e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.11, 2022
 UGC CARE Listed (Group -I) Journal

EFFECT OF SILVER NANOPARTICLES ON PLANT PATHOGENS AND SEED GERMINATION

Kavita P. Tambe[1]
[1]Department of Biotechnology, P.V.P. College of Arts,
Science & Commerce Pravaranagar, Loni, Ahmednagar (Dt.), Maharashtra, India

Ashok M. Bhosale[2]
[2]Department of Biotechnology, P.V.P. College of Arts, Science & Commerce Pravaranagar,
Loni, Ahmednagar (Dt.), Maharashtra, India

Sanjay P. Giri [3]
[3]Department of Biotechnology, P.V.P. College of Arts,
Science & Commerce Pravaranagar, Loni, Ahmednagar (Dt.), Maharashtra, India

ABSTRACT

Recently, concerns have been raised regarding antimicrobial activity of the nanoparticle against the plant pathogenic fungi and increases in seed germination indices. The biosynthesis of silver nanoparticle from *Trichoderma viride* was very rapid and took 12 h at 30 ^{0} c when cell free extract of the *Trichoderma viride* was used. The bio molecule secreted by *Trichoderma viride* was capable to synthesis and stabilize the particles. UV-Spectroscopy showed maximum surface Plasmon resonance at 444 nm. SEM showed polydisperse polyhedral and tetrahedral shape of nanoparticles (5-10µm). XRD pattern showed strong peak in entire spectrum at 1344. The biosynthesized silver nanoparticle proved efficient antifungal activity against plant pathogenic fungi *Fusarium oxysporum* which inhibit the 80-90% growth of fungal pathogen which causes *Fusarium wilt* in tomato. Biosynthesized silver nanoparticle proved significant impact on germination of Fenugreek seeds. The true benefit of the silver nanoparticle application it enhance the seed germination indices and resistance to plant pathogenic fungi.

Keyword: Silver nanoparticle, *Trichoderma viride*, plant growth promoter, Fenugreek **seed** and plant pathogenic fungi.

1. INTRODUCTION

Agriculture is considered as a backbone of developing countries. This agriculture sector is mainly affected by various types of bacterial and fungal pathogen (Bansal *et al.*, 2017). To control this pathogens repeated use of the conventional pesticide which is costly and may

disturb the environment and biological system (Zakharova *et al.*, 2017). To overcome this national and global problem new method fighting against plant pathogen hold great importance in the field of agriculture. Nano-agriculture is best solution to control this plant pathogen and improve the plant productivity.

Nanotechnology occupies very prominent position in the field of human welfare because of multifactorial role (Kumari *et al.*, 2017) and this technique control matter at molecular level. This strong correlation between size and property of nanoparticle offer opportunity of discovery to the scientist (Mishra *et al.*, 2014). The nanoparticle function depends on the nature of nanoparticle and method used for synthesis. There are many method for synthesis of nanoparticle but, biological method was very rapid and environmentally friendly which does not produce side product like physical and chemical method (Shelar & Chavan, 2015).

In biological method bacteria, fungi, actinomycetes and plant extract used for synthesis of nanoparticle (Shelar & Chavan, 2015). But fungal based synthesis of nanoparticle from *Trichoderma* adventitious over other bacteria and fungi (Elgorban *et al.*, 2016). Because *Trichoderma* is fast growing, non-pathogenic used as biocontrol agent (Elad *et al.*,1979 ; Grondona *et al*., 1997; Khabat *et al.*, 2011 ; Motlagh & Samimi, 2013). *Trichoderma* secret extracellular secondary metabolite having plant growth promoting and plant pathogen control abilities which serve as an efficient antimicrobial agent. (Saba *et al.*, 2012; Chitra & Annuadurai, 2013; Gherbawy *et al*., 2013; Mishra *et al.*, 2014).

