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# Antifungal activity of pharmacologically active silver nanoparticles (AgNPs) from *Nigella sativa* against *Aspergillus niger* and *Fusarium oxysporum*

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**Abstract**
Green synthesis method for nanoparticle production is gaining more important because of adverse effect of use of toxic chemicals for nanoparticles synthesis. An alarming increase in fungal strains resistant to existing antifungal agents demands a renewed effort to seek agents effective against pathogenic fungus resistant to antifungal agents. The aim of this study was to assess the antifungal activity of different concentrations of $AgNO_3$ solution, AgNPs synthesized by the wet chemical method and green method and aqueous extract of medicinal plant *Nigella sativa* seeds using disk diffusion assay on selected laboratory isolates of fungus. The aqueous extract of *Nigella sativa* were screened for their antifungal activity against laboratory isolates and the aqueous extract of *Nigella sativa* seeds showed pronounced dose dependant antifungal activity on laboratory isolates of *Aspergillus niger* and *Fusarium Oxysporum*.

**Keywords:** *Nigella sativa,* nanoparticles, antifungal activity

## Introduction
Currently, there is a worldwide growing interest in the use of medicinal herbs or plants in the treatment of various diseases due to their promising results and fewer side effects. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization (Ahmed *et al*., 1998). Following the advent of modern medicine, herbal medicine suffered a setback, but during the last two or three decades, advances in phyto-chemistry and in the identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines (Arora and Kaur, 1999) [4]. Plant products derived from barks, flowers, roots, leaves, seeds, fruits are the part of phytomedicines (Cragg and David, 2001) [6]. According to the World Health Organization (WHO), 60-80% of the world's population particularly in developing countries, depends on herbal remedies or traditional medicine for their primary health care and treatment. Moreover, the WHO has encouraged developing countries to use their medicinal plants as a resource to generate effective health care programs. One of the tops ranked evidence-based herbal medicines, which has been described as the "miracle herb of the century'' is *Nigella sativa* (Ahmad *et al*., 2013) [2].
*Nigella sativa*, a buttercup-like, the annual flowering plant which belongs to the botanical family of *Ranunculaceae*is native to the south and south west Asia and is cultivated in several countries in the Mediterranean region, South Europe, Syria, Turkey, Pakistan, India and Saudi Arabia. It is a small shrub with tapering green leaves and rosaceous white and purplish flowers. Its ripe fruit contains tiny black seeds, known as the black seed, black cumin, or black caraway in English. It is mainly cultivated around the world for its seeds which can be used as a spice or preservative agent. Today, however, the seeds are also used for the extraction of an essential oil that is used in traditional medicine (Zohary and Hopf, 2000) [9].

## Materials and Methods
### Experimental Site
The present study entitled "Assessment of Antifungal property of pharmacologically active nanoparticles from *Nigella sativa"* was conducted in the Nanotechnology Laboratory of Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad from January to May 2018.

### Materials
### Chemicals
All the chemicals were procured from Himedia Chemicals Pvt. Ltd. and Sigma Aldrich, USA and are as follows: Hydrochloric acid, Potato Dextrose agar powder, Polyethylene glycol, Sodium borohydride, Sodium hydroxide, Silver nitrate, Spirit.

### Plastic Wares and Glass Wares
Beaker, Boiling tubes, Centrifuge tubes, Conical flask, Funnel, Glass pipette, Measuring cylinder, Micropipettes, Morter and pestle, Test tubes, Test tube stand.

**Equipment**
B.O.D incubator of 25ºC Double distillation unit, Hot air oven, Hotplate, Laminar air flow chamber, Magnetic stirrer, Ultrasonic cleaner, UV-Visible Spectrophotometer, Vacuum oven, weighing balance.

**Collection and Preservation of Test Microorganisms**
Test microorganisms (*Aspergillus niger*and *Fusarium Oxysporum*) were collected from microbial culture collection bank of Department of Industrial Microbiology of Sam Higginbottom University of Agricultural, Technology and Sciences, Allahabad.
After the collection of the microorganisms (laboratory isolates) slants of potato dextrose agar were prepared, inoculated with organisms and incubated for 48 hours, then preserved in the refrigerator at 4°C.

