International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 6s, 2025
https://www.theaspd.com/ijes.php

Synthesis And Characterization Of Endophytic Fungal Silver Nanoparticles And Preparation Of Nano-Emulsions From Rhododendron Arboreum For Better Human Health

Vasudha Kak[1], Kunal Kishor[2*]

[1]*Research Scholar, Department of Microbiology, Sharda School of Allied Health Sciences, Sharda University, Greater Noida*
[2*]*Professor, Department of Microbiology, Sharda School of Allied Health Sciences, Sharda University, Greater Noida*

**Corresponding author: Kunal Kishor*
**Email: kunal.kishor@sharda.ac.in*

Abstract
Many researchers around the globe are interested in green nanobiotechnology. This domain encompasses the utilization of microorganisms or plants to create nanoparticles, specifically endophytic fungi. It is interesting because it is much more cost-effective and eco-friendlier than other nanoparticle synthesis methods that use toxic chemicals harmful to nature. It is also much easier to scale up the production of the nanoparticles using green nanotechnology. A sufficient number of studies have been conducted regarding the discovery of various beneficial microorganisms. Several recent studies have investigated the presence of endophytic microorganisms (including both bacteria and fungi) that reside asymptomatically within host cells, influencing the health of both humans and animals. They can also affect the total yield of crops. The total production of bioinoculants derived from endophytes, alongside their growing demand in agriculture, has focused attention on them and expanded their gross market size.
Our focus will also shift to a greater degree toward various underexplored areas and the identification and characterization of significant endophytes. This study will cover the microbiome of endophytes, different mechanisms, multiple factors involved in the actual recruitment of endophytes, and various strategies to enhance and improve the efficiency of shaping the microbiome of plants and crops. The effective utilization of nanoparticles can be integrated to address future requirements. This study will also emphasize the application of endophytic nanoparticles and their significance in medicine, environmental science, pharmaceuticals, and agronomy.

Keywords: *Green nano-biotechnology, nanoparticle, microbiome, endophytes*

Introduction:

Nowadays, the term called green nanobiotechnology has been of interest to many researchers worldwide. This field involves the use of microorganisms or plants to prepare nanoparticles; in this case, the use of endophytic fungi. The reason why it is of interest is that it is much more cost-effective and more eco-friendly when compared to other nanoparticle synthesis methods, which use toxic chemicals that pose a threat to nature. Green nanotechnology also allows the scale-up of production of the nanoparticles much more easily.
This type of symbiotic relationship between fungi and plants involves the interplay of their metabolisms; endophytic fungi are organisms that live in a plant host and do no harm to the plant. Additionally, they shield plants from disease attacks. It is also known that these organisms generate a variety of secondary metabolites, which are crucial for the creation of nanoparticles. The immediate need for new and novel compounds to provide assistance and relief in all aspects has increased tremendously. The uncontrolled and unregulated use of many chemical fertilizers has in return, degraded the quality of soil, thus making it unsustainable. To overcome this problem, eco-friendly strategies and applications should be implemented, and thus, we can fully explore the potential of microorganisms associated with plants as bioinoculants.
There have been sufficient studies done that are related to the discovery of several beneficial microorganisms. Many recent studies have explored that endophytic microorganism (both bacteria & fungi), reside asymptomatically within the host cell and they can influence the health of humans as well as animals. They can also impact the overall yield of crops. Endophytes and the associated studies are emerging as one of the most

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promising areas in the field of research & development. The overall production of bioinoculants of endophytes and their increased demand in the agriculture sector have channelled the attention in gaining prominence and enhanced the gross market scale.

Once we are at a stage where we can implement the actual use of these bioinoculants, the actual focus will shift to process development, optimization, and even production at a very large scale. These endophytic microbes tend to contribute to overall plant growth by helping in soil mineralization, production of many bioactive molecules, synthesis of phytohormones, etc. The association between the host plant and microorganisms can help in enhancing the health of a plant as it will uptake the nutrients and will overall improve the resistance of plants to pathogens as well as stress. These endophytic microorganisms contribute to the overall physiological and biochemical roles of plants. Under specific conditions, microbial activity can be affected by less phytohormone synthesis, which is very much essential for the nutrition of plants. In recent times, less study has been done in the context of several strategic methods to harness many endophytic microorganisms in the breeding of crops. So, in this regard, a wide range of techniques and methods can be implemented to determine the genotype of plants and to know the range of microorganisms that can be beneficial for crop production.

