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Green Synthesis Of Silver Nanoparticle Using Medicinal Fern Thuja Occidentalis Leaf Extract And Study Soil Test Activity

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Abstract
*In this paper we introduce green synthesis of Silver Nanoparticl**e.**The biological method of nanoparticle synthesis is a relatively simple, cheap, and environmentally friendly method than the conventional chemical method of synthesis. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. Here silver nanoparticles were synthesized using Thujaoccidentalisplant leaf extract. The leaf extract acts as both reducing and capping agent. The synthesized silver nanoparticles were confirmed by the change of colour after addition of leaf extract into the Silver Nitrate solution. Morphological studies were performed using scanning electron microscopy (SEM) and the elemental composition was determined using energy dispersive X-ray analysis. The biosynthesized Silver NPs were characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray analysis (EDX). The synthesized Silver NPs were in cubical structure with the average particle size in the range between 50-60nm.In soil activity tests it was found that the sample (soil) show 6.757 which indicates the soil is slightly acid.The Electrical conductivity of sample (ms/cm) 0.567 Which indicates the soil have strongly saline, only tolerant crops yield satisfactorily..*
Keywords: -*Nanoparticles (NPs), XRD, UV-vis, FTIR, SEM, EDX*

1- INTRODUCTION

The synthesis of nano structured materials, especially metallic [1,2] nanoparticles, has accrued utmost interest over the past decade owing to their unique properties that make them applicable in different fields of science and technology.The development of nanotechnology is making the interest of researchers towards synthesis of nanoparticles for the bio application.. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally, the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications. In the field of nanotechnology, the development of reliable and eco-friendly techniques [3] for the controlled synthesis of metal nanoparticles of well-defined size, shape and composition, to be used in many fields is a big challenge. ". Research on the synthesis of nanosized material is of great interest because of their unique properties like optoelectronic, magnetic, and mechanical, which differs from bulk [4]. To synthesize nanoparticles there are many ways such as sol gel method, chemical reaction, solid state reaction and co-precipitation. Compared to those methods green synthesis method is one of the best method for the production of nanoparticles in recent years. This green synthesis

method have several advantages over other methods namely cost effectiveness, simplicity, use of less temperature, the usage of less toxic materials, moreover it is compatible for medical and food applications. Biological methods seem to provide controlled particle size and shape, which is an important factor for various biomedical applications [5]. Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, leaf and skin of the fruits. The nanoparticles synthesized from plant extract were found to be covered by the medicinal properties of plant extract which could be used in drug, targeted drug delivery and cosmetic applications[6].Silver nanoparticles arises from the useful properties of this metal such as the good thermal and electrical conductivity. In particular, silver withits high conductivity is of great interest [7] and has led to much development with commercially available products. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [8].In the recent days, silver nanoparticles have been synthesized from the naturally occurring sources and their products like green tea (Camellia sinensis), Neem (Azadirachtaindica), leguminous shrub(Sesbaniadrummondii) , various leaf broth, natural rubber, starch , Aloe veraplant extract , lemongrass leaves extract, etc. [9].In reported method, the plant extract has been used as capping and reducing agent for the synthesis of silver nanoparticles due to their reducing properties present in the leaf extract[10,11].The current investigation focused on the aqueous leaves extract of Thujaoccidentalisused to synthesize AgNPs at various experimental conditions.Various Investigations were made through UV-Vis, SEM, FTIR, and X-Ray diffraction And EDX.Depending on pH of soil different soil reaction rating has been studied.

2- MATERIALS AND METHODS

All the reagents used in this experiment were obtained from Merck India Pvt Ltd. Double distilled water was utilized for this process. Filtration was established by using Whatman no.1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double distilled water and dried in hot air oven.

(i)Preparation of Thuja Leaf OccidentalisExtract

The thuja leaves were collected from SADTM campus, Jaipur National University, Jaipur (Rajasthan). These leaves were washed with distilled water, dried in oven at 50°C temperature for 3 hours. The dried leaves were crushed by using mortar pestle to get a powdered form. The 5gm powdered form of thuja leaves were added to 100 ml distilled water in 250 ml conical flask and boiled at 60°C for 30 minutes. The extract was filtered through Whatman filter paper and stored at -4°c.The extract was preserved in refrigerator for future use.

