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"Isolation and screening of endophytic fungi for biosynthesis of silvernanoparticles"

Sayyad Shahim[1], Gachande B D[1] and Shaikh N F[2]

[1]Post Graduate Department of Botany, N.E.S. Science College, Nanded.

[2]Government College of Arts and Science, Aurangabad.

Corresponding author's E-mail:- nazir.hingoli@gmail.com.

Abstract:-

Now a day's area of biosynthesis of nanoparticles via myconanotechnology increasing because fungi are ideal candidates in the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes with low expenses have been found to be capable of synthesizing nanoparticles. These myconanoparticles have its several applications in the field of medicine and agriculture. In present research we have isolated different endophytic fungi fromhost plant*Maytenus emarginata* (Willd.) Ding Hou.*"* from leaf and stem. Isolated endophytic fungi are *Nigrospora sphaerica, Alanphillipsia aloeigena, Colletotrichum gloeosporioides* and *Fusarium brachygibbosum.* The isolated endophytes were screened for biosynthesis of extracellular silvernanoparticles (AgNP) by using the mycelium free filtrate incubated with 1 mM silver nitrate solution. Then solution visually observed over a period of time indicates the bioreduction of silver ions to silver nanoparticles by change in colourless filtrate to brown colour. So from above investigation it is confirmed that isolated fungi can be used for further work to its potential activity in medicine and agriculture.

Keywords :- Endophyte, *Maytenus emarginata*, myconanoparticles & AgNP.

Introduction:-

Nanotechnology is an important emerging field of modern research helps in designing, synthesizing, and manipulation of particles structure ranging from 1-100 nm. Nanotechnology has now started new applications to change our lives. Now a day's growing field of nanotechnology has wide applications in various sectors of environment, energy, healthcare, agriculture, and consumer goods. Therefore, it has developed new idea in the academic community, investors, governments and industries.

Since plants have been a major source of bioactive compounds for drug discovery. Recently the attentions of many researchers increased towards nanoparticles due to their wide applications in industries, agriculture and medicine.

The applications of nanomaterials and nanoparticles are rapidly increasing in various fields. Metal nanoparticles have a high fraction of surface atoms and a high specific surface area. They are gaining the interest of scientists for their novel method of synthesis.

A wide variety of physical and chemical methods to synthesize nanoparticles are in practice but their inherent flaws that include contamination from precursor chemicals, use of toxic solvents and generation of hazardous by-products (Sunkar and Nachiyar, 2012). The present study focuses on the biosynthesis of silver nanoparticles using on faction of microbes, the endophytic fungi as a green alternative to the chemical method.

Plant endophytic fungi are important components of plant micro-ecosystems. It spends their whole life by colonizing intra or inter-cellularly within the healthy tissues of the host plants, without causing any symptoms of disease (Zhang *et al.* 2006). Endophytes are found in a wide variety of plant tissue types such as roots, stems, leaves, tubers, buds, ovules, seeds, fruits, xylem and bark (Tan and Zou, 2001).

The study revealed that approximately, there are near to 3,00,000 plant species on earth having host to one or more endophytes, and many of them may colonize different hosts. Plant endophytic fungi are novel and important for production of natural bioactive compounds with their potential use in agriculture, medicine and industry. The various important bioactive compounds from entophytic fungi isolated which shows antimicrobial, insecticidal, cytotoxic and anticancer activities (Gangadevi & Muthumary, 2008). (Yiing and Adeline, 2014). (Jingfeng *et al.*, 2013, Tanmayee *et al.*, 2015). (Praveen *et al.*, 2014). (Harper *et al.*, 2003). (Dhankhar and Yadav 2013).

