Biofabrication of iron oxide nanoparticles from green seaweed Ulva lactuca and screening for its insecticidal activity

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Research Article

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Abstract

The primary aim of the research was to utilize Ulva lactuca, green seaweed, to biofabricate iron oxide nanoparticles (FeNP) and evaluate their insecticidal effica absoluta, a pest that primarily impact tomato commercial crop. For the purpose of characterising biosynthesized nanoparticles, tools such as UV-Vis spectroscopy, FT-IR, XRD, SEM, and TEM were used. Inferred from the results is that, the aggregation of iron oxide nanoparticles is confirmed by the colour change from yellow to dark brown. Additionally, spectra from the 200–1000 range are absorbed by UV–Vis spectroscopy. The FT-IR peaks correspond to the band at 778 cm in the Ulva lactuca extract and correspond to F-O stretches of Fe_2O_3, confirming the production of nanoparticles. The band at 2851 cm may be due to C-N stretching from unreacted impurities or CO_2 in the sample compartment. According to a TEM image of iron nanoparticles, the majority of these particles are almost spherical in shape, and their average size was found to be between 50 and 250 nm. Pure iron oxide nanoparticles were recovered from Ulva lactuca by green synthesis. The prominent peaks in the range of 30 to 70θ indicated by XRD demonstrate the extraction of pure iron oxide nanoparticles from Ulva lactuca. The results of the insecticidal tests indicate that the synthesised iron oxide nanoparticles had a satisfactory death rate of 63% with an observed concentration of 77.32 ppm. The values for the LC_{50} are 97.25 and LC_{90} are 110.8 ppm. The values were compared one to the other with the control. The insect Tuta absoluta will be a possible target for control with iron oxide nanoparticles generated from Ulva lactuca.

Introduction

Numerous commercially significant crops, including cotton, corn, tomatoes, sorghum, soybeans, and tobacco, are susceptible to harm from the polyphagous, global, and cosmopolitan insect pest [1]. These pests are regarded as the most economically significant insect pests in a number of nations, including India, Japan, China, and Southeast Asia. They prey on more than 150 distinct host species [2]. The extremely dangerous leaf-mining parasitic Lepidoptera Tuta absoluta has a great predilection for tomatoes. It affects potatoes, sweet peppers, eggplants, and a number of other cultivated plants. It can also be found on weeds belonging to the Solanaceae family, such as Datura sp. and *Solanum nigrum*. *Tuta absoluta* has the potential to reduce tomato crop yields by 50–100%, and its presence may also prevent the product from being exported to certain countries.

Insecticides made from synthetic chemicals are used to control pests in agriculture; however, doing so causes severe environmental damage and soil contamination, in addition to being poisonous to non-target animals. Pesticides are substances that are either naturally occurring or artificially synthesized and are used to control a wide range of pests. Some of the chemical pesticides on the market are hazardous to people, plants, ecosystems, and the environment. They also cause the development of resistance and the deposition of leftover chemicals.

The development of environmentally friendly, non-toxic nanoparticle production techniques has received increased attention from researchers in recent years [3]. It also uses less energy, toxic chemicals, and high temperatures. Green nanoparticle production provides regulated growth, crystallization, greater influence, and increased stability. The production of shape- and size-controlled metal and metal oxide nanoparticles can be accomplished using green sources as a stabilizing and reducing agent [4], [5], [6]. Potential environmentally sustainable substitutes for risky and expensive chemical and physical procedures for the creation of nanoparticles, microorganisms, enzymes, and plants have been proposed [7].

Seaweed may contain many different compounds, including phlorotannins, flavoids, bromophenols, carotenoids, and phycobiliprotein, are thought to be abundant in seaweed that are essential for the reduction, capping, creation, and stability of nanoparticles [8].

Unlike most biomass, seaweed can be harvested multiple times in a single year. It is well known that certain algae species decrease metal ions [9]. Seaweeds are considered unsophisticated plants with limited benefits, such as growth rate and nutritional requirements, for creating nanoparticles when compared to higher plants. Numerous variables, such as seasonal change, geographic location, environmental conditions, species, and developmental phases, have an impact on the chemical makeup of macro algae [10].

