Applicant: NAVSARI AGRICULTURAL UNIVERSITY



Figure 1

Dated this 04th Day of July 2022

Signature:

Name: Bhavik Patel
Applicant's Agent: IN/PA-1379
INFINVENT IP



Figure 2

Dated this 04th Day of July 2022

Signature:

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INFINVENT IP

Applicant: NAVSARI AGRICULTURAL UNIVERSITY

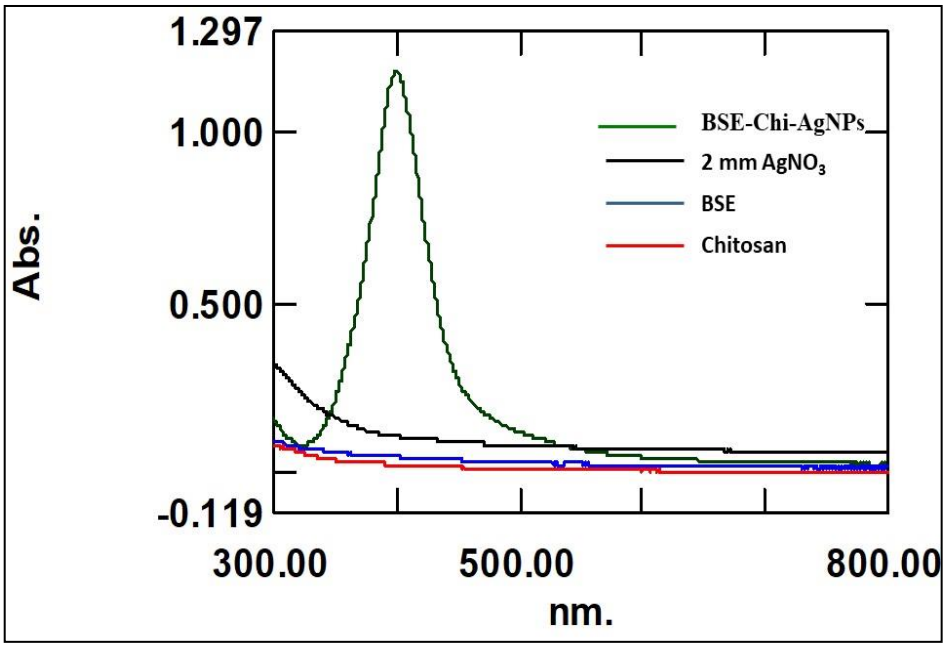


Figure 3

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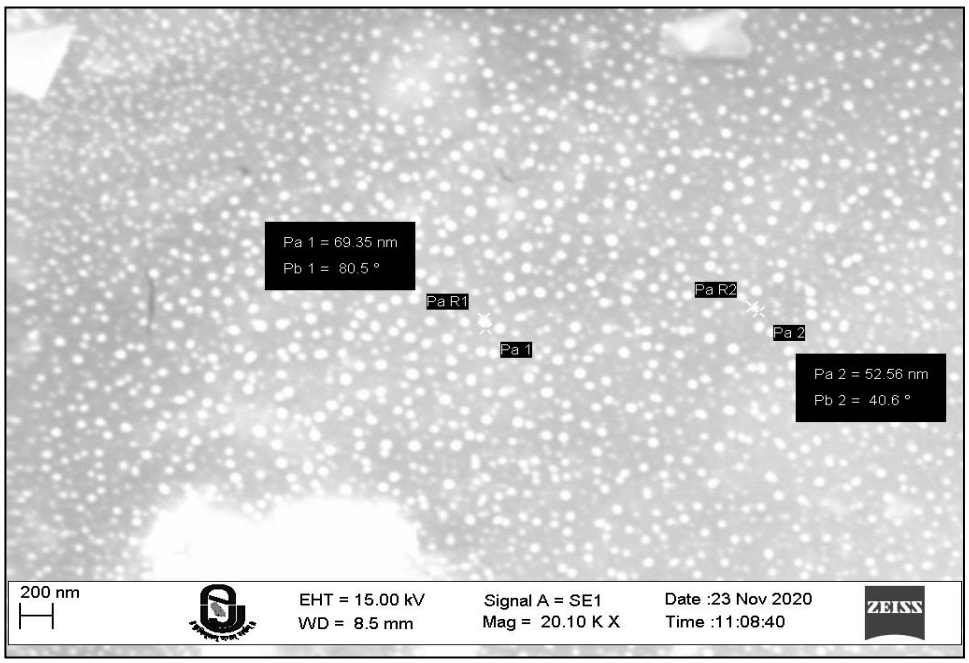


Figure 4

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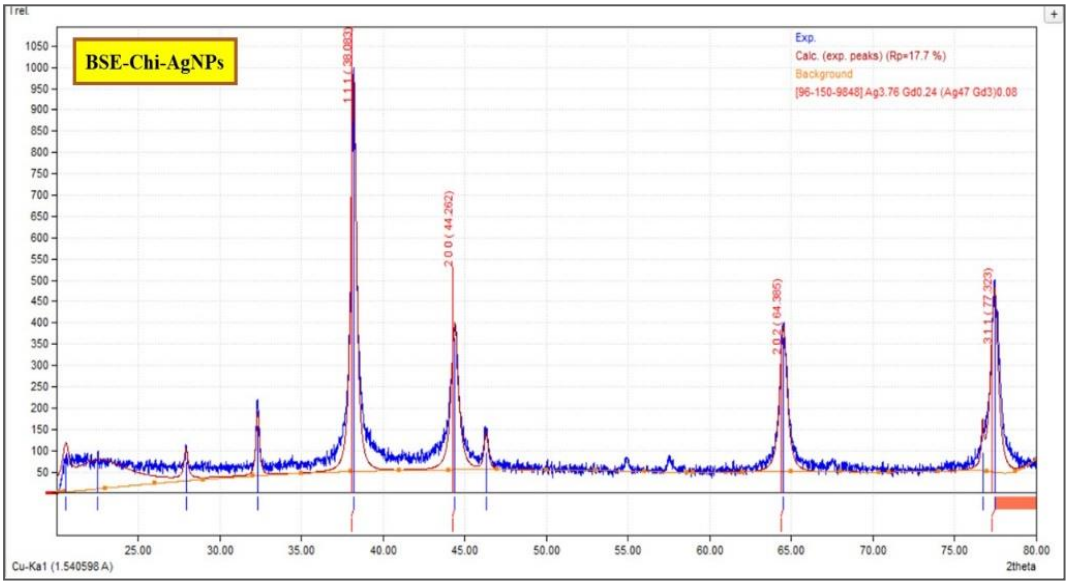


Figure 5

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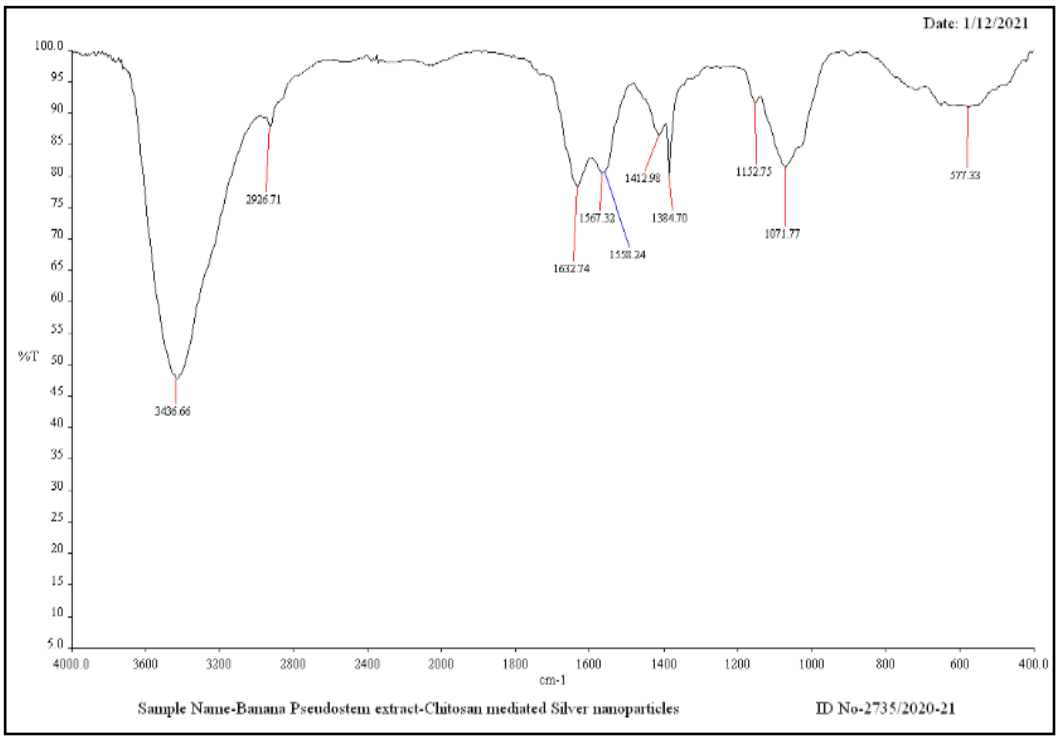


