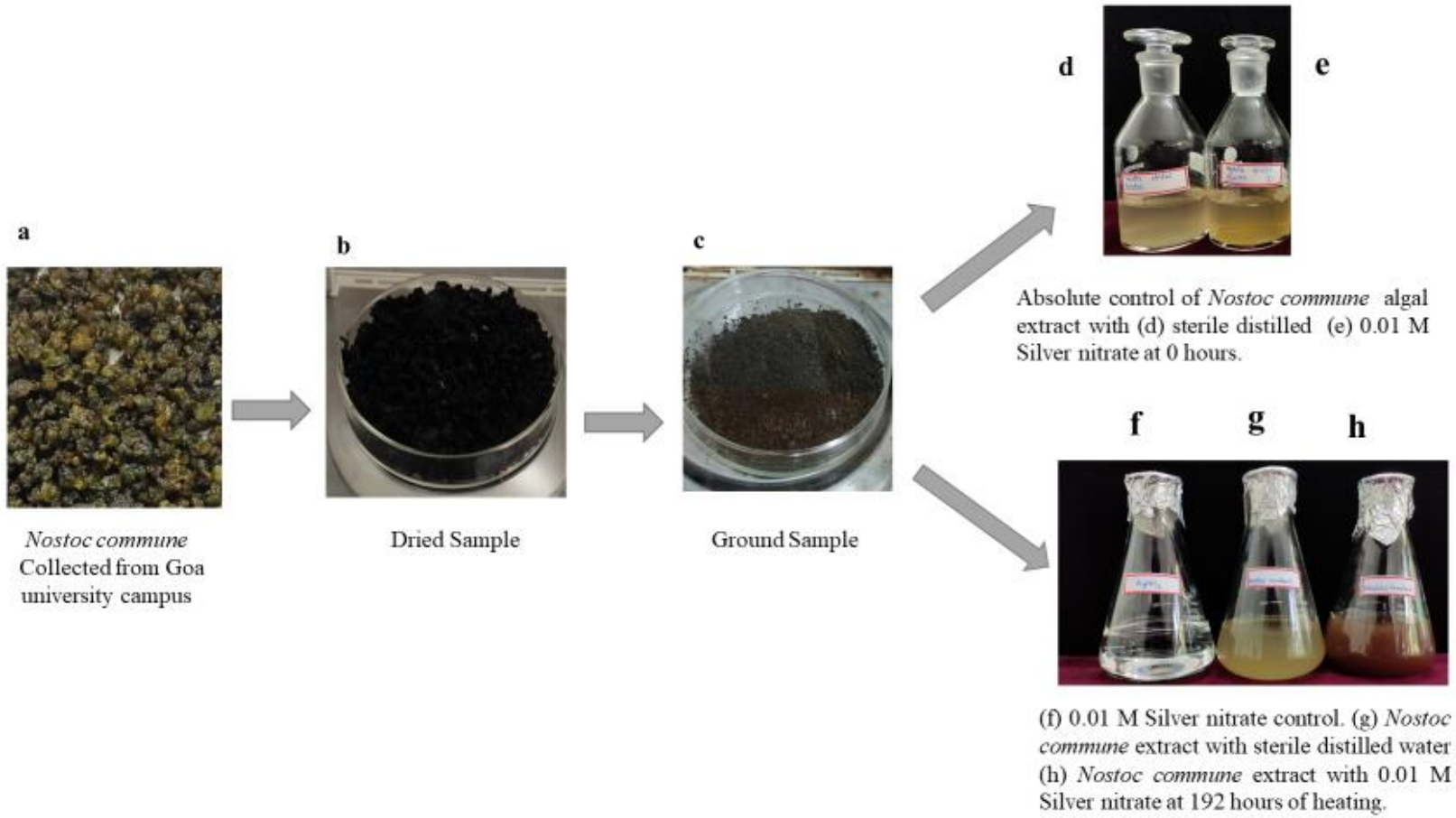

Fig. 1: Flow chart depicting (**a**) Collected Sample (**b**) Dried Sample (**c**) Ground Sample (**d**) Distilled water extract (**e**) 0.01M Silver nitrate at 0 hours, (**f**) 0.01M Silver nitrate control (**g**) Distilled water extract (**h**) 0.01M Silver nitrate after 192 hours of heating of Algae *Nostoc commune*

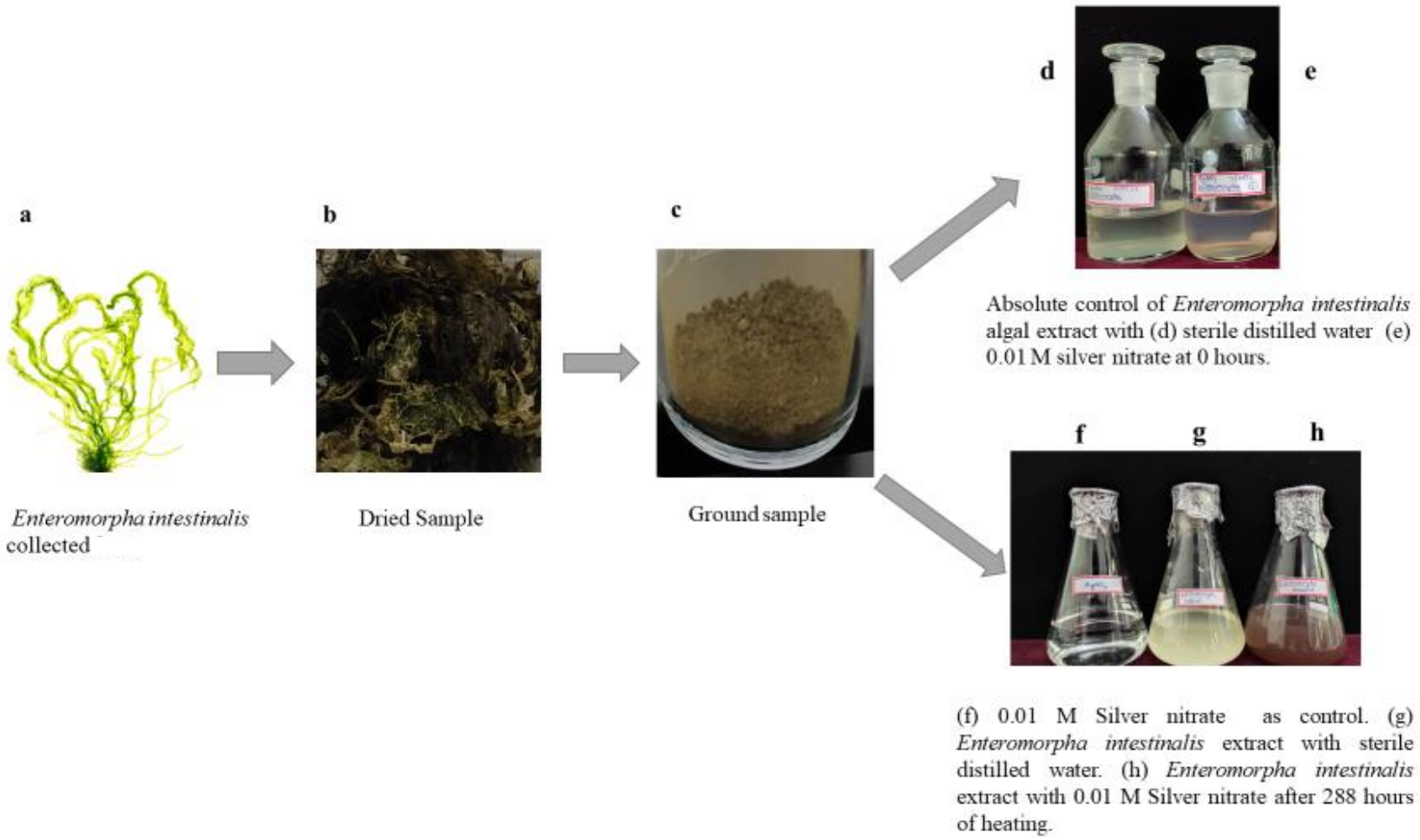

Fig. 2: Flow chart depicting (**a**) Collected Sample (**b**) Dried Sample (**c**) Ground Sample (**d**) Distilled water extract (**e**) 0.01M Silver nitrate at 0 hours, (**f**) 0.01M Silver nitrate control (**g**) Distilled water extract (**h**) 0.01M Silver nitrate after 288 hours of heating of Algae *Enteromorpha intestinalis*

