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In vitro Evaluation of the Antifungal Effect of Nanoparticles against *Fusarium sacchari* causing Pokkah Boeng Disease of Sugarcane

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ABSTRACT: Nanomaterials can contribute to the formulation of new, sustainable and effective fungicides for the control of fungi such as *Fusarium sacchari* causing Pokkah boeng disease of sugarcane. The present investigation was undertaken to screen different concentrations of engineered nanoparticles in the management of *Fusarium sacchari*. In this work, various engineered nanoparticles were evaluated at four different concentrations (50, 100, 150 and 200 ppm) through poisoned food technique to determine their antifungal activity in inhibiting the mycelial growth of *Fusarium sacchari*. The cent per cent mycelial growth inhibition was noticed with silver nanoparticles at 200 ppm concentration which was significantly superior over other nanoparticles tested. Whereas, the least mycelial growth inhibition was noticed in alumina (71.18 %) followed by titanium dioxide nanoparticles (74.71 %). According to our findings, the application of silver nanoparticles could be a viable alternative for the control of Pokkah boeng disease or even be integrated into novel disease management strategies.

Keywords: *Fusarium sacchari*, nanoparticles, poison food technique, mycelial growth inhibition.

INTRODUCTION

The sugarcane is one of the most important cash crop and bioenergy crops in India, but there are many fungal pathogens that affect it. Pokkah boeng (PB) is caused by *Fusarium sacchari* (E.J. Butler & Hafiz Khan) W. Gams, a fungal pathogen that causes twisting in young leaves and spindle changes, severely affecting growth and productivity of the cane crop. As a result of the severity of the disease, many commercial varieties were lost (Vishwakarma *et al*., 2016; Viswanathan *et al*., 2017; Viswanathan, 2020). Various agrochemicals have been tested in order to solve these problems, but the use of these chemicals can result in harmful side effects such as resistance, environmental degradation, and risks to human and animal health (Ashajyothi *et al*., 2016; Ballari *et al*., 2017).

Using nanotechnology to fabricate small particles below 100 nanometer is an alternative to the problems described above (Lira-Saldivar *et al*., 2018; Singh *et al*., 2019). Managing sustainable agriculture with nanoparticles can provide an excellent alternative, as well as increasing agri-food productivity, maintaining adequate levels of nutrients in agricultural products, as well as reducing the use of herbicides, fertilizers and pesticides (Beyene *et al*., 2017; Singh *et al*., 2019), which contain harmful chemical compounds. There is variety of applications for nanoparticles due to their chemical, physical and optical properties.

All this implies that there is an opportunity to investigate the antifungal effect of nanoparticles against plant diseases (Macías Sánchez *et al*., 2023). In order to control phytopathogens in agriculture, more sustainable alternatives must be investigated. There have been several studies reporting antifungal activity of nanoparticles against *Fusarium oxysporum*, but fewer studies have been conducted on *F. sacchari*. Therefore, an *in vitro* study was performed to evaluate the antifungal effect of nanoparticles against *Fusarium sacchari*, a fungus that causes Pokkah boeng disease of sugarcane.

MATERIALS AND METHODS

The antifungal activity of commercially available engineered nanoparticles from Shanghai Richem International Co., Ltd. was evaluated against *F. sacchari* using poisoned food technique (Macías Sánchez *et al*., 2015). Nanoparticles of known concentration (50, 100, 150 and 200 ppm) were added to melted potato dextrose agar (PDA), and about 20 ml of poisoned medium was added to each sterilized Petri plate. Afterwards, the plates were inoculated with agar discs of 5 mm diameter containing mycelia of *F. sacchari* (precultured for 7 days at 28 ± 2 °C). Suitable check was maintained without addition of nanoparticles. In each treatment, three replicas were run and after the colony reached maximum growth in control plates, the diameter of the colony was measured. The growth inhibition per cent was calculated using Vincent's formula (1947).

$$\text{Percent inhibition of mycelial growth} = \frac{\text{Growth of mycelium in control} - \text{Growth of mycelium in treatment}}{\text{Growth of mycelium in control}} \times 100$$

For analysing the experimental data, arcsin angular transformations were made and the data analyzed with ANOVA in factorial completely randomized design to test for its significant difference. There were significant differences in mycelial growth inhibition at 1% level of significance ($p<0.01$) for different nanoparticles used in the study.

