Green silver nano-particles: synthesis using rice leaf extract, characterization, efficacy, and non-target effects

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Abstract

Green synthesis of silver nano-particles (AgNPs) from silver nitrate was carried out using purple-colored rice leaves' extracts containing higher phenols, anthocyanins, and flavonoids. The efficacy of synthesized AgNPs was tested against rice diseases and investigation was carried out to check negative effect of AgNPs on soil microbes. Substantial reduction of total anthocyanins, total phenols, and total flavonoids was observed in reaction mixture during AgNP formation indicating the role of secondary metabolites on AgNP formation and stabilization. Scanning electron microscopy coupled with energy-dispersive spectroscopic images and FTIR spectral analysis of AgNPs confirmed the presence of elemental silver encapped by biomolecules. The optimized reaction parameters for synthesis of AgNPs from silver nitrate were (a) 48 h of incubation, (b) 9:1 (v/v) 1 mM AgNO3:plant extract, and (c) room temperature at 20–30 °C. Zeta potential and hydrodynamic particle sizes of synthesized AgNPs were ranged between − 16.61 to − 29.45 mV and 36–107 nm, respectively, at different time of incubation. AgNPs could control effectively *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *Oryzae* and *Helminthosporium oryzae*. AgNPs at higher concentration could cause negative effect on microbial biomass carbon and soil enzymes for distant future. But the negative effects of AgNP solution (10% of 1 mM AgNPs) were comparable to commercial fungicide, carbendazim. The synthesized AgNPs with desirable characters were effective against a number of disease-causing pathogens in rice, and it can be recommended as broad-spectrum pesticide.

Keywords Purple-colored rice leaves · Biomolecules · Particle sizes · Soil enzymes · Bacterial leaf blight · FTIR

Introduction

Nano-materials have attractive physio-chemical properties and can be used in agriculture for crop production. One of the most investigated nano-materials in agriculture is silver nano-particles (AgNPs). Research has undergone on

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different methods of synthesis of AgNPs. It could be synthesized by thermal decomposition (Adner et al. 2016), using microwave (Seku et al. 2018), electrochemically (Kuntyi et al. 2019), and different green methods utilizing plant extracts, microbes, etc. (Ahmad et al. 2019). Green synthesis of AgNPs usually is being carried out by reduction of silver ion from silver nitrate solution using a reducing biological agent. The reducing agent may be bacteria, fungi, plant, or animal extracts. Different plant extracts containing a wide range of different metabolites can produce nano-particles. Primary metabolites like carbohydrate, fat, and proteins and secondary metabolites like phenols, flavanoids, anthocyanins, alkaloids, and terpenoids can reduce silver nitrate to AgNPs (Manikandan et al. 2017). These molecules encapped the newly formed AgNPs and stopped the formation of agglomeration and thereby maintain uniform particle size. Green synthesis of nano-particles is preferable being environmental friendly as it does not require toxic solvents or high energy like in

other methods and it is easy and economical for a large-scale synthesis (Mittal et al. 2013).

Earlier rice leaf extract was avoided for synthesis of silver nano-particles due to its high silica content. There are rice genotypes with red, purple red, and purple color leaf sheaths beside normal green leaf sheaths. For example, wild rice and landraces have purple leaf sheath (Chin et al. 2016) and the character has been transgressed into few of the modern cultivars. The color may be due to the presence of anthocyanins, and flavonoids, etc. We are proposing for the first time the use of purple-colored rice genotypes with high content of antioxidants like phenol and anthocyanins to synthesize silver nano-particles.

Silver nano-particles could be used as fungicide, bactericide, and antiviral agents (Veerasamy et al. 2011). AgNPs have been used to control fungal pathogens, namely, *Rhizoctonia solani*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, etc. (Krishnaraj et al. 2012) and bacteria pathogens, namely, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* (Patra and Baek 2017). In rice, it has been utilized against a number of diseases namely blast (Elamawi and El-Shafey 2013), sheath blight (Soltani-Nejad et al. 2017), and bacterial blight (Annakodi et al. 2015). These diseases are yield limiting factors for rice and new management options are need of the hour. Indigenous and easy synthesis method will help to produce AgNPs for better rice disease management.

Bio-safety is an important issue on nano-silver uses and has been widely discussed in literature. It may pose a threat to non-targeted soil microbes in the environment. For example, bacteria were negatively affected by silver nano-particles (Sillen et al. 2015). This study investigated the effect of AgNPs on soil microbes by quantifying soil enzymes and microbial biomass carbon.

The present investigation was carried out to have a cheap and easy method to synthesize AgNPs. Experimental parameters like incubation temperature, $AgNO_3$ concentration, and rice leaf extract concentration were optimized to biosynthesis AgNPs. We investigated the biochemical changes taking place during the AgNP formation. The synthesized AgNPs were explored against rice diseases like sheath blight, bacterial blight, blast, and brown spot disease of rice. Investigation was also carried out to find out negative effect of AgNPs on soil enzymes, namely, dehydrogenase and fluorescein diacetate hydrolase activity and soil microbial biomass carbon.

Materials and method

Materials

Silver nitrate ($AgNO_3$) was supplied by Merck (India). Analytical grade chemicals were used in different experiments.

