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Production and characterization of Green Iron Oxide nanoparticles with antifungal properties against fungal pathogens

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ABSTRACT

In this study, iron oxide nanoparticles were synthesized through the green chemistry method. It involved the use of plant extract for synthesis of metal oxide nanomaterials. Aqueous extract of *Tridax procumbens* was employed for the production of iron oxide nanoparticles. *T. procumbens* is a weed and it has good medicinal properties. The green chemistry approach provides a simple, cost-effective and eco-innovative method to the synthesis of nanomaterials as compared to the other alternative methods. Phase, crystal, surface chemistry, size, shape and element composition of nanoparticles were assessed and determined by various techniques like Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy dispersion X-Ray (EDX). In addition, the antifungal properties of the biosynthesized nanoparticles were investigated against plant fungal pathogens. Spherical-shaped nanoparticles with a size of 26 $\pm$ 5 nm were obtained using the extract of *T. procumbens.* Bio-molecules from *T. procumbens* served as reducing, capping and stabilizing agents for the fabrication of nanoparticles. The highest zone of inhibition was obtained in *S. rolfsii*(16.5 $\pm$ 0.5)at 50 μg/ml of concentration of TFeONPs. Biosynthesized TFeONPs were capable of inhibiting the growth of fungal pathogens such as *S. rolfsii* and *F. oxysporum* and they might be employed as antifungal agents in agriculture to control fungal infections.

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INTRODUCTION

Nanotechnology is a growing innovative technology for manipulation, creation and application of nanomaterials. It has the combination of new ideas and knowledge from physics, material science, chemistry and biological sciences, has been applied in various research areas such as biomedical products, catalysis, energy, photocatalysis, electrochemical products, optics etc. (Song and Kim, 2009; Rajasekharreddy and Rani, 2014). Nanomaterials have been produced by different types of traditional methods such as physical and chemical science. Not with standing the fact that these production methods are innovative, they have some drawbacks like high usage of hazardous chemicals and high electric power in addition to controlling the formation of aggregation and crystal growth is very difficult in these methods (Izadiyan *et al.*, 2018). Green approach for synthesis of metal oxide and metallic composite is an inexpensive, non-toxic and environment-friendly technique (Vanathi *et al.*, 2014; Mukhopadhyay *et al.*, 2018). Several plants and their extracts have been employed for the production of metal oxide nanomaterials. Secondary metabolites from plant extracts play an important role during the formation of nanomaterials by serving as capping, reducing and stabilizing agents (Rajiv *et al.*, 2013; Prasad, 2014).

In the recent years, iron oxide nanoparticles have attracted the attention of researchers because of their interesting properties such as high stability, catalytic activity, magnetic and electrochemical power, crystal properties, photo-catalysis, thermal conductivity etc. Jagathesan and Rajiv (2018).

Several plants namely *Artemisia vulgaris* (Wang *et al.*, 2014), *Eichhornia crassipes* (Jagathesan and Rajiv, 2018), *Syzygium cumini* (Venkateswarlu *et al.*, 2014), *Jatropha gosspifolia* (Karkuzhali, 2015), *Lagenaria siceraria* (Kanagasubbulakshmi and Kadirvelu, 2017), *Couroupita guianensis* (Sathishkumar *et al.*, 2018), *Ficus carica* (Demirezen, 2019), *Albizia lebbeck* (Bharadwaj *et al.*, 2016), *Phyllanthus niruri* (Kumar and Prem, 2018), *Lantana camara* (Rajiv *et al.*, 2017), *Platanus orientalis* (Devi *et al.*, 2019) have been employed for the green synthesis of iron oxide nanoparticles. Devi *et al.* (2019) reported the production and characterization of iron oxide nanoparticles by green chemistry approach and concluded that iron oxide nanoparticles show excellent antifungal properties against fungal pathogens (*Aspergillus niger* and *Mucor piriformis*).

Sclerotinum rolfsii and *Fusarium oxysporum* are major plant pathogens for oil seed crops. Groundnut (*Arachis hypogaea*),a major oil crop, is mostly infected by *S. rolfsii* that causes diseases like sclerotial wilt, rot in root, pod and stem (Chohan, 1974; Mehan *et al.*, 1995; TH *et al.*, 2016). *F. oxysporum* is a plant fungal pathogen whose habitat is soil andis known tocause Fusarium wilt in *A. hypogaea* and other oil seed crops (Rajeswari, 2014).