Amongst fungi, not much work has been done on mycosynthesis of silver nanoparticles from endophytic fungi of different plants. A very few report such as *Penicillium* sp isolated from the medicinal plant *Centella asiatica* and *Curcuma longa* shows the antibacterial and antifungal activity (Devi, 2012, Dattu *et al.* 2014). Mycosynthesis of silver nanoparticles from endophytic fungi, *Penicillium* species of *Glycosmis mauritiana*, and its antioxidant, antimicrobial, anti-inflammatory and tyrokinase inhibitory activity (Govindappa *et al.* 2016). Endophytic fungi

namely, *Pencillium* sp., *Alternaria* sp., *Aspergillus* sp and *Cladosporium* sp from different parts of *Calotropis procera* for antimicrobial and antioxidant activities mediated by extracellular Synthesized Silver nanoparticles (Debjani *et al.* 2016). Silver nanoparticles from endophytic fungi of Leaf samples of *Garcinia xanthochyumus* and *Aravae lanata* as a effective antibacterial agents(Sunkar and Nachiyar, 2013). There is very little report on endophytic fungi from *Maytenus emarginata* (Willd.) Ding Hou includes *Phyllosticta capitalensis* isolated by (Naveen *et al.* 2014), *Xylaria* (Meenavalli *et al.* 2013), *Pestalotiopsis* sp. from *Maytenus ilicifolia* a medicinal plant from Brazil (Deeksha *et al.* 2016).

The present investigation focuses on isolation and screening of endophytic fungi for silver nanoparticles production from medicinal plant *Maytenus emarginata* (Willd.) Ding Hou.

There is no report on synthesis of endophytic fungal silver nanoparticles from *Maytenus emarginata* (Willd.) Ding Hou.

Materials and methods:-

Collection of plant materials:

Plant parts like stem and leaf of *Maytenus emarginata* (Willd.) were collected from different areas of Nanded and Hingoli districts. These plant materials were collected in sterile polythene bags and brought to the laboratory used for the investigation of endophytic fungi.

Isolation of Endophytic Fungi: Endophytic fungi were isolated by following methods employed by Hallman *et al.* (2007) and Selvakumar *et al.* (2014).

The collected plant samples were washed in running tap water to remove the debris and epiphytic microorganisms and soaked in 0.1 % mercury chloride subsequently surface sterilized by using ethanol. Followed by 2% Sodium hypochlorite solution for 2-4 minutes then dipped in sterile distilled water. The Sterile segments were then chopped into 3-4 mm x 0.1 cm lengths and inoculated in Petri dishes containing Czapadox agar and potato dextrose agar (PDA) medium supplemented with streptomycin. The Petri dishes were sealed and incubated at room temperature (28±2˚C) for 15 days. Fungal growths growing out of the pieces were sub cultured on separate Czapadox agar medium plates and slants before use for the identification.

The colonization frequency (CF) percentage of endophytic fungi was calculated using the method (Kumar & Hyde 2004).

Colonization frequency:-

$$CF\% = \frac{\text{No of species isolated}}{\text{No of segments screened}} \times 100$$

Molecular Identification of Isolated fungal organisms-

The identification of endophytic fungi was done using 18s rRNA ITS universal primers at Sai biosystems Pvt. Ltd. Nagpur. The pure culture of isolated fungal strains was maintained in Czapadox agar slants at 28^0C during the study.

Biosynthesis of Silver Nanoparticles:-

For screening of biosynthesis of extracellular silver nanoparticles (AgNPs) the isolated fungi were grown in the Czapadox broth supplemented with streptomycin at 28ºC with shaking at120 rpm for incubation period of 72 hrs. After incubation period biomass of fungi was harvested, filtered using Whatman filter paper number 1 and washed several times with sterile distilled water to remove any medium component. Harvested biomass was transferred into 100 ml of sterile distilled water and incubated for 48 hours. The cell-free filtrate was used in experiments. SNP was prepared by mixing AgNO3 (1 mM of final concentration) with cell-free extract. The above flask was then incubated at room temperature under dark conditions, and observed for color development. Control was also prepared, containing only the cell filtrate without silver nitrate solution. The formation of silver nanoparticle is usually confirmed by visual observation of color change from pale white to reddish brown. This appearance of color change from pale white to brown is a clear indication of the formation of silver nanoparticles by reduction of silver in the filtrate extracellularly.SNP synthesis was carried out by the method of (Prabavathy*et al.*,2015 and Kiran, 2017)

Results & Discussion:-

In present research a total 40 segments of plant material were screened for isolation of endophytic fungi on Czapex dox agar and Potato dextrose agar medium. The total 4 fungal endophytic species isolated and identified were screened for production of silver nanoparticles.