Plant samples and extraction

Seeds of 10 purple-colored rice germplasm (Harekrishna, Hempal, Badami dhan, Jaleshar, Kariglass 201/1674, Bygani Dhan, Baithani Dhan, Dharsal, Kamal Luchai, Dodana Dhan, Kari glass-2015/618, Laal Paana) were collected from rice gene bank of ICAR-National Rice Research Institute, Cuttack and were grown under field condition as puddled rice as per the agronomic practices. During the late tillering (50 days after sowing), the entire leaf including leaf sheath was collected and first washed with tap water, followed by double-distilled water to remove extraneous contamination. The cleaned rice leaves were dried at room temperature (28 ± 2 °C) in the absence of sunlight to achieve constant weight and dried leaves were grinded in common mixture grinder (Mishra et al. 2019). Ground leaves (5 g) of each sample were put into a beaker (250 mL) that contains 100 mL distilled water and the content was heated at 70 °C for 4 h. After 1 h of cooling at room temperature, the extracts were filtered through Buchner funnel under suction and final volume was made up to 100 mL for each extract.

Biochemical characterization of plant extract

For the biochemical analysis, 2 mL of each leaf extract was used for phenol and flavonoids estimation. Phenol (Zilic et al. 2011) and flavonoids (Eberhardt et al. 2000) was measured using catechine as standard. For phenol estimation, 0.2 mL supernatant was taken in centrifuge tube that contained 0.3 mL distilled water, followed by 0.25 mL folin ciocalteu reagent and 5 mL of sodium carbonate (20% w/v) were added. The content was vortexed for 45 min and total phenol content was measured in a spectrophotometer at 725 nm. For flavonoids, 1 mL of supernatant was mixed with 1 mL aluminum chloride (10% w/v). The mixture was incubated at room temperature for an hour and the color formation was read at 450 nm. The total amount of anthocyanins present in different plant extracts was determined as per Sutharut and Sudarat (2012). The difference in absorbance in samples was measured at two different spectral measurements, i.e., at 535 nm and 657 nm. Total anthocyanin content (TAC) was estimated according to the following formula:

$$TAC = \frac{A \times M_w \times DF \times 1000}{E_M \times 1}$$

where A = corrected absorbance (A = absorbance $\lambda_{vis\text{-}max\text{-}A535nm}$ − absorbance $\lambda_{vis\text{-}max\text{-}A657nm}$); M_W is the molecular weight (g/mol) (449.2 g/mol in the analysis); DF is the dilution factor; and E_M is the molar extinction coefficient (26,900).

Biosynthesis of silver nano-particles

Biosynthesis of silver nano-particles (AgNPs) mediated by rice leaf extracts was carried out as follows. Silver nitrate solution (1 mM in water) was mixed with different ratios of plant extract (silver nitrate:plant extract: 5:5, 7:3, 9:1, 9.5:0.5, and 9.9:0.1 v/v). Similarly, different temperatures (10, 20, 40, 60 °C) were imposed for synthesis of AgNPs for a fixed silver nitrate:plant extract (9:1 v/v) mixture. Incubation of a fixed silver nitrate:plant extract at 9:1 (v/v) mixture was done for 0, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h to find out optimum time. The reduction of silver ion to AgNPs was observed by the UV–vis spectral analysis in a spectrophotometer.

UV–vis spectroscopy

The formation of AgNPs was monitored based on change in absorbance using a UV–vis spectrophotometer (Evolution 300, Thermo Scientific, USA) as per Rasheed et al. (2017). The absorbance of AgNPs was recorded in the wavelength ranging from 300 to 800 nm at different reaction conditions.

FTIR analysis

The synthesized AgNPs were mixed uniformly with potassium bromide (KBr) to form pellets. FTIR spectra were recorded on a Bruker ALPHA FTIR-ATR system. AgNP pellets were scanned in the region of 4000–500 cm^{-1}.

SEM and EDS analysis

Surface morphology of AgNPs was examined using scanning electron microscopy (SEM). SEM analysis was performed using a Zeiss EVOMA10 scanning electron microscope, operating at 20 kV. To check the surface morphology, AgNPs were mounted onto the surfaces of clean silicon chips and the gold sputtering device was used to coat it with a thin film of gold. Elemental analyses of the AgNPs were accomplished using an energy-dispersive X-ray spectrophotometer (EDS).

Particle size analyzer

Dynamic light scattering (DLS) with dual optical probe technology at 780 nm (near infrared) wavelength was used in the particle size analyzer (ZetatracTM). AgNP solution was prepared in double-distilled water and it was sonicated for 5 min to form uniform distribution before measuring the hydrodynamic particle sizes. All measurements were done at 25 °C and a fixed angle light scattering was detected.

In vitro antimicrobial activity

The antimicrobial activity of AgNPs was evaluated against three pathogenic fungi, namely, *Magnaporthe grisea* (blast), *Rhizoctonia solani* (sheath blight), and *Helminthosporium oryzae* (brown spot) and one pathogenic bacteria, bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). Poisoned food technique was used to test the efficacy of silver nano-particles against rice pathogens (Nene and Thapliyal 2002). Potato dextrose agar (PDA) media was used to grow both *Rhizoctonia solani* and *Helminthosporium oryzae*, whereas, nutrient agar media and oat meal agar media was used to grow *Xanthomonas oryzae* and *Pyricularia grisea*, respectively. The nano-particles solution at different concentrations of 0, 1, 2, 5, and 10% of 1 mM AgNPs was mixed with the growing media, separately. ICAR-NRRI virulent isolates of *R. solani*, *H. oryzae*, *X. oryzae*, and *P. grisea* was inoculated in the middle portion of respective media. The plates were incubated in a biological oxygen demand (BOD) incubator at 27 ± 2 °C. Leaf extract, 1 mM AgNO$_3$ at 10% was used to check the efficacy along with absolute control. Growth was measured based on diameter of mycelia grown after 48 h of inoculation and compared with control for *R. solani*, *H. oryzae*, and *X. oryzae*, except for *P. grisea* where mycelial growth was measured after 10 days. After different days of incubation, the growth was measured as the colony diameter in mm (Singh and Tripathi 1999). Each treatment was replicated five times. The efficacy of the AgNPs was measured in terms of percentage growth inhibition using the following formula:

where C = Percent growth inhibition = $\frac{(C-T) \times 10}{C}$ mycelial growth in mm in control, and T = mycelial growth in mm in treatment.

