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Standardization, Characterization and Isolation of *Trichoderma*-Silver Nanoparticle-A Pharmaceutical Approach in Field of Nano-Medicine

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Silver is a well known antimicrobial agent. It is utilized in many antimicrobial and medication. The *Trichoderma* and its Metabolites have also been meant for antimicrobial activity against the various bacterial and fungal strain. In the present investigation Nanoparticle of silver is prepared by chemical method, green synthesis using plants and biosynthesis using microbes. *Trichoderma* secondary metabolite prepared by solvent extraction method from *Trichoderma harzianum* which act as a capping and reducing agent. The biosynthesised silver Nanoparticles were characterized by UV-Vis spectroscopy and TEM. UV-Vis spectra of silver Nanoparticle and *trichoderma* extract showed absorption spectra at 420nm & 430 nm respectively while the fused nanoparticle with *Trichoderma* secondary metabolite showed absorption spectra at 415 nm corresponding to the surface Plasmon resonance of silver Nanoparticle. It was determined the nanoparticle showed absorption spectra at 415 nm and morphology as spherical with size range 8 to 24 nm and providing good antimicrobial activity as *Trichoderma* silver fused nanoparticle against many microbial strain, so it can be prepared for pharmaceutical approach against the infectious disease caused by clinical pathogenic organisms.

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1. INTRODUCTION

Nanotechnology is basically the science that deals with the matter at the scale of 1 billionth of a meter. Means 10^{-9}m=1nm. Nanoparticle is having very important role in both biotechnology and pharmaceutical industries. Nanoparticle is the most fundamental component in the fabrication of a nanostructure. Basically the size of a Nanoparticles range between 1 nm to 100 nm. The metallic nanoparticles have different physical and chemical properties rather than its bulk form of metal. Also Nanoparticles of various metals have unique electrical, optical & biological properties. Due to preserving the qualities, play a very vital role in pharmaceuticals order too [1,2,3].

These Silver Nanoparticles can be prepared by microbiological synthesis, chemical method and green synthesis i.e using plant. For green synthesis plant like *Aloe barbadensis* is used. Also many microbes is used for synthesis of silver Nanoparticles like *Trichoderma viridae* [4,5]. Other microbes used are *Fusarium oxysparum, Alternaria alternate,Aspergillus terrus, Trichoderma virens, Trichoderma ressei, Trichoderma aspergillum,Phoma glamerata,Trichoderma longibrachitum.* For production of silver nanoparticle by chemical method like silver nitrate is used [6,7,8]

Pharmaceutical approach of these Silver Nanoparticle formed by green synthesis using *Aloe barbadensis* have been observed having antimicrobial activity against clinical pathogen like *E. Coli, S. Typhi* and many other organisms by agar well diffusion. So, it's the novel tool in the study of nanotechnology.

Silver nanoparticle prepared by chemical method using nutrient broth silver nitrate and *trichoderma* extract showed a good sensitivity against *Pseudomonas, Rhizopus* with Slight sensitivity against *Proteus and Fusarium* species [5,3].

2. LITERATURE REVIEW

The word Nano is referred to a Greek word which means dwarf or something which is very small. Nanotechnology also shortened to nanotech is the use of matter on an atomic, molecular and supramolecular scale. Nanoscience quantum leap in almost every field of science and nanotechnologies make life calm in this era [2,5].

The Nanoscience and nanotechnology represent an expanding research area and involve structures, devices and system with novel properties and function due to the arrangement of their atoms on the 1-100 nm scale. This field arouse much awareness and controversy in early 2000's and led to start of commercial application of nanotechnologies [1,2].

There is a difference between Nanoscience and nanotechnology. Nanoscience is the study of structure & molecules on a scale of nanometer ranging between 1 to 100 nm and in case of nanotechnology, technology is one that utilizes it in practical application such as devices, textiles, human health care etc [4,3].

2.1 Silver: Properties & Use

Silver is a chemical element with Ag as its symbol and belong to 11^{th} group in the periodic table and its atomic number is 47. Silver is lustrous, soft, very ductile and malleable metal. Silver have many application as in photography, dentistry, cutlery, mirror, as catalyst in oxidation reaction, coins and jewellery, In electrical & electronic industries for items such as printed circuits and computer [3,7].

