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Green synthesis, characterization and standardization of silver nanoparticles

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Abstract
The green synthesis of nanoparticles has received a lot of interest in recent years. In this context, researchers have explored the use of plant extracts, microorganisms and enzymes from natural resources as an excellent reagents for synthesizing nanoparticles. In this study, synthesis of silver nanoparticles was done through cumin (*Cuminum cyminum*) seed extract and through reduction method. The bio reduction of silver nitrate (1 mM) using the seed extract of cumin lead to change in colour from light brown to dark brown which confirmed the formation of silver nanoparticles. The synthesized nanoparticles were characterized through UV-Vis, PSA and SEM. Silver nanoparticles showed absorption peak at 420 nm in UV-Vis and average particles size of 64 nm in PSA, respectively. SEM images revealed silver nanoparticles were of spherical shape and smooth surface. Synthesized AgNPs standardized for priming duration in onion seeds. The experiment was conducted on 18 months aged onion seeds cv. Arka Kalyan with twenty nine treatments and three replications. Treatments involved priming seeds at different concentrations of AgNPs solution *viz*., 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 200 ppm, 1mM $AgNO_3$ and control for 1 h, 3 h, 6 h and 12 h respectively. Based on higher germination percentage, root length, shoot length and seedling vigour index- I, the seed priming duration was evaluated. Priming with synthesized AgNPs showed significant variation on seed quality parameters of onion seeds. AgNPs seed priming with 3 hour recorded higher performances for seed quality attributes and considered as best soaking duration for AgNPs priming.

Keywords: Cumin, silver nanoparticles, UV-Vis, PSA, onion, priming

Introduction

Nanotechnology is the branch of science. that deals with materials on the nanometer (10^{-9} m) scale. Nanoparticles refers to particles of diameters. ranging from 1 to 100 nm. These nanoparticles exhibit distinctive characteristics such as in their scale, dimension and morphology, which lead to entirely new or altered diagnostics. Different NPs (metal and metal oxide NPs) can decrease or increase the germination of many plant seeds (Feizi *et al*., 2013) [6]. However, silver (Ag), gold (Au), copper oxide (CuO), zinc oxide (ZnO), cerium oxide (CeO_2) and titanium dioxide (TiO_2) are the metal nanoparticles and metal oxides that have been extensively investigated by the majority of researchers in the field of. plant science (Maroufpoor *et al*., 2019) [9]. Silver nanoparticles in particular hold immense significance due to their exceptional and significantly altered physical, chemical and biological properties when compared to their larger-scale counterparts. Silver nanoparticles (AgNPs) currently represent one of the most commonly used commercial nanomaterials (Chen and Schluesener, 2008) [4]. Presently, silver nanoparticles are being explored for their potential to promote. seed germination, enhance plant growth, improve photosynthetic. quantum efficiency and serve as antimicrobial agents in managing plant diseases.

The green synthesis of nanoparticles has received a lot of interest in recent years. In this context, researchers have explored the use of plant extracts, microorganisms and enzymes from natural resources as an excellent alternative reagents for synthesizing nanoparticles.. In the biological reduction technique conventional reducing agents are replaced with naturally occurring product extracts possessing inherent stabilizing, growth-terminating and capping properties. The cumin (*Cuminium cyminium* L.) commonly known as *Jeera* is an important seed spice crop belongs to family Apiaceae of the order Apiales. The major volatile components found in cumin are cuminaldehyde, cymene and terpenoids. The primary components of aromatic compounds found in the essential oil of cumin are cuminaldehyde and cumin alcohol.

Keeping in view of above facts, the present investigation was carried out with an objective to synthesize and characterize silver nanoparticles through cumin seed extract and to standardize the nanopriming duration on onion seeds.

