Research Article

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Green synthesis of silver nanoparticles from *cymbopogon citratus* and their efficacy against grey blight disease of tea

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Abstract

Tea cultivation in India faces significant challenges from biotic and abiotic stresses, necessitating advanced research for sustainable production. Due to acute labour shortages, shearing is commonly practiced in southern tea plantations, often leading to severe grey blight and die-back of shoots caused by *Pestalotiopsis theae*. This study examines the bioefficacy of silver nanoparticles (AgNPs) green-synthesized from *Cymbopogon citratus* against grey blight disease in tea. The green synthesis was achieved by treating aqueous extracts of *C. citratus* with silver nitrate, confirmed through visual and spectrophotometric analysis. The scanning electron microscopy and X-ray diffraction characterization revealed that the nanoparticles were spherical in shape with an average size of 19 nm. The GC-MS chromatographic spectra of *C. citratus* extract highlighted the presence of hydrazine, 1-(5-hexenyl)-1-methyl-, citral, and geraniol as notable compounds. The *in vitro* bioefficacy proved that the synthesized AgNPs at 2.0 mL/L manifested complete inhibition of the grey blight pathogen under the Poisoned food technique. Further field evaluation revealed a significant reduction in disease incidence of 62% in the plots treated with AgNPs at 500 mL/ha, which was on par with the standard schedule of Copper oxychloride at 420 g/ha (68%). Therefore, it is concluded that the application of *C. citratus*-mediated silver nanoparticles is an effective alternative offering a greener strategy, replacing chemical fungicides.

***Keywords*:** Nanoparticles, green chemicals, grey blight of tea, bioefficacy, disease control

Introduction

Tea is consumed and cultivated worldwide over 60 countries notably, in India, China, Japan, Sri Lanka. FAO, (2024), data reveals that the global tea market produced 6.7 million tonnes of tea in 2022 among which India accounted for an annual production of 1.37 million tonnes (Tea Board of India, 2023).The tea production market holds a significant importance in the cultural and socio-economic aspects of the respective countries (Pandey *et al*., 2021). During the manufacturing process, the harvested tea leaves were subjected to fermentation and drying. The health benefits of tea consumption are well known besides their unique flavours and aromas (Huda *et al*., 2024). Samanta, (2022) have reported the health benefits of tea catechins, polyphenols and flavonoids in the reduction of cancer and cardiovascular diseases, and even improvement of mental health.

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The tea cultivation practices are affected by several biotic and abiotic factors resulting in the reduction of yield and quality. Among them, grey blight disease is a serious threat throughout the year (Wang *et al*., 2021). This disease is caused by a fungal pathogen called, *Pestalotiopsis theae* developing a greyish-brown lesion in the affected tea leaves. This ultimately leads to a reduced photosynthesis and defoliation (Y. Chen *et al*., 2018; Premkumar *et al*., 2009). Joshi *et al*., (2009) calculated the severe yield loss of 17% in India favouring the warm and humid regions. Additionally, the grey blight disease is widespread among the major tea manufacturing countries including China, India, Japan, Kenya and Sri Lanka —–(Chen et al., 2017; Maharachchikumbura *et al*., 2013; Nozawa *et al*., 2022). Premkumar *et al*., (2009) stated that Companion, a combination of Carbendazim (12%) and Mancozeb (63%) is used for the management of grey blight disease. However, the use of chemical fungicide possesses environmental risk and also health concerns. Sen *et al.*, (2020) reported a recent issue encountered in tea export due to Maximum Residue Level (MRL) of agrochemicals. To overcome this, Yang & Zhang, (2019) demonstrated the potential control of the disease with botanicals through host-pathogen interactions. In addition to this, Kellogg *et al*., (2019) highlighted the advantages of botanicals as less or nontoxic and biodegradable. In our previous study, lemon grass was identified to control grey blight pathogen when compared to other tested 15 plants (Personal communications)

Cymbopogon citratus commonly called as lemon grass belongs to the *Poaceae* family with medicinal values (Ekpenyong *et al., 2014)*. This perennial plant is widely grown for essential oil extraction containing citral, geraniol and flavonoids. Tibenda *et al*., (2022) reported the anti-microbial, anti-oxidant and anti-inflammatory activities of these compounds. Rahhal *et al*., (2024) have emphasised the positive impact of lemon grass in human diabetes and oxidative stress management. Having properties such as antifungal and antimicrobial, lemon grass can be employed as a sustainable alternative to chemical fungicides in agriculture (Mukarram *et al*., 2021). *C. citrates* was examined for its antagonistic effect against *Curvularia lunata*, causing Leaf Spotdisease in Maize (Mourão *et al*., 2017). ––Terumi Itako *et al*.,(2013) confirmed that the *C. citratus* oil develops an induced resistance against *Alternaria solani* in tomato plants. Kamsu *et al*., (2019) demonstrated the suppression of mycelial development of *Colletotrichum musae*, *Fusarium incarnatum* and *F. verticillioides* in banana treated with *C. citratus*. –Maheriya *et al.*, (2023) proved the repellent activity of lemon grass with increased crop yield and reduced pest incidence. Similarly, —Moustafa *et al*., (2021) demonstrated the allelopathic effects supporting weed management as well as integrated pest management (IPM) strategies. All these demonstrations make *C. citratus* a valuable candidate for plant health and environmental sustainability in modern agriculture.