In vivo bioassay

Rice (var. Tapaswani, *Indica* type) seeds were sown as puddled rice as per prescribed agronomic practices. Three rice seedlings (25 days old) were planted in pots (20 cm × 20 cm) containing 4 kg soil. Water was added regularly to keep the moisture at field capacity. Agronomic practices including recommended fertilizer (N:P:K 100:50:50) dose were applied to each pot. Small mycelium agar discs (5 mm diameter) of a virulent pathogen inoculum of *R. solani* from ICAR-NRRI were cut using a sterilized cork borer from the culture. This disc was kept on moist cotton and tied beneath the leaf sheath of rice plants at late tillering stage. One day after inoculation, different concentrations of AgNPs (2.5, 5, 10, and 20% of 1 mM AgNPs) along with silver nitrate and carbendazim at recommended dose (0.25 g L^{-1}) were sprayed using a hand sprayer (Mondal et al. 2012). The lesion length on the sheath of the inoculated plants was measured at different intervals (Adak et al. 2019).

Phytotoxicity data

Crop phytotoxicity was recorded 15 days after the application of AgNPs. Observations for the specific parameters like chlorosis, necrosis, and wilting were noted as per standard procedures. There were no phytotoxicity symptoms in rice plants at 20% AgNP treatments and AgNPs did not show any negative effect on rice plants.

Non-target effects on soil physico-chemical, microbial biomass and enzymes

Five hundred grams of soil samples from 0 to 15 cm rhizospheric depth was collected on 0 (2 h before AgNP application), 7, 15, and 30 days after AgNP application to assess the microbial activities. The soil:water (1:2.5 w/v) suspension was used to measure pH and electrical conductivity using a portable multi-parameter PCSTester™ 35 (Eutech Instruments, Oakton, Singapore). Soil organic carbon content was measured according to Walkley and Black (1934). Soil microbial biomass carbon (MBC) was quantified using chloroform fumigation extraction method with minor modification (Vance et al. 1987; Sahu et al. 2019). Soil enzymes, namely, fluorescein diacetate hydrolase and dehydrogenase were quantified as per the previous reports (Sahoo et al. 2017; Mahapatra et al. 2017).

Statistical analysis

Data were analyzed following analysis of variance (SAS Software packages, SAS EG 4.3) and means of treatments were compared based on Tukey's minimum significant difference test (MSD) at the 0.05 probability level. Error bars in figures represent standard deviation of five replications.

Results and discussion

Biochemical characterization of plant extract

Synthesis of silver nano-particles was carried out with 12 rice germplasm. Leaves of these rice genotypes were purple in color. They were supposed to have higher antioxidant activities to reduce Ag^+ to Ag^0. The estimated total anthocyanins, total phenols, and total flavonoids in the initial plant extracts were ranged between 35.56–59.29 mg L^{-1}, 461.07–832.95, and 1686.27–3433.51 μg equivalent catechine L^{-1}, respectively (Table 1). After standard 48 h of incubation, AgNPs were separated through centrifugation. Total anthocyanins, total phenols, and total flavonoids in the remaining reaction mixture were in the range of 12.27–15.22 mg L^{-1}, 289.86–363.67, and 288.06–482.71 μg equivalent catechine L^{-1}, respectively. Maximum concentration of total anthocyanins and

total phenols was obtained in Kariglass 2014/1674 and total flavonoids were obtained in Dodana dhan. Based on the differences in initial and final concentrations of total anthocyanins, total phenols, and total flavonoids in the reaction mixtures, we could conclude that Kariglass 2014/1674 and Dodana dhan will be most effective in reducing $AgNO_3$ to AgNPs. Similar to our study, different plant extracts, namely, *Camellia sinensis* (Nakhjavani et al. 2017), *Azadirachta indica* (Ahmed et al. 2016), *Aloe vera* plant extract (Tippayawat et al. 2016), *Citrus limon* (Vankar and Shukla 2012), *Euphorbia prostrata* (Zahir and Rahuman 2012), *Polyalthia longifolia* (Prasad and Elumalai 2011), *Cinnamomum camphora* (Huang et al. 2007), etc. were used to synthesize AgNPs. In all the above literatures, metabolites like phenols, terpenoids, and flavones were responsible for reduction of $AgNO_3$. Besides reduction, they are also responsible for the stabilization of synthesized nano-particles. There are reports that nano-particles of different shapes could also be formed by different plant extracts like irregularly shaped gold nano-particles were formed by clove buds extract (Raghunandan et al. 2010). In another report, stronger antioxidant properties were observed in synthesized nano-particles compared with original extracts, suggesting stabilized nano-particles with polyphenolic antioxidants on outer surface (Banerjee and Narendhirakannan 2011). Till date, rice leaves' extracts were not preferred due to high silica content and low polyphenols present in plant extracts, but the purple leaf sheath rice germplasm had adequate anthocyanins, flavonoids, and total phenolics responsible for the reduction of $AgNO_3$. This may be the first time we are utilizing these plants for AgNP synthesis.

