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Silver nanoparticles, a potential alternative to conventional anti-fungal agents to fungal pathogens affecting crop plants

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ABSTRACT

Metallic silver nanoparticles have been reduced from silver nitrate by employing the extracellular enzymatic machinery of edible White button Mushroom *(Agaricus bisporus).* The physical properties of these particles, size and shapes have been determined using techniques like TEM, FTIR and XRD and were reported in our earlier report. But, what stands of paramount importance in the present context is the ability of these nanoparticles to successfully combat and nullify the pathogenic fungal strains affecting crop plants. Our study has shown that these silver nanoparticles are successful antifungal agents and can replace the conventional antifungal synthetic chemicals as we tested their efficacy against different rot causative fungi in ground nut. The experimental pathogenic fungi include Stem rot causing diseases *Sclerotium rolfsii*, Dry root rot causing *Rhizoctonia bataticola*, Collar rot causing *Aspergillus niger* in agricultural plants.

Keywords: *Silver nanoparticles; Antifungal activity; Plant pathogens; Sclerotium rolfsii; Rhizoctonia betaticola; Aspergillus niger.*

INTRODUCTION

Nanotechnology, a brand new technology of the new millennium is anchoring strongly day by day by establishing a deep root system and by branching into many disciplines of science thus transforming itself into a new interdisciplinary science. Many new branches of the science are coming out with each passing day like nanomedicine, nanobiotechnology, nanorobotics, nanoelectronics etc, and it has made its potential felt even in the field of agriculture possibly coming out as what we can call "The Nano-agriculture". The antimicrobial activity of the nano particles has been well established in the field of medicine to fight pathogenic microbes. In the present study, we step forward by exploiting the antifungal activity of nanoparticles against the fungal plant pathogens to further the field of nano-agriculture.

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We have chosen one of the wide spread crops of India, namely Groundnut and scientifically called *Arachis hypogea* belonging to family Leguminosae and the largest oil seed crop of India. In an attempt to keep the environment intact and safe, we have chosen the biological synthesis of nanoparticles in *Agaricus bisporus* (White button mushroom) by utilizing its reducing ability of the noble metal. We furthered our studies by testing the efficacy of our silver nano particles against few of the most devastating fungi causing stem rot (*sclerotium rolfsii*), Dry root rot (*Rhizoctonia bataticola*) and collar rot (*Aspergillus niger*) of Groundnut, A major crop It is nearly produced in 6.80 m. ha of land in India of the total 25.02 m.ha in the world, and produces 5.2 m tons of the total 33.72 m tons in the world. India accounts for 33 and 44 per cent of the world's production and by area respectively [1]. The fungal pathogen stem rot alone accounts for a reduction of 10 to 25 percent [2]. Similar losses (25%) in the yield were also reported by Mayee and Datar, 1988 [3]. The pathogen is supposed to be a prevalent and major cause in the reduction of yield in at least 5,00,000 hectares of the land in India. On a global scale, the loss is around 25% and occasionally reaches an 80% mark when severely infected [4].

The causative organism of the stem rot is *Sclerotium rolfsii*, [5]. Shaw and Ajrekar,[6] isolated an organism from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. But, later studies revealed that, the fungus involved was *S. rolfsii* (Ramakrishnan, 1930[7]). Higgins, 1927[8] worked in detail on physiology and parasitism of *S. rolfsii*. Curzi, 1931[9] studied the perfect stage of the fungus in pure culture. Mundkur, 1934[10] successfully isolated the perfect stage of *S. rolfsii*. Mc Clintock, 1917[11] and Butler and Bisby, 1931[12] reported occurrence of stem rot of Groundnut for the first time in Virginia and India. The symptoms of the stem rot include mycelium covering the plant stem near the soil surface and produce organic acids that are toxic to living plant tissue (Wilson 1953[13]) as shown in Figure 1a. This is followed by the necrosis of plant cells. The mycelium invades the stem, gynophores and also pods causing the rotting of the tissues as in Figure 1b. The production of abundant white mycelium, and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease. Wheeler in 1969[14] observed the same symptoms and Siddaramaiah et al. 1979[15] observed foot rot symptoms accompanied by girdling of younger plants and later such plants were succumbed to death. Mehrotra and Aneja, 1990[16] noticed the cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debris. Symptoms of southern stem rot include wilting of individual stems, stem lesions, shredded stems and pegs, rotted pods, and plant death.

Fig. 1. a) Mycelium covering plant stems near the soil surface. b) Mycelium invading pods causing rotting of tissues

The next major disease that poses a serious threat to the Arachis production is Collar rot caused by the pathogen *Aspergillus niger*. It may cause an average 5 per cent loss in yield and the loss might go up to 40% in some areas. It is more serious problem in sandy soil (Chohan 1965[17]). The morbidity in Punjab region of India due to *A. niger* may amount to 40 to 50 per cent (Aulakh and Sandhu 1970 [18]). A survey report by Joshi, 1969 [19] regarding the growth of *A.hypogea* in the state of Gujarat (India) found as high as 50 per cent seedling blight in some fields. Ghewande *et al*. 2002[20] also reported that losses in terms of mortality of plants due to collar rot range from 28 to 50 per cent. Jochem, 1926[21] first actually reported the *A.niger* as causative organism of collar rot. However, Jain and Nema, 1952[22] first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India. The first symptom of collar rot in emerged seedling is usually a rapid withering of entire plant or its branches. In case of mature plants, infection lesions develop on the stem below the soil and spreads upward along. The dread branches were easily detached from collar regions.