The aquatic weed (*E. crassipes*) has been utilized in the synthesis of iron oxide nanoparticles and also to assess the antibacterial activity against waterborne pathogens (Jagathesan and Rajiv, 2018). *Tridax procumbens* is a weed that belongs to family Asteraceae and commonly termed as Common button and Coat button. It has anti-inflammatory, anti-microbial, hepatoprotective, insecticidal and wound-healing activity, (Vastrad and Goudar, 2016) due to the presences of flavonoids, alkaloids and tannins (Bhati-Kushwaha, 2014). In the present study, the aim is to explore the synthesis and characterization of iron oxide nanoparticles with its antifungal potent using the extract of weed (*T. procumbens*) serving capping, reducing and stabilizing agent at suitable ambience.

Figure 1: Images of *T. procumbens*

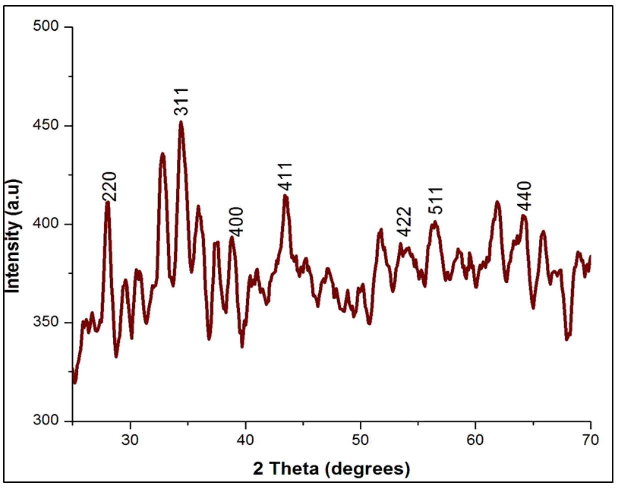

Figure 2: XRD pattern of TFeONPs

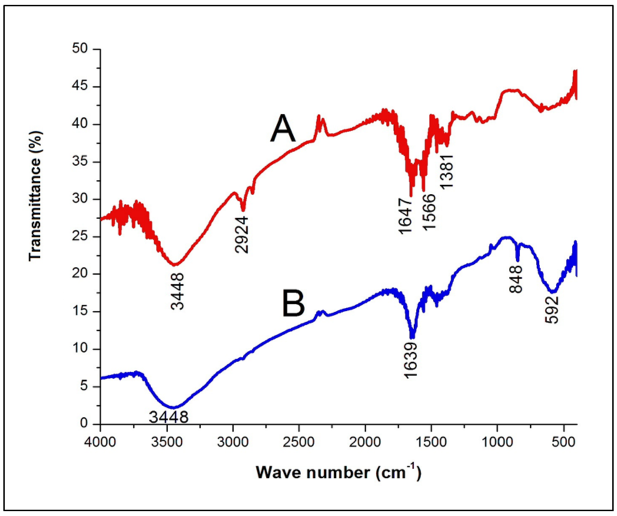

Figure 3: FT-IR spectrum of A) Plant Extract (aqueous) & B) TFeONPs

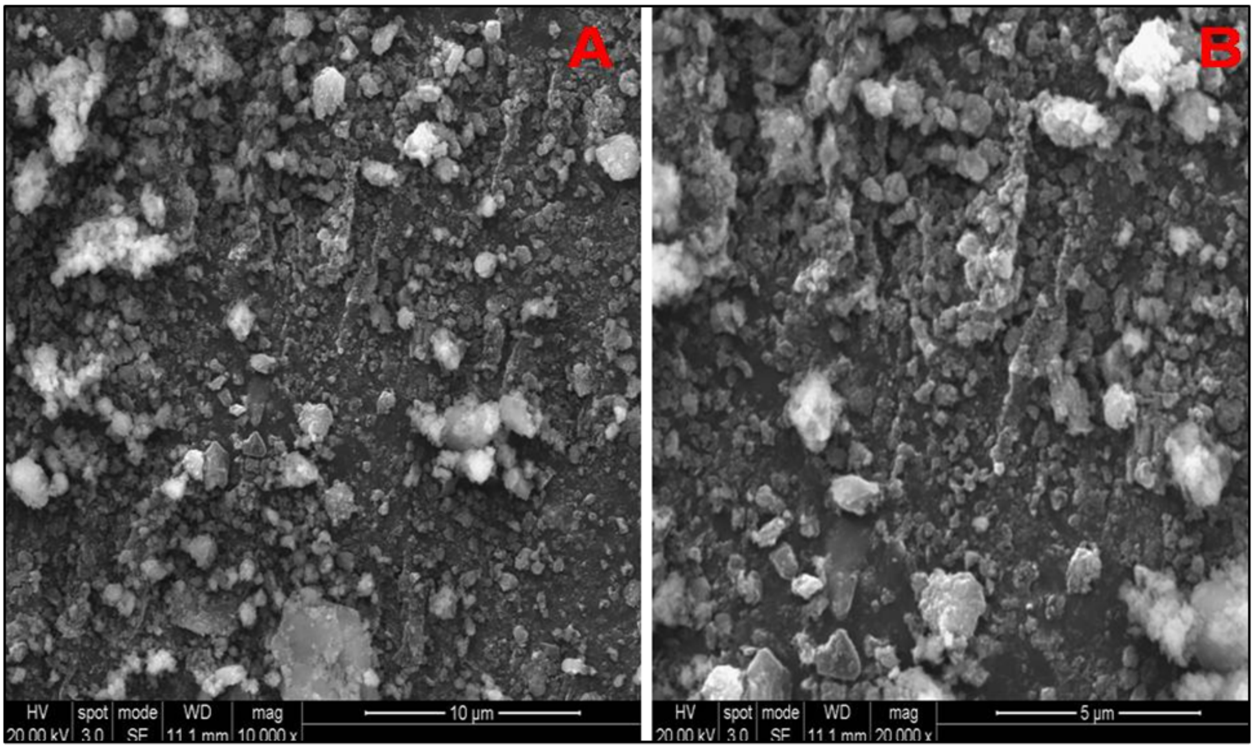

Figure 4: SEM images of TFeONPs

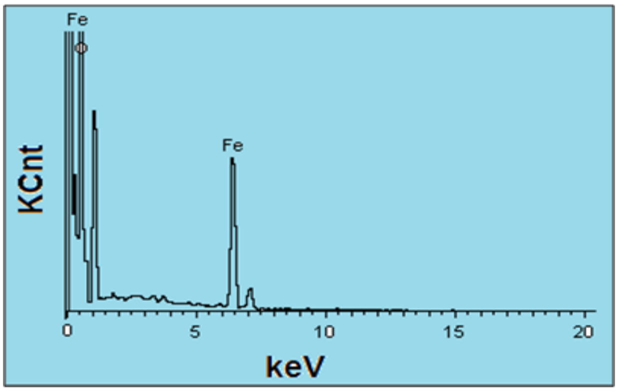

Figure 5: Elemental analysis of TFeONPs

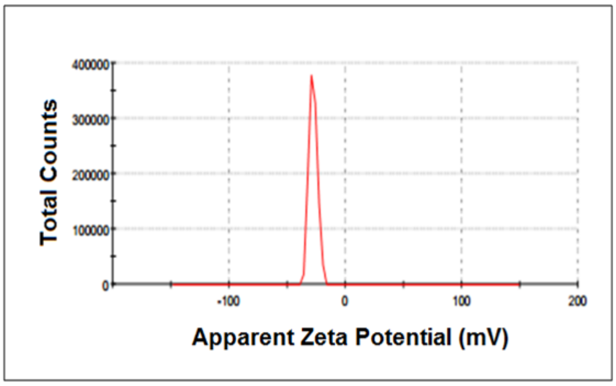

Figure 6: Zeta potential analysis of TFeONPs

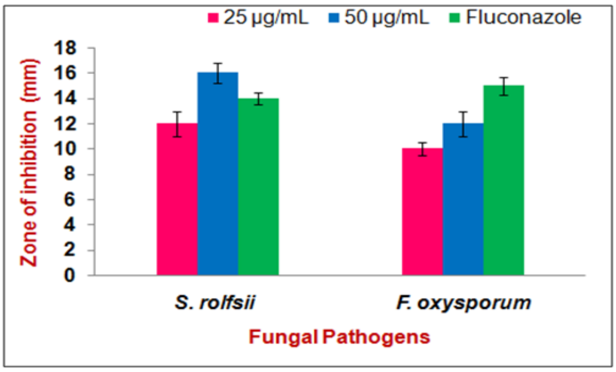

Figure 7: Antifungal activity of TFeONPs

MATERIALS AND METHODS

T. procumbens was acquired from the lands in and around Karpagam Academy of Higher Education, Coimbatore, India (Figure 1). It was then authenticated by Botanical Survey of India, Coimbatore. 99% pure reagents, chemicals and solvents were obtained from sigma-aldrich chemicals, India.

