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Research Paper

"Synthesis and characterization of silver nanoparticles from *P. hysterophorous* and *X. strumarium* and its effect on custard apple germination"

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ABSTRACT
The synthesis of silver nanoparticles from Parthenium hysterophorous and Xanthium strumariumundesirable plant. Silver nanoparticles (AgNPs) were synthesized by green synthesis method and characterized by UV-Vis spectrophotometer, SEM, FTIR.UV-Vis spectrophotometer showed peak at 450 nm of Parthenium leaf extract and 461 nm of Xanthium leaf extract, which confirmed the synthesis of silver nanoparticles.In SEM analysis, crystallite size of Parthenium AgNPs was observed in the range of 57.36 nm to 71.28 nm and Xanthium AgNPs was observed in range of 52.67 nm to 70.20 nm. These values confirmed that AgNPs formed were spherical in structure.Parthenium FTIR result showed that absorption bands at 2923 cm^{-1} it showed C-H stretching that reveled alkane functional group with strong intensity, 1737cm^{-1} showed C=O stretching that reveled carbonyl functional group with strong intensity, 1581 cm^{-1} showed N-H bending that reveled amide functional group. Xanthium FTIR result showed that absorption bands at 2976 cm^{-1} it showed skeletal vibration C-H stretching that reveled alkane functional groupwith strong intensity, 1217 cm^{-1} showed C-N stretching that reveled amine functional group with medium intensity, 1059 cm^{-1} showed =C-H bending that reveled alkane functional group,other peaks observed showed different functional groups.Silver nanoparticles were used to reducing the germination time and increased germination percentage of custard apple seeds. Parthenium AgNPs treated seeds germination percentage was found to be highest i.e. 100% at 2.5 mg/ml concentration and lowest 40% at 0.5 mg/ml concentration as compared to control and germination percentage of Xanthium AgNPs treated seed was found to be highest i.e. 60% from2.0, 2.5mg/ml concentration. Overall results indicated that Parthenium and Xanthiumsilver nanoparticles (AgNPs) were effective on Custard apple seed germination. P. hysterophorous AgNPs treatment was more effective, eco-friendly and beneficial than X. strumariumAgNPs that showed positive effect of custard apple seed germination
KEYWORDS: *Parthenium, Xanthium,custard apple, Silver nanoparticles, SEM, FTIR*

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I. INTRODUCTION

Nanotechnology is a branch of science that's associated with nanomaterials, which help overcome the limitation of size [1]. Nanotechnology could be a promising field of interdisciplinary research. It parades a large array of opportunities in exceedingly various fields like medicines, pharmaceuticals, electronics, and agriculture. Silver nanoparticles (AgNPs) are nanoparticles of silver of between 1 nm & 100 nm in size, some are composed of a percentage of silver oxide because of their large ratio of surface to the majority silver atoms [3]. The field of nanoscience and nanotechnology has provided an actuation within the development of varied high-resolution microscopy techniques so as to be told more about nanomaterials employing a beam of highly energetic electrons to probe objects on a really fine scale. Among various microscopy techniques, SEM can be fully capable of resolving different particles sizes and distributions.By Using SEM, one can study the morphology of particles and derive a histogram from the pictures by measuring the particles manually or by using specific software. The mix of SEM with energy-dispersive X-ray spectroscopy is often accustomed examine silver powder morphology and also conduct chemical composition analysis. SEM is unable to resolve the inner structure, but it can provide valuable information regarding the purity and degree of particle aggregation.The fashionable high-resolution SEM can be spotted in the morphology of nanoparticles below the extent of 10 nm [2].

Scanning microscope systems give various imaging and detecting techniques that are used to study different aspects of the composition of shale samples at very high resolution. Scanning microscopy works opposite conventional light microscopy produces images by recording various signals resulting from interactions of a beam with the sample because it is scanned during a raster pattern across the sample surface.A fine electron probe, size from few angstroms up to many hundred nanometers, is generated by focusing electrons emanating from an electron source onto the specimen surface employing a series of electro-optical lens elements.The mixture of the source and therefore the lens elements is named the electron column [5].

