JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Biogenic synthesis of silver nanoparticles from *Leucas aspera*, *Cyperus rotundus*, *Cynodon dactylon* leaf extract and their effects on chickpea germination

Ishwarya M S[1], N.C.Horti[2], M.D.Kamatagi[3], Lokesh S[1*]

[1]Department of Biotechnology, University of Mysore, Mysuru-570 005, Karnataka India.

[2,3]Department of physics, SS Government First Grade College and P.G study Centre, Nargund-582 207, Karnataka India.

[*]Corresponding author: e-mail: boramma@rediffmail.com

Abstract: In the current research**,** the leaf extracts of *Leucas aspera, Cyperus rotundus and Cynodon dactylon* were used to synthesize silver nanoparticles and their consequences on seed germination were examined. Synthesized AgNP's were subjected for the different characterization like X-ray diffraction, Scanning Electron Microscopy, UV-Vis absorption , Fourier Transform Infrared, and Raman spectroscopy. *Cicer arietinum* seeds were soaked in synthesized AgNP's for 24hrs and kept for germination under standard laboratory condition and germination was noted on daily basis. All the three AgNP's show the UV absorption spectra in wavelength range of 419-437nm. FTIR results show the presence and stretching vibration of different functional group of the samples. SEM examinations reveal the irregular shape with some spherical shape of the AgNP's. XRD graphs confirm the AgNP's crystallize in centered cubic phase and the change in peak intensity with different plant extracts were noticed. Treatment with the AgNP's increases the in seed germination and seedling growth rate compared with control. The biosynthesis AgNP's could be endorsed to improve germination rate of the seeds.
Index terms: Silver nanoparticles, biogenic synthesis, Germination study, Chickpea seeds

I. Introduction

During the previous two decades, nanomaterials are largely studied in numerous of disciplines notably pharmaceuticals, biosensors, catalysis, biomedicines, electrochemistry, food technology, and cosmetics. Nanomaterials are constricted to nanometer scale(less than 100nm); neoteric properties like smaller size and a higher surface-to-volume ratio are some of remarkable features which differs from their bulk materials. Biologically synthesized nanomaterials is one of the prominent choice by researchers due to its wide variety of applications like antibacterial activity, antifungal, antiviral, anti-inflammatory, catalytic, pharmacological components, diagnostic testing, biosensors, and numeric cancer therapy and others [1,2,3]. Sliver nanoparticles stand out among all other biologically synthesized metal nanoparticles because of its unique electrical, photochemical properties including distinctive optical properties linked with surface plasmon resonance,good ctalalytic activity, high electrical capacitance and many more [4, 5].

Chemical and physical methodologies have obstacles, such as cost, environmental friendliness, high energy consumption, high temperature, and nasty chemicals. Biological nanoparticles formation helps to overcome these barriers. For an improved experimental fabrication technique, combine ecofriendly resources such as bacteria, fungi, and plant extracts extends nanoparticles application in medical and agriculture fields [6, 7]. Plant-based nanoparticles production has an edge over other biological sources specially microorganism, due to monodispersed enhancement, more consistent and there is no need for frantic treatment (isolation, growing, and preservation of microorganisms) for extracting nanoparticles [8]. Accelerating the implementation of nanomaterials in most of the medical and agricultures industries owes greater impacts on economy, human health, agricultural products and environment. Currently its necessary to remodel the face of agriculture with the new tools of nanotechnology are explored to increase the ability to absorb nutrients in plants and also increase resistant to disease, pest attack as the continuous of insecticides, pesticides have left irreversible environmental and human health consequences.

