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Development Of Liquid Formulation Of Silver Nanoparticles Against Selected Fungal Disease

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ABSTRACT

The high protein yields, manageability, and low toxicity of the residues are all positives for the biogenic production of silver nanoparticles employing fungi as reducing and stabilizing agents. Additionally, the biomolecules generated from the fungus are coated onto the nanoparticles during this production process, which may increase stability and perhaps impart biological activity. AgNPs' biogenesis and recent instances of their use in plant disease control. AgNPs have shown promise as NPs for suppressing pathogen growth and managing plant diseases.

KEYWORDS silver nanoparticles, fungi, biological activity, AgNPs

INTRODUCTION

Silver nanoparticles' appealing and fascinating qualities make them a promising addition to a wide range of applications, including antimicrobial uses, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic items, and electrical components. Different chemical and physical methods may be used to create and stabilize silver nanoparticles. Electrochemical techniques, physicochemical reduction, radiolysis, and a wide variety of organic and inorganic reducing agents are often used in the production of silver nanoparticles. Nanoparticle synthesis has emerged as one of the cutting-edge fields of study, and efforts are being made to develop green chemical processes for their production. Advantages of green synthesis procedures over traditional methods utilizing chemical agents linked to environmental toxicity include utilization of polysaccharides, Tollens's technique, biological processes, irradiation, and mixed-valence polyoxometalates. In this review, the authors discuss the manufacture of silver nanoparticles using green synthesis techniques, such as the employment of fungus.

Fungi have a distinct advantage over bacteria in terms of nanoparticle production due to their superior protein secretion capabilities, which allows them to generate far more nanoparticles per unit of time. The suggested mechanism for the formation of silver nanoparticles in fungi includes the following steps: Enzymes in the fungal system remove silver (Ag+) ions that have been stuck on the surface of fungal cells. Naphthoquinones and anthraquinones, two extracellular enzymes, are credited with facilitating the reduction. When looking at Foxy Porum as an example, it is hypothesized that an extracellular shuttle quinine mechanism and the NADPH-dependent nitrate reductase are involved for nanoparticle synthesis. Silver

nanoparticle manufacturing by fungi is widely believed to be triggered by the aforementioned phenomenon, albeit the particular mechanism responsible has not yet been deciphered. Fungi, because of their abundant availability, low cost to cultivate, and strong enzyme secreting activity, are the ideal organisms for the creation of Silver nanoparticles.

LITERATURE REVIEW

Javad Abkhoo et.al (2016) Silver nanoparticles (AgNPs) typically vary in size from 1 to 100 nm. Different concentrations of AgNPs were tested for their ability to inhibit Fusarium oxysporum growth. The number of Petri dish colonies that were destroyed was used to calculate AgNPs' antifungal efficacy. The ability of AgNPs to prevent F. oxysporum from forming colonies is concentration dependent. Colony formation slowed with increasing AgNPs concentration. Even at the highest dose tested (5000 ppm), silver nanoparticles could not completely prevent F. oxysporum from forming colonies. After one hour of exposure to silver nanoparticles (5000 ppm), colonization of F. oxysporum drops to 35%; after three hours, it drops to 27%; and after five hours, it drops to 24%. Within an hour, it was clear that AgNPs had antifungal action, as measured by a decrease in colony formation. Colony development was drastically reduced when spores were exposed to AgNPs for five hours instead of only one. AgNPs' antifungal action might be very useful in preventing the spread of fungus spores.

Gehan AM Abdelmalek et.al (2016) Pathogenic The health and economic costs associated with fungal infections are quite high. The global citrus fruit industry has far-reaching consequences for people's health, well-being, and standard of living. Several diseases, both pre- and post-harvest, reduce citrus fruit output and drastically degrade fruit quality at every step of the citrus fruit's life cycle, from bloom through harvest. This research looked at the prevalence of citrus leaf spots and fruit degradation in select plantations in the Egyptian governorates of Menofiea and Beheira in 2014 and 2015. Three distinct fungal species were discovered in citrus fruit samples. Alternaria alternata, followed by Alternaria citri and Penicillium digitatum, was the primary causal agent of the spots seen on citrus leaves and fruits. Researchers looked at silver nanoparticles as a potential replacement for dangerous cytotoxic fungicides. The average size of the silver nanoparticles that were synthesized was 10.5 nm. Silver nanoparticles at 50, 100, and 150 ppm and two control conventional fungicides, iprodione and difenoconazole, were tested in vitro on Potato Dextrose Agar (PDA) medium at 150 ppm. The findings that silver nanoparticles at 150 ppm exhibit significant antifungal action against the isolated fungus pave the way for a new generation of fungicides that are less damaging to humans and the environment than the cytotoxic deadly fungicides now in use.