Also, nanotechnology has come up as one of the most promising fields. Nanotechnology is trying to contribute to and mitigate the problems that are associated with managing diseases in several crops and plants. They are helping in lowering the excessive usage of chemical substances as they are very harmful to humans as well as the environment. Many nanoparticles, as well as their derivatives, exhibit antimicrobial properties that are influenced by their morphology, size, and chemical composition.

The sudden onset of nanotechnology has paved the path in the field of science and technology and its application in several fields can thus help in overcoming the problems associated with sustainable agriculture and also managing the infestation of pests in the agricultural fields. Since these endophytes exhibit various properties, they can be environment-friendly, non-toxic, etc. These endophytes are non-pathogenic so the active metabolites present in them can be of great use. These metabolites can be used in the manufacturing and production of several nano pesticides in order to control the infestation of pests and the associated diseases.

The various nano-formulations can prove to be boon in this context. They can have targeted delivery of the most active compound and this will in return help the farmers to not use all these insecticides and harmful pesticides. The direct intervention of all the transfer of gene mediated by nanoparticle into the most active endophytic microorganism can bring out a sudden and highlighting change towards the resistance of all the pests and the diseases associated with it. Also, developing several biosensors from the endophytes can help in lot of ways. It will help farmers in order to observe and control the infestation of pests and diseases in the agricultural fields.

Various studies have highlighted the importance of interaction between nanoparticles and plants. Nanoparticles exhibit several properties which make them superior to others. But this fact cannot be denied that these nanoparticles do have some ill effects on the plants as well along with some positive effects. The extensive interaction of these nanoparticles with the environment as well is also responsible for affecting the different properties of these nanoparticles. Microorganisms that are living in the soil also affect the intake of the nanoparticles to the host plant.

We will also emphasize shifting our major focus to various underexplored areas and on the identification as well as characterization of important endophytes. We will discuss the microbiome of endophytes, various mechanisms, several factors responsible for the actual recruitment of endophytes, and various approaches to shape the microbiome of plants and crops in a much better and more efficient way. The efficient use of nanoparticles can then be incorporated to meet the needs of the future. We will also highlight the use of endophytic nanoparticles and their importance in the fields of medicine, environment, pharmaceuticals, and agronomy.

Tea tree oil (TTO) is an essential oil produced by the tea tree, *Melaleuca alternifolia*. Humans have been using it for many years and consider it to be the best antibacterial agent. Terpenes make up the majority of the oil's constituents. Since it can more readily pierce the lipid structures of bacteria, the primary terpene, Terpinen-4-ol, has been identified as the primary component responsible for the antibacterial capabilities.

When bacteria, viruses, fungi, and parasites change over time and become less susceptible to antibiotics, it's known as antimicrobial resistance (AMR). This makes illnesses harder to cure and increases the risk of infection, serious illness, and death. The problem of AMR is made even more severe by bacterial antibiotic resistance. Over

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several decades, bacteria that cause common or serious infections have developed varying degrees of resistance to every new antibiotic that leaves the market. It may be possible to reduce the incidence of infectious diseases and, thus, the need for antibiotics by creating innovative alternatives.

Methodology:

Preparation of fungal endophytic extracts from silver nanoparticles and nanoemulsions:

The strain isolated from *Rhododendron arboreum* was grown in potato dextrose broth for at least 14 days, approximately at 28 °C. Later on, the fungal mycelial was then sensibly taken with the help of sterile forceps. This was transferred to a conical flask with 100 ml of autoclaved distilled water. It was incubated for 48 hrs at 28 °C. The filtrate was obtained with the help of Whatman filter paper and it was used to synthesize the endophytic silver nanoparticles. 1 mM silver nitrate was added to the filtrate in a ratio of 1:5. The mixture was centrifuged at 13,000 rpm for about 10 mins to remove any excess silver ions. The pellet obtained was then dissolved in 2 % DMSO to make a colloidal solution. The endophytic fungal extract-mediated nanoemulsion was prepared using tea tree oil. The previously obtained colloidal solution was mixed with tea tree oil in a ratio of 1:1, sonicating it for 20 mins at 20 kHz with a time interval of 2 minutes between the cycles for 20 minutes to obtain an endophytic fungal nanoemulsion.

