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Indigenous green silver nano-particles

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Agricultural innovation is important to meet the growing food demand. Nanotechnology, in particular, has the potential to give effective solutions to a variety of agriculture-related issues. Nano-materials with attractive physio-chemical properties can be used to manage different pests. Silver nano-particles (AgNPs) are one of the most investigated nano-materials in agricultural sector. Different techniques of AgNPs synthesis have been reported. The reduction of silver ion from silver nitrate solution using a reducing biological agent is commonly used in the green synthesis of AgNPs. The reducing agent may be bacteria, fungi, plant or animal extracts. A wide range of metabolites from plant extracts can react with silver salts to prepare AgNPs. Silver nitrate can be reduced to AgNPs by primary metabolites such as carbohydrates, fats, and proteins, as well as secondary metabolites such as phenols, flavanoids, anthocyanins, alkaloids, and terpenoids. These molecules encapped the freshly generated AgNPs, preventing agglomeration and maintaining particle size uniformity. Green nanoparticle synthesis is desirable because it is ecologically benign, as it does not require hazardous solvents or a lot of energy, as other processes do, and it is simple and cost-effective for large-scale synthesis.

Rice leaf extract was previously avoided for the synthesis of silver nanoparticles because of its high silica concentration. There are rice genotypes with red, purple red and purple colour leaf sheaths beside normal green leaf sheaths. Purple colour rice genotypes having high anti-oxidants components like phenol, anthocyanins to reduce silver nitrate to silver nano-particles. Silver nano-particles could be used as fungicide, bactericide and anti-viral agents. It could be used to treat a variety of diseases in rice, including blast, sheath blight, and bacterial blight. These diseases impede rice production and new management methods are urgently needed. This technology proposes to synthesize AgNPs using an indigenous and simple approach for better rice disease management. Bio-safety is a major concern in nano-silver applications, and it has received a lot of attention in the literature. The non-target toxicity of AgNPs is also investigated to know it effects on soil microorganisms.

Synthesis

1. Collection of suitable rice genotypes

Rice genotypes namely, Kariglass 2014/1674 and Dodana dhan are the most effective in reducing $AgNO_3$ to AgNPs out of the 10 purple coloured rice germplasm tested.

2. Extraction of secondary metabolites

Five gram of cleaned, dried rice leaves (50 days after transplanting) is dispersed in 100 mL distilled water and the content should be heated at 70°C for 4 hours. After one hour of cooling at room temperature, the extracts should be filtered through Buchner funnel under suction and final volume should be made up to 100 mL for each extract.

3. Standardisation of reaction conditions

Silver nitrate solution (1mM in water) should be mixed with plant extract (silver nitrate: plant extract: 9:1 v/v) at room temperature. Incubation time should be maintained for 24 h.

4. Characterisation

Formation of AgNPs should be monitored based on change in absorbance using a UV–Vis spectrophotometer (Evolution 300, Thermo Scientific, USA). The synthesized AgNPs should be characterized using FTIR system, scanning electron microscope and particle size analyzer.

5. Bio-Efficacy

The anti-microbial activity of AgNPs is evaluated against three pathogenic fungi namely, *Magnaporthe grisea* (blast), *Rhizoctonia solani* (sheath blight), *Helminthosporium oryzae* (brown spot) and one pathogenic bacteria, bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). Poisoned food technique is preferred to test the efficacy of silver nano-particles against rice pathogens.

6. Non-target toxicity:

Crop phytotoxicity should be recorded 15 days after the application of AgNPs. Soil microbial biomass carbon (MBC), soil enzymes namely, fluorescein diacetate hydrolase and dehydrogenase should be checked for non-target toxicity.

Brief description of the technology:

Unique potential of purple colour rice leaves to synthesize the AgNPs is presented here. This may be the first time, we are reporting the purple colour rice leaves with high content of total phenol, anthocyanin and flavonoids to reduce the silver nitrate. The major advantages of the synthesis method are ease in use, eco-friendly and economic. The role of secondary metabolites on AgNPs formation and stabilization could be established. Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopic images of AgNPs and FTIR spectral analysis could confirm the presence of elemental silver and AgNPs may be encapped by biomolecules. The optimized reaction parameters for synthesis of AgNPs from silver nitrate are, a. 48 hours of incubation b. 9:1 (v/v) 1mM $AgNO_3$: plant extract c. room temperature 20-

