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Original Article

Weed Control of Cuscuta by Silver Nanoparticle Phytosynthesis Extract of *Rhazya stricta* Plant

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ABSTRACT

The study of Weed control of Cuscuta by silver nanoparticle photosynthesis extract of *Rhazya stricta* plant was conducted in two separate experiments as a completely randomized design in three replications in the Medicinal and Ornamental Plants Research Center of Sistan and Balochistan in 2022. The results of synthesizing these nanoparticles showed that the nanoparticles had a maximum absorption at a wavelength of 400-415 nm, an average size of 10-12 nm, and a spherical and uniform shape. The results of the effects of nanoparticle extracts on the germination and growth of Cuscuta weeds showed that 100% silver nanoparticle extract of *R. stricta*significantly decreased the germination percentage (50.66%), germination vigour (10.50) and germination rate (2.03) comparison with other treatments. The mean germination time was 2 days in control and increased to 4 days after applying 100% silver nanoparticle extract of *R. stricta*. Besides, rootlet length and plantlet length were affected by *R. stricta* extracts. The most reduction was related to 100% silver nanoparticle extract with 0.40 cm in rootlet length and 0.50 cm in plantlet length.

Keywords: Allelopathic properties, Cuscuta, Rhazya stricta, Silver nanoparticles

INTRODUCTION

Parasitic plants are widespread weeds that damage crops from an agronomic and economic point of view. For their survival, they need the host plants as a source of nutrients and they secrete their metabolites in the host plants. Parasitic plants have developed different ways to attack their host, such as using a special sticky organ called haustorium. The haustorium can penetrate the stem, leaf, or root of the host plant. Among the 4500 flowering parasitic plants (about 1% of all angiosperm species) [1], Cuscuta with the English name of "Dodder" belongs to the Cuscutaceae family and is represented by approximately 200 parasitic species that contain low amounts of chlorophyll and no Rubisco activity [2, 3]. Many species of Cuscuta reduce the performance of the host plant and carry diseases such as phytoplasma [4]. The natural hosts of Cuscuta are mainly dicotyledonous plants from the families Brassicaceae, Leguminosae, Solanaceae, and other species [5]. The formation of the haustorium is the first essential step for establishing mRNA binding between the parasite plant and the xylem and phloem of the host plant [1, 6]. Cuscuta plants emerge with filamentous hypocotyls that use nostic movements to identify the host and have neither roots nor cotyledons. Later, they develop filamentous ascending stems with scale-like leaves that are completely dependent on the host plant for water, photosynthetically absorbed substances, and nutrients [2].

Various methods have been proposed to control this parasitic plant, including mechanical methods (removal or deep plowing), chemical methods (use of herbicides), and biological control (biological herbicides, microbes, and genetically modified plants). Also, the use of nanomaterials as nano herbicides can be effective in controlling weeds [7]. The chemical method has disadvantages such as environmental pollution, threats to human and wildlife health, the phenomenon of resistance to herbicides, and changes in weed flora. All these things make successful management of this weed a problem. Therefore, we should look for an effective and alternative method to control it [8]. Among the alternative methods for chemical methods, biological control is

one of the most significant methods. In addition to all the advantages of the biological control method such as being healthy and economical, this method is effective in parasitic plants due to the close relationship between the host and the parasitic plant. In addition, the specificity of some feeding organisms on the selected host can be considered a useful factor, limiting weed populations and reducing their growth [9].

Silver nanoparticles (AgNPs) are used in biological systems as part of the development of bionanotechnology. It provides suppression of weed agents [10, 11]. The concentration, size, shape, chemical composition, reactivity, coating type, and aggregation levels of silver nanoparticles may affect plant growth [12]. However, the processes of absorption, transport, accumulation, and mode of action of AgNP in plants have received less attention. Silver nanoparticles have attracted a lot of attention among different metal nanoparticles due to their attractive shape, size, and environment-dependent properties [13]. Green chemistry, which uses plants as a source of AgNPs, has become increasingly popular because this method is cost-effective and environmentally friendly [14]. Plant extracts can be used to produce silver nanoparticles [15]. The three basic principles for producing nanoparticles using a green synthesis process are a solvent medium (ideally water), an environmentally friendly reducing agent, and a non-toxic chemical to stabilize the nanoparticles [16].

R. stricta is one of the most valuable medicinal plants found throughout South Asia and the Arabian Peninsula. Several studies have used different parts of *R. stricta* extract to screen phytochemical compounds [17]. By disrupting membrane function and photosynthetic ability, *R. stricta* leaf extract inhibited the growth and metabolic activity of Salsola villosa [18]. The methanolic extract of *R. stricta* inhibited the germination of *Phalaris minor*, *Chenopodium album*, and *Rumex dentatus* seeds by 43, 47 and 42%, respectively. *R. stricta* extract showed the ability to reduce the wet and dry weight of roots and shoots [19]. *R. stricta* extract caused mortality (91%) in Culex pipiens by reducing the expression levels of acetylcholinesterase and Glutathione-S-transferase [20].

Therefore, the aim of this study is the photosynthesis of silver nanoparticles by the aqueous extract of the leaves of the *R. stricta* plant and the comparison of its allelopathic potential with the extracts of this plant on the germination of seeds and the growth of Cuscuta seedlings that grow naturally with it.

MATERIAL AND METHODS

First Experiment

The*R. stricta* plant with herbarium code SH1128256.09FU was collected from Iranshahr-khash farms. *R. stricta* is an evergreen miniature shrub with thick foliage. These plants were completely washed with water and dried in the open air under natural conditions. Leaf and stem samples were powdered separately and stored in plastic bottles at room temperature (Fig. 1). This experiment was carried out in the biotechnology laboratory in the Medicinal and Ornamental Plants Research Center of Sistan and Balochistan in 2022.

Fig. 1 The Rhazya stricta plant that collected from Iranshahr-Khash farms

Extraction Process for Phytosynthesis of Silver Nanoparticles

All the materials utilized in this research were sourced with high purity from reputable suppliers. Silver nitrate salt (AgNO3), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were all obtained from Merck. About 5 grams of the powdered plant was weighed by a digital scale and transferred into an Erlenmeyer flask containing 100 ml of double distilled water. Next, it was heated at 40℃ for 30 minutes and stirred by an electric stirrer. After cooling at room temperature, the resulting mixture was filtered with Whatman 42 filter paper and centrifuged for 30 minutes at 10,000 rpm to remove suspended particles completely [21]. The obtained extract was stored in a refrigerator at 0-4℃ for further use.

