The biological processes eliminate the elaborate process of maintaining cell cultures and can also be easily scaled-up for large- scale production of nanoparticles (Veeraswamy et al. 2011). During synthesis of nanoparticles, the parameters such as pH, temperature, salt concentration and reducing agent have a significance influence on diameter , size distribution, shape, aggregation, state and stability. Thus, the optical properties of nanoparticles , conductivity and other characteristics may be changed (Kupiec et al., 2011).

Achyranthes aspera (Amaranthaceae) ; native to Africa and Asia, and found throughout India; is an erect, annual or perennial herb found on the road sides, field boundaries and waste lands.

The silver nanoparticle have a large area of interest as they have a large number of applications:- non-linear optics spectrally selective coating for solar energy absorption, biolabeling, intercalation material for electrical batteries as optical receptors, catalyst in chemical reactions, antibacterial material, chemically stable materials and good electrical conductors (Zargar et al., 2014).

It is well known that, silver is an effective antimicrobial agent and possesses a strong antimicrobial activity against bacteria, viruses and fungi. The antimicrobial activity of silver nanoparticles is a result of well- developed surface (Kaviya et al. 2011).

Phytochemical investigations showed that the presence of bioactive compounds like sterols, alkaloids , saponins and glycosides in leaves and roots are responsible for the reduction of silver ions to silver nanoparticles (AgNPs).

Numerous synthesis approaches were developed to obtain silver nanoparticles of various shapes and sizes, including laser ablation (Lee et al., 2001), gamma irradiation (Long et al., 2007), electron irradiation (Bogle et al., 2006), chemical reduction by inorganic and organic reducing agents (Bönnemann et al., 2001), photochemical methods microwave processing (Mallick et al., 2004), and thermal decomposition of silver oxalate in water and in ethylene glycol (Navaladianet al., 2007).

AgNPs enhances the seed quality such as germination percent, speed of germination , root length, shoot length etc.

Materials and Methods:

Collection and identification of the plant:- We collected Achyranthes aspera from our surrounding. We identified A. aspera by their morphological characteristics like it was a stiff annual herb , stems were angular , ribbed and woody from the base , often with tinged purple colour.

Preparation of plant extract:- The fresh A. aspera leaves and stems were washed to eliminate all dust. They were dried in Hot Air Oven and was converted into powdered form. Then 5gm. powered form of A. aspera was mixed with 150ml. of DIW and was stirred continuously. The solution was then filtered with the help of Whatman filter paper. Pale yellowish brown coloured plant extract was obtained.

Phytochemical screening of the plant:- Test for Phytochemical constituents were carried out for Alkaloids, Glycoside, Carbohydrates, Tannins, Saponins, Flavonoids, Quinines, Phenols, Proteins and Steroids.

Synthesis of silver nanoparticles:- In 1 ml. of DIW, 1 Or 2 tablet of AgNO3 was mixed and stirred continuously till a homogenous mixture of AgNO3 was obtained.

After that AgNO3 solution was mixed with 50 ml. aqueous extract of A. aspera in a conical flask and then the mouth of the flask was covered with aluminium foil.

Characterization of silver nanoparticles with the help of :-

UV-Vis spectroscopy:- UV-Vis spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. The reduction of AgNPs in the aqueous solution of the silver complex during the reaction with the leaf and root extract of A.aspera was confirmed by the UV-Visible spectra. The sample was prepared by diluting 1 ml of Ag NPs into 2 ml distilled water and measured the UV-Visible spectrum of Ag NPs solution (Habibi et al. 2017). The particle size measurement can be determined through UV-visible spectrophotometer (Joseph et al 2016).

Fourier-Transform Infrared Spectroscopy (FTIR):- FTIR provided the information about functional group present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic AgNO3 to elemental silver.

X-ray diffraction (XRD analysis):- XRD is a unique method for the determination of crystallographic structure of a material . The nanoparticles synthesized in this method are characterized using powder XRD to confirm the formation of nanoparticles and to know the structural information.

Application of silver nanoparticles:-

We used biosynthesized AgNPs on the plant Cicer arietinum to observe it's morphological and physiological change. There were different steps involved to observe morphological and physiological changes after AgNPs applied:-

Sterilization of seed:- Seeds were collected in bulk and washed under running tap water to remove any adhering dirt. Then sterilized (with0.5%HgCl2), dried in sunlight and store in empty, dry clean bottle.

Pre-Soaking of seeds:- Some seeds of chana were soaked in distilled water (control) and some in Silver nanoparticles with different concentrations of 100, 200 and 500 mg./L for 6 hours. Seeds were allowed to germinate for biochemical test.