3.2. Characterisation Of Synthesised Silver Nanoparticles

3.2.1. Ultraviolet-Visible (UV-Vis) Spectrophotometric Analysis

UV-Vis Spectrophotometry confirmed the silver ions' reduction and the silver nanoparticle's formation at different wavelengths at irregular intervals. The UV-Vis Spectrophotometry analysis depends on the generation of the colour in the reaction mixture due to the excitation of the Surface Plasmon Resonance (SPR) band, which was recorded as different functional times for both the algal samples.

There is no peak formation at the initial stage in both the sample reaction mixtures, indicating no silver nanoparticle synthesis at the initial stage. In the case of the *Nostoc commune* reaction mixture, the SPR vibration was observed at 440 nm, confirming silver nanoparticle synthesis. The silver SPR band occurred at 422 nm to 440 nm showing a steady increase in the absorbance with time until 192 hours (**Fig. 3a**).

In contrast, in the case of the *Enteromorpha intestinalis* reaction mixture, the SPR vibration was observed at 495 nm, which confirmed the synthesis of the silver nanoparticles, and the silver SPR band occurred at 443 nm to 495 nm showing a steady increase in the absorbance with time, until 288 hours (**Fig. 3b**). The peak broadening indicated the polydispersed nature of the particles. The intensity of the peaks in both cases increased when the samples were treated for a longer duration.

3.2.2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The Fourier Transform Infrared Spectroscopy (FTIR) investigation can estimate the mechanism of the synthesised silver nanoparticles. The FTIR was used to identify the biomolecules in the *Nostoc commune* and *Enteromorpha intestinalis* responsible for the

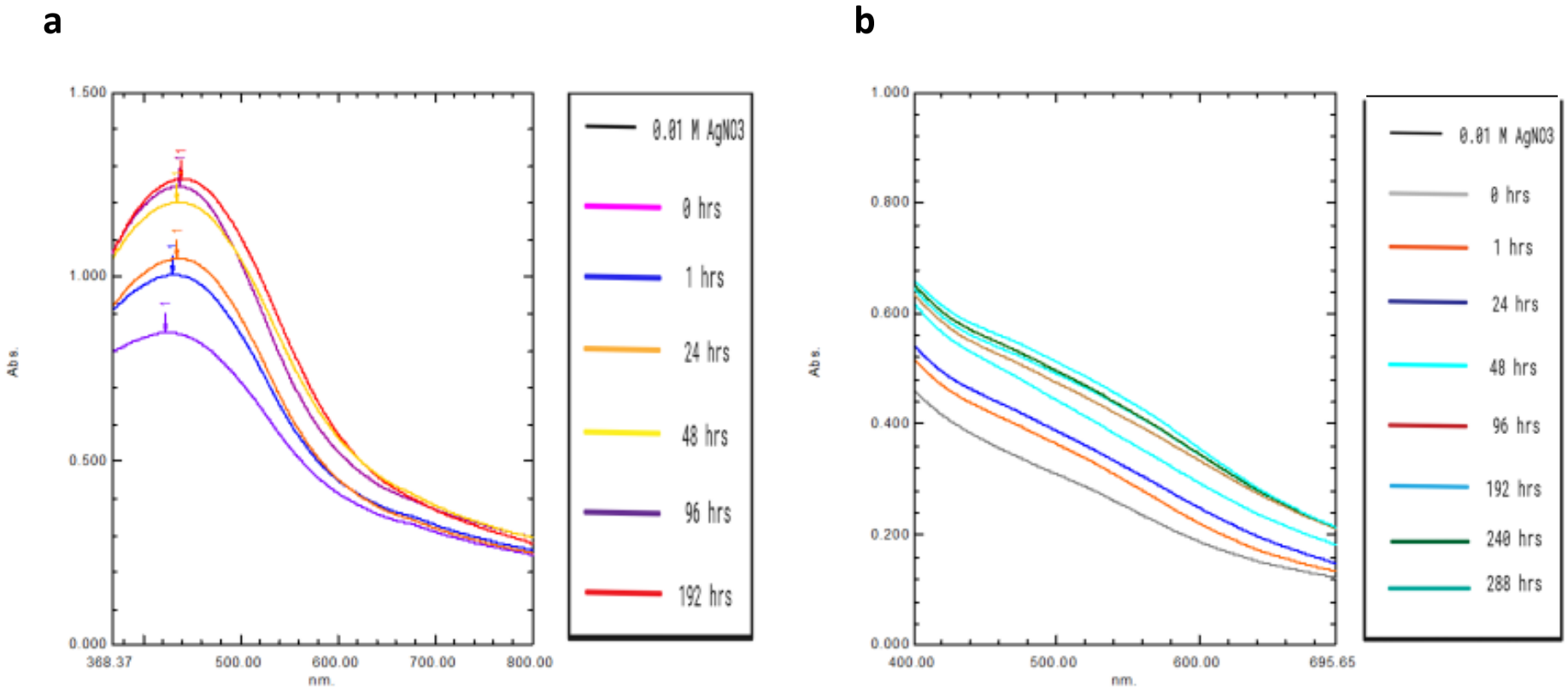

Fig. 3: UV-Vis absorption spectrum of silver nanoparticles synthesized; **a**- *Nostoc commune* extract; **b**- *Enteromorpha intestinalis* treated with 0.01 M Silver nitrate during different time intervals.

silver ion reduction and their stabilisation. The absorbed light indicates the fingerprint of the biomolecules.

FTIR spectra of the silver nanoparticles synthesised from *N. commune* showed bands at 3480.06 cm^{-1} and 3266.85 cm^{-1} due to the N-H stretch or O-H stretch corresponding to the primary, secondary amines, amides, carboxyl and phenols. The band at 2342.46 cm^{-1} is due to the carbon-carbon triple bonding corresponding to the alkynes the band at 1648.45 cm^{-1} is due to the N-H stretching corresponding to the primary amines. Whereas bands at 1482.11 cm^{-1} and 1366.55 cm^{-1} are assigned to the vibration of N-O nitro compounds, the bands at 668.25 cm^{-1} and 595.27 are allotted to the vibrations of C-Br stretching due to the alkyl halides (**Fig. 4a**).

