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Full Length Article

Phyto-Synthesized Silver Nanoparticles using Leaves Extracts of *Morus alba* and *Aegle marmelos* Inhibited Fusarium Wilt and Charcoal Rot in Tomato

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Abstract

Antifungal metal structures, using silver at nanoscale, were synthesized using leaves extract of two indigenous trees *i.e.*, *Morus alba* (white mulberry) and *Aegle marmelos* (bael fruit). The antifungal characteristics of synthesized silver nanoparticles (AgNPs) was verified against *Fusarium oxysporum*, the causal agent of Fusarium wilt and *Macrophomina phaseolina*, the pathogen of charcoal rot, taking tomato as a model plant. Synthesized nanoparticles were checked against selected fungi in concentrations of 25, 50, 75 and 100 μg/mL. Negative control received no nanoparticles but sterilized water, whereas positive control plants were treated with chemical fungicide (Nativo). Result of *in vitro* and greenhouse experiments confirmed the potential of synthesized nanoparticles to reduce fungal growth and disease incidence. However, the NPs synthesized using leaves extract of white mulberry were more effective. In green house trials, the plants treated with NPs in a concentration of 100 μg/mL showed parallel results as were recorded in plants receiving fungicide. Similarly, both selected fungi also depicted slight variation in their responses towards various treatments as percentage of growth inhibition as well as disease incidence was more in *F. oxysporum* than in *M. phaseolina.* UV-spectrophotometer ascertained the optimization conditions by adjusting concentrations of silver nitrate between 1, 1.5, 2, 2.5 and 3 m*M*, incubation period of 24, 48, 72 and 96 h and pH of biosynthesis system adjusted at 4, 6, 8, 10 and 12. FTIR confirmed the presence of different functional groups available both in plant extracts and nanoparticles while, SEM indicated spherical, polydisperse morphology of AgNPs with size ranges from 20–48 nm respectively. In pot experiment nanoparticles synthesized using white mulberry leaves reduced fusarium infection up to 97% and macrophomina rot up to 92%. Nanoparticles synthesized using bael leaves extract were comparatively less effective against selected fungi than those synthesized using white mulberry leaves extract. Hence these results provided basis for the use of green synthesized NPs using white mulberry leaves extract as an alternative to conventional fungicides to help reduce environmental pollution.

Keywords: Silver nanoparticles; Green synthesis; Fusarium wilt; Charcoal rot; Tomato

Introduction

Soil borne pathogens affect crop plants in different ways, resulting in heavy yield losses. The diseases caused by these microbes are often very difficult to manage because of their heterogeneous incidence. Mostly soil borne pathogens can survive in soil for long periods even in the absence of their host (Veena *et al.* 2014). These pathogens have a wide host range and chemicals often do not work well on these culprits (Veena *et al.* 2014). With over 120 strains, *F. oxysporum* Schlecht. emend. Snyder & Hansen is one of the most commonly occurring fungal pathogens in soil responsible for wilt in a range of agronomic and horticultural crops (Michielse and Rep 2009). *Macrophomina phaseolina* (Tassi) Goid. is another common soil borne fungi that is known to infect 500 plants belonging to 100 different plant families (Srivastava and Singh 1990). Both these pathogens are known to reduce yield in different crops between 30–80% (Mayek-Perez *et al.* 2003; Arturo and Karla 2017). Use of fungicides is a general method to control these pathogens. However, development of resistance in pathogen due to multiple applications of these fungicides; increasing awareness regarding effect of fungicidal residues on our ecosystem and human beings has lead researchers to focus on finding safe alternatives to reduce yield losses and to increase food security (Zubrod *et al.* 2019).

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Nanotechnology refers to the science of nanoscale objects that has been listed in European Commission's six "Key Enabling Technologies" recognised for their role in sustainable competitiveness and growth in various fields of industrial applications (EC 2012). Beside its known applications in the fields of medicine, environmental sciences, bio-engineering; cosmetics and other industries, nanotechnology possesses a range of potential applications for agriculture stor too. This has led to intense research on the use of nanotechnology in solving agricultural problems both at academic and industrial levels (Chen and Yada 2011; Dasgupta *et al.* 2015; Parisi *et al.* 2015). Special emphasis has been given to the development of nano-products for disease management and nano-fertilizers for improving soil fertility (Tolaymat *et al.* 2017). The uniqueness lies in the physical and chemical properties of NPs that includes their nano size, shape, increased surface area and catalytic reactivity that has opened new paradigms for agricultural sector.