Plant pathogens namely, *S. rolfsii* and *F. oxysporum* were collected from the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore, India.

Production and characterization of TFeONPs

Ten grams of fresh and healthy whole plant (*T. procumbens*) was taken and washed in tap water, and was further rinsed with de-ionized water. Plant

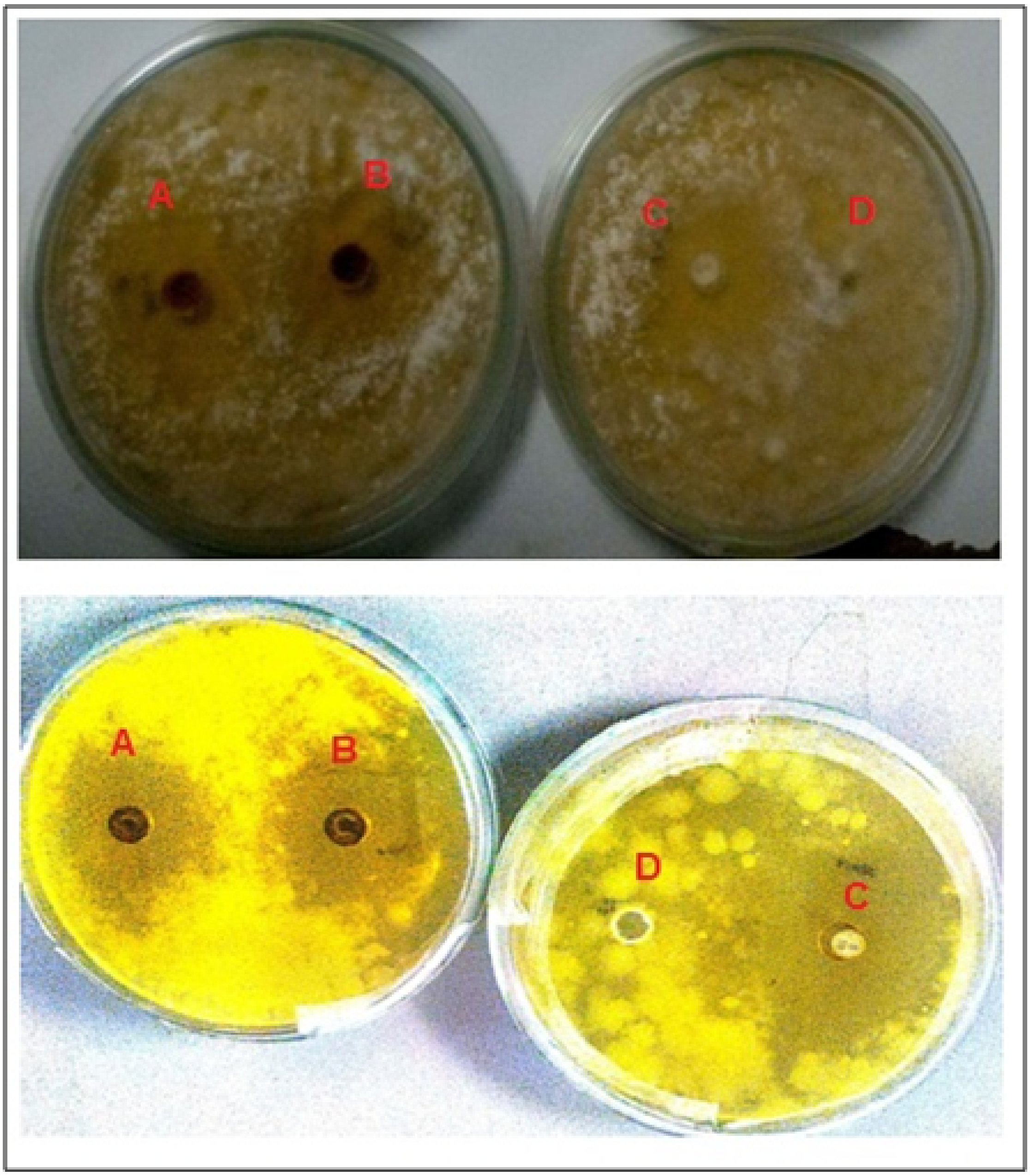

Figure 8: Antifungal activity of TFeONPs for fungal pathogens of oil seed crops. (A: 20 μg/mL of TFeONPs, B: 50 μg/mL of TFeONPs, C: Positive control–Fluconazole, D: Negative control- Plant extract)

samples were mashed into fine powder with the help of mortar and pestle using de-ionized water. 100 mL of crude extract was kept in water bath under 80°C for 30 min. Next, the crude extract was filtered, and the filtrate was kept at 4°C in store for use in the production of NPs.