The research is gradually gearing up on the application of nanomaterials in agriculture field. It is necessary to illuminate the mode of interaction, uptake, and accumulation in the ecological systems as agriculture is basic of any country. As nanoparticles and their effects on plants are contested in the academic papers since they are altered by nanoparticles size and concentration, plant variety and their lifespan, experimental parameters such as temperature, time, and exposure method, and nanoparticles size and concentration [9]. Some of the studies that support the positive influence of nanomaterials characteristics on crop plants include improved germination of seeds, root and shoot length, seedling biomass, photosynthetic capacity, and nutrient uptake [10,11]. At low concentrations of nanoparticles, nano-TiO_2 improves in photosynthesis and nitrogen metabolism, leads to increased spinach growth [12,13]. *Capsicum annum* L. seeds treated with nano-TiO_2 showed a considerable rise in seedling germination %, sprouting rate index, cotyledon fresh weight, and vigour index even at higher concentrations [14]. Abou-Zeid and Moustafa found that introducing AgNPs to barley grains heightened germination, as well as shoot length, fresh and dry weight, but decreased fresh and dry weight of the sprouts [15]. Srinivasan and his co-workers illustrated that diffusion of nanoparticles through thick seed is difficult when compared to movements of nanoparticles through plant cell membrane and cell wall [16]. In comparison to control seeds treated with deionized, carbon nanotubes were strong enough to break through the thick seed coat of tomato seeds, allowing them to germinate and develop rapidly reported by Khodakovskaya and his collaborators [17]. The blend of nano SiO_2 and nano TiO_2 promoted soy bean *(Glycine max)*, seed germination and growth by elevating nitrate reductase activity rate, is studied by Lu *et al* [18]. At optimum concentrations, Sharma *et al* demonstrated silver nanoparticles were capable of promoting fresh weight, root, and shoot length in *Brassica juncea* seedlings, and germination rate [19]. Kaveh *et al*. showed the treating *Arabidopsis thaliana* plants with sliver nanoparticles at low concentration increased seed biomass, while higher concentration inhibited the growth [20]. On the other hand, same studies have revealed, nanoparticles due asserts some of negatives effects on plants. The aquatic plant *Lemna gibba* was shown to be growth-restricted, with a significant AgNP concentration-dependent decline in the number of leaves [21]. In *Brassica nigra* it was observed that sliver nanoparticles inhibited germination, seedling growth, lipase activity, and uptake of soluble sugar [22]. In *Arabidopsis sthaliana*, the phytotoxicity of AgNPs was examined, and it was reported that AgNPs were apoplastically transported and aggregated at plasmodesmata [23].

The present study reports the synthesis of silver nanoparticles using *Leucas aspera*, *Cyperus rotundus* and *Cynodon dactylon* plant extract. These elegant perennial plants species are most sustainable, locally available, and they are competitive with most the crops for water, sunlight, nutrient and space which makes it a serious weed and also using of plants is cost effective, reproducible, environmental friendly. These plants rich in phytochemicals like flavonoids, alkaloids, tannins and triterpenoids. The consequences of the synthesized materials silver nanoparticles from the preceding plants on *Cicer arietinum* seed germination were investigated.

II. Materials and Methods:

Silver nitrate(($AgNO_3$), which was employed in this study was purshed by Sigma-Aldrich and then utilized, without any alteration *Leucas aspera*, *Cyperus rotundus and Cynodon dactylon* plant species were collected randomly from the University of Mysore in Mysuru, India.

2.1. Scheme of Plant Extracts Preparation

To begin, the plant material was sanitized until all surface-adsorbed contaminants were removed, tap water was used. As a consequence, they were deionized double distilled water rinsed twice, roughly chopped, and dried in the shade. Aqueous heat extraction was used on the plants. In a 500 mL Erlenmeyer flask, 25g of all three dried leaves smaples were combined with 100 mL distilled water and heated for 30 min with stirring using magnetic stirrer. After decanting the extract, Whatman filter paper was used to filter it before it was refrigerated at -4°C.

2.2. Biogenic synthesis of silver nanoparticles

In a standard synthesis approach, 1 mM $AgNO_3$ was added to 100ml of double distilled water. On constant stirring, 50 mL of leaves extract was added to 100 mL of 1 mM $AgNO_3$ solution at temperature 70ºC. Then reaction mixture was allowed to cool to room temperature. The variation in colour of the reaction mixture from pale yellow to reddish brown confirms the formation of AgNPs. Thereafter AgNP's were washed with distilled water and ethanol for 5-6 times and centrifuged at 10000 RPM for 10 min. The AgNPs in the form of pellet was freeze dried and stored at − 4 °C until use. Here we coded AgNPs synthesized using *L. aspera*, *C.rotundus* and *C. dactylon* plant extract as LA, CR and CD respectively. The flow diagram for synthesis of Ag nanoparticles is given in Figure 1.