**Preparation of Aqueous Extract of *Nigella Sativa* Seeds**
Seeds of *Nigella sativa* were purchased from the shops around Mahewa, Naini Allahabad, India. Prior to experimental processthe seeds of *Nigella sativa* were taken and washed with tap water for 20 minutes and were dried completely in the hot air oven for 40 minutes and weighed. 10 grams of seeds were grinned in fine powder by using mortar and pestle and was subjected to steam distillation using Clevenger- type apparatus for aqueous extract extraction. The contents were steam distilled for 3 to 4 h to obtain the aqueous extract with a pleasant smell. The aqueous extract was collected and stored at 4℃ for further synthesis of iron and silver nanoparticles (AgNPs).

**Synthesis of Silver Nanoparticles**
**Green Synthesis**
The green synthesis of silver nanoparticles was done using the aqueous extract of *Nigella sativa*. About 2 to 5 ml of aqueous extract of *Nigella sativa* was added to 100 ml of 0.001 M $AgNO_3$ solution at 70ºC to 80ºC with continuous stirring for 20 minutes. Rapid reduction of $AgNO_3$ ions to Ag occurred and the change of color of pale yellow was observed. The change of color indicates the formation of silver nanoparticles (Mazumdar *et al.*, 2011; Sivalingam *et al*., 2014) [8].

**Chemical Reduction**
The protocols according to Mazumdar *et al.,* 2011and Sivalingam *et al.,* 2014 [8] were modified as follows: silver nitrate solution of 0.001 M was prepared in 100 ml distilled water and 1 ml of 0.1 M polyethylene glycol and 1 ml of 0.01 M sodium borohydride was added to it and stirred while heating at 40 ºC for 20 minutes, the pale yellow color confirming the formation of silver nanoparticles.

**Characterization of Iron and Silver Nanoparticles**
**UV-Visible Spectroscopy**
The formations of AgNPs via green and chemical synthesis method were confirmed by the spectral analysis. The UV spectra of the synthesized silver nanoparticles were recorded using UV visible spectrophotometer from 200 nm to 700 nm.

**Antifungal Activity**
The crude extracts of *Nigella sativa,* Ag NPs, synthesized by wet chemical method and green method, and solutions of $AgNO_3$were prepared and diluted into different concentrations as follows: 4.4 mg/ml, 2.2 mg/ml, 1.76 mg/ml, 1.32 mg/ml, 0.88 mg/ml, 0.44 mg/ml to be used against the selected organisms.

**Preparation of Fungal Suspension**
New subcultures of the selected fungal strains (*Aspergillus niger*and *Fusarium oxysporum*) which was brought from microbiology department of Sam Higginbottom University of Agricultural, Technology and Sciences, Allahabad were inoculated into potato dextrose agar broth and incubated at 25ºC for 24 to 48 h.

**Assessment of Antifungal Activity**
The antifungal activity of AgNPs against *Aspergillus niger* and *Fusarium oxysporum* was analyzed by disk diffusion assay. At first, *Aspergillus niger* and *Fusarium oxysporum* was collected from microbial collection bank SHUATS as the test organisms. And was cultivated in potato dextrose agar plate (PDA) (Himedia, Mumbai) and from the cultivated agar plate standard fungal suspension was made by inoculating the fungi in potato dextrose broth incubated at 25℃ for 48 h. Six different concentrations (4.4 mg/ml, 2.2 mg/ml, 1.76 mg/ml, 1.32 mg/ml, 0.88 mg/ml, 0.44 mg/ml) of the crude extracts of *Nigella sativa,* AgNPs, synthesized by chemical reduction method and green method, and solutions of $AgNO_3$ were loaded in the sterile disc (5mm) which was placed on the potato dextrose agar plates and were incubated at 25º C for 24 h to 36 h. After incubation, the zone of inhibition was measured.

**Results and Discussion**
The fungi chosen for the study are harmful to human health, food, industry yield, agriculture production etc. Therefore these must be eradicated for healthy environment. Due to the small size and high surface to volume ratio of nanoparticles, these are finding important applications in the drug delivery antifungal preparation.