Fig1.1-ThujaOccidentalisPlant Fig1.2-Powdered form Fig1.3- Preparation of Leaf Extract

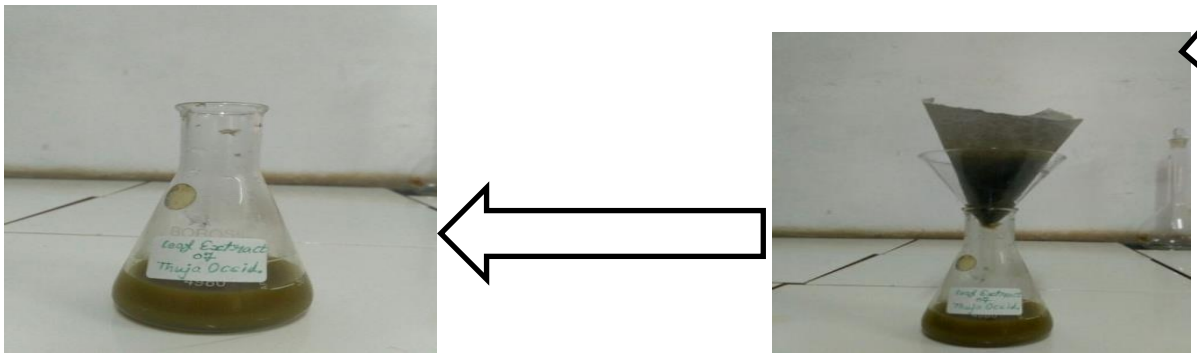

Fig1.4- Leaf extractsFig-1.5 Filter of plant extract

(ii) Preparation of Silver Nitrate Solution

A 0.1M stock solution of $AgNO_3$ in chloride-free distilled water was prepared. Therefore, 100 ml of 0.1M solution will contain 1.6 g of $AgNO_3$, then weighed amount of $AgNO_3$ was carefully transferred in a 100-ml volumetric flask and double distilled water was added drop-wise while stirring to dissolve the salt up to the mark. The solution were kept away from light (the containers were wrapped with carbon papers) and kept in dark.

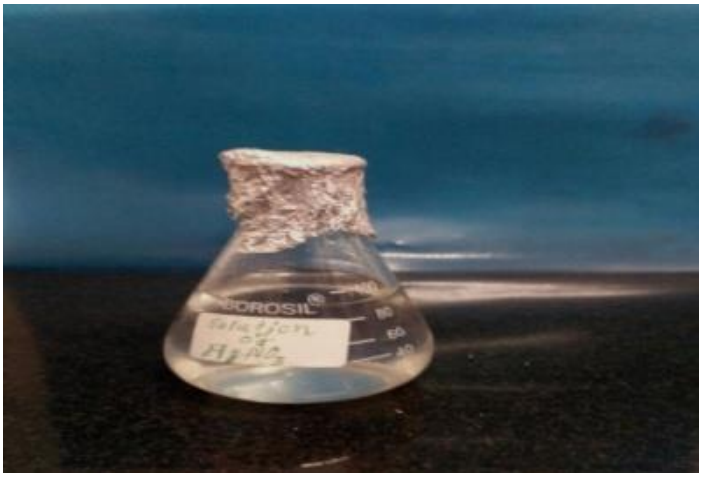

Fig-1.6 $AgNO_3$ Solution

(iii) Green Synthesis of Silver Nanoparticles

10 ml of leaf extract and 100ml of $AgNO_3$ solution was added in conical flask. Then, the solution was kept in the shaking incubator, color of experimental sample (mixture of silver nitrate and leaf extract) turned light dark- brown, and became deeper after 24 hours which clearly indicated the formation of silver nanoparticles. The experimental sample was centrifuged by using research centrifuge at 9000 rpm for 20 minutes at 0-4^{o}C.

