Proceedings of 15th Agricultural Research Symposium (2016) 140-143

UV Characterization of Green Synthesis of Silver Nanoparticles and its' Effects on Phytopathogenic Fungi

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ABSTRACT

Biological reduction of silver ions using *Azadiracta indica* (neem) aqueous leaf extract are used for the greener synthesis of silver nanoparticles (AgNPs). In this study, AgNPs were biosynthesized and characterized by UV- visible spectrum to confirm the formation of the AgNPs. Different incubation time periods from 2 h to 18 h of AgNPs solutions prepared by mixing 2.5 g/100 mL Neem leaf extract and 0.001 M silver nitrate were tested to determine the stable nature of AgNPs. Antifungal effect of AgNPs was also investigated with different amounts of AgNPs and tested to determine the inhibitory effect on phytopathogenic fungi namely *Mucor* and *Colletritichum*. The UV-visible spectrum of the biosynthesized AgNPs showed strong absorption peak ranged from 400-450 nm confirming the formation of AgNPs in the solution. Twenty five milligrams (25 mg) per 1.5 mL amount of AgNPs showed promising inhibitory effect against tested fungal pathogens. Silver nanoparticles can also be tested in controlling various plant diseases caused by phytopathogenic fungi.

KEYWORDS: Antifungal activity, *Azadirachta indica*, Silver nanoparticles, UV-visible spectroscopy

INTRODUCTION

Nanotechnology concerns with the fabrication of materials at nano level which have profound application in various areas. Owing to unique physical and chemical properties of nanoparticles, they are gaining the interest of scientist for their novel methods of synthesis. Biosynthesis of nanoparticles using plants provide a non-toxic and cost effective platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents (Singhal *et al.*, 2010).

Most recently, renewed interest has arisen in manufacturing silver nanomaterial which is one of the frequently used nanoparticles from *Azadirachta indica* leaf extract as it is commonly available medicinal plant and biosynthesized silver nanoparticles has efficient antibacterial activity and natural stabilization as it was capped with the neem leaf extract (Mukherjee *et al.*, 2008; Ahmed *et al.*, 2015).

Previously, synthesis of AgNPs from neem leaf extract and their characterization by color change, UV-visible absorption spectroscopy, X-ray diffraction (XRD), Fourier transform spectroscopy (FTIR), Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) have been reported (Thamer and Almashhedy, 2014; Vivehananthan *et al.*, 2015; Ahmed *et al.*, 2015). The antibacterial activity of silver nanoparticles was also detected against some pathogenic bacteria commonly found in wastewater (Kumar *et al.*, 2015). Further studies reported that phosphorus and sulphur compounds containing in fungi spores and cell membranes may be interacted with AgNPs and their interactions may affect to the destruction of DNA and proteins causing cell death (Krishnaraj *et al.*, 2012).

Present study focused on synthesis of silver nanoparticles, assessing the stability of aqueous AgNPs and evaluating antifungal activity of silver nanoparticles on selected phytopathogenic fungi. Further, this study was extended to determine the minimum concentration of AgNPs required to have complete inhibition on the selected phytopathogenic fungi.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Department of Biotechnology, Faculty of Agriculture and Plantation Management Wayamba University of Sri Lanka during the period of December 2015 to June 2016. Neem leaf samples were collected from Gampaha area.

Preparation of Azadirachta indica Leaf Extract and Silver Nanoparticle Solution

Crude neem leaf extract was prepared by taking 2.5 g of thoroughly washed and finely cut neem leaves in a 500 mL Erlenmeyer flask with 100 mL of sterile distilled water followed by boiling the mixture for 2 min. The mixture was then filtered using Whatman No. 1 filter papers (De Silva *et al.*, 2013).

Silver nanoparticles were synthesized by mixing neem leaf extract with 0.001 M Silver nitrate at 1:8 ratio and the reaction was allowed

to proceed in the dark at room temperature for the time period from 2 h to 18 h (Kumari *et al.*, 2014; Kumar *et al.*, 2015).

UV Characterization of Silver Nanoparticles

The synthesized AgNPs were initially characterized by color change and the maximum absorption was monitored by UV-visible Spectrophotometer from 200 to 800 nm on Shimadzu double beam spectrophotometer (model U-1800). The sterile distilled water was used as the blank. Progress in the reaction of the solution was monitored continuously at one-hour time intervals from 2 h to 18 h continuously.