Preparation of Silver Nitrate Stock Solution and Synthesis of Silver Nanoparticles

First, the initial stock solution was prepared with a concentration of 1 mM. Then, 2 ml of the extract was added to 4 ml of silver nitrate stock solution at room temperature. The pH of the solution was reported at 4.42. After changing the color of the solution to brown, the UV-Vis spectrum, HACH DR 5000 model, was taken from the solution containing the synthesized nanoparticles. Afterward, the effective parameters on the synthesis of silver nanoparticles (i.e., the effect of pH of the solution, the volume of the extract, the concentration of silver nitrate salt, and the reaction time) were evaluated to obtain nanoparticles with more uniform morphology and smaller size [22]. Finally, these parameters were optimized by UV-Vis spectrophotometry (Fig. 2).

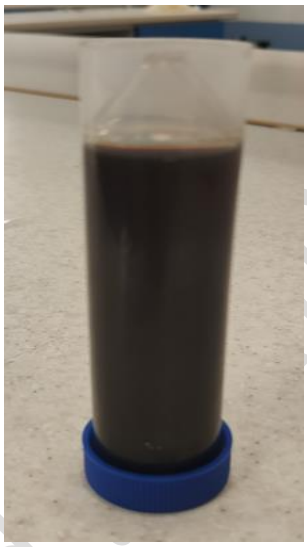

Fig. 2 Synthesis solution of silver nanoparticle from *R. stricta* plant.

Optimization of Effective Parameters in the Synthesis of silver Nanoparticles

Examining the Effect of pH

The pH values were optimized by preparing five series of solutions: 2 ml of extract and 4 ml of silver nitrate salt solution with a concentration of 1 mM with pH 2, 4, 6, 8, and 10. In the next step, their absorption spectra were analyzed by UV-Vis spectrophotometry, and the optimum pH was selected. The solution pH was adjusted using one of two sodium hydroxide or hydrochloric acid solutions with a concentration of 0.1 M [22].

Optimizing the Extract Volume

To optimize the volume of the extract, 1 to 4 ml of plant extract with a concentration of 5% w/v was added to 4 ml of silver nitrate salt with a concentration of 1 mM. The reaction pH was set equal to the optimal pH. The UV-Vis spectrum was taken from each solution separately, and the optimal extract volume was finally selected [23].

Optimizing the Concentration of Silver Nitrate Salt

The concentration of silver ion (I) was optimized by adding the optimized extract to 4 ml of different concentrations of silver nitrate salt solution (1, 2, 3, 4, 5 mM) and adjusting the pH of the reaction to the optimal pH. The UV-Vis spectrum was taken from the solutions, and the optimal concentration of silver nitrate salt was selected accordingly [24].

Optimizing Time and Checking the Stability of Silver Nanoparticles

To optimize the best time necessary to perform the reaction and check the stability of the produced silver nanoparticles, a solution containing a mixture of extract and silver nitrate salt was prepared with all the previous optimizations and at different times (from 1 hour to 72 hours after synthesis). UV-Vis spectrum was taken from each of them separately, and the effect of time was investigated. All optimizations were done at room temperature [25].

Characterization of Silver Nanoparticles

TEM Analysis (Transmission Electron Microscope)

The shape and size distribution of synthesized silver nanoparticles were investigated by applying effective factors by transmission electron microscope (Zeiss-EM10C-80 KV model). A few drops of the solution containing silver nanoparticles were placed on the grids of the device (made of copper coated with carbon). Eventually, its transmission electron microscope image was taken after evaporating the solvent at the laboratory temperature [22].

FT-IR Spectrum Analysis (Fourier Transform Infrared)

Fourier transform infrared spectrometry (PerkinElmer-FT-IR Spectrum two model) was performed to qualitatively identify the reducers and stabilizers around silver nanoparticles. For this purpose, first, the sample containing synthesized silver nanoparticles was mixed with potassium bromide after applying all the effective parameters. After drying and powdering the sample, the sample was used to make potassium bromide tablets. Finally, its spectrum was recorded along with the water extract used in the 400-14000-cm frequency range [22].

XRD Spectrum Analysis (X-ray Diffraction)

After optimizing the phytosynthesis of nanoparticles, the prepared sample was centrifuged and then smoothed and separated. The dried samples were subjected to XRD (Bruker-D8 advance model) for further investigation and crystal structure study [24].

Second Experiment

Cuscuta seeds were disinfected using 2% sodium hypochlorite for 15 minutes. They were kept in sterilized petri dishes for germination and moistened with 10 mL of prepared extracts. Sterile filter papers (Whatman No. 1) containing seeds were placed in each sterilized 9 cm Petri plate. The control included sterilized distilled water [4]. A completely randomized design was laid out with three replications, each with 50 seeds. After that, different concentrations of silver nanoparticles biosynthesized from the Eshvarak plant (20%, 40%, 60%, 80%, and 100%) along with control (distilled water) was conducted in a completely randomized design in three replications. The petri dishes were kept at a constant temperature (25 ± 1°C) with a 16 h/8 h (light/dark) cycle for 7 days. During these tests, no fungal contaminations have been detected.

Table 1 Equations of germination indices

Equation number	Index	Equation	References
1	Germination percentage	$GP=n/N\times100$	Panwar and Bhardwaj [26]
2	Germination rate	$GR=\Sigma n_i/t_i$	Kulkarni *et al*.,[27]
3	Mean germination time	$MGT=\Sigma\ (t_i\times n_i)/\ \Sigma n$	Kulkarni *et al*., [27]
4	Germination vigour	$GV=GR\times Mean_(PL+RL)/\ 100$	ISTA [28]

n=Total of germinated seeds during period, n_i=The number of germinated seeds at an interval of distinc period, t_i= The number of days after the start of germination, N= Number of sowed seeds, PL: Plumle length, RL: Radicle length.

After 7 days, the germination percentage, germination rate, and mean germination time were determined. The germinated seeds were counted and recorded daily for up to 7 days. The final count was performed after 7 days, and the final germination percentage was calculated using the following formula in Table 1 [26]. The mean germination time (MGT) was calculated as a measure of the speed of germination or emergence using the following formula in Table 1 [27]. Also, germination vigour was calculated based on the length of roots, and stems were measured (Table 1). On the last day of the experiment, 10 samples were randomly taken from each petri dish to measure the length of roots and stems. Afterward, the aerial part and the root were separated and

their lengths were measured. A ruler was used to measure plant height and root length and the results were stated in centimeters.

Statistical Analysis

The data obtained with a complete random design was analyzed using SPSS ver.16 software and an average comparison was done using Duncan's test at the probability level of 1%.