FTIR spectra of the silver nanoparticles synthesised from the *Enteromorpha intestinalis* showed bands at 3429.97 cm^{-1} and 3252.54 cm^{-1} are assigned to the N-H stretch or O-H stretch corresponding to the primary, secondary amines, amides, carboxyl and phenols. The bands at 2360.17 cm^{-1} and 2342.26 cm^{-1} are due to the carbon-carbon triple bonding corresponding to the alkynes, the bands at 1697.10 cm^{-1} and 1608.38 cm^{-1} are due to the N-H stretching corresponding to the primary amines. The band at 1383.72 cm^{-1} is due to the N-O stretching of the nitro compounds. While the bands at 1043.16 cm^{-1} and 921.53 cm^{-1} are allotted to the vibrations of the C-N stretching corresponding to the aliphatic amines, and the bands at 668.25 cm^{-1} and 585.55 cm^{-1} are due to the C-Br stretching corresponding to the alkyl halides (**Fig. 4b**).

3.2.3. Scanning electron microscopy (SEM) analysis of silver nanoparticles

SEM image shows the morphologies of the algal-mediated synthesised silver nanoparticles from *Nostoc commune* (**Fig. 5a and 5b**) and *Enteromorpha intestinalis* (**Fig. 5c and 5d**) at

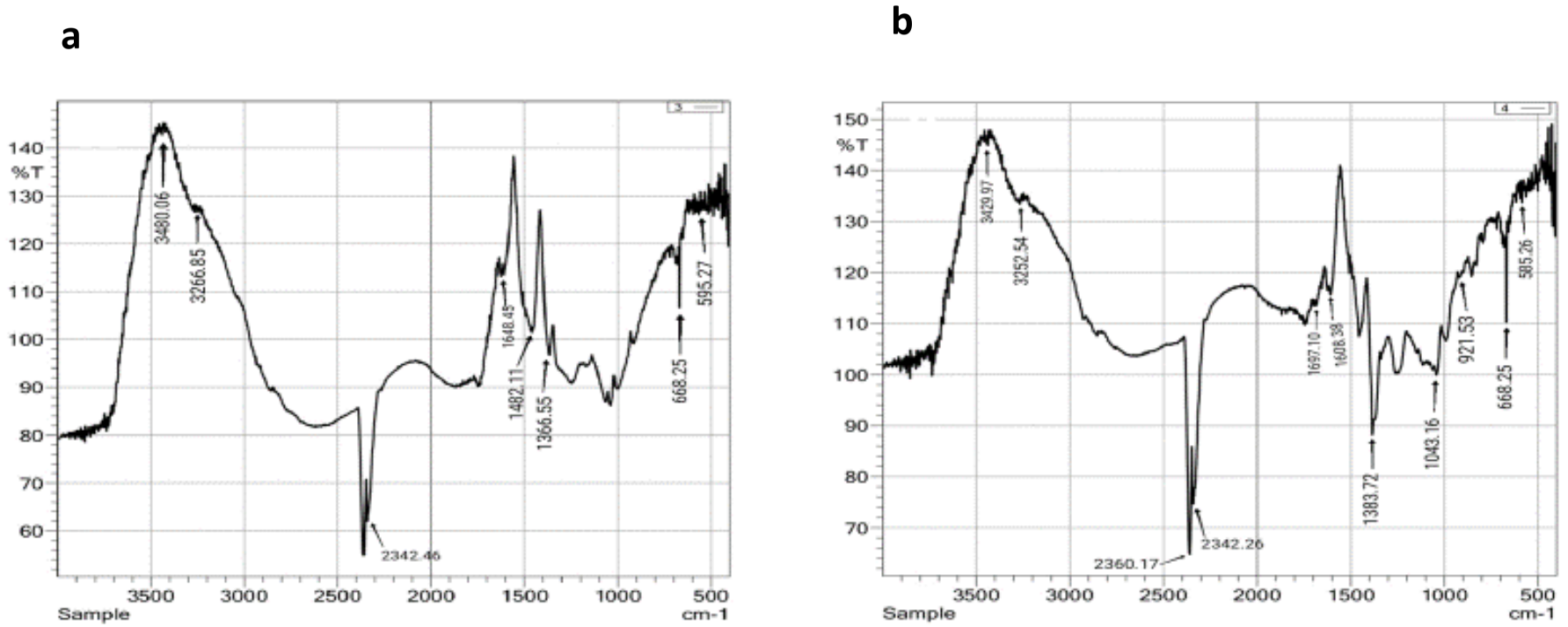

Fig. 4: FTIR spectra of silver nanoparticles (AgNPs) synthesized by the reduction of 0.01 M Silver nitrate; **a**- *Nostoc commune* extract; **b**- *Enteromorpha intestinalis* extract

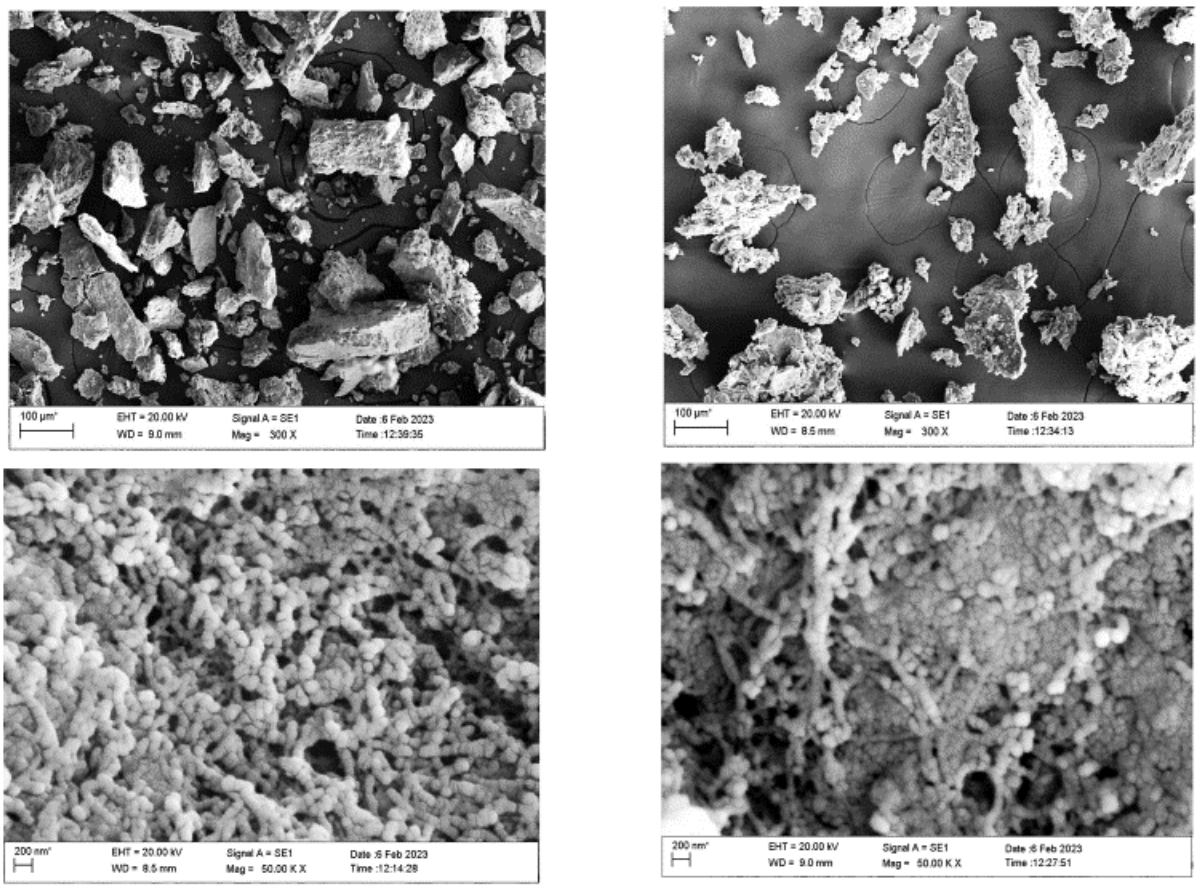

Fig. 5: SEM image at **(a)** 300 X **(b)** 50.00 KX magnification of silver nanoparticles synthesized by treating *Nostoc commune* and **(c)** 300 X and **(d)** 50.00 KX by using *Enteromorpha intestinalis* extract with 0.01 M Silver nitrate.