Seaweeds have a large role in industry and food sector items such as fish balls, meat balls, nuggets, broad bean cake, and stewed horse beans [11]. They have also been used as fertilizers, pharmaceuticals, anticancer, insecticides, and biofuel in many nations across the world, notably in Asia. As seaweeds offer more specialized benefits to people, this concept is commonly used in the creation of insecticidal activities, pharmaceutical medications, different nutraceuticals, the food industry, and the cosmetics industry [12]. Seaweeds such *Ptercladia capillacea, Turbinria turbinata, Chaetomorpha antennina, Sargassum muticum, Kappaphycus alvarezi*, and *Colpomienia sinuosa* are utilized for nanoparticle synthesis. All the seaweed nanoparticles were explored only for the antimicrobial and antifungal activity.

In comparison to other approaches, green synthesis has quick production periods, low prices, and environmental safety. In comparison to fungal and bacterial cultures, which can need for sterilized conditions and special preservation techniques, the creation of metal nanoparticles from seweed extracts is thought to be a simple procedure. The manufacture of iron nanoparticles utilizing green technology is the focus of several scientific investigations. The size and form distributions of the plant extracts employed to create the nanoparticles are also varied. The bioactive metabolites found in seaweed extracts have the potential to operate as reducing agents [13]. Among the existing green methods of synthesis for metal or metal oxide nanoparticles, the use of green extracts is a simple strategy for producing nanoparticles on a large scale as compared to bacteria and/or fungal-assisted synthesis. These substances are collectively referred to as biogenic nanoparticles [14].

When it comes to controlling and preventing the tremendous economic calamity that the tomato pest poses to the tomato crop, nonmaterials are thought to be a prominent, potent, dependable, cost-effective, and environmentally friendly technology. India's primary economic engine is its agriculture sector. One of the main issues facing agriculture is pest attacks.

To manage the pest, weed, and insects, a variety of strategies and control measures, including physical, chemical, and biological techniques, were put into place. Still now to our knowledge this is the first report on synthesis of iron oxide nanoparticles from the seaweed *Ulva lactcua* and its ability to target against the pathogenic pest Tuta absoluta were focused.

Materials and Methods

Seaweed

Ulva lactuca (green algae) was chosen due to its year-round availability and traditional understanding of its biological functions. Ulva also called Sea lettuce is the seaweed which has naturally abundant iron content. It was taken from the South East coast of India Gulf of Mannar coastal region at depths ranging from 1 to 5 metres. The selected seaweed was identified using a handbook approved by the Botanical Survey of India.

Preparation of aqueous extract

The obtained seaweed samples were rinsed in sea water and then fresh water to eliminate epiphytes and other contaminants. The seaweeds were then properly washed three times with distilled water and shade dried for ten days. The seaweeds were ground into a fine powder using a household blender. The seaweed powder was sterilized for 15 minutes at 120°C. In an Erlenmeyer flask, 20 g of seaweed powder was combined with 200 ml of distilled water and heated in a heating mantle at 60°C for 20 minutes. The extracts were filtered using Whatman No.1 filter paper and kept in a refrigerator at 4°C for future research.

Biosynthesis of Fe_3O_4 Nanoparticles

The seaweed extract was gradually dissolved in a 1:1 volume ratio of $FeCl_3$. $6H_2O$ (0.1 mol/L) with continuous stirring for 30 minutes. A buffer solution was used to adjust the pH of the solution, keeping it between 7 and 10 while vigorously swirling. The prepared mixture was agitated for 1 hour at room temperature using a magnetic stirrer. A precipitate of brown colour was generated. The mixes were then incubated for 72 hours at normal pH and stirred in the dark to minimize ferric chloride photo activation. Simultaneously, a negative control containing only ferric chloride aqueous solution was also maintained along with the experimental flasks under the same condition.

Characterization of Fe_3O_4-Nanoparticles

The biosynthesized nanoparticles were examined by monitoring the aqueous component using ultraviolet-visible spectroscopy and scanning the absorbance at 350–800 nm on a Perkin Elmer Lamda 25 spectrophotometer. The spectra of centrifuged lyophilized samples were obtained using the Thermo Nicolet Nexus 670 (FTIR) in the diffuse luminescence mode with a resolution of 4 cm. Transmission electron microscopy (TEM) was used to analyze the colloid's morphological structure and the size of the photogenic Fe_3O_4-NPs. XRD (X-ray diffraction) is used to examine physical parameters such as phase composition, crystalline structure, and sample orientation. The significant XRD peaks in the range of 30 to 70 show the extraction of unrefined iron oxide nanoparticles from Ulva lactuca

Insecticidal activity

Using the conventional topical administration protocol advised by Entomological Society of America (ESA) recommendations and the Robertson and Preisler method (1992), bioassay was carried out on two-day-old third-instar *Tuta absoluta* larvae. Different concentration of nanoparticles ranging from 5, 10, 25, and 100 ppm were created by dissolving in distilled water. For each concentration, three replicates were maintained, and twenty larvae were released into each replicate; larvae treated with distilled water alone were used as the control group. An insecticide combination droplet applied topically to the ventral area. After checking the larvae mortality after 24 hours, Abbott's formula (Abbott, 1925) was used to rectify the mortality. Using the Statistical Package for Social Sciences (SPSS), the values for LC_{50} and LC90 are determined.