The isolated endophytic species include fungi *Nigrospora sphaerica, Alanphillipsia aloeigena, Colletotrichum gloeosporioides* and *Fusarium brachygibbosum.* The species colonization consist of *Nigrospora sphaerica* 7.5%, *Alanphillipsia aloeigena* 5 %, *Colletotrichum gloeosporioides* 12.5% and *Fusarium brachygibbosum* 15%. (Table.1 and Fig.1)

The isolated endophytic species were screened for biosynthesis of silver nanoparticles. When silver nitrate (AgNO3), upon incubation with the fungal extracellular filtrates, turned dark brown colour, while the control flasks remained unchanged upto the 72 h incubation period. The formation of dark brown colour is due to the surface Plasmon resonance (SPR) exhibited by the nanoparticles indicates the formation of silver nanoparticles in the solution.

Similarly different researcher isolated endophytic fungi and preliminary screened for silver nanoparticles production by observing change in colour. Endophytic Fungi *Penicillium* sp. isolated from *Curcuma longa* (Turmeric) (Dattu Singh et.al., 2013). Silver nanoparticles using endophytic fungus *Pestalotiopsis versicolor (*Kavish Rajput et al., 2017).Silver Nanoparticles by Endophytic Fungus *Nemania* sp. isolated From *Taxus baccata* L. (*Iranian* Yew) (Mohammad and Saeed., 2018). Silver nanoparticles synthesized by endophytic *Aspergillus sp* isolated from *Justicia beddomei (* Prabavathy D.et al., 2015). Extracellular mycelium extract of *Penicillium oxalicum* isolated from *Phlogacanthus thyrsiflorus* to biosynthesize silver nanoparticles (Sukla Bhattacharjee et al., 2017). Fifteen endophytic fungi isolated from *Andrographis paniculata* and *Carica papaya* plant leaf, among, six genera used to induce the biosynthesize of silver nanoparticles (AgNO3) (Thaslimmunisha et al., 2016). *Isoptericola* endophytic fungus was isolated from the roots of *Borszczowia aralocaspica* screened for silver nanoparticles production. (Zhou Yan Dong et al., 2017).

Table.1. frequency colonization of endophytic fungal species from *Maytenus emarginata* (Willd.) Ding Hou.

Sr.No	Name of fungal Endophytes	Number of isolates	Frequency of colonization
1	*Nigrospora sphaerica*,	3	7.5%
2	*Alanphillipsia aloeigena*,	2	5 %
3	*Colletotrichum gloeosporioides*	5	12.5%
4	*Fusarium brachygibbosum*	6	15%

Conclusion:-

In present investigation a 4 species of endophytic fungi isolated from different parts of *Maytenus emarginata* (Willd.) Ding Hou indicate endophytic diversity of plant and biosynthesized silver nanoparticles can be used for study of its biological activity in terms of antimicrobial, antioxidants, antidiabetic and anticancer activity.