Among various nanoparticles, most widely studied one is silver nanoparticles because of its diverse antimicrobial application (Fayaz *et al.*, 2010). Silver is useful to human from ancient time because of its medicinal important characteristic of silver is in wound healing and burning (Panacek *et al.*, 2006). This biologically synthesized nanoparticle have antimicrobial activity of both silver and secondary metabolites secreted by *Trichoderma viride* which acquired during surface coating of particle (Elgorban *et al.*, 2016; Kumari *et al.*, 2017)

Fusarium wilt caused by *Fusarium oxysporum* one of the fungal pathogen of lycopersici family which causes wilting in various plants like potatoes, pappers, eggplant and legumes (Sanker *et al*., 2020, El-Sheekh *et al*., 2022). The pathogen is seed borne and also found in soil. The main symptom of the disease is clearing of the veinlets and chlorosis of the leaves followed by yellowing and wilting. The younger leaves may die in succession and the entire

may wilt and die in a course of few days. In field, yellowing of the lower leaves first and affected leaflets wilt and die. Plants become stunted and die.

Bhaskar *et al.,* (2016) synthesized silver nanoparticle checked antimicrobial activity against different plant pathogenic fungi like *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum*. Nano silver have been shown to be effective against phytopathogenic fungi and bacteria (Saravanakumar & Wang, 2018). Kaur *et al.,* (2012) synthesized nano-size silver/chitosan nanoformulations and checked antifungal activity against various seed borne plant pathogens especially *Rhizoctonia solani* and *Alternaria alterneta*. In addition, these nanoparticles have showed no severe toxic effect in the tested plant and recommended as a promising diseases control agent.

Seed germination is an important phenomenon in modern agricultural because it is thread of life of plant that guarantee its survival. So we have selected Fenugreek seed as a model system to study effect of silver nanoparticle plant growth and development. Fenugreek (*Trigonella foenum-graecum*) is a well-known spice which is annual plant in the family Fabaceae used commonly in most part of the word (Seyed & Hamidreza, 2015). However, there has been few report proved that impact of biochar nanoparticle on seed germination shoot and root development and changes in leaf area and biochemical attributes (Zhang *et al.,* 2019).

In this study, we report rapid biosynthesis of silver nanoparticle with cell free extract of *Trichoderma viride* and check its antimicrobial activity against plant pathogen *Fusarium oxysporum*. However, the statistical study of effect of silver nanoparticle on seed germination of Fenugreek seeds were carried out.

2. MATERIALS METHODS

Media were purchased from Himedia lab, Mumbai, India. All other chemical and reagent were highest purity. The fungal culture *Trichoderma viride* was obtained from Krishi Vigyan Kendra, Babhaleshwar, India and maintained on Potato Dextrose Agar (PDA) at 25±2°C.

2.1. Collection of sample

Infected leaves of tomato plants with Fusarium wilt symptoms were collected from various region of Pravaranagar and stored in the refrigerator at 4-6 ^{0} C.

2.2. Isolation and Identification

The infected leaf tissues were cut into small pieces then surface sterilized with 0.5% sodium hypochlorite solution for 1 min. Then the sample were washed thoroughly with SDW for several times (EL-Tanany *et al*., 2018). Then the tissue were dried using sterile filter paper transferred directly to the PDA medium and kept at 25±2°C for 12h light/dark photoperiod for 6–10 days (Meena *et al*., 2017). To avoid bacterial contamination, medium were supplemented with streptomycin. Colonies from each plate were sub-cultured by single spore isolation method on potato dextrose agar (PDA). Then microscopic examination was done by placing small portion of the fungal colony on slide by using sterile needle and mixing it with a few drops of water. According to Barnett and Hunter (1987) and Simmons (2007) observe the feature of the conidia like the length of the chains, type of branching in the chains, the number of sporadic spaces and width of the conidiogenous hyphae under light microscope.

2.3. Synthesis of silver nanoparticles.

For production of *Trichoderma viride* biomass for biosynthesis of silver nanoparticles it was grown in potato dextrose broth agitated on an orbital shaker at 27^0 C at 140 rpm for 72 h. After growth culture was filter though whatman filter paper No. 1 followed by three times washing with double distilled water to remove medium components. About 20 gm of biomass of *Trichoderma* added in 100 ml double distilled water and agitated on orbital shaker as described earlier. After incubation cell filtrate was filtered through Whatman filter paper No 1. In 100 ml filtrate 1mM $AgNo_3$ was added, and kept in dark. After 24 h incubation colour intensity and readings were taken on UV-Vis spectroscopy at 300-600 nm for five days (Fayaz *et al.*, 2009; Mishra *et al.*, 2014).

