Formulation and Evaluation of Selenium Nano Liquid Biofertilizer from brown algae *Lobophora variegata* for Eco-Sustainable Agriculture

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Abstract

Various types of biofertilizers can reduce the usage of chemical fertilizers, environmental contamination and farm management costs. Seaweed extract-based biostimulants are regarded as one of the best modern ecological plant growth enhancers. They have been demonstrated to maximize agricultural output by successfully eliminating plant diseases and abiotic stressors. Additionally, they have become known as environmentally benign biofertilizers that enhance plants' resistance to inanimate objects and support their development and general well-being. The purpose of this study is to create Selenium Nanoparticles (Se NPs) using the natural aqueous extract of the brown seaweed Lobophora variegata. Various analytical techniques have been used to study the properties of the synthesized Se NPs. The antimicrobial activity against particular harmful bacteria and fungi was analyzed. The green developed Selenium Nano Liquid Biofertilizer's (SNLB) larvicidal activity was examined.

The study's main objective was to determine whether it would be feasible to create Selenium Nano Liquid Biofertilizer (SNLB) using Selenium Nanoparticles (Se NPs) made from marine brown algae Lobophora variegata. The study also aimed to evaluate the effects of this SNLB on plant growth parameters and pigment levels, which are found to triple in plants treated with SNLB.

Keywords: Selenium nanoparticles, *Lobophora variegate*, biofertilizer.

Introduction

Groundwater contamination and global warming are the results of recent increases in greenhouse gas output. The future of the agricultural sector is directly impacted by these problems, which are primarily associated with climate change[31]. Chemical fertilizers are widely used which have serious polluting effects on humans as well as negative economic and environmental effects. Finding safe, healthy and economical sustainable procedures is essential in today's plant cultivation[47]. Formerly referred to as plant biostimulants, plant bio-effectors are part of revolutionary alternative strategy that has gained popularity and is currently widely employed in a variety of agricultural operations. This strategy has a number of benefits such as promoting plant growth and stress resistance[22].

Seaweed extracts are frequently employed as necessary and sustainable biostimulants[6]. Seaweed extracts have long been used as biostimulants in agriculture, going all the way back to the early days of plant breeding. Algal cells, such as those of microalgae and seaweeds, are abundant in a variety of vital nutrients, such as minerals, antioxidants, proteins, lipids, polyunsaturated fatty acids (PUFA), pigments and numerous other biological substances[5]. Furthermore, seaweeds perform vital environmental roles and are important to marine ecosystems. On the other hand, seaweed extracts may find application in several biological processes in the industrial domain. Additionally, adding algae cells and/or their extracts to aquatic organisms' diets boosts their immune systems and encourages growth [1].

Because of their biological effects and compatibility with plants due to their shared biological ingredients, seaweeds can be used as biofertilizers. Because of this important advantage, seaweeds are now considered leaders in the field of plant biostimulants and have significantly benefited a variety of plant treatment techniques, especially those that promote organic and sustainable agriculture. Despite the availability of numerous seaweed extract supplements for plant treatments, foliar spray administration has been sufficiently and extensively used in modern agriculture to increase the output of many commercial crops with remarkably promising results. Seaweed liquid fertilizers can improve the amount of chlorophyll, strengthen the root system and increase overall production. With a focus on encouraging growth and increasing the productivity of numerous important vegetable crops, research should be done on the use of seaweed extracts in foliar spraying, as well as its quick and easy handling process[2,36]. The brown marine algae species *Lobophora variegata*, also referred to as J.V. Clamour (Phaeophyceae), is a form of brown algae that is abundant and is widely distributed throughout the world. Additionally, the liquid extract from *L. variegata* demonstrated cytotoxic potential, antiviral qualities and antibacterial effects[34]. Additionally, seaweed was found to have the ability to repel mosquitoes.

Numerous phenolic compounds including polyphenols, bromophenol and lobophorone, have been reported to be present in *Lobophora variegata* by Arnold et al[3], Chung et al[14] and Gutierrez-Cepeda et al[21]. Furthermore, polysaccharide fractions from *Lobophora variegata* showed sulfoquinovosyl-diacylglycerol 1–3 [SQDGs 1–3][9], fucoidan[33] and fucans[12]. This specific seaweed[16] has also yielded a large number of bioactive compounds that have been found and documented. In addition to its antioxidant, anti-diabetic, anti-obesity and blood fat-reducing qualities, *Lobophora variegata* has several other physiological actions, including lowering blood sugar, preventing fatigue and providing neuroprotection.

According to a study by Teixeira et al[48], the acetone extract from *Lobophora variegata* effectively suppressed the activity of α-amylase, suggesting that it could be used as a treatment for hyperglycemia[32]. Furthermore, it has been noted that this variety of seaweed contains a vast amount of medicinal chemicals. Therefore, the seaweed species *Lobophora variegata* was utilized to create Se NPs, which were then used to create a biostimulant for plant development.

Even at trace amounts, selenium (Se), a crucial element, promotes the growth and development of plants. Additionally, it protects plants from a variety of abiotic stresses by acting as a dose-dependent stimulant or antioxidant. To benefit from the multiple benefits of selenium (Se), it is crucial to comprehend how it is absorbed, transported and stored in plants [27]. Due to their increased stability and decreased toxicity, selenium Nanoparticles (Se NPs) are now widely accepted and recommended by researchers for usage in a variety of scientific domains. According to Wadhwani et al[50], biologically generated Se NPs are safer, more environmentally friendly and more economically viable (both chemically and physically) than alternative methods.

Reports on biological processes for producing Se NPs from marine and plant sources are also accessible at the same time[45]. Plants' Se levels are effectively raised by the use of Se fertilizers. Additionally, the application of Se fertilizers might improve plant development and maturity, especially in harsh conditions such as excessively salty or extremely hot ones[16]. Selenium contributes to the protection of chlorophyll by improving photosynthetic pigments[49].

Recent research has demonstrated that adding small amounts of Selenium (Se) improves plant growth and yield. By interfering with a number of physiological processes, selenium may serve as an essential component. It is a remarkable antioxidant that aids plants in adjusting to a variety of abiotic stresses such as salt, drought, abrupt temperature changes, harmful metals and metalloids and other environmental contaminants and toxins. Promoting the synthesis of secondary metabolites, gas exchange, osmoprotectant residue, photosynthetic pigments and the net photosynthetic ratio during photosynthesis are all part of its defense mechanism. As an antioxidant, Se helps to reduce the accumulation of Reactive Oxygen Species (ROS) or free radicals and prevents oxidative stress[19].

In this instance, it is crucial to comprehend Se NPs' phyto-uptake and translocation. Although there is still much to learn about these occurrences, a number of approaches have been employed to investigate the absorption and entry of SeNPs into plant systems[23]. Se NPs traverse the cell wall and pierce the plasma membrane. NP aggregates smaller than the pore diameter[51] were the only particles that could successfully cross the cell membrane. The cell wall of the plant acts as a barrier, preventing Se NPs and other external substances from invading. These sieving properties are determined by the pore size of the cell wall, which can range from 5 to 20 nm[11]. According to the most widely recognized theory about the translocation of artificial nanomaterials, these molecules can move via different plant tissues both intracellularly and extracellularly before reaching the xylem[39].

The purpose of this work is to develop an environmentally friendly method for producing selenium nanoparticles (Se NPs) quickly from aqueous extracts of the marine seaweed *Lobophora variegata* and to verify the produced Se NPs using UV spectroscopy. Its characteristics are investigated using a variety of analytical techniques. The study also intends to look into the antifungal and antibacterial properties of Se NPs that have been biogenically produced from the brown algae *Lobophora variegata*. This project's primary goal is to obtain Selenium Nano Liquid Biofertilizer (SNLB) utilizing *Lobophora variegata* marine seaweed extract and to test the biofertilizer formulation's larvicidal properties on brine shrimp larvae.

Material and Methods

Sample Collection and Preparation of Aqueous Extract of *Lobophora variegate*: The seaweed samples (*Lobophora variegata*) were gathered from the Tamil Nadu coast at Rameshwaram and Mandapam. To remove any dead sections and epiphytes, the collected seaweeds were rinsed with water. After being cleaned, the seaweed was carefully allowed to dry for ten days at room temperature in the shade before being used right away for extraction. One gram of dried seaweed is broken up into tiny bits. A mortar and pestle are then used to grind these pieces with 50 milliliters of distilled water. After that, the extracts are cooked for five minutes. Whatmann filter paper no. 1 was used to strain the boiling extract and the liquid that remained after sedimentation was stored at 4°C for later use[35].

Preliminary phytochemical screening of the aqueous extract of *Lobophora variegate*: According to Rathee et al[41], a phytochemical screening procedure was applied to a part of the aqueous seaweed extract (*Lobophora variegata*). Proteins, phenolic compounds, amino acids, carbohydrates, alkaloids, saponins, sterols, tannins, terpenoids and

flavonoids were among the natural chemical groups it sought.

