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Antibacterial activity of gold nanoparticle against bacterial leaf blight of rice

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Globally bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo)is one of the major biotic threats to rice production causingyield loss of 12-75 per cent. In the present study, biologically synthesized gold (Au) nano-particle (NP) from *Paederia foetida* was evaluated against Xoousing disc diffusion method. Among the various concentration of Au NP used for *in vitro* studies, 200 ppm concentration was highly effective in inhibiting the Xoo growth up to 54.22 %. This was followed by 100, 50, 10 ppm. No growth inhibition was observed when the Au NP was used at 1 and 5 ppm concentration. *In planta* assay was carried outin pots under glasshouse conditions with 200 ppm of Au NP. The percent disease incidence on Au NP applied rice plants was found decreased by 26% as compared to inoculated but unsprayed control. Besides, application of Au NP increased the yield and plant growth in compared to control. We further demonstrated that application of nanoparticles increased the concentration of secondary metabolites like phenols, flavonoids, terpenoids and total soluble sugars and improves the resistance power of the plant.

Keywords : Bacterial blight, disease management, gold nanoparticles, secondary metabolites, sustainable agriculture, *Xanthomonas oryzae*

INTRODUCTION

Rice is a major staple food crop in the Asian continent but the productivity of this crop suffers substantially due to diseases. Among the diseases, bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is major one that causes yield losses up to 50% (Willocquet *et al.* 2000). *Xoo* is gram negative, rod shaped, non-spore forming, monotrichous bacterium of size ranging from 0.55 x 3.5-2.17 μm. It thrives best at temperature 25-30 ^{0}C and pH 6.5-7.5 (Saha *et al.* 2015). At the onset of the infection, water-soaked lesions appear from the leaf tip and gradually progress from tip to downwards along the leaf margin.The diseased leaves later turn into straw-coloured with wavy borders. At the *kresek* phase, the leaves turn greyish green, wilt, roll up and then eventually the entire plant will dry or die.The disease is favoured by high relative humidity (83-93%), moderate temperature (25-34^{0}C), prolonged rainfall, cyclone, flood and windy or stormy condition. The disease is additionally aggravated by a high nitrogen dose, extreme shade, closer planting, and late top dressing.

Conventional approaches to control the disease include use of resistant varieties having race specific *Xa* genes, through clean cultivation by removing the weeds, providing good drainage condition, seed treatment with chemicals like streptocycline, copper oxychloride, agrimycin, and foliar application of mixture of streptocycline and copper oxychloride. However, most of the strategies have limitations and thereby becomes less effective after a period of time. The resistant cultivars get prone to be susceptible due to emergence of new races, frequent application of pesticides results in development of pest and disease resistance, accumulation of residues in agricultural produce and environmental pollution. Application of nanotechnology in crop protection holds a significant promise in management of

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plant diseases(Borah and Dutta, 2021; Goswami *et al*. 2021; Dutta, 2024). Biological agents such as plants and microbes have emerged as a more cost effective and efficient possibilities for green synthesis of nanoparticles than traditional chemical approaches.

Gold nanoparticles (Au NP), also known as colloidal gold which is widely used in the field of diagnostics and therapeutics. It is also employed in genomics, immunoassays and clinical chemistry. In the field of medical science, it is used to identify the pathogen and the disease, photothermolysis of microorganisms and cancer cells and site specific delivery of peptides or DNA (Mody *et al*. 2010). Au NP are biocompatible with human cells and can be easily conjugated with variety of biomolecules such as DNA, RNA, antibodies, polyethylene glycol, and other biomolecules (Shah *et al*. 2014)..In plants, Au NP improves seed germination, leaf number, leaf area, plant height, chlorophyll content, and sugar content, all of which contribute to higher crop yields (Arora *et al*. 2012; Gopinath *et al*. 2014). Amongst the nanoparticles, Au NP have both antibacterial and antifungal activity(Das and Dutta, 2021) which is size and shape dependant. Smaller the size of Au NP, more will be its ability to penetrate the bacteria and cause damage (Bindhu and Umadevi, 2014). It was reported that triangular Au NP show better antibacterial activity than round shaped Au NP (Ahmad *et al*. 2013). Au NP also exhibits antifungal activity (Ahmad *et al*. 2013) which is also shape and size dependant. Au NP causes intracellular acidification due to inhibition of H^+-ATPase activity ultimately leading to death (Wahi and Ahmad, 2013). In the present study, gold nanoparticles (Au NP) was used to control bacterial blight disease.

