

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.610.211>

Green Nanoparticles - A Novel Approach for the Management of Banana Anthracnose Caused by *Colletotrichum musae*

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ABSTRACT

Keywords

Green nanoparticles,
Neem, Banana
anthracnose,
Colletotrichum musae,
Disease management,
*PDI: Per cent disease
index.

Article Info

Accepted:
17 September 2017
Available Online:
10 October 2017

Anthraco­nose of banana is caused by *Colletotrichum musae* and is one of the most serious diseases of ripe banana. Plant-mediated biological synthesis of nanoparticles is gaining importance in recent years due to its simplicity and eco-friendliness in the management of plant diseases. Present investigation was carried out during 2015-16 in department of plant pathology, UAS, Dharwad on management of banana anthracnose by using green nanoparticles. Efficacy of green nanoparticles (ajwain and neem leaf extract) of copper, silver, nickel and magnesium has been studied *in vitro* at different concentrations against *C. musae*. Silver-Neem nano particles (Ag-Neem Nps) were tested *in vivo* for their bio-efficacy against anthracnose as postharvest spraying at 0.01, 0.02, 0.05, 0.1 and 0.2 per cent concentrations. *In vitro* studies revealed that Ajwain-Mg NPs, Ajwain-Ni NPs at 0.2 per cent and Neem-Ag NPs at 0.1 and 0.2 per cent have shown complete inhibition of spore germination of *C. musae*. Spraying of Ag-Neem np at 0.2 per cent concentration recorded lowest disease severity (6.67 PDI)* which was on par with 0.1 and 0.05 per cent concentrations (8.89 PDI) and.

Introduction

Banana is one of the most popular fruits in India and many tropical countries and important dessert fruit in India. Major economic part of the banana plant is the fruit, suffers from many postharvest diseases. Anthracnose of banana is caused by *Colletotrichum musae* and is one of the most serious diseases of ripe banana. Symptoms of anthracnose include black and sunken lesions with spore masses or acervuli in the lesion. This has been a major constraint in the marketing of fruit intended for local as well as distant markets. Control of postharvest diseases has been traditionally achieved by pre and postharvest applications of

fungicides. However, at present situation producers have been compelled to seek alternative methods due to increased global demand for chemical-free fresh produce (Korsten, 2006) and due to issues like development of resistance in plant pathogens against currently used fungicides. Plant pathologists made a late start in harnessing the benefits of nanomaterials for management of plant pathogens. There have been some exciting results obtained especially in plant disease management aspects concerning fungi, bacteria and flowering plant parasite by developing nanoparticles of different metals, pesticides and growth promoters (Banik and

Sharma, 2011). Biogenic silver nanoparticles (Ag NPs) are proved to be potential antimicrobial agents against an array of pathogens [Elumalai and Vinothkumar, 2013]. In present study, Green nanoparticles of silver, copper, nickel and magnesium were evaluated at different concentrations for inhibition of mycelial growth and spore germination of *C. musae*. Postharvest spraying on fruits with Neem-Ag nanoparticles was also investigated for efficient disease management.

Materials and Methods

***In vitro* evaluation of green nanoparticles**

Agar well diffusion method

Present investigation was carried out during 2015-16 in department of plant pathology, University of Agricultural Sciences, Dharwad on management of banana anthracnose by using green nanoparticles. Green nanoparticles (68 nm) of different metals (copper, silver, nickel and magnesium) synthesized from ajwain and neem leaf extracts were screened for antifungal activity at different concentrations by agar well diffusion method according to Bobbaralal *et al.*, (2009) Procedure with modifications. The synthesized green nano particles were obtained from nanotechnology laboratory, University of agriculture sciences, Dharwad. Seven day old cultures grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (100 µl) of inoculums was introduced to molten PDA and poured in to a Petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar wells created, 30 µl nanoformulations of the test concentrations were loaded by using micropipet. Plates were incubated at $25 \pm 2^{\circ}\text{C}$ for 48-72 hr and inhibition zone around the

wells in the plates. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the wells filled with nano particles. Sterile water and a standard fungicide served as checks, the experiment was carried out in triplicates.