Optimization of silver nano-particles synthesis

Green synthesis of silver nano-particles (AgNPs) mediated by rice leaf extracts was undertaken to optimize the duration, volume of the plant extract, and temperature required. The reduction of silver (Ag^+ to Ag^0) was monitored by UV–vis spectrophotometer. Change in color was observed within 2 h of addition of plant extract in 1 mM $AgNO_3$ solution. The color of the reaction mixture was initially light brown which was changed to dark brown indicting the formation of AgNPs. Strong surface plasmon resonance of AgNPs was centered at 420–480 nm. The yellowish-brown color of AgNPs is due to excited surface plasmon vibrations (Krishnaraj et al. 2010). Absorbance of AgNPs became constant in between 36–48 h of incubation (Fig. 1a and S1Fig1). For further study, we took standard 48 h of incubation. Similarly, with the increase in temperature, the rate of AgNP formation was increased (Fig. 1b and S1Fig3). Among the different ratios of $AgNO_3$ and plant extracts, we could observe 9:1 (v/v) 1 mM $AgNO_3$:plant extract was optimum (Fig. 1c and S1Fig3). Based on the particle size and zeta potential, we found room

Table 1 Total phenols, total anthocyanins, and flavonoids content in different rice germplasms before and after silver nano-particles formation

Germplasm	Total anthocyanin (mg L^{-1})		Total phenol (µg equivalent catechine L^{-1})		Total flavonoids (µg equivalent catechine L^{-1})	
	Initial	After	Initial	After	Initial	After
Badamidhan	36.66	13.35	461.07[d]	314.26	1686.27[c]	288.06[d]
Baithani dhan	35.56	12.27	500.00[d]	308.41	2429.95[bc]	325.85[bcd]
Bygani Dhan	54.77	14.56	808.14[ab]	317.69	3298.04[ab]	479.06[a]
Dharshal	52.22	15.22	728.08[abc]	293.69	3249.55[ab]	322.28[cd]
Dodana dhan	51.25	14.36	745.22[abc]	289.86	3433.51[a]	482.71[a]
Harekrishna	39.89	11.23	652.25[c]	334.83	3070.23[ab]	367.91[bc]
Hempal	42.22	12.28	703.68[bc]	316.68	2951.16[ab]	362.21[bc]
Jalkeswar	40.22	13.02	677.06[c]	321.72	2943.32[ab]	377.18[b]
Kamal Luchai	46.77	13.25	746.23[abc]	291.47	3166.13[ab]	331.55[bcd]
Kariglass 2014/1674	59.29	15.14	832.95[a]	308.01	3123.01[ab]	477.01[a]
Kariglass 2015/618	49.58	14.22	697.43[bc]	298.53	2937.61[ab]	366.49[bc]
Laal Panna	38.25	13.25	675.65[c]	363.67	3085.56[ab]	342.25[bc]
p value	0.0689	0.0788	< .0001	0.1582	0.0002	< .0001
Tukey HSD at 5%	NS	NS	115.79	NS	971.81	53.338

*with at least one letter common (a, b, c as superscripts) that is not statistically significant using Tukey's honestly significant difference (HSD) test at $p \leq 0.05$

[#] *NS* non-significant differences among the treatments at $p \leq 0.05$

temperature was optimum for the nano-particles synthesis, which will be discussed in the "Characterization of synthesized silver nano-particles" section. Similar to our study, UV–vis spectrum of silver nano-particles developed from different plant extracts was around 400 nm (Nakkala et al. 2014; Zaheer et al. 2012; Thatoi et al. 2016). This is due to surface plasmon resonance of AgNPs. Higher peak height or absorbance of surface plasmon resonance indicates the yield of AgNPs and, similarly, larger peak width corresponds to polydispersity (Cao et al. 2014). In this study, we could observe higher peak height with increase in time and temperature. This indicates better yield of AgNPs. Similarly, in all reaction conditions, sharp peaks obtained indicate low polydispersity indices or uniform size of the molecules.
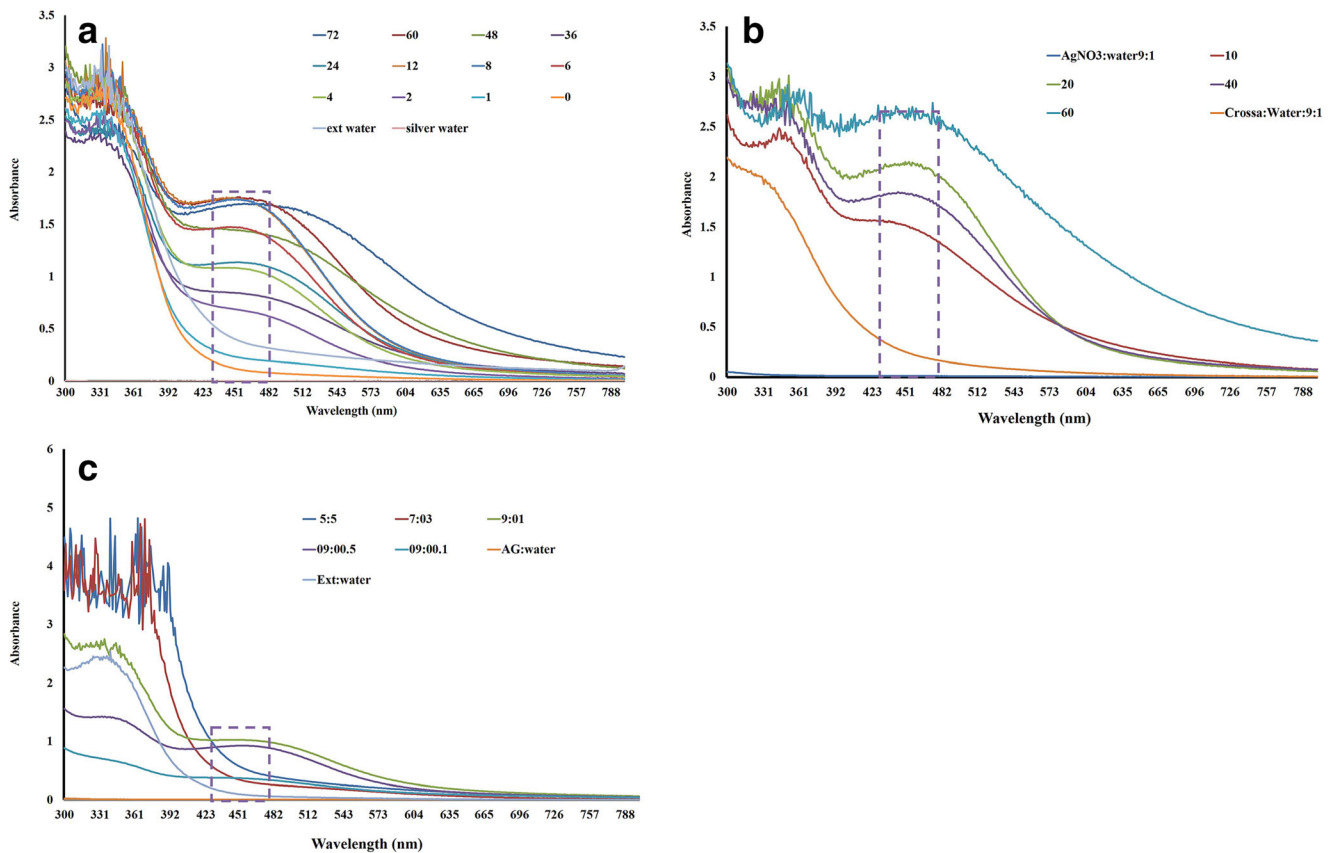
Characterization of synthesized silver nano-particles