Non target predator assay

The fish were acclimated for 14 days under laboratory conditions for a limit study and 14 days for a range finding research after the quarantine period. The feed was removed 24 hours before to exposure, and the fish were not fed during the exposure period. In tank nos. 1 for control, 2 for vehicle control, and 3, 4, 5, 6, and 7 for treatment group in range finding research, 10 fish per group, fish meeting the necessary length and weight requirements were released into the exposure medium. Following the measurement and mixing of 100 mg of iron oxide nanoparticles with 0.1 ml of ethanol, the mixture was dissolved in 999.9 ml of exposure medium. The solubility of the resulting mixture led to the selection of exposure medium as the vehicle. The stability analysis was conducted from 0 to 96 hours using the exposure medium (control), ethanol as the vehicle control, and 100 mg/l concentration of iron oxide nanoparticles in the exposure medium. A static test was run based on the findings of the stability study.

The hardness of the control and treatment groups was examined at the beginning and conclusion of the experiments for the limit research, and the physical chemical parameters of the exposure medium, such as pH (pH meter), dissolved oxygen (dissolved oxygen meter), temperature (digital thermometer), conductivity (conductivity meter) and hardness, were checked every 24 hours. Inside the testing facility, the calibration records are kept. With a concentration of 100 mg/l of nanoparticles, analytical verification was performed in the control vehicle. Analysis was done on the 0 to 96 hour sample. At 0 hours, the mean recovery is 100.24%, and at 96 hours, it is 99.69%.

Results and Discussion

In an effort to create novel materials at the nano-scale, nanotechnology has become a fast expanding field of study that interacts with many other areas of science and technology. Nanosized inorganic particles, whether simple or composite in nature, exhibit unique physical and chemical properties and represent an increasingly important material expressing significant advances; this is due to their numerous applications in the fields of biomedicine, sensors, antimicrobials, catalysts, electronics, optical fibers, environmental purification, pharmaceuticals, agriculture, bio-labeling, and other fields [15]. By employing atomic scale customization, nanotechnology is anticipated to offer up new avenues for the prevention and treatment of illness.

Tuta absoluta is the pest which mainly affects the tomato plants. The endophytic nature of the larvae in the root, stem and leaves prevent from the exposure of the chemicals and synthetic pesticides. The over usage of the pesticide create a negative impact to the environment. Significant research from the preceding investigations together suggests that the seaweed was cleaned, dried, ground, and combined with distilled water. To create the nanoparticles, the filtrate from the extract is combined with ferric chloride and heated to obtain particles. Similarly in this study, the aqueous extract of *U. lactuca* was used to create iron nanoparticles in a green manner.

Iron oxide nanoparticles are created when the phyto-chemical component of the seaweed extract acts as a reductant on ferric chloride. The presence of hydroxyl groups and sulphate in sulphated polysaccharides in marine macroalgae may lower the ferric ions and produce highly stabilized iron nanoparticles [16].Previous reports have described the molecular mechanism behind the production of ferric iron nanoparticles [17].

One of the most important instruments for characterizing the nanoparticles is UV-Vis spectroscopy. UV-visible spectrum measurement and colour changes in the reaction mixture were used in our investigation to track the reduction process. An SPR band was seen to form the nanoparticles. The high seaweed extract concentration increased the phyto-chemical content of the extract, which swiftly reduced the precursor and produced nanoparticles that immediately raised the absorbance value [18]. The location of the SPR band is dependent on the size, shape, and aggregation state of the nanoparticles as well as the dielectric properties of the surrounding medium.

