Original Research Article

Allelopathy effects **of Silver Nanoparticle synthesis by green method from *Pulcularia undulate***

Abstract:

Nanotechnology nowadays has good potential application in many fields, especially in agriculture and industries. This study investigated the allelopathic effects of aqueous extract of *Pulicaria undulate* and nanoparticle synthesis by eco-friendly method from silver nitrate and water extract *of Pulicaria undulate* on seed germination of two weeds species. Aqueous extract and silver Nanoparticles plant extract (AgNPs) had an inhibitory effect on seed germination of common (*Lepidium sativum,* and *Trifolium repens)* as compared with control. *Lpidum stavium* seed germination was inhibited by nanoparticle and water plant extract at 100% concentration at the end of 10 day incubation period, at low concentration 25% of aqueous extract and AgNPs the germination ratio of L. sativium reached (30% and 5%) where for *Trifolium repens* the germination ratio were (50% and 0% respectively). Nanoparticle are green synthesis by simple methods, using the aqueous extract of *pulclaria* leave and silver nitrate, the confirmation of nanoparticle was done by different methods UV-Vis spectroscopy (UV–Vis), Fourier transform infrared spectroscopy, (FT-IR), transmission electron microscopy (TEM). The UV-visible spectra revealed the absorption peak at 449 nm, while the emission peak of Ag was detected at 466 nm. SEM analysis revealed that the synthesis of Ag NPs was spherical in shape and has an average size (13.3-23.8 nm). The concentration of plant showed a dangerous role in size and dispersion of NPs, the decrease occurs at low concentration this confirms the plant are oxidizing in nature. Herein we suggest the use of nanoparticle synthesis from Ag nitrate and aqueous extract of *Pulicaria undulate* as herbicide instead of synthetic herbicide after further studies to confirm the results.

Kew words: Pulicaria undulate, herbicide, nanoparticles, TEM, allelopathy

1. Introduction

In the last 20 years nanoparticles have been studied by many researchers, photonic properties of silver particles are interesting in biochemical and biomedical usage. [1]

Nanoparticles play an important role in pharmaceutical, industrial, and biotechnological applications. Especially, silver nanoparticles have been studied and showed a potential antimicrobial, antiplasmodial and larvicidal activity [2.3].

The synthesis of the nanoparticle by biological methods using plant extract is eco-friendly alternatives method. Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the complicated process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis[4].

Nanotechnology has good potential for applications in agriculture, but there is still a long way down to reach the field. It is impossible to know all the details about how a nanotool will work in a particular crop, but we need to start with real field and in-plant tests. If we stay behind the limit of basic research only, the risk of agricultural nanotechnology becoming hype increases, hindering any further attempts for applications and research [5].

Pulcularia undulate is a traditional plant famous Southern Egypt and Saudi Arabia, people used to treat inflammation, and used as herbal tea. It is also used as an insect repellent [6] [7] studded the composition of oil of the fresh aerial parts of *P. undulata* (L.) kastel (from Saudi Arabia) by GC/MS. The oil is rich in phenolic compounds and monoterpene hydrocarbons and comparatively low in sesquiterpene hydrocarbons Arezou Ghahghaei 2014 **[**8], show that *P. undulata* extract has anti-aggregation properties, although *in vivo* the effectiveness of *P. undulata* extract remains to be investigated, it should be considered as a possible drug candidate or lead component of drugs to prevent or delay protein aggregation and amyloid disease. [8].

The role of allelopathy or secondary metabolites in overcoming arid conditions, in conjunction with their allelopathic behaviour, is still poorly understood. On the other hand, our current understanding of allelopathy has been successfully used to control weed populations via methods involving crop rotation, mixed cropping and essential oils [9].

The aim of the present study green synthesis silver nanoparticle from desert growing plant *Pulcularia undulate* and study the allopathic effect of Ag NPs (silver nanoparticles) on the germination of some harm weeds

Experimental

2.1. Materials and methods

Healthy leaves of githgath, scientific name *Pulcularia undulate* were collected from wade Arar from North region, (Figure 1). Arar is a region of Saudi Arabia, located in the North of the country. The plant was identified in the desert research centre and the authentic sample was kept in herbarium of DRC.