In the field of nanotechnology and Nanoscience, it is used in form of silver nanoparticle and are increasingly used in various field including medical, food, healthcare, consumer and industrial purposes due to their unique physical and chemical properties [9]. These include optical, electrical and thermal, high electrical conductivity and biological properties [10].

As silver exhibits a size and shape dependent optical effect known as localized surface Plasmon resonance (LSPR) at the nanoscale the ability to synthesize silver nanoparticles in different shape vastly increase ability to tune their optical behaviour [11].

So also it is well said sometimes a small things make a big impact. Ag*NP have a unique electrical, thermal, optical & biological properties [12,13]. It is the reason that silver Nanoparticle has been introduced in practical application like molecular diagnostics and a trial of its fused

version with natural antimicrobial agent is undergoing research to find out the pharmaceutical approach in the field of clinical sciences [14]. The basic chemistry behind Ag*NP is when this nanoparticle is in solution, molecules associated with the nanoparticle surface to establish a double layer of charge that stabilize the particle and prevent aggregation [15,16].

Silver nanoparticle are being used in numerous technologies and incorporated into a wide array of consumer products and take benefits of their desirable optical conductive and antibacterial properties [14,17]. Ag*NP have diagnostic application used as biological tags for quantitative detection, antibacterial application like Ag*NP are incorporated in apparel, footwear, wound dressing & bactericidal & bacteriostatic capability [16].

Advances in field of Nanoscience and nanotechnology have changed the way we diagnose, treat & prevent various diseases in many aspects of a human life. Ag*NP can be prepared by green synthesis that is plant extract like Aloe barbadensis, biological synthesis that is microbes & chemical synthesis like by using silver nitrate [1,2].

This Ag*NP also have antibacterial activity. When zeta potential determined by double beam UV-Vis spectrophotometer and FT-IR was measured, it was determined to be 194.2 nm [2,7]. Thus the final formation of Ag*NP was determined by colour change and differentiation pattern which occur from pale yellow to reddish brown [7,11,18]. Ag*NP formed by this seen having anti microbial activity against clinical pathogen *E.coli, S.typhi, S.aureus, B.thuringiensis, A.tumefaciens* by agar well diffusion method. So, it's also the novel tool in the study of nanotechnology [2,7].

For preparing green synthesised Ag*NP, nutrient broth, silver nitrate is used. Aqueous extract of *Aloe barbedensis* prepared as per description by Jenilia Rani Duraraj with some modification [1,2].

Trichoderma was first described by Christian Hendrik Person in 1794. There are total 89 species of *Trichoderma Hypocrea* [2,8]. Trichoderma is fast growing at temperature 25-30°C, exceptionally some species of Trichoderma grow at 45°C. In PDA it grow as shades of green. Conidium of *Trichoderma* is formed in one week as compact or loose tuft and as shade of green to yellowish, but sometimes white [4,7,11].

Yellow pigment is secreted in PDA and this yellow pigment is basically secondary metabolites. Microscopically conidiophores is branched with wider phialides and enlarged in middle also cylindrical. It is saprophytic belong to Ascomycota [19,20]. As per Gams and Bisset, *Trichoderma* comes under imperfect fungi. It is good spored, soil borne of have potential of degradation as not involved in sexual reproduction and can perform decomposition [21,22].

Trichoderma is also used as biological fungicides. The secondary metabolites produced by *Trichoderma* are kojic acid, citric acid and acetic acid. All of these work as a biocidal antibiotic against clinical bacterial & fungal pathogens [13,18] The Trichoderma have many useful properties, it control plant pathogenic fungi and play major role in nitrogen fixation which is important for plant growth. *Trichoderma* uptake the nutrients and promote the root & shoot growth and improve soil texture and help in control of phytopathogenic disease [4,11].

Due to the use of chemical method for protection of plant from phytopathogen have lead to decrease in product quality and soil texture. So to increase in demand of ecofriendly product replacement of chemical pesticides is done with biosynthesised based pesticides [1] The trichoderma species attack host by coil around the host hyphae and produce hydrolytic (fungitoxin) enzymes like chitinases, glucanases, proteases, peptaibol antibiotics and thus lead to phytopathogen control by biological method [2,8].