Usually nanoparticles are synthesized through reduction reactions using different reducing agents as tollens reagent, ascorbate, elemental hydrogen, sodium citrate etc. to reduce silver ions (Ag^+) in the reaction mixture. For stabilization of synthesized NPs different polymeric compounds as poly vinyl alcohol is used (Merga *et al.* 2007; Erdogan 2020). This chemical approach is considered as an eco-unfriendly and expansive method for nanoparticles' synthesis. Green synthesis using biological systems as plants and microbes provides a sustainable alternative method to synthesize nanoparticles with minimized generated waste. The processes are reproducible, cost effective and simple. Plant extracts provides both stabilizing and reducing agents for the nanoparticles' synthesis.

Several workers have tried different phyto-extracts to synthesise NPs possessing antimicrobial properties. Phenolic acids like caffeine present in *Camellia sinensis* were utilized for the production and stabilization of AgNPs (Vilchis-Nestor *et al.* 2008). Leaves extract of *Camellia sinensis* (black tea) also provided flavonoids and polyphenols for the formation of AgNPs (Begum *et al.* 2009). Huang *et al.* (2011) used *Cacumen platyclade* extract as a source of reducing sugars and flavonoids for reducing silver ions. The nanoparticles produced revealed substantial antibacterial activity against *S. aureus* and *E. coli.* Similarly, in a recent study Aritonang *et al.* (2019) used *Impatiens balsamina* and *Lantana camara* as a source for bioreducing agents and synthesized silver nanoparticles that were found active mutually against gram positive and gram-negative bacteria.

The present study explored the potential of two indigenous trees *i.e.*, *A. marmelos* L Correa (bael fruit); and *M. alba* L. (white mulberry) for synthesizing AgNPs to manage soil borne pathogens in our cropping system. Bael fruit belongs to the family Rutaceae and is native to the sub-continent. The plant is a rich source of furocoumarins, flavonoids and alkaloids that are well known for medicinal properties. White mulberry, a member of family Moraceae is native to northern China and India. The plant is helpful in treatment of digestive problems like cough, hepatitis and dyspepsia (Babu and Ammani 2009).

Chemically synthesized metallic NPs are used to control soil borne diseases but these have some drawbacks; therefore, search of bioactive green synthesized NPs is direly needed. In this context the present study was designed to investigate the potential of indigenous plant extracts including bael fruit and white mulberry in synthesizing antifungal AgNPs to manage soil borne diseases in tomato crop.

Materials and Methods

Preparation of phyto-extracts

For green synthesis of AgNPs, leaves extract of bael fruit (*A. marmelos* L. Correa); and white mulberry (*M. alba* L.) was prepared. Diseased free, middle aged leaves of selected plants were collected, washed and dried under shade. Powdered dried leaves were soaked in water at the rate of 100 g in 200 mL sterilized deionized water. The mixture was heated in a water bath covered with a plastic bag at 90°C for 10 min followed by its cooling and filtration using Whatman filter paper no. 1. The extract obtained was kept at 4°C until further use.

Synthesis of silver nanoparticles

In a 100 mL conical flask, each phyto-extract (5 mL) was supplemented with 45 mL of 2.5 m*M* $AgNO_3$. Sodium chloride (1 molar) was used to adjust pH of this solution to 8. After this the mixture was incubated in dark to prevent photo-activation of $AgNO_3$. The solution changed its colour from colourless to brown because of the silver reduction.

Optimized biosynthesis parameters for silver nanoparticles

Synthesis of AgNPs ($AgNO_3$) was optimized at various conditions including: (i) concentration of silver nitrate *i.e.*, 1, 1.5, 2, 2.5 and 3 m*M*; (ii) incubation time *i.e.*, 24, 48, 72 and 96 h and (iii) pH *i.e.*, 4, 6, 8, 10 and 12. These conditions were optimized to get maximum synthesis of NPs as this can reduce time in collecting maximum possible quantities of NPs to be used in bioassays and characterization. The synthesis of AgNPs at various conditions was confirmed by UV-VIS absorption spectra.

