
Research Article

Effect of silver nanoparticles, synthesized from fleshy stem and leaves extract of *Brassica campestris* L., on *Alternari lini* inhibition

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Abstract

Several fungal pathogens showed resistance against pesticides, plant mediated synthesized silver nanoparticles have been recognized as effective against them. The present research work was an attempt to synthesize silver nanoparticles by using fleshy stem and leaves extract of *Brassica campestris* L. from silver nitrate, which reduces it to synthesized silver nanoparticles. Biosynthesized AgNP's were characterized by different techniques like UV-visible spectrophotometry and Field Emission Scanning Electron Microscopy and fungal inhibitory activity by well diffusion method. The colour change of solution indicated the synthesis of silver nanoparticles. UV-visible spectrophotometer showed the peak between 415 to 455 nm for fleshy stem and leaves extract of *B. campestris* and Field Emission Scanning Electron Microscope analysis revealed that spherical shaped nanoparticles with size 20-65 nm. These synthesized nanoparticles showed inhibitory activity against fungi, *Alternaria lini*, a linseed blight pathogen which causes linseed blight disease in *Linum usitatissimum* L. The 30 µl concentrations of silver nanoparticles had showed significant inhibition to growth of *A. lini*. These findings may suggest AgNP's synthesized by *B. campestris* used against plant pathogenic fungi.

Introduction

Green synthesis of metallic nanoparticles is an emerging aspect of modern nanotechnology. It is an eco-friendly method of synthesis of nanoparticles (Raja et al., 2018). Besides this it is easy, economical and time saving method. It is reported by various researchers that Silver nanoparticles have great antibacterial (Asghar et al., 2018; Khatami et al., 2018; Gokak & Taranath, 2014), antifungal (Asghar et al., 2018) (Khatami et al., 2018), antiviral (Rogers et al., 2008), anticancer (Khatami et al., 2018), anti-

inflammatory (Nadworny et al., 2010) and antioxidant activity (Kharat & Mendhulkar, 2016; Sivakumar et al., 2015). Different parts of the plant such as: root, stem, leaves, fruit and bark are used for the production of nanoparticles (Shanmuganathan et al., 2017) due to the composition of various biomolecules (carbohydrates, proteins, phenols, ketones, etc) in them and capable to reduce, stabilize and maintain their shape and size (Mariselvam et al., 2014).

Brassica campestris L. is an important plant belongs to family Brassicaceae. Commonly

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known as sarso, yellow mustard, etc. It is used as a food for human and animals in Pakistan.

Linum usitatissimum L. known as common linseed or Flax, belongs to family Linnaceae, has cultivated world-wide due to its importance in medicine and industry as immunomodulatory, antioxidant, anti-tumor, antimicrobial, anti-inflammatory and analgesic, etc. (Ansari et al., 2019). At the time of its cultivation farmers face various problems like disease management. Linseed Blight disease that caused by *Alternaria lini* Dey, adversely effects and reduces the yield of linseed. This work was an attempt for the exploration of antifungal potential against linseed blight of phyto-synthesized silver nanoparticles from fleshy stem and leaves extract of *Brassica campestris* L.

Materials and Methods

Sample collection and identification

Fully young plants of *Brassica campestris* L. were collected from the fields of Sarhota No. 2, Tehsile and District Kotli AJK Pakistan, and then brought at University of Kotli for identification. Identification was done with the help of available literature and was confirmed from flora of Pakistan.

Preparation of fleshy stem and leaves extract

Brassica campestris were collected from the field and brought into the lab where they were rinsed with tap water for the removal of dust and other particles. Fleshy stem and leaves were then removed. To make aqueous extract of *B. campestris*, about 10 g of fleshy stem and leaf sample was mixed in 100 ml distilled water and boiled in water bath for 20 minutes at about 60 °C. Then, extract was cooled and filtered using Whatman No. 1, filter paper. Filtrate than stored and used for the further reactions.

Silver nanoparticles synthesis

Methods described by Meenakshisundaram et al. (2013) and Thilagam et al. (2013) were followed with appropriate changes for the synthesis of silver nanoparticles from the fleshy

stem and leaves extract of *B. campestris*, the extract was reacted with silver nitrate (AgNO_3) solution. About 10 ml extract added to the 90 ml silver nitrate solution. This reaction mixture was then kept in dark condition at room temperature. The bio-reduction of silver particles by fleshy stem and leaves extract was scrutinized by measuring the UV-visible spectra of the reaction medium at 200 to 800 nm. Complete bio-reduced sample was than centrifuged at 10000 rpm for 20 minutes at 4 °C. The pellet was collected and preserved for further reactions.

FE-SEM analysis

Field Emission Scanning Electron Microscope (Model: JSM-7800F) was used to observe the finest structural morphology of nano materials of Silver at 1,000,000x magnification with 1 nm resolution.