Inhibition of spore germination

Conidial suspension of *C. musae* was prepared in sterile water. Green nanoparticles from the stock were diluted in sterile water to get the required concentration; both spore suspension and green nanoparticles of different concentrations were mixed together in a cavity slide in equal proportions. Slides were incubated under room temperature for 24 hr in moist chambers. The observations on number of spores germinated and total number of spores in each microscopic field was recorded under compound microscope. Sterile water/fungicide/plant extract served as control. Per cent inhibition of spore germination was calculated by formula given by Vincent (1947) and data was later analysed statistically.

***In vivo* evaluation of green nano particles**

Fruits selected for *in vivo* studies were at mature green stage, free from blemishes and injuries. Banana fingers with intact crown portion were sterilized in 1.0 per cent sodium hypochlorite and rinsed with sterilized distilled water. Neem-silver nanoparticles were evaluated at different concentrations (0.01, 0.02, 0.05, 0.1 and 0.2 % v/v) as postharvest spray on fruits. Spray of AgNO_3 at 0.017 per cent, neem leaf extract at 20 per cent and sterilized water served as controls. Spore suspension of *C. musae* (1×10^5 spores/ml) was sprayed on the treated fruits and kept for incubation in moist chambers at room temperature. Observations were recorded on per cent area infected on fruit in 0-5 scale i.e. [0]- No symptoms on fruit

surface, (1) 0.1 – 5 % area covered by lesions, (2) 5.1 – 10 % area covered by lesions, (3) 10.1 – 25 % area covered by lesions, (4) 25.1 – 50 % area covered by lesions and (5) 50 % of area covered by lesions after eight days of inoculation. Per cent disease index was computed by using the formula given by Wheeler (1969) and analysed statistically. Per cent disease reduction in different treatments over control was derived by using the formula suggested by Vincent.

Statistical analysis

The experiment was laid out in a completely randomized design (CRD) with treatments in different concentrations in three replications. The experiment was conducted at room temperature in the laboratory. Statistical analysis was done as per the procedures given by Gomez and Gomez (1984). All comparisons of means were subjected to analysis of variance (ANOVA) and the significant differences among treatments were determined with a least significant difference (Lsd) separation test. Arcsine or square root transformation was used wherever required to normalize variance.

Results and Discussion

***In vitro* evaluation of green nanoparticles**

Green nanoparticles of silver, copper, nickel and magnesium were evaluated at different concentrations for inhibition of mycelial growth and spore germination of *C. musae* and results are shown in Table 1 and Fig 1. Green nanoparticles of silver, copper, nickel and magnesium have not considerably inhibited the mycelial growth however; spore germination inhibition was appreciable *in vitro* condition. Most of the treatments could show significant inhibition of spore germination. Ajwain-Mg NPs, Ajwain-Ni NPs (@ 0.2 %) and Neem-Ag NPs (@ 0.1 & 0.2 %) have shown complete inhibition which

was statistically at par with Ajwain-Ni NPs (99.05 %) and Ajwain-Mg NPs (97.09 %) at 0.1 per cent concentration. Metal nanoparticles probably cause damage on spores due to high penetration activity. Supporting to the current findings, antifungal property of ‘Chitosan-silver nanoparticle-composite as reported by Lamsal *et al.*, (2011) against mango anthracnose pathogen *C. gloeosporioides* (Chowdappa *et al.*, 2014). Similar reports were made in another *in vitro* assay of silver nanoparticle (WA-PR-WB13R [PR]) against isolates of *Colletotrichum* sp. These results have also validated the report of Aguilar-Méndez and co-workers (2011) who suggested that silver nanoparticles can significantly reduce the mycelial growth of *C. gloeosporioides* under *in vitro* condition.

***In vivo* evaluation of green nanoparticles**

Bioefficacy of silver neem-silver nanoparticles was investigated as postharvest spray on the fruits and results (Table 2 & Fig 2) indicated that all the treatments (0.2%, 0.1%, 0.05%, 0.02% and 0.01 % NeemAg-np) exhibited effective disease reduction over control, maximum disease reduction was found in fruits treated with 0.2 percent NeemAg-np (91.43%) (6.67 PDI) which was on par with 0.1 and 0.05 per cent NeemAg-np in which 88.57 per cent disease reduction (8.89 PDI) was noticed. NeemAg-np was found to be effective even at lower concentrations (0.02% and 0.01%) as well, showing 82.86 per cent disease reduction. Neem leaf extract (20.0% concentration) could give only 37.14 per cent disease reduction. Meanwhile, AgNO₃ showed 80 per cent disease reduction. Silver nanoparticles might have caused deformities in spores and the shape of hyphal walls. This is consistent with the findings of Lamsal and coworkers [2011] where in the efficacy of silver nanoparticles against pepper anthracnose (*C. gloeosporioides*) was well demonstrated at 50 ppm to prevent the disease outbreak.