Figure 6

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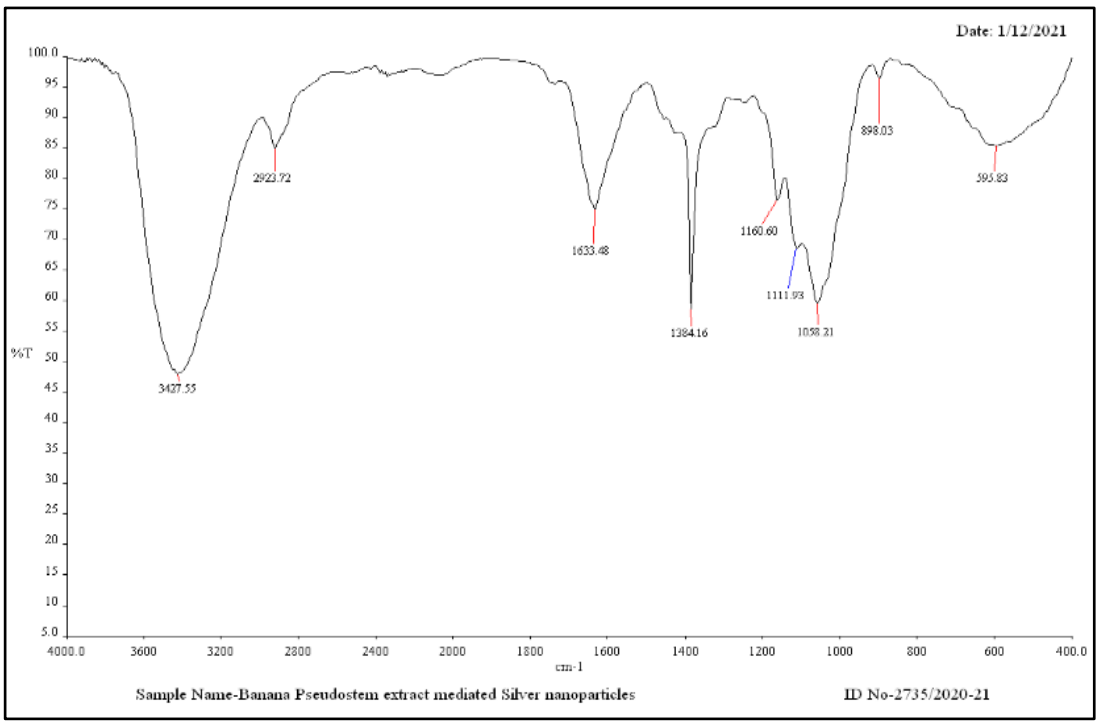


Figure 7

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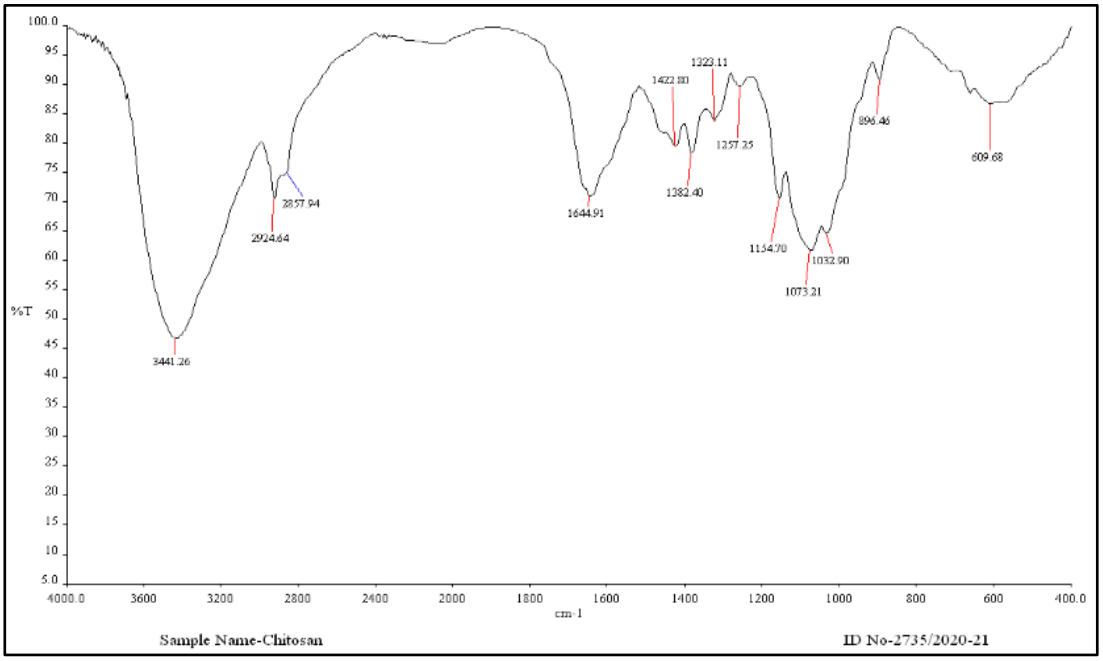


Figure 8

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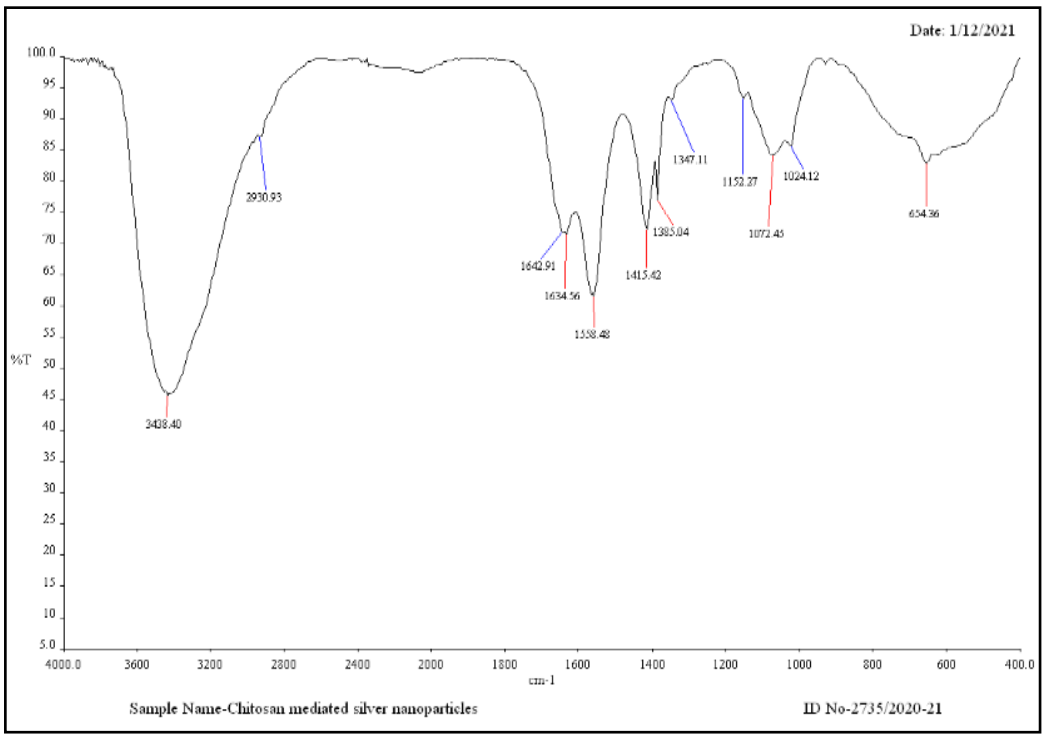


Figure 9

Dated this 04th Day of July 2022

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FORM 2
THE PATENTS ACT, 1970
(39 OF 1970)
&
The Patents Rules, 2003
COMPLETESPECIFICATION
(See section 10; rule 13)

Title of the invention – METHOD OF PREPARTION OF HYBRID SILVER NANOPARTICLES WITH BANANA PSEUDOSTEM EXTRACT AND CHITOSAN

2. Applicant(s)

(a) **NAME**: Navsari Agricultural University
(b) **NATIONALITY**: Indian
(c) **ADDRESS**: Navsari Agricultural University, Eru char rasta, Navsari 396450, Gujarat, India.