The green synthesis of nanoparticles (NPs) has been identified as one of the significant trend in modern-day science, attributing towards various advantages and its alignment with sustainable practices (Malik *et al*., 2023) Can, (2020) investigated the potential of polyphenols and proteins in plant materials to act as reducing agents replacing chemical reagents. The green synthesis of NPs is associated with several advantages such as pollution free process (Alsammarraie *et al*., 2018), non-toxicity (Devi *et al.*, 2019),eco-friendly and economic efficiency (Kataria & Garg, 2018). This green synthesis of silver nanoparticles (AgNPs) from *C. citratus* extracts makes a remarkable communion of biotechnology and sustainable agriculture

(Ajayi & Afolayan, 2017; Rakib-Uz-Zaman *et al*., 2022). In this context, this study concentrates on the green synthesis of AgNPs utilising *C. citratus* extract for the management of grey blight disease of tea. Subsequently, the use of plant-based synthesis of AgNPs is considered as an innovative strategy to minimize the adverse environmental impacts.

Materials and Methods

Isolation and identification of grey blight pathogen

The tea leaf samples infected with grey blight disease was collected from the F.No.1 (1961 area) of UPASI Tea Experimental Farm (N 10˚ 16' 10.1", E 76˚ 58' 2.6"). The leaf specimens were washed using tap water and subjected to surface sterilisation with 0.5% sodium hypochlorite. The leaves were then rinsed with deionised distilled water and cut into small pieces. The samples were placed in a potato dextrose agar (PDB) media aseptically and allowed for incubation at 28 ± 3°C for 7 days (Pallavi *et al*., 2012). The pathogenic culture was purified using the hyphal tip technique by transferring the emerged fungal hyphal tips to a fresh PDA plate. A well-grown slant of the pathogen was maintained at 4°C for further experiments (Gomaa *et al*., 2021).The pathogen was further confirmed through cultural and morphological characteristics as prescribed by Petch (1923).

Collection and preparation of plant extract of *C. citratus*

The plant specimen of *C. citratus* was collected from the Tea Experimental Farm of the UPASI Tea Research Foundation, Valparai. The leaf samples were rinsed thoroughly under tap water and shade dried. The dried samples were then finely chopped and 5g of the sample was added to 500 mL of distilled water and boiled for 15 minutes. The resulting solution was centrifuged for 15 minutes at 3000 rpm. The supernatant was filtered using Whatman filter paper No.1, obtaining in a clear dark greenish-yellow solution, which was stored at 4°C(Rakib-Uz-Zaman *et al*., 2022).

GC-MS analysis of *C. citratus* extract

For GC-MS analysis, methanol was added to the *C. citratus* extract at the ratio of 1:1 (v/v). The sample was analysed using Thermo Scientific Trace GC Ultra / ISQ Single Quadrupole MS with a TG5MS silica fused capillary column at TÜV SÜD South Asia Pvt. Ltd.The compounds were detected using a 70-eV electron ionisation system with helium supplied at a flow rate of 1 mL min^{-1}as carrier gas. The temperature of the sample injector was set at 280°C. The bioactive compounds were identified by correlating the spectra and retention time (RT) with National Institute of Standards and Technology (NIST) and WILLY libraries (Soundararajan *et al*., 2021). The classes of the identified compounds were determined using the web based tool known as Classy Fire (Djoumbou Feunang *et al*., 2016).

Green synthesis of *C. citratus* mediated silver nanoparticles

The green synthesis of AgNPs was performed by using silver nitrate ($AgNO_3$) from HiMedia as the precursor. 10% of the *C. citratus* extract was added drop wise to the 0.01 M of $AgNO_3$ solution under constant stirring using magnetic stirrer reaching a temperature of 80°C. The mixture was allowed for incubation at room temperature until a colour change indicating the formation of AgNPs (Tesfaye *et al.*, 2023).

UV–Visible spectrometric analysis

The primary detection of CSNPs was determined through visual observation of colour change. Further confirmation was carried out with analysing the spectra with UV-Visible spectrophotometer (Shimadzu UV-1780, Japan) within the range between 300 and 800 nm '(Ajayi & Afolayan, 2017; Win *et al*., 2020).

Scanning electron microscopic (SEM) analysis of CSNPs

For SEM analysis, nanoparticle suspensions were placed onto aluminium stubs and left to air dry. These air-dried particles were then sputter coated with gold under vacuum. The size of the synthesised CSNPs was examined using a SEM (SIGMA HV - Carl Zeiss, Germany) microscope, equipped with a Bruker Quantax 200 - Z10,an energy dispersive X-ray spectroscopy (EDS) detector (Marslin *et al*., 2015). The size and shape of the NPs were analysed visually using ImageJ v. 1.54g (Mazzoli & Favoni, 2012; Schneider *et al.*, 2012).