Characterization of Physio-chemical properties of endophytic silver nanoparticles & nanoemulsions:

The silver nanoparticles were synthesized using the aqueous extract of the isolated fungal endophytes. Silver nitrate was added to the extract and then incubated for 24 hours. It was incubated until the colour changed from yellow to dark golden brown. The colour change signifies that the silver ion was reduced to silver metal.
Scanning electron microscopy was also used to study the fungal endophytic nanoparticles and determine their surface morphology, aggregation, shape, and size.

Results:

Prepared endophytic nanoemulsions-

Endophytic nanoemulsions were obtained from the endophytic fungal extract isolated from *Rhododendron arboreum*.

	
(a)Endophytic nanoemulsion 1 obtained from *Rhododendron arboreum*	(b)Endophytic nanoemulsion 2 obtained from *Rhododendron arboreum*

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Characterization and Synthesis of endophytic nanoparticles and nanoemulsion-

Silver nanoparticles as well as silver nanoemulsions were synthesized with the help of an aqueous extract of the fungal endophyte. The colour was changed to dark golden brown signifying the reduction of silver ion to silver metal.

Scanning Electron Microscopy was done to study endophytic fungal nanoparticles and endophytic and endophytic fungal nanoemulsion. The overall result shows that the average nanoparticle size was ranging between 15-25 nm.

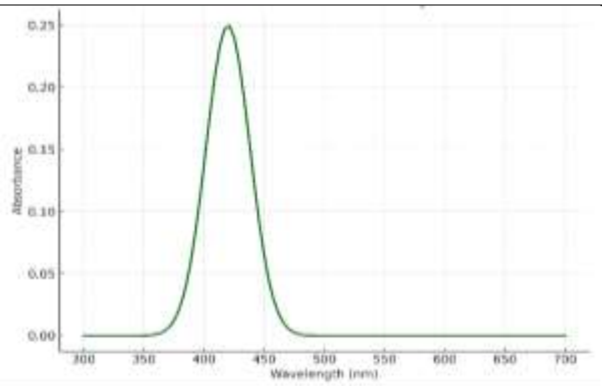
(a)

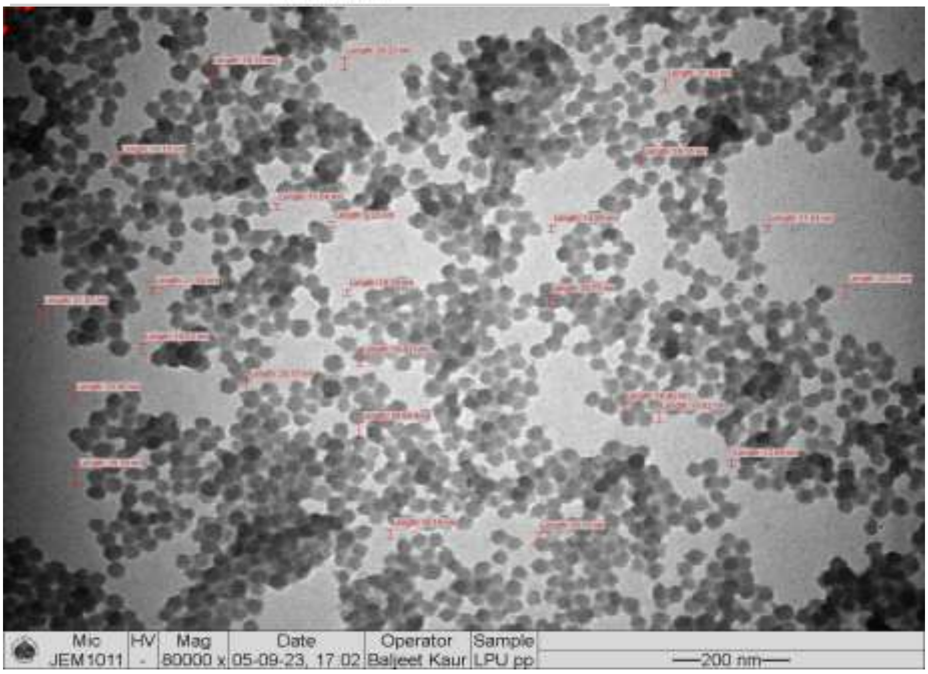
(b)