2.4.Characterization of silver nanoparticle

The reduction of silver ions was routinely observed by colour change as well as by UV-spectrophotometer for five days. Scanning electron microscopy (SEM) analysis of AgNPs was performed as described by (Elgorban *et al.*, 2016). Briefly, a drop of AgNPs solution was placed on the carbon coated copper grids followed by drying at room temperature. The AgNPs was analysed at the SPPU. A thin film coated on glass plate for measuring XRD. (Fourier transform infrared spectroscopy) ranged from 4000 to 600 cm^{-1} and was carried out at Department of physics Savitribai Phule Pune University. X-ray diffraction was carried out at Department of physics Savitribai Phule Pune University.

2.5.Antimicrobial activity by Pour plate method

The plant pathogenic fungi *Fusarium oxysporum* was used to determine the antifungal activity of the silver nanoparticles. The experiments were carried out by agar well diffusion method. To check antifungal activity on potato dextrose agar was supplemented with silver nanoparticle (10µg/ml). A bit of pathogenic fungi *Fusarium oxysporum* was placed at the centre of plate.

2.6.Seed germination

Seeds of Fenugreek were first washed with tap water then sterilized with 70% alcohol for 60 sec. then with 1% mercuric chloride solution for 1 min and rinsed several times with sterile distilled water. Sterile seeds were soaked in 3 days old broth of silver nanoparticles solution for 2 h and 4 h. Control seeds were simultaneously run by soaking in water. Treated and control seeds were placed equal distantly on 4-5 layered wet filter paper in petri plates. Plates were kept in dark for 48 h (Shelar & Chavan, 2015).

Statistical analysis

After 48 h of incubation shoot length and root length were measured (Seyed & Hamidreza, 2015).

Percent germination of seed was calculated by following Equation:

GP = Seeds germinated /Total seeds *100

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of *Fusarium Oxysporum*:

Fusarium species were isolated from tomato leaves on PDA and subculture. Microscopic observation shows mycelium septate and hyaline. They produce macro and micro conidia. Micro conidia are one celled, hyaline, ovoid to ellipsoid (Mohammed *et al*., 2016). Two races of pathogen have been identified.

3.2. Synthesis and characterization of AgNps:

Trichoderma viride is a biocontrol agent produces secondary metabolites which acquired during the coating of nanoparticle (Kumari *et al.*, 2017).

Fig 1. Biosynthesis of AgNPs: Color of solution change from colorless to brown within 24 h Incubation

Biosynthesis of silver nanoparticle was confirmed by the colour intensity and the optical properties. After addition of silver nitrate into culture the colour of the solution was changed from colourless to brown within 24 h (Fig 1). Silver nanoparticles was characterised by UV visible spectrometer at resolution 300 to 600 nm. Strong surface Plasmon resonance found at 444 nm (Fig 2). Which confirmed the synthesis of silver nanoparticles as is also similarly recorded for the *Trichoderma* sp. (Fayaz *et al.*, (2009, 2010); Khabat *et al.*, (2011); Devi *et al.*, (2013); Shelar & Chavan, 2015).

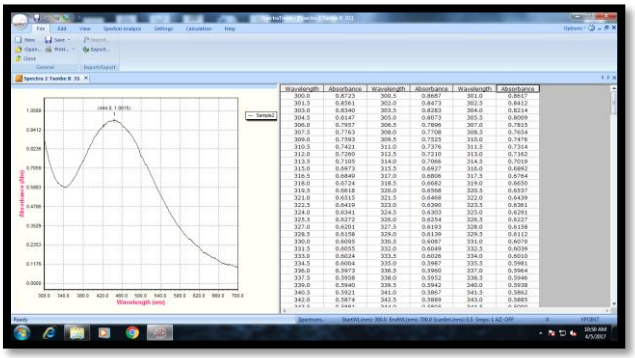

Fig. 2. Biosynthesis of AgNPs from *Trichoderma viride* and its characterization: A) UV-Visible Spectroscopy a sharp peak found at 444nm.