300 X and 50.00 KX magnifications. The high-density silver nanoparticles are shown in the polydisperse pattern in SEM images, further confirming the silver nanoparticles' development and formation by green synthesis. The silver nanoparticles obtained from both samples were spherical. The average size of the silver nanoparticles biosynthesised from the *Nostoc commune* was 89.37 nm, and the average size of the silver nanoparticles synthesised from *Enteromorpha intestinalis* was 76.42 nm.

3.3. Antibacterial Activity of AgNPs

The Silver ions and silver-based products are known to be toxic to microorganisms. The antibacterial activity of the biosynthesised silver nanoparticles was evaluated against the gram-positive *Bacillus cereus* and the gram-negative *Escherichia coli* bacteria by the disc diffusion method.

The silver nanoparticles were synthesised by *Nostoc commune* extract at 0.25 mg/mL concentration and showed the maximum zone of inhibition of about 6.14 mm against the gram-negative bacteria *Escherichia coli*. It also exhibited a zone of inhibition of about 5.89 mm at 0.25 mg/mL concentration against the gram-positive bacteria *Bacillus cereus* (**Table 3**).

The silver nanoparticles synthesised by *Enteromorpha intestinalis* extract at 0.25 mg/mL concentration showed a maximum zone of inhibition of about 5.98 mm against the gram-negative bacteria *Escherichia coli*. Furthermore, at the same concentration, it also showed a zone of inhibition of about 5.77 mm against the gram-positive bacteria *Bacillus cereus*. The *Escherichia coli* showed a zone of inhibition of 32.44 mm with 1 mg/mL ampicillin, whereas the *Bacillus cereus* showed a zone of inhibition of 5.6 mm (**Table 4**). This revealed that the silver nanoparticles synthesised from *Nostoc commune* and *Enteromorpha intestinalis* were

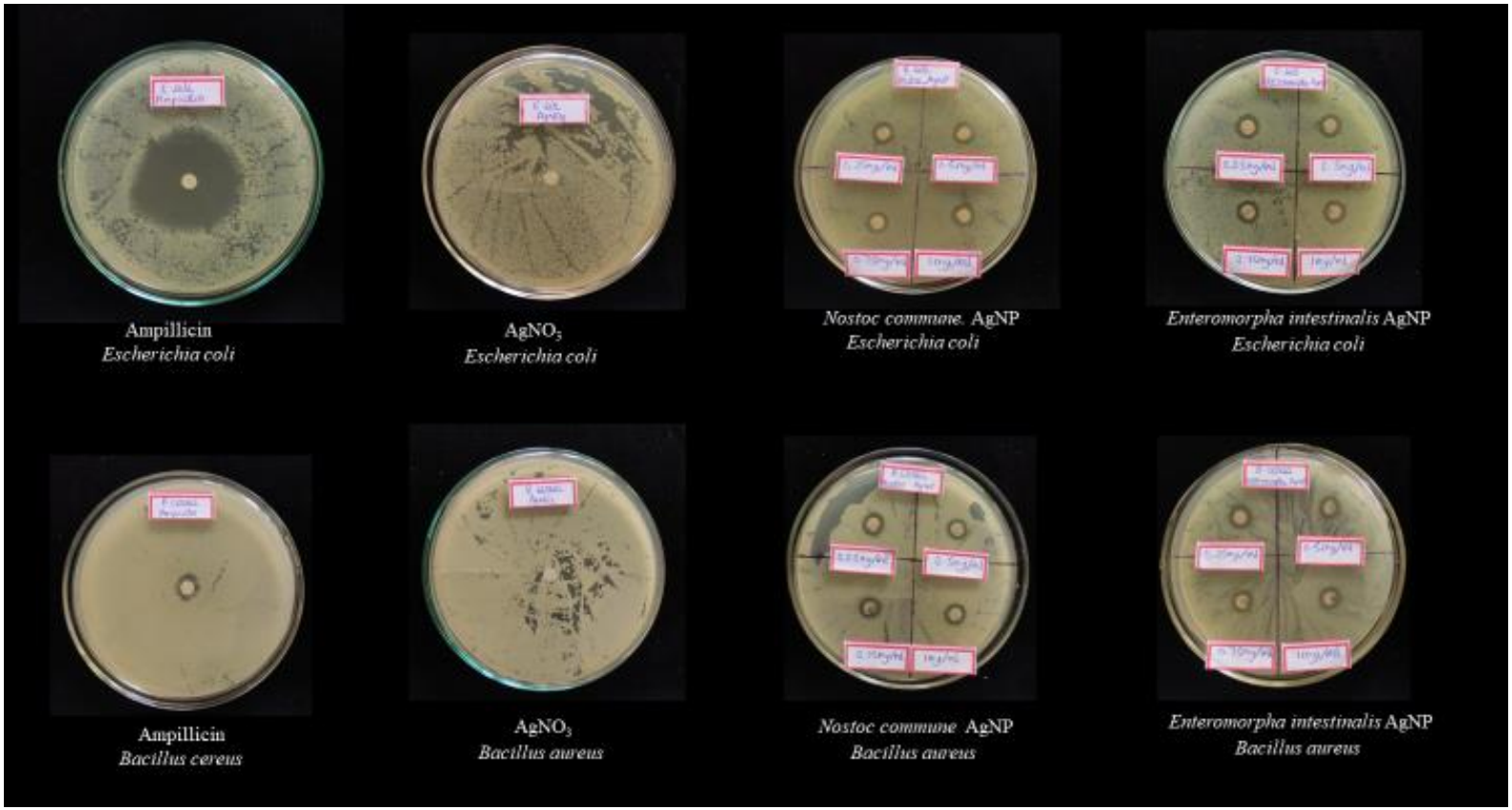

Fig. 6: Antibacterial activity of the synthesized silver nanoparticles against *Escherichia coli* and *Bacillus cereus*.

Table 3. Antibacterial activity: Inhibition zone (mm) of *Escherichia coli* and *Bacillus cereus* in presence of four different concentrations of biosynthesised silver nanoparticles from *Nostoc commune.* Data represent mean values ± standard deviation (n=3).

Concentration (mg/mL)	**Zone of inhibition (mm)**	
	Bacillus cereus	*Escherichia coli*
0.25	5.89 ± 0.74	6.14 ± 0.62
0.50	4 ± 0.63	5.28 ± 0.62
0.75	5.1 ± 1.16	4.64 ± 0.64
1.00	3.2 ± 0.74	4.82 ± 0.62
Ampicillin (1.00)	5.6 ± 0.47	32.44 ± 0.49
0.01 M $AgNO_3$	0	0

Table 4. Antibacterial activity: Inhibition zone (mm) of *Escherichia coli* and *Bacillus cereus* in presence of four different concentrations of biosynthesised silver nanoparticles from *Enteromorpha intestinalis*. Data represent mean values ± standard deviation (n=3).