UV-Vis spectroscopy useful and reliable technique for the first characterization of synthesized nanoparticles which is additionally accustomed monitor the synthesis and stability of AgNPs. AgNPs strongly interact with specific wavelengths of light with unique optical properties. Additionally, UV-Vis spectroscopy is fast, easy, simple, sensitive, and select for various varieties of NPs, needs only a brief period of time for measurement and at last, calibration isn't required for particle characterization of colloidal suspensions. In AgNPs, the conduction band and valence band lie very near one another within which electrons move freely. These free electrons bring about a surface plasmon resonance (SPR) optical phenomenon, occurring thanks to the collective oscillation of electrons of silver nanoparticles in resonance with the sunshine wave.Particle size, dielectric medium, and chemical surroundings are responsible for AgNPs absorption.Observation of this peak-assigned to a surface Plasmon is well documented for various metal nanoparticles with sizes starting from 2 to 100 nm. The soundness of AgNPs prepared from biological methods was observed for quite 12 months and an SPR peak at the identical wavelength using UV-vis spectroscopy was observed [2].

Fourier transform infrared (FTIR) spectroscopy technique majorly used for determines IR-active molecules in organic or inorganic solid, liquid or gas samples.It's a rapid and comparatively inexpensive method for the analysis of solids that are crystalline, microcrystalline, amorphous, or films. Samples are analyzed on the size of microns to the dimensions of kilometers and new advances make sample preparation, where needed, relatively straightforward. Another advantage of the IR technique is that it can also provide information about the "light elements" (e.g., H and C) in inorganic substances [7].

Parthenium weed (*Parthenium hysterophorus* L.) is a seriousweed of worldwide. Its main impacts are upon agricultural and natural ecosystem production and biodiversity and on human and animal health [6]. *Xanthium strumarium* is a major weed of soybeans and cotton within the US. It's recently become a hard weed in soybean fields in parts of southern Ontario. Worldwide, it's a minor weed of 1l crops in 28 countries. Infestations of *X. Strumarium* in soybeans can cause severe losses because of a decreased yield, increased moisture content of the beans at harvest, and also the presence of foreign material [14]. Custard Apple (*Annona squamosa* L.) belongs to the custard-apple family and is one of the best fruits introduced in India from tropical America [8]. It's found growing almost all told the tropical and sub-tropical regions mostly in the wild form [13]. Custard apple requires 35-50 days for potential germination [4]. It's adapted well in India where a substantial variability is found in Aravali hills and Southern India [9].

II. MATERIALS AND METHODS

Collection of sample

Parthenium hysterophorus and *Xanthium strumarium* leaves were collected from the campus of Modern College of Agricultural Biotechnology, Kule-Dakhane, Paud, Pune.Custard apple seeds of Balnagar variety were collected from Krishi Bhavan, Seed Research Laboratory, Shivajinagar, Pune-411005.

Preparation of leaf extract

Parthenium hysterophorus and *Xanthium strumarium* leaves weighing 25g separately and were thoroughly washed in distilled water for 5 min, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and were filtered for the removal of dust [10].

Synthesis of silver nanoparticles

1mM Silver Nitrateaqueous solution was prepared and 10 ml of plant extract added. Then the sample was incubated 24 hours in dark condition. After that sample was measured for its maximum absorbance using UV-Visible spectrophotometry. The sample was then heat dried to obtain the synthesized silver nanoparticles for characterization [10].

Seed treatment

Seeds were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility, they were soaked in distilled water for two hours, rinsed four times with distilled water; and then soaked in a series of prepared AgNP suspensions for approximately 2 hours. One piece of filter paper was placed into a 100 mm x 15 mm Petri dish and 5 ml of a test solution was added. Seeds were transferred onto the filter paper, with 10 seeds per dish and 1 cm or farther separating each seed. The Petri dishes were covered and sealed with tape and incubated at room temperature. The germination was halted after 30-50 days, except for custard plants, for which germination was halted after 09 days and 21 days. The seed germination rate and mean germination time were calculated and root length was measured [1].