Hydrodynamic particle sizes of synthesized AgNPs were ranged between 36–107 nm at different times of incubation (Table 2 and S1Fig4). The zeta potential was − 27.11 mV after 6 h of incubation. Thereafter, it was ranged from − 16.61 to − 29.45 mV. After 36 h of chemical reaction, the size of the AgNPs did not vary. Similarly, the zeta potential was also favorable for this period. Hydrodynamic size of AgNPs' different reaction mixtures of AgNO$_3$:extract after 48 h were ranged from 52.20 to 81.00 nm (Table 2). As there is increase in incubation time, the formation of AgNPs was increased, which could be seen by the increase in intensity. Besides that, there was a shift in absorbance maxima toward higher

wavelengths. This indicates a greater number of AgNPs were formed with incubation time.

The ratio of AgNO$_3$:extract at 9:1 was most favored for its hydrodynamic size (52.20 nm) and zeta potential (− 19.42 mV) (S2 Table 1). Rate of reaction was increased with temperature, but hydrodynamic particle size was also increased at higher temperature (69.30 nm at 60 °C) (S2 Table 2). The optimum temperature for uniform hydrodynamic particle size was 20–40 °C. AgNPs synthesized in this temperature range had optimum zeta potential (− 29.45 to − 21.87 mV) too. The negative zeta potential indicates repulsive forces among the nano-particles and thus increasing the stability of nano-particles. Overall, the green synthesis of AgNPs could be made with rice leaf extracts within 48 h of incubation at room temperature at a ratio of 1 mM AgNO$_3$:extract at 9:1 (v/v).

In this study, the absorbance due to surface plasmon resonance became higher and broader as the reaction temperature was increased to 60 °C. This denotes higher yield of AgNPs with a broad range of hydrodynamic particle sizes, which may not be suitable of agricultural application. The AgNPs synthesized at 20 and 40 °C had the sharpest surface plasmon resonance peak and it was observed within a shorter wavelength. These AgNPs were smaller in sizes and less polydisperse. Uneven particle sizes at higher temperature may be due to lack of stabilization of nano-particles. Rapidly synthesized AgNPs at higher temperature could not be encapped by the phenolics in the extract which might cause nano-particle agglomeration and flocculation (Liu et al. 2018).

Fig. 1 Ultraviolet-visible spectra of synthesized silver nano-particles at different time intervals (where, 0, 1, 2, ... 72 denote duration of reaction, ext water denotes plant extracts in water at a ratio of 1:9 (v/v), and silver water denotes AgNO$_3$ in water at a ratio of 9:1 (v/v)) (**a**); at different temperatures (where, 10, 20, 40, and 60 denote temperature of reaction)

(**b**); at different ratio of plant extract and AgNO$_3$ (where 5:5, 7:03, 9:01, 9:00.5, and 9:00.1 denote AgNO$_3$ and plant extract ratio in reaction mixture, Ext:water denotes plant extracts in water at a ratio of 1:9 (v/v), and Ag:water denotes AgNO$_3$ in water at a ratio of 9:1 (v/v)) (**c**)

A 9:1 ratio of 1 mM AgNO$_3$ and plant extract resulted in the sharpest peak with the highest intensity which denotes even AgNP formation with higher yield. Increase in dilution

Table 2 Particle sizes and zeta potentials of synthesized silver nano-particles (AgNPs) at different time of reaction

Time of reaction (h)	Size (nm)	Zeta potential (mV)
0	70.70	40.45
1	36.10	26.29
2	51.70	− 14.16
4	71.50	− 14.17
6	56.40	− 27.11
8	42.20	− 29.45
12	107.00	− 16.61
24	38.20	− 24.27
36	52.20	− 28.25
48	52.20	− 19.42
60	50.00	− 28.17
72	49.50	− 25.77

of plant extracts resulted in slow formation of AgNPs. Higher concentration of plant extracts also resulted poorly in formation of AgNPs. Increase in concentration of leaf extract of *Capparis spinosa* led to a greater number of biomolecules to reduce the silver nitrate to AgNPs (Benakashani et al. 2016).