For preparing Ag*NP, as given by Chikdu et al (3)0.1 M silver nitrate was prepared in water and distributed as 10 ml,15 ml & 20 ml of Ag*NO3 in 3 small beaker and kept in magnetic stirrer for 15 min at 65°C. To it 1 ml plant extract was added drop wise in all the 3 beaker of Ag*NO3 with continuous stir. The above solution was kept in stirrer for 15 min & colour change was observed to reddish brown which indicate synthesis of Ag*NP & formation of *Aloe barbadensis* extract-Ag fused nanoparticle. This was characterised UV-Vis spectroscopy for analysis of NP, the synthesised Ag* Nanoparticles showed highest absorbance peak at 420 nm [1,2,3].

Approx same absorbance peak observed by Jenelia that is 410 nm [7]. FT-IR analysis, peak

observed at 3271.91 showed C-H stretching indicate the presence of alkynes strong group & peak at 1633.49 showed N-H bond stretching indicate primary amines group. Both alkynes and amines group stabilize the synthesised Ag NP and also act as reducing agent [2,3]. The fusion of TR biofiltrate with silver nitrate can be observed by colour change from white to green [1].

Characterization and standardization done via UV- spectrometry showed the absorbance at 415nm of TR-Ag fused NP and size of nanoparticle of silver was 20 nm as when determined by TEM [6]. GC-MS spectra of metabolites of *T. Harzianum* showed presence of kojic acid,citric acid in very large amount which is assumed to have properties of antibiotic activities and also small amount of acetic acid.

Thus as per the different study done, these TR-Ag fused NP have a properties of antibiotic. And play an important role in pharmaceutics in field of nanoparticle & have a great role in nanotechnology.

As per Miko Yamada et al. not only silver, gold nanoparticle is also being used in clinical treatment as therapeutic drugs like treatment of tumour tissue [8,12].

According to Chikdu D et al., Miku Yamada et al. and R. M. Tripathi et al. Ag*NP have been used for wound healing properties & enhance tumour killing effect of anti cancer drugs. Thus Ag*NP act as catalyst [2,12,16]. As per the research the metal NP used have a bright future to act a therapeutics & also as catalyst for clinical treatment of various diseases.

In the research paper discussed by Miko yamada et al. AgNP give proof of it as a powerful antimicrobial agent. Basically the silver ions appear to block the resistor [11,12].

R.M. Tripathi et al. have described that *T. Konigii* can be used for biosynthesis of Ag*NP [16] *T. Konigii* secret Protein and enzyme that act as reducing agent and capping agent. The biosynthesised Ag*NP have the absorbance of 413 nm by U-Vis spectroscopy and size of nanoparticle is 8-24nm and morphology as spherical when determined by TEM [12].

The biosynthesis of nanoparticle by using fungi is more advantageous rather than plant because this fungus produce more protein that lead the increase production of nanoparticle and also provide longer stability. Also extracellular synthesis of Ag*NP is found in fungi like *Fusarium oxysporum, Aspergillus fumigates, Neurospora crassa* [23,24].

Ag*NP also perform application in the field of biolabelling, antimicrobial filters and bactericidal activity. *Trichoderma kongii* prepared Ag*NP treated against *S.typhimurium*, 45 µg/ml inhibit its growth [9]. Trichoderma first came into known as antimicrobial agent by Christian Hendrik Persoon and its quality is it is fast growing [12,14].

There are list of organism that have ability of forming Ag*NP that is *T. resai, T. viridae, T. harzianum, T. Koningii, F. oxysporangium, Alternana Alternata, A. Terrus, T. Virens, T. aspergillum, Phomaglamerata, T. longibraditum* [14,22]. There are many approaches of nanoparticle of silver antibacterial, antiviral, antifungal, anti inflammatory, antiangiogenic, antitumor. Thus we have many pharmaceutical use of Ag*NP. Also there are other uses of Ag*NP other than pharmaceutical like Bio sensing, Imaging, Textiles, Drug carrier, water treatment, cosmetics [16,14].

3. MATERIALS AND METHODS

A. Isolation of Trichoderma species from soil samples - To isolate *Trichoderma* from soil, selective medium was prepared [10]. The basal medium consisted of 0.2 g MgSO4 (7H2O), 0.9 g K2HPO4, 0.15 g KCl, 1.0 g NH4NO3, 3.0 g D. glucose anhydrous, 0.15 g Rose Bengal and 20g agar. These constituents were added to 950 ml of distilled water and autoclaved at 121°C for 30 minutes. The biocidal ingredients, 0.25g tetracycline were mixed in 50 ml of sterilized distilled water and added to the autoclaved basal medium where it cooled to 40 to 50°C. 10 grams of soil were suspended in 50 ml of sterile distilled water and agitated for 30 minutes at 200 rpm in a rotary shaker. Serial dilutions were made and 0.1 ml of each was spread on the Trichoderma selective medium plates with a glass rod. Three plates of each sample were prepared and incubated for 5 days at 30°C. *Trichoderma* isolates were collected and transferred onto potato dextrose agar (PDA) plates for maintaining pure culture [13].