Antifungal activity

Infected plant of linseed was collected from field and pathogen, *Alternaria lini* was screened and cultured on CDA media, according to method described by Savithamma & Rao (2011). Then, material from this culture media was used for the study of inhibitory action of silver nanoparticles. Well diffusion method described by Perez et al. (1990) was followed for the study of antifungal activity. The agar plates were inoculated by spreading the spores of the *Alternaria lini* over the entire agar surface. Then these 6 mm wells were punched and different concentrations viz 10 μl , 20 μl , and 30 μl of the nanoparticles were loaded in each plate. After incubation, twenty-four hours, zone of inhibition of *A. lini* was recorded.

Results and Discussion

Visual identification

Colour change of 1 mM silver nitrate (AgNO_3) solution indicated that the synthesis of nanoparticles, from colourless to blakish brown after reaction with fleshy stem and leaves extract of *B. campestris* (as shown in Figure 1).

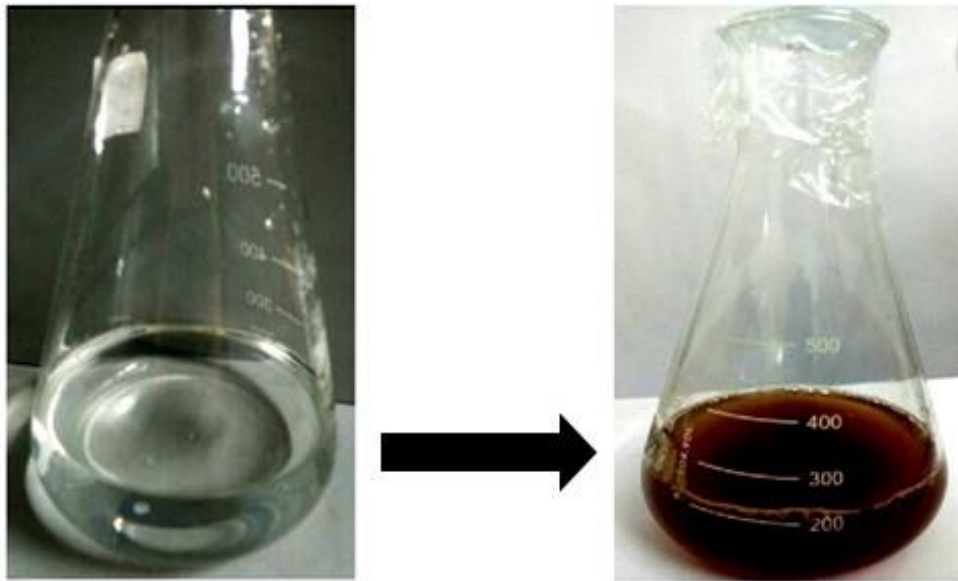


Figure 1. Visual identification of synthesis of AgNP ($AgNO_3$ solution colour changes from colourless to blackish brown after reaction with fleshy stem and leaves extract of *B. campestris*)

UV-Visible spectroscopy

Silver nanoparticles formation was confirmed by UV-visible spectrophotometer. Characteristics surface plasmon absorption peak was observed between 415 to 455 nm by UV-visible spectrophotometer (as shown in

Figure 2). In the literature, absorption peak of nanoparticles is observed at 400 to 450 nm (Ajitha et al., 2015) which is specific for silver nanoparticles (Dzul-Erosa et al., 2018; Patil & Kim, 2016).

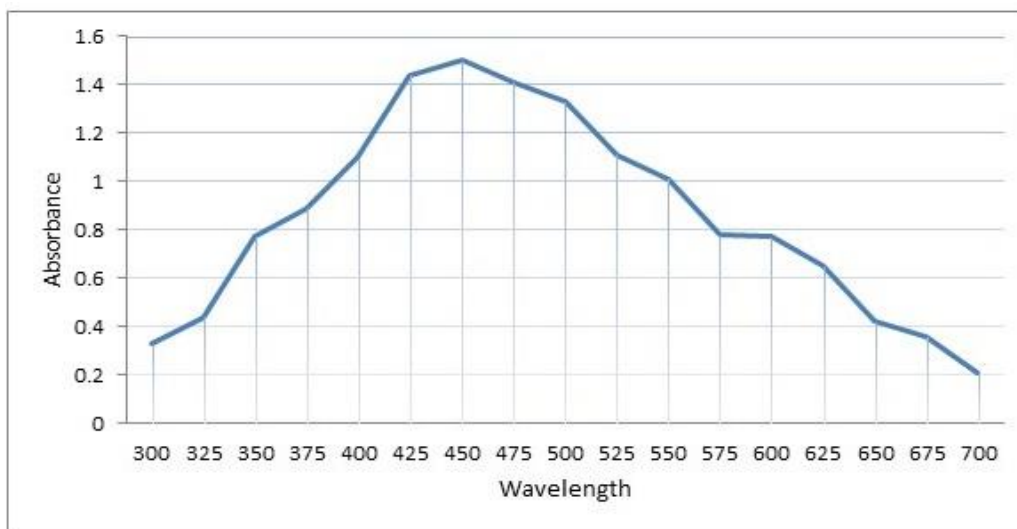


Figure 2. UV-visible spectrograph of silver nanoparticles

FE-SEM analysis

Size and shape of nanoparticles were confirmed through Field Emission Scanning Electron Microscope (FE-SEM) analysis. FE-SEM

analysis revealed that the shapes of nanoparticles were spherical and their size ranges between 20 to 65 nm (as shown in Figure 3). Most nanoparticles were distributed randomly in the form of clumps.