X-ray diffraction analysis (XRD) analysis of CSNPs

The confirmation of crystalline structure of synthesised CSNPs was analysed using an X-ray diffractometer (Shimadzu XRD-6000, Japan). The functioning parameters were set at 40 kV and 30 mA, with Cu Kα radiation source of λ = 1.5406 Å. Scanning was conducted over a 2θ range between 0˚ to 80˚, with 0.02˚ as step size and scan rate of 1˚ per minute ——(Anandalakshmi *et al*., 2016).

***In vitro* bio-efficacy of CSNPs against grey blight pathogen**

The synthesized CSNPs were tested against grey blight pathogenunder *in vitro*, employing two experimental methods following the poisoned food technique on PDA, where the radial mycelial growth was measured, and in PDB, where the fungal mat was weighe (Nepolean *et al*., 2014). A Completely Randomized Design (CRD) was followed with eight treatments replicated thrice. CSNPs were mixed with the respective media at the dosages ranging from 0.5, 1.0, 1.5, 2.0 and 5.0 mL/L. Copper oxychloride 50% WP (COC) at 6 g/L and Companion (Mancozeb 63% + Carbendazim 12% WP) at 0.2% were used as standards, while untreated controls were maintained with PDA and PDB alone. 5 mm of mycelial disc of 7-day old culture of *Pestalotiopsis* spp. was used in both the assays and incubated. After incubation in the PDA assay, the radial growth of mycelia was recorded. In the PDB assay, the mycelial fungal mat was weighed and compared with the untreated control, and the percentage of inhibition was calculated.

Field bioefficacy of CSNPs against grey blight disease in tea

A field study was conducted at UPASI Tea Experimental Farm, 1961 area (10° 16′ 8.8″ N, 76° 58′ 4.7″ E), at an elevation of 1150 MSL following Randomized block design (RBD). The experimental plot consisted of UPASI JAT – Assam seedlings spaced at 120 cm x 120 cm. A total of six treatments, including an untreated control with four replicates, were studied. The synthesized CSNPs were applied at dosages of 125 mL, 250 mL, 500 mL, and 1L/ha. The plots treated with COC at 420g/ha were taken as the standard control, while the plots without fungicide application were considered the untreated control. The sprays were conducted at 7-day intervals using a hand-operated knapsack sprayer (Aspee, India) with a water volume of 175 L/ha. The incidence of grey blight (GB) was assessed using a 1 sq. foot quadrate over the tea canopy, counting the infected and healthy intact leaves (IL), cut leaves (CL), and bare stalks (BS). The percent disease incidence (PDI) was determined using these counts with a modified formula, with assessments recorded pre-treatment and post-treatment every two consecutive sprays (Muraleedharan & Chen, 1997; Sanjay *et al.*, 2008)**.**

Statistical analysis

$$DI = \frac{IL + CL + BS}{3}$$

In vitro studies and field bioefficacy were conducted following CRD and RBD, respectively. ANOVA was conducted using R programming, and the mean values were

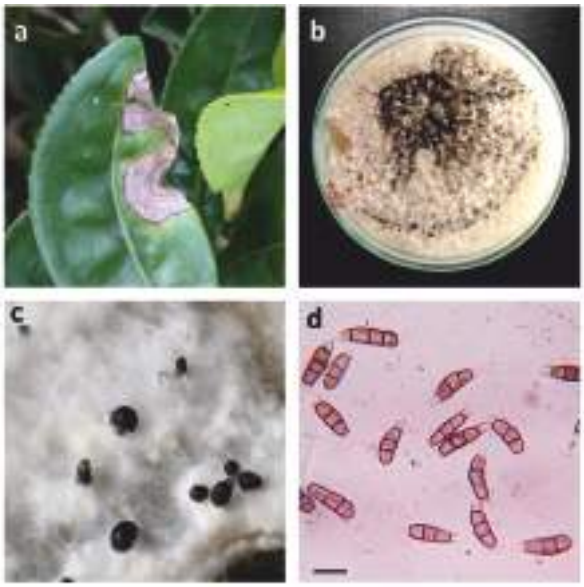
compared using the Duncan Multiple Range Test (DMRT) with a significance level of $p \leq 0.05$ (Schumacker & Tomek, 2013). Additionally, box plot analyses were performed using R to visualize the data distribution and variability.

Results and Discussion

Isolation and identification of grey blight pathogen

The colony characteristics were observed as whitish velvety appearance with blackish spots forming acervuli (fruiting bodies) overtime. The reverse view of the plate appeared to be pale yellowish orange in colour. The conidia were observed to be multicellular, four septate and are obclavate to ellipsoid in shape. Further two to three apical appendages with single basal appendages were observed. Therefore, the fungal pathogen was confirmed as *Pestalotiopsis* spp. through cultural observations and conidial morphology using microscopy. Slants of the *Pestalotiopsis* spp. was preserved in the UPASI TRF microbial germplasm (Figure 1).