B. Cultivation and culture conditions - *Trichoderma* cultures obtained were cultivated and maintained on slants of potato dextrose agar for 5 days at 28°C. Three hundred millilitres flasks were incubated for 14 days at 28°C on a laboratory incubator. The fungal biomass was collected for further use.

C. Microscopic and taxonomic identification of *Trichoderma c*ulture - Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of Trichoderma species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micro-morphological studies, a slide culture technique was used [14]. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of Trichoderma spp. Samples were compared to a taxonomic key for the genus Trichoderma [17] and further verified and confirmed by National Centre of Fungal Taxonomy, New Delhi, India

D. Preparation of *Trichoderma* filtrate- *Trichoderma* was inoculated in liquid nutrient medium containing consisted of 0.2 g MgSO4 (7H2O), 0.9 g K2HPO4, 0.15 g KCl, 1.0 g NH4NO3, 3.0 g D-glucose anhydrous, 0.15 g Rose Bengal and 0.25 g tetracycline per 50 ml of sterilized distilled water and added to the autoclaved basal medium where it is cooled to 40 to 50°C. The culture was then after placed in incubator shaker at 200 rpm at 280C for 5 days. Further filtered by Whatman's filter paper No. 42 to obtain filtrate. Then the mycelium and filtrate were separately subjected to solvent extraction.

E. Extraction of the filtrate- The filtrate of each fungus was extracted several times with ethyl acetate (v/v) in a separating funnel. The extracts from both mycelia and filtrate were evaporated under vacuum at 500C till dryness. The obtained solid material was dissolved in ethyl acetate to form the crude extract and tested for antimicrobial activity. For day optimization the fungus was grown in the malt extract medium at pH 6.2. Inoculated flasks were incubated at 270C on an incubator shaker for 8 days. The biomass production was determined each day for antibacterial activity.

F. Preparation of silver nanoparticles- For the preparation of silver nanoparticles two stabilizing agents, sodium dodecyl sulphate (SDS) and sodium citrate were used. For the synthesis of silver nanoparticles, silver nitrate solution (from 1.0 mM to 6.0 mM) and 8% (w/w) sodium dodecyl sulphate (SDS) were used as a metal salt precursor and a stabilizing agent, respectively. Hydrazine hydrate solution with a concentrate ranging from 2.0 mM to 12 mM and sodium citrate (1. 0 mM to 2.0 mM) were used as reducing agents. Citrate of sodium was used as stabilizing agent at room temperature. The transparent colorless solution was converted to the characteristic pale yellow and pale red colour, when citrate of sodium was used as stabilizing agent. The occurrence of colour was indicated by the formation of silver nanoparticles. The silver nanoparticles were purified by centrifugation. To remove excess silver ions, the silver colloids were washed at least three times with deionized water under nitrogen stream. A dried powder of the nanosize silver was obtained by freeze-drying [16,14,17,18,21].

G. Preparation of *Trichoderma* fused Silver nanoparticles The silver nitrate (1 mM) solution was prepared in 50 ml deionized water. Fungal biomass (5 g) was brought in contact with the silver nitrate solution in a 200 ml Erlenmeyer flask. The solution was then kept in dark condition at 29±1 0C under continuous shaking at 200 rpm for 72 h. After 72 h of reaction time the colour change was observed [25,26,19,22].

H. Characterization of prepared nanoparticles via UV- absorption spectra and Transmission electron microscopy (TEM) - The formation of Ag*NPs and TR-Ag*NPs by the bio-reduction of Ag+ to Ag0 was easily monitored using UV–Vis spectroscopy. The scanning was performed in the range of 200– 700 nm. The morphology and size were determined by TEM [27,20,23,24,28].