A scanning electron micrograph confirmed tetrahedral shape of silver nanoparticles with the average size of 5-10 µm (Fig 3A & Fig 3B).

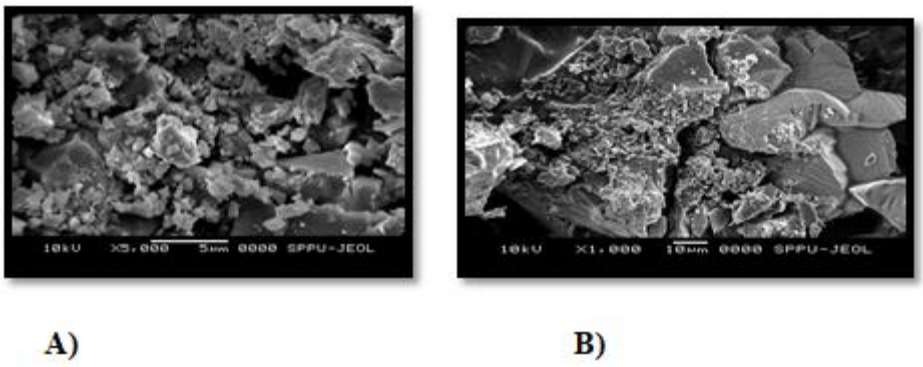

Fig. 3 Characterization of AgNPs by Scanning electron microscopy A) SEM images of the AgNPs size 0-5µm B) SEM images of the AgNPs size 10 µm

The SEM showed complex nanoparticles as well as number of aggregates. Fayaz *et al.*, (2009) also found size of silver nanoparticles in range 5-40 nm. Khabat *et al.*, (2011) in the range 5-50 nm and Chitra & Annuadurai, 2013) in the range at 28 nm. The shape and size of the silver nanoparticle varied according to use of microbes and method of synthesis. Further, the FTIR spectra displayed several alkaline, amine, proteins and aromatic peptide at the bands of 3291, 1647, 1392 and 1077 cm^{-1}. The band at 1647 cm^{-1} corresponds to primary amine NH band; similarly band at 1077 cm^{-1} corresponds to secondary amine (Fig 4).

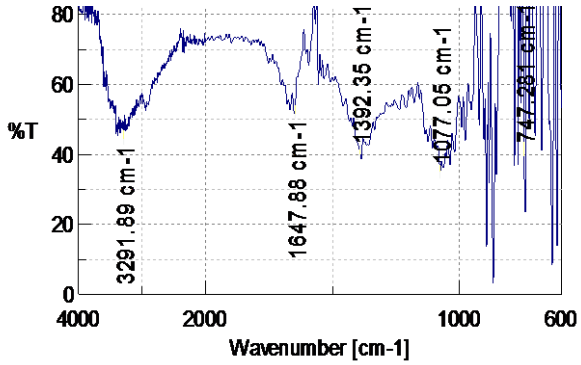

Fig.4. Characterization of AgNPs by FTIR pattern: FTIR spectra displayed several bands at the 3291, 1647, 1392 and 1077 cm^{-1}

The similar observation is made by Fayaz *et al.*, (2009), he found that band at 1650 cm^{-1} and at 1077 cm^{-1}. He also proved that because of the interaction with the Ag^{+} ion secondary structure of the protein does not affected. Further X-ray diffraction studies was carried out to determine crystalline nature of the nanoparticles. XRD pattern showed strong peak in entire spectrum at 1344 (Fig 5).