Fig1.7 Solution after incubated

Fig1.8 Solution in shaking incubator

3- CHARACTERIZATION

The formation of Silver nanoparticles was confirmed by UV- Visible spectroscopy using U5000 spectrophotometer instrument. Size of the AgNPs was analyzed with UV-Spectrometer in the range between 300-800nm. To determine the biomolecules present in the leaf extract, FTIR analysis was carried out which is responsible for the reduction of silver ions with the spectral range of 600-4000 cm-1. Here the sample was centrifuged at 9000 rpm for 20 min, dried using hot air oven to make powder form. The crystalline structure of the copper nanoparticles were determined by X-Ray diffraction analysis using BrukerD X-Ray diffractometer instrument operating at 40 kV with 2sec time interval at room temperature 27ºC. Morphology and mean particle size of the Silver were determined by SEM. The samples were prepared for SEM and TEM analysis. The SEM analysis was established by using Sigma Zeiss with 1nm resolution at 30 kV with 20 mm EDS detector. The elemental composition in the reaction mixture was determined by EDX analysis.

4- RESULT & DISCUSSION

(i) UV-Vis Spectroscopy Analysis

The result obtained from UV-Visible spectroscopy analysis of the sample is presented in Fig2.The AgNPs have absorbance at a characteristic wavelength between 300 and 800 nm. The Silver nanoparticles formation was confirmed from the peak at 444 nm, UV–visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 420-460 nm. [12]

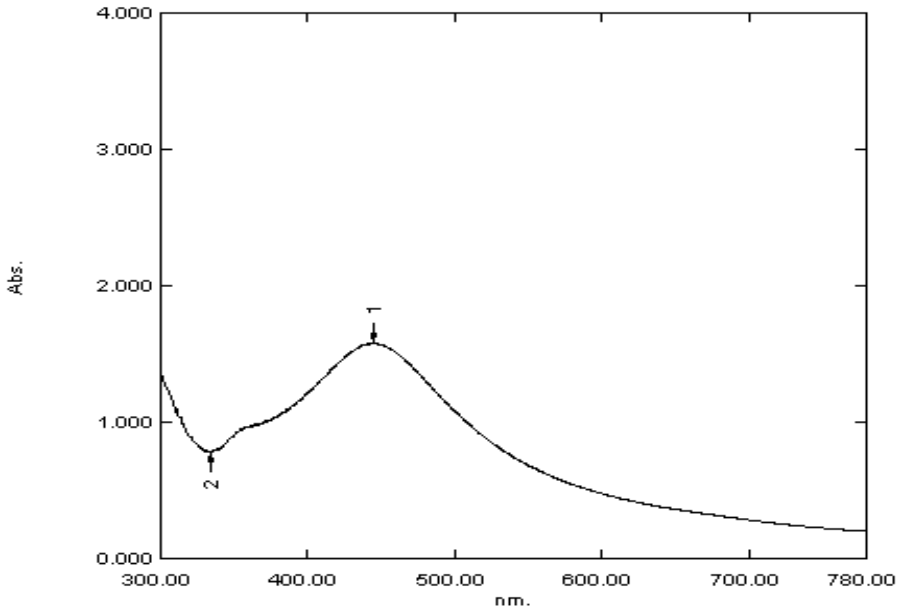

Fig2 UV-Vis Spectra of synthesized AgNPs of Thuja plant

(ii) Fourier Transform Infrared (FTIR) Analysis

FTIR measurements were carried out to identify the bio-molecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of aqueous silver nanoparticles showed the transmittance peaks between 3412cm^{-1}(O-H stretching due to alcohols group), 1384cm^{-1}, (-CH_2bending alkenes), 1121cm^{-1} (C-O stretch alcohols), 610cm^{-1} (C-H bend alkenes)and the peak at 1631cm^{-1}confirmed the formation of silver nanoparticles.