$FeCl_3$ was used as precursor materials for synthesis of TFeONPs. A 1:1 (v/v) of 0.1 M of $FeCl_3$ and 50 mL of plant extract was taken in a sterile conical flask and kept in water bath for 30 min at 80°C. Then, 20 mL of 0.2 M NaOH was mixed to the reaction mixture under the stirring condition of 60°C for 30 min. Finally, the end product (black colour) precipitate was obtained.

The pellets ware collected with the help of centrifugation method and washed with 80% ethanol followed by de-ionized water. The precipitate was then dried in hot air oven after which the fine powder was collected and stored in clean bottles for further analysis (Rajiv *et al.*, 2017; Jagathesan and Rajiv, 2018).

The properties of synthesized TFeONPs such as crystalline nature, surface chemistry, size, shape, purity, elemental compositions and stability were analysized by XRD, FT-IR, SEM, EDX and Zeta potential analyzer.

Assessment of antifungal properties

Antifungal properties of the synthesized TFeONPs were assessed using soil-borne fungal pathogens following well diffusion method (Bauer *et al.*, 1966). The pathogenic fungi were grown in potato dextrose broth. Potato Dextrose Agar (PDA) was prepared and 200 μL of broth culture was spread on it. After 10 min, wells of 5 mm were made on agar and Nano-solution of TFeONPs (25 and 50 μg/mL) was added.

Fluconazole was used as a positive control and a concentration of 10 μg/mL was employed. Plant extract was used as negative control. After the incubation of three days at 30°C, the zone of inhibition was measured, and the results are presented as mean with standard error.

RESULTS AND DISCUSSION

Analysis of crystalline nature

TFeONPs were synthesized using *T. Procumbens* extract and were proved by XRD analysis (Figure 2). The lattice planes of (220), (311) and (411) were obtained at 2θ: 30.47°, 35.75° and 43.51° respectively. The spectra were compared to JCPDS file (01-088-0315), which clearly revealed the formation of spinel single-phase with a cubic structure. The characteristic peaks show that the produced TFeONPs were crystalline in nature. The mean size of particles was deliberate by Debye-Scherer's formula. Mean size of TFeONPs was found to be about 26 nm. Several earlier reports showed similar XRD pattern for green synthesized nanoparticles (Yew *et al.*, 2016; Jagathesan and Rajiv, 2018).

Analysis of surface chemistry

FT-IR spectra of the as-synthesized nanoparticles and plant extract are given in Figure 3 A & B. It confirms that the functional groups are present on the surface of nanoparticles and are involved in the formation of nanoparticles. The IR peaks observed at 3448, 1647, 1639, 1566 and 1381 $cm^{-1}$are referring to the N-H bending, C=O stretching of amine and amide groups respectively. The peaks of 592 and 848 cm^{-1}refer to metal oxide group. This confirmed that the bioactive compounds from plant extracts were actively involved in the formation of TFeONPs. The FT-IR results are comparable to those determined in the previous investigations (Kanagasubbulakshmi and Kadirvelu, 2017; Kumar and Prem, 2018).

Morphology analysis

The surface morphology of TFeONPs is shown in Figure 4. SEM images showed mono-dispersed distribution with spherical shape with less aggregation. The aggregation may be due to a large surface area and a tendency to stick. Our result is in agreement with Karkuzhali (2015), where they produced spherical-shaped nanoparticles using Jatropha plants which was confirmed by SEM analysis.

Elemental analysis

Figure 5 determines the presence of various elements like iron and oxygen in as-synthesized nanoparticles. The EDX spectra determined the occurrence of iron (46.18%) and oxide (27.11%) and 26.71% of other elements were measured from the EDX analysis. Badni *et al.* (2016) performed the production of iron oxide nanoparticles using Roman nettle, and investigated the analysis of elemental compositions by EDX analysis, where they concluded that nanoparticles have 61.2% of iron, 17.2% of oxygen and 21.6% of other elements.