Figure. 1. Symptoms, colony and conidial features of *pestalotiopsis* spp.



a) Grey blight symptoms on tea leaf
b) *Pestalotiopsis* spp. grown on culture media (obverse)
c) fruiting body
d) Conidial characteristics (scale bar = 20 µm)

GC-MS analysis of aqueous extract of *C. citratus*

The GC-MS analysis, with a runtime of approximately 22 minutes, identified a total of 30 compounds. The Total Ion Chromatogram (TIC) provides a visual representation of these compounds, with key peaks at various retention times indicating their relative abundance (Figure 2). The most abundant compound was Hydrazine, 1-(5-hexenyl)-1-methyl- at a retention time of 7.442 minutes, comprising 46.65% of the area. Cyclohexene, 1,6,6-trimethyl- was prominent at a retention time of 7.631 minutes with 6.45% of the area. Notable compounds included 2,6-Octadienal, 3,7-dimethyl-, (E)- at 6.12% and trans-7-Oxabicyclo [4.3.0] nonane at 3.18%. The analysis also highlighted the presence of 2,6-Octadienal, 3,7-dimethyl-, (Z)- (5.17%), Heneicosane (2.69%), and Disulfide, di-tert-dodecyl (2.13%) (Table 1).

Figure 2. GC-MS spectral chromatogram of *C. citratus* aqueous extract

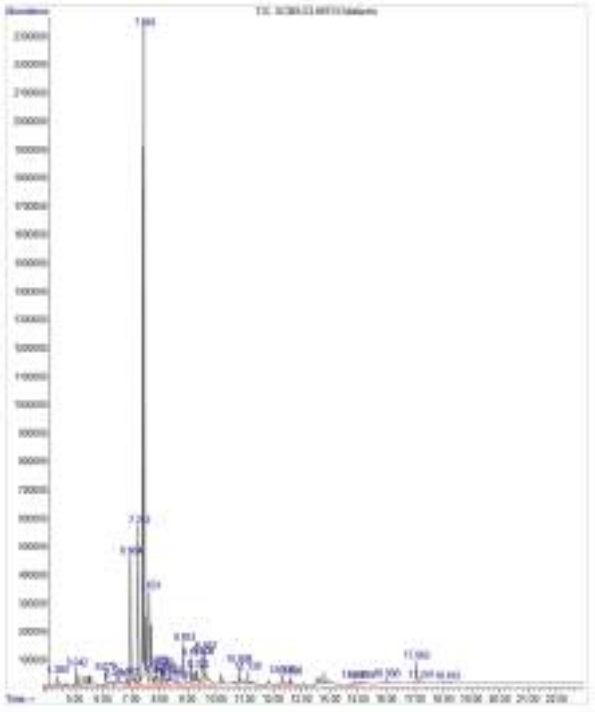
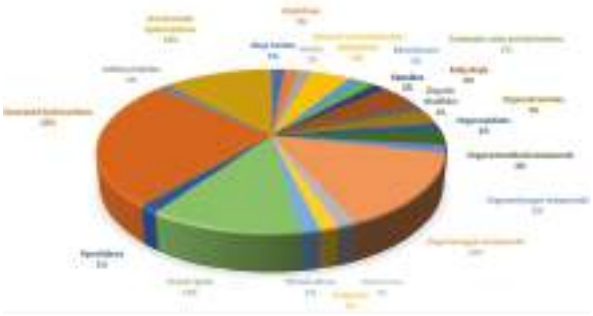


Table 1.Gas chromatography/mass spectrometry analysis of aqueous extract of *cymbopogon citratus.*

Peak No.	RT (min)	Compound name	Area (%)	Mol. Formula	Mol. Weight (g/mol)
1	4.365	5-Hepten-2-one, 6-methyl-	0.87	C8H14O	126.2
2	5.042	Undecane, 3,8-dimethyl-	1.25	C13H28	184.36
3	6.076	2-Cyclohexen-1-one, 3,5-dimethyl-	1.33	C8H12O	124.18
4	6.431	Butanamide, N-formyl-2-hydroxy-3-methyl-2-(1-methylethyl)-	0.81	C9H17NO3	187.24
5	6.875	(E)1-Allyl-2-methylcyclohexanol	0.85	C10H18O	154.25
6	6.964	2,6-Octadienal, 3,7-dimethyl-, (Z)-	5.17	C10H16O	152.23
7	7.242	2,6-Octadienal, 3,7-dimethyl-, (E)-	6.12	C10H16O	152.23
8	7.442	Hydrazine, 1-(5-hexenyl)-1-methyl-	46.65	C7H16N2	128.22
9	7.631	Cyclohexene, 1,6,6-trimethyl-	6.45	C9H16	124.22
10	7.842	2-Bromo dodecane	0.67	C12H25Br	249.23
11	7.909	Cyclohexene, 3,5,5-trimethyl-	1.6	C9H16	124.22
12	8.075	Cyclopentane, (2-methylpropyl)-	0.93	C9H18	126.24
13	8.164	6-Methyl-3,5-heptadiene-2-one	1.18	C8H12O	124.18
14	8.397	Disulfide, di-tert-dodecyl	2.13	C24H50S2	402.8
15	8.609	3,7-Nonadien-2-ol, 4,8-dimethyl-	0.67	C11H20O	168.28
16	8.853	trans-7-Oxabicyclo[4.3.0]nonane	3.18	C8H14O	126.2
17	9.131	Heneicosane	2.69	C21H44	296.6
18	9.32	3-Cyclohexene-1-acetaldehyde, .alpha.,4-dimethyl-	1.71	C10H16O	152.23
19	9.508	Dodecane	2.21	C12H26	170.33
20	9.597	Cyclohexanone, 2-ethyl-	3.87	C8H14O	126.2
21	10.808	Heptacosane	1.26	C27H56	380.7
22	11.13	Hexadecane	0.98	C16H34	226.44
23	12.308	10-Methylnonadecane	0.87	C20H42	282.5
24	12.586	Eicosane	0.76	C20H42	282.5