RESULTS

First Experiment

The addition of the extract to silver nitrate salt revealed that the solution color turned brown, proving the successful synthesis of these nanoparticles. The solution pH was read as 4.42. To investigate the effect of the pH factor on the synthesis process of nanoparticles, pH values higher and lower than the initial value were studied, and a UV-Vis spectrum was taken from all the samples (Fig. 3). The results showed a significant change in absorption at pH = 2. However, since the absorption was lower compared to other pHs, it is inferred that little synthesis has been done at this pH. On the other hand, with the gradual increase in the pH of the solution up to 8, the solution absorption increased significantly, which is a reason for the increase in the amount of silver nanoparticle synthesis. At pH = 8, a symmetrical spectrum with the highest amount of absorption compared to the rest of the pHs was observed. Nevertheless, a sharp and noticeable drop in absorption was observed at higher pH values (pH = 10). As a result, pH = 8 was chosen as the optimal pH.

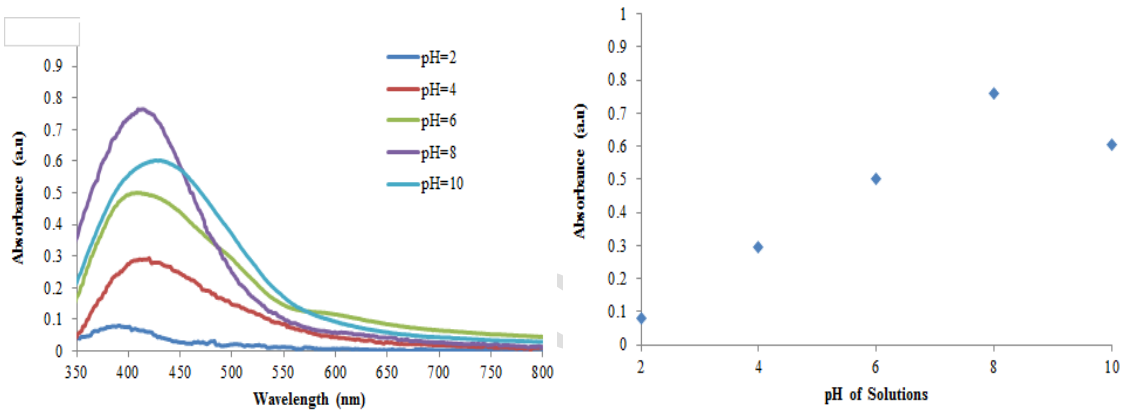

Fig. 3 Ultraviolet-visible spectrophotometric spectrum of phytosynthesized silver nanoparticles at different pH

Studying the effect of the amount of extract on the synthesis of silver nanoparticles revealed that with the increase in the amount of extract, the absorption of silver nanoparticles increased significantly. This result indicates an increase in the amount of synthesis of nanoparticles with the increase in the amount of extract (Fig. 4). This increase continues up to 3 ml of the extract, but in 4 ml of it, a sharp drop in the amount of absorption is observed. Hence, 3 ml of the extract was chosen as the optimal volume.

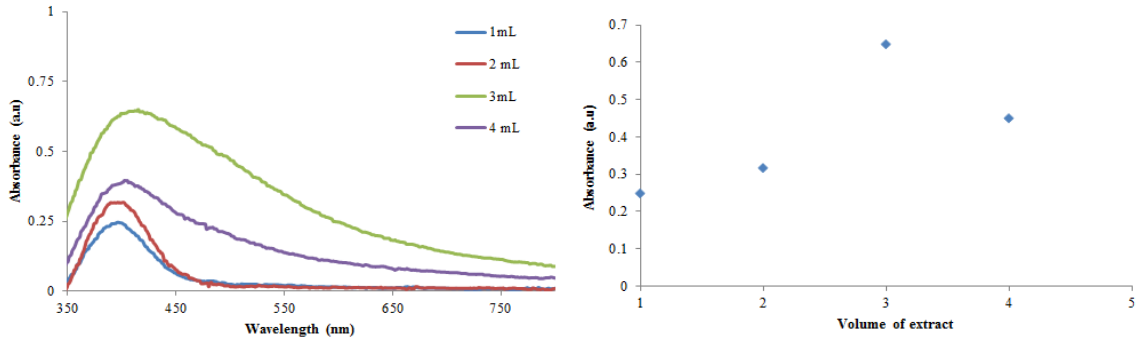

Fig. 4 Ultraviolet-visible spectrophotometric spectrum of phytosynthesized silver nanoparticles in different volumes of extract

The effect of metal ion concentration on nanoparticle synthesis is depicted in Fig. 5. According to Fig. 5, with the gradual increase in the concentration of silver ions, the absorption of the solution containing silver nanoparticles increased significantly. This increase continues up to a concentration of 4 mM of silver ion ($AgNO_3$). However, at a concentration of 5 mM, no significant increase in the amount of absorption related to nanoparticles was observed; instead, a sharp drop occurred in the amount of absorption. As a result, the concentration value of 4 mM of silver nitrate salt was chosen as the optimal concentration.

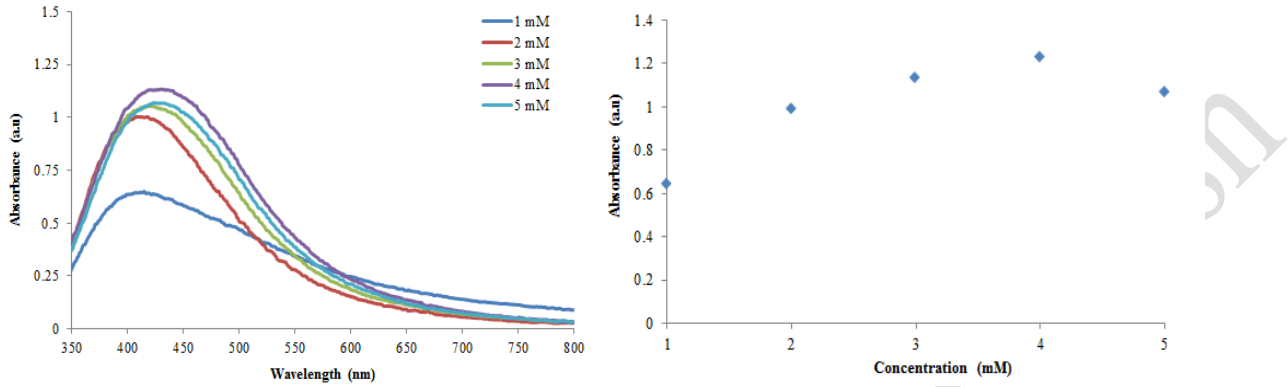

Fig. 5 Ultraviolet-visible spectrophotometric spectrum of phytosynthesized silver nanoparticles in different concentrations of silver nitrate salt