Seed Germination Measurement
The final germination percentage was calculated based on the total number of germinated seeds at the end of experiment. The measurements were carried out according to the International Rules for Seed Testing. Germination parameters were calculated using the following equations.

Germination Percentage (GP %) = (Gf/n) × 100

Where Gf is the total number of germinated seeds at the end of experiment and n is the total number of seed used in the test.

Characterization of the synthesized silver nanoparticles
UV-Vis spectrophotometry analysis
The sampleswere observed under UV-Vis spectrophotometer (UV-2450, Shimadzu)for its maximum absorbance and wavelength to confirm the reduction of Silver nitrate [11].

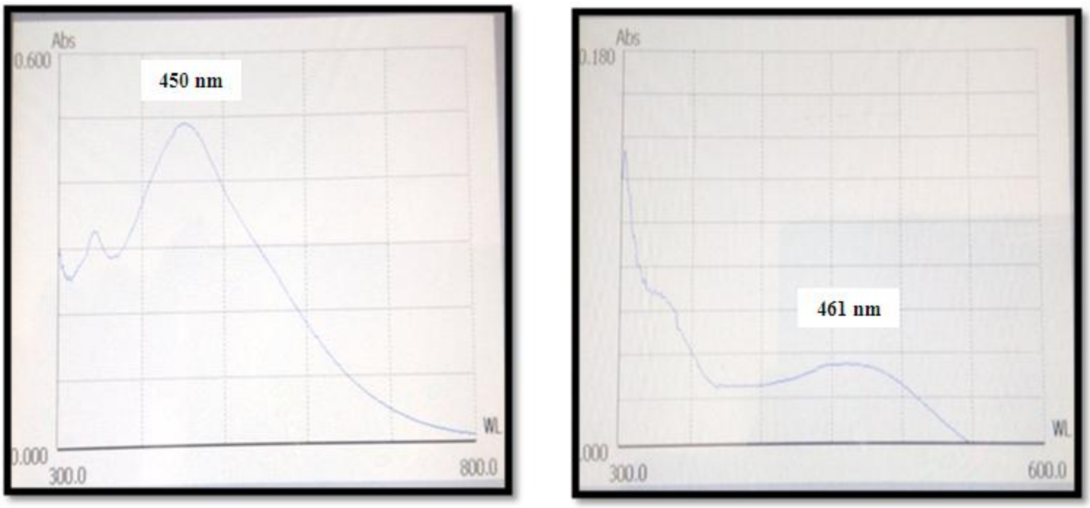
Scanning electron microscopy (SEM)
Parthenium and *Xanthium*NPs were loaded (individually) in the sample holder.SEM analysis was done at 20 kV in vaccume PHA mode. After secondary electron image (SEI) was obtained. Then morphology of the *parthenium* and *xanthium* AgNPs was analysed[12].

Fourier-Transform Infrared Spectroscopy (FT-IR)
Infrared Spectroscopy used for identification and characterization of a substance. FT-IR provide information of vibrational and rotational modes of motion of a molecule. The particles were analyzed under FT-IR for the size conformation [10].

III. RESULTS AND DISCUSSION

Visual detection of AgNPs
The *Parthenium* leaf extract sample was found to show change in color greenish to black and *Xanthium* sample was found to show change in color yellowish to black, indicated synthesis of *Parthenium and Xanthium* AgNPs.

UV-Vis spectrophotometry analysis
The *Parthenium* and *Xanthium* samples were found to show the peak at 450nm and 461nm respectively which confirms the reduction of silver nitrate to silver nanoparticle. The wavelength which had obtained varies slightly to the peak value mentioned in the work carried out by Sharma *et al*., 2009 in which the wavelength was found to be 450nm and 461nm respectively. The graph obtained is shown in graph1.



Graph. 1 Graph indicating the conversion of silver nitrate to silver nanoparticle

Scanning electron microscopy analysis (SEM)

SEM confered the shape and size of AgNPs. It revealed that the particles were spherical in shape and crystallite size of *Parthenium* AgNPs was observed in the range of 57.36 nm to 71.28 nm and *Xanthium* AgNPs was observed in the range of 57.67 nm to 70.20 nm.The SEM images obtained are shown in figure 1 and 2.