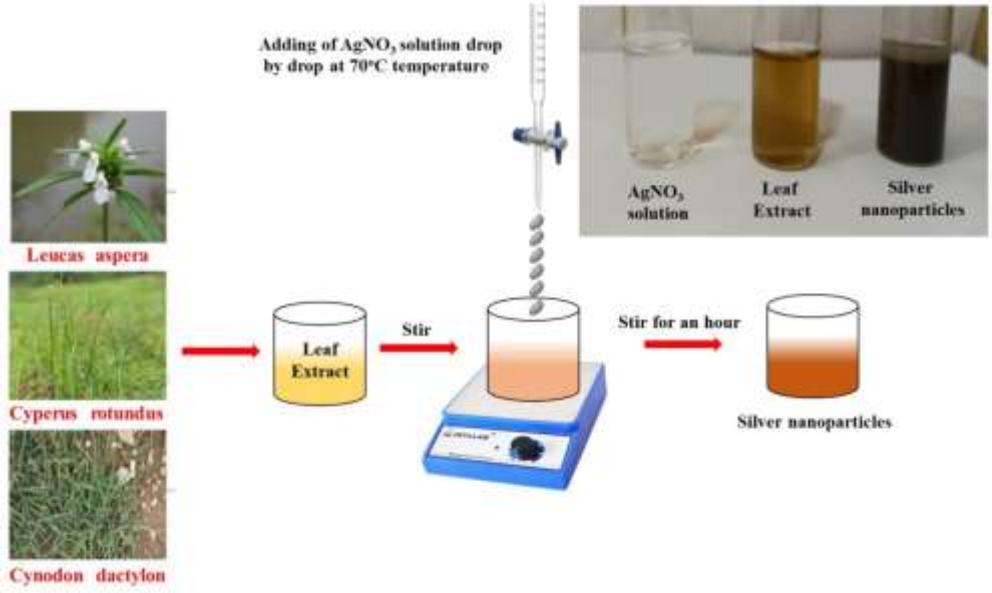

Figure 1. flow diagram for synthesis of silver nanoparticles.

2.3. Characterization Techniques

Scanning Electron Microscopy, Fourier Transform Infrared spectroscopy, X-ray diffraction and Raman spectrocopy were used to make structural and surface analysis of synthesised AgNP's. Optical characterization of samples were carried out by UV absorption spectrophotometer. The phase identification and crystallinity of the metal nanoparticles were made using X-ray diffractometer (Rigaku, BD63000074). External morphology and elemental analyses were evaluated using a Hitachi Japan-S-3400N Scanning Electron Microscopy with connected EDS. The elemental composition of silver nanoparticles and the availability of functional groups are investigated by FTIR spectrophotometer (Nicolet-6900, Model: 912A0637). Beckman-Coulter DU-730, USA, UV–vis spectrophotometer was used to measure the absorption spectra of samples. Raman spectra of sliver nanoparticles were measured by HORIBA, Xplora Plus- 42308.

2.4. Preparation and conditions for seed germination

Cicer arietinum L. seeds were obtained from a local vendor shop in Mysore district, Karnataka, India, and employed to measure the accuracy of sliver nanoparticles on seedling growth following design guideline with the appropriate modifications (International Seed Testing Association, 1976). Chickpea seeds were surface disinfected for ten mins by submerging them in a 10% sodium hypochlorite solution after which seeds were cleaned twice with deionized water to make sure that they were surface sterile. For about 2 hours, disinfected seeds were incubated in silver nanoparticles, plant extract, and distilled water. The seeds were therefore transferred to petri plates with filter paper that had been sprayed (control-5 ml deionized water, plant extract-5 ml, and Ag–NPs (1 mg / l, w/v) - 5 ml). As a treatment. the four seeds are kept in each plate and the experiment was done in triplicates. In a growth room the sealed petri plates were kept at 28±2° C. At periodic times, data on seed germination percentage and mean seedling length were recorded (2, 4, 6, 7days). The germination was interrupted on the seventh day, the seed germination rate was determined, and the seedling's root length was assessed. The germinated cotyledons with germination root and shoot were smashed using PBS. 1ml of PBS was used for 3 seeds and samples were prepared in triplicate in each groups of nanoparticles treatment with respected control.

III. Results and Discussion

3.1. UV-Visible Spectrometer analysis

To know the optical behaviour and stability of Ag NPs in dilute environment, the absorbance curves of biosynthesized nanoparticles was monitored and visualized in Figure 2(a-c). The absorption peak at 419 nm is found for LA sample and that of samples CR and CD at 421nm and 437nm respectively. The abundance of phytochemical compounds present in plants could be a contributor in the reduction of silver nitrate to silver nanoparticles in this process. The reduction of Ag^+ ions generates the surface plasmon resonance and holds good with findings by reference [24]. Similar absorption peak for biosynthesis of AgNPs was observed by Sudhakar et al [25].