Radical scavenging activity of *Lobophora variegata* extract: The DPPH Assay was used to assess the marine seaweed extract of *Lobophora variegata's* antioxidant capacity. One milliliter of 0.1 mM DPPH in methanol was made. Then, at concentrations ranging from 100 to 500µl, 0.95 mL of 1M HCl, 1 mL of ethanol and 1 mL of *Lobophora variegata* seaweed extract were added. The absorbance of the combination at 517 nm was measured after it had been stored for 30 minutes. The DPPH free radical scavenging activity was then assessed using Cho et al[13] methodology. Likewise, the Ferric Reducing Antioxidant Power assay (FRAP assay) was carried out using Jiménez-Estrada[25] methodology. 1.5 mL of the FRAP solution was mixed with samples of *L. variegata* marine seaweed aqueous extract (50 µL, 1 mg/mL) and the mixture was allowed to react for five minutes in the dark. It was then measured at 593 nm for the colorful product (ferrous tripyridyl triazine complex).

The findings were expressed in milligrams of trolox equivalents (mg TE/g). By adding 0.5 mL of *L. variegata* methanol extract at different doses (range from 100 to 900 µg/mL) to 0.3 mL of ABTS solution, the percentage inhibition of the ABTS radical was measured. After measuring the absorbance at 745 nm, the % inhibition was calculated. Superoxide scavenging activity was assessed using the Kunchandy and Rao[30] methodology. Together with 2.64 mL of phosphate buffer, 0.05 mL of riboflavin, 0.1 mL of nitroblue tetrazolium and 0.2 mL of EDTA, 0.2 mL of the *L. variegata* extract (or 20 mg of extract) was placed in the assay tubes. Algal extracts were substituted with DMSO (dimethyl sulfoxide) solution in the control tubes. For 30 minutes, the tubes were evenly lit by a fluorescent lamp while the initial optical densities of the solutions were measured at 560 nm.

By comparing the O.D. of the algal extract samples with that of the control tubes, the percentage of inhibition of the algal sample was ascertained. The quantity of nitric oxide was estimated using the Griess reaction and nitric oxide radical scavenging. For five hours at 25 ºC, different concentrations (100 to 900 µg/mL) of *L. variegata* methanol extract in phosphate buffer (0.025 M, pH 7.4) were incubated in a standard phosphate buffer solution containing 5 mM sodium nitroprusside. 0.5 mL of the solution was extracted and mixed with 0.5 mL of Griess reagent (1% Sulfanilamide, 2% Orthophosphoric Acid and 0.1% Naphthalene Diamine Dihydrochloride) during the course of a 5-hour incubation period. 546 nm was used to evaluate the absorbance of the chromospheres created by mixing naphthalene diamine with sulfanilamide and diazotizing nitrite.

By assessing the competition between deoxyribose and the aqueous extract of *L. variegata* for the hydroxyl radical generated by Fenton's reaction, the hydroxyl radical scavenging activity was ascertained. There are 2.8 mM of deoxyribose in the reaction mixture, KH_2PO_4-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), EDTA (0.1 mM) and varied volumes of sample extracts with a 0.1 mL final volume. The reaction mixture was incubated at 37ºC for one hour. Using thiobarbituric acid reactive substances (TBARS), the percentage of inhibition in the deoxyribose degradation process was measured and computed.

Biogenic synthesis of Selenium Nanoparticles using aqueous extract of *Lobophora variegate*: Aqueous marine seaweed extract of *L. variegata* and sodium selenite were used in the synthesis of selenium nanoparticles. To put it briefly, 5 g of the plant material was dissolved in 100 ml of distilled water and heated to 60 °C for 20 minutes. The extract was filtered through Whatmann no. 1 filter paper and distilled water was used to dissolve 0.1 M sodium selenite (pH 8). The ratios of sodium selenite to *L. variegata* aqueous extract were 5:5, 6:4, 7:3, 8:2 and 9:1. Because it exhibits greater output than the other ratios, the 5:5 ratio concentration was chosen for the bulk preparation out of all of them. A magnetic stirrer set at 800 rpm was used to continually stir the reaction mixture. The mixture turned pale brown in under one hour. The entire reaction happened in total darkness. The resultant suspension was centrifuged at 10,000 rpm for 10 minutes.

The pellet containing selenium nanoparticles was rinsed three or four times in deionized water to remove impurities. After being dried, the Se NPs were kept in a dark, dry and cool environment until additional analysis was done. To improve yield, selenium nanoparticles were optimized at different pH levels[38]. The biogenic synthesis of Se Nanoparticles using an aqueous extract of *L. variegata* is shown in fig. 1. The color of the fluid turned from colorless to brick red as the Se Nanoparticles developed. The solution containing selenium nanoparticles was produced following separation, concentration and drying. The resultant nanoparticle was retained and used in additional research.

Characterization of biogenically produced Selenium Nanoparticles: Aliquots of the reaction solution were measured using a UV Spectrophotometer. To identify the probable biomolecules responsible for the reduction of the ions and the capping of the bio-reduced selenium nanoparticles, FTIR analyses were performed. The crystalline structure of the Se-NPs was determined using X-Ray Diffraction (XRD) analysis. Energy-dispersive X-ray spectroscopy (EDAX) in conjunction with a 10kv scanning electron microscope (SEM) was used to investigate the size, shape and presence of selenium ions in the selenium nanoparticles. Thermogravimetric analysis (TGA) was performed using N_2 atmosphere between 30 and 550°C. The Zeta potential measurements and particle size analysis of Selenium nanoparticles were carried out by a particle size analyzer system[35] and the quality, shape and size can be examined using an HR-TEM (High-resolution Transmission Electron Microscopy).

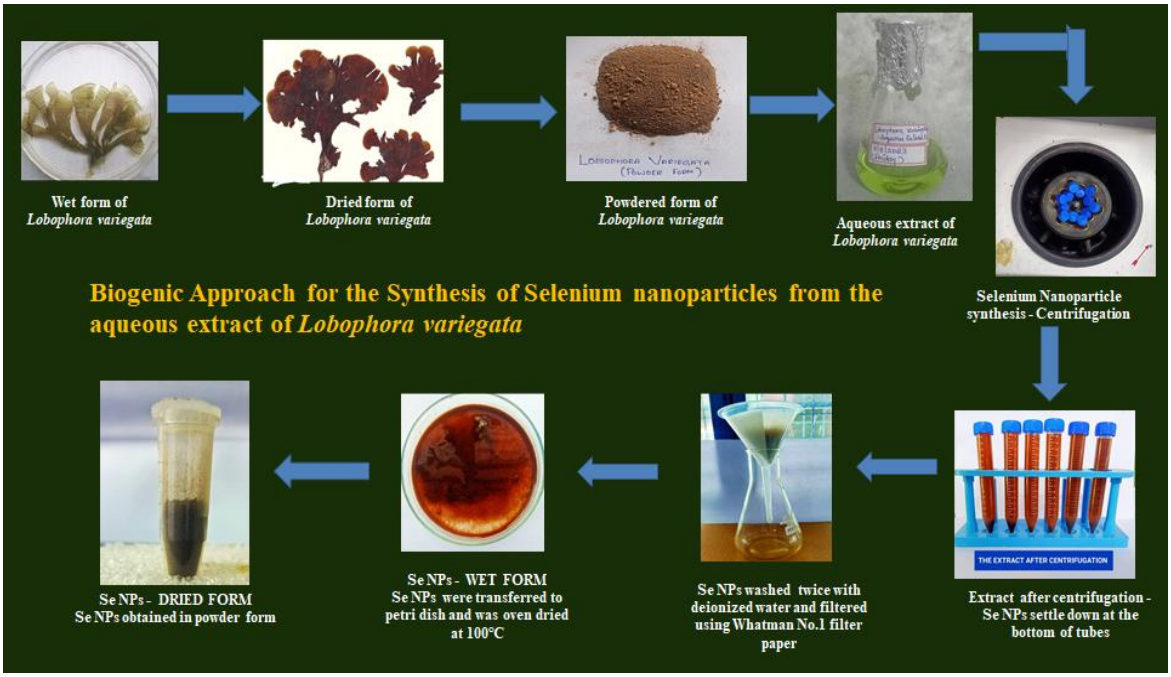

Fig. 1: Biogenic approach for the synthesis of Selenium Nanoparticles from the aqueous extract of *Lobophora variegata*

Determination of Antimicrobial activity of biogenically produced Selenium Nanoparticles

Bacterial strains: For the antimicrobial test, Gram-positive bacterial strains, *Bacillus sp. and* Gram-negative bacterial strains, *Proteus sp*., *Klebsiella sp*. and *E. coli*, were acquired from the DKM College Microbiology Laboratory. A nutritional broth medium comprising of beef extract, peptone, sodium chloride and yeast extract at pH 7.0 was used to inoculate the chosen bacterial strains, which were subsequently incubated at 37°C. The antibacterial activity of Se nanoparticles was examined using an overnight broth culture.