The presence of elemental silver was confirmed from the images of scanning electron microscopy and energy-dispersive spectroscopy of the synthesized AgNPs (Fig. 2a and b). There was a strong signal at approximately 3 keV. It is due to the surface plasma resonance of silver nano-particles. There was also carbon, oxygen, and chloride signals in the images. This indicates the presence of the other organic moieties coming from the rice leaf extract.

The IR spectra of synthesized nano-particles showed distinct peaks at 3432, 2924, 2853, 1638, 1165, and 1037 cm^{-1} (Fig. 3). The N–H or O–H stretching vibration corresponded to strong broad peak at 3432 cm^{-1}. Besides, C–N stretching vibrations were observed at 1165 cm^{-1} and 1037 cm^{-1}. Peaks at 2924 and 2853 cm^{-1} corresponded to aliphatic and aromatic C–H stretching vibrations, respectively. Peak at 1638 cm^{-1} corresponded to C=O group and it might denote the presence of amide bond. The peak at 1037 cm^{-1} can be assigned for C–

O stretching vibration. AgNPs synthesized and stabilized by different functional groups like phenols, amines, and carbonyl could be identified by FTIR analysis (Rasheed et al. 2017). For example, bands at 1620 to 1650 cm^{-1} were assigned to –C(O)– of peptide bonds and those at 1380–1030 cm^{-1} to C–N bonds (Ballottin et al. 2016). Role of protein or cysteine residues in the proteins or free amino acids on nano-particles binding and stabilization is already documented (Guilger-Casagrande and de Lima 2019)

## Efficacy of nano-silver against rice diseases

The antimicrobial activity of AgNPs was tested against three disease-causing fungi, namely, *Rhizoctonia solani* (sheath blight), *Magnaporthe grisea* (blast), and *Helminthosporium oryzae* (brown spot) and one pathogenic bacteria, *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight). Effects of different treatments of AgNPs against *R. solani* and *X. oryzae* pv. *oryzae* were significantly different. Among the different concentrations tested, AgNPs at 5% (v/v) could control *R. solani* (81% growth inhibition) and it was better when compared with 10% (v/v) AgNO$_3$ (64 % growth inhibition) (Table 3 and S1Fig5). There was no growth of *R solani* when we added AgNPs at 10% (v/v) in the media. Similarly, growth of *X. oryzae* pv. *oryzae* was inhibited by AgNP application and AgNPs at 5% (v/v) and AgNO$_3$ at 10% (v/v) were at par (growth inhibition of 11 mm) (Table 3 and S1Fig6). AgNPs at 10% (v/v) could inhibit 28.5 mm of the growth of *X oryzae* pv. *oryzae*. AgNPs was effective against brown spot disease but was not effective against blast in *in vitro* condition. Application of 10% (v/v) AgNP solution could inhibit 42% growth of *H. oryzae*. Under *in vivo* condition, AgNPs had shown better activity compared with silver nitrate solution against *R. solani*. Infection of sheath blight was only 3.4% in treatment of 20% (v/v) AgNPs compared with control which had 13.89% of infection (Table 4).

There are many studies regarding the effectiveness of AgNPs as a bactericidal agent (Rai et al. 2012; Lara et al. 2010). AgNP efficacy against *X. oryzae* pv. *oryzae* was

reported earlier (Cui et al. 2016). Nano-silver is an effective broad spectrum fungicide against common fungi. Soltani-Nejad et al. (2017) reported the efficacy of AgNPs against *R. solani*. Researchers have proposed various mechanisms responsible for the antibacterial effect of silver nano-particles. Most of the researchers are in opinion that larger surface area of AgNPs is responsible for antimicrobial properties owing to their better interaction with pathogens (Panacek et al. 2006; Morones et al. 2005). Besides, particle size, shape, and preparation method are responsible for its efficacy. The smaller size of AgNPs with the spherical shape had a better antibacterial efficacy (Simon-Deckers et al. 2009). The gram-negative bacteria like *X. oryzae* pv. *oryzae* is more sensitive to AgNPs due to the presence of thinner cell wall and limited quantity of peptidoglycan in its cell wall (Ankanna et al. 2010). Inside the cell, AgNPs may interact with sulfur or phosphorus-containing constituents of cell (Nayak et al. 2016) or by producing reactive oxygen species (ROS) which eventually could demonstrate bactericidal effect (MubarakAli et al. 2011; Logeswari et al. 2015) etc.