2.2. Preparation of Plant Extract

The Leaves of plant sample were dried in shade at room temperature for 3 days, then powdered using kitchen blender. Plant powder of dried clean sample 10 gm was mixed in 500 ml of distilled water and the mixture was boiled for 30 minutes then filtered by Whatman filter paper, the filtrate was kept under 4C° for further use .

***2.3.* Synthesis of silver nanoparticles**

For Synthesis silver nanoparticles, 1.5 ml, 1ml, 0.8 ml, 0.6 ml and 0.5 ml and 0.2 ml of plant extract was added to 5ml of 1 m $MAgNO_3$ solution in 250 ml flask and shaking for the bioreduction of Ag^+ to Ag^0. The formation of nanoparticles was preliminarily confirmed by color change from faint yellow to dark brown. The Mixture was incubated in the dark at 37°C until used for analysis.

2.4. Characterizations of Silver nanoparticles

The confirmation of Silver Nanoparticles synthesis was conducted at Advanced Materials and Nanoscale Research Laboratory**,** Ain Shams university by UV–visible spectra were recorded at room temperature using a Shimadzu 2600 spectrophotometer. UV-vis spectra of AgNPs and Ag ions were measured at 200 to 800 nm. [10]

***2.5.* TEM Analysis of silver Nanoparticles Analysis**

The size and morphology of the nanoparticles were examined by TEM images were obtained on a JEOL-1200JEM for the TEM measurements.

2.6. Allelopathic study

Germination tests were carried out in clean Petri dishes, according to the guidelines of the Association of Official Seed Analysis (AOAC, 1988).

The following parameters were measured:

Germination percentage (G%): calculated as the number of germinated seeds/total number of seeds× 100 according to ISTA, 1999).

The experiment was performed according to the methods described by (11), *Lepidium sativum,* and *Trifolium repens* seeds were germinated in Petri dishes 10 seeds were placed in each plate. The Petri dishes divided into two groups, one group was irrigated by 4 ml from AgNPS extract and other group irrigated by 4 ml plant aqueous extract, then incubated in the dark at 27C° for (48h), as control, the germinated seed was irrigated by distilled water. Each treatment was tested in three replicates. After incubation, the number of germinated seed was count and growth parameters were recorded.

***2.6.* Statistical analysis**

The data analyzed using analysis (ANOVA) test, the means of treatment were grouped on the basis of LSD at the 0.05 probability level.

3. Results

Pulcularia undulate plant extract was used for the synthesis of AgNPS under unsophisticated conditions. It was observed that the mixed of 0.5 ml of a plant extract with 5 ml of mM AgNO3, the colour begin to change after 2 hours at room temperature (27 C°) from yellow to brown which indicate the formation of AgNPs Fig 2

Under the similar condition, the change in the colour of other concentration occurs after 48 hours This colour change occurred also faster at 60 C° (in less than 30 minutes) than at room temperature. This observation agrees with data reported by (Mohan Kumar et al., 2012 **)** [12], where the speed of formation nanoparticles have been found to be increased by increasing temperature and incubation period [12].

The formation of silver nanoparticles (AgNPs) was monitored by UV-VIS analysis where AgNPs show absorption at visible range 380-450 nm, according to the shape and size of [13].