Fungal Strains: The antimicrobial test was conducted using fungal strains from the DKM College Microbiology Laboratory, including *Aspergillus sp., Rhizopus sp., Mucor sp., Penicillium sp. and Fusarium sp.* After being inoculated in a medium containing Sabouraud dextrose broth, the chosen strains were allowed to incubate at room temperature. The antifungal activity of Se Nanoparticles was examined using an overnight broth culture.

Agar Well Diffusion Technique: The well diffusion technique was used to screen for antibacterial activity. Using a sterile swab, 0.1 ml of the standardized inoculum from each test organism was seeded into Muller Hinton agar plates, distributing the inoculum evenly across the plate. An 8 mm conventional cork borer was used to drill a consistent hole in the MHA's surface. As controls, 100 µl of selenium nanoparticles made from *L. variegata* aqueous extract, standard antibiotics and sodium selenite solution were added to the well. The inhibition zone was evaluated following a 24-hour incubation period for the infected plates at 37°C. Using a sterile swab, 0.1 ml of the standardized inoculum from each test organism was seeded into Sabouraud dextrose agar plates, distributing the inoculum evenly across the plate. An 8 mm standard cork borer was used to drill a consistent hole in the SDA's surface. For the infected plates, the inhibition zone was evaluated using standard antibiotics, sodium selenite solution and 100 µl of selenium nanoparticles made from *L. variegata* at room temperature[40].

Preparation of Selenium Nano Liquid Biofertilizer (SNLB) from aqueous seaweed extract of *Lobophora variegate*: Selenium nanoparticles made from the aqueous extract of *L. variegata* are used in the biogenic method to prepare selenium nano liquid biofertilizer (SNLB), as illustrated in fig. 2. Ten milligrams of biogenic Se NPs and ten mL of *L. variegata* aqueous extract were dissolved in one thousand milliliters of distilled water to create the SNLB (Selenium Nano Liquid Biofertilizer). A surfactant (Tween-20; Sigma-Aldrich) was added to the solution containing selenium NPs before application[37] to guarantee the maximum fixation of Selenium NPs on plants.

Brine Shrimp lethality Test (Larvicidal activity): The toxicity of selenium nano liquid biofertilizer (SNLB) for larvae nauplii was assessed using a brine shrimp lethality test. Seawater in a beaker was used to hatch the brine shrimp eggs. After 48 hours, the active free-floating phototrophic nauplii were harvested using a pipette from bright light and utilised in the test. Under bright light, the nauplii were poured into a sterile watch glass filled with two millilitres of seawater. Each watch glass containing 10 nauplii was filled with several concentrations of selenium nano liquid biofertilizer (SNLB) (100, 250, 500, 1000 and 2000 µg/ml) and the glasses were left to incubate for 24 hours at room temperature in the dark[37].

Fig. 2: Formulation of Selenium Nano Liquid Biofertilizer (SNLB) from the marine brown algae *Lobophora variegata*

As a control, seawater without SNLB was employed. Over 24 hours, the macroscopic count of live Nauplii (Brine Shrimp larvae) was taken every three hours and the corresponding mortality percentage was computed. The percentage of mortality (% M) was calculated as:

% M = percentage of survival in the control - a percentage of survival in the treatment

Experimental Design to investigate the effect of Selenium Nano Liquid Biofertilizer (SNLB) formulated from the marine seaweed extract of *Lobophora variegata* in enhancing Plant Growth

Pot Assay: An experiment employing a randomised design was conducted in pots to assess the function of selenium nano liquid biofertilizer (SNLB) as a biostimulant for sustainable plant growth. For this experiment, five pots (P1 through P5) are needed in total. One therapy is represented by each pot. Commercial biofertilizer (CB) was applied to the seeds in P1 at a concentration of 10 millilitres per day with SNLB, P2 received 10 millilitres per day of commercially available biofertilizer (CB), P3 received 10 millilitres per day of SNLB, P4 received 10 millilitres per day of Tap water and P5 received 10 millilitres per day of sodium selenite solution. The experiment and pattern of treatment were continued for 15 days and the results were calculated and tabulated[44].

Experimental Setup

P1 – The seeds treated with a combination of Commercial Liquid Biofertilizer (CB) and Selenium Nano liquid bio-fertilizer (CB+SNLB),
P2 –The seeds treated with Commercial Liquid Biofertilizer (CB)
P3- The seeds treated with Selenium Nano liquid bio-fertilizer (SNLB),
P4 – The seeds treated with Tap water (Control),
P5– The seeds treated with Sodium Selenite solution (Control).

Growth Characteristics and Leaf Area Analysis: Growth parameters such as the fresh and dry mass of the root and shoot, as well as the length of the root and shoot, were measured after 15 days. The method employed by Khan et al[26] was taken into consideration for measuring the plant growth characteristics. The area of the leaves was measured using a leaf area meter.

Chlorophyll Content Value Analysis: The amount of chlorophyll in intact plant leaves was measured using a Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502; Konica, Minolta Sensing, Inc., Japan).

Analysis of Biochemical Parameters required for Plant Growth

a) Carbonic Anhydrase (CA) activity: In C3 plants, photosynthesis is facilitated by the carbonic anhydrase enzyme found in the chloroplast matrix, which catalyzes a reaction between bicarbonate and CO_2, the substrate of the immobilized enzyme RuBisCO. The carbonic anhydrase (CA) activity was measured using the technique proposed by Dwivedi and Randhava[17].

b) Nitrate Reductase (NR) activity: The reduction of nitrate's NAD(P)H to nitrite is catalyzed by nitrate reductase. To test nitrate reductase (NR) activity, the Jaworski[24] protocol was executed.

Estimation of Endogenous Proline Levels: Free proline level measurement is a useful assay for assessing physiological condition and stress tolerance in higher plants. Proline content was estimated from fresh plant leaves using the method developed by Bates et al[9].

Results and Discussion

Preparation of Plant Extract: Figures 3 and 4 display the cleaned, fresh and healthy *Lobophora variegata* seaweed as well as the shade-dried and powdered varieties. To create the aqueous extract, 50 mL of distilled water was combined with one gram of dried *Lobophora variegata* powder (Fig. 5 and 6).

Phytochemical screening of aqueous extrcat of *Lobophora Variegata*: Table 1 presents the findings of an investigation into the phytochemical components of the *L. variegata* stem extract. The phytochemical analysis revealed a wide range of constituents such as triterpenoids, flavonoids, anthraquinones, alkaloids, polyphenols, saponins and phytosterols.

Biogenic synthesis of Selenium Nanoparticles using aqueous extract of *Lobophora variegate*: A discernible color shift from pale yellow to reddish brown signified the successful biogenic synthesis of selenium nanoparticles (Se NPs), demonstrating the creation of the nanoparticles. Se NPs were detected by UV-visible spectroscopy, which showed a distinctive absorption peak at about 270–280 nm. During synthesis, the *Lobophora variegata* marine seaweed extract served as a stabilizing and reducing agent.

Characterization of biogenic Selenium Nanoparticles

UV-Visible Spectral analysis: With a size range of 250 nm to 700 nm, selenium nanoparticle formation was determined by UV-visible spectral analysis. A colloidal solution displayed an absorption maximum at 350 nm. Initially appearing white, the colloidal solution turned reddish-brown after a full day of incubation. The gradual formation of selenium nanoparticles throughout the course of the incubation time is demonstrated by the building of the absorbance maximum at 350 nm (Fig. 7).

FTIR Analysis: FTIR spectroscopy is used to analyze the chemical composition of the capping agents and SeNPs during NP synthesis. Figure 8 displays the results of an FTIR analysis of synthetic Se nanoparticles using an aqueous extract of *L. variegata*.

The peak at about 3431 cm^{-1} can be used to identify the O-H and N-H absorption peaks. The peaks that are located between 2920 cm^{-1} could be the result of the presence of the C-H group. The peaks between 2300 cm^{-1} and 2000 cm^{-1} can be recognized as C-O peaks. The peaks, which are between 1500 cm^{-1} and 1800 cm^{-1}, indicate the C=O/C=N/C=C stretching. Peaks at around 1200 cm^{-1} and 1100 cm^{-1} were attributed to the stretching vibration of the carboxyl group (C=O). The peaks at 1100 cm^{-1} and 1000 cm^{-1} can be due to the presence of the C-O-C group in the polysaccharides of the algae.

The development of peaks at around 800 cm^{-1}, 600 cm^{-1} and 500 cm^{-1} indicates the presence of the minerals in algae and the Se metal. As a result, the SeNPs' FTIR validates that the bioactive substances fucoidan and alginate effectively cap the Se nanoparticles.