3. **PREAMBLE TO THE DESCRIPTION**:

The following specification particularly describes the invention and the manner in which it is to be performed.

FIELD OF INVENTION

The present invention relates to the field of nanotechnology to provide a method of prepartion of nanoparticles. The present invention particularly relates to a method of prepartion of hybrid silver nanoparticles with banana pseudostem extract and chitosan. The present invention further relates to hybrid silver nanoparticles provide better fungicidal activity at low concentration.

BACKGROUND OF THE INVENTION

Nanotechnology is gaining popularity as researchers create novel nanomaterials for applications in chemistry, biology, electronics, physics, and other domains. Nanotechnology has the potential to improve the agriculture and food industries by providing unique Nanotools for disease management, nutrient absorption capacity enhancement, and other applications. Specific applications like Nano fertilizers and Nano pesticides to trail products and nutrient levels to increase productivity without decontaminating soils, waters, and protection against several insect pests and microbial diseases are among the major interests of using nanotechnology in agriculture.

Biological systems of microbial and plant origin have developed eco-friendly techniques of nanoparticle (NP) synthesis throughout the last decade. Nanostructures of consistent size and shape are frequently produced using bio-inspired approaches. Plant-mediated biological synthesis of metal nanoparticles, as opposed to microbes, offers a promising eco-friendly alternative for realizing large quantities of nanomaterials because it does not involve harmful chemicals, eliminates the time-consuming process of maintaining cell cultures, is simple to recover products, and has a dual nature that allows it to cap and reduce at the same time.

Agriculture is always the most important and stable sector because it produces and provides raw materials for food and feed industries but the phytopathogens that cause plant illnesses are well-known for severe damage to agroforestry. Therefore, we should have to take one bold step for preventing the damage to plants caused by

the fungi. The most common plant pathogens are fungi, and they cause a wide spectrum of significant plant diseases. Fungi are responsible for the majority of vegetable illnesses. Plants are harmed by them because they kill cells and/or put them under stress. Chemical pesticides were the first-ever unique choice under the traditional concept. However, in light of the expanding environmental and medicine resistance issues, new eco-friendly fungistats are urgently needed. The rapid advancement of nanotechnology has given rise to the prospect of resolving such a problem. Silver's antibacterial action was greatly improved at the nanoscale, and it was found to be more broadspectrum for AgNPs against bacteria, fungi, viruses, and pathogenic cells. Chitosan is a linear polymer of b-(1-4)-linked Dglucosamine and N-acetyl-Dglucosamine derived from chitin, and it demonstrated potential properties with nanoparticles. It showed low toxicity, biocompatibility, biodegradability, and immunogenicity.

Nanomaterials with a smaller size procure a larger surface area and exhibits more activity. Nanotechnology is knocking on the doors of perception with this strategy. Nano fertilizers have become widely available in the market in recent years, but large chemical corporations have been unable to form agricultural fertilizers. Nano fertilizers may contain nano zinc, silica, iron, and titanium dioxide, as well as ZnCdSe/ZnS core-shell QDs (Quantum Dots), InP/ZnS core-shell QDs, Mn/ZnSe QDs, gold nanorods, core-shell QDs, and core-shell QDs, etc.

Nano fertilizers are being used in agriculture with the goal of reducing mineral losses in fertilizing, increasing yield during mineral management, and assisting plant development. Nano fertilizers are being utilized to boost plant nutrition and increase nutrient efficiency. They have several unique features that are now being investigated for new agricultural prospects due to their exceedingly small size. Metal nanoparticles have been synthesized using a variety of chemical techniques. The majority of these methods cause serious environmental and ecological hazards. The development of eco-friendly technologies in material synthesis is of considerable importance to expand their biological applications. Nowadays, a variety of inorganic nanoparticles with well-defined chemical composition, size, and morphology have been synthesized by using different plant extracts, and their applications in many cutting-edge technological areas have been explored.

Various mechanical, chemical and biological methods have been employed to synthesize nanomaterials. Examples of mechanical processes for producing nanoparticles include mechanical attrition (e.g., ball milling), crushing of sponge iron powder, and thermal quenching. Examples of chemical processes for producing nanoparticles include precipitation techniques, sol-gel processes, and inverse-micelle methods. Chemical synthesis methods (such as sol-gel process, micelle, chemical precipitation, hydrothermal method, pyrolysis, chemical vapor deposition, etc.), however, lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. Some reactions require high temperature and/or high pressure for initiating the reaction, while other reactions require inert atmosphere protection, and/or require noxious H_2S gas, toxic template and stabilizers. Thus, chemical methods used for synthesis and stabilization of nanoparticles are costly, toxic and potentially hazardous besides difficult separation procedure, high pressure and energy requirement.

Synthesis of hybrid silver nanoparticles by using green synthesis process involves the use of plant or plant parts for the bioreduction of metal ions into their elemental form. The process of green synthesis is more efficient, simpler, and economical, and can easily be scaled up to perform larger operations. Synthesis of hybrid silver nanoparticles aims at implementing sustainable processes. In recent years, green processes using mild reaction conditions and nontoxic precursors have been emphasized in the development of nanotechnology for promoting environmental sustainability.

One of the prior arts WO2012161603A2 discloses a method of hybrid material containing silver nanoparticles this hybrid material is composed of calcium carbonate with polyelectrolyte and silver nanoparticles. Wherein the cationic and anionic surfactants are used for surface modification. This invention also covers the antifungal and antibacterial application of the hybrid material. But in this invention hybrid material synthesis by ultrasound-assisted extraction method by using ultrasonic waves.

Another prior art CN105379758A discloses a preparation method for the hybrid antimicrobial agent of silver-loaded illite-smectite clay/chitosan quaternary ammonium salt. Where in illite-smectite clay is use as a carrier and chitosan is use as a quaternary ammonium salt and utilizes the interaction between the chitosan and illite-smectite clay.

The prior arts mentioned above discloses chemical and physical methods for hybrid silver nanoparticles synthesis. None of the prior art discloses method of prepartion of hybrid silver nanoparticles with banana pseudostem extract for fungicidal activity at low concentration. The prior arts also do not disclose the method of prepartion of hybrid silver nanoparticles by using the green synthesis method.

The present invention describes a novel hybrid silver nanoparticles and the methodology for the prepartion of hybrid silver nanoparticles.

There is therefore a need for a Nano fertilizer in the agricultural input sector which will be an advantageous and a noteworthy invention.

SUMMARY OF THE INVENTION

The main objective of the present invention is to provide a method of prepartion of hybrid silver nanoparticles with banana pseudostem extract (BSE) and chitosan by using green synthesis method.

The another objective of the present invention is to provide a hybrid silver nanoparticles of Banana pseudostem extract-Chitosan-Silver (BSE-Chi-AgNPs).

The other objective of the present invention is to provide green process for the preparation of BSE-Chi-AgNPs wherein first prepare a) BSE-AgNPs by mixing banana pseudostem extract dropwise in silver nitrate. b)

The other objective of the present invention is to provide a green process for the preparation of hybrid silver nanoparticles with banana pseudostem extract as a active ingredients.

The other objective of the present invention is to provide a BSE-Chi-AgNPs by using the silver as a metal ions.

Another objective of the present invention is to provide a BSE-Chi-AgNPs by using the chitosan as a carrier.

Another objective of the present invention is to provide a hybrid silver nanoparticles comprises BSE- AgNPs with chitosan in ratio 1:1 (v/v).