Fig. 1. Plant Picture of Plant

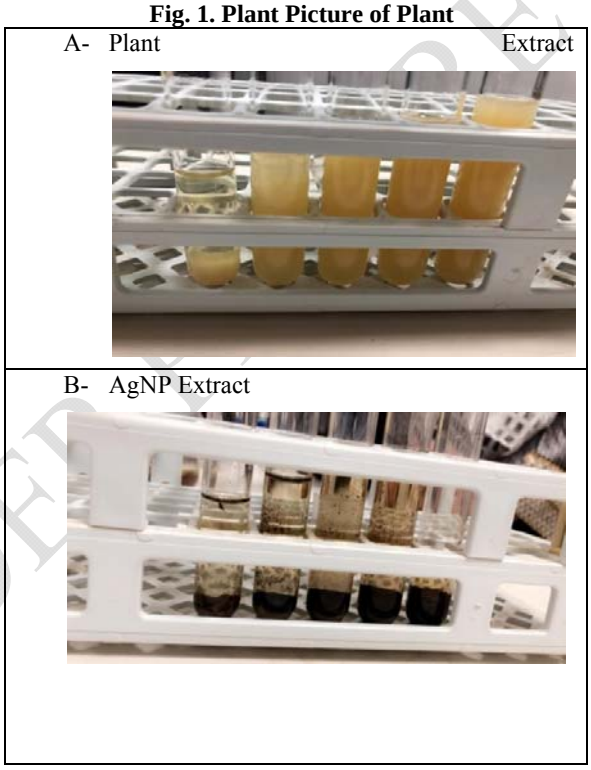


Fig 2: Colore change of Plant Extract before and after addition of AgNPs

3.1.2. **UV-VIS –Spectroscopy*:*** UV-Vis absorption spectroscopy was considered as an important tool in confirmation and monitor the synthesis process and stability of NPs solution. The absorption spectrum of NPs metals is affected by many factors, including particle size, shape and particles–particles particle–particle interaction with the medium. thus, the aqueous bioreduction of Ag+ ions can be efficiently monitored by UV-VIS. FIG 3, demonstrate the absorption spectra of AgNPs synthesis at different concentration, The results reveal that the beast concentration for synthesis AgNPs is 0.5 ml of plant extract with 5 ml $AgNO_3$, synthesis of AgNPs from *P. undulata* is

significantly faster at 0.5 ml and after 2 hours this the beast condition, which is visibly reflected by regular increase in the intensity of the surface plasmon resonance (SPR) and after 120 min there is no further colour change this indication for the completion of reaction. As the concentration of the plant extract increases, the absorption peak gets more sharpness and the blue shift was observed from 458 to 446 nm. The blue shifted and sharp narrow shape SPR band indicating the formation of a spherical and homogeneous distribution of silver nanoparticles was observed **[13].** It should be mentioned that the extract absorption has a maximum at about 400 nm and could contribute to the absorption of AgNPs at high extract concentration.