Zeta potential analysis

Dynamic light-scattering has been utilized to find out the surface zeta potential of the as-synthesized nanoparticles. It is used to determine the stability and surface charge of the nanomaterials. According to Figure 6, the zeta potential for TFeONPs was -27.9 mV. In addition, the TFeONPs was negatively charged on their surface. The values of zeta potential between -20 to -30 mV indicated that particles were moderately stable (Ardani *et al.*, 2017). Hence, the result clearly reveals that as-biosynthesized TFeONPs were moderately stable in nature.

Analysis of antifungal properties

Figure 7 and Figure 8 represent the antifungal activity of as-synthesized TFeONPs. Plant extracts did not show any antifungal activity against the used plant fungal pathogens. The highest zone was obtained in *S. rolfsii*(16.5 $\pm$ 0.5 mm) at 50 μg/mL concentration of TFeONPs, which was compared to standard antibiotic. *F. oxysporum* shows less zone of inhibition against the TFeONPs. The inhibition of fungal growth occurred due the destruction of cell membranes inducing the reactive oxygen, which caused cell death. This analysis proves the antifungal properties of TFeONPs.

CONCLUSION

Green synthesis of nanoparticles using plants is considered a biological, non-hazardous and eco-friendly method. The present investigation, which is a first attempt was used to determine a green chemistry technique for yield of iron oxide nanoparticles with antifungal properties by means of weed plant. The synthesized TFeONPs were spherical-shaped with size of 26 $\pm$ 5 nm. TFeONPs have an extremely potent antifungal properties, which inhibit the growth of plant fungal pathogens and can be utilized for the management of *F. oxysporum* and *S.*

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REFERENCES

Ardani, H. K., Imawan, C., Handayani, W., Djuhana, D., Harmoko, A., Fauzia, V. 2017. Enhancement of the stability of silver nanoparticles synthesized using aqueous extract of Diospyros discolor Willd. leaves using polyvinyl alcohol. *IOP Conference Series: Materials Science and Engineering*, 188:012056–012056.

Badni, N., Benheraoua, F. Z., Tadjer, B., Boudjemaa, A., Hameur, H. E., Bachari, K. 2016. Green synthesis of α-Fe2O3 nanoparticles using Roman nettle. In *Proceedings of the Third International Conference on Energy*, pages 30–31.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, 45(4_ts):493–496.

Bharadwaj, M. S., Prem, K., Satyanandam, K., C, E. 2016. Green synthesis of iron nanoparticles using Albizialebbeck leaves for synthetic dyes decolorization. *International Journal of Science, Engineering and Technology Research (IJSETR)*, 5:3429–3434.

Bhati-Kushwaha, H. 2014. Biosynthesis of silver nanoparticles using fresh extracts of Tridaxprocumbenslinn. *Indian Journal of Experimental Biology*, 52(4):359–368.

Chohan, J. S. 1974. Recent advances in diseases of groundnut in India. In SP, R., JP, V., editors, *Current Trends in Plant Pathology*, pages 171–184. Lucknow University Press.

Devi, H. S., Boda, M. A., Shah, M. A., Parveen, S., Wani, A. H. 2019. Green synthesis of iron oxide nanoparticles using Platanus orientalis leaf extract for antifungal activity. *Green Processing and Synthesis*, 8(1):38–45.

Izadiyan, Z., Shameli, K., Hara, H., Taib, S. H. M. 2018. Cytotoxicity assay of biosynthesis gold nanoparticles mediated by walnut (Juglans regia) green husk extract. *Journal of Molecular Structure*, 1151:97–105.

Jagathesan, G., Rajiv, P. 2018. Biosynthesis and characterization of iron oxide nanoparticles using Eichhornia crassipes leaf extract and assessing their antibacterial activity. *Biocatalysis and Agricultural Biotechnology*, 13:90–94.

Kanagasubbulakshmi, S., Kadirvelu, K. 2017. Green synthesis of Iron oxide nanoparticles using Lagenaria siceraria and evaluation of its Antimicrobial activity. *Defence Life Science Journal*, 2(4):422–422.

Karkuzhali, A. Y. 2015. Biosynthesis Of Iron Oxide Nanoparticles Using Aquous Extract Of JatrophaGosspifolia As Source Of Reducing Agent. *International Journal of NanoScience and Nanotechnology*, 6(1):47–55.