Incubation:- Seeds of *Cicer arietinum* and of both treated and untreated NPs were grown in Petri plates with cotton bed and allow to germinate under a sunlight (minimum 8-12 hr) for 6-7 days. After sowing the seeds, each seed was treated with 5ml. of 100mg./L, 200mg./L and 500mg./L concentrations of NPs. Distilled water was sprayed in the control treatment. Seed germination was monitored daily for 6 days and were considered germinated when radicle was extended upto more than 6 mm.

Transfer of seedlings into soil:- When the plants reached upto the height of 5-10 cm, it was transferred into the pot. At the end of the of germination experiment, length and weight of the seedlings were measured .

Morphological parameters:-Physical parameter generally deals with the plant growth and morphological characteristics of the plant.

Final Germination Percentage (FGP): Germination percentage was observed in Cicer *arietinum* by the given formula:-

GP = Number of seeds germinated /total number of seeds sown for germination x 100

Seedling Vigour Index: Biomass of seedling was measured after 14 days of germination. Seedling vigour index = [root length (cm.) + shoot length (cm.)] x germination (%)

Day of Emergence of 1st leaf: Once the seedling started to photosynthesize, it was no longer dependent on seed's energy reserves.

The 1st "true" leaves expand and could often be distinguished from the round cotyledons through their species – dependent distinct shapes.

Total Number of Leaf: Count the total number of leaves at the last day of experiment . Number of leaves indicates a plant's physiological age .

Plant Height: Plant height was recorded from base of plant to the uppermost node of main shoot of plant at 14th days of germination and height was expressed in cm.

Root Length: Root length was measured from tip of root to just below the bottom of shoot with the help of thread and scale. It was expressed in cm.

Shoot Length: Shoot was measured from tip of shoot to just above of starting of root, with the help of thread and scale. It is measured in cm.

Plant Biomass

Plant Fresh Weight: Take sample plant and blot the plant gently with soft paper or towel to remove any free surface moisture.

Weigh immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data).

Plant Dry Weight: Take sample plant and blot the plant to remove any free surface moisture. Dry the plant in an oven set to low heat (100°F) overnight.

Let the plant cool in a dry environment (a Ziplock bag will keep moisture out), in a humid environment the plant tissue will take up water.

Once the plants have cooled, weigh them.

Results and Discussion :

Various test were done to find the presence of phytochemical in the plant extract. The results are shown in Table 1.

Phytochemical study of *A.* aspera leaf extract shows the positive results for carbohydrates, tannins, phenols, flavonoids , proteins, alkaloids, saponins and glycosides (Table 1).

Phytochemical investigations were revealed that the presence of bioactive compounds like sterols, alkaloids, saponins, sapogenins, cardiac and glycosides in leaves and roots are responsible for the reduction of silver ions to silver nanoparticles (Ag NPs) (Triguna et al. 1992).

Table 1. Phytochemical screening of *A.* aspera.

Test for Phytochemicals	Aqueous extracts
Saponins :- Foam test	+
Flavonoids :- Sulphuric acid test	+
Alkaloids :- Mayer's test	+
Quinines :- Sulphuric acid test	+
Glycoside :- Sulphuric acid test	+
Phenol :- Ferric chloride test	+
Proteins:- Ninhydrin test	+
Steroids :- Sulphuric acid test	+

During AgNPs synthesis, colour formation occurred within 2 days with the appearance of dark brownish black colour from pale yellowish brown colour solution.

The appearance of brown colour indicated the formation of silver nanoparticles (Kalidasan and Yogamoorthi 2014).

This might be due to the reduction of Ag+, indicating the formation of AgNPs.

Characterization of nanoparticles was done by using different methods, which include:-

Ultraviolet – visible spectroscopy (UV-Vis):- The aqueous extract – based AgNPs showed prominent peak around wavelength max 448 nm.

Similar results were reported by Hafez et al., (2017), Halawani (2017) and Sivakumari et al., (2018) reported SPR (Surface plasmon resonance) band for biosynthesized silver nanoparticles using *Morus nigra leaf* extract (425 nm), *Zizyphus spinachristi* L. leaf extract (414 nm) and *Achyranthes Aspera* (450 nm).

So the reduction of AgNPs in the aqueous solution of the silver complex during the reaction with the leaf and root extract of *A. aspera* was confirmed by the UV-Visible spectra (Fig. 1).