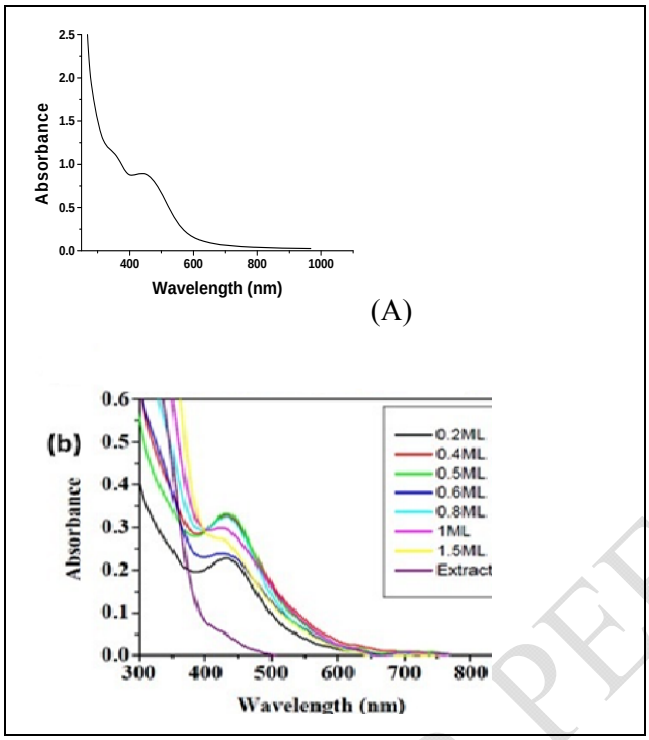
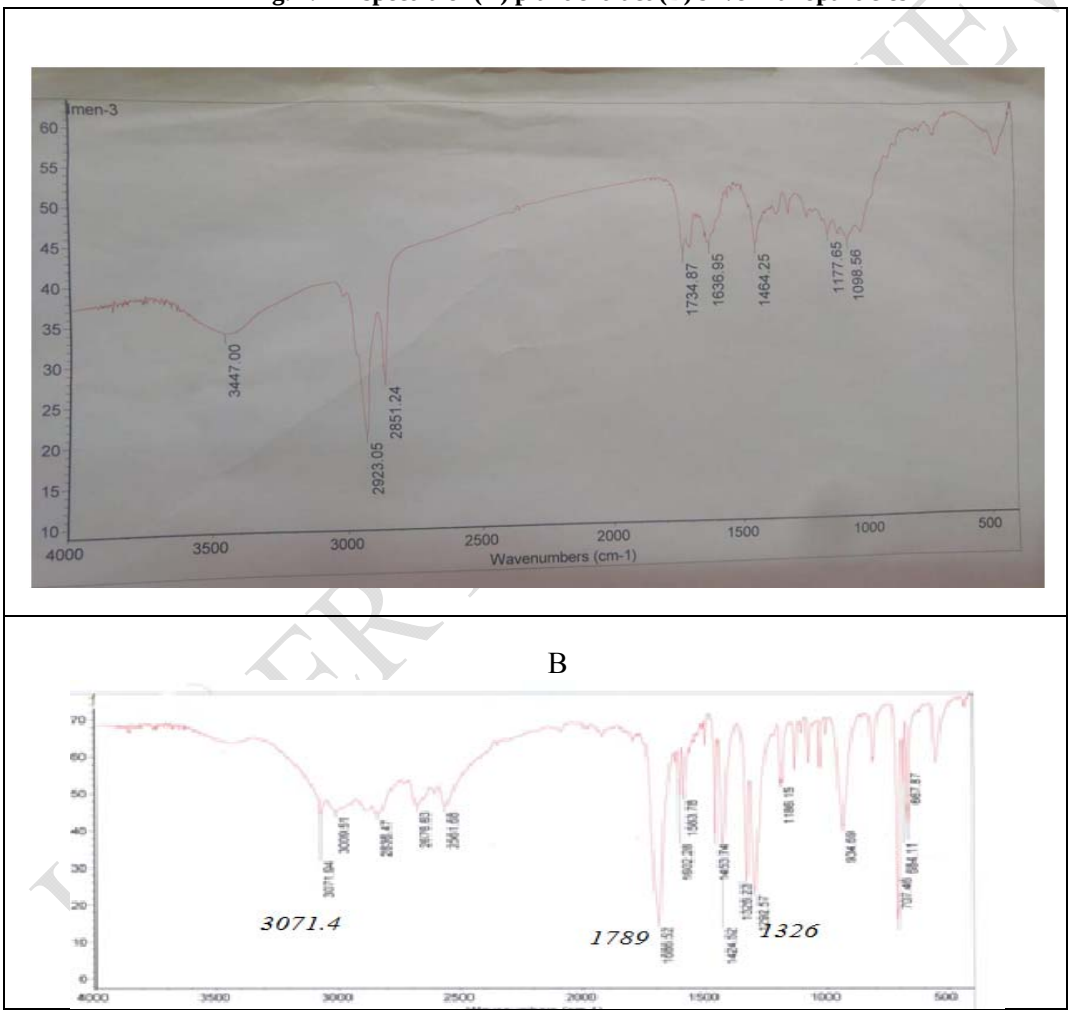


Fig. 3. (a) The colour change of silver solution formed using different concentrations of plant extract, (b)UV–vis spectra of silver nanoparticles using constant concentrations of extract 0.5 ml.

3.1.3. Fourier transform infrared spectroscopy (FTIR): FTIR spectrum was used to identify the possible functional groups of biomolecules of plant extract, that might be responsible for bioreduction figure 4, illustrated the spectrum of IR of plant extract which showed a broadband at 3447 cm^{-1}. This band attributed to the OH groups in the biomolecules. The IR bands at 2837 and 2852 cm^{-1} due to C–H stretching vibration modes in hydrocarbon chains. The IR bands at 1326 and 1789 cm^{-1}were characterized as C–O and C=O stretching modes of the carbonyl functional group. The stronger band at 1686 cm^{-1} was characterized as C=O of the amide groups of protein in the plant, Medium bands at 1072 cm^{-1} due to the C–O–C and C–OH vibrations are observed. In the case of AgNPs, a large shift in the absorbance peak with decreased band intensity was observed from 3071.4 to 3447 $cm^{-1}$and 1382.2 to 1320 cm^{-1}. The spectra also illustrate a prominent shift in the wave numbers corresponding to amide (1652.5–1600 cm^{-1}), validates that free amino (NH_2) groups in compounds of the plant extract have interacted with AgNPs surface making AgNPs highly stable.