Fig. 3: Marine seaweed *Lobophora variegata*

Fig. 4: Dried powdered form of *Lobophora variegata*

Fig. 5: *Lobophora variegata* extract filtered after placing in water bath

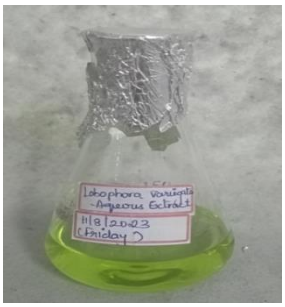

Fig. 6: Aqueous extract of *Lobophora variegate*

Table 1
Results of Phytochemical screening of *Lobophora variegate*

S.N.	Phyto Constituents	Result
1	Alkaloids	Positive
2	Carbohydrates	Negative
3	Test for starch	Negative
4	Reducing sugar	Negative
5	Cardiac glycosides	Negative
6	Protein and amino acids	Negative
7	Flavonoids	Positive
8	Tannin	Negative
9	Phlobatannins	Negative
10	Lignin	Negative
11	Anthraquinones	Negative
12	Anthocyanin	Negative
13	Phytosterols	Positive
14	Coumarins	Positive
15	Saponins	Positive

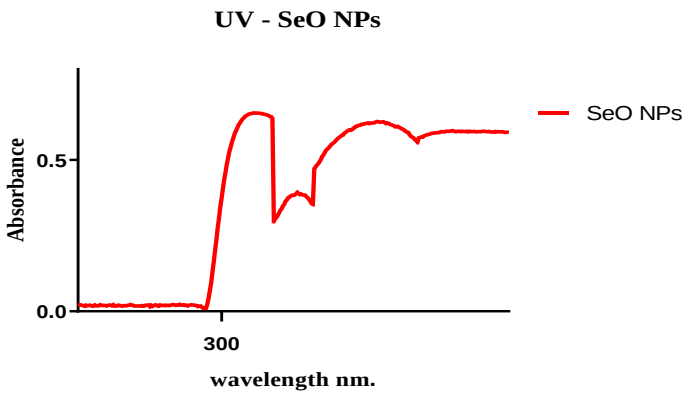

Fig. 7: UV-VIS spectral analysis of Selenium nanoparticles

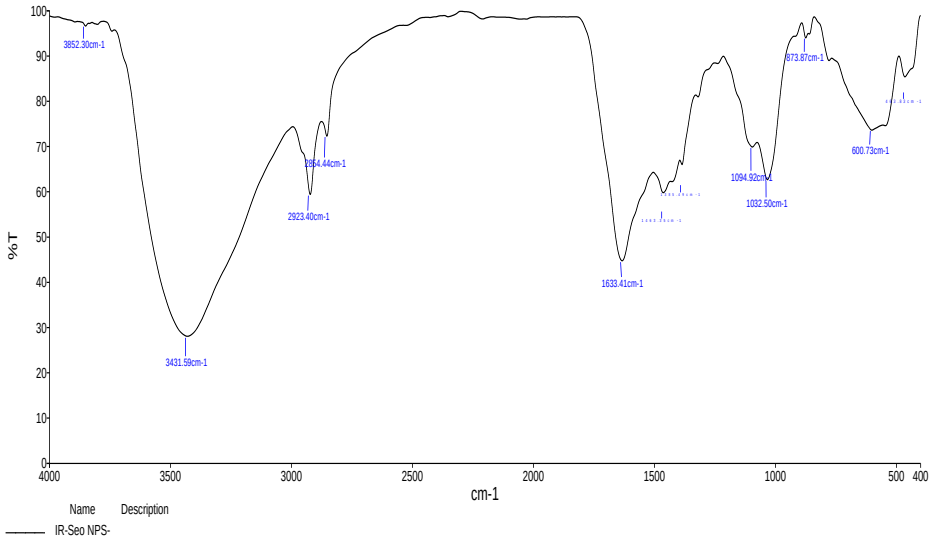

Fig. 8: FT-IR analysis of Selenium nanoparticles

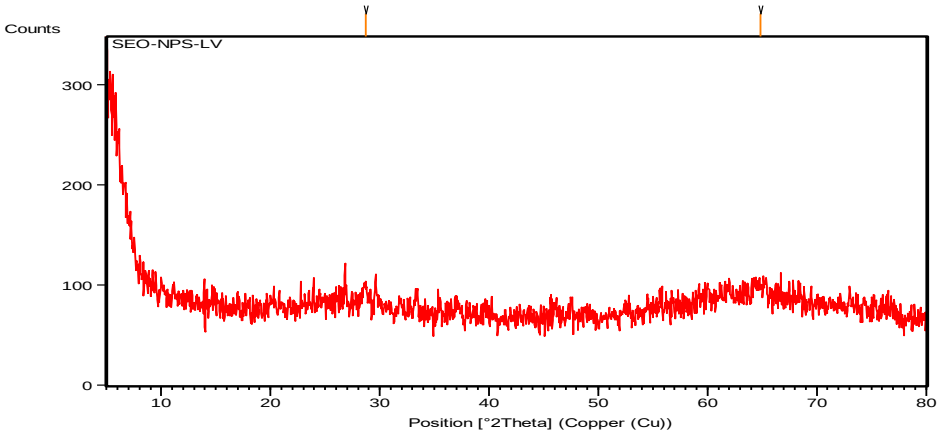

Fig. 9: XRD analysis of Selenium nanoparticles

XRD analysis: The content and crystal structure of the produced selenium nanoparticles were ascertained within the 10º–90º range using X-ray diffraction analysis. The identification of narrow and sharp peaks suggests the creation of very pure and well-crystallized selenium nanoparticles. The selenium peaks centered at 2θ of 23.5º, 29.7º, 41.4º, 43.6º, 45.4º, 51.7º, 55.9º and 61.5º are represented by the crystal planes (100), (101), (110), (102), (211), (201), (112) and (202) of the standard. According to the standard (JCPDS card No. 06-362), the lattice constants were a = 4.36 Å and c = 4.95 Å and the hexagonal-shaped Se NPs were successfully synthesized. The Se NPs had been growing more favorably in the (202) direction, as indicated by the increased peak intensities in the (100) and (101) planes (Fig. 9).

Zeta Potential with DLS analysis: Zeta potential is the measurement of an effective electric charge on the surface of a nanoparticle. The zeta potential is a vital indicator of the stability of the colloidal dispersion of nanoparticles. Zetasizer assessed the purified Se NPs and determined that their ZP was −26.9 (mV) when dried Se NPs were suspended in deionized water. Higher Zeta potential nanoparticles' enhanced stability can be ascribed to their stronger electrostatic repulsion with one another. It also showed that the intensity of the Se NPs was greatest at 100 nm, indicating that the maximum amount of Se is present in this range. In contrast, 530 nm was found to be the typical particle size (Fig. 10). This could be a result of the phytoconstituents in the plant extract encapsulating the nanoparticles.

SEM-EDAX Analysis: The size and shape of SeNPs were ascertained by SEM. The size range for Se NPs is 74.29 nm and the morphology and size of the particles are shown to be rod-shaped using SEM.

EDAX analysis provides the qualitative and quantitative status of the components that may be involved in the formation of nanoparticles. Fig. 11 shows the elemental profile of the generated nanoparticles. The study found that the highest amount of selenium (25%) in nanoparticles was followed by oxygen (20%), P (10%), sodium (10%), S (8%) and so on.

TGA Analysis: Thermogravimetric analysis (TGA) was used to examine the Se NPs' heat stability. The Se NPs exhibited two phases of weight loss when heated to 900 °C. A 5-6% weight loss was observed up to 220 °C, most likely due to the evaporation of volatile components (mainly adsorbed moisture) (Fig. 12). At 450°C, the samples lost almost all of their weight.

TEM Analysis: TEM analysis of the green synthesis of selenium nanoparticles revealed that the particles were within the nanometer range and were virtually spherical, with an average diameter of 66.55 ± 8.46 nm. (Fig. 13).

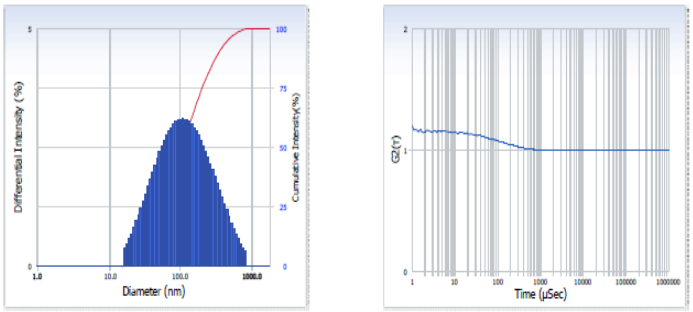

Fig. 10: Zeta Potential and Particle size of Selenium Nanoparticles

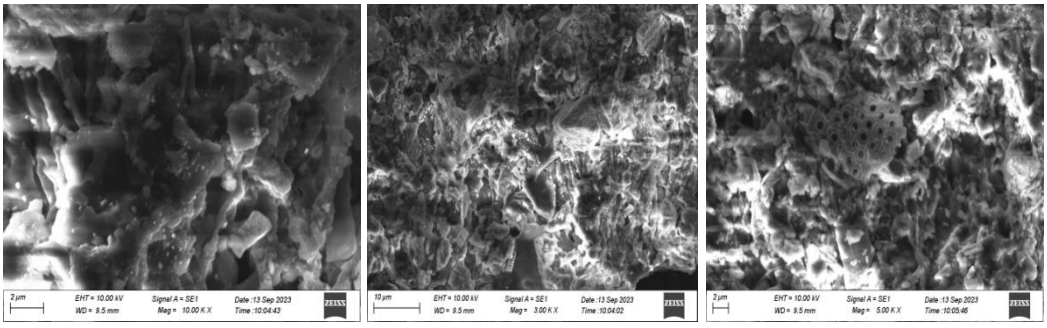

Fig. 11: SEM-EDAX analysis of Selenium Nanoparticles

Antibacterial Activity of Selenium Nanoparticles: In comparison to aqueous seaweed extract, antibiotics and sodium selenite solution, the antibacterial activity of biogenic Se NPs made from the extract of *L. variegata* was examined. Biogenic Se nanoparticles had the strongest antagonistic action among the aforementioned. *E. coli* (25 mm), *Pseudomonas sp*. (20 mm), *Staphylococcus sp.* (20 mm) and *Klebsiella sp.* (20 mm) were all significantly inhibited by biogenic Se nanoparticles (Fig. 14a).

