Original Research Article

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

Abstract:

Nanotechnology nowadays has good potential application in many felid especially in agriculture and industries. This study investigated the allelopathic effects of aqueous extract of *Pulicaria undulate* and nanoparticle synthesis by ecofriendly 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 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 to (30% and 5%) where for *Trifolium repens* the germination ratio were (50% and 0% respectively). Nanoparticle are green synthesis by simple methods, using 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 average size (13.3-23.8 nm). The concentration of plant showed dangerous role in size and dispersion of NPs, the decrease occur at low concentration this confirm the plant are oxidizing in nature. Herein we suggest the use of nanoparticle synthesis from Ag nitrate and aqueous extract of *Pulicaria undulate* as herbside instead of synthetic herbicide after further studies to confirm the results.

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

1. Introduction

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

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

The synthesis of 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 a 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 nano tool 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 behavior, 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 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 desert research center and 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 whattman filter paper, the filtrate was kept under 4C° for further use .

2.3. Synthesis of silver nanoparticules

For Synthesis silver nanoparticules , 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$AgNO_3$ solution in 250 ml flask and shaking for the bioreduction of Ag^+ to Ag^0. The formation of nanoprticles was preliminary 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 Nano scale 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. Allophathic 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 number of germinated seeds/total number of seeds× 100 according to ISTA, 1999).

The experiment was performed according 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 two group, 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, germinated seed was irrigated by distilled water. Each treatment was tested in three replicates. After incubation, number of germinated seed were count and growth parameters were recorded.

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

Under similar condition, the change in the color of other concentration occur after 48 hours This color change occurred also faster at 60 C° (in less than 30 minutes) than at room temperature. This observation are agree with data reported by (Mohan Kumar et al., 2012 **)** [12], where the speed of formation nanoparticles have been found increase 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 shape and size of [13].

Fig. 1. Plant Picture of Plant

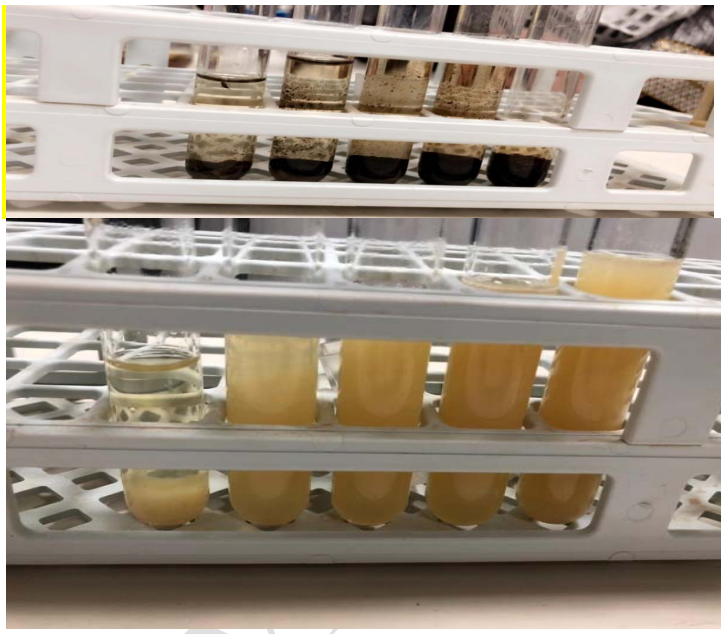


Fig 2: Colore change of Plant Extract before and after addition of AgNPs

3.1.2. **UV-VIS –Spectroscopy*:*** UV-Vis absorption spectroscopy was considered as 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 Ag NPs is 0.5 ml of plant extract with 5 ml $AgNO_3$, synthesis of Ag NPs 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 plasmaon resonance (SPR) and after 120 min there is no further color change this indication for the complete 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.