Preparation of Silver Nanoparticles

AgNPs were pelleted down by centrifugation at 12 000 rpm for 15 min at 4 °C. Pelleted down AgNPs were mixed with 100% acetone and the mixture was air dried for two days to prepare dry AgNPs.

Preparation of Fungal Lawn

In this experiment, *Mucor* and *Colletrtichum* were cultured in potato dextrose agar (PDA) medium by inoculating fungi spores in the center of sterilized and solidified medium. Then, the plates were incubated at 37 °C for 7 days to obtain a pure fungal lawn.

Preparation of Fungal Spore Suspension Culture

The pure fungal lawn was taken and 10 mL of autoclaved distilled water was poured onto the surface of the fungal lawn. The mycelium was scraped using a sterile inoculation loop and poured the liquid into a conical flask and filtered it through gauze. Remaining mycelium was also scrapped, rinsed with additional 20 mL of autoclaved distilled water and filtered by pressing the gauze.

At the end of the filtration, fungal spores in 30 mL of distilled water was collected. Fungal suspension culture was diluted 10, 100, 150 and 200 times by adding autoclaved distilled water.

Effects of AgNPs on Fungal Growth

Different amounts of AgNPs (0.5, 1, 2, 3, 5 and 10 mg) prepared after 2 h incubation period of the AgNPs solution, were directly added to the series of diluted fungal spore suspensions separately. Control was used without adding AgNPs.

In addition, different concentration of AgNps (5, 10, 12.5, 15, 17.5 and 25 mg) were tested directly to the original fungal cultures of the selected fungi (Figure 3).

All the samples were incubated at room temperature under constant agitation at 120 rpm in an orbital shaker for 3 h. Two hundred micro liters (200 µL) from each sample was spread on same diametric PDA plates after 3 h shaking and incubated at 37 °C overnight.

RESULTS AND DISCUSSION

UV Characterization of Silver Nanoparticles

The present study demonstrated the rapid formation of silver nanoparticles from neem leaf extract which acted as a natural stabilizer, reducing and capping agent. Reduction of the silver ions to AgNPs during exposure to the neem leaf extracts was evident by color change from pale yellow to ruby red. This is due to the excitation of surface plasmon vibrations in AgNPs (Chandran *et al.*, 2006; Krishnaraj *et al.*, 2012).

Surface plasmon resonance (SPR) band reported in the visible range from 400-450 nm indicated the formation of silver nanoparticles (Singh *et al.*, 2010; Ahmed *et al.*, 2015; Vivehananthan *et al.*, 2015). UV-visible spectra of the present study also showed the SPR band within this range confirming the formation of AgNPs in the solution.

In previous studies different parameters were optimized for synthesizing silver nanoparticles including concentration of silver nitrate, mixing ratio of leaf extract and silver nitrate. Time is also found to be one of the key factors playing a major role in nanoparticles synthesis (De Silva *et al.*, 2013; Krishnaraj *et al.*, 2012; Shankar *et al.*, 2004).

In this study, formation of AgNPs was initially characterized by color change within 30 min of incubation period. Biosynthesized silver nanoparticles were then characterized by UV-visible spectra as depicted in Figure 1 where the reaction between neem leaf extract and silver nitrate was monitored from 2 h to 18 h at one hour time interval continuously. Research in the past revealed that the intensity of the SPR band is initially proportional to the concentration of AgNPs (Mukherjee *et al.*, 2008). It has also recorded that the intensity of SPR band increases with number of days of incubation of silver nanoparticle solution due to the rapid rise in AgNPs concentration in the solution (Mukherjee *et al.*, 2008; Krishnaraj *et al.*, 2012; Shirvastava *et al.*, 2007).

Similarly, present study exhibited the increase in intensity with number of hours of incubation as a result of increasing amount of synthesized AgNPs. Absorbance values corresponding to the SPR peak increased linearly for first 2 days due to rapid formation of silver nanoparticles (Mukherjee *et al.*, 2008).