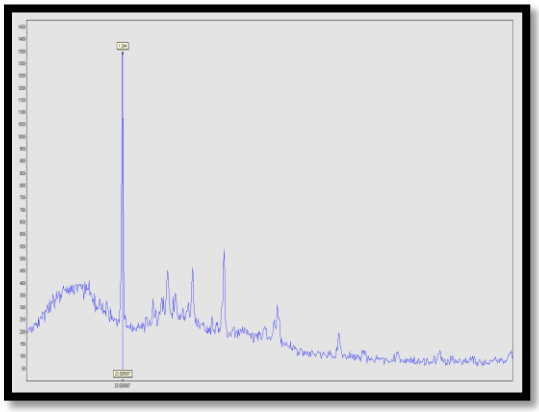

Fig. 5 Characterization of AgNPs by XRD pattern: XRD pattern showed strong peak in entire spectrum at 1344

The assigned peak in spectrum indicates the presence of inorganic matter which is present in fungal filtrate. (Chitra & Annuadurai, 2013) found XRD pattern strong peak in entire spectrum of 2Θ ranging from 20 to 80. Fayaz *et al.*, (2010) also proved XRD pattern peak in between 20-90 range.

3.3.Antifungal activity:

From result it was found that 80-90 % inhibition of *F. oxysporum* plant pathogen (Fig 6).

Fig. 6 Effect of Ag NP against plant pathogens A) Growth of *F. oxysporum* on PDA without nanoparticle. B) Growth of *F. oxysporum* on PDA with nanoparticle

This result proved that silver nanoparticles from *Trichoderma viride* was significantly inhibit the growth of fungal pathogen. This finding also supported by Bhaskar *et al.*, (2016). He synthesize silver nanoparticles from *Trichoderma* species and proved effective against *A.niger* causing collar rot of groundnut. Kaur *et al.*, (2012) proved Ag/Ch, exhibit higher inhibition against *Aspergillus* followed by *Alternaria* and *Rhizoctonia* species.

e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.11, 2022
 UGC CARE Listed (Group -I) Journal

3.4.Seed germination:

Silver nanoparticle treated seeds show long shoot and root length then the control seeds (Fig 7 & Fig 8).

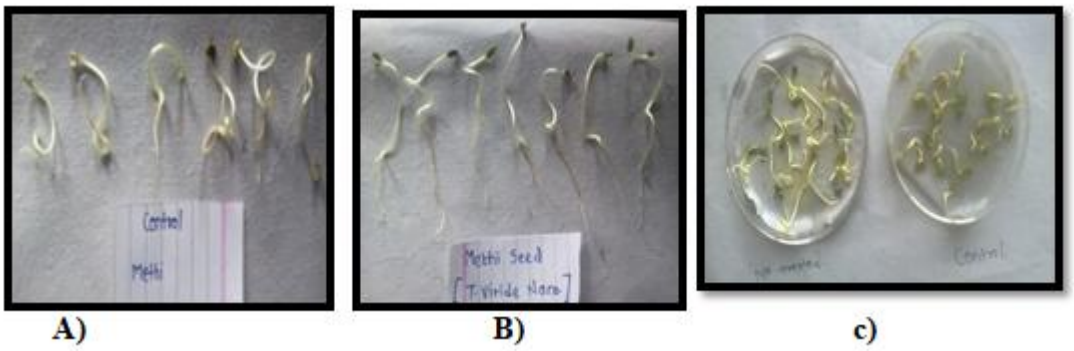

Fig. 7. Effect of AgNPs on seed germination: A) Nontreated Fenugreek seed B) Treated Fenugreek seed C) Treated and non treated seeds of the Fenugreek seed in petri plate