Dry Root rot is another major concern or ailment that affects the Groundnut productivity worldwide which is also known as dry wilt rot or charcoal rot. It infects at all stages of crop starting from seedling to harvest stage. The lesions become dark as the infection spreads to upward and down words. Wilting occurs due to girdling of initial infection as shown in Figure 2a. The infected root and stem portion is shredded and appears as black due to the development of sclerotia shown in Figure 2b.

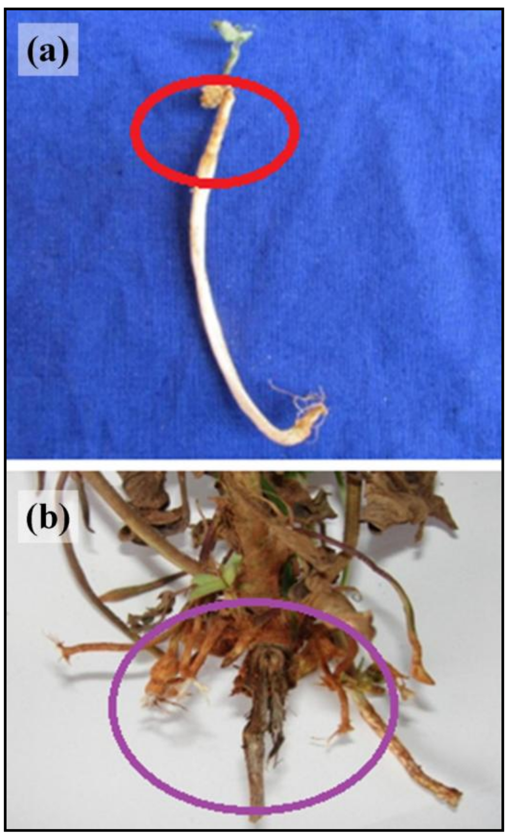

Fig. 2. a) Seedling wilt. b) Shredding of root

EXPERIMENTAL

Collection of nanoparticles

The Silver nanoparticles used in this study were synthesized from locally available edible white button mushroom (*Agaricus bisporus)* and their size and shapes were characterized. Narasimha *et.al*, (2011) [23].

Sub culturing of fungi and Agar well plate assay

The pure cultures of the pathogenic fungi affecting Groundnut crop namely *Sclerotium rolfsii* causing stem rot, *Rhizoctonia bataticola* causing Dry root rot and *Aspergillus niger* causing Collar rot were obtained from Agricultural Research Station, Kadiri in Andhra Pradesh, India. The pure cultures were then sub-cultured on Czapek-Dox Agar (Hi-Media laboratories Pvt Ltd, Mumbai - India) medium. The antifungal property of the nanoparticles is studied using Agar well plate method by using different volumes viz. 25µL, 50 µL, 100 µL and 150 µL of single concentration stock of 1mM silver nanoparticles. In the agar well plate method four wells are bored into the agar medium each having a 1cm diameter and into which the silver nanoparticles of the above said volumes are loaded.

RESULTS AND DISCUSSION

The results substantiating the antifungal activity of silver nanoparticles from mushrooms tested on plant pathogens results were shown in Figures 3a, 3b and 3c, and Table 1. The silver nanoparticles synthesized from *Agaricus bisporus* have the ability to suppress the propagation and proliferation of a wide range of fungal pathogens. We can also deduce from the results that the efficacy of the nanoparticles went increasing with increasing concentrations and high efficacy is found at 100 µL and 150 µL (Figures 3a, 3b and 3c) and it seriously emphasizes the concentration required for effective killing of the plant fungal pathogens. The major antifungal agents used against the Stem Rot and other diseases include Chlopyrifos, PCNB .The antibacterial properties of silver nanoparticles is a well known fact and many evidences are present to reconfirm so. The silver ions are being used as wound disinfectants in various forms from more than a thousand years.