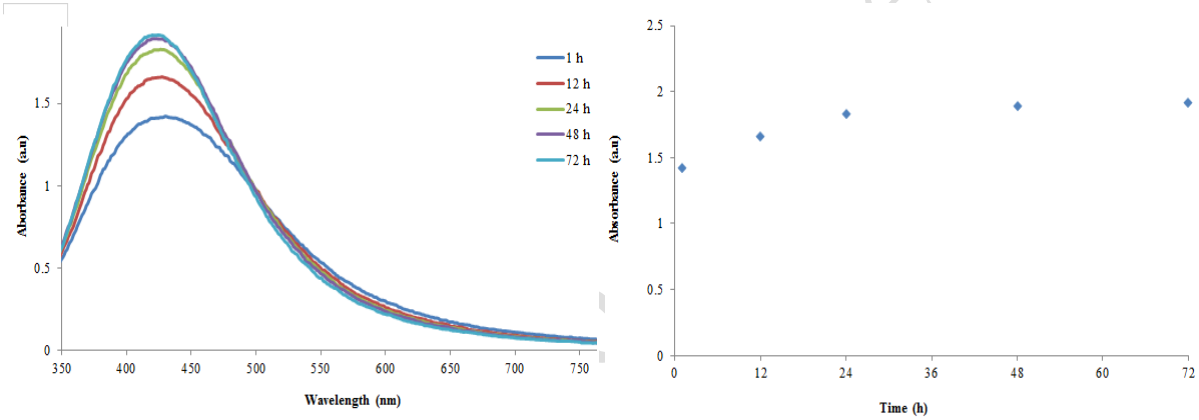

Fig. 6 Ultraviolet-visible spectrophotometric spectrum of phytosynthesized silver nanoparticles and their maximum absorption at different times

Studying the effect of the time of proximity of silver nitrate solution with the aqueous extract of the plant on the reaction process shows that with an increase in the interaction time between the reactants from the first moment, the absorption increases. This increase in absorption up to the first 24 hours is completely upward and occurs as a straight line. However, after 24 hours, there was no noticeable change in the amount of this absorption, proving the resulting nanoparticles' stability. As a result, 24 hours was chosen as the optimal and appropriate time (Fig. 6).

TEM images are among the effective tools for finding silver nanoparticles' characteristics, such as shape and size. The TEM image obtained from silver nanoparticles made under optimal conditions shows the accurate and complete synthesis of silver nanoparticles. The resulting nanoparticles' size was 8-10 nm (Fig. 7). It is possible to establish a relationship between TEM images and the UV-Vis spectrum taken from nanoparticles. In general, the higher and sharper the peak intensity, the better the synthesis of nanoparticles, the smaller their size, and the more uniform their shape [22]. Research also shows that factors such as reaction pH, amount of extract, metal ion salt concentration, and reaction time affect the size and shape of nanoparticles [23].

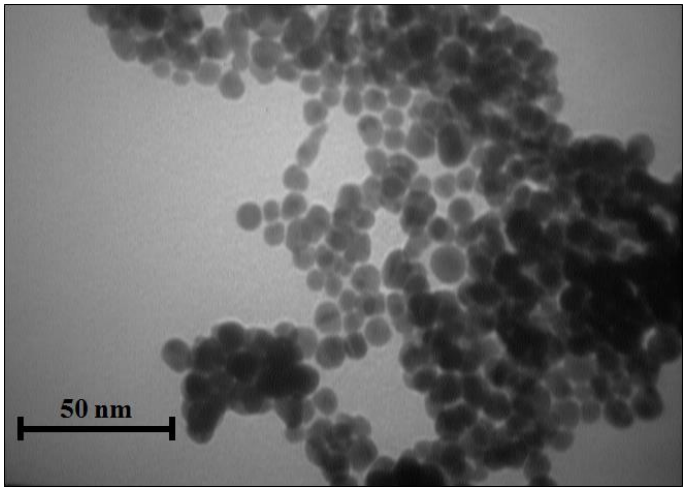

Fig. 7 Transmission electron microscope image of phytosynthesized silver nanoparticles

An XRD pattern was used to investigate the crystal structure of synthesized silver nanoparticles (Fig. 8). The average size of the crystalline grains was estimated by calculating the width of the peaks formed in the samples using the Debye-Scherrer formula (Eq. 1):

$D = 0.9\lambda / \beta \cos\theta$

where β is the peak width at half of the maximum height, λ is the X-ray wavelength equal to 1.54 Angstroms, θ is the diffraction angle, and D is the average crystal grain size (nm). Also, 0.9 is Scherer's constant value, which is assumed for crystal structures with cubic symmetry.

As shown in Fig. 8, there are clear peaks in the 2θ range of 38.13°, 44.13°, 64.46°, and 77.42° of silver nanoparticles, proving the successful synthesis of nanoparticles. Structural analysis shows that silver nanoparticles have a crystalline structure with Miller indices (111), (002), (022), and (113) in a cubic lattice. In a similar research, the structural analysis of nanoparticles synthesized by plants showed that the obtained nanoparticles have a crystal structure in a cubic lattice, the highest degree of crystallinity of which is the Miller index (111). Meanwhile, the crystal plates of nanoparticles are mostly formed in this direction, which is completely consistent with the results of previous studies [24].