Another objective of the present invention provides a BSE-Chi-AgNPs Nano-particles for fungicidal activity and preventing the plant from damage by the fungi at low concentration.

In one aspect of the present invention BSE-Chi-AgNPs shows the fungicidal activity against *S. rolfsii* and *P. Aphanidermatum* at low concentration.

In another aspect of the present invention is to develop a process for the preparation of hybrid BSE-Chi-AgNPs nanoparticles having a particle size in the range of 50 to 80 nm.

In another aspect, the present invention provides a crystalline form of BSE-Chi-AgNPs nanoparticles characterized according to the powder X-ray diffraction data.

Yet another objective of the present invention is to provide a novel environmental friendly, time- and energy-saving biological process for the preparation of BSE-Chi-AgNPs nanoparticles with a minimized agglomeration of the primary particles. As the aggregation/agglomeration of nanoparticles reduces the potential enhancement of mechanical properties in nanocomposites, due to the restriction of interfacial area.

These and other features of the present disclosure will become readily apparent upon further review of the following specification and drawings.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Illustrates the In-vitro efficacy result of the BSE-Chi-AgNPs against *S. rolfsii*.

Figure 2: Illustrates the In-vitro efficacy result of the BSE-Chi-AgNPs against *P. aphanidermatum*.

Figure 3: Illustrates the UV-visible spectra image of BSE-Chi-AgNPs.

Figure 4: Illustrates the Scanning Electron Microscopes (SEM) image of the BSE-Chi-AgNPs.

Figure 5: Illustrates the X-ray Powder Diffraction (XRD) image of the BSE-Chi-AgNPs.

Figure 6: Illustrates the Fourier transforms infrared spectra (FTIR) image of the BSE-Chi-AgNPs.

Figure 7: Illustrates the Fourier transforms infrared spectra (FTIR) image of the BSE-AgNPs.

Figure 8: Illustrates the Fourier transforms infrared spectra (FTIR) image of the chitosan.

Figure 9: Illustrates the Fourier transforms infrared spectra (FTIR) image of the Chi-AgNPs.

DETAILED DESCRIPTION OF THE INVENTION

A detailed description of the preferred embodiments of the present invention is disclosed herein; however, it is to be understood that the disclosed embodiments are merely exemplary of the invention, which may be embodied in various forms. Therefore, specific functional and structural details disclosed herein are not to be interpreted as limiting, but merely as a basis for the claims and as a representative basis for teaching one skilled in the art to employ the present invention in virtually any appropriately detailed structure.

"BSE-Chi-AgNPs" means a small particle comprising Banana pseudostem extract, chitosan and Silver as the major component."Nanoparticles" have a diameter ranging from 1 nm to several hundred nm. BSE-Chi-AgNPs hybrid nanoparticles of many of the embodiments described herein will have a diameter of about 50 nm to 80 nm.

BSE, as the term used herein, is a banana pseudostem extract for the extraction selected fresh and healthy pseudostem of banana sword sucker.

Mud crab (*Scylla serrata* Forskal)shells as a term used herein are seafood used for the extraction of chitin and chitosan.

Centrifugation, as the term used herein, is a technique used for the separation of particles from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

The supernatant, as the term used herein, is a liquid lying above a solid residue after centrifugation.

PDA, as the term used herein, is a potato dextrose agar used for the inoculation fungi.

Nanotechnology monitors a leading agricultural controlling process, especially by its miniature dimension. Additionally, many potential benefits such as enhancement of food quality and safety, reduction of agricultural inputs, enrichment of absorbing nanoscale nutrients from the soil, etc.

Banana

A banana is an elongated, edible fruit botanically a berry produced by several kinds of large herbaceous flowering plants in the genus Musa. Banana is the common name for herbaceous plants of the genus Musa. Binomial nomenclature of banana

plant is Musa acuminata. Locally known as 'unere', bananas come in a variety of sizes and colours when ripe, including yellow, purple, and red. The banana plant is the largest herbaceous flowering plant. Bananas and plantains constitute the fourth most important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes.

Botanical Classification	
Kingdom	Plantae
Order	Zingiberales
Family	Musaceae
Genus	Musa

Chitin and Chitosan

Chitin is the most common biopolymer found in nature. Chitin and its derivatives have great economic value because of their biological activities and their agriculutre and pharmaceutical applications. Chitin, a homopolymer of N-acetyl-D-glucosamine (Glc- NAc) residues linked by β-1-4 bonds and it is the most prevalent renewable natural resources. Chitin is found as organised crystalline microfibrils creating structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also found as a key component in the shell of shrimps, crabs, squid cartilage, and the outer cover of insects.

Chitosan is chemically defined as a copolymer of α-(1,4) glucosamine $(C_6H_{11}O_4N)_n$, with a varying content of N-acetyl groups. It is produced by deacetylation of Chitin. Chitosan is obtained from the mud crab (*Scylla serrata* Forskal) shells.

Botanical Classification	
Kingdom	Animalia
Order	Decapoda
Class	Malacostraca
Family	Protunidae
Genus	Scylla
Species	S. serrata

Nano silver is the most recognized and employed nanoparticle in biotechnology. It is known to have potent inhibitory and bactericidal properties, as well as a wide range of antibacterial properties. In comparison to bulk silver, silver nanoparticles with a large surface area and a high fraction of surface atoms have a strong antibacterial effect. Furthermore, AgNPs have long been known for their antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory activities. AgNPs have been investigated as a possible contender for increasing crop productivity by improving seed germination and plant growth. The concentration of Ag nanoparticles (AgNPs) has a positive or negative impact on plant growth response.

The invention is best practiced with the following embodiments:

The main embodiment of the present invention is to provide a method of prepartion of hybrid silver nanoparticles with banana pseudostem extract (BSE) and chitosan by using green synthesis method.

The another embodiment of the present invention is to provide hybrid silver nanoparticles for fungicidal activity comprising extract of banana pseudostem, chitosan and silver.

In yet another embodiment, hybrid silver nanoparticles wherein the banana extract obtained from the fresh and healthy pseudostem of banana sword sucker.

In yet another embodiment, hybrid silver nanoparticles wherein the chitin and chitosan extract obtained from the mud crab (*Scylla serrata* Forskal) shells.

Another embodiment of the present invention provides a green process for the preparation of Banana pseudostem- Chitosan- Silver nanoparticles (BSE-Chi-AgNPs) comprising steps of:

1. The freshly prepared 2 ml BSE was mixed dropwise in 98 ml of 2.0 mM $AgNO_3$ conc. of 11 pH with constant stirring on the magnetic stirrer (Make: Neuation Technologies Pvt. Ltd., Gandhinagar, Gujarat, India).

2. The mixture was incubated at 80ºC for 20 min in a water bath (Make: Labman Scientific Instruments Pvt. Ltd., Chennai, India).

3. Then 0.2% solution of chitosan (0.2 g chitosan in 1% acetic acid solution) 100 ml was added in a 1:1 (v/v) ratio and the mixture was heated in the autoclave (Make: Equitron Medica Pvt. Ltd., Mumbai, Maharashtra) at 121.6ºC and 15 psi pressure for 45 min.

4. Synthesization of nanoparticles was indicated with the colour change. The colour of BSE-Chi-AgNPs showed a light yellow to dark brown change.

5. The reduction of pure Ag+ ions was monitored by measuring the UV-vis spectrum (Make: SHIMADZU UV-2600 visible absorption spectrophotometer) of the reaction.

6. Separation of nanoparticles:
   Separation of synthesized nanoparticles from the solution was done by centrifugation (Chilling centrifuge - CR21GIII, Make- Hitachi Koki Co. LTD, Japan) at 18,000 rpm for 20 min and the supernatant was discarded. The pellet was collected and washed three times with Milli-Q water and centrifuged to ensure better separation of free entities from the AgNPs. Finally, the purified suspension of the AgNPs was dried in a hot air oven at 200ºC for 3 h.

Another embodiment of the present invention provides hybrid silver nanoparticles of BSE-Chi-AgNPs having the fungicidal activity and preventing the plant from damage by the fungi at low concentration.