Consequently, the UV-Vis examination showed that $Fe_3\ O_4$-NPs had a strong absorbance at about 300 nm, demonstrating the synthesized particles' photosensitivity in the UV range. The iron nanoparticles were found to have unique functional groups by FT-IR analysis, and the presence of a sulphated polysaccharide group was indicated by the wave number at 778 cm − 1. The shifted absorption b and observed at618cm^{-1}, for Ulva lactuca extract band at778 cm^{-1} corresponds to F–O stretches of Fe2O3, confirming the formation of nanoparticles. The b and at 2851 cm^{-1} may be duet o C ≡ N stretching frogman reacted impurities or due to CO2 in the sample compartment. FT-IR research revealed that the seaweed extract polysaccharide component is essential for reducing ferric chloride and stabilizing the particle. The band with OH groups indicates water-soluble polyphones compound that have capped these face of the prepared Fe NPs. Van der Waals forces of interaction between the nitrogen and carbon atom of the bioactive compounds have previously been hypothesized [19]. This sulphate group has been implicated in $FeCl_3$ reduction and stabilization [20]. Therefore, the infrared data showed that metabolites found in the marine alga's aqueous extract encapsulated the iron nanoparticles. The production of iron-NPs was revealed by a large absorption peak in the UV-visible spectrum of the green synthetic iron nanoparticles.

To ascertain the exterior morphology and verify the nano-structure, SEM examination was employed. The sample was 30–40 nm in size, according to the SEM pictures. Seaweed sulfate polysaccharides plays a pivital role in controlling oxidation and aggregation over time, iron nanoparticles exhibit high reactivity and a greater potential for stabilization both before and after synthesis. Despite the fact that several stabilizing agents made chemically are suggested and used earlier. The seaweed's inherent polysaccharides function as a stabilizing factor to regulate the size and amount of nasnoparticle production. The previously reported green synthesized iron nanoparticles were found in the form of nanospheres [21]. Because iron nanoparticles are magnetic, they can come into contact with one another [22].

The crystal structure of the Ironoxide nanoparticles was analyzed by X-ray diffract meter. The synthesized iron oxide nanoparticles showed distinct diffraction peaksas shown in the figure. The experimental XRD pattern agrees with the XRD pattern ofironoxidenanoparticles other literature .The strong's peaks at 30 to 70thetawhichconfirmsthatpure Iron oxide nanoparticles are extracted from the *Ulva lactuca*.

The dispersion, sticking, and shape of the synthesized nanoparticles were observed under scanning electron microscopy at different magnifications. The manufactured nanoparticles were made in the size range of 21–82 nm. Because of the altered reactant content of iron chloride and seaweed extract, the surface morphology of the nanoparticles revealed unevenly shaped particles. But the majority of the synthetic nanoparticles were discovered to have a rod- and circular-shaped structure. Strong dispersion was revealed by the SEM investigation, which improved the properties of the nanoparticles.

The seaweed derived bio synthesized iron oxide nanoparticles were used in insecticidal bioassays, and they proved to be very efficient against *Tuta absoluta* larvae in their second instar; the LC_{50} and LC_{90} values were 97.25 ppm and 110.8 ppm, respectively. After 48h of treatment the larvae showed 50% mortality was observed. The control and the values were compared, accordingly [23]. The efficaciousness of iron oxide nanoparticles against insects was recorded. Previous researchers highlighted the insecticidal toxicity of each specific metal and metal oxide nanoparticle will depend on their releasing capacity of metal ions, stability, size of the particle and charges etc. Similarly [24] observed zinc oxide will have potential to fight against *Spodoptera frugiperda* a pest affect the economic crop.

Based on the stability analysis iron oxide nanoparticles was stable in exposure medium for 96 hours. Hence the study was conducted as static test. Acute toxicity of nanoparticles was tested in fresh water fish. The study was conducted as per the standard protocol. The solubility and stability of the iron oxide nanoparticles were analyzed by 96 hour exposure. The result of range finding study in five different concentrations of 1, 10, 25, 50 and 100 mg/l along with control and vehicle control (ethanol). Seven group of fresh water fish (Brachdanio rerio) each group containing 10 fishes was exposed for 96 hours at five selected test concentration. The fishes were acclimatized prior to exposure. The fishes were observed daily during the acclimation and were found to be normal. No mortality was observed in the control group throughout the experimental period. At the end of 3, 6, 24, 48, 72 and 96 hour no mortality and clinical signs of toxicity was observed in fish exposed to control, vehicle control and treatment. At the end of study all the exposed live fishes were euthanized and safely disposed.