Materials and Methods

The nanoparticles synthesis and characterization were carried out at the Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Standardization of AgNPs priming duration on onion seeds was carried out at the Seed Quality Research Laboratory, College of Agriculture, Dharwad. Onion seeds were collected from the Seed Unit, University of Agricultural Sciences, Dharwad. For green synthesis of silver nanoparticles, silver nitrate ($AgNO_3$) was used as a precursor and cumin extract was used as both reducing and capping agent. The cumin extract was prepared by a fine powder of cumin seeds (15 g) which was prepared through the kitchen blender. The seed powder (10 g) was weighted and added to 100 ml of nano pure water followed by heating at 65 °C for 15 min. Then after, by using Whatmann No.1 filter paper the seed extract was filtered. Finally, the filtered extract was preserved at 4 °C for further studies in order to store the sample from environmental conditions. Green synthesis of silver nanoparticles was made by reducing the reaction mixture by stirring in a magnetic stirrer at 60 °C for an hour. The green synthesized nanoparticles were characterized in order to know the shape, size and other parameters by using UV-Visible spectrophotometer (UV-Vis), Particles Size Analyzer (PSA) and Scanning Electron Microscope (SEM). The experiment conducted for standardization of silver nanoparticles consists of twenty nine treatments and three replications. The experiment was laid out in Completely Randomized Design. In this experiment onion seeds were primed with different concentrations of AgNPs at 25, 50, 75, 100, 125, 150 and 200 ppm along with other two treatments which were 1 mM $AgNO_3$ and control (untreated). The priming was carried for a period of 1 h, 3 h, 6 h and 12 h. Later, seeds were shade dried to bring back to original moisture content under ambient conditions and seed quality parameters such as Germination percent, shoot length, root length and Seedling vigour index were studied.

Observations recorded

Seed germination (%)

The germination test was conducted in four replicates of 100 seeds in onion by following between paper method and are incubated in the walk-in seed germination room maintained at 25 ± 5 °C temperature and 90 ± 5 percent relative humidity. The number of normal seedlings in each replication was counted on 12th day. The germination was calculated based on the number of seedlings and expressed in percentage

Root length (cm)

From the germination test, the same ten normal seedlings which are used for measuring the shoot length was used for measuring the root length. The root length was measured from tip of root to the hypocotyl point and the mean length was calculated and expressed in centimeter.

Shoot length (cm)

From the germination test, ten normal seedlings were randomly selected from each treatment on twelfth day and the shoot length was measured from the tip of shoot to the hypocotyl point and the mean length was calculated and expressed in centimeter.

Seedling Vigour Index (SVI-I)

The Seedling Vigour Index was calculated by using the formula suggested by Abdul-Baki and Anderson (1973) [2].

Seedling Vigour Index- I = Germination (%) × Total seedling length (cm)

Results and Discussion

In the present experiment, silver nanoparticles were synthesized using aqueous extract of cumin seeds. The reaction mixture was prepared by adding 25 ml cumin seed extract to 50 ml of 1 mM $AgNO_3$ solution in the ratio 1:2. The mixture of aqueous seed extract and $AgNO_3$ solution was stirred in a magnetic stirrer and boiled at 60 °C for an hour. After an hour of reaction, the colour of the reaction mixture changes to dark brown colour indicating the reduction of Ag^+ ions to AgNPs. The development of dark brown colour owing to the surface plasmon resonance confirmed the synthesis of the silver nanoparticles. *Cuminum cyminum* is a medicinal plant that contains several secondary metabolites such as terpenoids, polyphenols and flavonoids. The cumin extract's potential functional groups such as alcohols and alkenes are responsible for capping and reducing silver nanoparticles. However, the significant flavonoid and phenolic content of *Cuminum cyminum* extract greatly supported its ability to bioreduce Ag^+ to Ag^0 as reported by Karamian and Kamalnejad (2019). To confirm the formation of the silver nanoparticles, AgNPs were characterized through PSA, SEM and UV-Vis Spectrophotometer.

The UV-visible spectrophotometer (UV-Vis) was used to characterise the green-synthesized silver nanoparticles. The silver nanoparticles developed a noticeable peak at 420 nm. In this study, a sharp and broad surface plasmon resonance peak at 420 nm in UV–Vis spectroscopy obtained confirmed the successful synthesis of AgNPs using cumin seed extract. Because of their surface plasmon resonance (SPR), metallic nanoparticles exhibit UV-Vis spectrograph peaks in a certain range of electromagnetic wave. Jahan *et al*. (2021) [7] stated that silver nanoparticles provide the characteristic sharp peak in the range of 400-475 nm.