Alia Servin et.al (2015) When it comes to global food production, food security, and food safety, nanotechnology might play a pivotal role. Fertilizers, insecticides, and sensors are all examples of nanotechnology's usefulness in the agricultural sector, where it is used to improve plant growth and productivity and to control pests and diseases. Nanopesticides, nanofertilizers, and nanosensors are just a few examples of the many patents and products produced over the last decade that use nanomaterials in farming. All these strategies aim to make farming more resource-efficient and environmentally friendly by replacing wasteful, time-consuming, and costly methods with ones that are more efficient and produce less waste

than the status quo. This study examines the current literature on the issue of nanoscale nutrients, which have been explored for their potential to decrease crop disease and boost growth and productivity. Important micronutrients needed for host defense may be provided by the nanoparticles themselves, which may explain why production was up even if hazardous organisms were not reduced. We further argue that these advantages are due, in large part, to the ''nano'' form's improved availability of the nutrients. At last, we discuss the regulatory outlook in the present day for such applications.

Sahar M. Ouda (2014) The present study evaluated the effectiveness of silver (AgNPs), copper (CuNPs), and silver/copper (Ag/CuNPs) nanoparticles against the plant-killing fungi Alternaria alternata and Botrytis cinerea. Different concentrations of metal nanoparticles were used to test their antifungal efficacy in vitro. Maximum anti-fungal hyphal growth suppression was achieved with a 15 mg L-1 dose of silver nanoparticles. Combining silver and copper nanoparticles was also tested for efficacy. The nanoparticles had a damaging impact on the hyphae and conidia of the fungus, as seen under the microscope. Sugar, protein, n-acetyl glucosamine, and lipid levels were all affected by Ag-nanoparticles in the culture filtrate, in addition to the cell wall components of both plant pathogens.

Wioletta Wrótniak –Drzewiecka et.al (2014) Here, we describe how Myxococcus virescens cell filtrate may be used to create silver nanoparticles outside of the cell. Human pathogens have also been investigated for sensitivity to silver nanoparticles and their ability to kill them, including Staphylococcus aureus (ATCC 6538), Bacillus subtilis subsp. spizizenii (ATCC 6633), and Pseudomonas aeruginosa (ATCC 10145). The silver nanoparticles isolated from the M. virescens cell filtrate were analyzed by UV-Vis Spectroscopy, Nanoparticle Tracking Analysis (NTA) with LM 20, Zeta Potential analysis, Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). The impact of silver nanoparticles on the viability or mortality of human pathogenic bacteria was evaluated to determine their antibacterial activity. The cell lethality of silver nanoparticles produced via biosynthesis was promising against human pathogenic bacteria. Protein caps make the silver nanoparticles produced with M. virescens very stable. This method offers a green, straightforward, and sustainable way to create silver nanoparticles with antimicrobial characteristics useful in therapeutic settings. Importance and Influence of the Research: Synthesized nanoparticles mediated by M. virescens may provide a starting point for developing novel antibacterial compounds effective against a wide range of antibiotic-resistant pathogens.

METHODS

Sample collection: From various locations in the Gulbarga district, After collecting infected samples, we placed them in sterile plastic bags and transported them to the Mycology and Plant pathology Laboratory for further study. We also collected wilted chickpea, pigeon pea, and sorghum seeds, Pongamia leaves and seeds, groundnut seeds, and pea pods.

Storage of sample: Samples were cleaned by running water and mercuric chloride to eliminate any external contaminants before being stored in a laboratory.

Identification of isolates: After preparing microscope slides of pure isolates and observing them under a microscope, the following fungi were identified by comparing the structure of the microscopic field to a Barnett book diagram. Peas in all their forms: seeds, leaves, and pods. Liquid nutrient media was used to cultivate pure fungal cultures that had previously been identified.