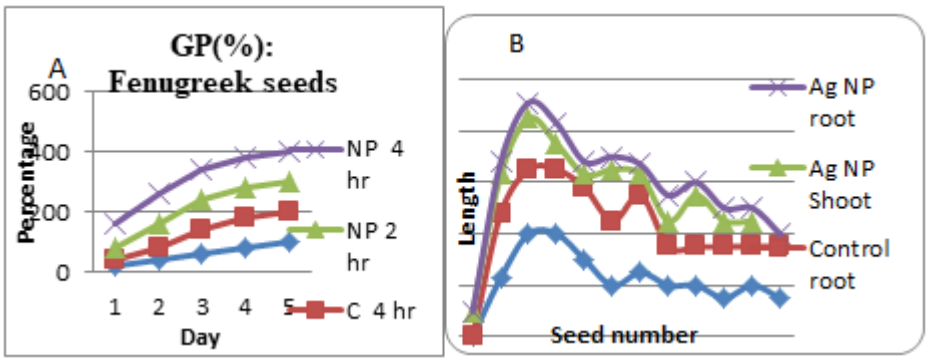

Fig. 8. Effect of AgNP on seed germination A) AgNPs enhance shoot and root length compared to control. B) GP of treated & Non-treated seeds for 2 h and 4 h incubation

From Table 1b it was clear that as soaking period increase the seed germination percentage increasee. 4 h and 2 h treated seeds showed 100 % germination on 2nd day and 3rd day respectively however in control 100 % germination was observed on 4 th day. Silver nanoparticles treated seed show optimistic effect on germination of the Fenugreek seeds then the control (Table 1 & Table 2).

Table. 1. Effect of silver nanoparticles on Fenugreek seed sgermination

No. of	Ag NP Shoot	Control Shoot	Ag NP Root	Control Root
1	2.3	1	2.5	0.5
2	4	1.5	2.5	0.6
3	4	2	2.5	0.8
4	3	1	2.8	0.5
5	2	0.5	2.5	0.5
6	2.5	2	3	0.4
7	2	0.8	1.5	1
8	2	1	1.5	0.5
9	1.5	2	2	0.5
10	2	1	1	0.5
11	1.5	1	1	0.5
Mean	2.43 *	1.25	2.07 *	0.57
SD=	0.461		0.56	

*Significant at 0.05 level of significance

Table. 2. Effect of silver nanoparticles on seed Germination Percentage

	Fenugreek seeds			
	2 H		4 H.	
	NP	Control	NP	Control
1	40	20	80	60
2	80	40	100	65
3	100	60	100	68
4	100	80		
Mean	80 *	50	93.3*	46.66
SD	29.76		17.66	

The results were proved significant at 0.05 percent level of significant. Therefore this finding proved that silver nanoparticles played significant role in improvement in percent seed germination. Similar finding also supported by (Shelar & Chavan, 2015) as soaking period increases the seed germination indices increases. (Mahajan *et al*., 2011; Seyed & Hamidreza., 2015) studied at lower concentration silver nanoparticle

enhance seed germination and early seedling growth in Fenugreek seed and adverse effect at high concentration. Saba *et al.*, (2012) proved *Trichoderma viride* produces secondary metabolite as growth promoter and bio control agent (Błaszczyk *et al.*, (2014) which acquired during the surface coating of the nanoparticle (Kumari *et al.*, 2017) which enhance seed germination and inhibit the plant pathogen growth.

CONCLUSION

The present investigation concluded that biosynthesized silver nanoparticles play a significant role in antibacterial, antifungal activity and enhancement in seed germination. Therefore present finding advice to the farmers use biosynthesized silver nanoparticles to control of plant diseases, improvement in crop productivity.

ABBREVIATIONS

Ag/Ch- silver/chitosan

Ag^{+}- Silver Ion

AgNPs- Silver Nanoparticle

$AgNo_3^{-}$ Silver Nitrate

Cm - Centimetre

Fig- Figure

FTIR-Fourier transform infrared spectroscopy

GP- Germination Percentage

h- Hour

µm- Micrometre

mM- Milimeter

Nm- Nanometre

NH- Nitrogen-Hydrogen bond

%- Percentage

PDA-Potato Dextrose Agar

Rpm-Revolution per minute

SEM-Scanning Electronic Microscopy

SDW-sterile distilled water

SPPU-Savitribai Phule Pune University

UV-Ultraviolet

XRD- X-ray diffraction

Ethical Approval and Consent to participate

Not applicable

Availability of supporting data

Shelar & Chavan, (2015) studied effect of AgNPs on seed germination of soybean and sunflower. But I had studied effect of silver nanoparticle on various types of seed germination as follow.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contribution

All authors have read and agreed to the published version of the manuscript and contribute during the work.