Non-target effect of silver nano-particles on soil microbial properties

The pH, electrical conductivity (EC), and organic carbon content of soil were ranged 6.2–6.6, 0.19–0.26 ds min^{-1} and 0.50–0.58%, respectively. No significant difference was observed among the treatments including control during the entire experimental period. Negative effect of AgNPs on soil microbial biomass carbon was observed after 7 days of its application. AgNPs applied at 10% (v/v) or above negatively influenced the MBC content (Table 4). MBC content was 63.38 μg g^{-1} soil in 20% AgNP treatment after 7 days after application compared with control which had 73.28 μg g^{-1} of MBC. The negative effect was comparable with carbendazim as well as 10% AgNO$_3$ application. The negative effect on MBC was recovered thereafter. There were no significant differences among the treatments including control after 15 days of application. FDA activity did not vary significantly among
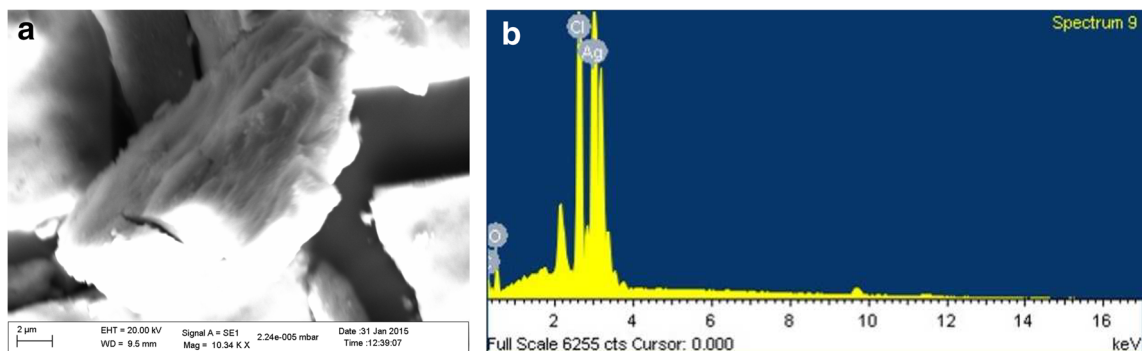
Fig. 2 Representative scanning electron microscope (**a**) and energy-dispersive spectroscopic (**b**) images of synthesized silver nano-particles
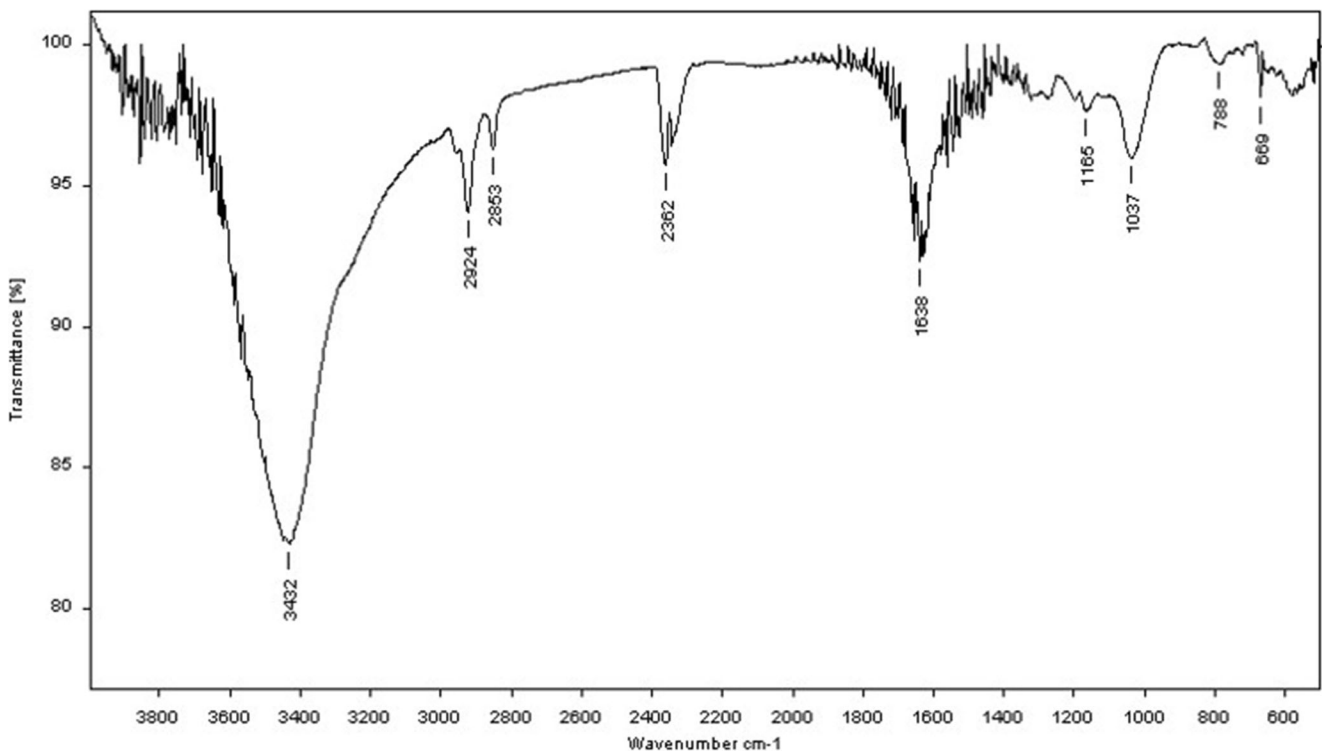
Fig. 3 Representative infrared spectrum of synthesized silver nano-particles

different treatments (Table 4). There was reduction in FDA activity in all treatments except control on day-7 soil sample. Reduction in FDA activity was observed in 20% AgNP treatment compared with other treatments across the duration. DHA did not vary among treatments on day-7 soil sample (Table 4). But there was reduction in DHA activity on day-7

Table 3 *In vitro* bio-efficacy of silver nano-particles (AgNPs) against rice diseases