Antifungal Activity of Selenium Nanoparticles: In comparison to aqueous seaweed extract, antibiotics and sodium selenite solution, the antifungal activity of biogenic Se nanoparticles made from the extract of *L. variegata* was examined. Biogenic Se nanoparticles had the strongest antagonistic action among the aforementioned. *Aspergillus sp.* (25 mm), *Mucor sp.* (20 mm), *Rhizopus sp.* (22 mm), *Penicillium sp.* (20 mm) and *Fusarium sp.* (18 mm) were all significantly inhibited by Se nanoparticles (Fig. 14b).

Free Radical Scavenging Activity: Hydrosoluble radicals (DPPH, FRAP, ABTS, Hydroxyl, Superoxide and Nitric oxide radicals) were used to evaluate the various free radical scavenging properties of *L. variegate* methanol extract. Table 2 and figure 15 provide a summary of the findings.

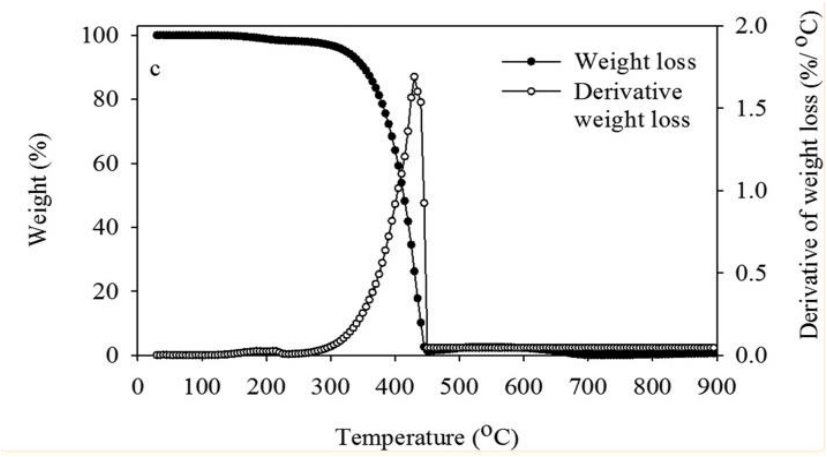

Fig. 12: Thermogravimetric analysis of Selenium nanoparticles

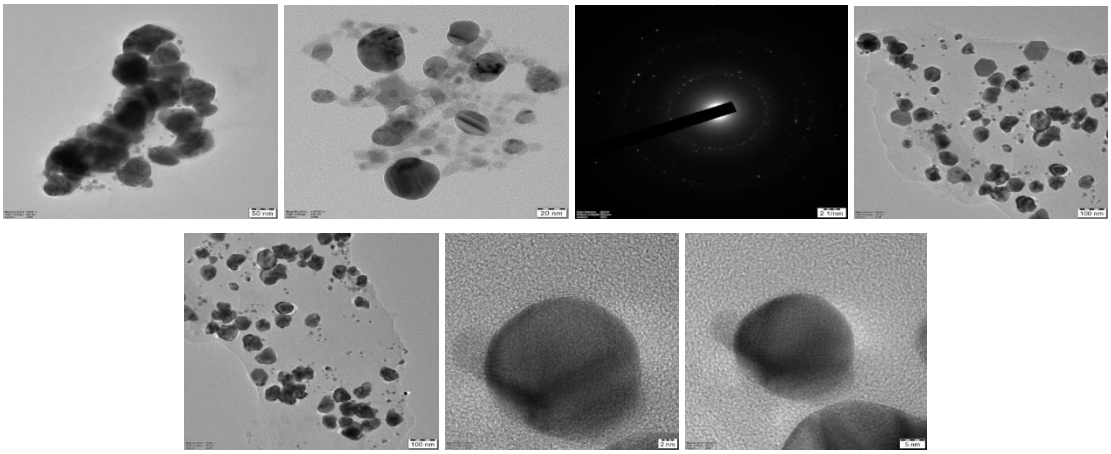

Fig. 13: TEM analysis of Selenium Nanoparticles

Table 2
Effect of methanol extract of *Lobophora variegata* on different antioxidant models- inhibition percentage (%)

S.N.	Conc. (µg/ml)	Free radical scavenging activity of *Lobophora variegata* (inhibition %)					
		DPPH radical	FRAP radicals	Superoxide radicals	ABTS	Nitric oxide radical	Hydroxyl radical
1	100	20.92±1.00	23.92±1.00	58.92±0.40	67.92±1.00	60.92±0.20	38.92±0.31
2	200	25.85±0.12	25.85±0.13	59.85±0.22	76.85±0.16	65.85±0.54	46.85±0.37
3	300	27.13±0.15	29.13±0.17	67.13±0.26	80.13±0.35	67.13±0.49	49.13±0.45
4	400	36.17±0.15	35.17±0.19	76.17±0.45	93.17±0.54	73.17±0.39	57.17±0.33
5	500	39.85±0.35	39.75±0.36	79.85±0.50	98.85±0.21	74.85±0.25	60.85±0.22
P-Value		0.000	0.000	0.000	0.000	0.000	0.000
F-Value		6.244	8.543	9.345	4.573	6.556	5.443

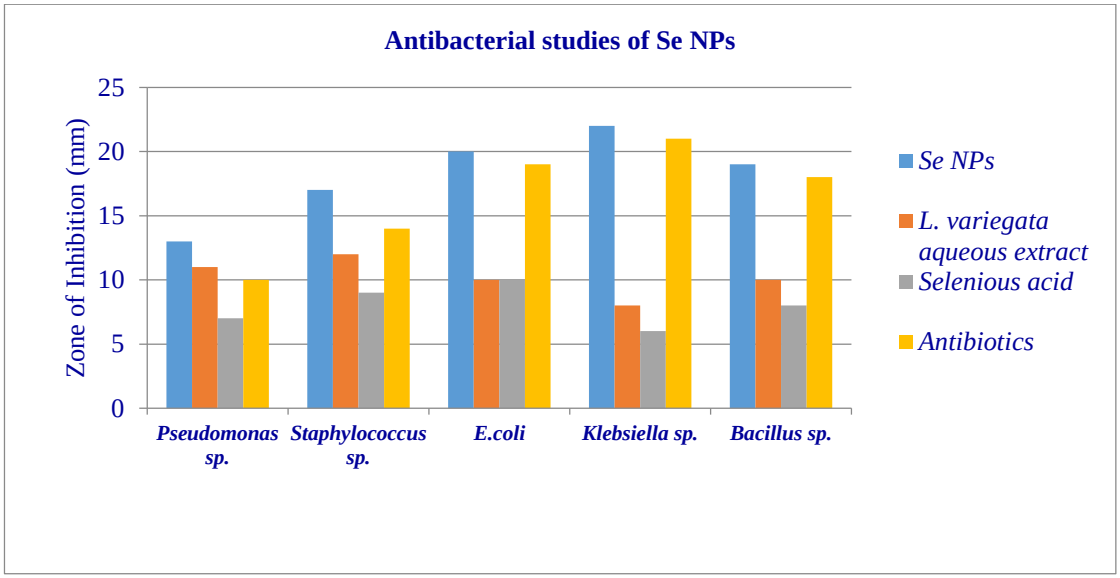

Fig. 14(a): Antibacterial activity of *Pseudomonas sp., Staphylococcus sp., E. coli, Klebsiella sp., Bacillus sp.*