Based on the result of range finding limit study was conducted with 100 mg/l concentration. A concurrent control and vehical control group was also maintained along with the selenium concentration. No mortality was observed in control group and fish exposed to 100 mg/l concentration throughout the study period. There is no change in the weight, height pH, temperature, dissolved oxygen and electrical conductivity in all the treatments and in the control and vehicle control. All the fishes of control, vehicle control and treated group appeared normal and throughout the exposure period. At the end of the study all the exposed live fishes were euthanized and safely discarded. So The LC_{50} value of nanoparticles was > 100mg/l for 96h exposure based on cumulative mortality of fresh water fish (*Brachydanio rerio*). The weight and length of the fishes used in the study was within the limit. The Physico-chemical parameter of exposure medium viz pH, dissolved oxygen, temperature and conductivity was analyzed from start to end with 24 hour interval and hardness was analyzed at start and end of the experiment for control and treatment group in the limit study.

Conclusion

The current research concludes that green mediated synthesis of iron oxide nanoparticles from seaweed have the ability to destroy *Tuta absoluta* larvae in an efficient manner. The findings indicate that bioinsecticides, a viable substitute for traditional synthetic pesticides in pest control management, are a possibility.. Moreover, the current study's findings may lead to a decrease in the use of synthetic pesticides, which would raise the chance that botanical pesticides might naturally manage a variety of medically significant pests. Future research aims to create biogenic nanoemulsion to shield the environment from hazardous chemical pollutants. Additionally, it lessens the possibility of negative ecological impacts and prevents from developing a resistance to pesticides.

Declarations

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Consent to Participate:

Not Applicable.

Consent to Publish:

The manuscript has been approved by all authors, and they consent to its submission to Applied Biochemistry and Biotechnology.

Ethics Approval:

The ethics committees of the university and the department have cleared this study because it does not include any humans or animals.

Contributions:

The research was conceptualized by all of the authors. The tests were overseen by R.S & N.M, who also drafted the original draught of the manuscript. R.S was in charge of keeping daily track of the project's development. The experimental work was being assisted by EE.G.L, and C.S. assisted in the execution of the experiment as well as the interpretation of the findings. Throughout this investigation, P.V. has assisted with the instrumentation facilities and has offered helpful comments on a number of occasions. All authors read and approved the manuscript.

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Conflict of interest:

The authors declare that they have no competing interests.

Availability of Data and Material:

On reasonable request, the corresponding author will provide the datasets created and/or analysed during the current work.

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Figures

Figure 1

Seaweed *Ulva lactuca* used for the iron oxide nanoparticles

Figure 2

Iron oxide (Fe_3O_4) nanoparticle synthesis (Brown colour)