Fig. 4. IR spectra of (A) plant extract (B) silver nanoparticles



3.1.4. TEM analysis:

Transmission Electron Microscope (TEM) analysis method used for determined the shape and size of biosynthesis NPs, from the image of silver NP at Fig 5 revealed that AgNPs have spherical shape and size range from (13.3-23.8 nm) are) in which few nanoparticles are agglomerated. The TEM images confirm the formation of the nanoparticles and the size also comes in agree with UV data.

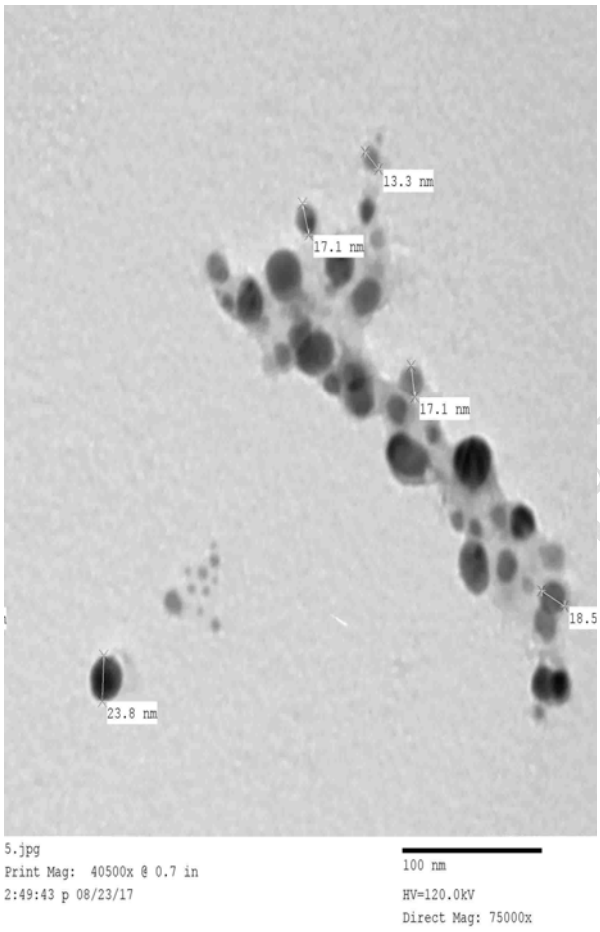


Fig. 5. TEM of AgNPs using plant *Pulcularia undulate*

3.4.5. In–vitro Allelopathic effect and seed germination:

The experiment was carried out to study the allelopathic effect of silver nanoparticle, and aqueous extract *Pulicaria undulate* on the germination of two weeds (*Lepidium sativum,Trifolium repens*). The effects of aqueous extract and nanoparticles on the germination and growth parameters of two harm weeds after 10 days in Petri dishes are shown in **Table 1 and Table 2**.

Both aqueous extract and AgNps, have been shown inhibition of seed germination compared with control, the inhibition ratio was time dependent on the concentration, where the 100% of aqueous extract and AgNPs give 100% inhibition of seed germination of both weeds. Nanoparticle (AgNPs) showed strong inhibition of two weeds *Lepidium sativum, Trifolium repens*), furthermore at low concentration.