RESULTS AND DISCUSSION

In the present experiment, five commercially available engineered nanoparticles were evaluated against *F. sacchari* and results obtained are presented in Table 1, Plate 1 and Fig. 1. Five test nanoparticles reduced the mycelial growth at 50, 100, 150 and 200 ppm concentrations. At 50 ppm concentration, maximum mycelial growth inhibition was noticed in silver (67.06 %) which was significantly superior over other nanoparticles tested followed by zinc oxide with 51.41 per cent mycelial growth inhibition. The least mycelial growth inhibition was noticed in alumina (32.94 %) followed by silicon carbide (39.53 %).

At 100 ppm concentration, maximum mycelial growth inhibition was noticed in silver (72.94 %) which was significantly superior over other nanoparticles tested. Next best was titanium dioxide (63.18 %) followed by silicon carbide (61.18 %) whereas, least mycelial growth inhibition was noticed in alumina (46.71 %). At 150 ppm concentration, significantly highest mycelial growth inhibition was noticed in silver (81.18 %) compared to other nanoparticles tested. Next best was titanium dioxide (70.00 %) followed by silicon carbide (69.41 %) whereas, least mycelial growth inhibition was noticed in alumina with 61.18 per cent. At 200 ppm concentration, cent per cent mycelial growth inhibition was noticed in silver which was significantly superior over other nanoparticles tested followed by silicon carbide with 81.76 per cent mycelial growth inhibition. The least mycelial growth inhibition was noticed in alumina (71.18 %) followed by titanium dioxide (74.71 %). Irrespective of nanoparticles concentrations tested, silver (80.30 %) found to be the best in inhibiting the mycelial growth of *F. sacchari* and it was significantly superior over other nanoparticles tested however, mean least mycelial growth inhibition was noticed in alumina (53.00 %).

In several studies, smaller silver nanoparticles generate more toxicity by being absorbed into cells owing to their intracellular permeability (Zhang *et al*., 2014; Qing *et al*., 2018; Lekamge *et al*., 2020). The results of the present study are consistent with other studies that have shown silver nanoparticles to have antifungal properties. It was observed that, silver nanoparticles at 200 ppm concentration showed 100% inhibition like that found in higher concentrations in the literature. For example, Ashraf *et al*. (2020) found that 80-100 mg/L has 85-90% inhibition over the *Fusarium oxysporum* f.sp. *lycopersici* with silver nanoparticles (AgNPs). Kim *et al*. (2012) obtained 94.10 % inhibition on the same fungus but at a concentration of 100 mg/L of commercial AgNPs that were 7-25 nm in particle size. As a result, engineered silver nanoparticles exhibit a comparable antifungal effect on *Fusarium sacchari* to previous studies. These were similar to work of Ashajyothi *et al*. (2016) who reported 72.8% of inhibition to AgNPs at 60 mg/L over *F. oxysporum* MTCC 284 (from an Indian collection).

Metal-protein interactions in cells make it difficult to study the antifungal mechanism of AgNPs. There has been speculation that AgNPs have antifungal effects on Fusarium, destabilizing the cell membrane and enzymes, resulting in the death of the cell membrane. Besides their small size, silver ions also interact with DNA and stop replication (Kim *et al*., 2012). Certain genes associated with carbohydrate, fatty acid, amino acid and nucleotide metabolism that were not expressed resumed their expression after exposure to AgNPs. AgNPs are energy-intensive, so it costs a high level of energy for the cell to survive the stress from them (Shen *et al*., 2020).

Table 1: Efficacy of nanoparticles on inhibition of mycelial growth of *Fusarium sacchari.*

Sr. No.	Nano chemicals	Mycelial growth in diameter (cm)				Mean	Mycelial growth inhibition (%)				Mean
		Concentrations					Concentrations				
		50ppm	100ppm	150ppm	200ppm		50ppm	100ppm	150ppm	200ppm	
1.	Zinc oxide (~30 nm)	4.13	3.80	3.20	1.65	**3.20**	51.41 (45.81) *	55.29 (48.04)	62.35 (52.15)	80.59 (63.86)	**62.41**
2.	Silver (<90 nm)	2.80	2.30	1.60	0.00	**1.68**	67.06 (54.97)	72.94 (58.66)	81.18 (64.29)	100.00 (90.00)	**80.30**
3.	Alumina (15 nm)	5.70	4.53	3.30	2.45	**4.00**	32.94 (35.03)	46.71 (43.11)	61.18 (51.46)	71.18 (57.53)	**53.00**
4.	Silicon carbide (~50 nm)	5.14	3.30	2.60	1.55	**3.15**	39.53 (38.96)	61.18 (51.46)	69.41 (56.42)	81.76 (64.72)	**62.97**
5.	Titanium dioxide (~7 nm)	4.53	3.13	2.55	2.15	**3.09**	46.71 (43.11)	63.18 (52.64)	70.00 (56.79)	74.71 (59.81)	**63.65**
6.	Control	8.50				**8.50**	-	-	-	-	-
		SE.m. ±		C.D. (P=0.01)			SE.m. ±		C.D. (P=0.01)		
Nano chemicals (N)		0.07		0.27			0.52		1.99		
Concentration (C)		0.06		0.22			0.46		1.78		
N × C		0.14		0.54			1.04		3.97		