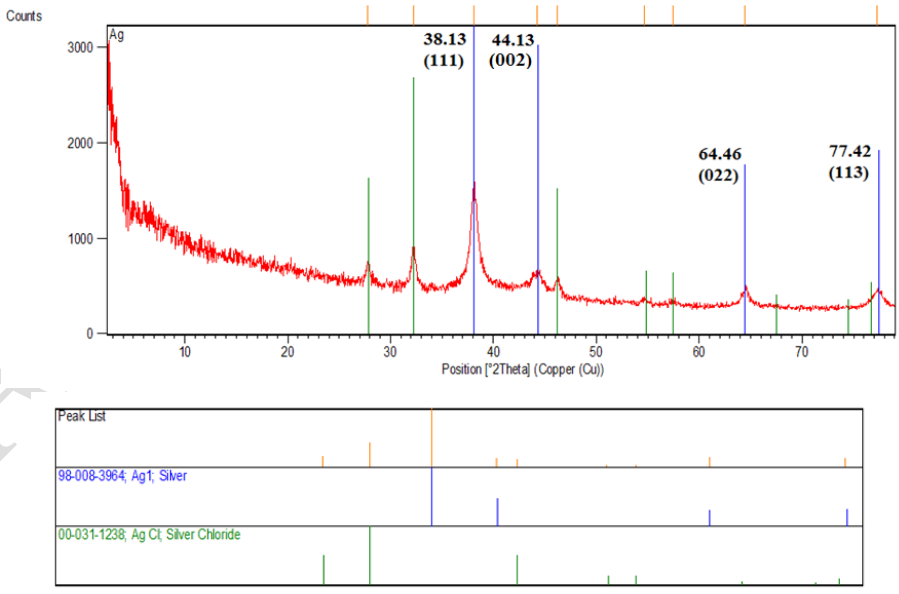

Fig. 8 X-ray diffraction pattern of phytosynthesized silver nanoparticles

FT-IR was used to identify reducing and stabilizing compounds around the nanoparticles (Fig. 9). The results show clear bands in the region of 3434.01, 2828.02, 2751.04, 1620.01, 1410.15, 1078.00, and 620.05 for aqueous extract and 3415.96, 2818.12, 2748.45, 1635.02, 1399.55, 1070.00, and 618.05 for silver nanoparticles. These peaks show O—H and N−H stretching vibrations, aromatic compounds, C—H alkanes,

and Also, the groups C=O, N=O, C=C, C=N, C—O, and C—N due to the presence of protein compounds, terpenoids, flavonoids, and vitamins in the plant extract [22]. In addition to reducing silver ions, these compounds cover the surroundings of the related nanoparticles, contribute to the stability of these particles, and prevent their aggregation and aggregation. As can be seen, the intensity of some peaks in silver nanoparticles has been reduced or completely removed. Thus, it can be claimed that the compounds in question have been completely consumed in biosynthesis or their amount has been reduced.

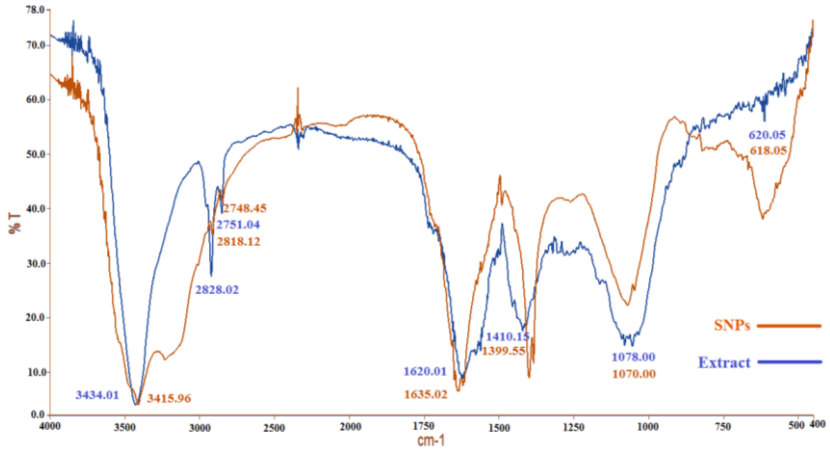

Fig. 9 FTIR spectrum of photosynthesized silver nanoparticles versus aqueous plant extract

Second Experiment

The results of the analysis of variance of the effect of different silver nanoparticle extracts of *R. stricta* plant on the characteristics of germination and growth of Cuscuta seedlings showed that all the measured characteristics were significant at the 1% level with a probability of 99% (Table 2).

Table 2 ANOVA (Mean squares) for the effect of different silver nanoparticle extracts of *R. stricta* plant on the seed germination of cuscuta weed

S.O.V.	df	Mean squares					
		Germination percent	Germination vigour	Germination rate	Mean germination time	Shootlet lenght	Rootlet lenght
Treatments	5	899.289 **	553.700 **	10.729 **	1.658 **	4.597 **	4.280 **
Error	12	4.788	0.210	0.002	0.010	0.011	0.200
Total	17	--	--	--	--	--	--

ns, ** and *: non-significant, significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

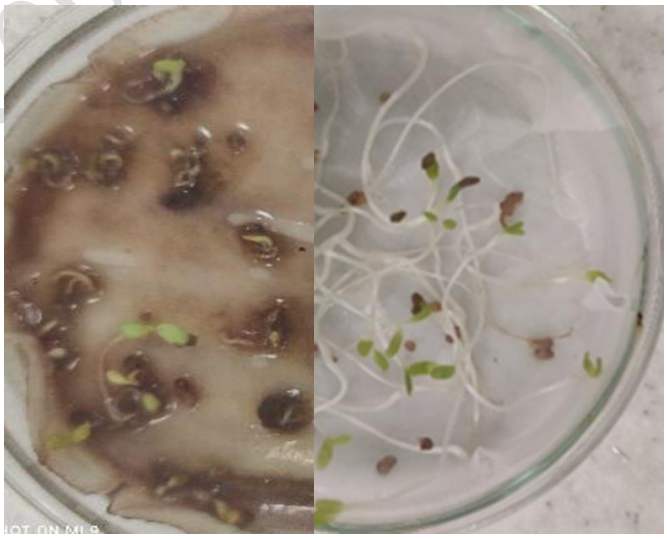

Fig. 10 The germination rate in cuscuta seeds with application of extracts of *R. stricta* (from left to right: 100% silver nanoparticle, control, respectively).

This study showed that the all of extracts decreased the germination percentage of Cuscuta seeds but the most reduction was related to 100% silver nanoparticle extract of the leaves of *R. stricta* plant that showed significant inhibitory effects on seed germination and seedling growth of the tested species. This indicates the availability of inhibitory chemicals in leaf and stems of *R. stricta*. Therefore, the 100% silver nanoparticle extract of *R. stricta* significantly decreased the germination percentage (50.66%) of Cuscuta seeds compared with other treatments (Table 3 and Fig. 10).

Also, germination vigour (10.50) and germination rate (2.03) of Cuscuta seeds decreased with using of 100% silver nanoparticle extract of *R. stricta* compared with other treatments. The mean germination time was 2 days in control and increased to 4 days with the application of 80 and 100% silver nanoparticle extract of *R. stricta*. Besides, rootlet length and plantlet length were affected by *R. stricta* extracts and the most reduction was related to 100% silver nanoparticle extract with 0.40 cm in rootlet length and 0.50 cm in plantlet length (Table 3).