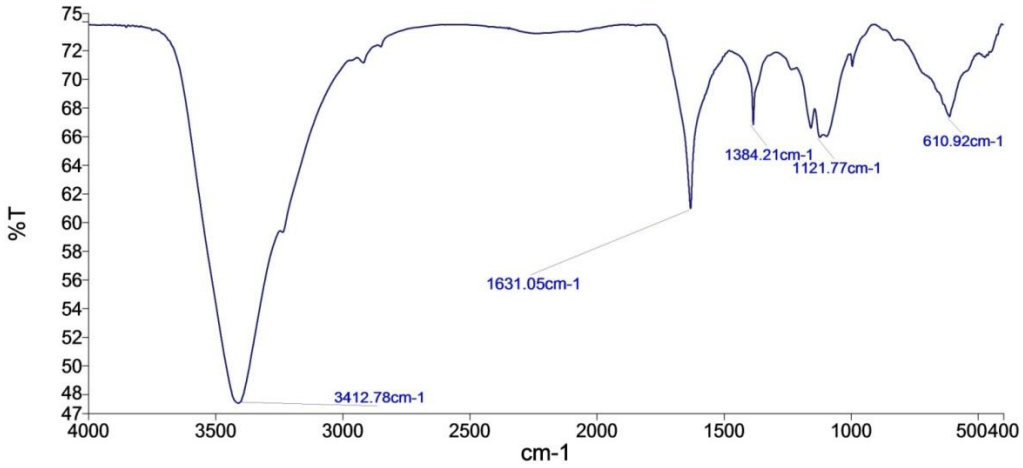

Fig: 3FTIR Spectral representation of synthesized silver Nanoparticles

(iii) Scanning Electron Microscopy (SEM) Analysis

SEM analysis shows high-density silver nanoparticle synthesized by the leaf extract of thujaoccidentalis. SEM (Scanning Electron Microscopy) used to determine the morphological character, shape, size and surface of silver nanoparticles. SEM images revealed that the silver nanoparticles in the approximately in the size range 54nm with clearly observed spherical and undifferentiated shape.

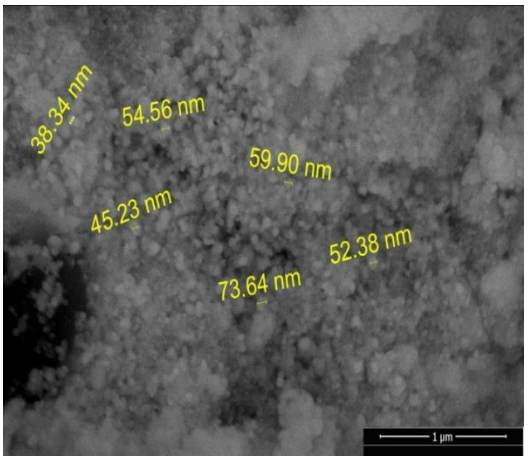

Fig-4.1SEM Image of synthesized silver NPs

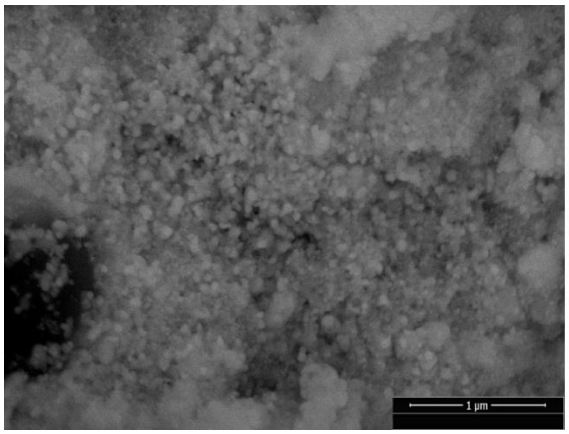

Fig-4.2SEM Image of synthesized silver NPs

(iv) Energy Dispersive X-Ray (EDX) Analysis

In Figure 4.3, a standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum one can clearly see peaks located between 2 kV and 4 kV.The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed.