The extract of *C. citratus* was identified to have a diverse array of classes of chemical compounds within a runtime of approximately 22 minutes. The largest class is saturated hydrocarbons, making up 25% of the total composition. This is followed by organooxygen compounds at 15%, and prenol lipids and unsaturated hydrocarbons each at 12%. Other distinguished classes include organobromides and organometalloid compounds, each comprising 3%, and benzene and substituted derivatives, and fatty acyls, each at 4%. Smaller categories, each contributing 1%, include sulfonyl halides, azetidines, alkyl halides, azoles, benzofurans, epoxides, organic disulfides, organoiodides, organonitrogen compounds, pyrrolidines, phenol ethers, oxazinanes, and oxepanes. This analysis highlights the complex and varied chemical makeup of the sample, revealing significant contributions from multiple classes of compounds (Figure 3).

Figure 3. Chemical composition of *cymbopogon citratus* extract



The *C citratus* plant extract was processed for green synthesis of AgNPs by amending with $AgNO_3$. The plant extract was subjected to GCMS analysis which revealed the presence of potential bioactive compounds which plays roles in antifungal activity (Subramaniam *et al.*, 2020). The compound Hydrazine, 1-(5-hexenyl)-1-methyl- was found to be the most abundant which was studied by Bibi *et al.* (2020), targeting azo dye

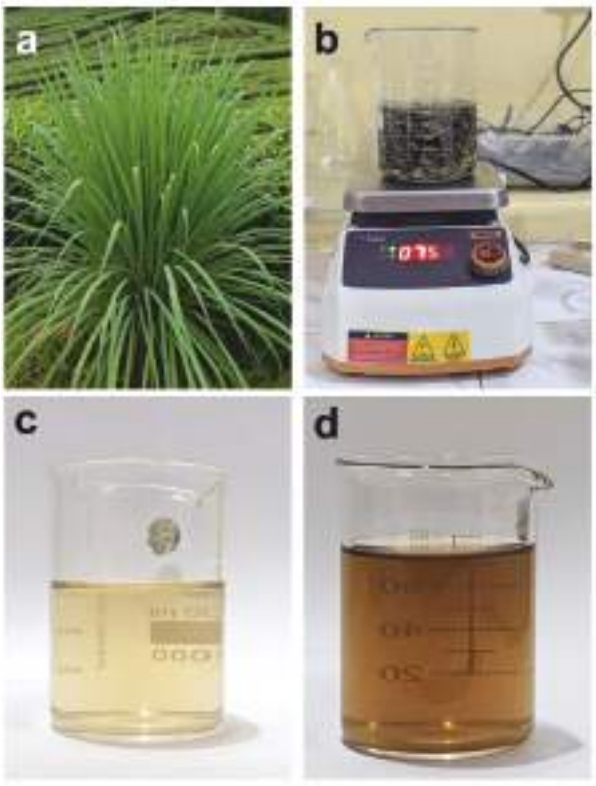
degradation using *Proteus mirabilis*, facultative microbe. Following this, Cyclohexene, 1,6,6-trimethyl-, and 2,6-Octadienal, 3,7-dimethyl-, (E)- were identified as notable compounds. The latter compound known as Citral, which has been extensively reported to exhibit potent antifungal activity against various plant pathogens (Menezes et al., 2020). Recent studies have demonstrated that citral inhibiting the growth of pathogens such as *Phytophthora capsica (Song et al., 2023)*, *Aspergillus flavus*, and *A. ochraceus* (Tang et al., 2018). In addition, citral has been shown to improve plant resistance to pathogens by activating the jasmonic acid pathway and enhancing the accumulation of phenylpropanoid compounds (Bin Duan *et al*., 2024). Osei-Obeng *et al*., (2024) demonstrated that citral significantly reduced the severity of *Fusarium* wilt in tomatoes by up regulating genes responsible for plant defence mechanism.