Acknowledgements

The author convey their thanks to the Head of the Department of the Biotechnology Loni, India for providing laboratory facility and equipment. Also thanks to Savitribai Phule Pune University, Pune, India and Pravara Medical Trust Loni, India for providing the UV-spectroscopy, SEM, XRD and FTIR facility and microbial pathogen respectively. Also thanks to the A. M Bhosale sir and Nikhil Ghate for supporting me how to write and publish the paper.

Statements and Declarations

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

REFERENCES

1. Bansal, P., Kaur, P., Surekha, Kumar A., & Duhan, J. (2017). Microwave assisted quick synthesis method of silver nanoparticles using citrus hybrid "Kinnow" and its potential against early blight of tomato. *Res. on Crops*, 18 (4), 650-655.
2. Bhaskar B., Ahammed K. S., Chaitanya B. H., Rasheed V. A. & Prasad T.N.V.K.V, (2016). silver Nanoparticles: Mycogenesis, characterization and its anti-plant pathogenic application *Impact: International Journal of Research In Applied, Natural*, 4(10), 105-114.
3. Błaszczyk L., Siwulski M., Sobieralski K., Lisiecka J. and Jedryczka M. (2014). *Trichoderma* spp.-application and prospect for use in organic farming and industry. *Journal of plant protection research*, 54 (4), 309-317.
4. Chitra K. & Annuadurai G. (2013). Bioengineered silver Nanobowles using *Trichodema viride* and its antibacterial activity against gram-positive and gram –negative bacteria. *Journal of Nanostructure in Chemistry*, 3(9), 1-7.
5. Devi P., Kulanthival S., Kamil D., Borah J., Prabhakaran N. and Srinivasa N. (2013). Biosynthesis of silver nanopartcle from *Trichoderma* spacies. *International Journal of Experimantal Biology*, (51), 543-547.
6. El-Sheekh. M., Deyab. M., Hasan. R., Ahmed. S. & Elsadany. A. (2022) Biological control of Fusarium tomato-wilt disease by cyanobacteria Nostoc spp. *Archives of microbiology*, 204 (116), 2-14.
7. Elad Y., Chet I., & J. ketan, (1979). *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Disease control and pest management*, 70 (2), 191-121.
8. EL-Tanany, M., Hafez, M., Ahmed, G., & El-Mageed, M. (2018). Efficiency of biotic and abiotic inducers for controlling tomato early blight disease. *Middle East Journal of Agriculture Research*, 7(2), 650-670.
9. Elgorban E. M., Al-Rahmah A. N., Sayed S. R., Hirada A., Mostafa A. F. & Bahkali A. H., (2016). Antimicrobial activity and green synthesis of silver Nanoparticles using *Trichoderma viride*. *Biotechnology & biotechnological equipmentI*, 30 (2), 299-304.
10. Fayaz M., Balaji K., Girilal M., Kalaichelvan P., Venkatesan R. (2009). Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vegetable and fruit preservation. *Agricultural and Food Chemistry*, 57(14), 6246-6252.

