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In vitro evaluation of biosynthesized silver nanoparticles (Ag NPs) against *Fusarium oxysporum* f. Sp. *Capsici* causing wilt of chilli

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Abstract
Efficacy of biosynthesized silver nanoparticle at different concentration (250 ppm, 500 ppm and 750 ppm) was tested against *Fusarium oxysporum* f. sp. *capsici* causing wilt of chilli by poisoned food technique and the result showed that the *Trichoderma harzianum* silver nanoparticles @ 750 ppm was found most effective with least mycelial growth (20.23 mm) and numerically highest mycelial inhibition (77.52%), followed by *T. harzianum* silver nanoparticles @ 500 ppm (35.73 mm and 60.30%), *T. harzianum* silver nanoparticles @ 250 (46.30 mm and 48.55%), *T. hamatum* silver nanoparticles @ 750 ppm (46.96 mm and 47.82%), *T. hamatum* silver nanoparticles @ 500 ppm (58.16 mm and 35.37%) and *Trichoderma asperellum* silver nanoparticles @ 750 ppm (61.66 mm and 31.48%), respectively over control.

Keywords: Biosynthesized silver nanoparticles, *Fusarium oxysporum* f. Sp. *Capsici* causing wilt, chilli

Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop grown in almost all parts of tropical and subtropical regions of the world. It belongs to the family Solanaceae and originated from South and Central America where it was domesticated around 7000 BC. Nanotechnology ("nanotech") is the manipulation of matter in an atomic, molecular and supramolecular scale. The earliest, widespread description of nanotechnology referred to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macroscale products, also now referred to as molecular nanotechnology. A more generalized description of nanotechnology was subsequently established by the National Nanotechnology Initiative, which defines nanotechnology as the manipulation of matter with at least one dimension sized from 1 to 100 nanometres. Nanotechnology is an emerging field in the area of interdisciplinary research especially in biology. The advancement of nanotechnology mainly requires the development of reliable and eco-friendly protocols for the synthesis of nanomaterial over a range of biological composition, sizes, shapes and high monodispersity. Species of *Trichoderma* were used as biological control agents against soilborne plant pathogenic fungi (Kucuk and Kivank, 2003) [5]. Mycoparasitism and excretion of lytic enzymes. The antifungal enzyme system of *Trichoderma* species played an important role for detection and destroying the host cell wall (Schirmbock *et al.*, 1994) [7].

Source of Silver Nanoparticles
Biologically synthesized silver nanoparticles from *Trichoderma asperellum*, *T. harzianum* and *T. hamatum* was collected from Department of Plant Pathology, College of Agriculture, Latur for *in vitro* evaluation against soil borne pathogens. A characteristic surface plasmon resonance band was observed at 380 nm for *Trichoderma asperellum*, at 420 nm for *T. harzianum* and at 380 nm for *T. hamatum* after 24 hrs. of incubation. Maximum intensity of synthesized silver nanoparticles wasobserved with six days incubated culture filtrate of *Trichoderma asperellum*, *T. harzianum* and *T. hamatum* treated AgNO3 solution followed by four days incubated culture filtrate treated AgNO3 solution.Similar to the present study, UV absorption peak of silver nanoparticles synthesized from *T. viride* and *T. koningii* (Tripathi *et al*., 2013), *T. harzianum* (Shelar and Chavan, 2015) [8], *T. reesei* (Khabat *et al*, 2011) [4] was observed at 400 nm, 413 nm, 440 nm and 420 nm, respectively. Transmission Electron Microscopy studies showed the synthesized silver nanoparticles were spherical in shape with

the average size of *Trichoderma asperellum* silver nanoparticles i.e. 07.09 to 12.18 nm, *T. harzianum* silver nanoparticles as 08.45 to 15.03 nm and *T. hamatum* silver nanoparticles as 22.93 to 35.66 nm, respectively.Results of the present study on Transmission Electron Microscopy (TEM) analysis are in consonance with those reported earlier by several workers on size 5-50 nm (Khabat *et al*., 2011) [4], 10-20 nm (Kaur *et al*., 2012) [3], 8-24 nm (Tripathi *et al*., 2013) [9] and 19-63 nm (Shelar and Chavan, 2015) [8], respectively.

Antifungal Activity of Silver Nanoparticles: The suspension of silver nanoparticles was used to study the antifungal activity against *Fusarium oxysporum* f. sp. *capsici* by poisoned food technique. Effect of silver nanoparticles were compared with the effect of *Trichoderma* culture filtrate. The per cent inhibitions were increased with increase of the concentrations. Results (Table 1) revealed that, *Trichoderma asperellum, T. harzianum* and *T. hamatum* culture filtrates treated with silver nitrate solution @ 250, 500 and 750 ppm concentrations and *Trichoderma asperellum, T. harzianum* and *T. hamatum* culture filtrates @ 250 ppm evaluated *in vitro* by poisoned food technique and exhibited fungistatic/ antifungal activity against *Fusarium oxysporum* f. sp. *capsici* and numerically inhibited its growth, over untreated control (PLATE I).

However, *Trichoderma harzianum* silver nanoparticles @750 ppm was found most effective with least mycelial growth (20.23 mm) and numerically highest mycelial inhibition (77.52%), followed by *T. harzianum* silver nanoparticles @ 500ppm (35.73 mm and 60.30%), *T. harzianum* silver nanoparticles @ 250 (46.30 mm and 48.55%), *T. hamatum* silver nanoparticles @ 750 ppm (46.96 mm and 47.82%), *T. hamatum* silver nanoparticles @ 500 ppm (58.16 mm and 35.37%) and *Trichoderma asperellum* silver nanoparticles @ 750 ppm (61.66 mm and 31.48%), respectively.(Table 1 and Plate I).