Concentration (mg/mL)	**Zone of inhibition (mm)**	
	Bacillus cereus	*Escherichia coli*
0.25	5.77 ± 0.63	5.98 ± 0.21
0.50	4.38 ± 0.44	5.52 ± 0.33
0.75	4.22 ± 0.56	4.54 ± 0.48
1.00	4.3 ± 0.50	4.3 ± 0.50
Ampicillin (1.00)	5.6 ± 0.47	32.44 ± 0.49
0.01 M $AgNO_3$	0	0

more effective against the gram-positive bacteria *Bacillus cereus* than the conventional antibiotic ampicillin having a concentration of 1 mg/mL (**Fig. 6)**.

3.4. Effect of AgNP on salt-stressed wheat

3.4.1. Determination of Biomass and Shoot/Root length

The shoot/root lengths and plant biomass of the plants from the experimental set - Control (Hoagland solution), control + *Nostoc* AgNP, control + *Enteromorpha* AgNP, 200 mM NaCl, 200 mM NaCl + *Nostoc* AgNP and 200 mM NaCl + *Enteromorpha* AgNPs + 200 mM NaCl were evaluated (**Fig. 7a and 7b**). The per cent increase or decrease was calculated by comparing with the control (**Table 5**). 200 mM NaCl-treated plants showed a 44.41 % and 83.4 % decrease in shoot and root lengths. Whereas, the control + *Nostoc* AgNP treated plants showed a 38.03 % and 17 % increase in shoot and root lengths. Furthermore, 200 mM NaCl + *Nostoc* AgNP treated plants showed a 9.02 % increase and a 6 % decrease in the shoot and root lengths. On the other hand, the control + *Enteromorpha* AgNP treated plants showed a 59.92 % and 18 5 % increase in shoot and root lengths. 200 mM NaCl + *Enteromorpha*-treated plants showed a 7.58 % increase and a 19.4 % decrease in the shoot and root length (**Fig. 8**).

200 mM NaCl-treated plants showed a 62.9 % and 83.33 % decrease in shoot and root biomass, respectively. The control + *Nostoc* AgNP treated plants showed a 4.24 % and 50 % decrease in shoot and root biomass, respectively. Furthermore, 200 mM NaCl + *Nostoc* AgNP treated plants showed a 6.36 % and 58.33 % decrease in shoot and root biomass, respectively. The control + *Enteromorpha* AgNP-treated plants showed a 40.64 % increase and a 2.78 % decrease in shoot and root biomass, respectively. 200 mM NaCl +

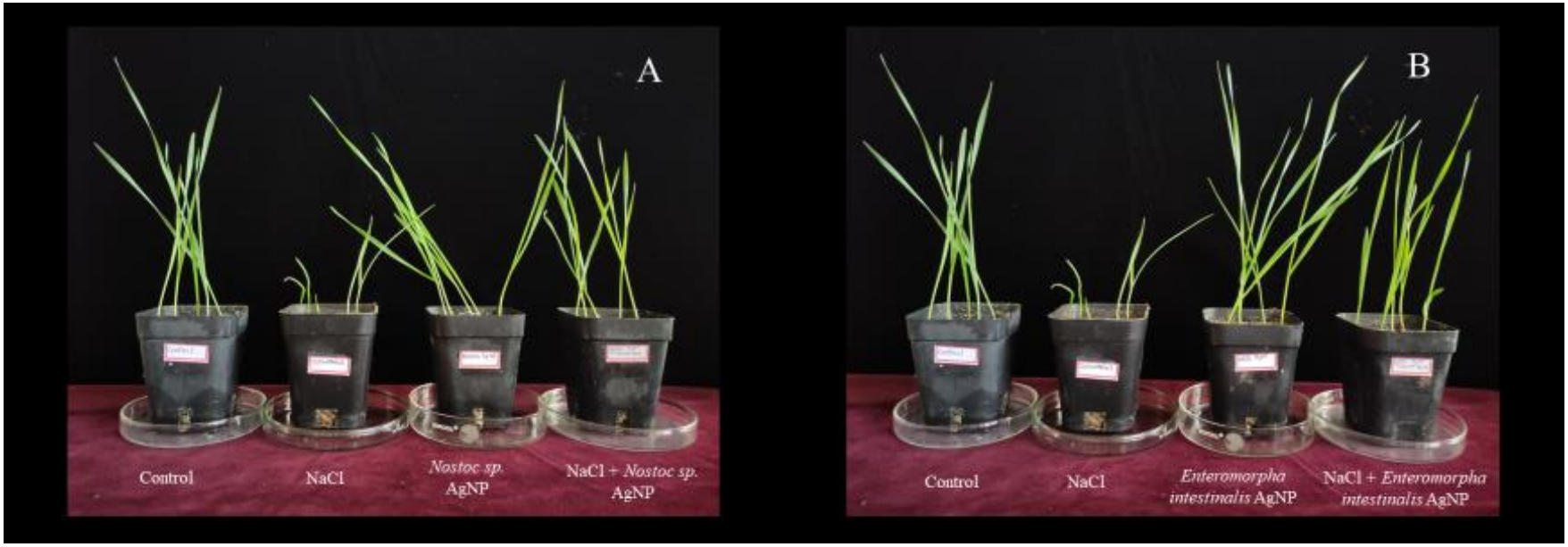

Fig. 7: Experimental setup to study the plant growth with Control, NaCl, synthesized silver nanoparticles and NaCl + synthesized Silver nanoparticles using (**a**) *Nostoc commune* and (**b**) *Enteromorpha intestinalis*

Table 5. Interactive effect of silver nanoparticles on the shoot length, root length and biomass (shoot and root) of wheat. Data represents mean values ± standard deviation (n=3).

Sr. No.	Treatment	Shoot length (cm)	Root length (cm)	Biomass (grams)	
				Shoot	Root
1.	Control	13.85±0.65	7.5±0.2	0.283±0.059	0.036±0.018
2.	200mM NaCl	7.7±1	1.25±0.25	0.105±0.002	0.006±0.002
3.	*Nostoc* NP	19.2±0.6	8.8±0.8	0.271±0.011	0.018±0.000
4.	*Nostoc* NP+ NaCl	15.1±0.1	7.05±0.85	0.265±0.035	0.015±0.002
5.	*Enteromorpha* NP	22.15±0.15	8.85±0.55	0.398±0.046	0.035±0.011
6.	*Enteromorpha* NP+ NaCl	14.9±0.4	6.05±0.05	0.247±0.042	0.013±0.002