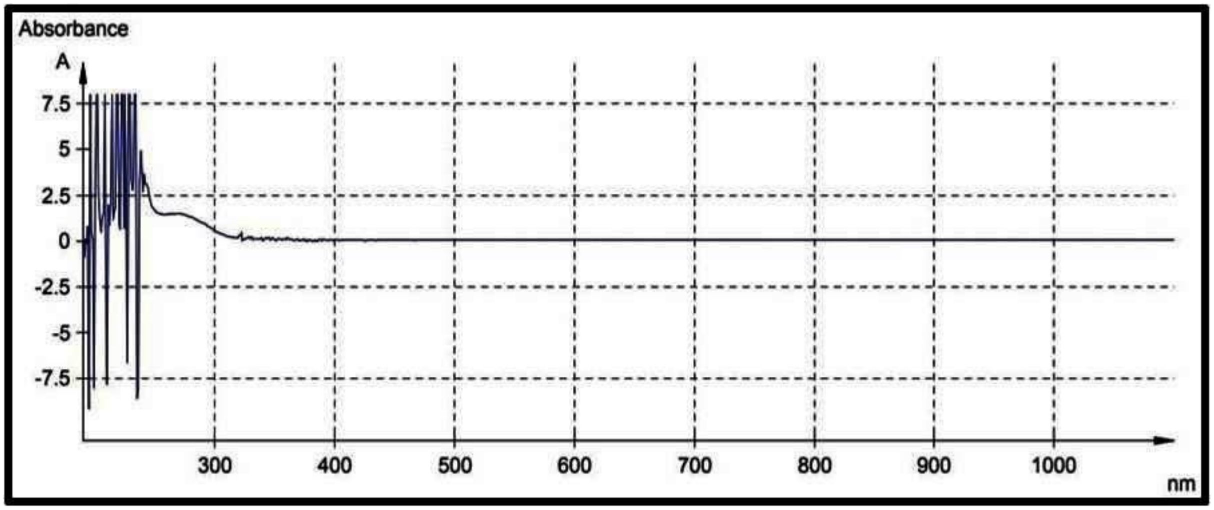

Figure 3

UV- Vis spectrophotometer showing the spectra of the Iron oxide nanoparticles

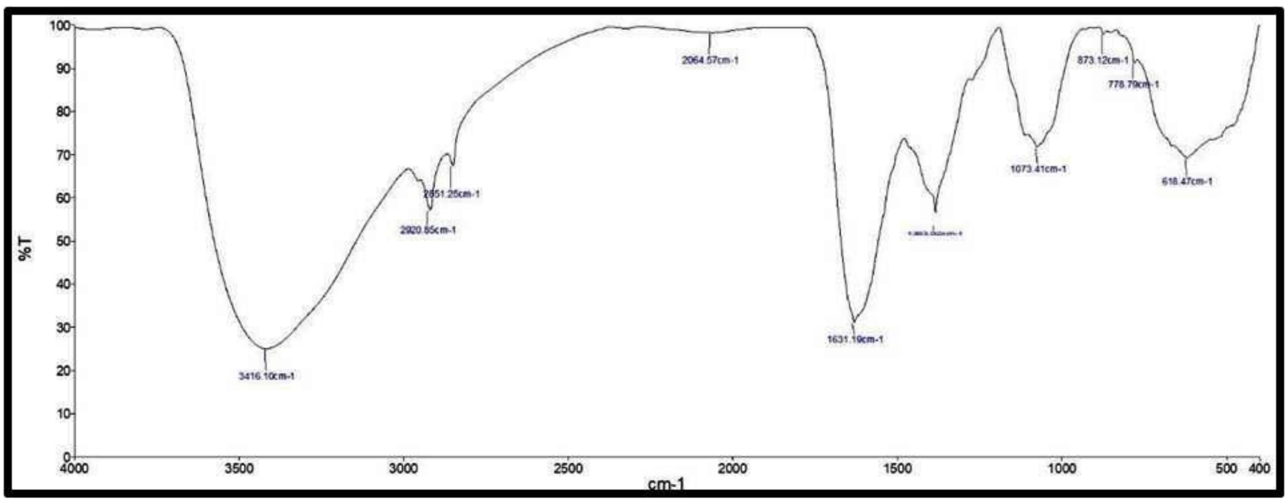

Figure 4

FT-IR with the peaks of Ulva lactuca &Fe_3O_4 nanoparticles

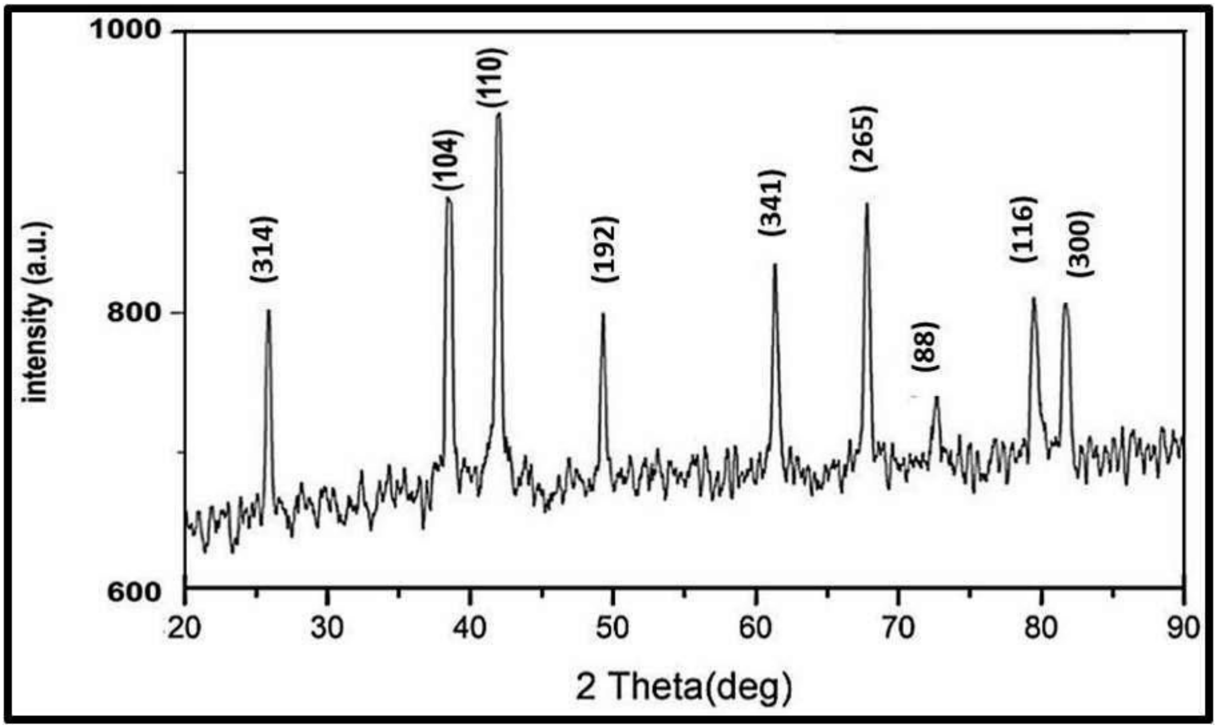