AgNPs synthesized using white mulberry plants showed maximum absorption peak at 2.5 m*M* of silver nitrate after 48 h of incubation and at pH 8. Whereas, in case of bael fruit maximum absorption was recorded at 2 m*M* of silver nitrate after 72 h of incubation at pH 10.

Sample preparation for AgNPs characterization

The synthesized nanoparticles' solution was centrifuged at 6000 rpm for 30 min at 4°C. The supernatant was discarded

and pellet was transferred to a china dish using deionized water. The material was dried by placing these china dishes in a hot air oven at 40°C for fortnight. The parched surface was scratched and stored for further characterization.

Characterization of synthesized AgNPs

The synthesis of AgNPs was determined through UV-VIS spectrophotometer (DeNovix DS-11). The absorption spectrum was taken between 200–800 nm. Fourier Transform Infrared (FTIR) spectra were acquired using Nicolet 800 spectrophotometer in concurrence with MTech PAS cell. The spectra were recorded between at 4–6 cm^{-1} with a resolution average of 128 scans. Magnesium perchlorate was used as the drying agent, whereas, in PAS cell Helium gas was used. SEM micrographs were obtained using scanning electron microscope with 25 kV accelerating voltage. Thin films of the samples were prepared using carbon coated grid. For this the prepared samples were dropped on the gird in a very small amount followed by removal of the extra solution using blotting paper. Prepared thin films were dried by placing them under mercury lamp for 5–6 min.

***In vitro* antifungal assay**

Antifungal activity of synthesized nanoparticles was investigated against two soil borne phytopathogenic fungi *i.e*., *M. phaseolina* (Tassi) Goid. and *F. oxysporum*i (Sacc.) W.C. Snyder & H.N. Synthesized nanoparticles were added in Malt Extract Agar (MEA) media in different concentrations of 25, 50, 75, 100 μg/mL. All treatments were replicated thrice where control received no NPs. The selected fungi were inoculated in the centre of each media plate followed by their incubation at 25 ±1°C for seven days. The antifungal activity was recorded in terms of fungal growth inhibition in treatments in comparison to the control. The assay was performed in completely randomized design.

***In vivo* antifungal assay**

The effectivity of synthesized NPs was checked through a greenhouse trial. Earthen pots of ~25 cm diameter were filled with sandy loam soil containing 0.69% organic matter; 6.3 ppm available phosphorus; 100 ppm available potassium and pH 7.8. The soil was treated with methylene bromide for sterilization and leaving for 4 days to eliminate residues of methylene bromide. Conidial suspensions of *F. oxysporum* and *M. phaseolina* adjusted at final concentration of 10^6 spores/ mL were prepared in distilled water and added at the rate of 30 mL per pot. Roots of 20-days old tomato (*Solanum lycopersicum* variety Rio Grande) plants were dipped in a concentration range of synthesized AgNPs *i.e*., 25, 50, 75 and 100 μg/mL for 2 h. Roots of the negative control plants was dipped in sterilized water and those of positive control were dipped in fungicide (Nativo) for same time period. Four plants in each pot were transferred. Each treatment was replicated thrice. Plants were fertilized after every two weeks with a 20:20:20 NPK soluble fertilizer (1 g/L) and the pots were irrigated with tap water when required. Hand weeding was done to remove any appearing weeds in pots. The plants were regularly checked for disease symptoms. The trial was conducted in a completely randomized design with three replications. After 6 weeks the plants were harvested and data for their root and shoot lengths; fresh and dry weights and disease incidence was taken. Disease incidence was calculated by diving number of infected plants by total number of plants and multiplying the resultant by 100 (Vincent 1947).

Data analysis

Collected data were statistically analysed using analysis of variance technique by statistical analysis software (SAS) and Microsoft Excel program. In case of significance, treatments means were separated using LSD (Least Significant Difference) test at $P \leq 0.01$.