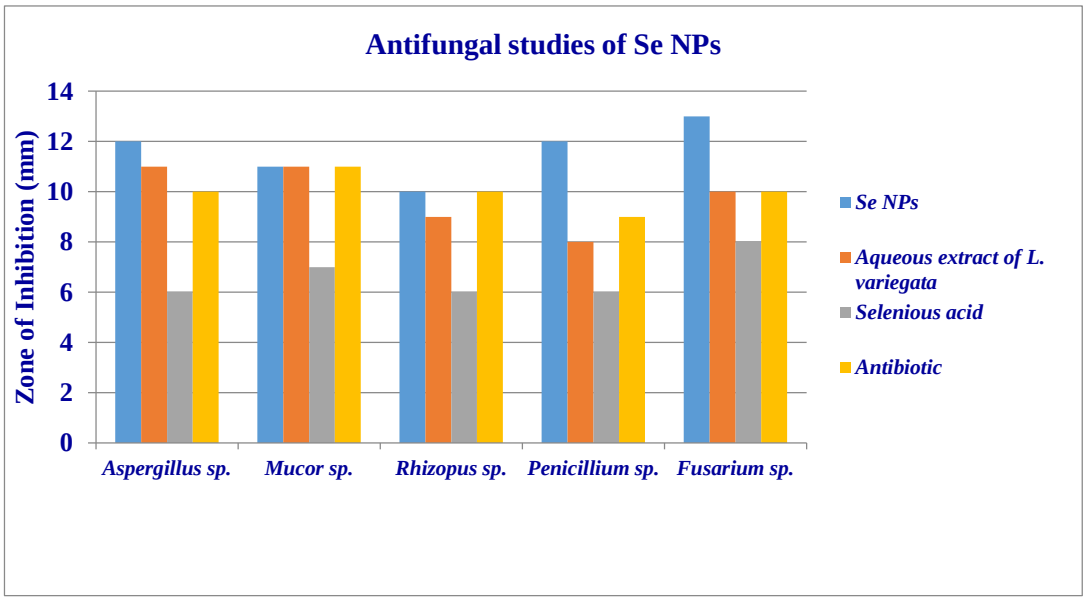

Fig. 14(b): Antifungal activity of *Aspergillus sp., Mucor sp., Rhizopus sp., Penicillium sp., Fusarium sp.*

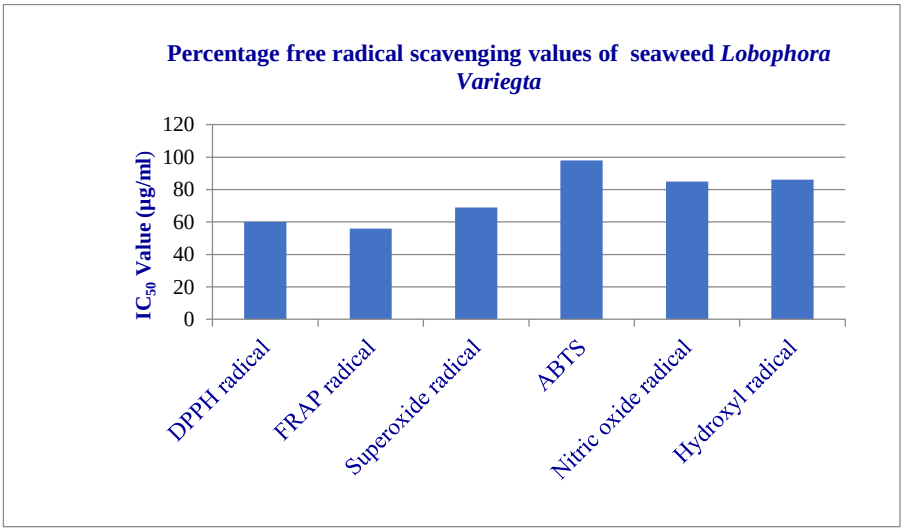

Fig. 15: Free radical scavenging system of brown algae *Lobophora variegata*

Antioxidant assay: DPPH is frequently used as a material to evaluate antioxidant capacity. According to the results of the present study, *Lobophora variegata* extract at 500µg/mL demonstrated a higher capacity to scavenge DPPH radicals with a scavenging activity of 39.85±0.35% (Table 2). The results suggest that *L. variegata* can donate hydrogen and also exhibits compounds that have the most scavenging power of radicals. According to DPPH free radicals, *L. variegata* exhibits potential as a useful natural antioxidant source. The findings of this study are in line with those of previous studies by Athukorala et al[7] who also found that brown algae were more capable of scavenging DPPH radicals and had higher levels of polyphenols than red and green algae. However, at a dosage of 1000 µg/mL, Roginsky and Lissi[42] found that brown seaweeds had comparatively modest levels of DPPH radical scavenging activity, ranging from 18.69 to 24.26% (Fig. 15).

Likewise, the ferric-reducing antioxidant power assay (FRAP assay) verifies that the reduction in absorbance is connected with the quantity of antioxidants (reductant). Compounds that are active in Fe^{3+} reduction promote peroxidation activity, or the generation of $OH^{\bullet}$. A pH of 3.6 is optimal for the process. According to the results, *L. variegata* exhibited the highest FRAP activity (39.75±0.35%) at a dose of 12 nM (Table 2). Even at a concentration of 100µg/mL, the algal sample was able to scavenge more ABTS than DPPH, with a difference of more than two times, according to the ABTS radical scavenging activity. In the end, the algal preparation outperformed the other free radicals examined in terms of its capacity to scavenge ABTS[43]. More specifically, at a concentration of 100µg/mL, the activity was measured at 67.38%.

When the algal sample was treated with 500 µg/mL of *L. variegata*, its superoxide anion scavenging activity peaked at 79.85±0.50% inhibition. *N. decipiens*, a tasty brown seaweed, has a good ability to scavenge superoxide anion, according to Kuda et al[28]. Additionally, the current study found that the methanol extract of *L. variegata* has a significant superoxide anion inhibitory effect, suggesting that it can be used as a natural antioxidant source[47].

In the same way, nitric oxide free radicals scavenging activity of *L. variegata* was 74.85 ± 0.22%, comparable to lower values. The IC_{50} value for methanol extracts of brown algae *L. variegata* was 82µ g/mL. By reducing the quantity of nitrite generated during the *in vitro* breakdown of sodium nitroprusside, direct scavenging by L. variegata extracts may be partly to blame for the decrease in nitric oxide emission. Yangthong et al[54] discovered that brown seaweeds had a much higher phenolic content and antioxidant activity than red and green seaweeds, as consistent with the findings of this study.

To estimate the hydroxyl radical scavenging activity of *L. variegata*, the scavenging impact of OH (hydroxyl) was investigated using the Fenton process. The radical was inhibited at a percentage of 37.86% by the 100 µg/mL algal sample, which was somewhat higher than the DPPH scavenging percentage. Even so, the measured activity was significantly lower than that of other radicals. The HO scavenging ability of the brown seaweed *L. variegata*[20] has been linked to phenoltannins, a polyphenolic compound that can act as an electron trap and is responsible for multifunctional antioxidant properties such as hydroxyl radical, peroxy radical and superoxide scavenging. Additionally, it was found that the primary component in *L. variegata* that facilitated HO scavenging activities, was ascorbic acid.

Toxicity testing [Brine shrimp lethality test (Larvicidal activity)] for Selenium Nano Liquid Biofertilizer (SNLB): The zebrafish embryos were used to investigate the toxicity of samples of Selenium Nano-Liquid Biofertilizer (SNLB). The study looked at how SNLB treatments affected the zebrafish embryo survival rate during the course of their incubation periods, which ran from 0 to 24 hours after exposure. The Artemia cytotoxicity (Brine Shrimp Assay) is one of the most accurate methods for assessing the cytotoxicity of bioproducts. Figs. 16a and b show the results of an investigation into the lethality of various doses of selenium nano liquid biofertilizer (SNLB), prepared using the aqueous extract of *Lobophora variegata*, ranging from 100 to 2000 µg/ml.

As the incubation period and nanoparticle concentration increased, the lethality of selenium nano-liquid biofertilizer (SNLB) increased as well. After 24 hours of exposure, the mortality reached a maximum level of 92% in the presence of 2000 µg/ml of Se NPs (Fig. 16a and b). On the other hand, the lethality of 100µg/ml of the SNLB was just 10%. After 24 hours of exposure to 250 µg/ml, 500 µg/ml and 1000 µg/ml of SNLB, the lethality was determined to be 59%, 67% and 82% respectively. Wan-Mohtar et al[52] and Wu et al[53] reported similar findings.

Effect of Selenium Nano Liquid Biofertilizer (SNLB) in Enhancing Plant Growth: An approach that shows promise for determining the effectiveness of the manufactured biofertilizer is the examination of plant development. The following figures show the growth performance in terms of fresh and dry root and shoot mass, leaf area, chlorophyll content, carbonic anhydrase and nitrate reductase activity and proline content. In the current study, *Vigna radiata*, also known as mung bean seeds, were used and inoculated in the biofertilizer mixed soil. Fig. 17 depicts the pot experimental setup.