Experimental Procedure

Isolation of the pathogen

The pathogen *X. oryzae* pv. *oryzae* was isolated from the freshly infected leaf sample showing typical symptom of bacterial leaf blight of rice in Nutrient Agar media. Pure culture of the pathogen was maintained by transferring in fresh NA medium by single colony isolation method (Fig. 1).

Source of nanoparticles

Gold nanoparticle (size: 30 nm, shape: face centric cubic) synthesized from *Paederia foetida* was collected from the Department of Plant Pathology, AAU, Jorhat.

In vitro efficacy of gold nanoparticle (Au NP) against Xanthomonas oryzaepv. oryzae

In vitro evaluation was performed to check the efficacy of biologically synthesized Au NP against *X. oryzae* pv. *oryzae* at six different concentrations i.e. 1, 5, 10, 50, 100 and 200 ppm by disc diffusion method. The comparison was made with a recommended chemical i.e.1000 ppm Streptocycline and the observation was recorded on percent inhibition by using the following formulae (Padmapriya and Poonguzhali, 2015).

$$\text{Percent inhibition} = \left(\frac{\text{Zone of inhibton}}{\text{Diameter of petriplate}}\right) \times 100$$

Inoculation of pathogen and application of Au NP

Rice seedlings (cv. TN-1) were raised in soil mixed with manure (2.5 g farm yard manure, 2 g vermicompost), 0.11 g of Urea, 0.15 g of Single super phosphate and 0.04 g of Muriate of potash. After 30 days, the seedlings were transplanted to plastic pots of 5 kg capacity and grown in net house condition. Leaf clip method (Kauffman *et al*. 1973) was followed for inoculation of *X. oryzae*pv. *oryzae*. A pair of sterilized scissors was dipped in bacterial suspension (10^8 CFU mL^{-1}) prepared in sterile distilled water which was then used for clipping off the leaves approximately 2-3 cm from their tip. Sometimes the clipped leaves were immersed in the bacterial suspension to get more inoculum. The plants were covered with plastic bags to conserve moisture and incubated at 25-27ºC. Au NP at its best dose (200 ppm) were applied to the plants by spraying with the help of a sprayer. Observations were recorded on per cent disease incidence, plant growth parameters like effective tiller, plant height, yield, panicle length, root length, 1000 grain weight, total no. of grains per panicle, fresh and dry weight and secondary metabolites of plants.

Per cent disease incidence (PDI) was recorded using the following formula (Rao *et al.* 2016).

$$\text{PDI} = \left(\frac{\text{No. of infected plant}}{\text{Total no. of plant assesed}}\right) \times 100$$

Effective tiller (%) {ET} or Total Productive Tiller (%) {TPT} was recorded using the following formula (Kareem *et al.* 2013).

$$\text{ET or TPT} = \left(\frac{\text{No.of panicle bearing tillers}}{\text{No. of tillers per plant}}\right) \times 100$$

Estimation of vital secondary metabolites

To study the effect of Au NP (in challenged inoculated plants) on biochemical defence mechanism of rice, vital metabolite like phenol, flavonoid (Woisky and Salatino, 1998), terpenoid (Ghorai *et al*. 2017), total soluble sugar (Yemm and Willis, 1954) content was estimated.

RESULTS AND DISCUSSION

In vitro efficacy of Au NP against Xoo

Growth of *X. oryzae* pv*. oryzae* was significantly decreased with increase in concentration of Au NP from 10 ppm to 200 ppm (Table. 1, Fig. 2). Chemical check @ 1000 ppm streptocycline showed the highest zone of inhibition (61.10 mm with 67.89% of inhibition) however, when comparisons were made amongst the six concentrations of Au NP against *Xoo*, 200 ppm was found best in inhibiting the growth by 54.22%. This was followed by 100 ppm, 50 ppm, 10 ppm with per cent growth inhibition of 54.22%, 47.77%, 38.78% and 32.00% respectively.No growth inhibition was recorded at 1 ppm and 5 ppm concentration of Au NP found statistically at par with control. Antibacterial activity of Au NP against both gram positive (*Bacillus subtillis* and *Staphylococcus aureus*) and gram negative (*Klebsiella pneumonia* and *Escherichia coli*) bacteria were reported earlier (Shamaila *et al*. 2016). It was also reported that Au NP were more influential against Gram-negative bacteria because of their thin peptidoglycan layer. Similarly, Au NP synthesized from *Pergularia-daemia* was reported to possess antibacterial property against *E. coli*, *Pseudomonas aeruginosa* and *B. subtilis* at 300 μl/mL (Senthilkumar *et al*. 2017).Inhibitory effect of Au NP against bacterial pathogen might be due to increase in oxidative stress of microbial cells (Zheng *et al*. 2017) and attachment of Au NP to the membrane of the bacteria and there by disturbing the integrity of the cell (Tiwari and Lee, 2013).