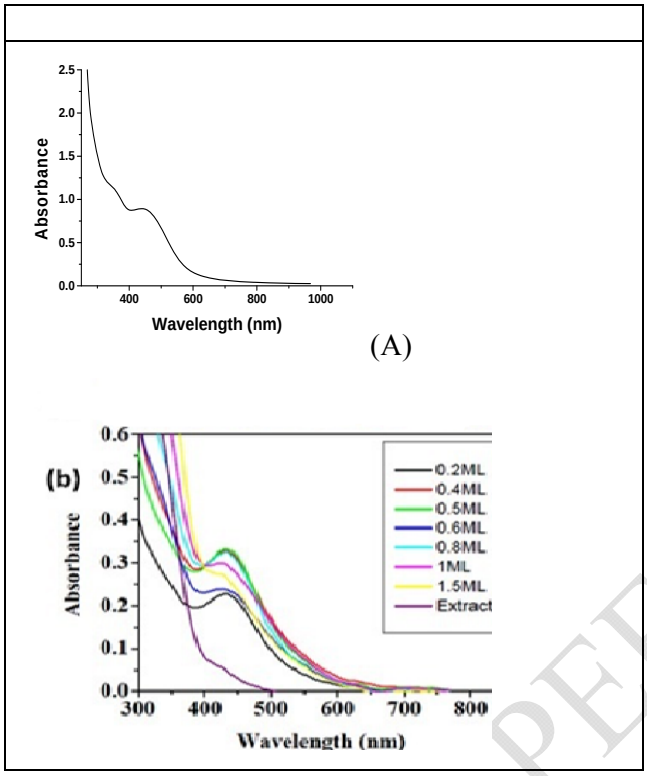
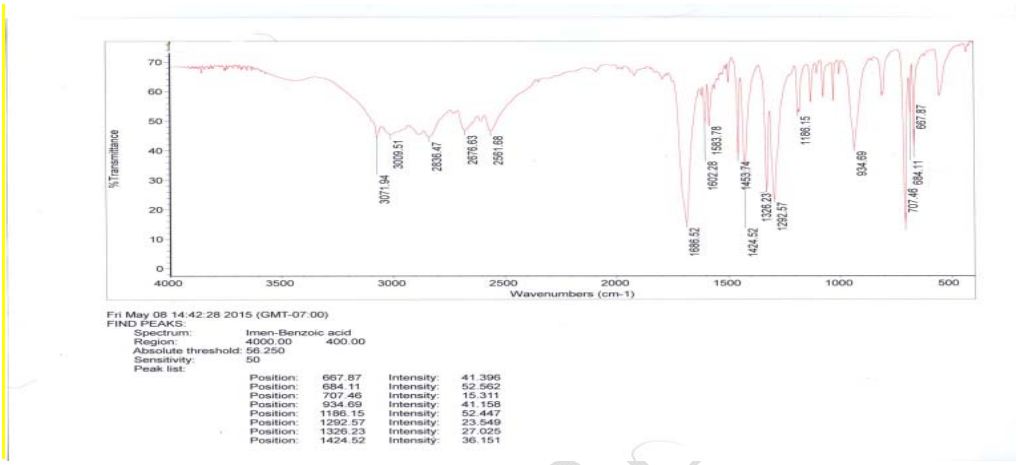


Fig. 3. (a) The color change of silver solution formed using different concentrations of plant extract, (b)UV–vis spectra of silver nanoparticles using constant concentrations of extract 0.5 ml

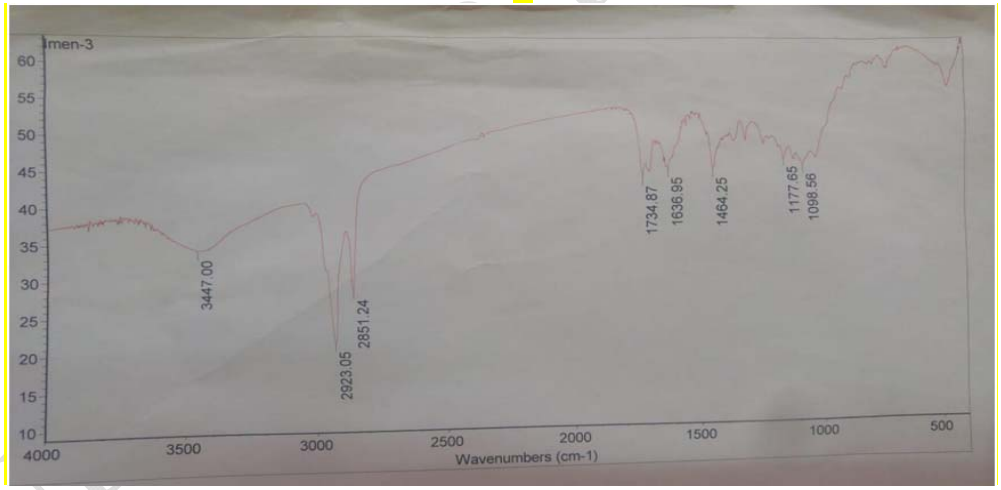
3.1.3. Fourier transform infra-red spectroscopy (FTIR): *FTIR spectrum was used to identify the possible function groups of biomolecules of plant extract, that might be responsible for bioreduction figure 4, illustrated the spectrum of FTIR of plant extract which showed a broad band at 3071cm^{-1}. This 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 plant, Medium bands at 1072 cm^{-1} due to the C–O–C and C–OH vibrations are observed. In the case of Ag NPs , a large shift in the absorbance peak with decreased band intensity was observed from 3071.4 to 3404.7 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) sliver nanoparticles