I. Determination of antimicrobial activity of Trichoderma-Ag* fused nanoparticles against the pathogens- The antimicrobial activity of *Trichoderma* extract and *Trichoderma* fused silver nanoparticles was determined at different concentrations

against local isolated pathogenic cultures viz. *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus luteus, Leuconostoc mesentroides, and Aspergillus niger* by well diffusion method [29,30-35]

4. RESULTS

The present study showed the isolation and characterization of Trichoderma spp. culture isolated from soil. Further the culture was determined taxonomically and was determined as *Trichoderma harzianum* and was denoted as per the accession no- NCFT.9153.17. The culture photograph is shown in Fig. 1.

On microscopic and taxonomic identification of trichoderma culture the colony was observed as green colour with compact conodiophore. *Conidia* is compact or loose tuft in shades of green. . On microscopy septate hyaline hyphae seen along with branched phialids, were hyaline, flask shaped and inflated at the base. Colony is green colour with sweet or coconut smell. Thus the culture was identified as *T. Harziamun* (Fig. 2).

Extraction of secondary metabolite extract from *T. harziaum: T. harzianum* extracted prepared and filtered by solvent extraction method to determine secondary metabolite. And then this solvent extract was further vacuum dried, to evaporate the solvent and obtain the powder form having secondary metabolite for further identification and preparation of nanoparticle in fusion with silver Fig. 3. The GC-MS was done to identify the secondary metabolite and it was found to be kojic acid, citric acid & acetic acid Fig. 4.