The alginate fibers embedded with silver ions are found to be very effective in wound management according to Yimin Qin 2005 [24]. Similarly, artificially synthesized silver nanoparticles through inert gas condensation and co-condensation techniques were found to be effective antibacterial compounds by turning completely cytotoxic to *E.coli* at as low as 8 µg of Ag/cm^2 by Baker C et al. 2005 [25]. The fungal (*Aspergillus niger*) mediated synthesized silver nanoparticles have been found to be effective against gram positive *Staphylococcus sp.* and *Bacillus sp.* and also gram negative *E.coli* by Jaidev and Narasimha, 2010 [26]. Similar observations were also made by Malliszewska et al. 2008 [27]. The silver nanoparticles synthesized using white button mushrooms are also found to effective against bacteria like *E.coli*, *Staphylococcus sp.*, *Bacillus sp. and Pseudomonas sp.*by Narasimha et al. 2011 [23]. The antifungal properties of silver nanoparticles are also established but however the data is minimal and especially against the plant fungal pathogens is very rare. Silver nanoparticles synthesized using Aspergillus niger have found to effective against the same fungus (*Aspergillus niger*). Jaidev and Narasimha, 2010 [26]. Silver nanoparticles have shown potential antifungal activity against clinical isolates of dermal pathogens *Trichophyton mentagrophytes* and *Candida sp.* according to Kim, Keuk-Jun et al. 2008 [28]. However the following results as evident from Figures 3a, 3b and 3c and as delineated from Table 1 bring evidence to not just the antifungal ability of silver nanoparticles but with special emphasis on their ability to fight out the major fungal pathogens affecting plant crops.

Table 1. Antifungal activity of silver nanoparticles at different concentrations

Name of fungi	Zone of Inhibition (in cm)			
	25 µL	50 µL	100 µL	150 µL
Sclerotium rolfsii	0.25	0.3	0.35	0.38
Rhizoctonia bataticola	0.2	0.23	0.25	0.3
Aspergillus niger	0.25	0.33	0.38	0.38

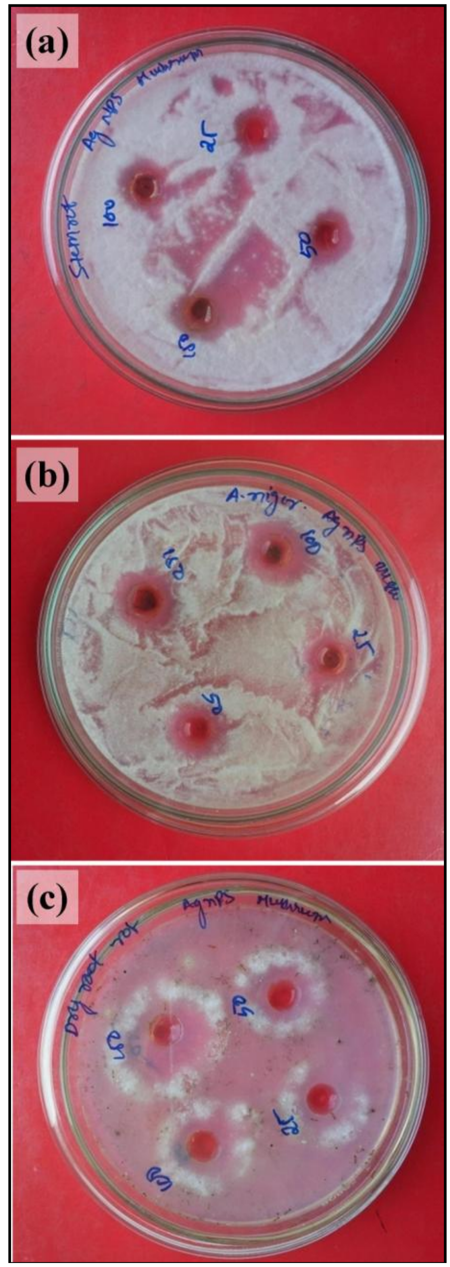

Fig. 3. (a): Stem Rot *(Sclerotium rolfsii)*. (b): *Collar Rot (Aspergillus niger)*. (c): *Dry Root Rot (Rhizoctonia bataticola)*

Note: The values shown in the above table are a mean of triplicates

But, these compounds are found to have environmentally toxic effects and are toxic to animals. Chlorpyrifos treated rats at concentration of 9mg/Kg/day for 90 days have shown piloerection, diarrhoea, nose and eye bleeding, reduced body weight followed by the death of animals Nahid Akhtar 2009 [29-31]. Hence, we suggest and we propose that our research would further the interest and objectivity of nano-scale particles and especially silver nanoparticles to replace the conventional antifungal drugs to which the pathogens are gaining resistance and also keep the environment intact. This research can be effective as it decreases the redundancy of using multiple numbers of drugs by reinstating the statement of one cure for all. The *Agaricus bisporus* that is used for the production the nanoparticles in the present experiment is also a fungus and how these nanoparticles could affect the survival of other fungal pathogens is a point of interest for further research in our lab. It is also a point of quest to check whether the extra-cellular machinery of the mushrooms used for the nanoparticle synthesis contribute any special properties to the nanoparticles by imparting any additional anti-fungal properties or is it just a mere reduction of the ionic metal and so it furthers our interest on the topic.

CONCLUSIONS

Silver nanoparticles synthesized from white mushrooms (*Agaricus bisporus*) are act as effective antifungal agents against plant fungal pathogen especially on those infecting crop plants especially Groundnut (*Arachis hypogeal)*. These nanoparticles could be replacing conventional antifungal agents like chlorpyrifos, PCNB etc, whose adverse effects on the environment are profoundly worse. However, it is necessary to escalate our laboratory studies to field level to arrive at a picture as a whole.

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