DATA ANALYSIS

Fungus colonies were seen on the surface of diseased pongamia leaves and fruits. We employed ground nut and sorghum seeds, onion arils, chickpea and pigeon pea roots and stems, and ground nut hulls to study and isolate saprophytic fungi. Brown necrotic lesions of a round or angular form were seen on the pongamia leaves and fruits, indicating the presence of fungal colonies. We also noticed white faded irregular lesions on the onion's aerial parts, we began by isolating the saprophytic fungus that had colonized the seeds and interior tissues of these plants. After two days, we saw the development of concomitant fungus. These fungi ranged in color and texture from white cotton to wet cream to yellow to brown. Rhizopus, Fusarium, Colletotrichum, Alternaria, Fusicladium, Cladosporium, Pythium, Pencilium, Stemphylium, and Aspergillus were among the 20 fungal isolates found in the collected samples. Morphological and genetic data were used to place these isolates onto a Barnet book diagram.

Fusarium oxysporum, Rhizopus nigricans, and Pencilium notatum are just some of the fungal species that have been isolated as saprophytes on sorghum seeds and are listed in Table 1: Fungi Isolated from Sorghum Seeds at Gulbarga University Campus. Fusarium oxysporum has a cotton-like white tint and displays a yellow color when seen from the back, whereas Rhizopus nigricans has a dark brown color and has a fibrous exterior. The morphology of Pencilium notatum is that of a dark yellow color. Fusarium semi tectum, Colletotrichum gloeosporioides, Aspergillus terreus, Aspergillus nidulans, Aspergillus clavatus, and Aspergillus flavous are only few of the fungi that have been isolated from pongamia fruits. Colletotrichum gloeosporioides is a dark gray when seen from the front, but looks almost black when viewed from behind. The exterior of Aspergillus nidulans is crystalline and brown, while the underside is a subtle crimson. Aspergillus flavous has a green, powdery morphology. Ground nut seeds were cultured to yield the fungi Fusicladium venturia and Cladosporium hebarium. The underside of a fusicladium venturia flower is a cottony white, belying its dark brown morphology. Cladosporium hebarum has a dark, wet brown color, with a very subtle white color on the back. Two different species of Fusarium were recently discovered from the roots of chickpeas and pigeon peas, respectively. Stemphylium varicans is a dark, wet black in front and a dark, white back. Alternaria alternata has a powdered brown tint and a pale yellow morphology. The morphology of Alternaria porri is that of a damp, black cottony look with a dark white color.

Table – 1: Fungal colonies morphological feature and their Colour

Fungi	Source of isolation	Morphological feature	Colonies colour
Fusarium oxysporum	Sorghum seeds	Cotton white in color	Yellow
Rhizopus nigricans	Sorghum seeds	Fibrous and brown in color	Yellow
Pencilium notatum	Sorghum seeds	Dark yellow in color	Yellow
Fusarium semitectum	Pongamia fruits	Whitish in color	Pink
Colletotrichum gloeosporioides	Pongamia fruits	Light black in color	Dark white
Aspergillus nidulans	Pongamia fruits	Crystallized brown in color	mild red
Aspergllus flavous	Pongamia fruits	Green powdery in color	Yellowish Green
Aspergllus terreus	Pongamia fruits	Brown in color	Brown
Aspergllus clavatus	Pongamia fruits	Brown in color	Brown
Fusicladium venturia	Ground nut seeds	Dark brown in color	Cotton whitish
Cladosporium herbarum	Ground nut seeds	Moist brown in color	Mild white
Fusarium oxy.ciceri	Chick pea	Cotton white color	Red
Fusarium udum	Pigeon pea	Cotton white in color	Yellow
Pythium debaryanum	Bean pods	White in color	White
Pythium ultimum	Bean pods	White in color	White
Cladosporium cladosporioides	Ground nut seeds	Creamy color	Mild white
Stemphylium varicans	Onion	Moist black in color	Dark white
Alternaria alternata	Onion	Powdery brown in color	Mild yellow
Alternaria porri	Onion	Moist, cottony, black in color	Dark white
Aspergillus vaericolor	Pongamia fruit	Brown in color	Light red

AgNPs in Nematode Disease Management

More than 4100 species of plant pathogenic nematodes cause yearly harm to various crops. This highlights the need of finding ways to reduce the negative impact of current nematicides on non-target creatures and the environment. The nematicidal efficacy of AgNPs has only recently been shown. Pre-treating seedlings with AgNPs has been shown to reduce nematode infection, according to many research. Researchers have speculated that AgNPs provide a direct threat to nematodes, which might limit their ability to reproduce. Intense oxidative stress production may play a significant role in the toxicity of AgNPs to nematodes. AgNP poisoning led to cell wall degradation in laboratory-reared Meloidogyne incognita larvae during the J2 stage.