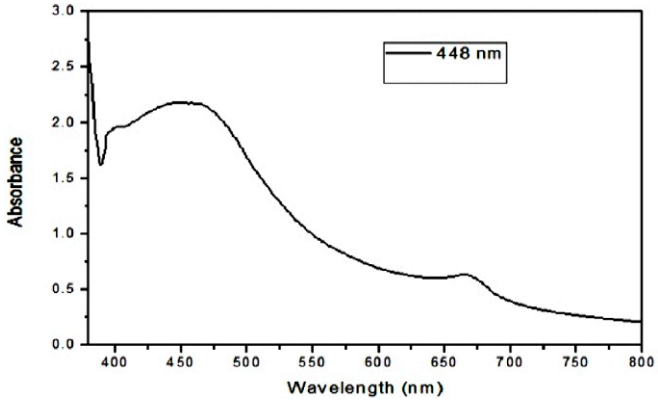

Fig. 1. UV-Vis spectral image of aqueous extract-based synthesized AgNPs.

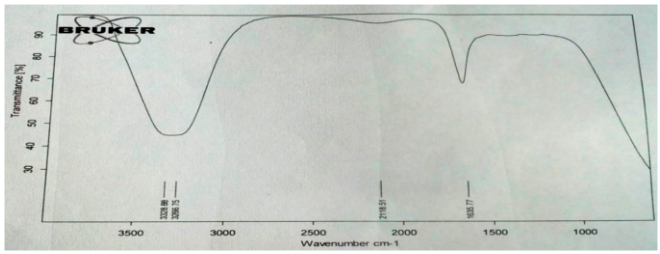

Fig. 2. FTIR spectral image of A. aspera aqueous extract-based synthesized AgNPs.

X-ray diffraction (XRD analysis):- Crystalline nature of the silver nanoparticles was measured on X- ray diffraction instrument (M/S Rigaku, Ultima 4, Tokyo , Japan) operated at 30 KV and 100 mA. Spectrum was recorded by CuK radiation with wavelength 1.5406 A° in the 2θ range 20-80°.

Silver nanoparticles (app. 1g.) were uniformly spread on glass sample holder and placed in scanner chamber . The set scan speed and step size of 0.30° / min. and 0.00 s., were fixed respectively.

The XRD pattern was recorded for phase identification of silver nanoparticles (Djangang et al., 2015). The patterns clearly shows (Fig. 3) the main peaks at (2 θ) 31.87, 38.53, 48. 25.

The peaks observed during XRD analysis were due to the presence of organic compounds in the extract and intensity of the peaks denoted the degree of crystallinity of the particles (Halawani, 2017).

By comparing JCPDS (file no:- 89-3722), the typical pattern of green synthesis AgNPs was found to possess an fcc structure .

So, XRD is a unique method for determination of crystallographic structure of a material.

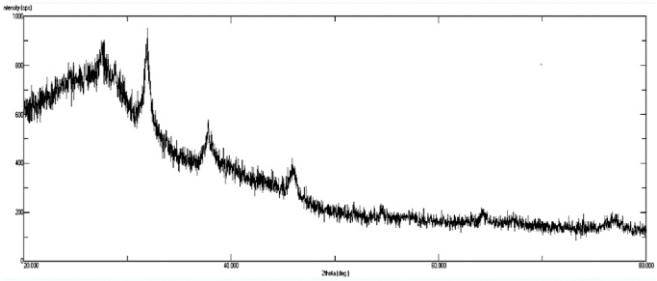

Fig. 3. XRD pattern for A. aspera mediated AgNPs.

Results obtain synthesized silver nanoparticles of different concentrations and their effect on the growth of chana plant are discussed below:-

Seeds of *Cicer arietinum* are incubated for 6 h in synthesized nanoparticles and then transfer to cotton fixed petriplate. After when the seeds were germinated and reach upto the height of 5-10 cm., then it was transferred into the soil for proper growth and nourishment. The transferred seedling of the soil observed significant changes in shoot of the plant.

The leaf colour was changes into bright green, the number of leaves increased, and the shoot height were increased, and it became thicker.

Plant growth parameter:-

Plant of maximum growth was selected and transferred from Petriplate to the soil and tagged for recording various morphological observations at different concentration of nanoparticles .

Result obtained (Table 2) signifies that silver nanoparticles treated with Chana plant at different

concentrations such as 100 mg./L, 200mg./L and 500mg./L exhibited significant effects on seed germination. We observed that % of germination of the seed treated with control was 65% and those treated with AgNPs at concentration 100mg./L was 50%, 200mg./L was 70% and 500mg./L was 80%.