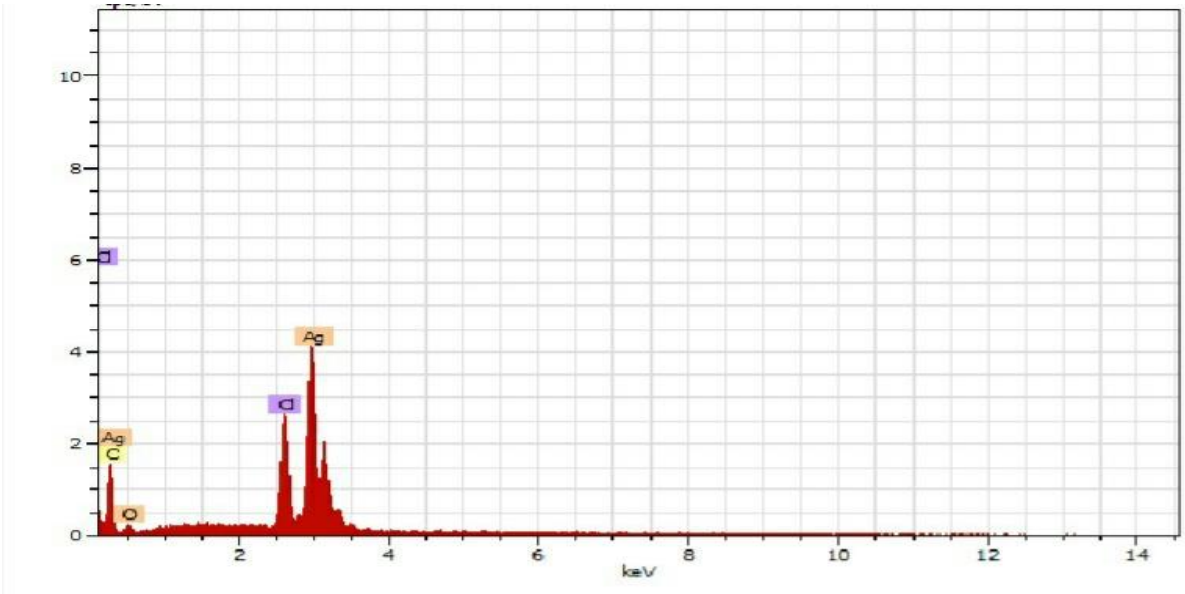

Fig- 4.3 EDX spectra of synthesized silver Nanoparticles

(v) X-RAY Diffraction (XRD) Analysis

Figure 4.4 shows the XRD pattern of the produced Ag nanoparticles which represents the formation of Ag nanoparticles with a face centered cubic structure (JCPDS 036-0664 & 08-01268) with lattice parameter 4.0732. The diffraction data exhibits that the silver nanoparticles have characteristic diffraction peaks 37.23º, 44.44º, 64.58º and 77.58º corresponding to the crystal planes (111), (200), (220) and (311). . Using Scherrer's formula, the grain size of silver nanoparticles was obtained. The average size of obtained silver nanoparticles found to be 54 nm.

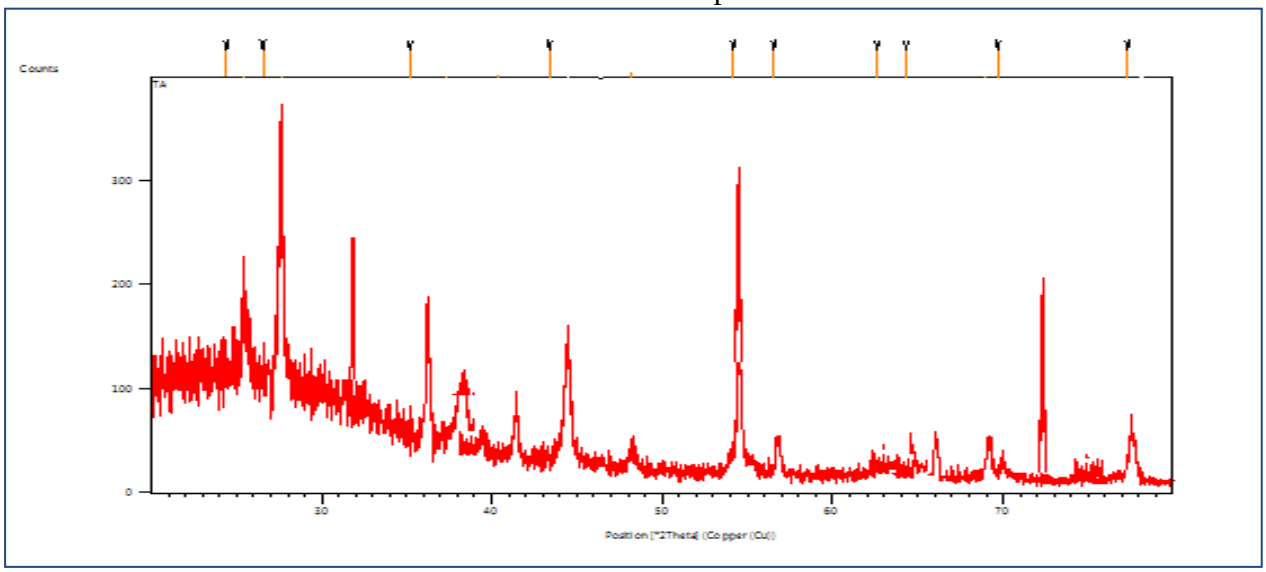

Fig- 4.4 XRD Spectra of synthesized silver Nanoparticles

5- SOIL ACTIVITY TEST

Determination of pH & Electrical Conductivity

For black soil take 5ml soil and 30 ml distilled water. Stir the suspension for 2 minutes and then measure PH and electrical conductivity as follows.