Results from table 1 also revealed that, all concentration of *T. harzianum* i.e. 250, 500and 750 ppm were effective against *Fusarium oxysporum* f. sp. *capsici* infecting wilt in chilli than other treatments. *Trichoderma asperellum*, *T. harzianum* and *T. hamatum* silver nanoparticles and culture filtrates at different concentrations were reported as efficient against many *Fusarium* spp. including *F. oxysporum* by earlier worker, Kaman and Dutta (2017) [2].

Javad and Naser (2017) [1]. Observed the efficacy of silver nanoparticles (AgNPs) against the *Fusarium oxysporium* by poisoned food technique and showed, highest colony inhibition at 5000ppm, followed by 2500ppm and 1000ppm, respectively.

Table 1: *In vitro* evaluation of antimicrobial activity of silver nanoparticles of *Trichoderma asperellum*, *T. harzianum* and *T. hamatum* and their culture filtrate against *Fusarium oxysporum* f. sp. *capsici* by using poisoned food technique

Tr. No.	Treatments at different conc.	Col. Dia. *(mm)	% Inhibition*
T1	*Trichoderma asperellum* silver nanoparticles @ 250 ppm	80.20	10.88 (19.25)
T2	*T. harzianum* silver nanoparticles @ 250 ppm	46.30	48.55 (44.16)
T3	*T. hamatum* silver nanoparticles @ 250 ppm	76.80	14.66 (22.51)
T4	*T. asperellum* silver nanoparticles @ 500 ppm	76.40	15.11 (22.87)
T5	*T. harzianum* silver nanoparticles @ 500 ppm	35.73	60.30 (50.94)
T6	*T. hamatum* silver nanoparticles @ 500 ppm	58.16	35.37 (36.49)
T7	*T. asperellum* silver nanoparticles @ 750 ppm	61.66	31.48 (34.12)
T8	*T. harzianum* silver nanoparticles @ 750 ppm	20.23	77.52 (61.69)
T9	*T. hamatum* silver nanoparticles @ 750 ppm	46.96	47.82 (43.75)
T10	*T. asperellum* culture filtrate @ 250 ppm	85.16	05.37 (13.39)
T11	*T. harzianum* culture filtrate@ 250 ppm	82.83	07.96 (16.38)
T12	*T. hamatum* culture filtrate@ 250 ppm	85.30	05.22 (13.20)
T13	Control (Untreated)	90.00	0.00 (0.00)
	S.E. ±	0.44	
	C.D. at 1%	1.79	

*: Mean of three replications, Dia.: Diameter, Conc.: Concentration, Figures in parentheses are arc sin transformed values

The results were confirmed with the similar findings of Lamsal *et al.* (2011) [6]. In poisoned food method, AgNPs exhibited highest inhibition against *Colletotrichum* species at concentrations 100, 50, 30, and 10 ppm with 90, 84.56, 84.50 and 11.33% inhibition, respectively.

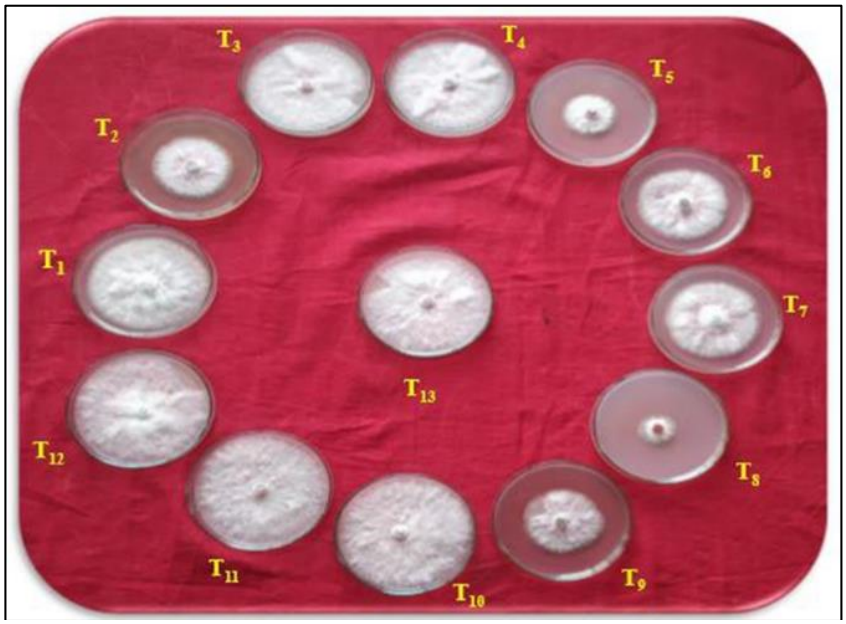

Plate I: *In vitro* evaluation of antifungal activity of silver nanoparticles of Trichoderma asperellum, *T. harzianum* and *T. hamatum* and their culture filtrate against *Fusarium oxysporum* f. sp. *capsici* by using poisoned food technique

Conclusion

The present study showed that myconanoparticle synthesized from *Trichoderma harzianum* silver nanoparticles found highly effective at a concentration of 750 ppm for suppression of mycelia growth of the *Fusarium oxysporum* f. sp. *capsici* causing wilt of chilli.

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