Table 3 Mean comparison of effects of different silver nanoparticle extracts of *R. stricta* plant on the seed germination of cuscuta weed

Treatments	Germination percent	Germination vigour	Germination rate	Mean germination time	Shootlet lenght	Rootlet lenght
Control	99.66 a *	49.50 a	7.11 a	2.10 d	3.80 a	3.65 a
20% Silver nanoparticle	88.00 b	38.50 b	5.50 b	3.80 b	2.30 b	2.10 b
40% Silver nanoparticle	80.00 c	30.50 c	4.40 c	3.31 c	1.40 c	1.20 c
60% Silver nanoparticle	72.00 d	25.50 d	3.20 d	3.76 b	1.10 d	1.00 c
80% Silver nanoparticle	65.00 e	21.50 e	2.80 e	4.10 a	0.70 e	0.73 d
100% Silver nanoparticle	50.66 f	10.50 f	2.03 f	4.00 a	0.50 f	0.40 e

*Means followed by the same letters in each column are not significantly at 1%.

DISCUSSION

Based on other research on plant synthesis of nanoparticles, the plant can act as a regenerating and stabilizing agent. Research has shown that molecules such as phenols, flavonoids, and proteins play this role in plants [21], where flavonoids are no exception. Some studies considered the existence of this category of secondary compounds as the reason for synthesizing metal nanoparticles [22]. As stated, the plant used in this research also has many natural compounds such as antioxidants, phenols, flavonoids, etc. In this respect, the FT-IR spectrum obtained from the aqueous extract of this plant in this research proves this claim. Therefore, these compounds contributed to the regeneration and stability of synthesized silver nanoparticles. In this study, silver nanoparticles were produced by a biological and environmentally friendly method without using any hazardous chemicals. Using the high capacity of plants enables us to obtain nanoparticles with much more stability than chemical methods. The biosynthesis of nanoparticles produced by medicinal plants can be used in various cases, including the delivery of drugs to the body. Among the advantages of this method are its facile setup and low cost. Also, it is possible to produce nanoparticles with different shapes and the same size. Compared to the other two nanoparticle synthesis methods (physical and chemical), the mentioned method has higher efficiency and almost no disadvantages. One of the limitations of most nanoparticle synthesis methods is the large dispersion of nanoparticles in a wide range of sizes in nanometer dimensions. This major limitation can be dealt with by different methods such as optimum pH, changes in different concentrations of salt solution and duration, and reducing the range of changes in the size of nanoparticles to achieve an optimal and suitable condition [24]. The optical absorption spectrum of metal nanoparticles is sensitive to several factors such as particle size, shape, interaction of particles with each other, and the refractive index of the medium. In this regard, pH is one of the most important parameters affecting the synthesis of silver nanoparticles. Previously, some reports have been presented about the effect of reaction pH on the size and shape of nanoparticles. According to these reports, pH does not significantly affect the shape of nanoparticles and only affects their size [22]. Findings related to optimum pH showed that at pH = 2, no significant change in absorption was observed. Therefore, it can be claimed that no significant synthesis took place. However, with the gradual increase in the pH of the solution up to 8, the absorption of the resulting solution increases, which is related to

the increase in the synthesis of nanoparticles. Finally, at pH = 8, a sharp peak is observed. It seems that at a pH above 8 (pH=10), silver ions are hydrolyzed, forming stable species of silver ion hydroxides and finally preventing the entry of this ion into the biological and biological reduction reaction [29]. As a result, pH = 8 was chosen as the optimal acidity. In the synthesis of nanoparticles using plant extracts, the extract plays the role of reducing and regenerating metal ions and stabilizing these nanoparticles [30]. After adding the reagents and increasing the solution's acidity to 8, it was observed that the solution color turned dark brown, indicating the successful synthesis of silver nanoparticles. Reports show that the increase in absorption due to the increase in metal ion concentration is because the ions will be more exposed to reduction and more nanoparticles will be produced [23]. Several studies have proven the effect of metal ion concentration on the synthesis of nanoparticles. Some studies show that with the increase in metal ion concentration, the observed absorption also increases. The explanation is that with the increase in metal ions, more ions are regenerated and, thus, more nanoparticles are produced [31]. However, the excessive decrease or insignificant increase in the amount of absorption related to nanoparticles due to the excessive increase in the silver ion concentration can be due to the adhesion of nanoparticles and the synthesis of nanoparticles with a larger size [24]. According to the mentioned reasons, a 4 mM solution of silver ion was chosen as the appropriate concentration. Like the other discussed factors, time has a significant effect on the synthesis and stability of nanoparticles [32]. Therefore, in such reactions, in the case of incomplete synthesis, nanoparticle production will increase with time. Also, time is the most important factor in proving the stability of the synthesized nanoparticles [22]. If there is no significant increase in the amount of silver nanoparticle absorption with time, the resulting nanoparticles are quite stable over time. Accordingly, the reduction reaction of silver ions by the aqueous extract of the stork plant was completed in 24 hours.

In the investigation of allelopathic effects of *R. stricta* plant on the germination and growth of Cuscuta seeds observed from the initial screening, it was found that leaf and stem extracts had the strongest allelopathic effect on seed germination. Its effectiveness on germination and growth suggests that the leaves and stems of this plant may act as a source of allelochemicals after being released into the soil or after decomposition, which negatively affects nearby plants [33]. Allelopathic substances released by plants accumulate in the soil to physiologically active levels [34]. The plants release phytochemicals from dead tissues and their incorporation into the soil can be accelerated by leaching, thereby facilitating their harmful effects in the field [35]. This aspect, when studied using the leaves and stems of *the R. stricta* plant in this experiment, significantly inhibited the Cuscuta species. The effectiveness of *R. stricta* plant on the germination and growth of Cuscuta shows that the leaves and stems of this plant can act as a source of allelochemicals after decomposition, which in turn negatively affects adjacent or successive plants. The mean germination time was 2 days in control and increased to 4 days with the application of 80 and 100% silver nanoparticle extract of *R. stricta*. The different phytotoxicities observed from the *R. stricta* plant can be attributed to the presence of variable amounts of phytotoxic substances in different parts that are washed under natural conditions. Some recent studies have shown the phytotoxic/allelopathic effect of aqueous extracts of weeds including *Parthenium hysterophorus L.* and *Ageratum conyzoides L.* [36]. It has been reported that *Scrophularia Striata*aqueous extract had anti-parasitic effects on the Leishmania major [37]. Also, the methanolic extract of the aerial parts of *Verbascum speciosum L.* had significant antibacterial activity against *L. monocytogenes*, *B. cereus*, *S. aureus*, and *S. thyphimurium* [38]. Besides, *Phoenix dactylifera* extract may be considered as an alternative to acyclovir and a potential phototherapeutic against herpes simplex virus type 1 (HSV-1) [39]. The rootlet length and plantlet length were affected by *R. stricta* extracts and the most reduction was related to 100% silver nanoparticle extract with 0.40 cm in rootlet length and 0.50 cm in plantlet length. Also, Other studies strongly demonstrated the release of phototoxic chemicals during the preparation of aqueous extracts. The allelopathic effect of different plants significantly affects seed germination and seedling growth of several crops and weed species [40], and these studies showed that the leaf extract of *E. camaldulmensis* reduced the root growth of most crops.