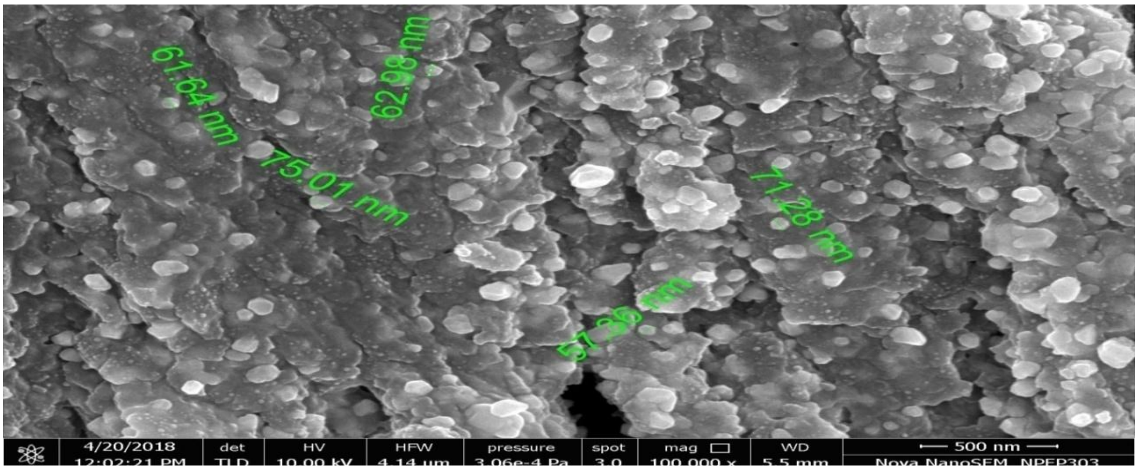


Figure. 1 Image indicating the size and shape of silver nitrate to silver nanoparticle (*Parthenium*)

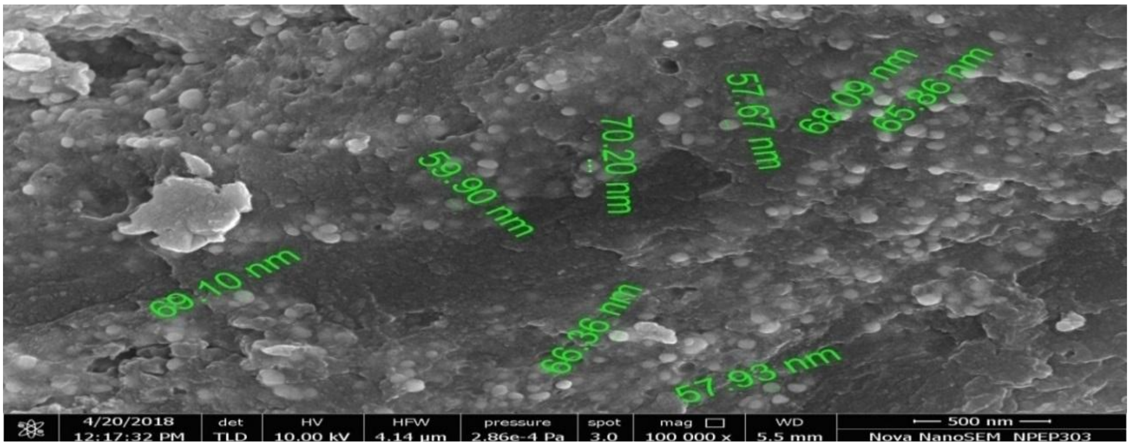
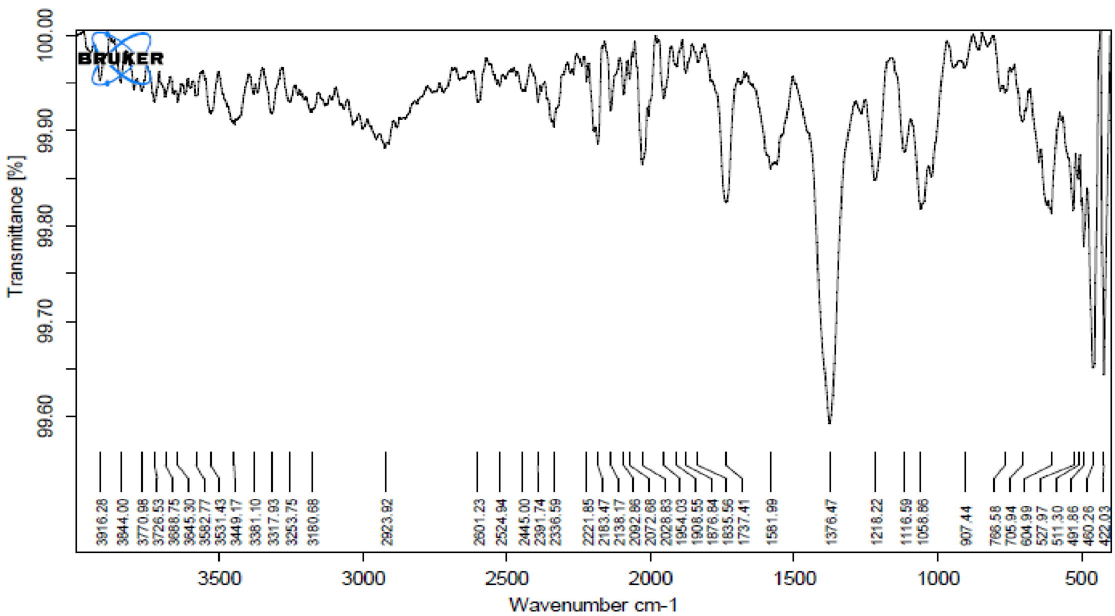