The scope of the present invention is best describe by the following examples:

Example 1: Preparation of Silver Nitrate Solution

The $AgNO_3$ stock solution of 10 mM was prepared by dissolving 340 mg of $AgNO_3$ (Electronic balance: Settra Scale Industries, Mumbai, India) in 200 ml of deionized water. The working solutions of 2.0 mM $AgNO_3$ were prepared freshly from the stock solution by dilution with deionized water and the 11 pH of the $AgNO_3$ was set by using a freshly prepared 0.1 N NaOH solution using a pH meter (pH Meter: Toshneuation Technologies Pvt. Ltd, Ajmer, Rajasthan, India).

Example 2: Preparation of Aqueous Banana Pseudostem Extract (BSE)

The fresh and healthy pseudostem of banana sword sucker was selected for the preparation of aqueous banana pseudostem extract. The BSE was prepared by boiling 100 g of freshly cut pseudostem pieces in 100 ml of double-distilled water (1:1 w/v) at 60ºC for 30 min. Then BSE was filtered through a 0.2 μm nylon mesh followed by Whatman No.1 filter paper using the Millipore vacuum filtration unit. The filtered extract was subjected to centrifugation in the centrifuge (Centrifuge 3-30KS, make: Sigma Laborazentrifugen, Germany) at 10,000 rpm for 5 min. After centrifugation, the supernatant was collected and stored at 4ºC as a stock solution for the green synthesization of BSE-Chi-AgNPs.

Example 3: Extraction of Chitin and Chitosan

The mud crab (*Scylla serrata* Forskal) shells were autoclaved at 121.6ºC for 5 min and air-dried under sunlight. Dried shells were then ground in small particles and sieved by passing through a 0.3-0.5 mm sieve. Then shell particles were subjected to deproteinization, demineralization, decolourization and deacetylation for preparation of chitosan powder as given below:

Deproteinization:

The mud crab shell waste from the carotenoid extraction process was treated with 4.0% of NaOH solution with a ratio of ground shell to the solution of 1:20 (w/v) with constant stirring for 2 h at 90ºC to remove the protein. The shell particles were then filtered and the filtrates were washed with tap water for 30 min until the pH becomes neutral. The deproteinized shells were dried in the oven at 60ºC for 24 h.

Demineralization:

The deproteinized mud crab shells were demineralized with 2.5% (w/v) of hydrochloric acid (HCl) at room temperature for 6 h to remove the mineral content with a ratio of ground shell to the solution of 1:20 (w/v). Then filtered and washed for 30 min with tap water until the pH becomes neutral. The demineralized shells were then dried in the oven at 60ºC for 24 h.

Decolouration and Dewatering:

Decoloration was achieved by treating the samples with acetone for 10 min and drying for 2 h at room temperature and removed the residues of acetone. The decolourized shell particles were then washed in running tap water, rinsed, filtered, and dried at 60ºC for 24 h in the oven to obtain crab chitin.

Deacetylation of Chitin:

The chitin obtained was treated with 50% (w/v) aqueous NaOH with a ratio of chitin to the solution of 1:15 (w/v) at 105ºC for 2 h. Then, the chitin was filtered and washed with deionized water until the pH becomes neutral to obtain the chitosan. The chitosan obtained was then dried at 60ºC for 24 h in the oven. Finally, the product was pulverized and packed as chitosan (chi).

Example 4: Evaluation of Antifungal Activity

In-vitro efficacy of hybrid silver nanoparticles against fungi:

In this study data presented in **Table 1.** and **Fig. 1 & 2** for optimization of BSE-Chi-AgNPs dose were tested with different conc. 10, 50, 100, 150, 200, 250, and 300 ppm against *S. rolfsii* and *P. aphanidermatum.*

The per cent growth of inhibition against *S. rolfsii* were recorded 5.59%, 21.47%, 75.00% respectively at 10, 50, 100 ppm conc. while 100 per cent inhibition was observed above 150 ppm conc. Similarly, the per cent growth of inhibition showed at different conc. of BSE-Chi-AgNPs against *P. aphanidermatum* were 0%, 22.94%, 49.41%, 90% respectively compared with the control while 100% inhibition was observed above 200 ppm conc. Based on per cent growth of inhibition BSE-Chi-AgNPs showed 100 per cent growth of inhibition against S. rolfsii above 150 ppm conc. and against *P. aphanidermatum* above 200 ppm conc.

Table 1: *In-vitro* efficacy of BSE-Chi-AgNPs against fungi

Contractions of BSE-Chi-AgNPs (ppm)	% growth of inhibition of fungi on PDA	
	S. rolfsii	***P. aphanidermatum***
10	5.59	0.00
50	21.47	22.94
100	75.00	49.41
150	100.0	90.00
200	100.0	100.0
250	100.0	100.0
300	100.0	100.0
Control	0.00	0.00

Table 2: *In-vitro* efficacy of BSE-AgNPs against fungi

Contractions of BSE-AgNPs (ppm)	% growth of inhibition of fungi on PDA	
	S. rolfsii	***P. aphanidermatum***
10	0.00	0.00
50	1.76	0.00
100	10.88	6.47
150	39.12	36.76
200	69.12	71.76
250	100.0	91.76
300	100.0	100.0
Control	0.00	0.00

Table 3: *In-vitro* efficacy of Chi-AgNPs against fungi

Contractions of Chi-AgNPs (ppm)	% growth of inhibition of fungi on PDA	
	S. rolfsii	***P. aphanidermatum***
10	0.00	0.00
50	8.24	10.30
100	69.41	24.41
150	86.47	76.47
200	100.0	94.71
250	100.0	100.0
300	100.0	100.0

Control	0.00	0.00

Conclusion:

From the above result of In-vitro efficacy study can conclude that the BSE-Chi-AgNPs shows the fungicidal activity against *S. rolfsii* and *P. aphanidermatum* at low concentration as compare with BSE-AgNPs and Chi-AgNPs. The above result indicate that the BSE-Chi-AgNPs shows the maximum growth of inhibition at concentration 10 ppm, 50 ppm, 100 ppm as compare with BSE-AgNPs and Chi-AgNPs.

Example 5: Evaluation by UV–visible spectroscopy:

The formation of silver nanoparticles was monitored with color change and UV (ultraviolet)-Visible spectroscopy. The colour of BSE-Chi-AgNPs showed a light yellow to dark brown, which indicated that the nanoparticles were formed. due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag^{o} via the active molecules present in the BSE and Chitosan. This color is attributed to the excitation of SPR.

As shown in **Figure 3,** a characteristic and well-defined SPR band for BSE-Chi-AgNPs was showed, the maximum absorbance in UV-vis spectra, λmax and AUAWC were 1.179, λmax 400 nm and 63.024, respectively. Control silver nitrate solution, chitosan and BSE neither developed the reddish-brown colour nor did they display the characteristic band, indicating that abiotic reduction of silver nitrate did not occur under the used conditions.

Table 4: UV–visible spectroscopy

COMPOUNDS	UV-VIS SPECTRA	λmax	AUAWC
BSE-Chi-AgNPs	1.179	400 nm	63.024
BSE-AgNps	1.617	404.5 nm	96.756
Chi-AgNPs	0.318	421.5 nm	16.455

Example 6: Evaluation by Scanning Electron Microscopy:

The morphological features of the synthesized hybrid nanoparticles were characterized by a scanning electron microscope (SEM) and the result obtained were as per **Figure 4.** The sample was analyzed under the microscope at a 10.02-20.10 KX magnification scale. It was observed that hybrid nanoparticles (BSE-Chi-AgNPs) were near to spherical.

Table 5: Scanning Electron Microscopy

COMPOUNDS	PARTICLE SIZE
BSE-Chi-AgNPs	50 to 80 nm
BSE-AgNps	50 to 92 nm
Chi-AgNPs	20 to 40 nm

Example 6: Evaluation by X-ray Diffraction Analysis:

X-ray Diffraction Analysis of BSE-Chi-AgNPs:

The crystalline nature of BSE-Chi-AgNPs was confirmed from the XRD pattern. The BSE-Chi-AgNPs showed reflection peaks (see, **Fig. 5**) at 38.08º, 44.26º, 64.38º and 77.32º in the 2Θ range between 20º–80º which could be indexed to (111), (200), (202) and (311) planes of face-centered cubic crystal were obtained and indicated that the crystalline nature of BSE-Chi-AgNPs. The additional peaks were also observed at 27.94º, 32.34º and 46.32º. The additional peaks were due to the organic compound which was present in the extract and responsible for silver ions reductions and stabilization of nanoparticles.