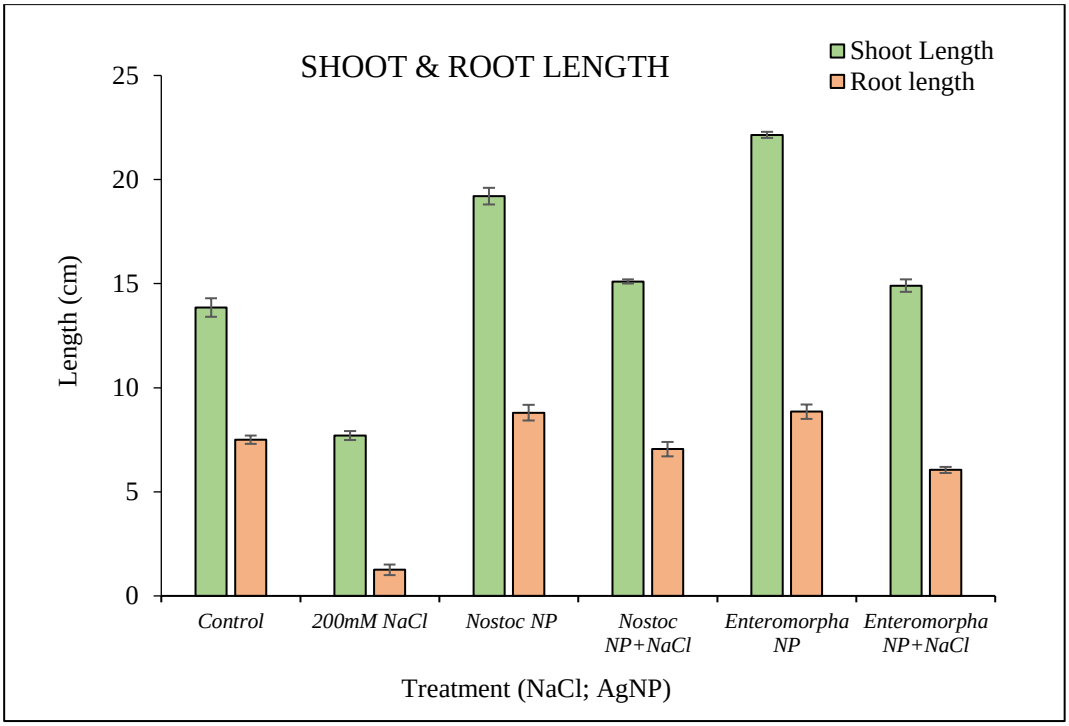

Fig. 8. Interactive effect of the silver nanoparticles and salinity on the shoot and root lengths of wheat.

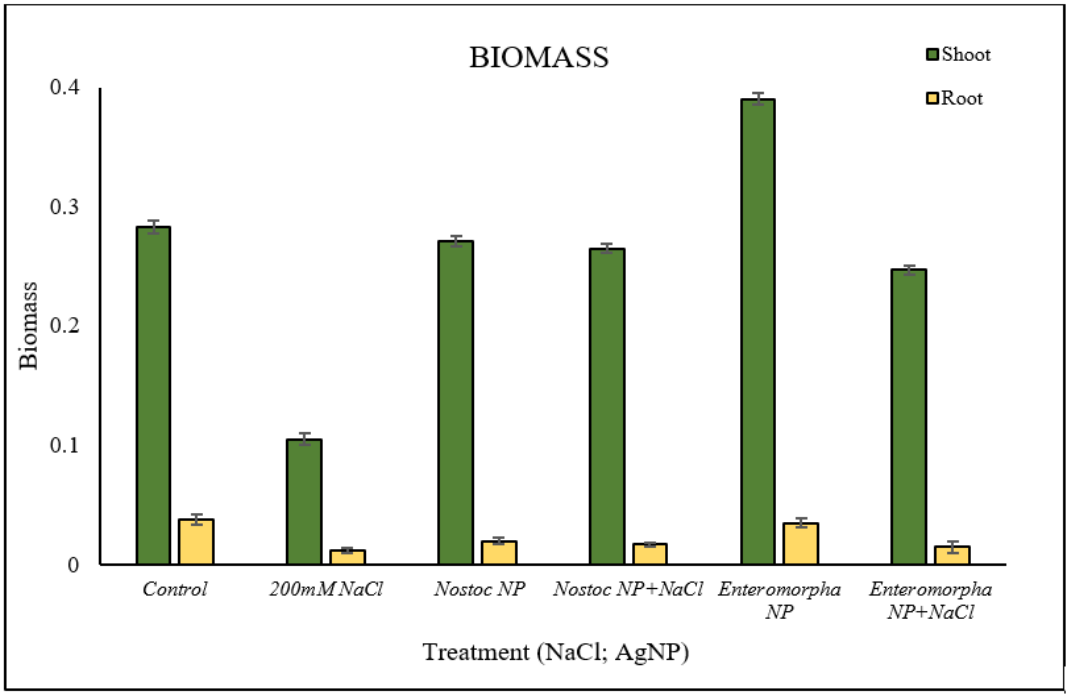

Fig. 9. Interactive effect of the silver nanoparticles and salinity on the biomass (shoot and root) of wheat.

Enteromorpha treated plants showed a 12.72 % and 63.89 % decrease in shoot and root biomass, respectively (**Fig. 9**).

3.4.2. Estimation of Photosynthetic pigments

Different pigments such as Chlorophyll a, Chlorophyll b and the total Carotenoids were estimated in control and the treated plants (**Fig. 10 and 11, Table 6**). 200 mM NaCl treated plants showed a 48.41 % decrease in chlorophyll a with respect to their control plants. The control + *Nostoc* AgNP treated plants showed a 5.45 % increase in chlorophyll a with respect to their control plants. 200 mM NaCl + *Nostoc* AgNP treated plants showed a 30.42 % decrease in chlorophyll a with respect to their control plants. The control + *Enteromorpha* AgNP-treated plants showed a 7.91 % increase in chlorophyll a with respect to their control plants. 200 mM NaCl + *Enteromorpha* AgNP treated plants showed a 30.15 % decrease in chlorophyll a with respect to their control plants.

200 mM NaCl treated plants showed a 37.88 % decrease in chlorophyll b with respect to their control plants. The control + *Nostoc* AgNP treated plants showed an 8.97 % increase in chlorophyll b with respect to their control plants. 200 mM NaCl + *Nostoc* AgNP treated plants showed a 26.23 % decrease in chlorophyll b with respect to their control plants. The control + *Enteromorpha* AgNP-treated plants showed a 13.17 % increase in chlorophyll b with respect to their control plants. 200 mM NaCl + *Enteromorpha* AgNP treated plants showed an 8.09 % decrease in chlorophyll b with respect to their control plants.

200 mM NaCl-treated plants showed a 42.48 % decrease in carotenoids with respect to their control plants. The control + *Nostoc* AgNP treated plants showed a 2.75 % increase in carotenoids with respect to their control plants. 200 mM NaCl + *Nostoc* AgNP treated plants showed a 27.5 % decrease in carotenoids with respect to their control plants. The control + *Enteromorpha* AgNP-treated plants showed a 8.34 % increase in carotenoids with respect to

their control plants. 200 mM NaCl + *Enteromorpha* AgNP treated plants showed an 11.01 % decrease in carotenoid with respect to their control plants.

Table 6. Interactive effect of silver nanoparticles on chlorophyll a, chlorophyll b and total carotenoids wheat. Data represent mean values ± standard deviation (n=3).