Effect of Au NP on per cent disease incidence, yield and plant growth parameters.

Percent disease incidence was found decreased after application of Au NP in compared to inoculated control of *Xoo.* Per cent disease incidence of 26.00% was recorded where Au NP was applied in *Xoo* inoculated plants while 98.00% disease incidence were recorded in inoculated control of *Xoo* and application of only Au NP (without challenged inoculation of the pathogens) and absolute control showed no disease incidence (Fig.3). The highest effective tiller was recorded only in Au NP applied plants i.e. 49.33%. Effective tiller of 42.20% was recorded in Au NP applied *Xoo* inoculated plants (Table 2). This was followed by absolute control (34.46%) and lowest effective tiller was recorded in inoculated control (18.76%). Highest yield attributing characters were recorded in treatment where only Au NP was applied i.e. 72.00 q/ha yield, 1000 grain weight of 24.70g, and highest no. of grains per panicle of 88.00 (Table 2). This was followed 62.69 q/ha yield, 23.16 g 1000 grain weight, 87.40 no. of grains per panicle where Au NP were applied in *Xoo* inoculated plants. The lowest yield, 1000 grain weight and lowest no. of grains per panicle i.e. 34.46 q/ha, 20.14 g, 65.00 were recorded in pathogen inoculated control as compared to absolute control where yield recorded was 46.56 q/ha, 1000 grain weight recorded was 21.06 g and total no. of grains per panicle was 77.00.

Observation on plant height, panicle length, root length, fresh and dry weight of shoot and root follows the same trend (Table 2). The highest plant height of 81.56 cm, panicle length of 22.66 cm and root length of 23.04 cm were recorded in plants treated with Au NP, followed by application

Table 1: Effect of gold nanoparticle on growth of *Xanthomonas oryzae* pv. *oryzae*

Treatment	Zone of inhibition (mm)	Growth inhibition (%)
T_1: Control (*X. oryzae* pv.*oryzae* alone)	0.00	0.00 (0.95)^{f*}
T_2: 1 ppm Au NP + *X. oryzae* pv.*Oryzae*	0.00	0.00 (0.95)^{f**}
T_3: 5 ppm Au NP + *X. oryzae* pv.*Oryzae*	0.00	0.00 (0.95)^{f}
T_4:10 ppm Au NP + *X. oryzae* pv.*Oryzae*	28.80	32.00 (34.44)^{e}
T_5: 50 ppm Au NP + *X. oryzae* pv. *Oryzae*	34.90	38.78 (38.51)^{d}
T_6: 100 ppm Au NP + *X. oryzae* pv.*Oryzae*	43.00	47.77 (43.72)^{c}
T_7: 200 ppm Au NP + *X. oryzae* pv.*Oryzae*	48.80	54.22 (47.42)^{b}
T_8: 1000 ppm Streptocycline+*X. oryzae* pv.*Oryzae*	61.10	67.89 (55.48)^{a}
S.Ed (±)	1.09	1.18
CD (p=0.05)	2.24	2.43

* Data in parentheses are angular transformed value** Letter followed by same alphabets are statistically at par

of Au NP in*Xoo* inoculated plant. The lowest plant height, panicle length and root length were recorded in *Xoo* inoculated plant i.e. 64.94 cm, 16.37cm, 8.20 cm. Similarly, the highest fresh and dry weight of shoot (41.50 g, 13.20 g) and root (24.08 g, 3.66 g) were recorded where only Au NP was applied without the inoculation of *Xoo* followed by (30.52 g, 9.53 g) and (21.68 g, 2.72 g) respectively where Au NP was applied in*Xoo*inoculated plants. In case of absolute control, fresh and dry weight of shoot and root were (24.62 g, 9.32 g) and (14.53 g, 2.73 g) respectively. The lowest observation on fresh and dry weight of shoot (10.78 g, 6.02 g) and root (8.73 g, 1.35 g) were recorded in pathogen inoculated plants. Thus, it can be concluded that application of nanoparticles without the pathogen helps in improving the yield and plant growth parameters which may be due to better nutrient absorption by plant cells resulting in optimal plant growth (Benzon *et al.* 2015; Das and Dutta, 2022). In case of PDI, it was recorded lowest in the treatment, where highest secondary metabolites in the present study were recorded which might play a role in reducing the disease. Similarly application of biosynthesized nanoparticle was found to reduce the per cent disease incidence of faba bean, tomato and barley caused by *Fusarium oxysporium* (Elamawi and Al-Harbi, 2014; Das and Dutta, 2021, 2022).