Figure 5

X-Ray diffract meter showing the peaks of pure iron oxide np's extracted from seaweed (*Ulva lactuca*)

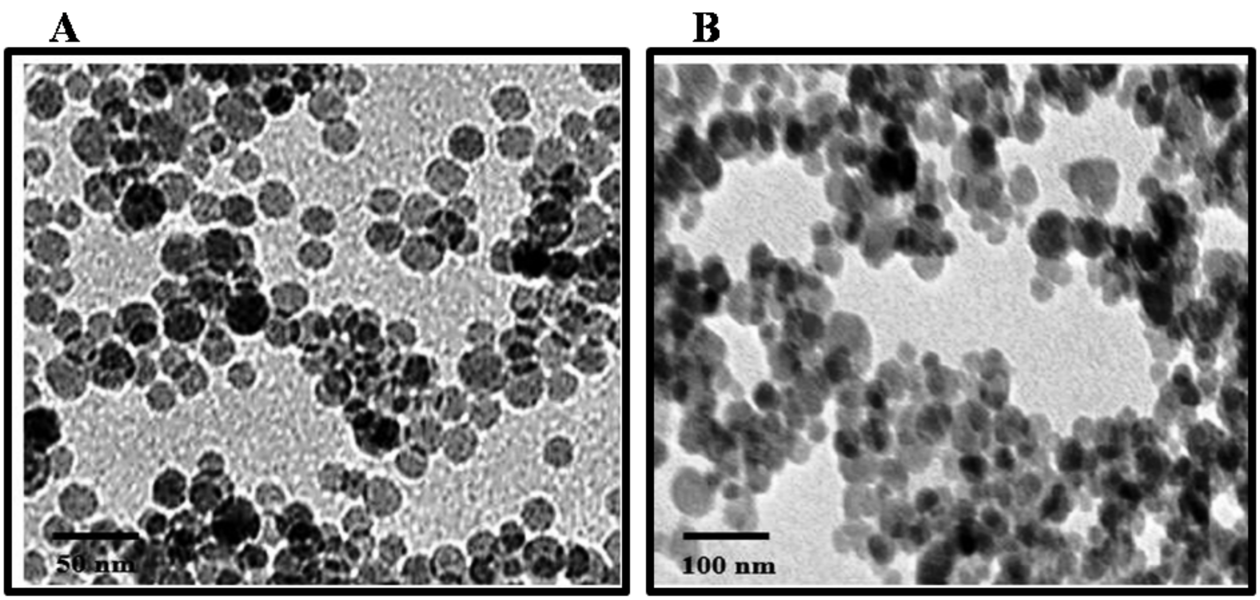

Figure 6

Image of iron oxide nanoparticles from TEM (Transmission electron microscopy)