CONCLUSION

Nanotechnology is rapidly developing in virtually all areas of science and technology due to the unique chemical, physical, and biological properties of nanoparticles (NPs). Nowadays, green synthesized nanoparticles are environmentally friendly and affordable. In our study, after preparing the extracts by maceration method, silver nanoparticles were synthesized by aqueous extract of the leaves of the *R. stricta* plant. Then, in order to obtain more uniform and homogeneous nanoparticles, parameters such as reaction pH, volume of extract used, concentration of silver nitrate salt, and reaction time were studied and optimized by ultraviolet-visible spectrophotometry (UV-Vis) technique. Nanoparticles had a maximum absorption at a wavelength of 400-415 nm, an average size of 10-12 nm, and a spherical and uniform shape. Parasitic plants include a diverse group of plants, and among them, the Cuscuta weed is an obligate parasite of many plant families that has a global spread. After 48 h of incubation, treatment of 100 % silver nanoparticles of *R. stricta*aqueous extract inhibited the growth of cuscuta seeds. *R. stricta* extracts demonstrated the ability to suppress Cuscuta density, length, and weight (fresh and dry) of the root and shoot. The effectiveness of *R. stricta* plant on the germination and growth of Cuscuta showed that the leaves and stems of *R. stricta* plant can act as a source of allelochemicals after decomposition, which in turn negatively affect adjacent or successive plants.

REFERENCES

1. Yoshida S., Cui S., Ichihashi Y., Shirasu, K. The haustorium, a specialized invasive organ in parasitic plants. Annu. Rev. Plant Biol. 2016; 67: 643-667. https://doi.org/10.1146/annurev-arplant-043015-11.
2. Tešitel J. Functional biology of parasitic plants: A Review. Plant Ecol. Evol. 2016; 149: 5-20. https://doi.org/10.5091/plecevo.2016.1097.
3. Wikipedia. The free Encyclopedia. Cuscuta. http://www.wikipedia.org/. 2010.
4. Saric-Krsmanovic M.M., Bozic D.M., Radivojevic L.M., Umiljendic J.S.G., Vrbnicanin S.P. Effect of Cuscuta campestris parasitism on the physiological and anatomical changes in untreated and herbicide-treated sugar beet. J. Environ Sci Health B. 2017; 52: 812-816. http://doi.org/10.1080/03601234.2017.135.
5. García M.A., Costea M., Kuzmina M., Stefanovic S. Phylogeny, character evolution, and biogeography of Cuscuta (dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. Am. J. Bot. 2014; 101: 670-690. DOI: http://doi.org/10.373
6. Kim G., Westwood J.H. Macromolecule exchange in Cuscuta–host plant interactions. Curr Opin Plant Biol. 2015; 26: 20-25. http://doi.org/10.1016/j.pbi.2015. 05.012
7. Westwood J.H., Charudattan R., Duke S.O., Fennimore S.A., Marrone P., Slaughter D.C., Swanton C., Zollinger R. Weed Management in 2050: Perspectives on the Future of Weed Science. Weed Sci. 2018; 1-11. https://doi.org/10.1017/wsc.2017.78
8. Bastiaan L., Zhao D.L., Denhollander N.G., Bamann D.T., Kruidhof H.M., Kropff M.J. Exploiting diversity to manage weeds in agro-ecosystems. In: Spiertz J.H.J., Struik P.C., van Laar H.H. (eds.) Scale and Complexity in Plant System Research. Gene-Plant-Crop Relat. 2007; 267-284.
9. Sauerbon J., Muller-Stover D., Hershenhorn J. The role of biological control in managing parasitic weeds. J. Crop Prot. 2007; 26: 246-254. http://doi.org/10.1016/j.cropro.2005.12.012
10. Mousavi S.R., Rezaei M. Nanotechnology in agriculture and food production. J. Appl. Environ. Biol. Sci. 2011; 1: 414-419
11. Pokhrel L.R., Silva T., Dubey B., El Badawy A.M., Tolaymat T.M. Scheuerman P.R. Rapid screening of aquatic toxicity of several metal-based nanoparticles using the MetPLATE™ bioassay. Sci. Total Environ. 2012; 426: 414-422. http://doi.org/10.1016/j.scitotenv.2012.03.049
12. Pradas del Real A.E., Vidal V., Carriere M., Castillo-Michel H., Levard C. Chaurand, P. Silver nanoparticles and wheat roots: A complex interplay. Environ. Sci. Technol. 2017; 51: 5774-5782. http://doi.org/10.1021/acs.est.7b00422
13. Anwar N., Wahid J., Uddin J., Khan A., Shah M., Shah S.A. Phytosynthesis of polyethylene glycol methacrylate-hybridized gold nanoparticles from C. tuberculata: Their structural characterization and potential for in vitro growth in banana. In Vitro Cell Dev. Biol. 2021; 57: 248-260. http://doi.org/10.1007/s11627-020-10150-4
14. Acharya P., Jayaprakasha G.K., Crosby K.M., Jifon J.L., Patil B.S. Green-synthesized nanoparticles enhanced seedling growth, yield, and quality of onion (Allium cepa L.). ACS Sustain. Chem. Eng. 2019; 7 (17): 14580-14590. http://doi.org/10.1021.