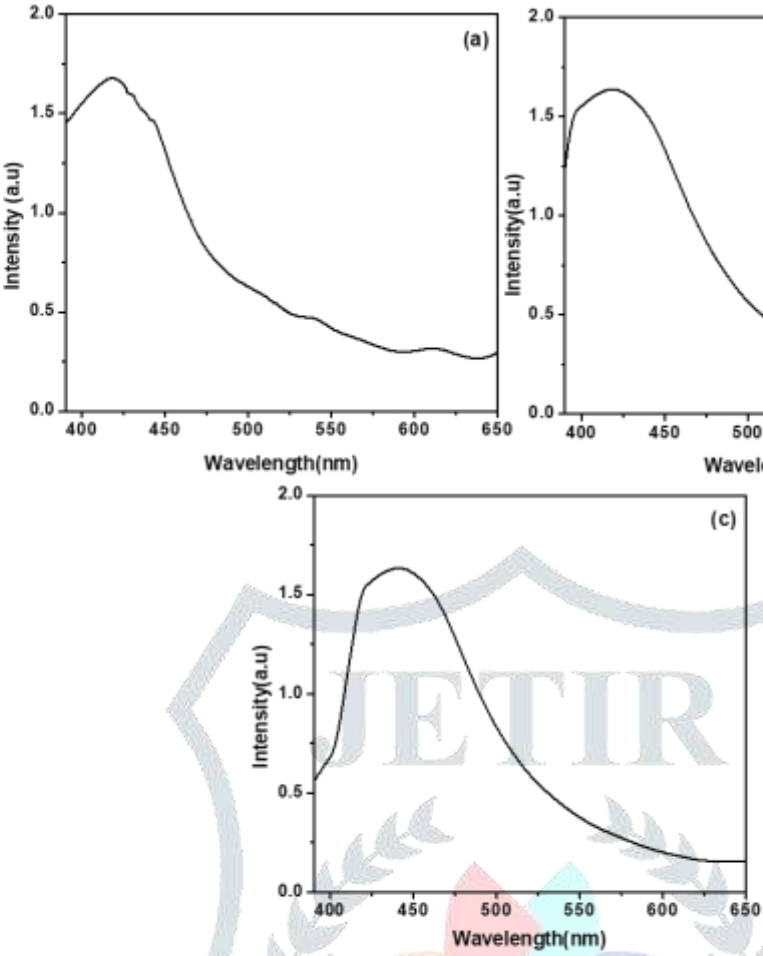

Figure 2. UV-Visible spectrometric analysis of AgNP's, (a) LA (b) CR and (c) CD samples

3.2. Dynamic scattering light analysis

The histogram of DLS analysis for particle size distribution of *L. aspera*, *C. dactylon*, *C.rotundus* AgNPs and are presented in Fig.3 and Table 1. DLS analysis being one of the reliable technique for the characterization of nanoparticles in solution for particle size, distribution and zeta potential as well. The interaction of biosynthesized nanoparticles with the biological systems is controlled by the surface charge distribution (zeta potential). In the present study the biosynthesized AgNPs shows an average diameter of 89, 21.49 and 181.5 nm, respectively, in the aqueous colloidal solution.

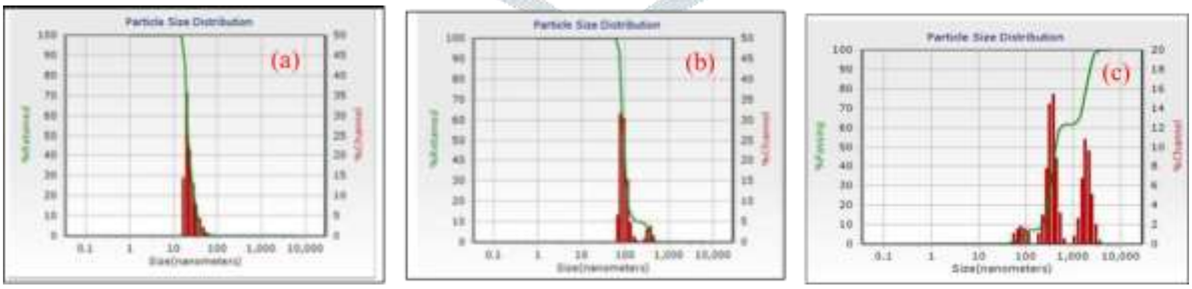

Fig 3(a-c). DLS analysis of silver nanoparticles.