Phenotypic Character: When compared to CB, SNLB, tap water and sodium selenite solution at various stages of plant growth (5 days, 10 days and 15 days), plants grown with commercial biofertilizer (CB) and selenium nano liquid biofertilizer (SNLB) demonstrated a positive increase in growth biomarkers (root length, shoot fresh mass, root fresh mass, shoot length, root dry mass and shoot dry mass).

Furthermore, it was observed that plants treated with CB and SNLB at a dose of 10µg/ml daily for 15 days showed the greatest increase in growth parameters (Fig. 18).

The findings demonstrate that *Vigna radiata* grows well in CB and SNLB-treated plants in terms of the fresh and dry mass of root and shoot after 15 days. This is because SNLB contains biostimulants. Enhancing glucose metabolism, repairing the ultrastructure of chloroplasts, speeding up the generation of chlorophyll and halting the breakdown of chlorophyll are some of Se's vital roles in supporting plant development and boosting plant growth[55].

Leaf area, SPAD value: Leaf area and SPAD chlorophyll levels significantly increased when the exogenously delivered CB and SNLB were compared to the other treated solutions, which included CB, SNLB, tap water and sodium selenite solution. Conversely, the values of SPAD and the maximum leaf area were assessed in plants treated with CB after they were given 10µg/ml of SNLB every day for 15 days (Fig. 19). Likewise, Se-NP concentrations between 50 and 100 mg kg^{-1} markedly improved organogenesis 48=64 and the root system (>40%).

Activity of Nitrate reductase (NR) and Carbonic Anhydrase (CA): For 15 days, the plant treated with 10µg/ml of CB with SNLB showed the highest levels of RA and CA activities compared to the control (Fig. 20 and 21).

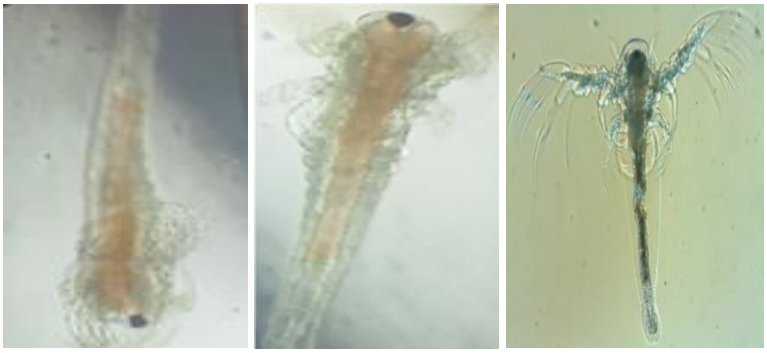

Fig. 16a: Morphological variations of brine shrimp during the Treatment

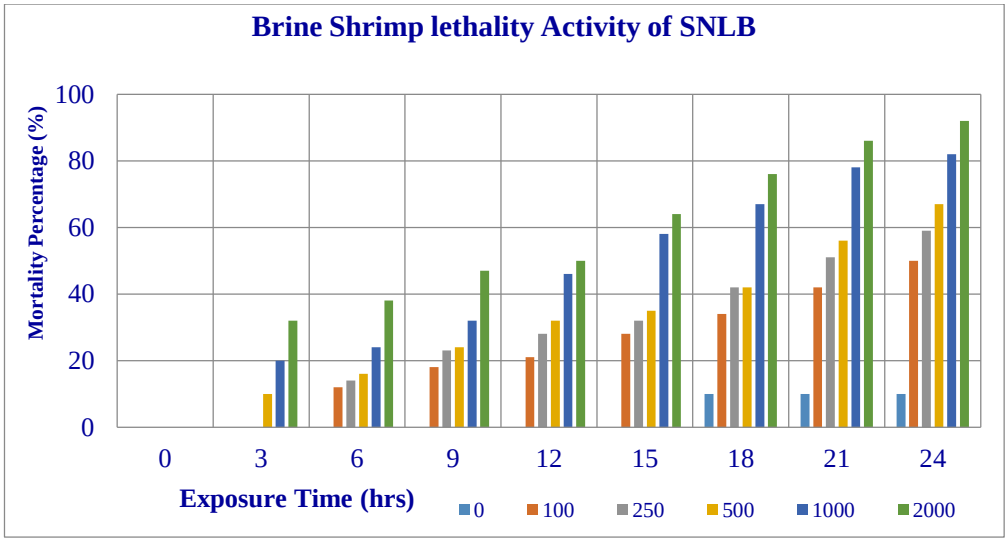

Fig. 16b: Brine shrimp larvicidal activity (lethality) of SNLB

Fig. 17: Plant Growth after 15 days of exposure to treatment solutions (*Vigna radiata* seeds treated with SNLB+CB, SNLB, CB, Tap water and Sodium Selenite solution)

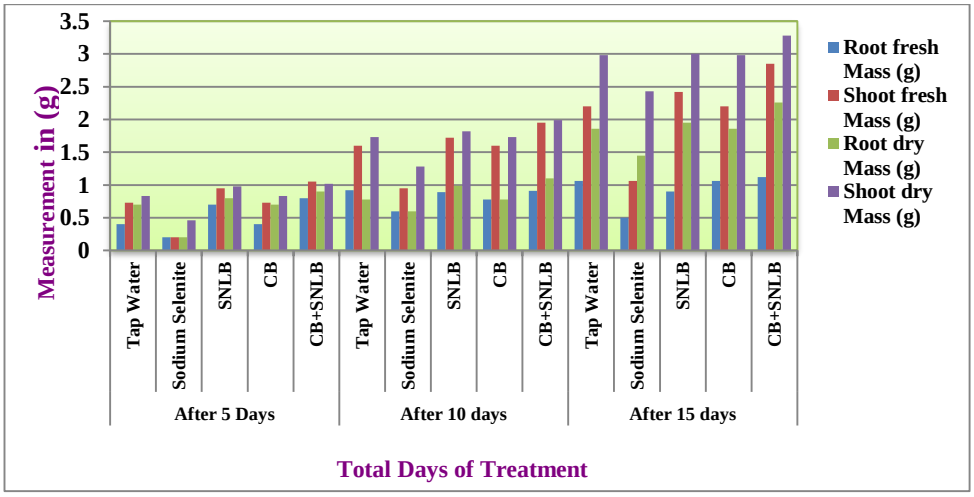

Fig. 18: Growth Characteristics of *Vigna radiata* treated with CB+SNLB, CB, SNLB, TAP WATER, Sodium Selenite Solution (Leaf area)