Table.1 *In vitro* evaluation of green nano particles against *Colletotrichum musae*

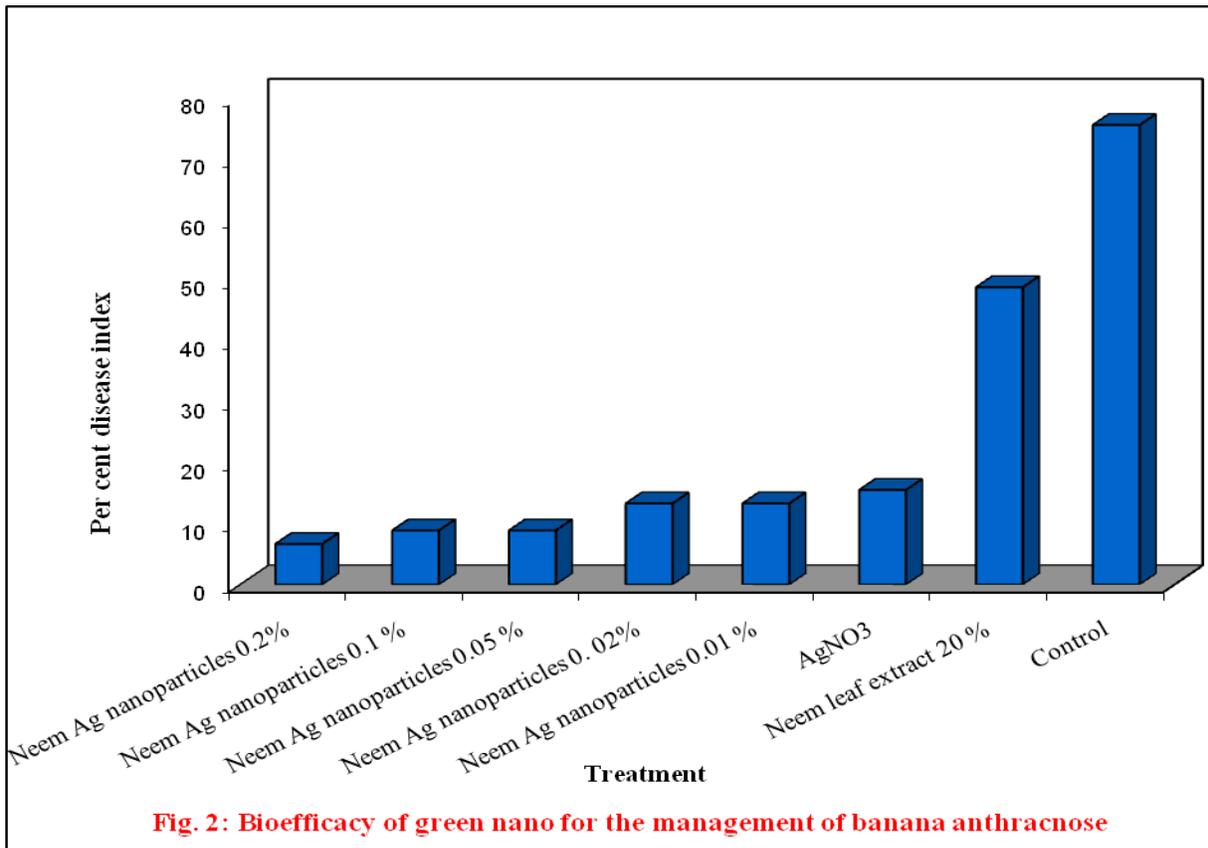
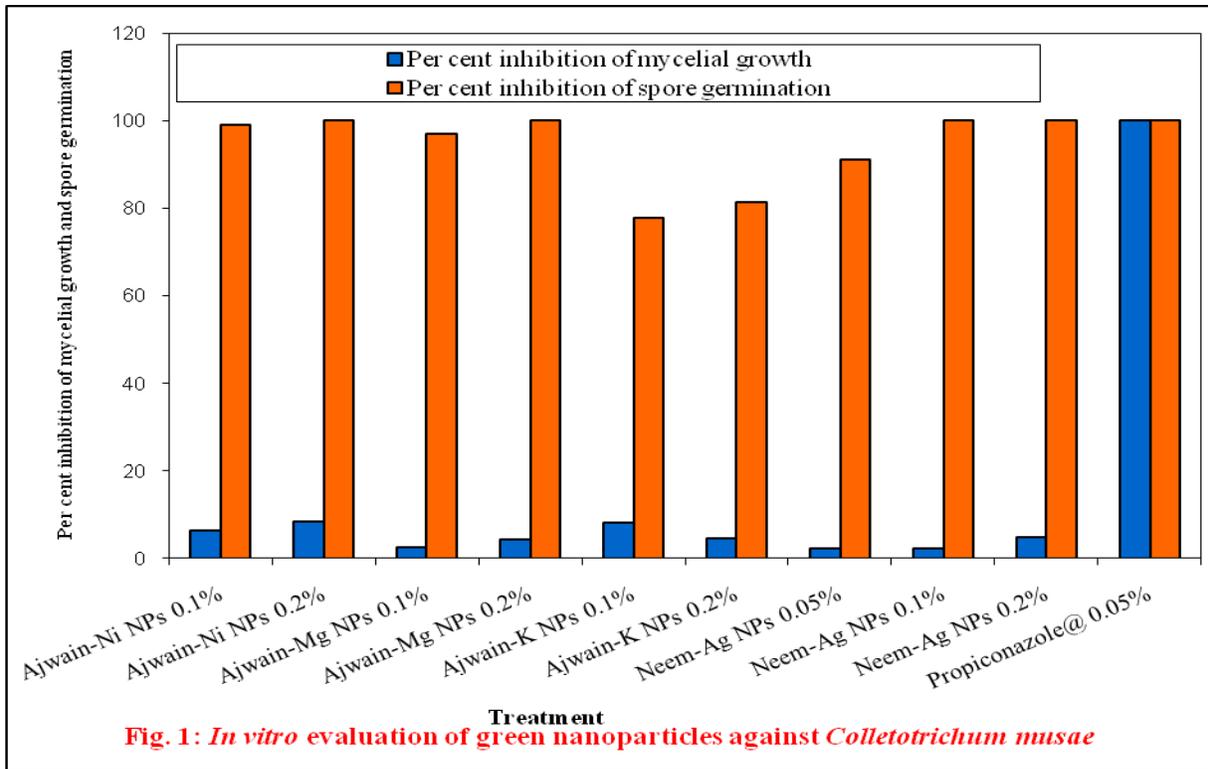
Treatment	Concentration (%)	Per cent inhibition of mycelial growth	Per cent inhibition of spore germination
Ajwain-Ni NPs	0.1	6.37 (14.60) *	99.05 (86.76)*
Ajwain-Ni NPs	0.2	8.46 (16.91)	100.00 (90.00)
Ajwain-Mg NPs	0.1	2.47 (9.04)	97.09 (82.06)
Ajwain-Mg NPs	0.2	4.35 (12.03)	100.00 (90.00)
Ajwain-K NPs	0.1	8.09 (16.52)	77.76 (61.95)
Ajwain-K NPs	0.2	4.64 (12.43)	81.29 (64.43)
Neem-Ag NPs	0.05	2.32 (8.75)	90.98 (72.60)
Neem-Ag NPs	0.1	2.32 (8.76)	100.00 (90.00)
Neem-Ag NPs	0.2	4.87 (12.73)	100.00 (90.00)
Propiconazole@ 0.05%	0.1	100.00 (90.00)	100.00 (90.00)
S.Em±		0.35	1.31
CD at 1%		1.39	5.28
CV %		2.97	2.40