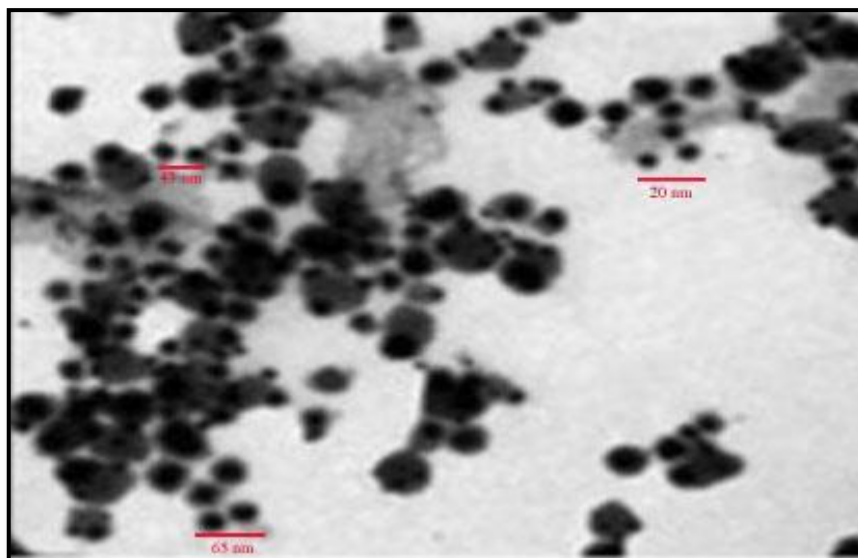


Figure 3. FE-SEM micrograph of silver nanoparticles

Antifungal activity

Silver (Ag) nanoparticles synthesized from fleshy stem and leaves extract of *B. campestris* had showed the potential to inhibit the growth of fungal strain, *Alternaria lini*. Different concentrations i.e. 10 μ l, 20 μ l, 30 μ l of nanoparti-

cles were applied on culture media of fungi, *Alternaria lini*. It was observed that 30 μ l concentrations of nanoparticles were more effective in inhibiting the growth of *Alternaria lini* (as shown in Table 1).

Table 1. Fungal zone of inhibition by applying different concentration of AgNP's

| Sr. # | Concentration of AgNP's used | Inhibition zone |
|-------|------------------------------|-----------------|
| 1 | 10 μ l | 7 mm |
| 2 | 20 μ l | 11 mm |
| 3 | 30 μ l | 13 mm |

In the last few decades, silver nanoparticles are widely used against various pathogens including bacteria and fungi. Due to their diverse applications in certain other fields such as: agriculture, drug development and in clinical research, much attention has been given on their eco-friendly and economical synthesis. Plant based approaches for their synthesis are eco-friendly and economical (Prabhu & Poulouse, 2012; Kathiresan, et al., 2019; Pasa et al., 2019). Phytochemicals in plants acts as reducing agent. They reduce the toxic Ag to nontoxic Ag nanoparticles (Kumar et al., 2017). In present work, Silver nanoparticles were synthesised from *B. campestris* and from UV-visible spectroscopic analysis maximum absorption peak was

observed at 415 to 455 nm. Our result, resembles with the results of Asha et al. (2017), Rajeshkumar et al. (2017), Iqbal and Bakht (2019) and Pasa et al. (2019), their results showed maximum absorption peak between 400 to 450 nm.

The productivity of linseed crop had reduced due to various diseases caused by various pathogens. In sub-continent especially in Pakistan and India the productivity of linseed crop is very low due to pathogenic attack. A fungal species, *Alternaria lini* reduced the yield of linseed crop 25 to 60% alone annually of the total yield (Chauhan et al., 1975). The silver nanoparticles synthesized from fleshy stem and leaves extract of *B. campestris* are useful in

management of the *A. lini*, pathogen and boosting the yield of linseed crop. These nanoparticles might be used against various other pathogens and in agricultural industries for the development of fungicides.

Conclusion

Silver nanoparticles were synthesized from fleshy stem and leaves extract of *Brassica campestris* L. and confirmed by UV-visible Spectroscopy and FE-SEM analysis. The inhibitory activity of biosynthesized silver nanoparticles against *Alternaria lini* was then evaluated. Our results showed significant inhibitory activity against *A. lini*. Thus, the findings of this study could be adopted as a tool for green synthesis of silver nanoparticles, having significant fungal inhibiting potential. These nanoparticles may be used against various fungal pathogens to enhance the yield quantity and quality of cereal crops.

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Conflict of Interest

Author declared no conflict of interest.

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