i- pH Measurement

Put on the STFR meter and press the enter button which will show the pH on the screen. Put the pH electrode in pH 7 buffer solution enter and go to STD PH 7 and calibrate with pH 7 buffer solution and then go to STD pH 4 and calibrate with pH 4 buffer by dipping pH electrode in pH 4 buffer solution. Then go to sample and enter to see the sample pH by dipping pH electrode in soil suspension. Depending on

pH see the soil reaction rating as given below:

Based on soil pH values, following types of soil reactions are distinguished:

Table- 1.1 Soil pH test rating

S. No.	PH Range	Soil Reaction Rating
1.	<4	Extremely acid
2.	4-5	strongly acid
3.	5-6	moderately acid
4.	6-7	slightly acid
5.	7.0	Neutral
6.	7-8	moderately alkaline
7.	>8.5	strongly alkaline

The strongly acidic soils (pH <5) need to be limed; will be indicated in the meter before they can be put to normal agriculture production. The strongly alkaline soils (pH >9.2) need to be treated with gypsum to remove the excessive content of sodium. In that case the instrument will automatically calculate gypsum requirement and will show in the Display.

ii-Determination of lime requirement

Take 2.5 ml soil in a 15ml centrifuge tube and add 2.5 ml distilled water and 5.0 ml LR solution. Close the tube and shake for 10 minutes and dip the pH electrode in the tube and enter in the lime requirement option for STFR meter.

Table 1.2- pH value

S. No.	Solution	pH reading
1.	pH -4	7.00
2.	pH -7	4.00
3.	pH -9.2	9.2
4.	Sample	6.757

The sample (soil) show 6.757 which indicates the soil is slightly acid.

iii-Electrical conductivity measurement

Put on the STFR meter and press the enter button which will show the electrical conductivity on the screen. Enter too see the "put standard ". Dip the electrode in the standard solution and enter. Then go to sample menu by DOWN button and dip the electrode in sample suspension. And press the enter button and note down the reading. The instrument has been calibrated according to table.

Relationship between EC and salinity effect for alluvial soil (silt loam), soil: water =1.2 and for Black soil (clay loam) soil: water 1:5

Table-1.3 Relation between EC and salinity effect for alluvial soil

EC of extract (ds/m)	Salt (%) in soil	Salinity effect
0-0.4	0-0.05	Non saline salinity effect mostly negligible
0.4-0.8	0.05 -0.1	Very slightly saline .yield of very sensitive crops may be restricted
0.8- 1.6	0.1-0.2	Moderately saline. Yield of many crops restricted. Cotton, sugar beet, cereals, grain sorghum may be taken

>1.6	>0.2	Strongly saline ,only tolerant crops yield satisfactorily

From above table the result obtained is given below-
Standard specific conductivity = 0.407
Electrical conductivity of sample (soil) EC (ms/cm) = 0.567
Which indicates the soil have strongly saline, only tolerant crops yield satisfactorily

6- CONCLUSION

In the present study, The Silver nanoparticles were obtained from Thuja (Morpunkhi) leaf extract using biosynthesis method. This method is very easy toperform in University laboratory.It was confirmed by initial color change from pale yellow to brown. UV-VIS spectra of Ag nanoparticles were observed at 444nm. The functional group present in the leaf extract was confirmed by FTIR analysis. These functional groups were mainly responsible for the reduction of silver metal ions into AgNPs. FTIR spectra of AgNps showed the presence of the IR peaks band at around 3415cm^{-1}, 1384cm^{-1}, 1631cm^{-1},1114cm^{1} and 621cm^{-1}.The synthesized silver nanoparticles were analyzed using UV spectrophotometer , FTIR, SEM with EDAX, and XRD. Using Scherrer's formula, the grain size of silver nanoparticles was obtained. The average size of obtained silver nanoparticles found to be54 nm. The benefits of this biological method is an ecofriendly and easy to obtain method is affordable and pollution free. In the soil test activity the sample (soil) show 6.757 pH which indicates the soil is slightly acid.

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