Fig. 1. Isolated *Trichoderma* culture from soil

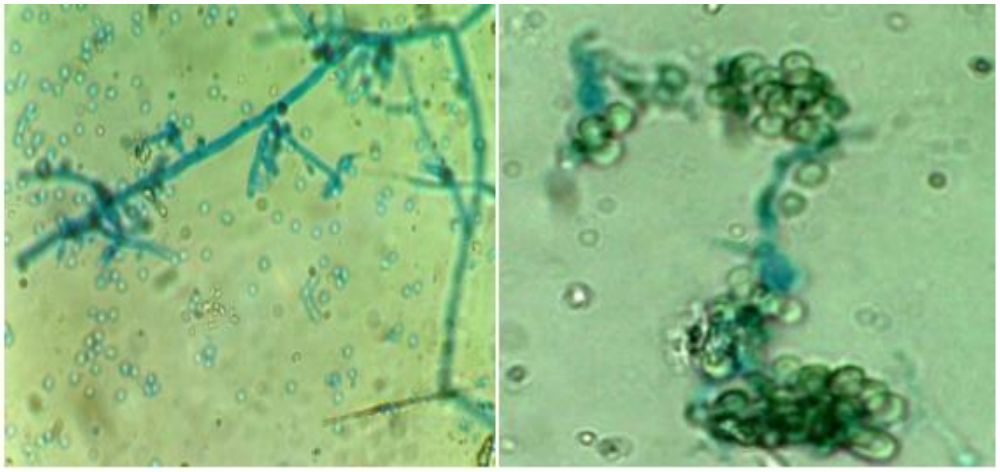

Fig. 2. Conidia and spores in *Trichoderma harzianum*

Fig. 3. Preparation of extract & solvent extract by *T. harzianum*

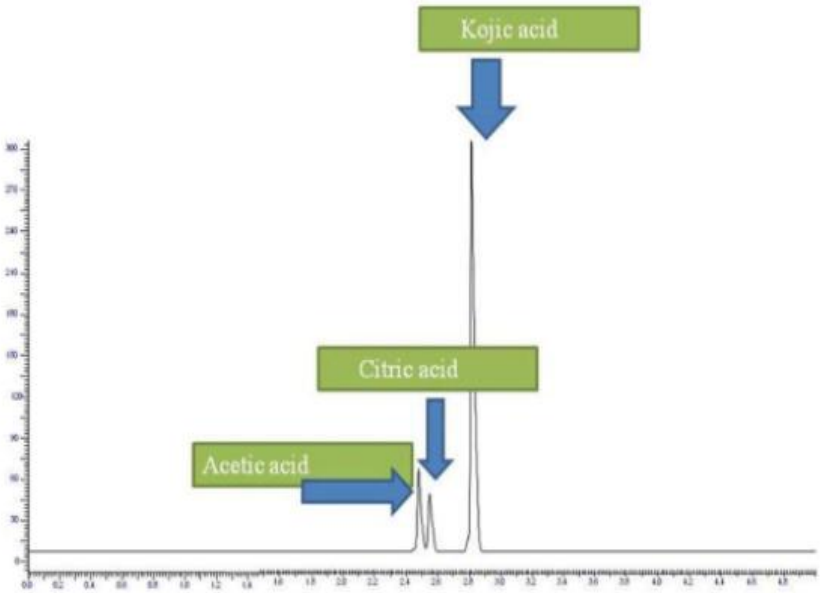

Fig. 4. GC-MS spectra of metabolites extract

3.1 Preparation & Characterization of *Trichoderma fused* Silver Nanoparticle (TR-Ag NPs)

In the present investigation Tr-Ag*NPs were produced (Figs. 5 & 6). The particle size was determined by SEM. And the absorption spectrum was determined at 200-700 nm. It was found the TR-Ag*NP were very fine shape and size having optimal size 20 nm same as in case of green synthesised nanoparticle or *T.viridae* synthesised nanoparticle and are having slight rough spherical structure.

The UV absorption spectra of the fused nanoparticle had the maximum wave length 415 nm (Fig. 7).

On determination of antimicrobial activity of *trichoderma harzianum* solvent extract (Fig. 8a) and Trichoderma silver fused nanoparticle (Fig. 8b) against clinical pathogen gave significant result. Also the antimicrobial sensitivity was observed more in case of trichoderma silver fused nanoparticle against clinical pathogen rather than sensitivity test with Trichoderma extract only.

Fig. 5. (Preparation of silver nanoparticles)

Fig. 6. (Preparation of TR-Ag Fused NP)

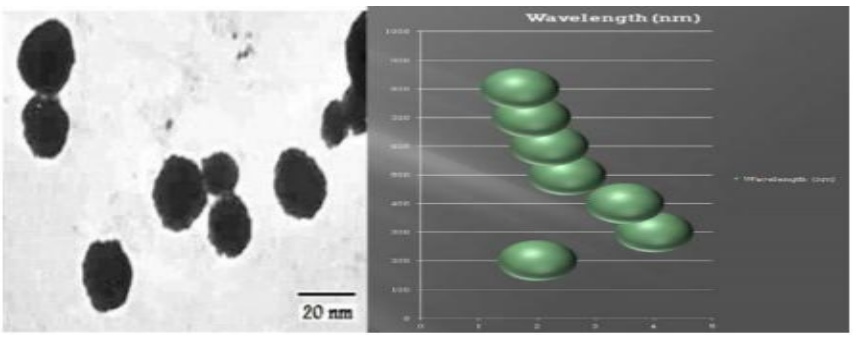

Fig. 7. Characterization of TR-Ag Np by SEM and determination of absorption maxima

Table 1a. *T. harzianum* extract (TR 2°metabolite) and its antimicrobial activity

	10µl	20 µl	5ppm	10ppm	25ppm	1ml	Ethyl alcohol(control)
PA	25	30	_____	_____	_____	45	_____
BS	26	29	_____	_____	_____	38	_____
ML	29	33	_____	_____	_____	47	_____
LN	32	36	_____	_____	_____	42	_____
AN	0	____	_____	_____	_____	43	_____