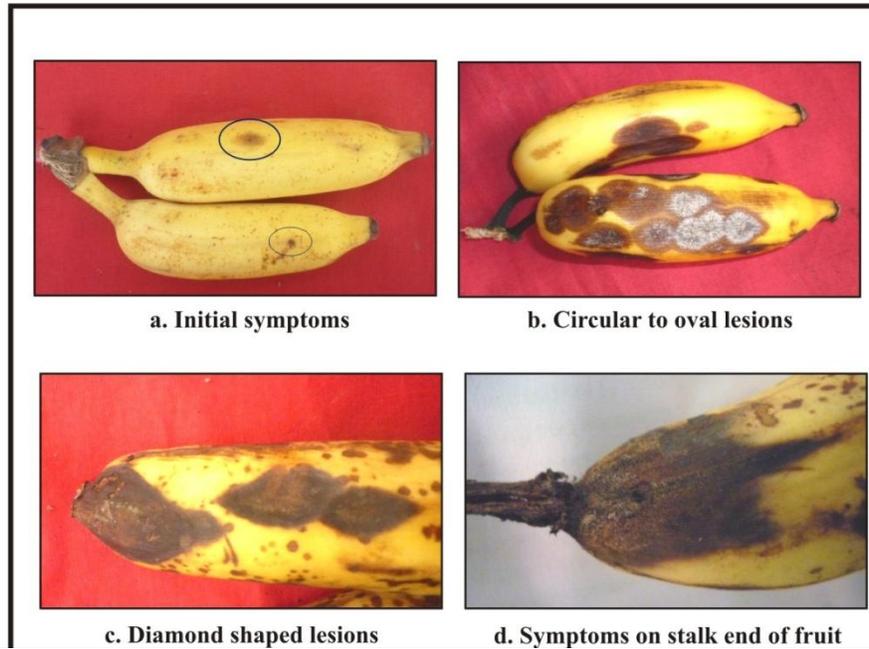
*Arcsine transformed values

Table.2 Bioefficacy of green nano for the management of banana anthracnose

Treatment	Per cent disease index
Neem Ag nanoparticles @ 0.2%	6.67 (14.96) *
Neem Ag nanoparticles @ 0.1 %	8.89 (17.35)
Neem Ag nanoparticles @ 0.05 %	8.89 (17.35)
Neem Ag nanoparticles @ 0.02%	13.33 (21.42)
Neem Ag nanoparticles @ 0.01 %	13.33 (21.42)
AgNO ₃ @ 0.017%	15.56 (23.23)
Neem leaf extract @ 20 %	48.89 (44.36)
Control	75.56 (61.88)
S.Em±	1.28
CD at 5%	3.88