Sr. No.	Treatment	Chlorophyll a	Chlorophyll b	Total Carotenoids
1.	Control	5.132±0.009	1.571±0.061	1.090±0.007
2.	200mM NaCl	2.648±0.119	0.976±0.0295	0.627±0.032
3.	*Nostoc* NP	5.412±0.482	1.712±0.136	1.120±0.117
4.	*Nostoc* NP+ NaCl	3.571±0.316	1.159±0.096	0.793±0.074
5.	*Enteromorpha* NP	5.538±0.125	1.778±0.027	1.181±0.030
6.	*Enteromorpha* NP+NaCl	3.585±0.011	1.444±0.005	0.970±0.041

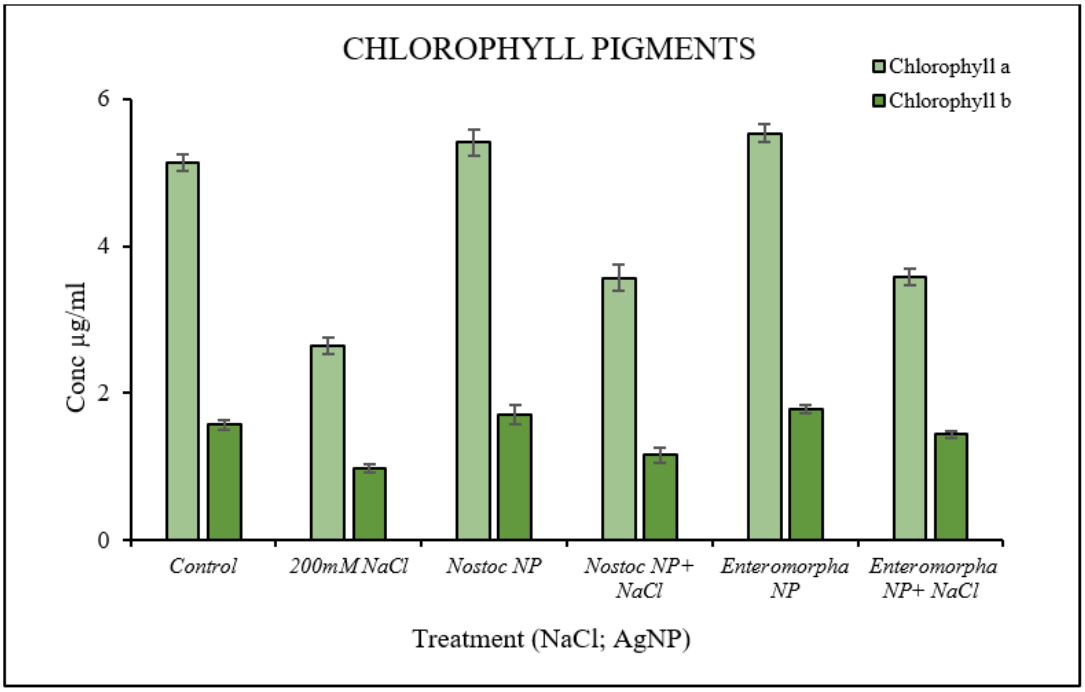

Fig. 10. Interactive effect of the silver nanoparticles and salinity on the Chlorophyll pigments of wheat.

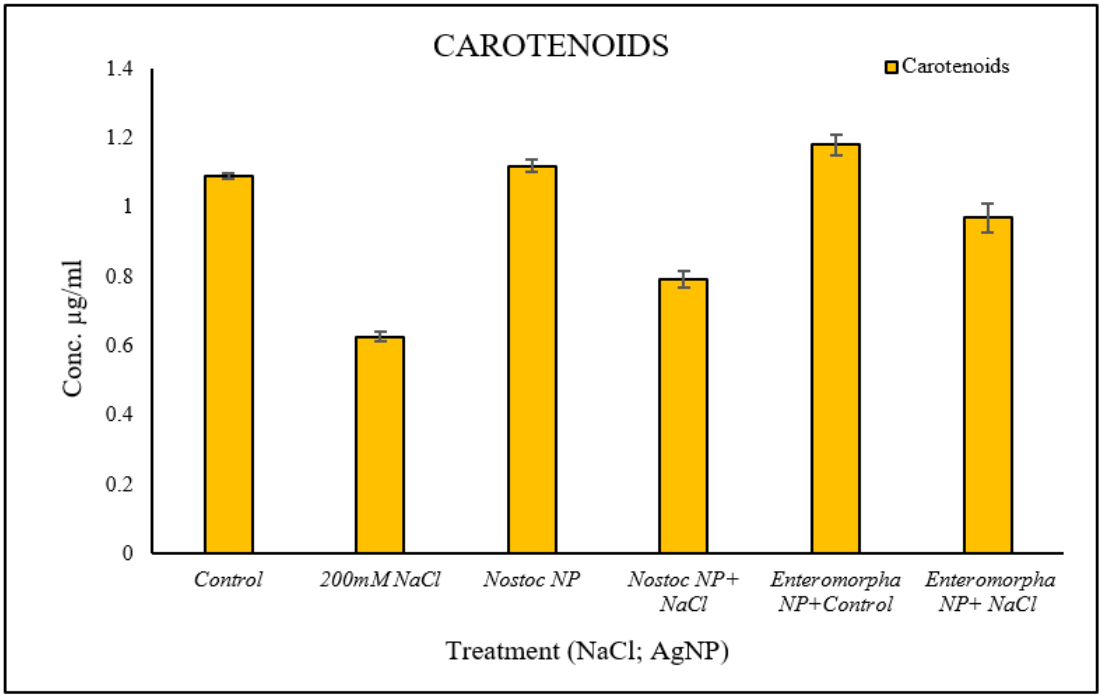

Fig. 11. Interactive effect of the silver nanoparticles and salinity on the Carotenoids in wheat.

Chapter-4

DISCUSSION

4. DISCUSSION:

The results obtained from our present study on the green synthesis of silver nanoparticles using cyanobacteria, *Nostoc commune*, and seaweed *Enteromorpha intestinalis* were similar to the work done by Shivaraj (2012). The formation of the silver nanoparticles was indicated by the reduction of the aqueous Ag^+ ions during exposure to the aqueous extracts of *Nostoc commune* and *Enteromorpha intestinalis*. The reduction was visually seen by the colour change from yellowish green to dark reddish brown and dark brown upon 192 h and 288 h of incubation period in *Nostoc commune* and *Enteromorpha intestinalis,* respectively. The fact that silver nanoparticles exhibit reddish brown in the synthesis medium is widely known due to the excitation of the silver nanoparticles' surface plasmon resonance (SPR) effect. This thus confirms that the silver nanoparticles were formed by the reduction of Ag^+ into the Ag^0 when the extracts from the respective samples were added to the solution of 0.01 M Silver nitrate (Rajeshkaran, 2012). The intensity of the colour did not change remarkably after 192 h in the case of *Nostoc commune* and after 288 h in the case of *Enteromorpha intestinalis*. This indicates that the reaction had reached saturation. This may confirm that the silver nanoparticles might be well dispersed in the synthesis medium showing mild agglomeration (Nabikhan, 2010).

The size and shape of the metal nanoparticles and the dielectric constant of the metal and the surrounding medium are given by the width and the frequency of the surface plasmon resonance (Mukherjee *et al.,* 2001). Our study showed that the surface plasmon resonance was obtained at 440 nm for the *Nostoc commune* and at 495 nm for *Enteromorpha intestinalis*. Similar surface plasmon resonance has also been observed in previous studies using *Nostoc linckia* (Vanlalveni *et al.,* 2018), *Enteromorpha intestinalis* (Haglan *et al.,* 2020), *Padina* sp. (Bhuyar *et al.,* 2020).

The images from the Scanning Electron Microscopy of the nanoparticles revealed that the silver nanoparticles synthesised from the present study using both samples were highly distributed with aggregation, spherical shaped and polydispersed (Puchalski *et al.,* 2007). The synthesised silver nanoparticles showed an average size of 89.37 nm and 76.42 nm in the case of *Nostoc commune* and *Enteromorpha intestinalis,* respectively.