X-ray Diffraction Analysis of BSE-AgNPs:

The XRD patterns of synthesized BSE-AgNPs showed Bragg reflection peaks at 38.064º, 44.24º, 64.35º and 77.28º in the 2Θ range between 20º–80º which could be indexed to the (111), (200), (202) and (311) planes of face-centered cubic (fcc) crystal, respectively. These Bragg peaks might have resulted due to the capping agent (BSE) present in the nanoparticles revealing that the synthesized AgNPs were composed of pure crystalline silver. The peak corresponding to the (111) plane was more intense than the other planes, suggesting that the (111) plane was in the predominant orientation.

X-ray Diffraction Analysis of Chi-AgNPs:

The XRD patterns of synthesized Chi-AgNPs showed peaks at 27.73º, 32.13º, 46.07º, 56.63º, 57.28º, 67.20º, 74.18º and 76.45º in the 2Ɵ range between 20º–80º which could be indexed to the (111), (200), (202), (311), (222), (400), (313) and (402) planes of face-centered cubic crystal, respectively. These Bragg peaks might have resulted due to the capping agent chitosan present in the nanoparticles and additional peaks were also observed at 38.16º, 44.32º and 64.48º.

Example 7: Evaluation by Fourier Transforms Infrared Spectroscopy Analysis:

Fourier Transforms Infrared Spectroscopy Analysis of BSE-Chi-AgNPs:

FTIR spectral analysis demonstrates the characteristics of surface structure and functional group present in the reduction of Ag+ ions and possible interaction between organic compounds and AgNPs.

The hybrid BSE-Chi-AgNPs showed different absorbance peaks (see, **Fig. 6**) at 346.66, 2926.71, 1632.74, 1562.32, 1412.98, 1384.70, 1152.75, 1071.77 and 577.33 with the significant peaks at 3436.66 cm-1 (corresponding to OH groups), 2926.71 cm-1 (CH2 stretching), 1632.74 cm-1 =(-C=O) stretching amide I group, 1071.77 cm-1 (C-O-C group stretching). The peak at 1567.32 cm-1 of BSE-Chi-AgNPs and 1422.80 cm-1 (see, **Fig. 8**) of chitosan correspond to N-H stretch which was characteristic to amide II. The presence of amide II proving the presence of chitosan in BSE-Chi-AgNPs and the strong intense peak located at around 1384.16 cm-1 in BSE-AgNPs (see, **Fig. 7**) and the same peak at 1384.70 cm-1 (see, **Fig. 6**) could be assigned for the carboxyl group (-CH3 bending) were present in BSE-Chi-AgNPs means the presence of BSE in hybrid.

Fourier Transforms Infrared Spectroscopy Analysis of BSE- AgNPs:

The FTIR spectrum of BSE-AgNPs presented in **Fig. 7** showed the presence of distinct peaks in the range of 3427.55, 2923.72, 1633.48, 1384.16, 1160.60, 1111.93, 1058.21, 898.03 and 595.83. The strong intense peak located at around 1384.16 cm-1 (-CH3 bending) could be assigned for the carboxyl group. The medium bans appear at 1058.21 cm-1 (C-O-C stretching) and 1633.48 cm-1 (-C=O stretching) were due to fatty acid and flavanone, respectively. The weak absorption

peaks at 1111.93 cm-1 and 2923.72 cm-1(-CH2 stretching) were attributed to amide bond of proteins and carboxylic acids present in BSE. Also, there was a broad peak located at 3427.55 (-OH stretching) which could be assigned to the O-H stretching vibration indicating the presence of alcohols and phenols. As per the FTIR results, it was understood that some proteins and metabolites such as flavones, polyphenols, etc., present in the BSE might be responsible for the reducing and capping of AgNPs.

Fourier Transforms Infrared Spectroscopy Analysis of Chitosan:

The FTIR spectrum of extracted chitosan from crab shells showed a peak at 3441.26 cm-1 that indicated stretching vibration of hydroxyl (-OH) group, -NH group of amines and hydrogen bonding. 1644.91 cm-1 peak in extracted chitosan indicated the vibrations of (-C=O)) carbonyl group (amide band I). The peak at 1422.80 cm-1 showed the presence of amide II (N-H) bending band of -CONH-. For -CH group in CH2OH peak were observed at 2924.64 cm-1 (-CH2 Stretching), -CH3 group of NHCOCH was shown at 1382.40 cm-1 and oxygen stretching of glycoside linkage was 1073.21 cm-1 (C-O-C stretching bridge of O-H groups). The pyranose ring was found at 896.46 cm-1.

The present method of preparation of Hybrid Silver Nanoparticles with banana psedostem extract and chitosan was found to be:

- eco friendly
- can be used for large scale synthesis
- low cost
- no need to use high pressure, energy, temperature, and toxic chemicals
- good stability
- high antifungal activity at lower concentration.

CLAIMS

We claim,

CLAIM 1. The method of preparation of hybrid silver nanoparticles of banana pseudostem extract-Chitosan-Silver nanoparticles comprises step of:

i. The freshly prepared 2 ml BSE was mixed dropwise in 98 ml of 2.0 mM $AgNO_3$ conc. of 11 pH with constant stirring on the magnetic stirrer;
ii. mixing dropwise 2ml of freshly prepared BSE in 98 ml of 2.0 mM $AgNO_3$ having pH of 11 with constant stirring on magnetic stirrer;
iii. incubate the mixture formed in step ii at 80ºC for 20 min in a water bath;
iv. addition of 0.2% chitosan solution in acetic acid in mixture formed in step ii followed by heating in autoclave at 121.6ºC with pressure of and 15 psi for 45 min.
v. centrifugation of solution at 18,000 rpm for 20 min and the supernatant was discarded;
vi. collection and washing of pellets with Milli-Q water;
vii. drying purified suspension formed in step vi of banana pseudostem extract-chitosan-silver nanoparticles in a hot air oven at 200ºC for 3 h.

CLAIM 2. The method of preparation as claimed in claim 1, wherein chitosan solution and BSE-AgNPs are in ratio of 1:1 (v/v).

CLAIM 3. The method of preparation as claimed in claim 1, wherein particle size of hybrid silver nanoparticles of banana pseudostem extract-Chitosan-Silver nanoparticle is in the range of 50nm to 80 nm.

CLAIM 4. The method of preparation as claimed in claim 1, wherein chitosan solution is a carrier.

CLAIM 5. The method of preparation as claimed in claim 1, wherein the active ingredient is banana pseudostem extract.

CLAIM 6. The method of preparation as claimed in claim 1, wherein crystalline form of BSE-Chi-AgNPs X-ray diffraction spectrum comprising characteristic peaks, in terms of 2θ angle (±0.2), at 38.08º, 44.26º, 64.38º and 77.32º which could be indexed to (111), (200), (202) and (311).

CLAIM 7. The method of preparation as claimed in claim 1, wherein absorbance peaks through Fourier Transforms Infrared Spectroscopy for BSE-Chi-AgNPs are at 346.66, 2926.71, 1632.74, 1562.32, 1412.98, 1384.70, 1152.75, 1071.77 and 577.33 with the significant peaks at 3436.66 cm-1 (corresponding to OH groups), 2926.71 cm^{-1} (CH_2 stretching), 1632.74 cm^{-1} =(-C=O) stretching amide I group, 1071.77 cm^{-1} (C-O-C group stretching).

CLAIM 8. The method of preparation as claimed in claim 1, wherein λmax through UV (ultraviolet)-visible spectroscopy for BSE-Chi-AgNPs is at 400 nm.