Results

Confirmation and characterization of silver nanoparticles

A clear change in colour of the mixture (plant extract + $AgNO_3$) was recorded in both plant extracts. Also, appearance of a clear peak after 400 nm in both cases *i.e*., at 413 nm for the extract of white mulberry (Fig. 1A) and at 410 nm for bael fruit (Fig. 1B) also confirmed presence of AgNPs in both cases.

***In vitro* antifungal bioassay**

Nanoparticles synthesized using plant extracts were checked for their potential to inhibit two soil borne fungi in invitro conditions. Silver NPs synthesized using both plant extracts significantly reduced growth of the tested fungi (Fig. 2). However, NPs prepared from the extract of white mulberry leaves extract showed better inhibition in comparison to the NPs prepared using extract of bael fruit (Fig. 2). The percentage of inhibition increased with increase in concentration of NPs used. Among the two selected soil borne fungi, *F. oxysporum* showed more inhibition percentages when compared to the *M. phaseolina*. The highest tested concentration of NPs *i.e*., 100 μg/mL, synthesised from leaves extract of white mulberry decreased growth of *F. oxysporum* by 84% and of *M. phaseolina* by 77%. Whereas, particles synthesized from leaves extract of bael fruit reduced mycelial growth up to 68% in *F. oxysporum* and 63% in *M. phaseolina* hence showed 14–16% lower antifungal potential than leaves extract of white mulberry (Fig. 2).

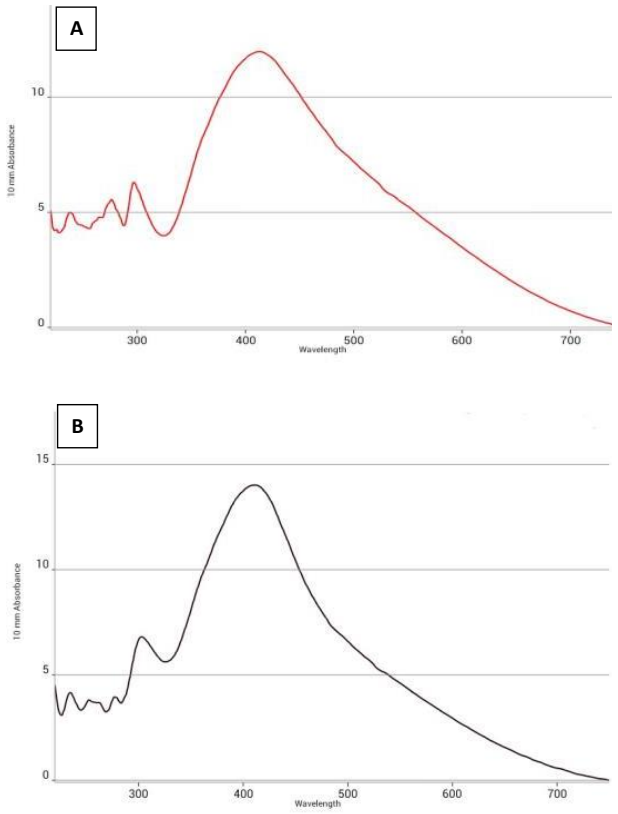

Fig. 1: UV-Vis graphs of AgNPs synthesized from leaves extract of **A**: *M. alba*; **B**: *A. marmelos* after incubation time of 48 hours, using 2.5 m*M* of $AgNO_3$ and at pH 8

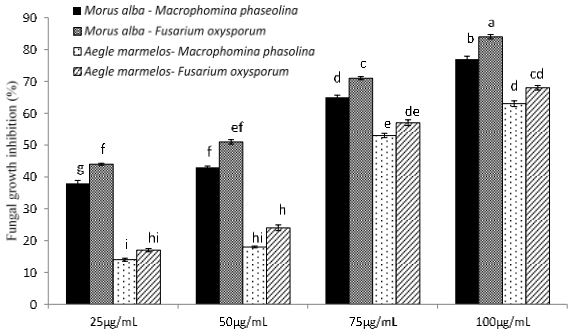

Fig. 2: *In vitro* effect of AgNPs synthesized using *Morus alba* and *Aegle marmelos* leaves extract in concentrations of 25, 50, 75, 100 µg/mL against *F. oxysporum* and *M. phaseolina*. Vertical bars show standard error of means of three replicates. Means not sharing the same letters within a column differ significantly from each other at $P \leq 0.01$