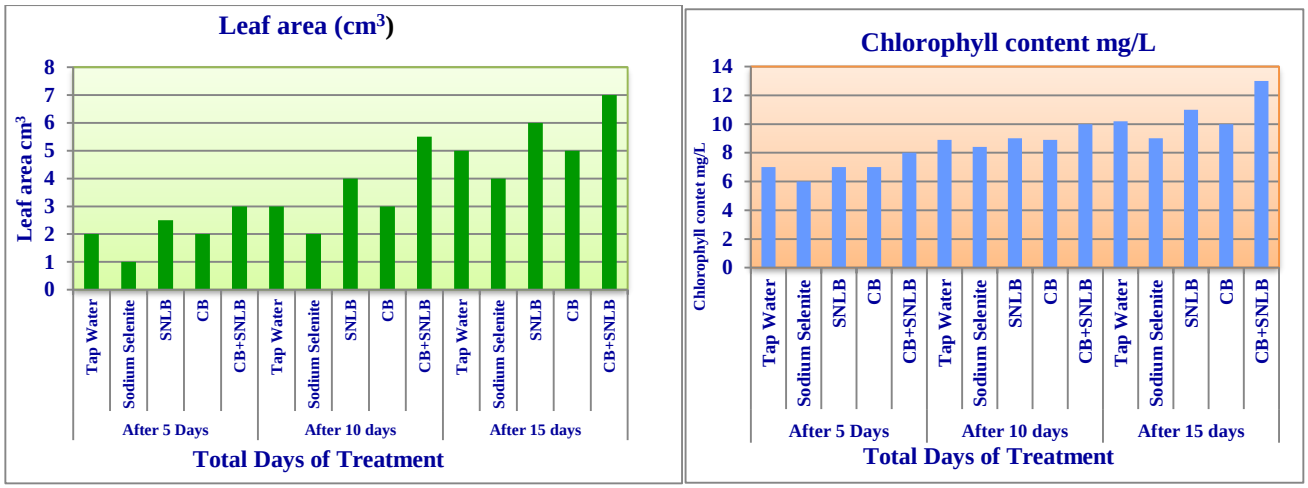

Fig. 19: Measurement of Leaf area and Chlorophyll content

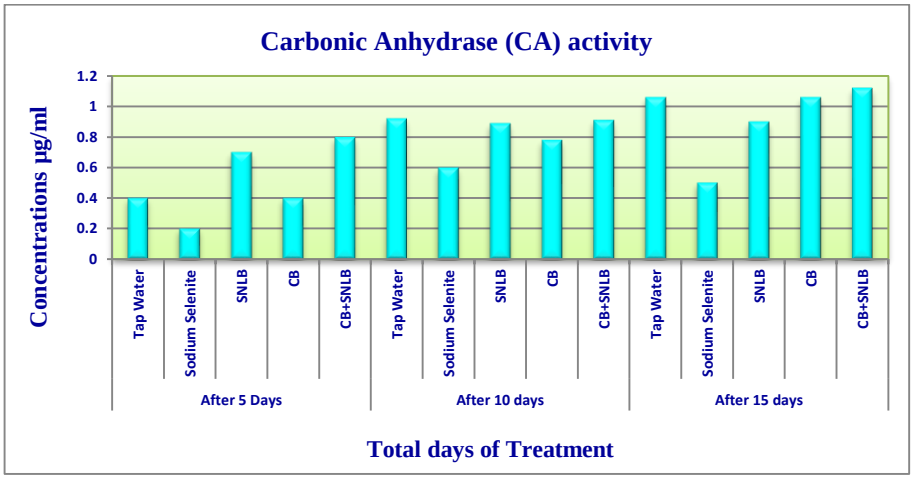

Fig. 20: Carbonic Anhydrase (CA) activity

Proline content: As the plant grew older, the amount of proline in its leaves increased. The plant with the highest proline content in its leaf was the one that was grown with 10µg/ml of CB and SNLB during the 15-day growth stage (Fig. 22). Thus, figures 20-22 show the effect of various treatments such as sodium selenite, SNLB, CB and SNLB+CB compared with tap water and record the highest growth-promoting effect of CB+SNLB among others[46].

Dai et al[15] in their investigation of the beneficial effects of selenium (Se) on *Brassica campestris sp*. Pekinensis i.e. *Brassica* grown under two distinct treatments of selenium

and zinc accumulation, found that the presence of Se increased the concentrations of proline, glutathione peroxidase (GR), ascorbate peroxidase (APX), SOD, POD and catalase (CAT). After testing the effects of nSe (0, 1, 4, 10, 30 and 50 mg L^{-1}) or bulk (selenate) treatments on bitter melon seedlings, Rajaee Behbahani et al[39] found that these treatments significantly boosted leaf nitrate reductase activity, with an average increase of 52% above the control. The biogenic Se in the biological medium like SNLB surpassed normal Se in plant growth[18].

When the number of treatment days grew, the plants' growth in terms of biomass, leaf area and other enzymes rose progressively. After the maximal exposure duration of 15 days of therapy, maximum parameters were observed. Additionally, when comparing the growth parameters for the various treatment compositions, the plants treated with CB+SNLB benefited the most because all the parameters were greater in this treatment than in CB, sodium selenite and plain tap water. The findings also demonstrate that plain SNLB had a noteworthy effect on plant development, indicating that SNLB had a considerable effect on plant growth and functions as an appropriate biostimulant for plant growth.

This indicates that the biostimulant also helps the plant overcome stressors like heavy metals, chemical fertilizers and other pollutants, drought, salinity, extreme temperatures and other environmental factors. Proline, an amino acid that is used to combat oxidative stress in plants, also increased. The most popular extract for making plant biostimulants is seaweed extract. There is evidence that the many chemical components of seaweed extracts, including primary metabolism, antioxidant action and up- and down-regulation of phytohormone signalling, operate in concert to boost agricultural productivity[29].

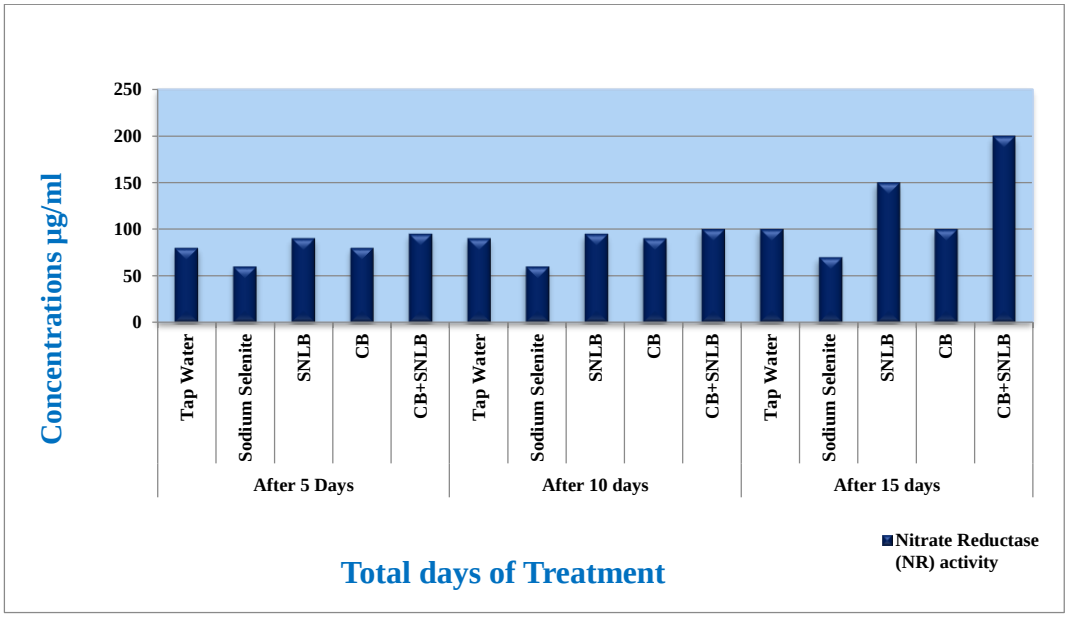

Fig. 21: Nitrate Reductase (NR) activity

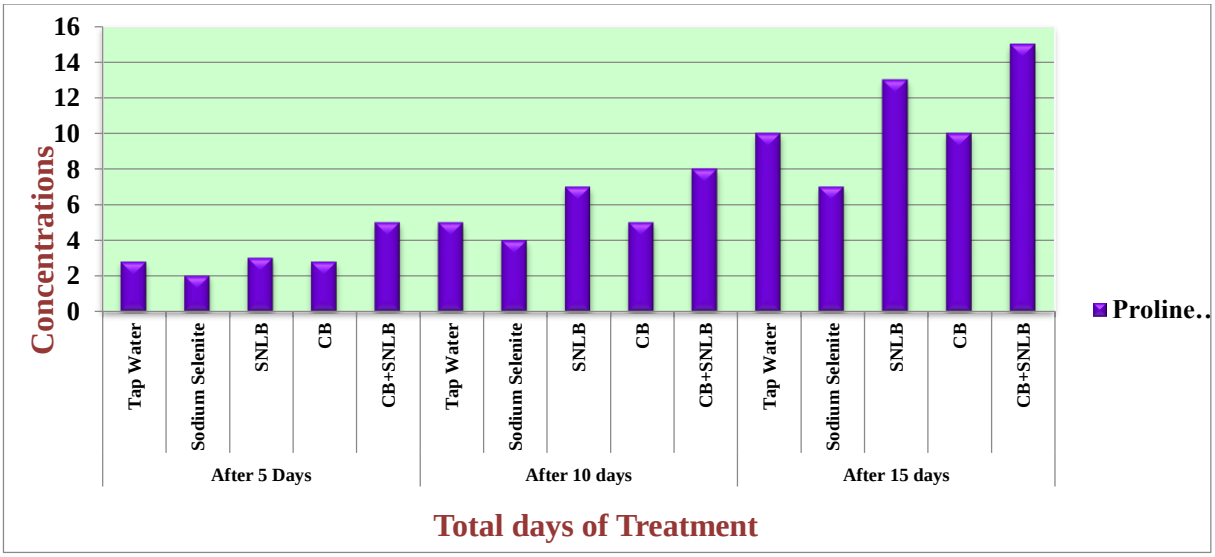

Fig. 22: Proline content

Conclusion

We have successfully synthesised selenium nano liquid biofertilizers (SNLB), which are useful because they release nutrients and urea into the soil gradually, enhancing plant growth. The maximum nutrient utilization efficiency and higher crop yields were achieved when *Vigna radiata* was treated with a combination of commercial biofertilizer (CB) and selenium nano liquid biofertilizers (SNLB). The slow-release characteristics of SNLB were investigated when SNLB was mixed with CB, SNLB alone and CB alone, together with various control solutions during the experiment, which had a maximum duration of 15 days.

According to this study, there are a number of advantages to using SNLB and commercial biofertilizer (CB) together as fertilizer. These include little land damage, cost, the sustained and progressive release of nutrients and the need for only a tiny quantity (10 mg/ml). According to the current investigation, the concentration affected the reaction that SNLB mediated. Furthermore, the leaves of *Vigna radiata* plants treated with 10 mg/ml SNLB with CB showed the most promising response, leading to better plant development and increased photosynthetic capacity. When SNLB and CB were used together, the physiological parameters improved, which in turn improved the growth of *Vigna radiata* plants as a whole. The process of photosynthesis is advanced and more biomass and crop yield are eventually produced when stomatal conductance is raised because it speeds up the exchange of gases.

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