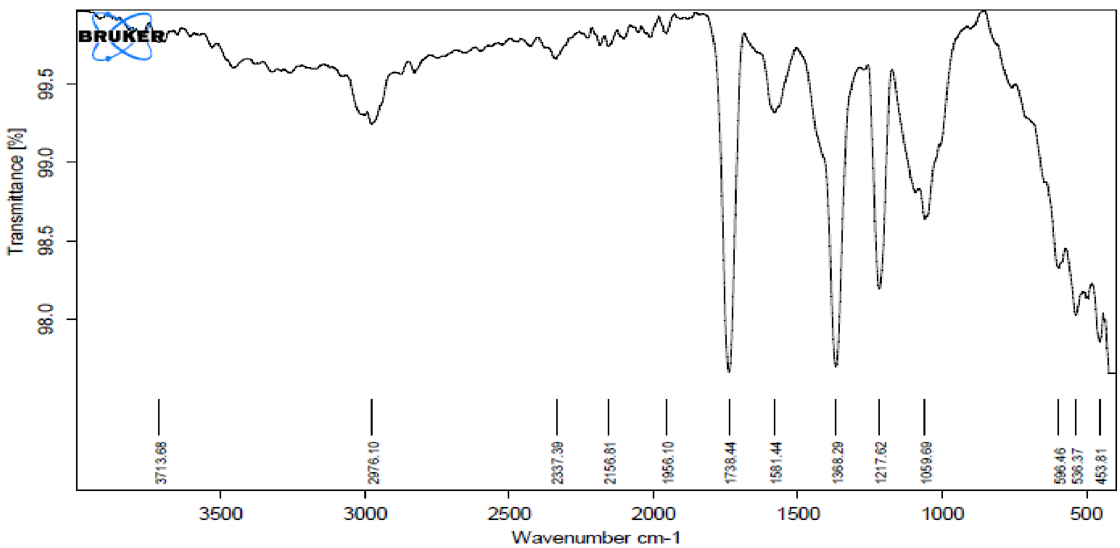
Figure. 2 Image indicating the size and shape of silver nitrate to silver nanoparticle (*Xanthium*)