In vivo antifungal bioassay

Results of greenhouse experiment also followed similar trend as was observed in *in vitro* assays (Fig. 3). Nanoparticles synthesized from white mulberry leaves extract decreased disease incidence more significantly than those synthesized using bael leaves extract. Highest disease reduction was

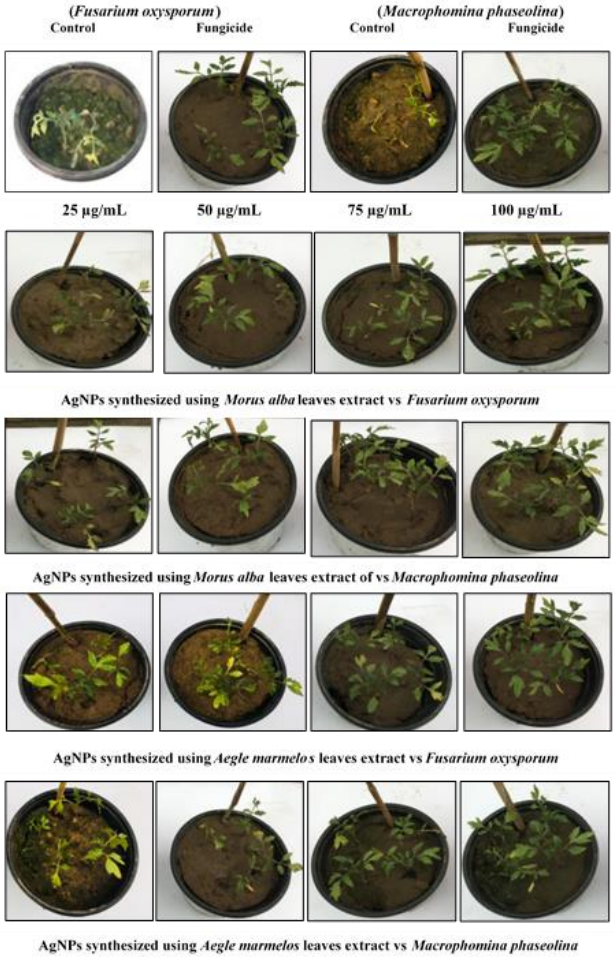

Fig. 3: *In vivo* effect of various treatments on disease development in tomato pots

recorded at 100 μg/mL of NPs which was equivalent to the disease reduction of fungicide used. Nanoparticles extracted from white mulberry leaves decreased incidence of Fusarium wilt up to 96.99% when used in 100 μg/mL. Nanoparticles synthesized using bael leaves extract reduced wilt by Fusarium up to 87.53% that is 9.46% lower than the AgNPs synthesized by using white mulberry leaf extract. Both of these treatments reduced infection by *M. phaseolina* by 92.39 and 81.58% respectively (Table 1). Reduction in disease incidence was found correlated with plant height and biomass confirming the positive effect of various treatments on crop health and physiology.

Characterization of AgNPs synthesized using leaves extract

FTIR analysis of leaves and NPs synthesized from *M. alba*: The possible biomolecules/phytochemicals present in white mulberry dried leaves and AgNPs biosynthesized using its leaves extract were identified through FTIR analysis (Fig. 4A and B). In case of dried leaves, the bands

Table 1: *In vivo* effect of phyto-synthesized silver nanoparticles on disease development in tomato plants