Dated this 04th Day of July 2022

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Applicant's Agent: IN/PA-1379
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Figure 1

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Figure 2

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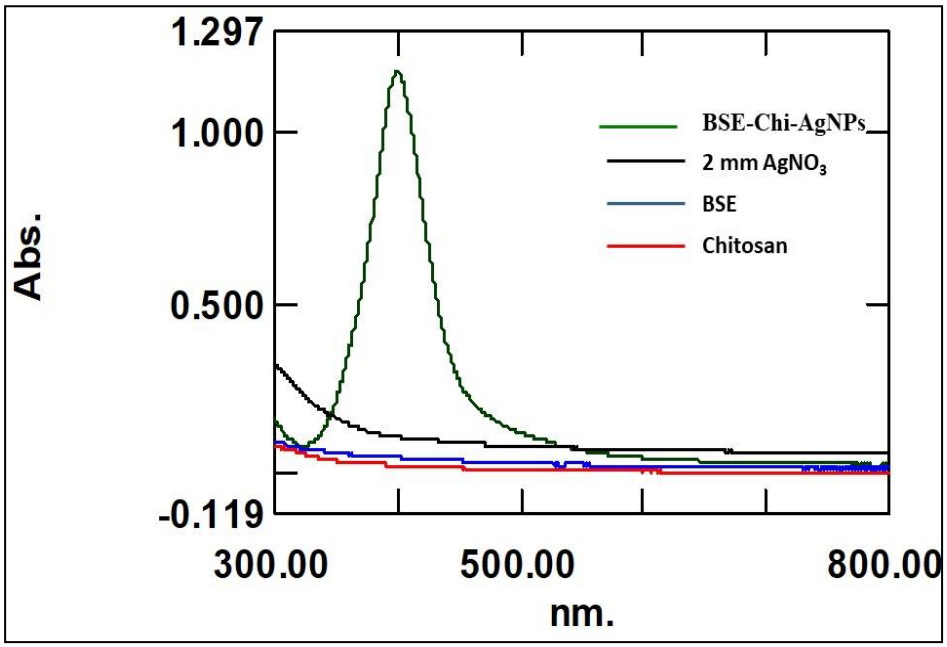


Figure 3

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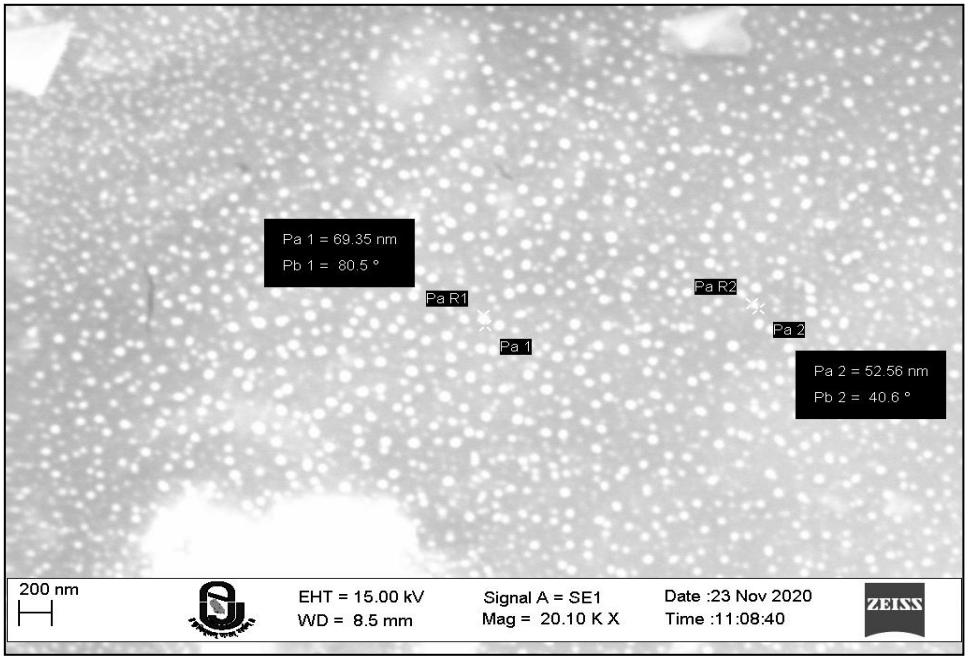


Figure 4

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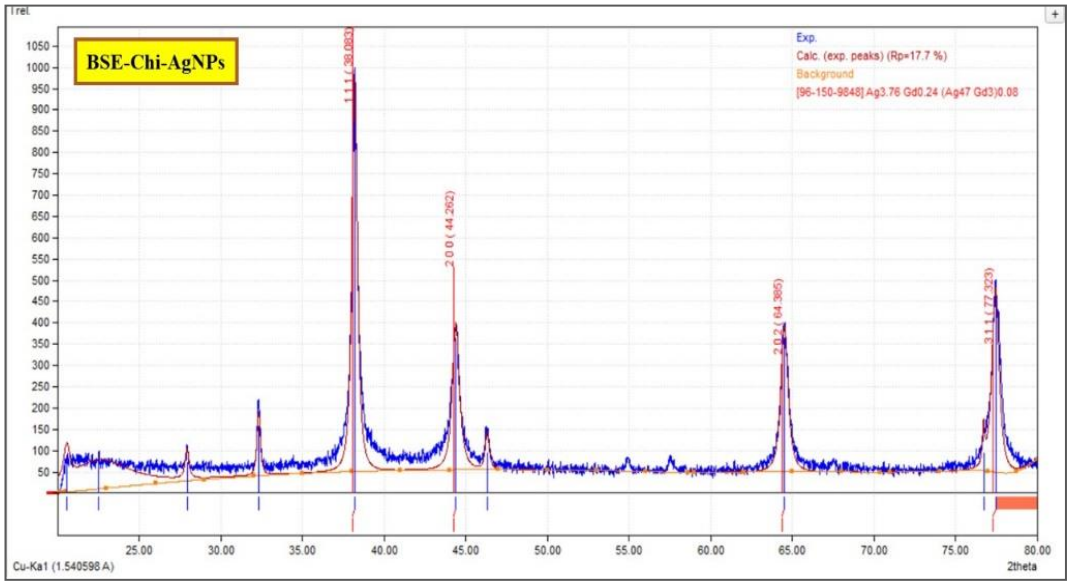


Figure 5

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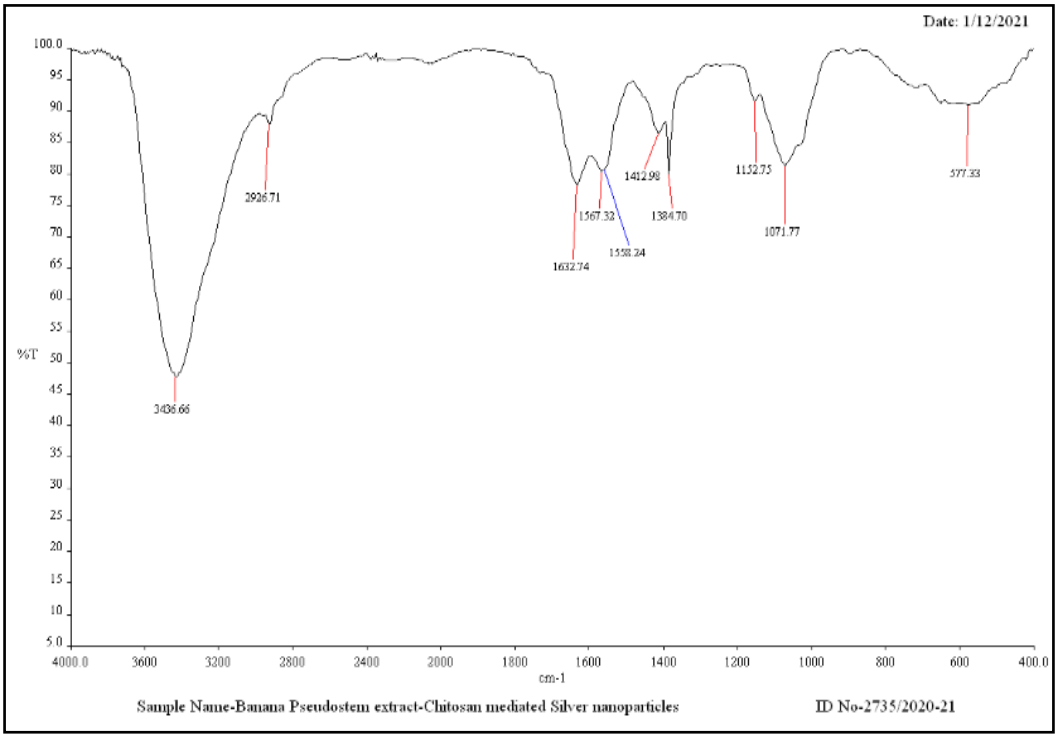