The presence of 2,6-Octadienal, 3,7-dimethyl-, (Z)-, commonly known as geraniol and a stereoisomer of Citral, has been detected in the results. Kaur *et al.,* (2019) revealed that plant-derived geraniol effectively inhibits *Sclerotium* rolfsiileading to the development of promising biocide. Geraniol has been studied to inhibit the growth of *C. albicans* and *A. flavus*, by inducing the accumulation of intracellular reactive oxygen species (ROS) and altering cell membrane permeability (Song *et al.,* 2022; Tang *et al.,* 2018). Furthermore, the studies of 'Da Silva et al., (2024) and Leite *et al.,* (2015) clearly proved the potential to enhance plant resistance to fungal infections by activating defence pathways. —Vanitha *et al.,* (2020) identified heneicosane as the effective bioactive compound exhibiting antimicrobial activity against *S. pneumoniae* and *A. fumigatus*. Prathipkumar *et al.,* (2023) utilised the aqueous extract of heneicosane to synthesise plant-based AgNPs through a cost-effective approach, enhancing antimicrobial, anticancer and photocatalytic capabilities. Disulfide, di-tert-dodecyl has been recognized for its multifaceted biological activities, including antimicrobial, antidote, coronary dilator, digestive, and diuretic agent, as well as its ability to increase superoxide dismutase (SOD) activity (El-Shahir *et al.,* 2022; Sivakumaran *et al.,* 2020). According to Mirghani *et al.* (2012), *C. citratus* contains a significant amount of phenols and terpenes supporting its antifungal potential —(Parveen *et al.,* 2010; Wu *et al.*, 2013).

Green synthesis of *C. citratus* mediated silver nanoparticles

The green synthesis of AgNPs was successfully obtained utilizing the *C.citratus* extract as the reducing agent. The AgNPs formation was confirmed by observing the colour change of the solution from light yellow to darkish brown colour. This colour change indicates the conversion of silver ions into AgNPs (Fig. 4).In general, the green synthesis of AgNPs is performed by treating silver nitrate ($AgNO_3$) solution with reducing agents derived from plant materials (Ying *et al.,* 2022). The synthesis of AgNPs is evident by the appearance of solution into brown in colour (Hemmati *et al*., 2019). This aligns with the study of Jain & Mehata, (2017) confirming the synthesis through the colour change to brown colour over time, signifying the reduction of silver ions by phytochemicals in the extract of *C. citratus* (Chitra & Annadurai, 2014). No colour change was observed after 24 hours, consistent with findings by Rakib-Uz-Zaman *et al.*, (2022). Khatami *et al.,* (2018) proven that green synthesis can be performed with not only plant extracts but also plant waste materials, such as hay. Additionally, Roopan *et al.,* (2013) synthesised AgNPs from coconut coir extract at a low cost and successfully examined their larvicidal activity for pest management.

Figure 4. Green synthesis of *C. citratus* mediated silver nanoparticles a) *C. citratus*; b) Aqueous extraction; c) Synthesis of AgNPs at 10 minutes; d) Synthesis of AgNPs after 2 hours

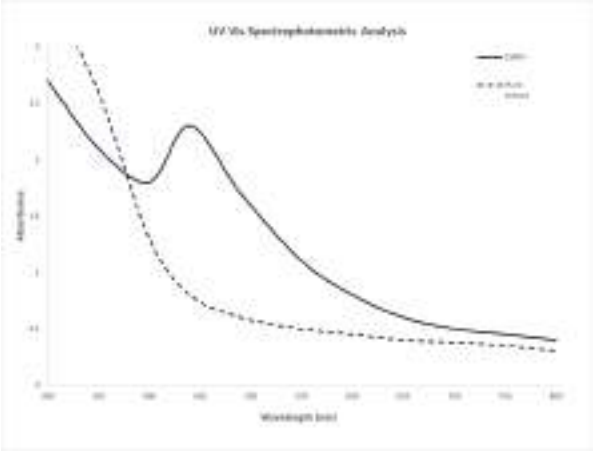


Characterization of *C. citratus* mediated silver nanoparticles

UV–Visible spectrophotometric analysis

The absorption spectra of CSNPs displayed a peak at 440 nm with an absorbance of 2.2, followed by a gradual decrease. This observed peak in the spectra confirms the presence of AgNPs in the *C. citratus* extract (Figure 5). In contrast to CSNPs, the spectra of *C. citratus* extract showed a steady decline in absorbance starting from 400 nm.Subsequent UV-Vis spectrophotometric analysis exhibited a distinct peak at 440 nm, thereby confirming the presence of AgNPs. This result positively correlates with earlier studies that reported a wavelength shift at 424 nm by Anandalakshmi *et al*., (2016). Njagi *et al.*, (2011) identified colloidal AgNPs with a band formed within the 400-450 nm region. Similarly, Nakkala et al., (2014) recorded an individual peak at λ_{max} 420 nm, demonstrating the stability of AgNPs synthesised from *Acorus calamus* rhizome extract.

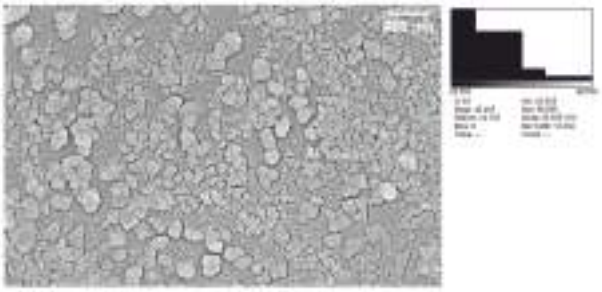
Figure 5. UV–Vis spectra of synthesized CSNPs and plant extract



Scanning electron microscopic (SEM) analysis

The result of the SEM analysis revealed that the nanoparticles were predominantly spherical in shape with a size range of 25-100 nm. The high-resolution image confirms the uniform distribution and stability of the nanoparticles, ranging from a minimum of 25.57 nm to a maximum of 99.56 nm. The surface appears covered with irregularly shaped particles. The accompanying histogram and statistical data, displaying 50 measurements, reveal that the nanoparticles have an average size of 45.41 nm (Figure 6).