The silver nanoparticles were examined by a Particle Size Analyzer (PSA) to determine several parameters, including average diameter and percentage distribution based on intensity-weight, volume-weight and number-weight of the nanoparticles. However, for the purposes of this specific study, only the percentage distribution in relation to intensity-weight was considered. The results of the analysis revealed that the average size of the AgNPs ranged from 60 to 70 nm, as determined by the particle size analyzer. The findings presented herein are supported by the report given by Jahan *et al*. (2021) [7], wherein, they have synthesized silver nanoparticles from cumin seed extract and have indicated an average particle size of 14.3 nm.

Scanning Electron Microscopy (SEM) is a surface imaging technique that provides valuable information regarding material size, shape and morphology. Nanoparticles of various sizes were identified with smooth shiny surfaces and spherical shapes which was confirmed through SEM analysis using different magnifications. A similar report was made by Dinparvar *et al*. (2020) [5], in which silver nanoparticles synthesized from cumin seed extract exhibited spherical shapes upon SEM characterization. Abdulazeem *et al*. (2021) [1] findings also supported the results in the study, where the presence of spherical-shaped green-synthesized nanoparticles with dimensions of 48 nm.

For standardization of priming duration of silver nanoparticles, onion seeds were primed with green synthesized silver nanoparticles at different concentrations *i.e.*, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 200 ppm and control for durations of 1 hour, 3 hour, 6 hour and 12 hours soaking period respectively. Observations of seed quality parameters such as seed germination (%), shoot length (cm), root length (cm) and Seedling Vigour Index (SVI-I) were recorded and shown in Table 1.

The data on seed quality parameters in onion were significantly influenced by priming with silver nanoparticles. The higher germination percentage was found in seed primed with silver nanoparticles at 100 ppm for 3 hour soaking (T_{14} - 85.67%) followed by seeds primed with silver nanoparticles at 75 ppm for 3 h soaking (T_{10} - 84.00%), while remained significantly varied with treatments, where lower germination percentage was recorded in control (T_{29} - 76.33%), respectively. Higher shoot length was recorded in AgNPs at 100 ppm (T_{14} - 12.21 cm) primed seeds for 3 hours, which was followed by AgNPs at 75 ppm (T_{10} - 12.06 cm) for 3 hours when compared to control (T_{29} - 10.41 cm). Significantly higher root length was recorded in AgNPs at 100 ppm (T_{14} - 7.18 cm) primed seeds for 3 hours followed by AgNPs at 75 ppm (T_{10}- 6.89 cm) for 3 hours compared to control (T_{29} - 5.81 cm). Similarly, Significantly higher Seedling Vigour Index-I was recorded in AgNPs at 100 ppm (T_{14} - 1661) primed seeds for 3 hour duration, which was followed by AgNPs at 75 ppm (T_{10} - 1592) for 3 hours when compared to control (T_{29} - 1238).

Results recorded on seed germination, shoot length, root length and Seedling Vigour Index-I clearly indicated that between 1 h, 3 h, 6 h and 12 h soaking duration, seeds primed for 3 hours observed higher results as compared to seeds primed for other durations. The significant impact of priming with green synthesized AgNPs on seed quality parameters in onion was recorded compared to the control. The potential explanation for this phenomenon is supported by findings of Savithramma *et al*. (2012) [10]. According to their study, the nanoparticles could have induced the creation of novel pores on the seed coat upon infiltration. This activation of phytohormones subsequently facilitates the process of water absorption, which in turn may promote the influx of nutrients into the seed or the transportation of nutrients alongside the water. These mechanisms ultimately contribute to a marked enhancement in both germination and seedling growth rate. It is justified that the practice of utilizing nanoparticles for seed priming may facilitate the infiltration of water through the seed coat, resulting in increased water imbibition and hastened seed germination as well as improved establishment and increased emergence rate. The findings of this current study are akin to the research conducted by Acharya *et al*. (2017) [3] on onion seeds, where the implementation of seed priming with photosynthesized AgNPs solutions led to a more rapid uptake of water after 4 and 12 hours of imbibition. This, in turn corresponded with early protrusion of radicles and higher germination percentages as well as longer shoot and root lengths in nanoprimed seeds as compared to other priming treatments and control.