TR extract Zone of inhibition in "mm" by Trichoderma Extract

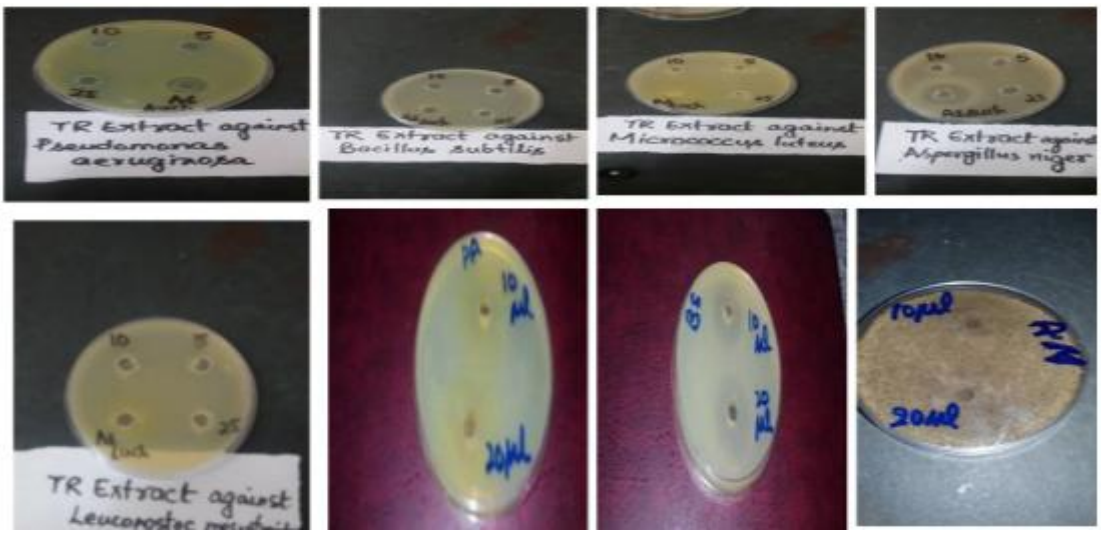

Fig. 8a. Antimicrobial activity determination by well diffusion method of solvent extracts of *Trichoderma*

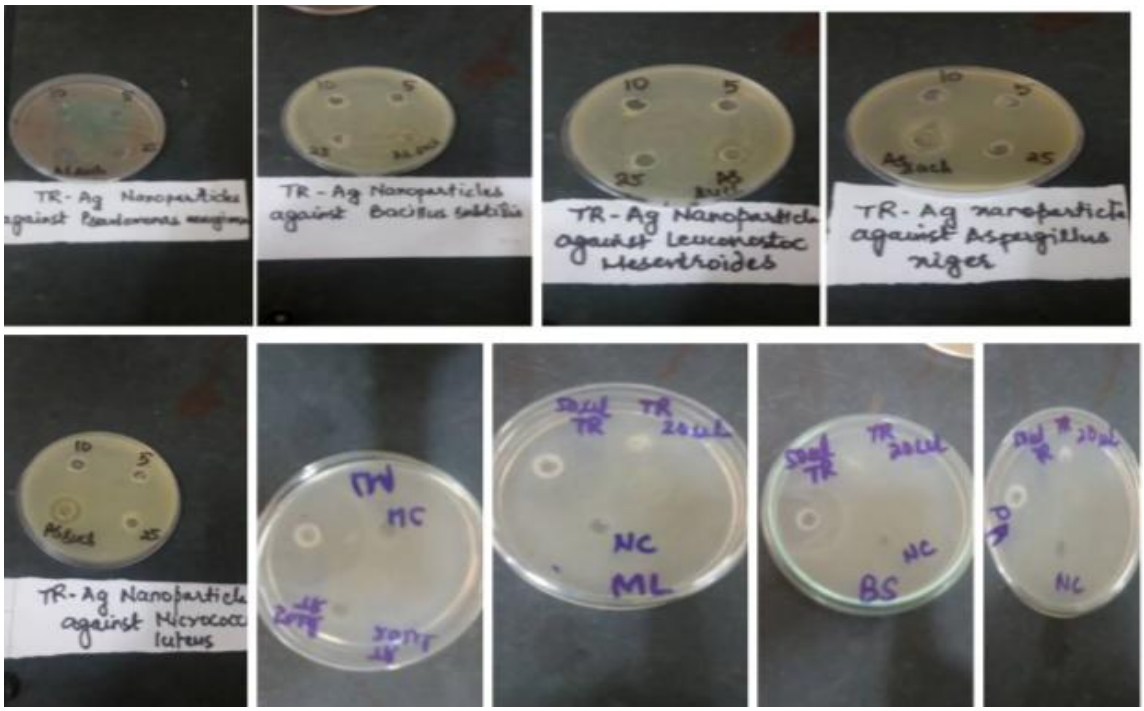

Fig**. 8b. Antimicrobial activity determination by well diffusion method of *Trichoderma fused* silver nanoparticles (TR-Ag Np)**

Table 1b. *T. harzianum* –Ag* fused NP and its antimicrobial activity

	10 µl	20 µl	5ppm	10ppm	25ppm	1ml	Ethyl alcohol(control)
PA	33	40	____	_____	_____	57	_____
BS	34	37	_____	_____	_____	58	_____
ML	35	39	_____	_____	_____	49	_____
LN	35	39	_____	_____	_____	48	_____
AN	___	___	_____	_____	_____	48	_____

TR-Ag fused NP Zone of inhibition in "mm" by TR-Ag* fused NP*

As per plant extract fused Ag*NP described by Chikdu D et al. [2] organism like Bacillus (2514), *S. aureus* (2078), *E. coli* (1692), *S. typhi* (2501), A.tumefaciens all show sensitivity at 10 µl & 20 µl (Table 2).