Table 2: Effect of Au NP on per cent disease incidence, yield and plant growth parameters against *Xoo* causing bacterial leaf blight of rice

Treatment	Per cent Disease Incidence (PDI)	Effective tiller (%)	Yield (q/ha)	1000 grain weight (g)	Total no. of grains per panicle	Plant height (cm)	Panicle length (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T_1: Control (No pathogen, No NP)	0.00 (0.95)^{c**}	34.46 (35.94)^{c}	46.56^{c}	21.90^{b}	77.00^{b}	71.50^{c*}	17.51^{c}	16.36^{c}	24.62^{c*}	9.32^{c}	14.53^{c}	2.73^{b**}
T_2: *Xoo* alone	98.00 (81.86)^{a}	18.76 (25.62)^{b}	34.46^{d}	20.14^{c}	65.00^{c}	64.94^{d}	16.37^{d*}	8.20^{d}	10.78^{d}	6.02^{d}	8.73^{d**}	1.35^{c}
T_3: Au NP @ 200 ppm without *Xoo*	0.00 (0.95)^{c}	49.33 (44.60)^{a**}	72.00^{a}	24.70^{a}	88.00^{a**}	81.56^{a}	22.66^{a}	23.04^{a*}	41.50^{a}	13.20^{a}	24.08^{a}	3.66^{a}
T_4: Au NP @ 200 ppm + *Xoo*	26.00 (30.66)^{b}	42.20 (40.51)^{a}	62.69^{b}	23.16^{b**}	87.40^{a}	77.18^{b}	19.93^{b}	19.14^{b}	30.52^{b}	9.53^{b}	21.68^{b}	2.72^{b}
S.Ed (±)	2.47	3.36	0.67	0.61	2.70	1.51	0.26	0.57	1.15	0.33	0.66	0.14
CD(p=0.05)	4.13	6.00	1.34	1.28	5.46	3.13	0.54	1.19	2.33	1.19	1.34	0.29

* Data in parentheses are angular transformed value, **Letter followed by same alphabets are statistically at par

Estimation of vital secondary metabolites

Biochemical defence in rice against *Xoo*was recorded highest in plants treated with Au NP along with challenged inoculation of*Xoo*(Table 3). Highest content of the vital secondary metabolite i.e. phenol (19.47%), flavonoids (5.59 %), terpenoids (26.05 %), TSS (15.40 %) recorded when Au NP was used at 200 ppm. This was followed by application of Au NP alone without the pathogen where in phenol content recorded was 18.15 %, flavonoids was 4.23 %, terpenoids was 16.54 % and TSS was 12.38%. This was subsequently followed by inoculated control of *Xoo*where phenol content was 16.35 %, flavonoid 1.62 %, terpenoid 10.49 % and TSS was 11.04 %. However, the lowest secondary metabolites were recorded in absolute control i.e. phenol (14.68 %), flavonoids (0.57 %), terpenoids (9.92 %), TSS (9.94 %). Plant tend to activate and stimulate the anti-oxidant system that helps in the production of defence related compounds like phenols, flavonoids, terpenoids, TSS and protects the plant from adversities when treated with NP (Krishnaraj *et al*. 2012; Dutta *et al*. 2023). The effect of Zn NP on antioxidative system of potato plants was studied and found that foliar sprays of Zn NP at 100, 300 and 500 ppm concentration increased the total phenolics, anthocyanins, catalase, peroxidase, starch and total soluble sugars as compared to control plants (Raigond *et al*. 2017). Similarly effect of biosynthesized Ag NP on physiological parameters of *Vicia faba* infected by Bean Yellow Mosaic Virus were assessed and they reported that total phenolic content was lowest in pathogen inoculated plant and highest in leaves treated with Ag NP (Elbeshehy *et al*. 2014).