15. Ahmad N., Rab A., Sajid M., Ahmad N., Fazal H., Ali M. Sucrose-dependent production of biomass and low caloric Steviol glycosides in adventitious root cultures of Stevia rebaudiana (Bert.). Ind. Crops. Prod. 2021b; 164: 113382. http://doi.org/10.1016/j.indcrop.2021.113382
16. Song K., Zhao D., Sun H., Gao J., Li S., Hu T. Green nanopriming: Responses of alfalfa (Medicago sativa L.) seedlings to alfalfa extracts capped and light-induced silver nanoparticles. BMC Plant BioL. 2022; 22: 323.
17. Bukhari N.A., Al-Otaibi R.A., Ibhrahim I. Phytochemical and taxonomic evaluation of Rhazya stricta L. in Saudi Arabia. Saudi J. Biol. Sci. 2017; 24: 1513-1521.
18. Alqarawi A.A., Hashem A., Kumar A., Al-Arjani A.B.F., Abd-Allah E.F., Dar B.A., Wirth S., Davranov K., Egamberdieva D. Allelopathic effects of the aqueous extract of Rhazya stricta on growth and metabolism of Salsola villosa. Plant Biosyst. 2018; 152: 1263-1273.
19. Iqbal J., Zahra S., Ahmad M., Shah A., Hassan, W. Herbicidal Potential of Dryland Plants on Growth and Tuber Sproutingin Purple Nutsedge (Cyperus rotundus L.). Planta Daninha. 2018; 36.
20. Al-Solami H.M. Larvicidal activity of plant extracts by inhibition of detoxification enzymes in Culex pipiens. J. King Saud Univ. Sci. 2021; 33: 101371.
21. Azizian-Shermeh O., Valizadeh M., Taherizadeh M. Phytochemical investigation and phytosynthesis of eco-friendly stable bioactive gold and silver nanoparticles using petal extract of saffron (Crocus sativus L.) and study of their antimicrobial activities. Appl. Nanosci. 2020; 10: 2907-2920. https://doi.org/10.1007/s13204-019-01059-5
22. Azizian-Shermeh O., Jalali-Nezhad A.A., Taherizadeh M. Facile, Low-cost and rapid phytosynthesis of stable and eco-friendly silver nanoparticles using Boerhavia elegans (Choisy) and study of their antimicrobial activities. J. Inorg. Organomet. Polym. Mater. 2021; 31: 279-291. https://doi.org/10.1007/s10904-020-01691-7
23. Basiratnia E., Einali A., Azizian-Shermeh O. Biological Synthesis of Gold Nanoparticles from Suspensions of Green Microalga Dunaliella salina and Their Antibacterial Potential. J. Bionanosci. 2021; 11: 977-988. https://doi.org/10.1007/s12668-021-00897-4
24. Azizian-Shermeh O., Einali A., Ghasemi A. Rapid biologically one-step synthesis of stable bioactive silver nanoparticles using Osage orange (Maclura pomifera L.) leaf extract and their antimicrobial activities. Adv. Powder Technol. 2017; 28 (12): 3164-3171. https://doi.org/10.1016/j.apt.2017.10.001
25. Bahador E., Einali A., Azizian-Shermeh O., Sangtarash M.H. Metabolic responses of the green microalga Dunaliella salina to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment. Aquat. Toxicol. 2019; 217: 105356. https://doi.org/10.1016/j.aquatox.2019.105356
26. Panwar P., Bhardwaj S.D. Handbook of practical forestry, Agrobios (India). 2005; 191.
27. Kulkarni M.G., Street R.A., Staden J.V. Germination and seedling growth requirements for propagation of Diosscorea dregeana (Kunth) Dur. and Schinz-A tuberous medicinal plant, S Afr J Bot. 2007; 33: 131-137. https://doi.org/10.1016/j.sajb.2006.09.002
28. ISTA. ISTA Handbook on Seedling Evaluation. 3rd Ed. Zurich, International Seed Testing Association. 2009;117.
29. Shenya D.S., Mathewa J., Philip D. Phytosynthesis of Au, Ag and Au–Ag bimetallic nanoparticles using aqueous extract and dried leaf of Anacardium occidentale. Spectrochim Acta A Mol Biomol Spectrosc. 2011; 79: 254-262.
30. Shankar S.S., Rai A., Ahmad A., Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Neem (Azadira chtaindica L.) leaf broth. J. Colloid Interface Sci. 2002; 275: 496-502.
31. Dwivedi A.G., Gopol K. Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract. Colloids Surf. A: Physicochem. Eng. Asp. 2010; 360: 27-33.
32. Philip D. Rapid Green synthesis of spherical gold nanoparticles using Mangifera indica Leaf. Spectrochim. Acta Mol. Biomol. Spectrosc. 2010; 77: 807-810. http://dx.doi.org/10.1016/j.saa.2010.08.008.
33. Khan M., Musharaf S., Ibrar M., Hussain F. Allelopathic effects of Rhazya stricta dence on seed germination and seedling growth of Pennisetum typhoides. Proc. Int. Techno. Edu. Environ. Conf. (c). Afr. Soc. Sci. Res. (ASSR), 2015.
34. Hussain F., Niaz F., Jabeen M., Burni T. Allelopathic potential of Broussonetia papyrifera Vent. Pak. J. Agric. Sci. 2004; 10(2): 69-77.
35. Inderjit M., Duke S. Ecophysiological aspects of allelopathy. Planta J. 2003; 217 (4): 529-539. http://doi.org/10.1007/s00425-003-1054-z
36. Singh H.P., Batish D.R., Pandher J.K., Kohli R.K. Assessment of allelopathic properties of Parthenium hysterophorus residues. Agr. Ecosyst. Environ. 2003; 95: 537-541. https://doi.org/10.1016/S0167-8809(02)00202-5
37. Nokhodi F., Bandani, E., Kooshki H., Eftekhari M., Mahmoudi R., Mansouri M., Jafari A.A. Medicinal plant Scrophularia striata evaluation anti-parasitic effects on Leishmania major: In vitro and In vivo Study. Biosci., Biotech. Res. Asia. 2014; 11 (2): 627-634. http://dx.doi.org/10.13005/bbra/1315

38. Nofouzi K., Mahmudi R., Tahapour K., Amini E., Yousefi K. Verbascum speciosum Methanolic Extract: Phytochemical Components and Antibacterial Properties. J. Essent. Oil-Bear. Plants. 2016; 19 (2): 499-505.
39. Allahyari S., Pakbin B., Amani Z., Mahmoudi R., Hamidiyan G., Peymani A., Qajarbeygi P., Mousavi S. Antiviral activity of Phoenix dactylifera extracts against herpes simplex virus type 1: an animal study. Comp Clin Pathol. 2021; 30: 945.951. https://doi.org/10.1007/s00580-021-03293-22021; 30: 945-951.
40. Khan M.A., Marwat K.B., Hassan Z. Allelopathic potential of some multipurpose trees species (MPTS) on the wheat and some of its associate's weeds. Int. J. Biol. Biotec. 2004; 1(3): 275-278.