A



B



3.1.4. TEM analysis:

TEM (transmission electron microscope) analysis method used for determined the shape and size of biosynthesis NPs, from 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 nanoparticle are agglomerated . The TEM images confirm the formation of the nanoparticles and the size also come in agree with UV data.

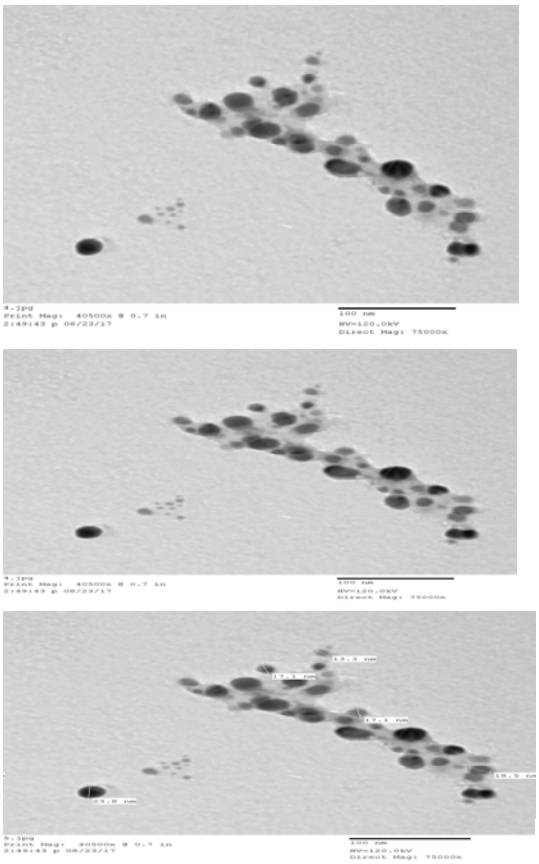


Fig. 5. TEM of Ag NPs using plant *Pulcularia undulate*

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

3.4.5. In–vitro Allophathic effect and seed germination:

The experiment was carried out to study the allophathic 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 Ag Nps, 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 strongly inhibition of two weeds *Lepidium sativum, Trifolium repens*), furthermore at low concentration.

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 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 give indication the plant affect on the enzyme responsible on shoot elongation, the inhibition of shoot elongation of Lepidum sativum by plant extract are 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/tumor 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 herbside by study the role of nanoparticle in the inhibition of germination of two weeds which grow in crops and affect the production of crop. Allophathy effect is phenomena can used in inhibition of germination of weeds, some plant have allophathic 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 **Ag NPs** than aqueous extract, whereas with 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 clear reduce in shoot and root length of two weeds compared by control.

Our study are agree 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 color change after addition of aqueous plant extract to silver nitrate solution, the color 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 were studied on two weeds ***Lepidium sativum, Trifolium repens*** and revealed 100% seed germination inhibition for synthesis Ag NPs, and the inhibition go in parallel with the concentration. Present study assessed the potential of nanoparticale for allelopathy on harm weeds. Many further study recommended for identification of some bioactive compounds

References

1 Krenn, J. R., Leitner, A., Aussenegg, F. R Metal Nano-Optics. In Encyclopedia of Nanoscience and Nanotechnology Nalwa, H. S., Ed American Scientific Publishers: Los Angeles, CA, 2003.