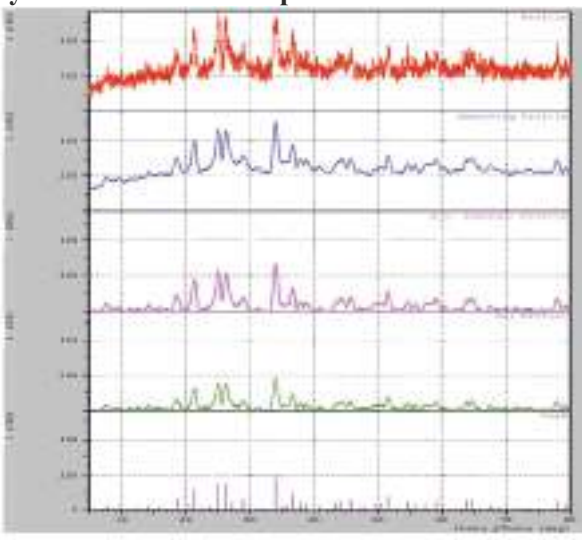
Figure 6. Scanning electron microscope image of *C. citratus* mediated AgNPs



X-ray diffraction analysis (XRD)

The blue profile, generated by smoothing the data, revealed more distinct peaks after noise reduction, facilitating better identification of the crystalline structures. Anandalakshmi *et al.,* (2016) described that silver being a key component of the phytochemicals present in the plant extracts with the appearance of respective planes. The XRD pattern clearly indicated primary peaks at 21.62˚, 25.04˚, 26.27˚, 34.01˚, and 36.86˚, which correspond to the 389, 459, 452, 500, and 372 planes, respectively (Figure 7). This was confirmed with the study of Sougandhi & Ramanaiah, (2020) that the crystal structure of AgNPs was studied as face-centred cubic, corroborating four peaks observed at 38.1˚ (111), 44.3˚ (200), 64.4˚ (220), and 78.2˚ (311). Addition to the Bragg peaks, the presence of other peaks not contributing to the spectrum was observed. Roopan *et al.,* (2013) explained that these peaks arise from the compounds participating in the reduction and stabilisation of the AgNPs.Using the Debye–Scherrer equation, the average size of the nano particles was calculated to be 19 nm.

Figure 7. XRD pattern of *C. citratus* extract synthesized silver nanoparticles



***In vitro* bioefficacy of CSNPs against *pestalotiopsis* spp.**

The impact of CSNPs on the colony growth rateand the dry weight of the fungal growth of the *Pestalotiopsis* spp.was evaluated using the food poisoning technique. The results revealed that treatments with CSNPs showed dose-dependent inhibition, with CSNPs at 2.0 mL/L and 5.0 mL/L completely inhibiting radial growth (100% inhibition). The percentages of inhibition were similar to those observed with the standard chemicals used. The efficacy in liquid culture indicated that the same dosages resulted in nearly complete suppression of 99% and 100% inhibition of fungal mat, respectively (Figure 8). The results of the study clearly indicated that CSNPs at 2.0 mL/L is the optimum dosage to control *Pestalotiopsis* spp. under *in vitro* conditions. The data were statistically analysed and confirmed significant differences among treatments (Table 2).The antifungal efficacy of *C. citratus* has been further improved with its use in the green synthesis of AgNPs (Masurkar *et al*., 2011; Paulkumar *et al*., 2014). The antifungal activity of AgNPs synthesised from *C. citratus* against *Candida* spp. was validated by Al-Otibi *et al*., (2023).It was demonstrated through *in vitro* bioassay studies that AgNPs mediated by *C. citratus* provided significant control under *in vitro* conditions. Moreover, –Vanti *et al*., (2019) highlighted the antifungal activity of AgNPs synthesised using the stem extract of *Gossypium hirsutum* against *Xanthomonas* spp. All these evidences support the potential of CSNPs for the use in controlling grey blight disease in tea as an alternative to conventional chemical fungicides.

Figure 8. *In vitro* bioassay of *C. citratus* mediated silver nanoparticles (CSNPs) against *pestalotiopsis* spp.