The corresponding growth of Cicer arietinum in 15 days are shown which demonstrate that by increasing the concentration of AgNPs, it caused more growth in the Chana plant as compared with the plant treated with control.

So , the silver nanoparticles shows fast growth in root length and shoot length as compared with control.

Plant colour:- The colour of plant leaves estimated that by increasing the concentration of AgNPs, plant shows more healthier colour as compared to the plant treated with control was yellowish green.

Further, plant treated with AgNPs at 100 mg./L was light green, at 200mg./L was dark green and 500 mg./L was more dark green then 200 mg./L.

It shows that by increasing the concentration of AgNPs we can obtain more healthier plant.

Root and shoot height:- There was significant improvement in shoot height of *Cicer arietinum.* Shoot height ranges from 5.5 to 9 cm.

Plant treated with 500mg./L AgNPs had highest shoot length.

The root of plant treated with control (4.5) has recorded the lowest root length followed by different concentrations of AgNPs such as 100mg./L (4.9 cm.), 200 mg./L (5cm.), 500 mg./L (6cm.).

So, by increasing the concentration of AgNPs we observed more root and shoot growth.

Plant height:- It shows that the plant treated with AgNPs of concentration 500 mg./L had highest plant height which is 14 cm., plant treated with AgNPs of concentration 200 mg./L had plant height which is 12 cm., plant treated with AgNPs of concentration 100 mg./L had plant height which is 10 cm. While plant treated with control had lowest plant height which is 9 cm.

So, by increasing the concentration of AgNPs we observed maximum rise in the plant height.

Plant Biomass:- There was a significant change in plant biomass treated with different concentrations of nanoparticles .

Plant fresh weight:- The fresh weight of plant ranges from 25 mg.- 29 mg., among them the untreated control (25mg.) had lowest plant fresh weight followed by weight of plants treated with AgNPs at different concentrations are 100 mg./L (26mg.), 200mg./L (27mg.) and 500 mg./L (29 mg.).

Plant dry weight:- For remaining any surface moisture, plants were allowed to dry in a hot air oven. Then weighing was performed. The dry weight of control (20 mg.), plant treated with AgNPs with concentration 100 mg./L (22 mg.), 200 mg./L (24 mg.) and 500 mg./L (26 mg.) significantly observed. It shows by increasing the concentration of AgNPs, plant's fresh weight and dry weight are increased.

Table 2. Morphological result of *Cicer arietinum*

Sl. No.	Physical parameter	Non treated control	Treated silver nanoparticles at different concentration		
			100 mg./L	200 mg./L	500 mg./L
1	% of germination	65%	50%	70%	80%
2	Seedling vigour index	1200 cm.	1000 cm.	900 cm.	800 cm.
3	Day of emergence of 1st leaf	6th day	5th day 4	4th day	3rd day
4	Number of leaves	5	6	7	8
5	Colour of leaf	Yellowish green	light green	dark green	dark green
6	Plant height (cm.)	9cm.	10cm.	12cm.	14cm.
7	Shoot length (cm.)	5.5 cm.	5.8 cm.	6 cm.	9 cm.
8	Root length (cm.)	4.5 cm.	4.9 cm.	5 cm.	6 cm.
9	Plant fresh weight(mg.)	25 mg.	26 mg.	27 mg.	29 mg.
10	Plant dry weight (mg.)	20 mg.	22 mg.	24 mg.	26 mg.

Above morphological results signify that by increasing the concentration of AgNPs we can obtain more healthy plant, having qualities such as more root and shoot growth, fast plant growth and increasing biomass of plant such as fresh weight and dry weight. So, we can use AgNPs in agricultural field as eco-friendly fertilizers which can increase the yield and develop the seed qualities.

Conclusion :

The present study shows that the leaves and stems extract of *A. aspera* can be used as an excellent source for synthesizing the silver nanoparticles . Green synthesis of nanoparticles can be ecofriendly, involved in many applications of clinical and biomedical sectors.

UV-Vis spectroscopy revealed the surface plasmon property. The structural analysis by XRD strongly suggest the formation of elemental silver NPs instead of their oxides in biosynthesized NPs.The XRD structural analysis of AgNPs showed that they were crystalline in nature, which might be due to the presence of bioreduction of silver. Biomolecules were responsible for reducing and capping of silver, which were confirmed by FTIR measurements.

This green method resulted many advantages such as being ecofriendly, low cost production and large scale synthesis of silver nanoparticles.

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