Table.1: DLS particles size analysis

Compounds	Peaks Summary		
	Dia(nm)	**Vol%**	**Width**
L. aspera	89	90	32.6
C. dactylon	21.49	100	12.01
C.rotundus	81.2	8.2	48.3

3.3. SEM analysis

Surface roughness and composition of samples is studied using Scanning Electron Microscopy attached with EDX spectroscopy. SEM images of LA, CR and CD samples are shown in figure 4(a-c). SEM micrograph of all samples demonstrates the particles are irregular shape and some spherical like structures are noticed. This may be due handiness of different quantity and nature of capping agent present in plant extract.

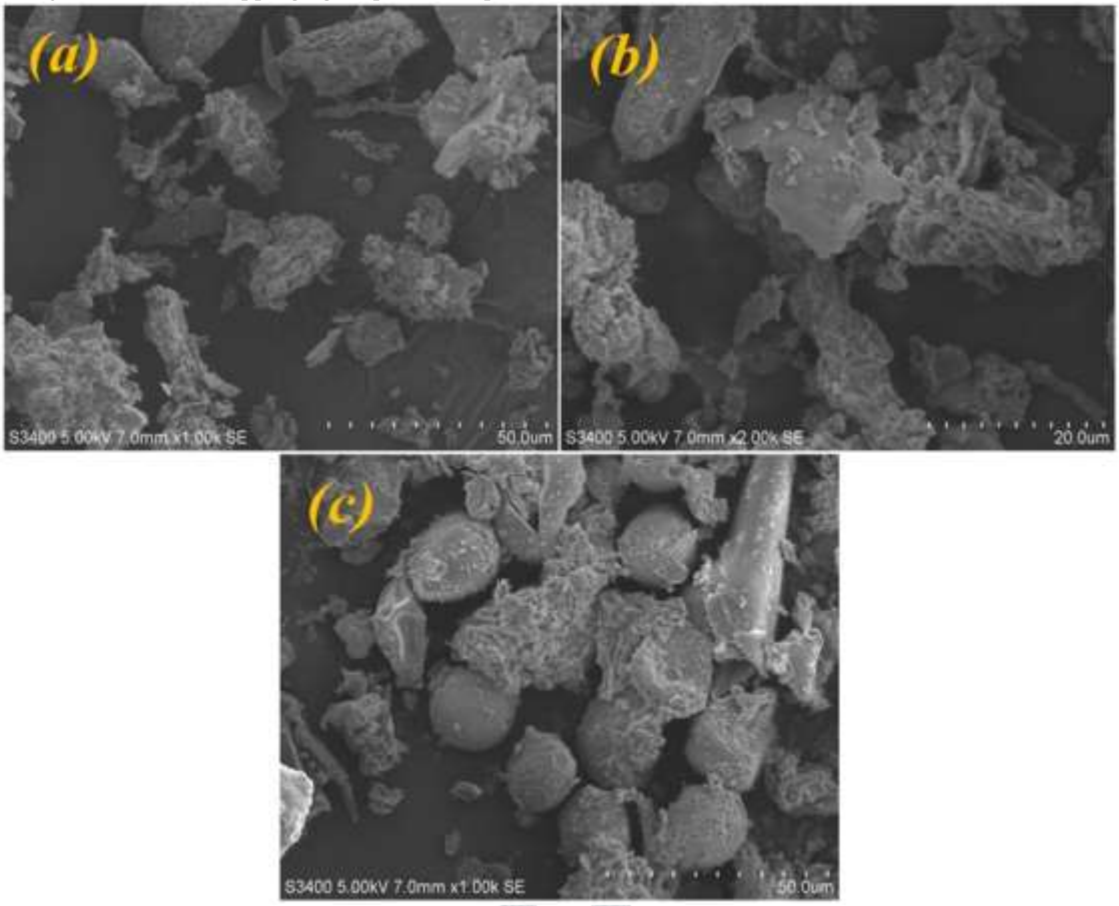

Figure 4 (a-c).SEM micrographs of silver nanoparticles.