*Angular transformed values

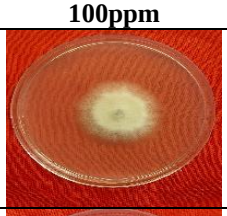
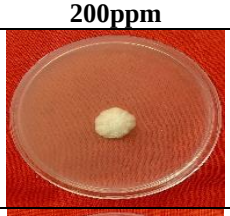
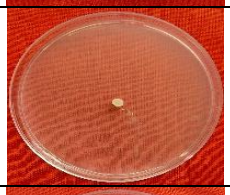
Nanoparticles	Concentrations			
	50ppm	100ppm	150ppm	200ppm
Zinc oxide				
Silver				
Alumina				
Silicon carbide				
Titanium dioxide				
Control				

Plate 1: Mycelial growth inhibition of *Fusarium sacchari* in different nanoparticles.

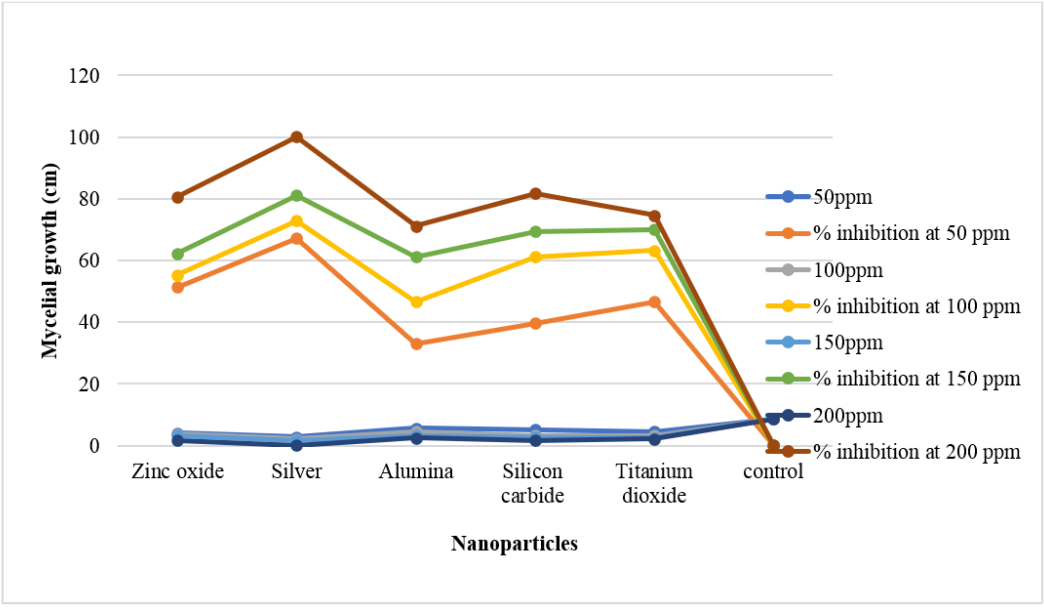


Fig. 1. *In vitro* efficacy of nanoparticles on mycelial growth of *Fusarium sacchari.*

CONCLUSIONS

Among five commercially available engineered nanoparticles tested, cent per cent mycelial growth inhibition was noticed with silver followed by silicon carbide (81.76 %) whereas, least inhibition was observed with alumina (71.18 %) at 200 ppm concentration. It is possible to use silver nanoparticles as fungicides to control *Fusarium sacchari* with relative safety. Despite this, regulation of nanoparticle use is still pending among the scientific community. It is essential to conduct more research into the toxic potential of silver nanoparticles and the possible risks they pose. A further assessment of their fungicide potential would require additional *in vivo* research.

FUTURE SCOPE

A variety of engineered nanoparticles are now being developed and available worldwide for the use as a viable alternative to fungicidal formulations. The present finding is based on results of *in vitro* experiments. Therefore, practical applicability of the nanoparticles must be assessed in the field and should be evaluated in conjunction with other management strategies used for the plant disease management.

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Conflicts of Interest. None.

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