11. Fayaz M., Tiwary C., Kalaichelvan P. & Venkatesan R. (2010). Blue and orange light emission from biogenic synthesized silver nanoparticles using *Trichodrma viride*. *Colloids and Surface B: Biointerfaces*, (75), 175-178.
12. Gherbawy Y., Shalaby I., Sadak Abd M., Elhariry H. & Banaja A. (2013). The anti-fasciolasis properties of silver nanopartcle produced by *Trichoderma herzianum* and their improvement of the anti- Fasciolasis drug Triclabendazole, *International Journal of Molecular Science*, (14), 21887-21898.
13. Grondona I., Hermosa R., Tejada M., Gomis G.M., Mateos P.F., Bridge P.D., Monte E. & Garcia-acha I. (1997). Physiological and Biochemical Characterization of *Trichoderma harzianum*, a Biological Control Agent against Soilborne Fungal Plant Pathogens. *Applied And Environmental Microbiology*, 63(8), 3189–3198.
14. Kumari, M., Shukla, S., Pandey, S., Giri, V.P., Tripathi, T., Bhatia, A., Kakkar, P., Nautiyal, C.S., Mishra, A., (2017). Enhanced cellular internalization: a bactericidal mechanism more relative to biogenic nanoparticles than chemical counterparts. *ACS Appl. Mat. Interfaces* 9, (4519–4533).
15. Kaur P., Thakur R. & Choudhary A. (2012). An In Vitro Study of The Antifungal Activity of silver/Chitosan Nanoformulations Against Important Seed Borne Pathogens. *International Journal of scientific & technology research*, 1(6), 83-86.
16. Khabat V., Mansoori G., and Karimi S., (2011). Biosynthesis of silver nanoparticles by Fungus *Trichoderma Reesei*, *Inscience Journal*, 1(1), 65-79.
17. Mahajan, P., Dhoke, S., & Khanna, A. (2011). Effect of Nano-ZnO Particle Suspension on Growth of Mung (Vigna radiata) and Gram (Cicer arietinum) Seedlings Using Plant Agar Method. *Journal of Nanotechnology*, (1), 1-7.
18. Mishra A., Kumari M., Pandey S., Chaudhy V., Gupta K. C., Nautiyal C. S. (2014). Biocatalytic and antimicrobial activities of gold Nanoparticles synthesized by *Trichoderma* sp. *Bioresour Technol*, (166) 235-242.
19. Mohammed. A., Kadar. A., Kihal, M., Henni. J., Sanchez, J., Gallego, E., & Garrido-Cardenas. J. (2016). Characterization of Fusarium oxysporum isolates from tomato plants in Algeria. *African Journal of Microbiology Research*. 10(30), 1156-1163.
20. Motlagh M. R. & Samimi Z. (2013). Evaluation of *Trichoderma* spp., as biological agents in some of plant pathogens, (Scholars Research Library). *Annals of Biological Research*, 4 (3), 173-179.

21. Panacek A., Kvıtek L., Prucek R., Kolar M., Vecerova R., Pizurova N., K. Sharma., Nevecna T. & Zboril R. (2006). Silver Colloid Nanoparticles: Synthesis, Characterization, and Their Antibacterial +Activity. *Journal of Phys.Chem*, (110), 16248-16253.
22. Saba H., Vibhash D., Manisha M., Prashant K.S., Farhan H., Tauseef A. (2012). *Trichoderma* – a promising plant growth stimulator and biocontrol agent. *Mycospher*, 3(4), 524–531.
23. Saravanakumar K. & Wang, M. (2018). *Trichoderma* based synthesis of anti-pathogenic silver nanoparticles and their characterization, antioxidant and cytotoxicity properties. *Microbial Pathogenesis,* (114), 269-273.
24. Sanker. P., Shanmugapackiam. S. and Ramyabharatis. (2020). Fusarium wilt of Tomato (Fusarium Lycopersicum L.,). *Lambeart Academic publishing*, 1-150.
25. Seyed H. & Hamidreza H. (2015). Effect of Nano Silver on Seed Germination and Seedling Growth in Fenugreek Seed. *International Journal of Food Engineering*, 1(2), 106-110.
26. Shelar G. and Chavan A. (2015). Myco-synthesis of silver nanoparticles from *Trichoderma harzianum* and its impact on germination status of oil seed. *Biolife*, 3(1), 109-113.
27. Zhang, K., Yaofeng, W., Mao, J. & Chen, B. (2019). Effects of biochar nanoparticles on seed germination and seedling growth. *Environmental pollution*, (256), 1-11.
28. Zakharova, O., Gusev, A., Zherebin, P., Skripnikova, E., Skripnikova, M., Ryzhikh, V., Lisichkin, G., Shapoval, O., Bukovskii, M., & Krutyakov, Y. (2017). Sodium Tallow Amphopolycarboxyglycinate-Stabilized Silver Nanoparticles Suppress Early and Late Blight of *Solanum lycopersicum* and Stimulate the Growth of Tomato Plants. *BioNanoscience*, 7(2), 692-702.