2 Saxena, A., Tripathi, R.M., Singh, R.P: Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity. *Dig J Nanomater Bios.,* 2010, **5** (2): 427-432.

3 Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T: Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surf B Biointer*., 2010, **76**: 50-56.

4 Sankar, R., Rizwana, K. Ultra-rapid photocatalytic activity of Azadirachtaindica engineered colloidal titanium dioxide nanoparticles. App nanosci. 2014.

5 Pérez-de-Luque A. Interaction of Nanomaterials with Plants: What Do We Need for Real Applications in Agriculture? Front. Environ. Sci. 2017, **5**:12. doi: 10.3389/fenvs.2017.00012

6. Elshiekh, Y.H, Abd. E.l. Moniem, M.A: Gas chromatographyemass spectrometry analysis of Pulicaria crispa (whole plant) petroleum ether extracts. Am J Res Commun., 2005, **3**:58e67.

7 Mossa, J.S., Hifnawy, M.S., Al-Yahya, M.A, Al-Meshal, IA., Mekkawi, AG: GC/MS analysis of essential oils of *Pulicaria arabica* and *P. undulata*. Pharm Biol. **1987**, 25:113–119.

8 Ghahghaei,A., Valizadeh, J., Nazari, S., Ravandeh, M: Chaperone Potential of Pulicaria undulata Extract in Preventing Aggregation of Stressed Proteins, AAPS PharmSciTech, 2014, **15**, No. 3.

9 Schulz, M., Marocco, A., Tabaglio, V., Macias, F.A., Molinillo, J.M.G: Benzoxazinoids in rye allelopathy–from discovery to application in sustainable weed control and organic farming. J Chemical Ecol. 2013, **39**: 154–174.

10. Masoud, E., A.Al-Hajry, M., Al-Marrani, A: Antibacterial Activity of Silver Nanoparticles Synthesized by *Sidr (Ziziphus spina- Christi)* Leaf Extract against Pathogenic Bacteria, *Int.J.Curr.Microbiol.App.Sci.,* 2016, ***5***(4): 226-236 226.

11 Wu H, Pratley J, Lemerle D, Haig T, An M (2001) Screening methods for the evaluation of crop allelopathic potential. Botanical Review 64: 403–415.

12 Mohan Kumar K, Sinha M, Mandal BK, Ghosh AR, Siva Kumar K, Sreedhara Reddy P. Green synthesis of silver nanoparticles using Terminalia chebula extract at room temperature and their antimicrobial studies. *Spectrochim Acta A Mol Biomol Spectrosc*. 2012;91:228–233.

13 Sathishkumar, G., Gobinath, C., Karpagam, K., Hemamalini, V., Premkumar, K., Sivaramakrishnan, S: Phyto-synthesis of silver nanoscale particles using Morinda citrifolia L. and its inhibitory activity against human pathogens. *Colloids Surf B Biointerfaces*. 2012, **95**:235–240.

14 Sardans, J., Peñuelas, J : The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. Plant Physiol., 2012, **160**: 1741–1761.

15 Teerarak, M., Laosinwattana, C., Charoenying, P., Kato-Noguchi, H : Allelopathic activities of *Jasminum officinale* f. var. grandiflorum (Linn.) Kob.: inhibition effects on germination, seed imbibition, and α-amylase activity induction of *Echinochloa crus-galli*(L.) Beauv. African J Biotechnol., 2012.,. **11**: 7850–7854.

16 Hussain, M., Farooq, M., Shahzad, M., Basra, A., Lee, D.J : Application of *Moringa* allelopathy in crop sciences. Allelopathy., 2013, 469–483.

17 Ashfaq, M., Ali1, A., Haider, M.S : Allelopathic Association Between Weeds Extract and Rice (Oryza sativa L.) Seedlings, Journal Of Pure & Applied Microbiology,. 2014 8(Spl. Edn. 2), p.573-580

18 Khalil, M.M.H: Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem., 2014, **7**: 1131-1139.