Table 1: Influence of seed priming with AgNPs on germination percentage, shoot length (cm), root length (cm) and Seedling Vigour Index (SVI-I) of onion seeds

Treatments	Germination (%)	Shoot length	Root length	SVI-I
T_1- SP with AgNPs @ 25 ppm for 1 h	78.00 (62.03)	11.21	6.12	1323
T_2- SP with AgNPs @ 25 ppm for 3 h	81.67 (64.65)	11.56	6.31	1459
T_3- SP with AgNPs @ 25 ppm for 6 h	80.00 (63.43)	11.06	5.98	1346
T_4- SP with AgNPs @ 25 ppm for 12 h	79.33 (62.96)	10.86	5.75	1290
T_5- SP with AgNPs @ 50 ppm for 1 h	80.00 (63.43)	10.89	6.21	1356
T_6- SP with AgNPs @ 50 ppm for 3 h	82.33 (65.14)	11.76	6.61	1513
T_7- SP with AgNPs @ 50 ppm for 6 h	81.00 (64.16)	11.38	6.35	1412
T_8- SP with AgNPs @ 50 ppm for 12 h	80.33 (63.67)	11.14	6.17	1339
T_9- SP with AgNPs @ 75 ppm for 1 h	81.67 (64.65)	11.53	6.59	1437
T_{10}- SP with AgNPs @ 75 ppm for 3 h	84.00 (66.42)	12.06	6.89	1592
T_{11}- SP with AgNPs @ 75 ppm for 6 h	82.33 (65.14)	11.78	6.67	1500
T_{12}- SP with AgNPs @ 75 ppm for 12 h	81.00 (64.16)	11.27	6.37	1377
T_{13}- SP with AgNPs @ 100 ppm for 1 h	82.33 (65.14)	11.81	6.85	1511
T_{14}- SP with AgNPs @ 100 ppm for 3 h	85.67 (67.76)	12.21	7.18	1661
T_{15}- SP with AgNPs @ 100 ppm for 6 h	84.00 (66.42)	11.92	7.01	1559

T_{16}- SP with AgNPs @ 100 ppm for 12 h	81.33 (64.40)	11.57	6.72	1445
T_{17}- SP with AgNPs @ 125 ppm for 1 h	81.00 (64.16)	11.31	6.29	1384
T_{18}- SP with AgNPs @ 125 ppm for 3 h	82.67 (65.40)	11.88	6.94	1568
T_{19}- SP with AgNPs @ 125 ppm for 6 h	82.00 (64.90)	11.54	6.49	1461
T_{20}- SP with AgNPs @ 125 ppm for 12 h	79.67 (63.92)	11.20	6.45	1400
T_{21}- SP with AgNPs @ 150 ppm for 1 h	81.00 (64.16)	11.47	6.34	1395
T_{22}- SP with AgNPs @ 150 ppm for 3 h	83.00 (65.65)	11.74	6.67	1491
T_{23}- SP with AgNPs @ 150 ppm for 6 h	78.67 (62.49)	11.42	6.38	1370
T_{24}- SP with AgNPs @ 150 ppm for 12 h	78.00 (62.03)	11.17	6.19	1331
T_{25}- SP with AgNPs @ 200 ppm for 1 h	78.33 (62.26)	11.14	6.09	1309
T_{26}- SP with AgNPs @ 200 ppm for 3 h	80.33 (63.67)	11.43	6.51	1424
T_{27}- SP with AgNPs @ 200 ppm for 6 h	77.67 (61.80)	10.89	5.73	1274
T_{28}- SP with AgNPs @ 200 ppm for 12 h	77.00 (61.34)	10.77	6.18	1306
T_{29}- Control (Untreated)	76.33 (60.89)	10.41	5.81	1238
S.Em (±)	0.73	0.10	0.06	16.71
C.D (1%)	2.35	0.27	0.19	62.92

*Figures in the parentheses indicate arcsine root transformed values

Conclusion

In conclusion, this study demonstrates that silver nanoparticles was successfully synthesized through *cuminum cyminum* seed aqueous extract by reduction method. By characterization, silver nanoparticles showed absorption peak at 420 nm in UV-Vis and average particles size of 64 nm in PSA, respectively. SEM images revealed green synthesized silver nanoparticles were of spherical shape and smooth surface Among different AgNPs priming duration seed priming with 3 hour recorded higher performances for seed quality attributes and considered as best soaking duration for AgNPs priming.

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