*Arcsine transformed values





Symptoms of banana anthracnose caused by *Colletotrichum musae*

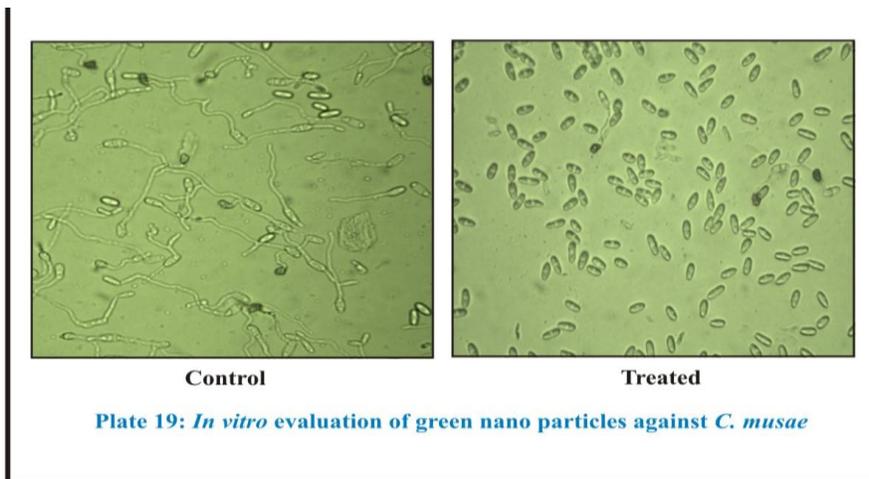


Plate 19: *In vitro* evaluation of green nano particles against *C. musae*

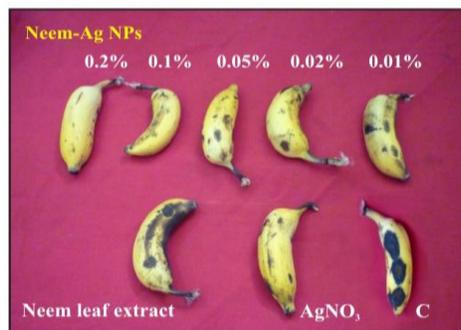
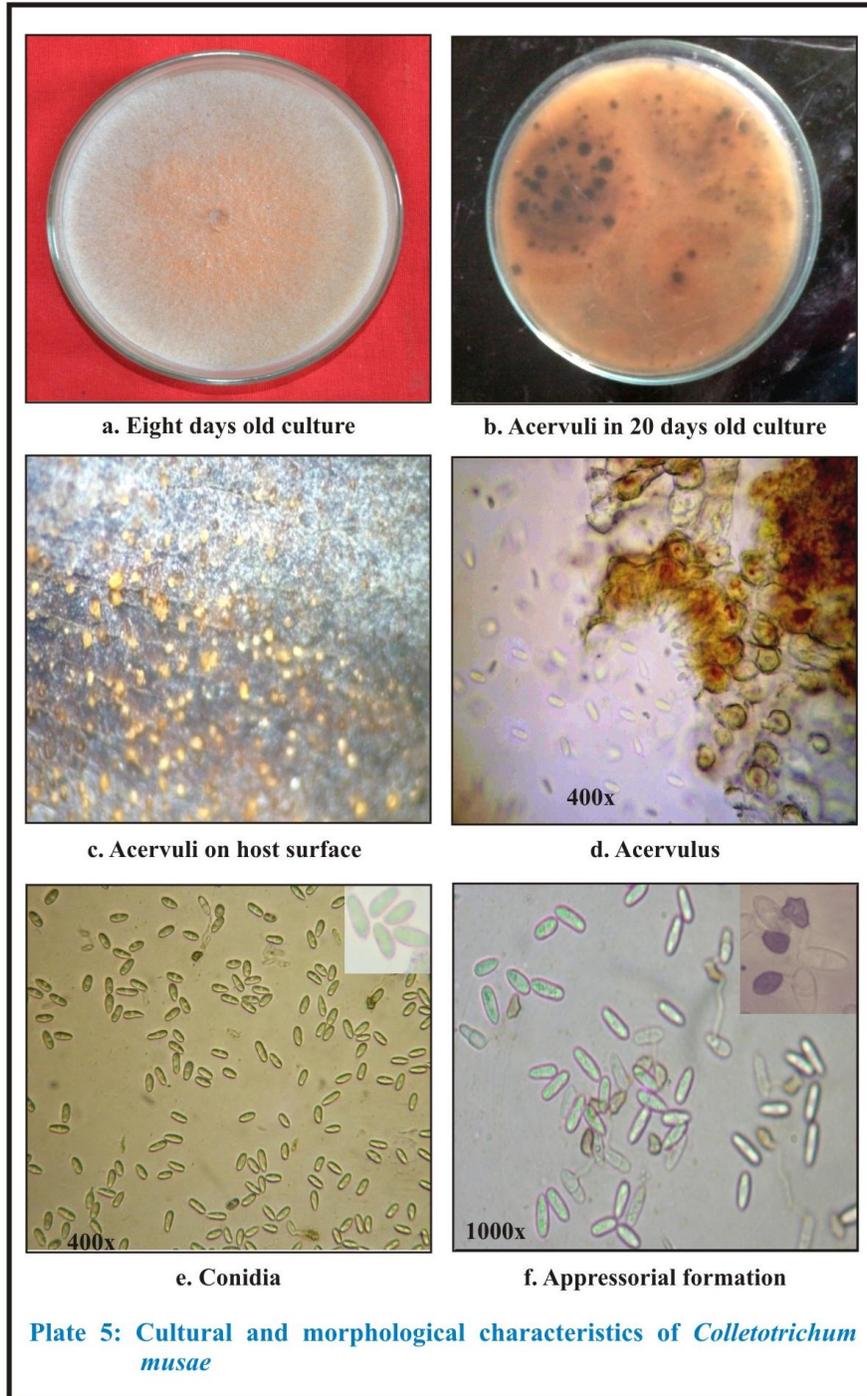


Plate 24: Bioefficacy of green nano particles against Anthracnose



Mode of action of nanoparticles was explained by different researchers. Samuel and Guggenbichler (2004) shown that the nanoparticles efficiently penetrate into microbial cells, which implies lower concentrations of nano-silver would be sufficient for microbial control.

The present study emphasizes the use of plant material for the synthesis of AgNPs with antifungal effect. It is clear that green nanoparticles have a great potential even at very low concentrations in the management of post-harvest anthracnose and future research may have to focus on the consumer

acceptability of nano treated fruits in the market.

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How to cite this article:

Divya Jagana, Yashoda R. Hegde and Rajasekhar Lella. 2017. Green Nanoparticles - A Novel Approach for the Management of Banana Anthracnose Caused by *Colletotrichum musae*. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 1749-1756. doi: <https://doi.org/10.20546/ijcmas.2017.610.211>