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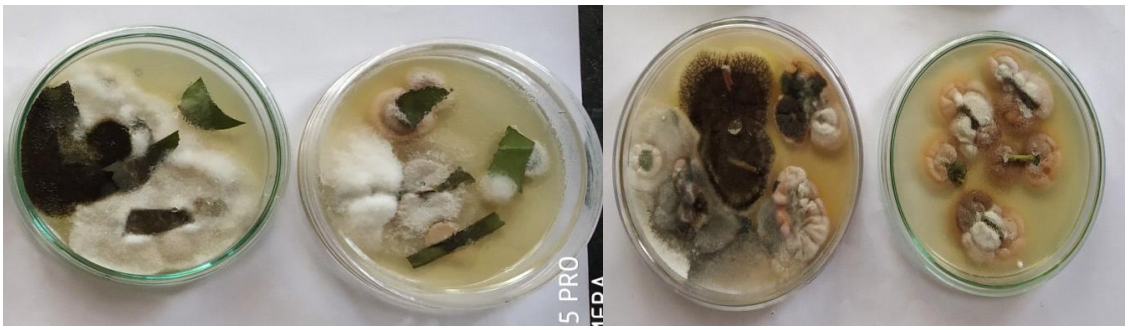
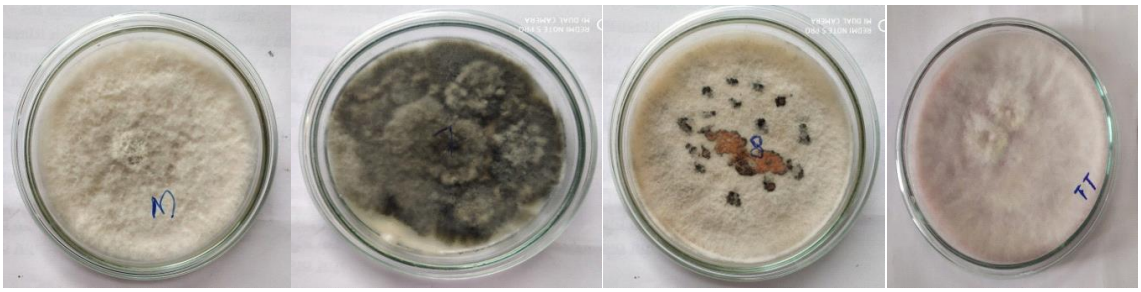
Plate 1 : - Endophytic fungi isolation, pure culture and screening of silver nanoparticles production

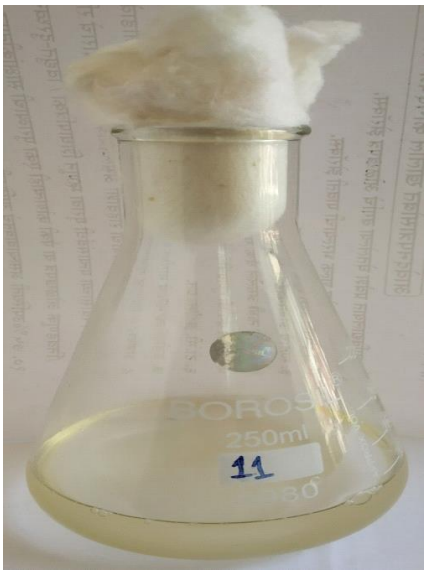


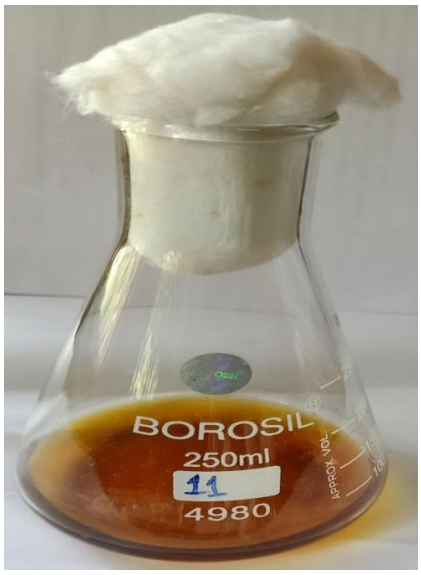
a) Plant segment leaf and stem showing endophytic fungi



N. sphaerica *A. aloeigena* *C. gloeosporioides* *F.brachygibbosum*

b) Plate containing pure culture of endophytic fungi





c) Fungal extracellular filtrate **d) Filtrate with silver nitrate**

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