Kumar, V. G. V., Prem, A. A. 2018. Green Synthesis and Characterization of Iron Oxide Nanoparticles Using Phyllanthus Niruri Extract. *Oriental Journal of Chemistry*, 34(5):2583–2589.

Mehan, V. K., Mayee, C. D., McDonald, D., Ramakrishna, N., Jayanthi, S. 1995. Resistance in groundnut toSclerotium rolfsii-causedstem and pod rot†. *International Journal of Pest Management*, 41(2):79–83.

Mukhopadhyay, R., Kazi, J., Debnath, M. C. 2018. Synthesis and characterization of copper nanoparticles stabilized with Quisqualis indica extract: Evaluation of its cytotoxicity and apoptosis in B16F10 melanoma cells. *Biomedicine & Pharmacotherapy*, 97:1373–1385.

Prasad, R. 2014. Synthesis of Silver Nanoparticles in Photosynthetic Plants. *Journal of Nanoparticles*, pages 1–8.

Rajasekharreddy, P., Rani, P. U. 2014. Biofabrication of Ag nanoparticles using Sterculia foetida L. seed extract and their toxic potential against mosquito vectors and HeLa cancer cells. *Materials Science and Engineering: C*, 39:203–212.

Rajeswari, P. 2014. Management of Wilt of Arachis hypogea (Groundnut) Caused by Fusariumoxysporum with Trichoderma spp. and Pseudomonas fluorescens. *Journal of Biological Control*, 28(3):151–159.

Rajiv, P., Bavadharani, B., Kumar, M. N., Vanathi, P. 2017. Synthesis and characterization of biogenic iron oxide nanoparticles using green chemistry approach and evaluating their biological activities. *Biocatalysis and Agricultural Biotechnology*, 12:45–49.

Rajiv, P., Rajeshwari, S., Venckatesh, R. 2013. Bio-Fabrication of zinc oxide nanoparticles using leaf extract of Parthenium hysterophorus L. and its size-dependent antifungal activity against plant fungal pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 112:384–387.

Sathishkumar, G., Logeshwaran, V., Sarathbabu, S., Jha, P. K., Jeyaraj, M., Rajkuberan, C., Senthilkumar, N., Sivaramakrishnan, S. 2018. Green synthesis of magnetic Fe3O4 nanoparticles using Couroupita guianensis Aubl. fruit extract for their antibacterial and cytotoxicity activities. *Artificial Cells, Nanomedicine, and Biotechnology*, 46(3):589–598.

Song, J. Y., Kim, B. S. 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and Biosystems Engineering*, 32(1):79–84.

TH, B., Nath, K., DA, C. 2016. Effect of Age on Susceptibility of Groundnut Plants to Sclerotium rolfsii Sacc. Caused Stem Rot Disease. *Journal of Plant Pathology & Microbiology*, 7(12).

Vanathi, P., Rajiv, P., Narendhran, S., Rajeshwari, S., Rahman, P. K., Venckatesh, R. 2014. Biosynthesis and characterization of phyto mediated zinc oxide nanoparticles: A green chemistry approach. *Materials Letters*, 134:13–15.

Vastrad, J. V., Goudar, G. 2016. Green Synthesis and Characterization of Silver Nanoparticles Using Leaf Extract of Tridax Procumbens. *Oriental Journal of Chemistry*, 32(3):1525–1530.

Venkateswarlu, S., Kumar, B. N., Prasad, C. H., Venkateswarlu, P., Jyothi, N. V. V. 2014. Bio-inspired green synthesis of Fe3O4 spherical magnetic nanoparticles using Syzygium cumini seed extract. *Physica B: Condensed Matter*, 449:67–71.

Wang, Z., Fang, C., Megharaj, M. 2014. Characterization of Iron–Polyphenol Nanoparticles Synthesized by Three Plant Extracts and Their Fenton Oxidation of Azo Dye. *ACS Sustainable Chemistry & Engineering*, 2(4):1022–1025.

Yew, Y. P., Shameli, K., Miyake, M., Kuwano, N., Khairudin, N. B. B. A., Mohamad, S. E. B., Lee, K. X. 2016. Green Synthesis of Magnetite (Fe3O4) Nanoparticles Using Seaweed (Kappaphycus alvarezii) Extract. *Nanoscale Research Letters*, 11(1).