3.4. Impact of silver nanoparticles on seed germination

To study the effect of synthesised nanoparticles on germination rate of seed, we have soaked seeds into the solution of plants extract and sliver nanoparticles. The seeds were tracked for 7 days to see how they grew in the seedlings. Seedling growth of seeds treated with plant extract began on the third day of observation, just as it did in the control group. (untreated seeds). In figure 5(a-c) and (e-f), we show the effect of synthesized nanoparticles and LA, CR, and CD plant extracts on seed germination rate.. The LA and CD (Fig.5(a,c)) samples have favourable influence on the growth and development of chickpea, while seeds treated with CR sample germination was slowed down in specific (Fig.5(b)). Our studies affirm that chickpeas seeds treated with LA and CD samples have influence that promotes growth by including lengthened roots and initation of shoots formation early. The higher seedling length, seed germination percentage (93%) and seedling vigor index may be due to the absorption of nutrients and the diffusion of nanoparticles into the seed accelerated the growth. Similar studies were reported by Pandey et al [26]. They elaborated the influence of carbon nanotubes on growth rate of *Cicer arietinum* seeds Tripathi and co-workers [27] studies shows the zinc is an important nutrient for plants and ZnO has a significant effect on plant development rate. The seeds treated with CR samples results in twisted and root deformation indicates the misperception of nanoparticles toxicity. Similar conclusions have been drawn by Navarro et al.,2008, plant toxicity is caused by AgNP interactions with cells, which are altered by nanoparticle size and coating [28]. The synthesized nanoparticles promotes growth rate of seeds as compared to plants exctract because of higher rate of biomass production and water uptake. The effect of nanoparticles on growth rate varies with nature of plant, the particles physico-chemical properties (size and shape), and experimental settings (concentration, exposure methods, exposure times). Figure 6 depict the plot of seed treatments versus root length. The seeds were divided into 3 sets with 4 seed each, first set of seeds were treated with only double distilled water, 2nd set were treated with plant extracts, 3rd set with sliver nanoparticles The seeds treated with plant extracts detectable decrease in growth in root length while sliver nanoparticles show significant increase in length of roots.

To detect the existence of the silver nanoparticles inside seed embryos, we made Raman measurement. In figure 7, Graphs (a-c) and (d-f) respectively, represent the Raman spectra of Ag NPs synthesized using plant exctract and plant extracts(LA,CR and CD). The Raman analysis of seeds treated with plant extracts show the peaks of aromatic and alkenes present in the sample. The peaks observed at 2791.93cm^{-1} and 1500 cm^{-1} in Raman spectra of the seeds treated with silver nanoparticles confirm the absorption of nanopaticles into seeds , intensity of peaks relates to the number of nanoparticles present and have impact on seed germination. Chickpea seeds were found to be capable of uptaking sliver nanoparticles and influencing their biological activity by increasing moisture penetration during the germination period, The detailed mechanism is unclear yet. In detail investigation of influence of synthesized nanoparticles on germination rate is underway.