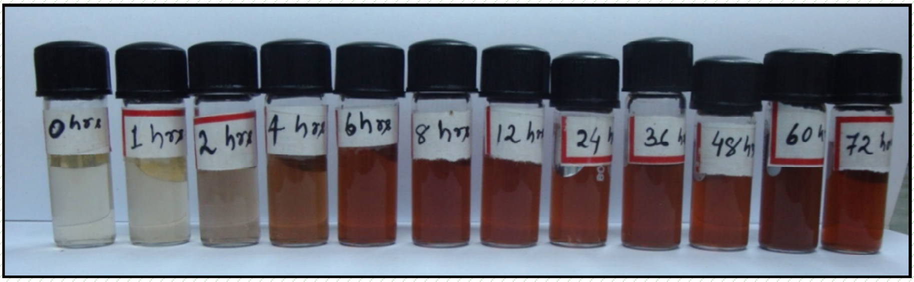

Fig1. Transformation in colour of synthesized Ag-NPs at different time intervals

30°C. Zeta potential and particle sizes of synthesized AgNPs are ranged between -16.61 to -29.45 mV and 36-107 nm, respectively at different time of incubation. The size and related properties of synthesized AgNPs are very much suitable for its use as an effective antimicrobial agent. AgNPs could control effectively *Rhizoctonia solani* and *Xanthomonas oryzae* pv. oryzae and *Helminthosporium oryzae*. 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 do not have substantial differences in its effect on soil microbes as compared to existing most popular fungicide, carbendazim. The novel and green synthesis of AgNPs will help further for its effective use as antimicrobial agent.

Salient features

- First time, use of purple colour rice leaves for silver nanoparticle synthesis
- Role of phenol, anthocyanin and flavonoids to synthesize silver nanoparticle
- Synthesis method is ease in use, eco-friendly and economic
- It could be recommended as a broad-spectrum rice fungicide
- Negative effects of AgNPs are comparable to existing fungicide, carbendazim

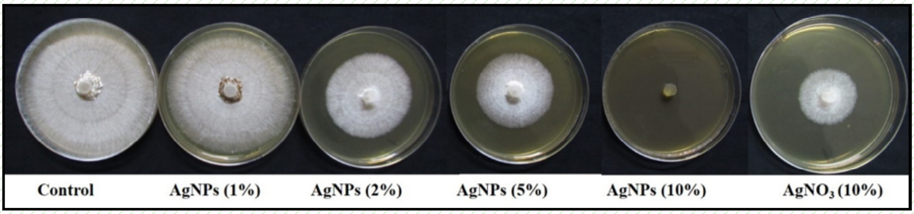

Fig 2. Efficacy of different concertation of 1mM AgNPs against *Rhizoctonia solani*

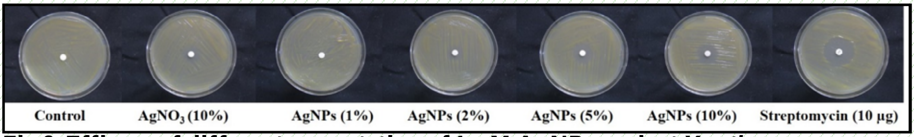

Fig 3. Efficacy of different concertation of 1mM AgNPs against *Xanthomonas oryzae pv. Oryzae*

Precautions

1. Synthesis time, plant extract and $AgNO_3$ ratio and temperature should always be uniform to get similar size nanoparticles
2. Efficacy depends on size and shape of the particles
3. Toxicity studies and field trial are yet to be undertaken

Country context of potential industry

Reduction of synthetic chemical pesticides and utilization of novel method is a priority in pest management. The limited choice of pesticides in sheath blight and bacterial blight management is a challenge. The offered technology will fulfil the needs of pest management issues. Pesticide manufacturers, start-up can take up the technology for upscaling.

Upscaling

Nano-pesticide can alter the dynamics of pesticide industry due to their higher efficacy and targeted action. Present study is a complete report of indigenous synthesis of silver nanopesticides, their efficacy and non-target actions. Unique, low-cost, environmental friendly AgNPs will have greater future.

FAQS

- ***Where from I will get the genotypes?***
  All purple coloured rice genotypes are available at ICAR-NRRI gene bank.
- ***What is the maximum time it takes to synthesis the AgNPs?***
  AgNPs formation starts at 4 hours. We standardised it at 24 hours at room temperature to achieve uniform size.
- ***Is the toxicity study undertaken?***
  No, we have not done yet. We did phytotoxicity and soil microbial toxicity with regards to few enzymes and soil microbial biomass carbon.
- ***Is the technology under patent?***
  No, it is not covered under patent. A simple MOU is required for technology transfer.
- ***Is any field study undertaken?***
  No, we did screenhouse experiment to understand the efficacy of the AgNPs.

N.B. - The technology is approved by the institute and uploaded in the Krishi Portal (201637146663129).

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