(c)

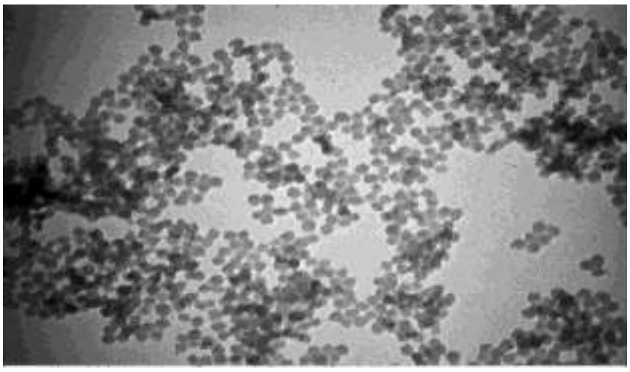
(d)

Figure 1: (a)Growth of endophytic fungus in PD Broth (b) Fungal mycelium obtained after 2 weeks of growth (c) $AgNo_3$ solution (d) $AgNo_3$ solution mixed with the obtained fungal biomass





(a)



(b)

Figure 2: (a) UV–vis absorption spectrum of EFNP (b) SEM image of synthesized EFNP displaying size, shape, and surface

With the help of UV- Vis spectroscopy, a prominent peak was also observed between the range 350-500 nm, which thus confirms the formation of nanoparticles. The endophytic nanoparticle is spherical, as seen by SEM analysis.

FTIR also determined the various functional groups. The absorption peaks ranged from 4000 to 400 cm^{-1}. The peaks were specifically observed at 3295.81 cm^{-1}, 2922.55 cm^{-1}, 2105.79 cm^{-1}, 1620.82 cm^{-1}, 745.280 cm^{-1}, and 1192.70 cm^{-1}. These different values helped determine the presence of various functional groups, such as alcohol, alkane, alkyne, conjugated alkene, amines, and arenes, respectively.

Characteristic absorption (cm^{-1})	**Functional groups**
3295.81 cm^{-1}	O-H (alcohol)
2922.55 cm^{-1}	C-H (alkane)
2105.79 cm^{-1}	C≡C (alkyne)
1620.82 cm^{-1}	C = C (conjugated alkene)
745.280 cm^{-1}	C–N (amine)
1192.70 cm^{-1}	C–H (arenes)

Table 1- FTIR analysis which is showing the wavelength of the absorption peak and corresponding functional groups that are present in the endophytic fungal nanoparticles

Discussion:

The overall synthesis of the endophytic nanoparticle was thus confirmed by the colour change that was seen by reducing the silver ions to silver metal. The phenomenon that is responsible for this is known as surface plasmon resonance. The overall intensity of the colour change that occurs is then increased as time passes, indicating that the Ag ion has been reduced with the help of reductants found in the secondary metabolites of the fungal extract, and thus silver nanoparticles were formed. The underlying reason behind the colour change is the excitation of the silver nanoparticles' surface plasmon, which is considered the primary and major indicator for the formation of endophytic nanoparticles.

The important step was confirming the formation of nanoparticles, which was done using UV-vis spectroscopy, where peak formation was observed. As per the research done and previous studies, the major reason behind this is that there are free electrons present in silver nanoparticles. The FTIR spectroscopy also helped in confirming the endophytic fungal nanoparticle showing various functional groups present. The study also helped in highlighting that the functional groups present in the extract of fungus can actually act as a capping agent as well as reductants which can thus help in the synthesis of nanoparticles.

Conclusion:

Silver nanoparticles and a nanoemulsion were prepared using an endophytic fungal extract \ This research confirms that this method for synthesizing endophytic nanoparticles and endophytic nanoemulsion is greener, more sustainable, and more efficient. Numerous additional studies have demonstrated that TTO, made up of inherently hydrophilic components with sufficient lipophilicity, can facilitate oil dispersion into the membranes of organisms. Another reason for the nano emulsion's ease of entry into the cell through the cell membrane is its size, which is under 100 nm (around 81.242 nm).

So the research work concludes that endophytic fungal nanoemulsions could be a more effective antifungal agent against a wide range of microorganisms and thus can help in the betterment of good human health.

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