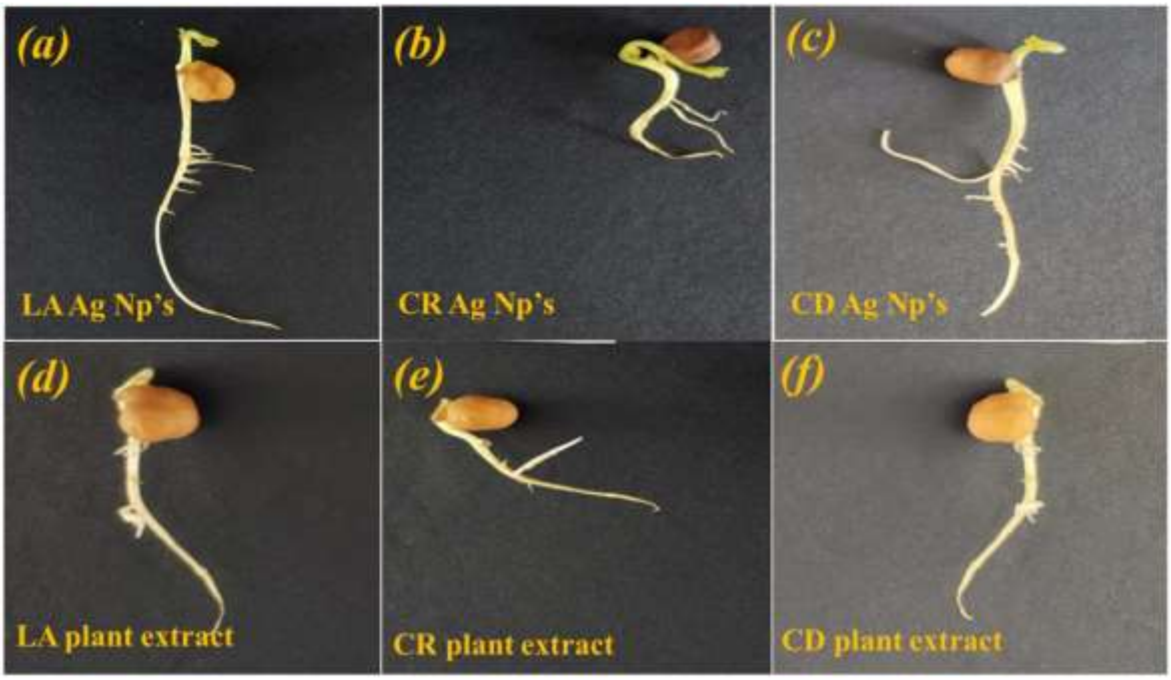

Figure 5. Effect of synthesized AgNP's on germination of chickpea seed. Images (a-c) and (e-f) respectively, represent the effect of silver nanoparticles and plant extract on germination of seed.

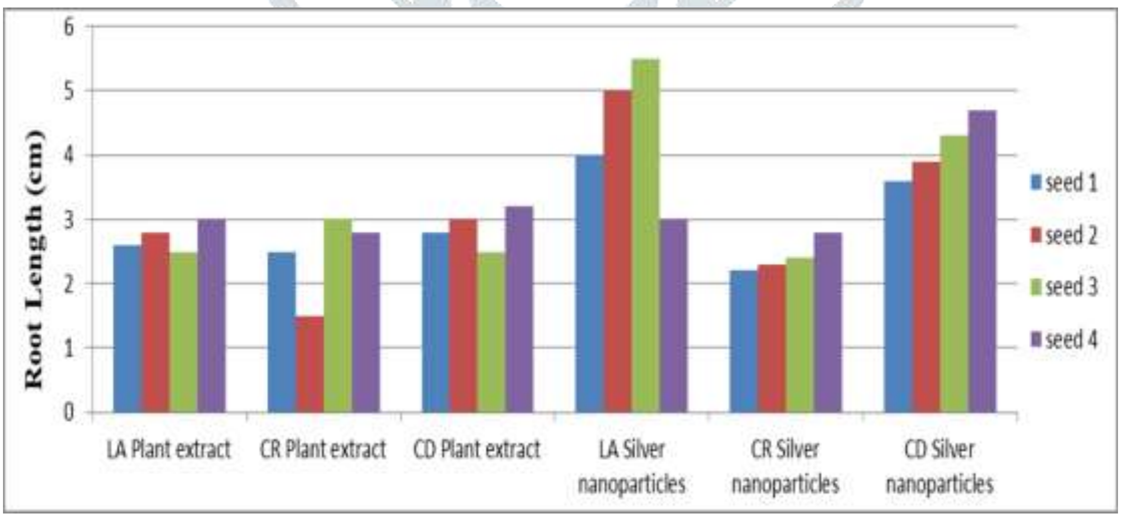

Figure 6. Histogram of root length (cm) of *Cicer arietinum* seed germination.