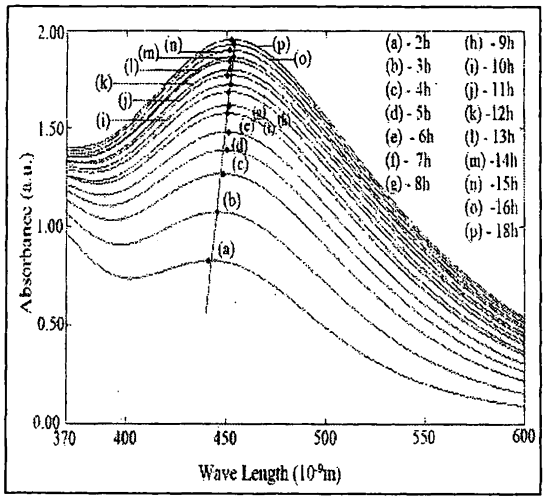

Figure 1. Ultra violet-visible spectra of aqueous silver nitrate with neem leaf extract at different time intervals

However, the study illustrated that the intensity of the SPR band within 400-450 nm range rises linearly from 2 h to 6 h due to rapid formation of AgNPs and then increases slowly until the graph flattens out after 6th hour indicating slower reduction rate of silver ions (Figure 2).

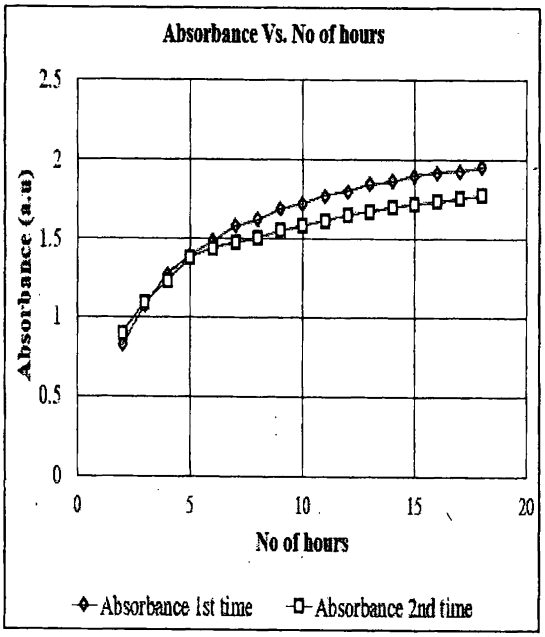

Figure 2. Absorbance values of reaction mixture at different time intervals

From UV-visible spectroscopy, it is clear that first 6 h of the incubation period is effective for the rapid formation of silver nanoparticles and therefore minimum time period of this range (2 h) was selected to synthesis AgNPs to test the antifungal activity.

UV-visible spectroscopic analysis study was proven to be effective in synthesizing silver nanoparticles. This study clearly showed no alteration in the peak at 400-450 nm even after 10 h of incubation period, indicating strong stability of biosynthesized silver nanoparticles in the solution. Therefore, it is clear that monitoring of different time period required for the synthesis of AgNPs by UV characterization process will give a better idea of minimum incubation period related to rapid formation of silver nanoparticles.

Antifungal Activity

The antifungal activity of biosynthesized silver nanoparticles was performed against phytopathogenic fungi, *Mucor* and *Colletritichum.*

Maximum level of fungal inhibition was observed with increasing the amount of silver nanoparticles. Moreover, minimum 3 h agitation period is proven to be better before culturing of phytopathogenic fungi treated with AgNPs.

Interestingly, this study showed that 25 mg/1.5 mL of AgNPs can greatly suppress the fungal growth in original microbial culture (Table 1) and these results are more or less similar observations with the previous investigations with 15 mg/10 µL to control the fungal growth of *Botrytis cinerea* (Krishnaraj *et al.*, 2012).

Earlier studies have reported that AgNPs have an ability to destroy fungal spores by destructing the membrane integrity and AgNPs which caused to damage DNA and proteins of fungi resulting in cell death (Krishnaraj *et al.*, 2012).

Table 1. Inhibited dilution level of phytopathogenic fungi treated with AgNPs

AgNPs (mg) in 1.5 mL	Dilution of fungal suspension for complete inhibition
0.5	200
1	100
2	10
3	10
5	10
25	0

AgNPs- Silver nano particles

Present study revealed that AgNPs have substantial potential applications to be used to control the selected fungi effectively.

CONCLUSIONS

Present study clearly concluded that the rapid formation of AgNPs within 400-450 nm required 2-6 hour of incubation period. Also, it can be concluded that AgNPs have good antifungal activity against all the tested fungal pathogens. Therefore, AgNPs may have the potential in controlling plant diseases caused by phytopathogenic fungi.

ACKNOWLEDGEMENTS

The authors would like to offer their sincere thanks to all the academic and non-academic staff members of the Department of Biotechnology and all the members of Nanotechnology Laboratory Specially Dr. U. S. Liyanaarachi and Mr. G. G. Chathuranga, Department of Electronics, Faculty of Applied Science, Wayamba University of Sri Lanka.

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