Our results from the Fourier Transform Infrared Spectroscopy (FTIR) were also similar to the previous reports using *Padina* sp. (Sahayaraj *et al.,* 2012), *Enteromorpha intestinalis* (Haglan *et al.,* 2020) and *Nostoc linckia* (Vanlalveni *et al.,* 2018). Fourier Transform Infrared spectroscopy results for the silver nanoparticles synthesised by the *Nostoc commune* showed bands and stretches at 3480.06 cm^{-1}, 3266.5 cm^{-1}, 2342.46 cm^{-1}, 1648.45 cm^{-1}, 1482.11 cm^{-1}, 1366.55 cm^{-1}, 668.25 cm^{-1} and 595.27 cm^{-1}. These bands correspond to the vibrations of the primary and secondary amines, amides, carboxyl, phenols, alkynes, nitro compounds and alkyl halides. Fourier Transform Infrared Spectroscopy results for the silver nanoparticles synthesised by the *Enteromorpha intestinalis* showed bands and stretches at 3429.97 cm^{-1}, 3252.54 cm^{-1}, 2360.17 cm^{-1}, 2342.26 cm^{-1}, 1697.10 cm^{-1}, 1608.38 cm^{-1}, 1383.72 cm^{-1}, 1043.16 cm^{-1}, 921.53 cm^{-1}, 668.25 cm^{-1} and 585.55 cm^{-1}. These bands correspond to the vibrations of the primary, secondary and aliphatic amines, amides, carboxyl, alkynes, nitro compounds, alkyl halides and phenols.

Our results from the antibacterial analysis showed that both the gram-positive bacteria, *Bacillus cereus* and the gram-negative bacteria, *Escherichia coli* were susceptible to the biosynthesised silver nanoparticles from both, *Nostoc commune* and *Enteromorpha intestinalis*. This result is similar to the earlier studies (Rajeshkaran *et al.,* 2012). The

maximum zone of inhibition against the gram-negative bacteria *Escherichia coli* of about 6.14 mm was recorded when treated with *Nostoc commune* synthesised silver nanoparticles at a concentration of 0.25 mg/mL. The *Nostoc commune* synthesised silver nanoparticles also showed a high zone of inhibition of about 5.89 mm against the gram-positive bacteria *Bacillus cereus* at 0.25 mg/mL concentration. A high zone of inhibition of about 5.98 mm was observed against the gram-negative bacteria *Escherichia coli* when treated with *Enteromorpha intestinalis* synthesised nanoparticles at a concentration of 0.25 mg/mL. The *Enteromorpha intestinalis* synthesised silver nanoparticles also showed a high zone of inhibition of about 5.77 mm against the gram-positive bacteria *Bacillus cereus* at 0.25 mg/mL concentration. To our surprise, no zone of inhibition was obtained for the 0.01 M silver nitrate solution for both bacteria. 1 mg/mL. Both the gram-positive and gram-negative bacteria were susceptible to the antibiotic ampicillin, and the *Escherichia coli* showed a large zone of inhibition of about 32.44 mm whereas the *Bacillus cereus* showed a zone of inhibition of about 5.6 mm. Unlike *Escherichia coli, Bacillus cereus* was comparatively less susceptible towards ampicillin (Bhuyar *et al.,* 2018).

There is no confirmed or well-established mechanism for the bactericidal effect of the silver nanoparticles. Previous studies suggest that the silver nanoparticles might attach to the cell membrane surface, disrupting permeability and affecting respiration (Slavin *et al.,* 2017). With the help of previous literature, it can be stated that the binding of the silver nanoparticles onto the surface of the bacterial cell membrane depends on the surface area available for the interaction. The essential metabolism of the bacteria can be disrupted when the nanoparticles penetrate deep into the cell and bind with the DNA, thus interrupting some gene expression. The silver nanoparticles have a higher bactericidal effect due to the higher surface area to volume ratio than the larger particles (Darroudi *et al.,* 2014). There is a high

demand for developing new antibacterial substances to overcome the increasing resistance of bacteria to the existing antibacterial agents.

Wheat is a staple food consumed by more than 36 % of the world's population since it is rich in protein and carbohydrates. The present study has shown silver nanoparticles' effects in mitigating wheat's inherent stress traits under salinity stress. The results from our study suggest that salt stress decreases the biomass and shoot and root lengths significantly in wheat seedlings (**Fig. 7**). Several previous studies have also reported that salt stress reduces the growth and biomass in wheat seedlings (Rizwan *et al.,* 2015; Rehman *et al.,* 2016). The reduction in nutrient uptake or the higher translocation of sodium from roots to shoots might be a reason for the reduction in the growth of the plants (Shafi *et al.,* 2010). In the present study, silver nanoparticles might increase the plants' growth by reducing sodium translocation from roots to shoots (Zhu *et al.,* 2016). This is similar to the study where the *B. juncea* biomass and growth were increased with the application of silver nanoparticles (Sharma *et al.,* 2012).

Our study also revealed that the plants treated with the biosynthesised silver nanoparticles showed increased chlorophyll a, chlorophyll b and total carotenoids. The increase in the photosynthetic pigments might result from the lower uptake of sodium by the plants in the presence of silver nanoparticles (Latef *et al.,* 2017). Our results revealed a reduction in the photosynthetic pigments, possibly due to increased oxidative stress or damage to the chloroplast structure (Wu *et al.,* 2015). We can state that the biosynthesised silver nanoparticles impart salt tolerance to the wheat plants showed better growth than the salt-treated plants. The results thus indicated that the silver nanoparticles could be used to overcome the salt stress. This method can be accepted by farmers in order to obtain a higher yield.

Chapter-5

CONCLUSION

5. CONCLUSION:

The development of a simple, low cost and eco-friendly method for metallic nanoparticles is of crucial importance in the field of nanotechnology. The present study showed that the silver nanoparticles synthesised using the *Nostoc commune* and *Enteromorpha intestinalis* extracts were stable and thus can be an alternative method to the chemical synthesis since green synthesis is an eco-friendly, pollutant-free and cost-effective approach. The silver nitrate ($AgNO_3$) reduction was visually confirmed by the colour change from pale yellowish-green to dark reddish-brown in the case of *Nostoc commune* and from yellowish-green to dark brown in the case of *Enteromorpha intestinalis*. The synthesised silver nanoparticles were further characterised using UV-Vis, FTIR, and SEM imaging. The nanoparticles synthesised from the algal samples showed excellent antibacterial efficiency against the gram-positive and gram-negative bacteria. Future research on the cyanobacteria *Nostoc commune* and the seaweed *Enteromorpha intestinalis* as a nanofactory can be crucial for medicine applications and biology. The antibacterial activity of the silver nanoparticles can be well-compatible with pharmaceutical and other applications. Future research can be carried out to evaluate the cytotoxicity of the silver nanoparticles on the human cell line. The plants grown in Hoagland solution and treated solely with silver nanoparticles showed increased shoot and root length as well as chlorophyll a, b and total carotenoids. The silver nanoparticle-treated non-stressed plants showed an increase in the well-watered control. The results showed that the combination of salt and silver nanoparticles showed better growth and more chlorophyll a, chlorophyll b and total carotenoids than salt-treated plants. Therefore, proving that silver nanoparticles can overcome salt stress to a certain extent. And thus, silver nanoparticles can be used to tolerate salt in wheat plants. However, there is a need to study the role and dosage of silver nanoparticles and the mechanism behind silver nanoparticle-mediated salt tolerance in plants.

Chapter-6

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6. REFERENCES

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