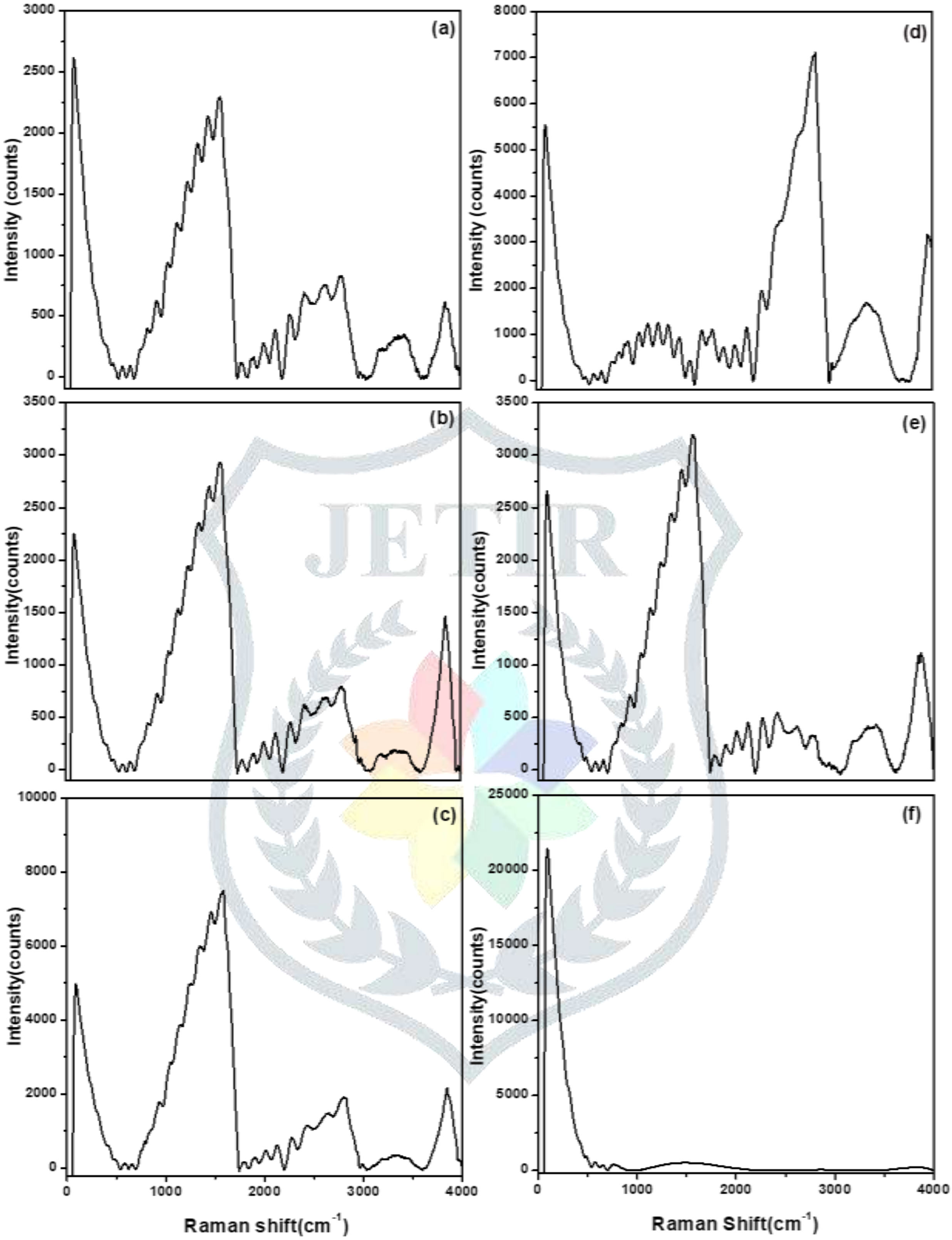

Figure 7. Raman spectra of effect silver nanoparticles (a-c) and of plant exctrat (d-f) on germination of seed.

IV. Conclusion

In summary, *L.aspera*, *C.dactylon* and *C.rotundus* extract of leaves were used to successfully preparation of environmentally sustainable green synthesis of Ag NPs. Scanning Electron microscopy (SEM), Fourier transforms IR (FTIR) and UV−Vis spectroscopy were used to characterize the samples. SEM images show the formation of irregular and some spherical nanostructures. The UV-vis absorption peaks at the range of 419-437nm. FTIR verifies the formation and composition of components, and the findings of DLS are highly correlated.

The results of our seed germination study show the all three samples (LA, CR, and CD) have considerable seedling development and sprouting on *Cicer arietinum* L seeds. Synthesized AgNPs have been able to penetrate the seed's outermost layer and increase moisture levels, perhaps resulting in faster germination and increased biomass production. Among three samples, LA and CD samples have a greater impact on seed germination compared to CR sample. The positive effect of silver nanoparticles could have impact on economic importance for agriculture production. The use of nanoparticles in plant growth could be particularly beneficial in dry agricultural areas where water is scarce and must be conserved and used efficiently as they help in absorption of nutrients and transport within the plant cells. The mechanism involving progress of nanoparticles across the xylem, as well as the improved flow of fluid and ions either by the nanoparticles or through the porous external walls of linked nanoparticles,still remain unexplored. Hence the further studies are required for various agricultural applications.

V. Acknoweledgment

The authors are grateful to instrument facility center, Mysore University, Mysuru for providing characterization facility.

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