Treatments	Disease incidence (%)				Disease reduction (%)			
	M. alba AgNPs		*A. mamelos*AgNPs		*M. alba* AgNPs		*A. mamelos* AgNPs	
	F. oxysporum	*M. phaseolina*	*F. oxysporum*	*M. phaseolina*	*F. oxysporum*	*M. phaseolina*	*F. oxysporum*	*M. phaseolina*
Control	100a	100a	100a	100a	-	-	-	-
Fungicide	5.33e	7.58d	5.33e	7.58e	94.76a	92.42a	94.76a	92.42a
25 μg/mL	94.15b	97.06a	98.04a	99.15a	5.85d	2.94d	1.96e	0.85e
50 μg/mL	73.28c	80.18b	81.59b	86.63b	26.72c	19.82c	18.41d	13.37d
75 μg/mL	43.81d	52.83c	55.58c	63.51c	56.19b	47.17b	44.42c	36.49c
100 μg/mL	3.01e	7.61d	12.47d	18.42d	96.99a	92.39a	87.53b	81.58b
LSD value at $P \leq 0.01$	2.53	11.19	2.62	1.70	3.26	2.36	3.54	1.80
	Shoot length (cm)				Root length (cm)			
Control	-	-	-	-	-	-	-	-
Fungicide	38.62a	35.85b	38.62a	35.85a	14.15a	12.27a	14.15a	12.27a
25 μg/mL	2.35d	1.21e	1.65d	0.38c	1.02c	0.98d	0.94c	0.52c
50 μg/mL	11.53c	13.82d	13.42c	12.82b	3.64c	2.71c	2.62c	2.01c
75 μg/mL	20.53b	17.41c	18.47b	13.49b	8.74b	6.72b	7.63b	5.61b
100 μg/mL	41.01a	39.91a	40.18a	33.41a	14.65a	12.46a	13.38a	10.47a
LSD value at $P \leq 0.01$	2.78	1.82	3.06	2.61	2.89	1.51	2.39	2.07
	Fresh weight (g plant^{-1})				Dry weight (g plant^{-1})			
Control	-	-	-	-	-	-	-	-
Fungicide	24.91a	20.83a	24.91a	20.83a	4.09b	3.94a	4.09a	3.94a
25 μg/mL	2.18d	1.02c	1.13d	0.98c	0.68d	0.53b	0.55b	0.43b
50 μg/mL	5.68c	3.74c	3.01d	2.15c	1.13d	1.03b	1.10b	0.89b
75 μg/mL	11.58b	9.68b	8.16c	7.77b	2.69c	1.47b	1.54b	1.03b
100 μg/mL	25.74a	21.19a	22.45b	18.94a	5.63a	4.08a	4.65a	3.11a
LSD value at $P \leq 0.01$	2.31	3.19	2.32	2.74	1.01	1.52	1.12	1.18

Means not sharing the same letters within a column differ significantly from each other at $P \leq 0.01$

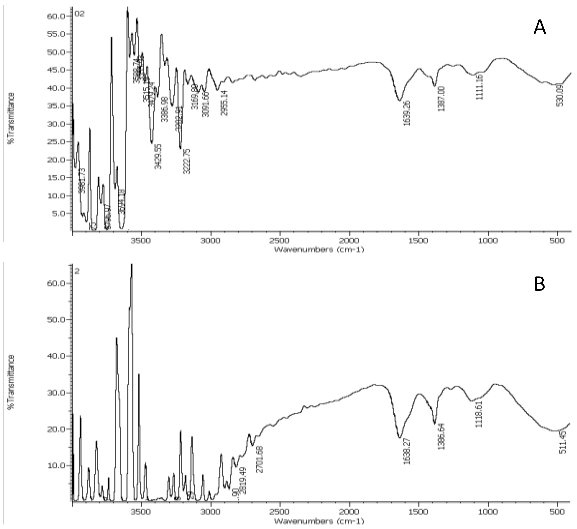

Fig. 4: FTIR spectra **A**: *M. alba* dried leaves; **B**: AgNPs synthesized using leaves extract of *M. alba*

recorded at 2819.49 and 3091.66 cm^{-1} are representing stretching vibrations of alkenes and alkyls (C-H). Whereas, the bands recorded at 1639.26 and 1638.27 cm^{-1} are showing C-N stretching (Fig. 4). Comparatively weak bands appearing between 511.45 and 530.09 cm^{-1} in FTIR spectra of dried leaves and AgNPs synthesized from leaves extract of white mulberry respectively showed the presence of halogen compounds. Bands at 1387 and 1386.64 cm^{-1} are indicating C-H bond with alkane's functional group. As can be seen in the Fig. 4A and B the peaks recorded at 1118.61 and 1111.16 cm^{-1} are representing the C-O stretching.