Table 1. Effect of plant extract and silver nanoparticle on the germination of Lepidum sativum and *Trifolium repens*

Conc% **(1g /100 ml H_2O)**	***Lepidum sativum***		***Trifolium repens***	
	Germination % Plant extract	Germination % Nanoparticle	Germination % Water extract	Germination % Nanoparticle
100	0%	0	0%	0
75	13%	0	5 %	0
50	18%	0	25%	0
25	30%	0	50%	0

Table 2: Effect of plant extract and silver nanoparticle on the growth parameters of weeds

	Lepidum sativum		***Trifolium repens***	
CONC % **(1g /100 ml H_2O)**	Germination water extract %	Germination Nanoparticle %	Germination % water extract	Germination Nanoparticle %
	Shoot length			
100 %	0	0	0	0
75%	0	0	28%	0
50%	84%	0	83%	0
25%	88%	0	94%	0
	Water extract %Germination %	Germination Nanoparticle %	Water extract Germination %	Germination Nanoparticle %
	Root length			
100 %	0	0	0	0
75%	0	0	0	0
50%	68%	0	76%	0
25%	84%	0	88%	0

3.4.6. Seedling growth

The effect of aqueous extract and AgNPS on root and shoot length of two species under study showed the same effect on germination, the reduction in shoot and root length come in parallel with the increasing concentration as illustrated in

Table 2, The root length of Lepidum sativum and Trifolium repens, were significantly reduced by aqueous plant extract, which completely inhibited the root growth. In the application of low concentration, 25% of plant extract showed significantly increased root length, whereas the higher concentrations induced significant gradual reductions in root length. The effect of aqueous extract on shoot length show strongly reduction in the length of shoot, which gives indication the plant affect on the enzyme responsible on shoot elongation, the inhibition of shoot elongation of *Lepidum sativum* by plant extract is more strongly than *Trifolium repens*, the degree of inhibition of shoot and root increased gradually in parallel with increasing concentration of plant extract.

3. Discussion

Nanoparticles have been considered as an important area of research due to their unique and tunable surface plasmon resonance (SPR) and their applications in biomedical science including tissue/tumour imaging, photothermal therapy and antimicrobial [2]. So in this study we aimed to add other application of silver nanoparticles in industrial and agriculture field we aimed to synthesis nanoparticle, prepared by green method from aqueous extract of Pulclaria undulate and silver nitrate, and use as herbicide by study the role of nanoparticle in the inhibition of germination of two weeds which grow in crops and affect the production of crop. Allelopathy effect is phenomena can be used in inhibition of germination of weeds, some plant has allelopathic effect, inhibit the germination of some seed and stimulate the germination of other, as found by [14,15]. Hussain et al. reported that Allelopathy is a well-studied phenomenon in which chemicals released by one plant may have detrimental or inhibitory effects on the germination, Weeds interfere with crops by inhibiting their germination and seedling establishment [16].

Seed germination and seedling growth were more inhibited by **AgNPs** than aqueous extract, whereas, with an increase in concentration, there was a decrease in germination percentage and seedling growth. Germination of two weeds was affected slightly by the lower concentration of extracts, the shoot length, and root length also affected by the highest concentration. There is a clear reduction in shoot and root length of two weeds compared by control.

The study agrees with **(Ashfaq et al., 2014**) [17] who reported that the interaction between *C. esculentus* and *P. hysterophorus* showed a significant effect on germination rate, plumule length, radical length, fresh weight, and dry weight of seeds.

The formation of NPs was preliminary showed initial by colour change after the addition of aqueous plant extract to silver nitrate solution, the colour change from pale–yellow, then light brown to dark brown with reaction time due to excitation of surface plasma [18] the formation of NPs which confirmed also by (Uv-visible spectrophotometer and TEM).

4. Conclusion

The biosynthesis of Ag NPs using traditionally medicinal plant Bioreductive synthesis of Ag and Au NPs using a medicinally *Pulcularia undulate*, yielded spherical nanoparticles with average size (13.3-23.8 nm) with average size 18.5 nm. The inhibition of germination of Ag NPs and aqueous extract was studied on two weeds ***Lepidium sativum, Trifolium repens*** and revealed 100% seed germination inhibition for synthesis Ag NPs, and the inhibition goes in parallel with the concentration. The present study assessed the potential of nanoparticle for allelopathy on harm weeds. Many further studies recommended for identification of some bioactive compounds

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