Treatment name	*Rhizoctonia solani* (% inhibition)	*Helminthosporium oryzae* (% inhibition)	*Xanthomonas oryzae pv. oryzae* (growth inhibition in mm)
AgNPs at 1%	22.07[d]	19.25	3.50[d]
AgNPs at 2%	54.48[c]	34.13	4.00[d]
AgNPs at 5%	81.03[ab]	39.33	11.50[c]
AgNPs at 10%	100.00[a]	48.02	28.50[b]
AgNO₃ at 10%	64.25[bc]	28.28	11.00[c]
Streptomycin	–	–	31.00[a]
p value	< .0001	0.1745	< .0001
Tukey HSD at 5%	19.794	NS	2.3101

*with at least one letter common (a, b, c as superscripts) that is not statistically significant using Tukey's honestly significant difference (HSD) test at $p \leq 0.05$

NS non-significant differences among the treatments at $p \leq 0.05$

soil sample in AgNP treatments along with carbendazim and AgNO₃ treatments, whereas, treatments differed significantly on day-15 soil sample. Lowest DHA activity was observed in 20% AgNP treatment on day-30 soil sample. AgNPs at higher doses had negative effects on soil enzymes and MBC. As soil is elastic, it was recovered over time. On day 30, AgNP solution at 20% might impart negative effect on soil enzymes and MBC but AgNP solution at 10% was comparable to commercial fungicide, carbendazim.

In this experiment, AgNPs did not have any negative effect on soil physico-chemical properties like pH, EC, and organic carbon, but there are reports that higher doses of AgNPs could affect the soil physico-chemical properties (Hänsch and Emmerling 2010; Antisari et al. 2013). However, AgNPs had negative effects on the soil microbial biomass in this study. Higher doses of AgNPs had momentary negative effects on soil enzymes (Peyrot et al. 2014; Asadishad et al. 2018; Grün et al. 2018). The effect of AgNPs on the soil microbial properties was recovered over time. The extent of negative effect may vary according to microbial biomass and diversity of a particular ecosystem (Sillen et al. 2015). Soil properties like organic matter and clays can moderate the effect of AgNPs due to adsorption or complex formation of AgNPs with organic matter and clay, which shield the microbes from AgNPs (Peyrot et al. 2014; Asadishad et al. 2018; Schultz et al. 2018). This may be the reason behind absence of significant differences on microbial activity among the treatments and control 30 days after application of AgNPs.

Table 4 *In vivo* efficacy of silver nano-particles (AgNPs) against *Rhizoctonia solani* and non-target effect on soil microbial properties

Treatment	Efficacy against *Rhizoctonia solani* (% infection)	Fluorescein diacetate hydrolase (µg fluorescein g^{-1} dry soil h^{-1})				Dehydrogenase (µg triphenyl formazan g^{-1} dry soil h^{-1})				Microbial biomass carbon (µg g^{-1} soil)			
		0	7	15	30	0	7	15	30	0	7	15	30
20% AgNPs	3.4[c]*	3.10	2.81	3.20	3.36	2.30	2.12	1.91[b]	1.83	67.73	63.38[c]	67.46	67.91
10% AgNPs	4.3[bc]	3.15	2.91	3.39	3.45	2.24	2.19	1.97[ab]	2.04	65.39	64.42[c]	68.65	69.88
5% AgNPs	5.3[bc]	3.01	2.91	3.42	3.44	2.27	2.14	1.95[ab]	2.13	70.03	69.98[ab]	70.44	68.88
2.5% AgNPs	5.8[b]	3.03	2.91	3.36	3.38	2.22	2.10	1.98[ab]	2.08	66.42	66.56[bc]	70.75	73.21
AgNO$_3$ 10%	12.75[a]	3.15	2.91	3.40	3.46	2.27	1.97	1.92[b]	2.09	70.33	68.82[abc]	70.82	69.83
Carbendazim	1.22[d]	3.11	2.81	3.36	3.31	2.23	1.97	1.97[ab]	2.04	69.67	66.47[bc]	71.13	72.27
Control	13.89[a]	3.09	3.12	3.42	3.46	2.20	2.37	2.34[a]	2.34	68.18	73.28[a]	74.27	75.36
p value	0.042	0.941	0.709	0.072	0.497	0.815	0.103	0.041	0.124	0.816	0.001	0.411	0.575
Tukey HSD at 5%	1.333	NS#	NS	NS	NS	NS	NS	0.409	NS	NS	5.455	NS	NS

*with at least one letter common (a, b, c as superscripts) that is not statistically significant using Tukey's honestly significant difference (HSD) test at $p \le 0.05$

#*NS* non-significant differences among the treatments at $p \le 0.05$

Conclusions

The present study reports unique potential of purple color rice leaves to synthesize the AgNPs. This may be the first time we are reporting the purple color rice leaves with high content of total phenol, anthocyanin, and flavonoids to reduce the silver nitrate to AgNPs. The major advantages of the synthesis method are ease in use, eco-friendly, and economic. The size and related properties of synthesized AgNPs are very much suitable for its use as an effective antimicrobial agent. The AgNPs could control three rice pathogens and could be recommended as a broad spectrum rice fungicide. The negative effect of AgNPs depended on dose; the effective fungicidal doses did not have substantial differences in its effect on soil microbes compared with existing most popular fungicide, carbendazim. The novel and green synthesis of AgNPs will help further for its effective use as antimicrobial agent.

Availability of data and materials All data generated or analyzed during this study are included in this published article and its supplementary information files. For more information, the datasets used and/or analyzed during the current study can be obtained from the corresponding author on reasonable request.

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Compliance with ethical standards

Ethics approval and consent to participate Not applicable

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