FTIR analysis of leaves and NPs synthesized from *A. marmelos*: FTIR spectra were acquired to trace potential biomolecules liable for effective stabilization and capping of inorganic (metal) nanoparticles synthesized by leaf extract of bael. The IR bands (Fig. 5A and B) recorded at 3196.78 and 344.51 cm^{-1} confirmed the presence of alkenes C=C and alcohol (-OH). The band appearing at 2346.58 and 2344.11 cm^{-1} is assigning to C-O. The transmission bands at 1638.22 and 1630.89 cm^{-1} are corresponding to alkenes in aromatic compounds and amides (N-H). Whereas, the peak recorded at 1385.89 cm^{-1} shows stretching of iso-propyl group. The bands appearing at 1109.52 and 1065.83 cm^{-1} are confirming the existence of polysaccharides (Fig. 5A).

Merely few minor changes in the position of transmittance band between FTIR spectrum of AgNPs and phyto-extract were observed. Compounds present in phyto-extract and participating in biosynthesis of AgNPs got confirmed with shifting of peaks. Reduction and stabilization of AgNPs were affected by plant extracted compounds comprising CO and OH groups that play a dynamic part in AgNPs' synthesis.

SEM Analysis of synthesized AgNPs

SEM image of the green synthesized AgNPs using leaves extract of white mulberry clearly indicates poly disperse spherical morphology. The average diameter of AgNPs ranged between 20–40 nm. However, in case of AgNPs synthesized using bael leaves are predominately spherical. The bio synthesized AgNPs are of comparatively larger size ranging up to 25–48 nm (Fig. 6).

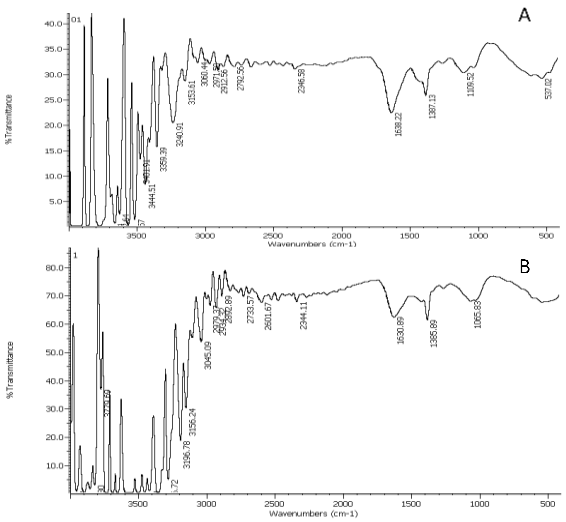

Fig. 5: FTIR spectra **A**: *A. marmelos* dried leaves; **B**: AgNPs synthesized using leaves extract of *A. marmelos*

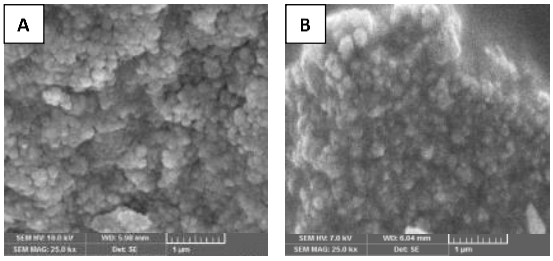

Fig. 6: Scanning electron microscope analysis of AgNPs synthesized from **A**: *M. alba* and **B**: *A. marmelos*

Discussion

This study confirms the potential of phyto-synthesized silver nanoparticles using bael and white mulberry leaf extracts in controlling soil borne fungal diseases in crops like tomato. Selection of white mulberry was carried out due to its excellent antimicrobial activities reported by many earlier workers (Ayoola *et al.* 2011; Zheng *et al.* 2013). It's extract has also shown significant inhibition of *Fusarium oxysporum* (Sharma and Trivedi 2002). Choice of bael fruit was also made on similar grounds as many earlier workers have validated antimicrobial potential in its different parts against both fungi and bacteria (Dhankhar *et al.* 2011; Rahman and Parvin 2014).