The hatchability and survival of embryonic eggs in J2 of M. incognita were found to be affected by a dosage of 20-40 ppm of AgNPs embedded in micro-crystalline cellulose. Another study found that AgNPs made from Artemisia Judaica extracts increased juvenile mortality and decreased egg hatch. In addition, AgNPs inhibited the growth of M. graminicola in rice while having no influence on seed germination or development. Produced AgB-NPs' major method of action as an anti-nematode involves blocking many physiological processes in nematode cells, including those involved in membrane permeability, Structure of adenosine triphosphate

and its response to oxidative stress. Proteins involved in pathogenesis and the production of phytochemicals, as well as differences in cell wall composition, may all have major impacts on structural immunization. Additionally, phytoalexin structure is often linked to the subsequent resistance phases of manufactured AgB-NPs. Some plants create secondary metabolites like phenol and other phytochemicals in response to nanoparticle stimulation. Potentially useful in warding off pests like root-knot nematodes, they may be an integral element of a plant's defensive system. Table 2 is a summary of a few research that looked at how AgNPs affected nematodes.

Table 2. AgNPs in nematode disease management

Nanoparticles	Size (nm)	Target Nematode	Test Crop	Effect
AgNPs	100	*Heterodera sacchari*	*Oryza sativa*	Decreased nematode population in the root and soil, improved vegetative development of the rice plant
AgNPs	15	*Meloidogyne incognita*	*Solanum nigrum*	Apart from nematode movement, impacts on production, embryogenesis, hatchability percentage, and larval stages were evident
Et-AgNPs	20–30	*M. incognita*	*S. lycopersicum*	Inhibition of J2 worms and prevention of egg hatching (in vitro). In vivo infestation of tomato roots was considerably decreased when a root dip therapy with AgNPs was used
AgNPs	30–100	*M. incognita*	*S. melongena*	Inhibition of eggs and 2nd juvenile (J2) stage of *M. incognita*
AgNPs	2	*M. incognita*	*Arachis hypogea*	Vegetative growth and fruit weight were increased to varying degrees when the nematode population was diminished
AgNPs	50–150	*M. incognita*	*S. lycopersicum*	Antagonistic effect on the nematode eggs and larval stages
AgNPs	5–50	*M. incognita*	*S. lycopersicum*	Highest increase in growth parameters, as well as the minimum galls and egg masses
AgNPs	20	*M. graminicola*	*O. sativa*	A substantial reduction in the formation of root galls
AgNPs	16	*M. javanica*	Faba bean	Drastically decreased egg hatching, increased larval mortality, diminished root galling, and J2 population in soils
Green Silver Nanoparticles (GSN)	8–19	*M. javanica*	*S. melongena*	Reduced second-stage juveniles (J2s), nematode population in soil, and enhanced growth characteristics
AgNPs	-	*M. incognita*	Bermuda grass	Increased turfgrass productivity in one year and reduced root gall development in two years without phytotoxicity
AgNPs	5–10	*M. incognita*	*S. lycopersicum*	Significant reduction in the number of galls, egg masses, developmental stage, rate of build up, and nematode population in soil
AgNPs	13.09 and 10.51	*M. javanica*	*S. lycopersicum*	Increased the plant defense gene's expression (chitinase gene)
AgNPs	20	*M. incognita*	*S. lycopersicum*	Second-stage juvenile immobility and mortality
Ag-BNPs	29.55	*M. incognita*	*S. lycopersicum*	Reduced the level of second-stage juveniles, females, and developmental stages while improving the host plant's resistance and immunity
AgNPs	25 to 55	*M. incognita*	*S. lycopersicum*	Galls, egg masses, females per root system/plant, and juvenile mortality were all reduced, and the immune system was induced to resist against nematode infection

CONCLUSION

There are a number of ways to produce silver nanoparticles, but because biological synthesis is both more environmentally friendly and cheaper, we choose to use fungi as our means of production. Research using UV-Vis spectroscopy and subsequent analysis using XRD and TEM established that the silver nanoparticles were indeed fungal in origin. More study is needed to determine what concentrations of AgNPs would most effectively reduce disease incidence or severity in plants without having a negative effect on off-target species or soil fertility.

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