Fourier - Transform Infrared Spectroscopy analysis

Parthenium FTIR result showed that absorption bands at 2923 cm^{-1} it showed skeletal vibration C-H stretching that reveled alkane functional group and with strong intensity, 1737cm^{-1} showed C=O stretching that reveled carbonyl functional group with strong intensity, 1581 cm^{-1} showed N-H bending that reveled amide functional group, 1218 cm^{-1} showed C-N stretching that reveled amine functional group with medium intensity, other peaks observed around 2601 cm^{-1}(0-H), 2183 cm^{-1}(C-C), 1376 cm^{-1} (O-H).The graph obtained is shown in graph 2.*Xanthium* FTIR result showed the absorption bands at 2976 cm^{-1} it showed skeletal vibration C-H stretching that reveled alkane functional group and with strong intensity, 1738 cm^{-1}showed that C=O stretching that reveled carbonyl functional group with strong intensity, 1217 cm^{-1} showed C-N stretching that reveled amine functional group with medium intensity, 1059 cm^{-1} showed =C-H bending that reveled alkane functional group,other peaks observed around 1581 cm^{-1}(C=O), 1368 cm^{-1} (O-H). The graph obtained is shown in graph2 and 3.



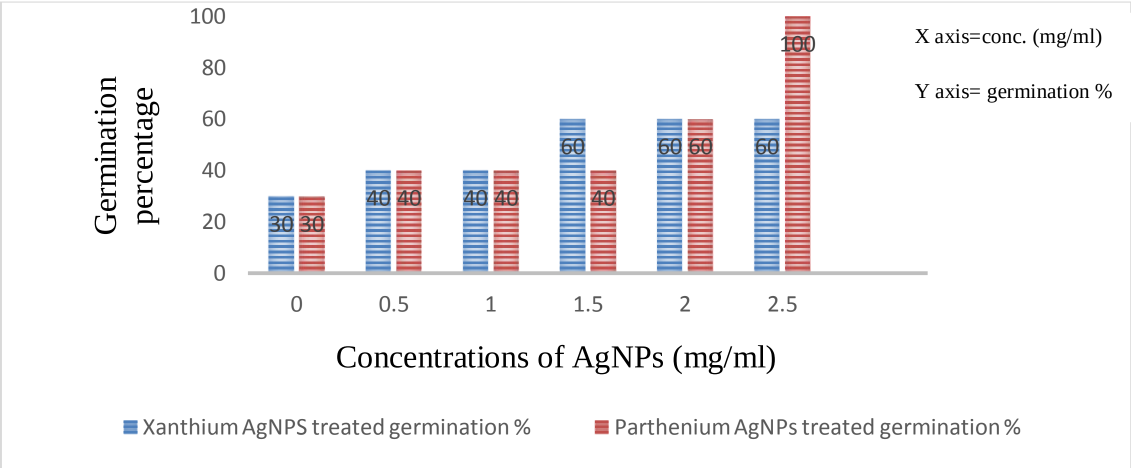
Graph. 2 FT-IR image of the synthesized *Parthenium*silver nanoparticles



Graph. 3 FT-IR image of the synthesized *Xanthium*silver nanoparticles

Germination percentage (%)

Parthenium AgNPs treated seeds germination percentage was found to be highest i.e. 100% at 2.5 mg/ml concentration and lowest 40% at 0.5 mg/ml concentration as compared to control (30%) and germination percentage of *Xanthium* AgNPs treated seed was found to be highest i.e. 60% from 2.0, 2.5mg/ml concentration as compared to control (30%).The percentage obtained is shown in graph 4.



Graph 4. Effect of Parthenium and Xanthium AgNPs on seed germination percentage

Synthesized silver nanoparticles from *Parthenium hysterophorous* and *Xanthium strumarium* shows the effectively increasing germination capability in a custard apple seed. AgNPs from *Parthenium hysterophorous* showed 100% germination at 2.5 mg/ml while *Xanthium strumarium* showed max 60% germination at 1.5 mg/ml. On the basis of this result, Parthenium AgNPs are best suited for caster apple seed germination than Xanthium AgNPs. It is also clear from this study *Parthenium hysterophorous* and *Xanthium strumarium* both are useful for the nanoparticles synthesis. There is a further detailed study required.

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