UV-Vis spectrophotometer was used to first confirm synthesis and presence of nanoparticles in the reaction mixture. Earlier literature showed that absorption between 400 – 450 nm is usually characteristic of silver NPs in the UV-Vis region (Sathishkumar *et al.* 2009; Ashraf *et al.* 2020). Clear peaks recorded at 413 and 410 nm in case of reaction mixture containing extracts of white mulberry and bael leaves thus followed the trend. Optimization trials showed maximum synthesis in reaction mixture possessing white mulberry a little earlier than in the reaction mixture supplemented with bael leaves extract. Optimum concentration of silver nitrate and pH were also found different for both plant extracts. The results were found consistent with the reports of Singh *et al.* (2009). Variation in parameters of NPs synthesis may be due to the variation in active biomolecules of both plants involved in reduction of silver ions into AgNPs. The mechanism and the cause of the conversion of Ag^+ into Ag nanoparticles are not very well understood. However, it is strongly anticipated that various functional groups existing in plant extracts might be the cause of this bio reduction of Ag^+ into AgNPs (Biswal and Misra 2020). Raja *et al.* (2012) and Rathi *et al.* (2015) documented the role of biomolecules such as C-O and -OH in this kind of reduction during NPs synthesis. The presence of such related biomolecules was confirmed through FTIR. The size of AgNPs synthesized by using leaves extracts of white mulberry and bael fruit was recorded between 20–48 nm respectively with spherical morphology. Earlier findings also confirm variation in particle size with change in the source of nanoparticles' synthesis (Geethalakshmi and Sarada 2010).

Synthesized NPS showed significant antifungal potential against both selected soil borne phytopathogens with little variation. AgNPs synthesized using white mulberry leaves extract showed better results than that of synthesized using bael leaves. Size of synthesized NPs could be a reason for this variation, as small sized NPs are more efficient due to their easy uptake by the plants and their translocation in whole living system (Wang *et al.* 2013; Lv *et al.* 2018). Further reason of this variation might be the difference in attached biologically active components on the surface of synthesized NPs from the biological source. Hence selection of biological source for synthesis of NPs affects their antifungal potential. Both tested fungi also showed slight variation in their responses towards various treatments.

A reason for selecting nanoparticles to control soil borne pathogens in this work was the interesting mode of action of these nano sized particles. The efficacy of nano sized particles in comparison to their bulk materials is always higher due to the fact that the number of surface atoms rises with decrease in the particle size that in return increases the reactivity and hence several physical and chemical properties (Maurice and Hochella 2008; Hochella *et al.* 2008). The mechanism of Ag^+ ions to inhibit microbial growth is not well understood; however according to some scientists the negative charge present in the cell membrane of microbes interact with the positive charge of the Ag^+ ions in NPs (Dragieva *et al.* 1999; Stoimenov *et al.* 2002; Dibrov *et al.* 2002; Rawashdeh and Haik 2009). In a study conducted in 2004, Sondi and Salopek-Sondi (2004) established that the antimicrobial potential of AgNPs is dependent on their concentration and they also found this directly related to the pits formation in the cell walls of gram negative bacteria. Similarly, Kim *et al.* (2009) also validated the effect of nano Ag^+ on cell

membranes of microbes, thus hampering their function and leading to cell death. Feng *et al.* (2000) reported that treatment of cells with Ag^+ results in loss of DNA ability to replicate, hence inactivation of expression of ribosomal subunit proteins, along with certain other cellular proteins and enzymes important for ATP production (Yamanaka *et al.* 2005). It is also known that plants treated with silver nanoparticles accumulate silver in form of highly stable nanoparticles that do not release ionic silver within the plant cells. Hence AgNPs are least toxic than its ionic form (Pak *et al.* 2017). Keeping all this information, the present study is providing a basis for the use of bio-synthesized NPs in disease management in fields. This will decrease reliance on synthetic pesticides providing a better eco-friendly approach.

Conclusion

Results of this study confirmed the potential of bael and white mulberry leaves extracts to synthesize antifungal, uniform and stable metallic nanoparticles that can significantly lower disease incidence of soil borne fungi in crops like tomato. Use of such phyto-synthesized silver nanoparticles can lower down the application of synthetic chemical fungicides in our agricultural soils.

Author Contributions

TA planned the whole work and provided lab facilities. NH and HA performed experimental work and WA helped in write up and statistical analysis.

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