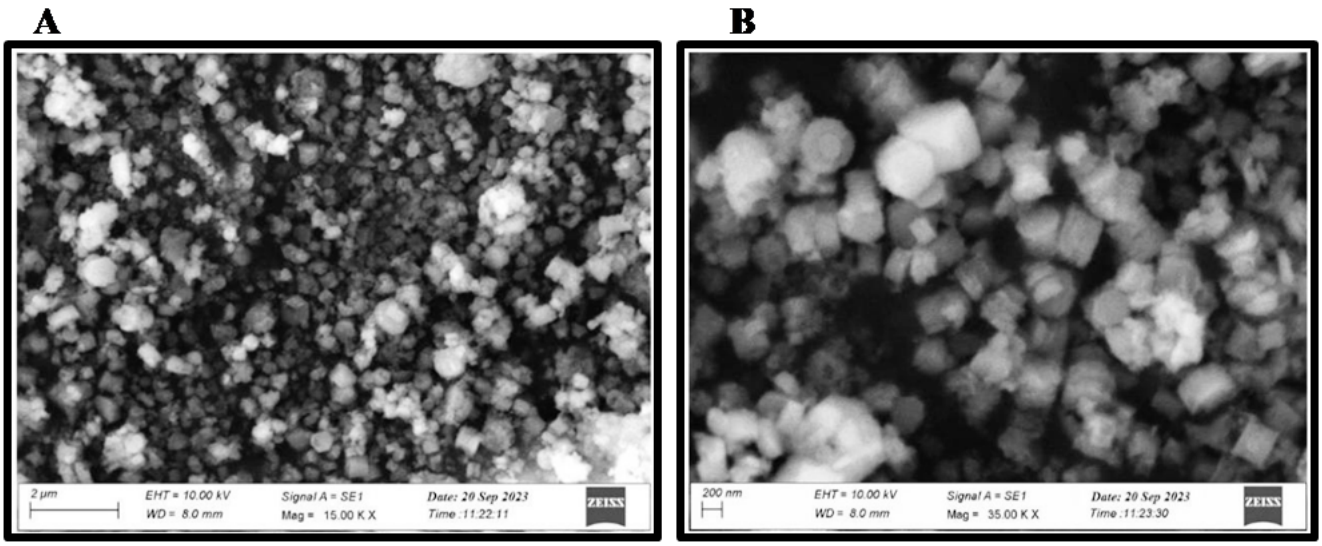

Figure 7

Scanning electron microscopy images with iron oxide nanoparticles

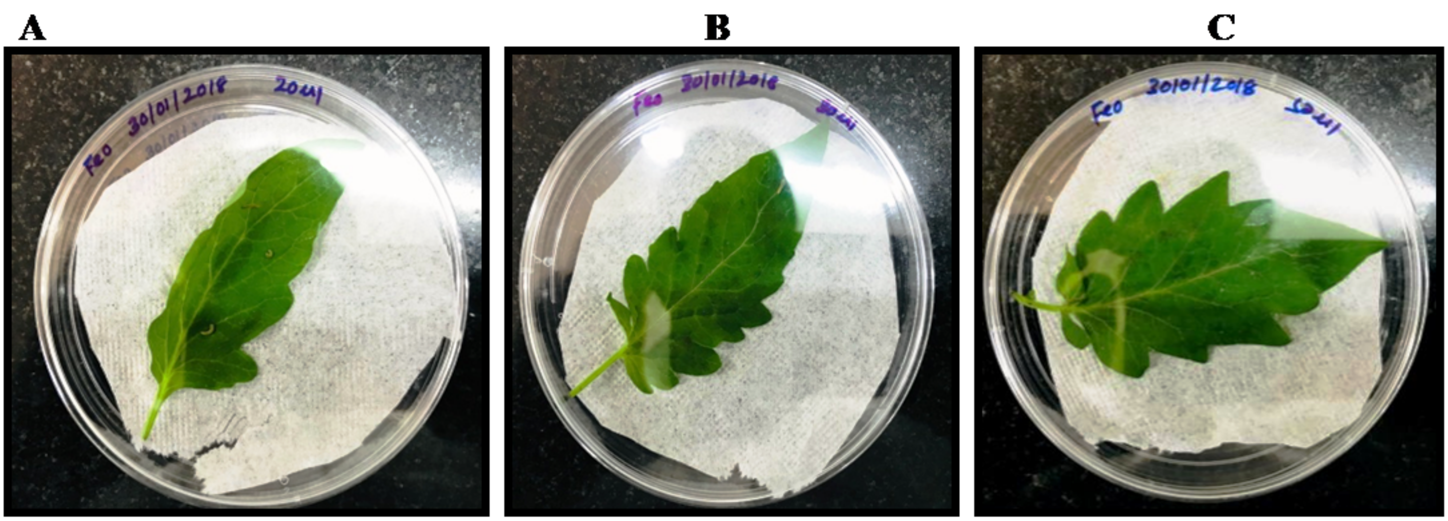

Figure 8

Synthesized iron oxide nanoparticles against the insecticidal activity