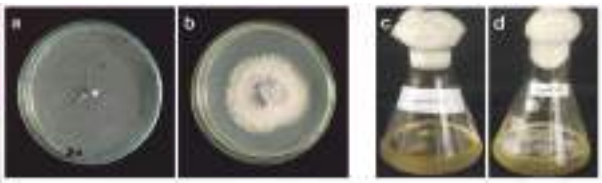


Plate culture a) CSNPs treated at 2.0 mL/L; b) Control; Broth Culture c) CSNPs treated at 2.0 mL/L; d) Control

Table 2. *In vitro* bioefficacy of *C. citratus* mediated silver nanoparticles (CSNPs) against grey blight pathogen

			Food poisoning technique			
			Plate culture			Broth
Sl. No.	Treatments	Dosage/L	Radial growth of mycelia (mm)*	Percentage of inhibition over Untreated control (%)	Dry weight of fungal mat (g)*	Percentage of inhibition over Untreated control
T1	CSNPs	0.5 mL	78±4[b]	0	3.9±0.4[a]	15
T2	CSNPs	1.0 mL	59±4[c]	27	2.8±0.3[b]	32
T3	CSNPs	1.5 mL	19±4[d]	74	1.0±0.3[c]	75
T4	CSNPs	2.0 mL	2±1[e]	100	0.0[d]	99
T5	CSNPs	5.0 mL	0[e]	100	0.0[d]	100
T6	Copper oxychloride 50% WP(COC)	6 g	0[e]	100	0.0[d]	100
T7	Companion (Mancozeb 63% + Carbendazim 12% WP)	0.2 %	0[e]	100	0.0[d]	100
T8	Untreated Control	-	92±2[a]	-	3.9±0.2[a]	-
		CD @ 0.05	4.23	-	0.32	-
		CV	7.54	-	12.79	-
		F value	788.15	-	277.54	-
		Se(m)	1.41	-	0.11	-

**Mean of three replicates. Treatments with same letters are not significantly different at P ≤ 0.05 as per DMRT*

Field bioefficacy of CSNPs against grey blight diseases in tea

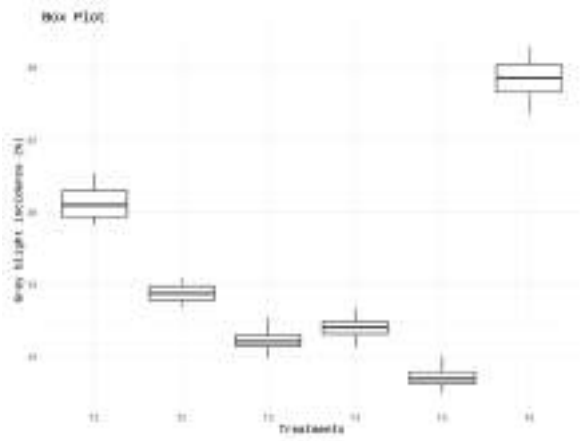
The effectiveness of CSNPs on grey blight disease in tea plants was evaluated under field conditions. The results from the RBD trial demonstrated that CSNPs at a dosage of 500 mL/ha successfully reduced grey blight incidence by 62%, while a dosage of 1000 mL/ha achieved a 53% reduction. Copper oxychloride (COC) at 420 g/ha provided superior control, with a 68% reduction in disease incidence compared to all other treatments. The untreated control plots experienced an 11% increase in grey blight incidence (29.2%), underscoring the necessity of applying treatments (Table 3). Statistical analysis confirmed significant differences among the treatments, which were visualised in the following box plot (Figure 9). These findings highlight the potential of CSNPs at a dosage of 500 mL/ha as an effective treatment for managing grey blight in tea plants.

Table 3. Field evaluation of *C. citratus* mediated silver nanoparticles (CSNPs) against grey blight disease

S. No.	Treatments	Dose / ha.	Grey blight / Die–back incidence		Grey blight control over pre-treatment (%)
			Pre - treatment	Post-treatments*	
1.	CSNPs	125 mL/ha	27.7	20.7[b]	25
2.	CSNPs	250 mL/ha	24.1	14.4[c]	40
3.	CSNPs	500 mL/ha	29.5	11.2[d]	62
4.	CSNPs	1000 mL/ha	25.6	12.0[d]	53
5.	Copper oxychloride (COC)	420 g /ha	26.9	8.6[e]	68
6.	Control (Untreated)	-	26.3	29.2[a]	-
		CD @ 0.05		1.98	
		F value		132.26	
		SE(m)		0.67	
		CV		8.30	

**Mean of four post-treatment assessments. Treatments with same letters are not significantly different at P ≤ 0.05 as per DMRT.*

Figure 9. Boxplot showing the efficacy of *C. citratus* mediated silver nanoparticles under field conditions



T1) CSNPs @ 125 mL/ha; T2) CSNPs @ 250 mL/ha; T3) CSNPs @ 500 mL/ha; T4) CSNPs @ 1000 mL/ha; T5) Copper oxychloride @ 420 g /ha; T6) Untreated Control

Conclusion

The validation of *C. citratus* mediated silver nanoparticles (CSNPs) was carried out under both *in vitro* and *in vivo* conditions. This study proved the potential of CSNPs at the dosage of 500 mL/ha exhibiting an effective control over grey blight disease in tea plants. Further strategies such as developing an Integrated Disease Management (IDM) package helps to align with the principles of sustainable agriculture with reduced inputs of chemical fungicide.

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Author contributions: CRediT

FLM: Investigation, Data curation, Software, Writing – original draft; NP: Conceptualization, Funding acquisition, Supervision, Validation, Writing – review and editing; BJ: Investigation, Visualization, Writing – review and editing; RS: Software, Writing – review and editing.

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Data availability

The data generated during the research work will be made available on request.

Declaration of competing interests

The authors declare that they have no conflict of interest.

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