Table 2. Plant extract-Ag* fysed NP & its antimicrobial activity

	10 µl	20 µl
Bacillus	5	11
S.aureus	10	13
E.coli	13	13
S.typhi	3	6
A.tumefaciens	11	11

**Zone of inhibition in mm*

The other species of Trichoderma that is biomass of *T.viridae* –Ag * fused NP when subjected to anti microbial sensitivity test, it showed good sensitivity against *MRSA, Acinetobacter baummamni, Shigella sonnei, S.boydii, Salmonella typhi* Table 3a.

Table 3a. Microbiological synthesized NP. *T. viridae*-Ag* fused NP & its antimicrobial activity

	Ag*NP
MRSA	20
Acinetobacter baummamni	23
Shigella sonnei	21
S.boydii	28
Salmonella typhi	25

The Biomass of TR- viridae silver fused nanoparticle when subjected to antibiotic sensitivity test along with other antibiotic agent , TR viridae-Ag* fused nanoparticles showed comparable good sensitivity against organism like Shigella sonnei, S.boydii, Salmonella typhi. [Table 3b].

The TR extract & TR extract silver fused NP when subjected to antimicrobial sensitivity test by agar gel well diffusion technique as described by Bina Pani Gupta et al. also presented a zone of inhibition against organism like *P.aeurogenosa, B.subtilis, Micrococcus, Leuconostoc, Aspergillus niger* at different quantity of TR extract & its fused form with AgNP. It was also observed the TR-Ag fused NP (Table 3a) Show more zone of inhibition rather than TR extract alone (Table 3b).

5. DISCUSSION

When green synthesised Ag*NPs was prepared and fused plant *Aloe barbadensis* extract silver fused nanoparticles finally formed was known by colour change to reddish brown whereas when *Trichoderma harzianum* secondary metabolite silver fused nanoparticle formed was known by colour change to greenish colour of solvent extract which was fused by silver. Also, a per research *T.konigii* secrete protein amd enzymes which act as a reducing agent and capping agent [9]. AgNP synthesised by *T. konigii* also gave a comparable good result as antimicrobial agent against clinical pathogen and it was also found the absorbance by UV spectroscopy as 413 nm which was approx same range as 415nm for the *T. harzianum* silver fused nanoparticle and also the absorbance UV spectroscopy by Aloe barbadensis extract silver fused nanoparticle as 420 nm. Same in the case of *T. viridae*, it also has the ability of biosynthesis of Ag*NP and when the *T. viridae* silver fused nanoparticle is reacted against clinical pathogenic organism give the remarkable anti microbial sensitivity report. Even this is sensitive toward MRSA strain too.

Thus the green synthesised and biosynthesised Ag*NP and the Trichoderma extract when fused together give a remarkable result against many clinical pathogenic strain and can be a good scope for diagnostic & pharmaceutics in the field of nano-medicine.

Table 3b. Microbiological synthesized NP. *T. viridae*-Ag* fused NP & its antimicrobial activity

*Zone of inhibition in mm	Tetracyclin	Ampicillin	Gentamycin	Neomycin	Ag*NP
MRSA	26	0	10	0	20
Acinetobacter baummamni	26	0	23	0	23
Shigella sonnei	17	0	17	20	21
S.boydii	15	19	17	11	28
Salmonella typhi	16	11	23	23	25

6. CONCLUSION

Thus as per the different studies done, these trichoderma silver fused nanoparticle have the properties of antibiotics and play an important role in pharmaceutics in the field of nanoscience and have a great role in nanotechnology. Silver nanoparticles have been used for wound healing and enhance tumour killing effect of anti cancer drugs. The biosynthesis of Nanoparticles by using fungi is biocompatible, biodegradable and ecofriendly advantageous rather than chemical or plant based. Ag*NP synthesis because these fungus produce more protein that lead the increase production of Nanoparticles and also provide long stability. It can thus be used to prepare potent biocidal product against clinical pathogen.

Further different-different dose based study is required to determine and analyse the antimicrobial sensitivity produced of Ag*NP produced by different method is green synthesis, microbial synthesis and chemical synthesis of Ag*NP and its AST against same pathogenic strain at different doses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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