Silver nanoparticles were prepared by chemical and green synthetic methods and characterized through UV-Visible spectrophotometer analysis. Peaks were observed at 268 nm, 404 nm, 408 nm, 292 nm and 384 nm for pure extract, silver nanoparticles via chemical reduction method, silver nanoparticles via green synthesis method. Antifungal efficiency testing was done by disk diffusion test. On the basis of Dilution test the minimum inhibitory concentration of each nanoparticles was calculated for fungi culture used. Our experimental values shows that for *A. niger* MIC of pure aqueous extract of concentration 4.4 mg/ml, silver nanoparticles via chemical synthesis, and green synthesis of concentration 4.4 mg/ml is more effective as shown in Figure 1.

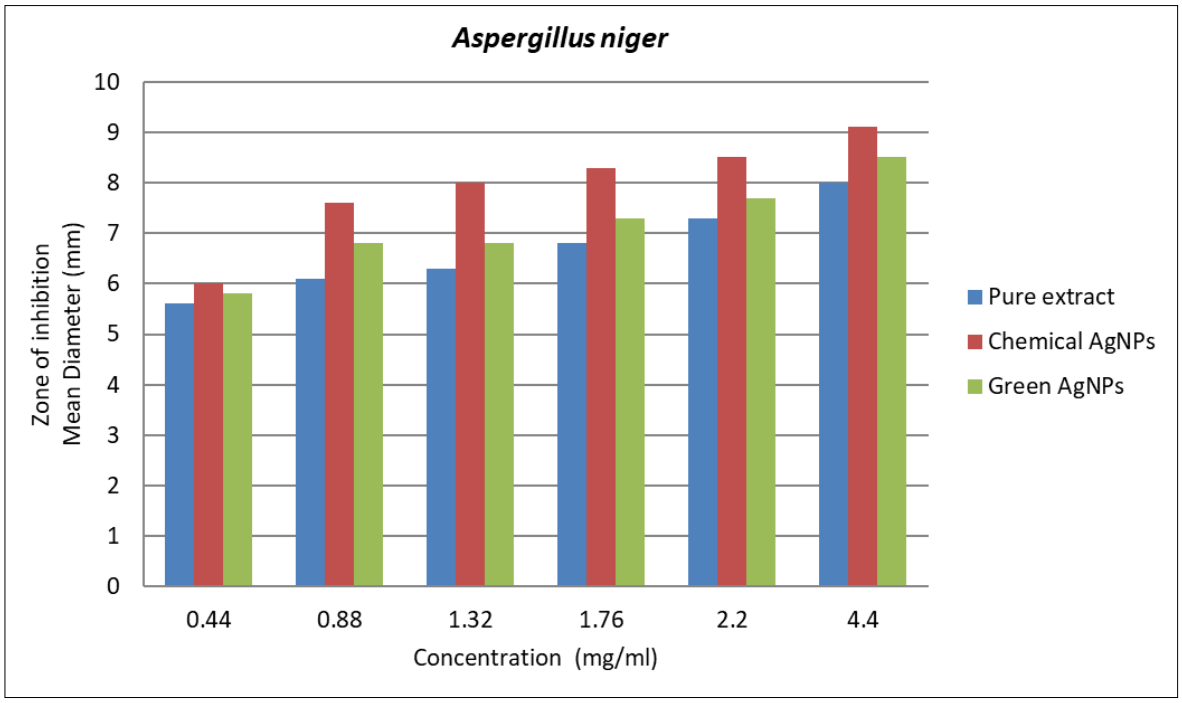


**Fig 1:** Effect of all treatments on *Aspergillus niger*

Whereas experimental values shows that for *F. oxysporum* MIC of pure aqueous extract of concentration 4.4 mg/ml, silver nanoparticles via chemical synthesis, and green synthesis of concentration 4.4 mg/ml is more effective as shown in Figure 1.