Figure 6

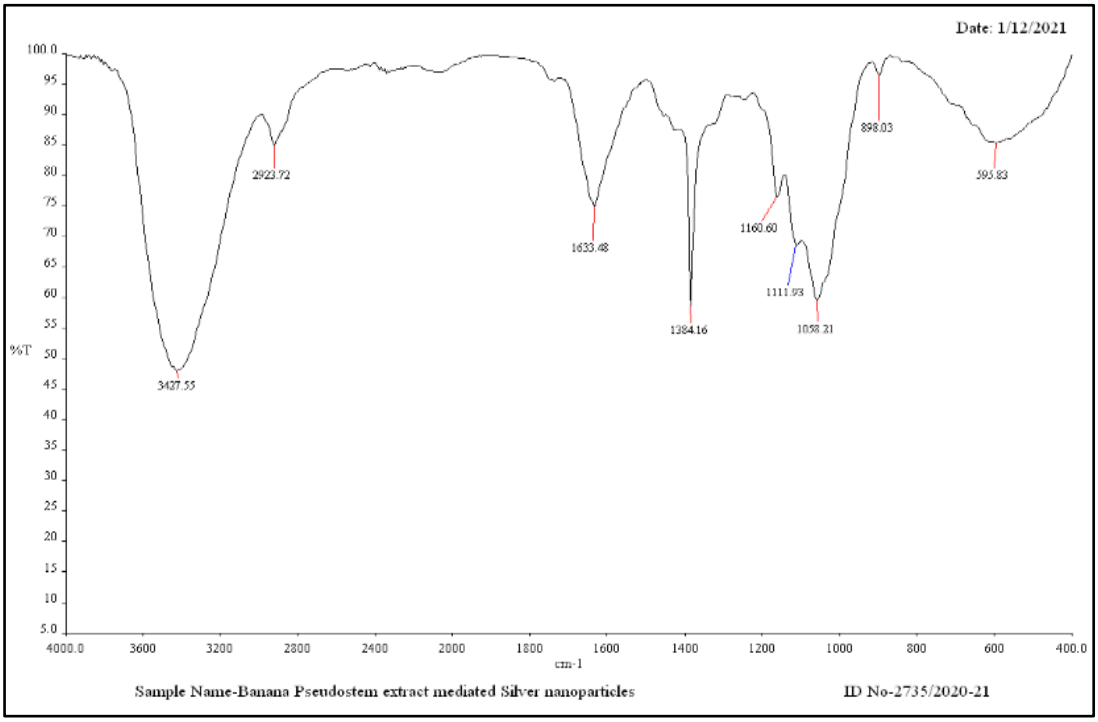
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Applicant: NAVSARI AGRICULTURAL UNIVERSITY



Sheet 7 of Sheet 9

Figure 7

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Applicant: NAVSARI AGRICULTURAL UNIVERSITY

Sheet 8 of Sheet 9

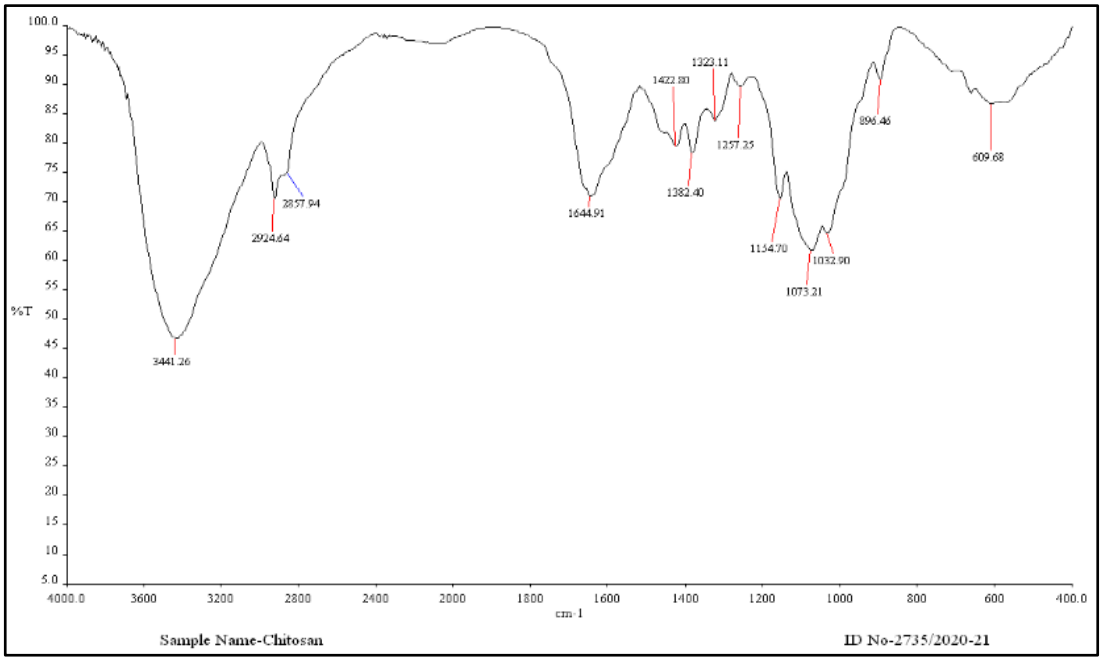


Figure 8

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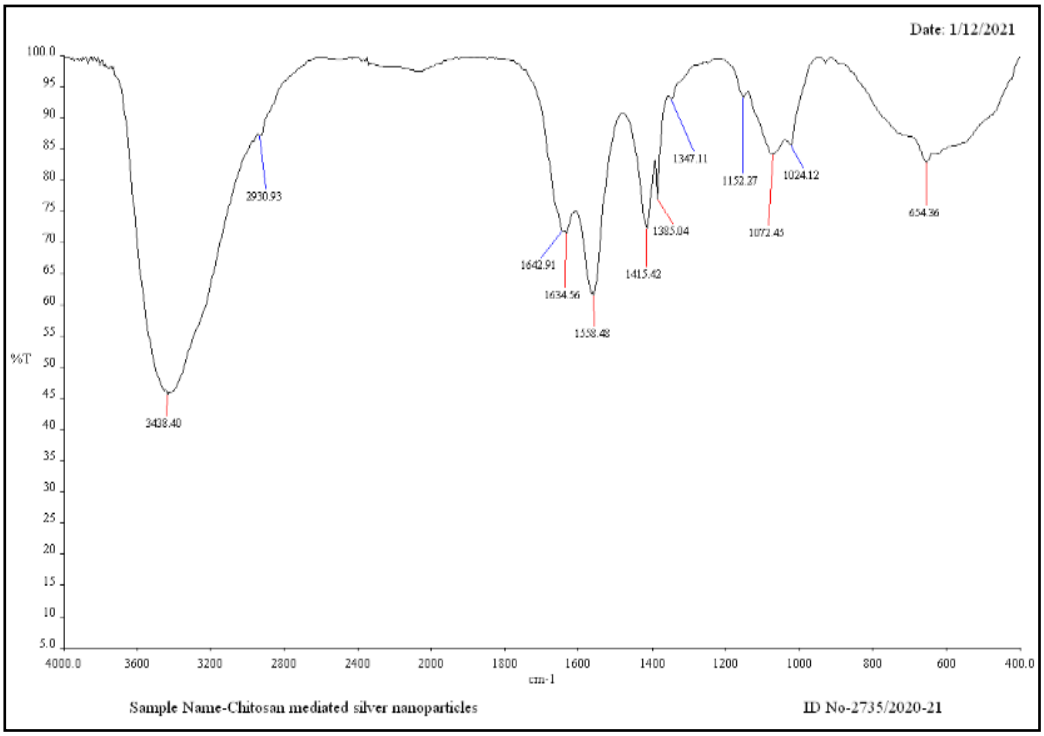


Figure 9

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TITLE: METHOD OF PREPARTION OF HYBRID SILVER NANOPARTICLES WITH BANANA PSEUDOSTEM EXTRACT AND CHITOSAN

ABSTRACT

The present invention relates to the method of preparation of hybrid silver nanoparticles. The invention more particularly relates to the method of preparation of hybrid silver nanoparticles with banana pseudostem extract and chitosan. The hybrid silver nanoparticles shows the fungicidal activity against *S. rolfsii* and *P. Aphanidermatum* the nanoparticles showed good fungicidal activity at low concentration.