CONCLUSION

Results from the present study showed that Au NP synthesized from *Paederia foetida* is an effective antibacterial agent which could give a lead way in controlling plant pathogenic bacteria like *X. oryzae* pv. *oryzae*. Apart from combatting the disease, it also helps in increasing the yield

Table 3: Vital secondary metabolites in control, inoculated and treated plants

Treatment	Phenol (%)	Flavonoid (%)	Terpenoid (%)	TSS (%)
T_1: Control (No pathogen, No NP)	14.68 (22.52)^{f*}	0.57 (4.20)^{g}	9.92 (18.34)^{g}	9.94 (18.37)^{g}
T_2: *Xoo* alone	16.35 (23.84)^{e**}	1.62 (7.30)^{f}	10.49 (18.89)^{f}	11.04 (19.40)^{f}
T_3: Au NP @ 200 ppm without *Xoo*	18.15 (25.21)^{d}	4.23 (11.86)^{d}	16.54 (23.98)^{d}	12.38 (20.59)^{d}
T_4: Au NP @ 200 ppm + *Xoo*	19.47 (26.17)^{b}	5.59 (13.66)^{b}	26.05 (30.68)^{b}	15.40 (23.11)^{b}
S.Ed (±)	0.143	0.291	0.167	0.089
CD (p=0.05)	0.297	0.605	0.347	0.185

* Data in parentheses are angular transformed value **Letter followed by same alphabets are statistically at par

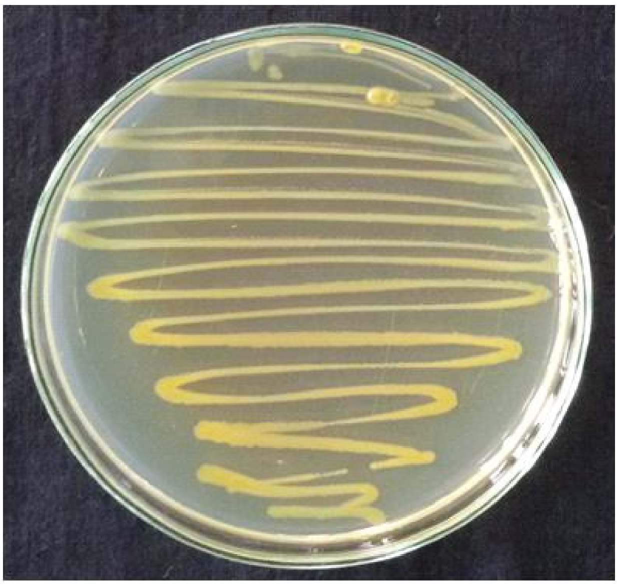

Fig. 1: Pure culture of *Xanthomonas oryzae* pv. *oryzae* in nutrient agar plate

and growth parameters as well as enhancing the biochemical defence mechanism thereby reducing the disease incidence of bacterial leaf blight of rice.

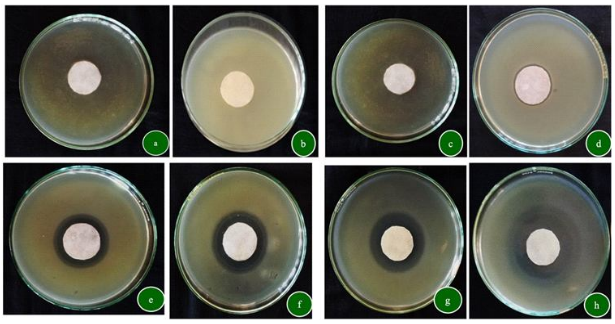

Fig.2: (a-h)*In vitro* efficacy of Gold nanoparticle at different concentration against the *Xanthomonas oryzae* pv. *Oryzae* (Growth of *Xanthomonas oryzae* pv. *oryzae* in a. Control, b. 1 ppm Au NP, c. 5 ppm Au NP, d. 10 ppm Au NP, e. 50 ppm Au NP, f. 100 ppm Au NP, g. 200 ppm Au NP, h. 1000 ppm Streptocycline**)**

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Fig 3. (a-e). Effect of Gold nanoparticle on bacterial leaf blight of rice. a. General view of the experiment, b. Control (No Pathogen, No NP), c. *Xanthomonas oryzae*pv. *oryzae* alone, d. Au NP @ 200 ppm without *X. oryzae* pv. *oryzae*, e.Au NP @ 200 ppm + *X. oryzae* pv. *oryzae*

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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