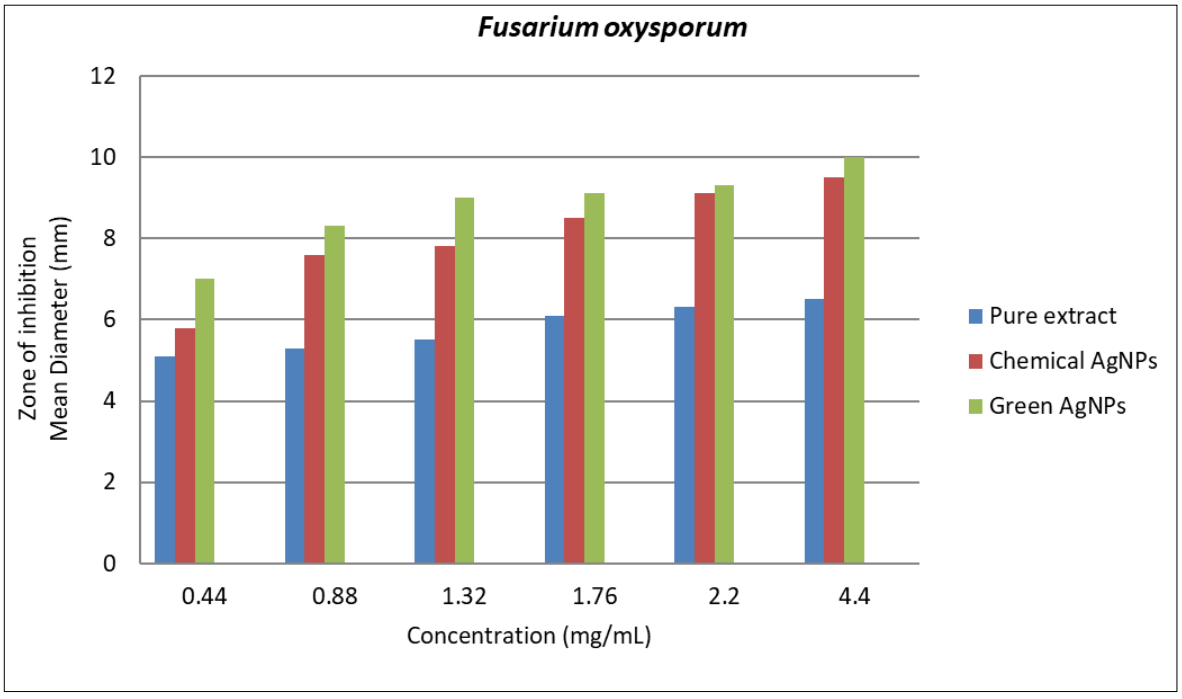


**Fig 2:** Effect of all treatments on *Fusarium oxysporum*

In *vitro* comparison of antifungal activity of thymoquinone and amphotericin B against *Fusarium solani* and *Aspergillus niger* showed complete inhibition of growth at 1.0 and 2.0 mg/ml respectively. (Naeem Akhtar *et. al*., 2007; Abdul Rahman Al-Qurashi *et. al*., 2007) [1, 7, 3].

Antifungal activity of methanolic and ethanolic extracts of the seeds of *Nigella sativa* was investigated on different pathogenic fungal strain such as *Aspergillus, Candida, Cryptococcus* and *Issatchenkia* species. Activity of *Nigella sativa* oil, ether extract and some of its active principles have been reported in the literature against a number of bacteria and *Candida albicans*. The effect of thymoquinone, an active constituent of *N. sativa* and amphotericine B was determined against *Aspergillus niger* ATCC 16404. (Al-Qurashi *et al.*, 2007; Raval *et al*., 2010; Bita *et al*., 2012; Abdel Azeiz *et al.*, 2013) [1, 3, 7, 5].

**Conclusion**

In this study *Aspergillus niger* and *Fusarium oxysporum*were collected from microbial culture collection bank of Department of Industrial Microbiology of Sam Higginbottom University of Agricultural, Technology & Sciences, Allahabad. Further these fungal strains were tested for antifungal activity and determined the efficacy of various formulated nanoparticles against *Aspergillusniger* and *Fusarium oxysporum*.

The aqueous extract of *Nigella sativa* was found to be effective on both the laboratory strains of *Aspergillus niger*and *Fusarium oxysporum.* Whereas, in case of formulated nanoparticles, silver nanoparticle showed antifungal activity against both the laboratory strains of *Aspergillus niger*and *Fusarium oxysporum*. The efficiency of the antifungal activity of aqueous extract of *Nigella sativa* and formulated nanoparticles was found to increase when increasing the extract and formulated nanoparticles concentration.

To conclude, the results of this study indicated inhibitory activity of *Nigella sativa* crude extractand pharmacologically active nanoparticles from *Nigella sativa*against *Aspergillus niger* and *Fusarium oxysporum* that revealed the potential of *Nigella sativa* as a natural source for production of a new fungicidal agent for use in preventing many infectious diseases.

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