

Full Length Research Paper

# Antifungal activity of nanofungicide Trifloxystrobin 25% + Tebuconazole 50% against *Macrophomina phaseolina*

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This study was aimed to develop the nanoform of a commercial fungicide Trifloxystrobin 25% + Tebuconazole 50% (75 WG) with broad spectrum of action for improving its antifungal activity against *Macrophomina phaseolina*. The fungicide commercially available as Trifloxystrobin 25% + Tebuconazole 50% (75 WG) was converted into its nanoform using ball milling method and assessed for its efficacy against the soil borne fungal pathogen *M. phaseolina* at various concentrations, namely 5, 10, 15, and 25 ppm using poisoned food technique. Nanoform of the fungicide was characterized using Scanning Electron Microscopy (SEM) and Particle Size Analyzer (PSA). The average particle size of nano Trifloxystrobin 25% + Tebuconazole 50% (75 WG) was about 108 nm. Fungicidal potential of nanoform was better in comparison to the conventional ones. Nanoform of the fungicide was effective at 10 ppm and it exerted hyphal abnormality, hyphal lysis and abnormality of sclerotial formation on *M. phaseolina* when tested under *in vitro* than control. This study suggests the possibility to enhance the antifungal activity of fungicide Trifloxystrobin 25% + Tebuconazole 50% (75 WG) towards the control of *M. phaseolina*.

**Key words:** Antifungal, nanoformulations, chilli, Trifloxystrobin 25% + Tebuconazole 50% (75 WG), *Macrophomina phaseolina*.

## INTRODUCTION

Soil-borne fungal pathogens cause diseases in economically important crops resulting in huge monetary losses to the farmers. Among them, *Macrophomina phaseolina* has very wide host range in the tropics and subtropics that causes charcoal rot, seedling blight, dry root rot, wilt, leaf blight and ashy stem blight in more than

500 cultivated and wild plant species including economically important crops as soybean, common bean, sorghum, maize, cotton, peanut, and cowpea (Hall, 1991; Diourte et al., 1995; Javaid and Saddique, 2011). It forms microsclerotia in senescing shoot tissues and survive in the soil for a long period (Mayek-Pérez et al., 2002).

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Changes in agricultural technology have been a major factor shaping modern agriculture. The development of nanodevices and nanomaterials could open up novel applications in plant biotechnology and agriculture (Scrinis and Lyons, 2007). Nanotechnology permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful by-products through enzymatic nano-bioprocessing, disease prevention and treatment in plants using various nanofungicides (Patolsky et al., 2006). Improved properties of the nanoparticles compared to the application of bulk materials have greater opportunity to reduce the load of unwanted chemicals especially plant protectants. The fungicidal efficacy of nano-sulphur and commercial sulphur at 1000 ppm against *Erysiphe cichoracearum* of okra has been studied. The sulphur fungicides significantly reduced the germination of conidia of *E. cichoracearum* than the control. Nano-sulphur was more effective than the commercial formulations and could be applied at lower amount for controlling powdery mildew disease for its better efficacy (Gogoi et al., 2013). In the present scenario, *M. phaseolina* is managed by seed and soil application of benzimidazole group fungicides. Bulk use of fungicides, not only causes environmental pollution, but also results in the development of resistance in pathogens.

To avoid the risk of phytotoxicity, threat to non-targeted organisms and the environment, an idea was conceived to have better control of *M. phaseolina* using lower or safe doses of fungicides. Recently, nanoformulations (particle size <100 nm) of fungicides have been able to draw much attention due to their higher efficacy even at very low doses, because nanoparticles could be more chemically reactive and bioactive than larger particles (Gogoi et al., 2009).

Hence, the present study was aimed to increase the efficacy of commercially available Trifloxystrobin 25% + Tebuconazole 50% (75 WG) by converting into nanoform and nanoform of fungicide had higher antifungal activity against *M. phaseolina* even at low concentration.

## MATERIALS AND METHODS

The experiment was carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, during 2015 under *in vitro* condition.

### Chemicals

Commercially available Trifloxystrobin 25% + Tebuconazole 50% (75 WG) fungicide of Bayer Crop Science Ltd. was purchased from the pesticide shop from Coimbatore, Tamil Nadu, India.

### Synthesis and characterization of nanofungicide

The nanofungicide was size reduction using FRITTSCH-High Energy

Ball Milling Method (Shyla, 2014). Characterization of the synthesized nanoparticles was performed by the techniques described subsequently.

### Particle size analyzer (PSA)

Particle size and the distribution pattern of synthesized sample suspensions were determined using Horiba Scientific Nanopartica SZ-100 (Nanoparticle analyzer), Japan. MALVERN, Zetasizer Ver.6.01. particle size analyzer was used accurately, 0.5 mg sample was dispersed in 20 ml distilled water, sonicated for 15 min and the suspension was analyzed under dynamic light scattering method using 90° or 173° at 25°C (Anandraj, 2013).

### Zeta potential

Zeta potential measurement for synthesized inorganic NPs was determined using a zeta analyzer (Horiba, SZ-100). In the zeta analyzer, zeta potential measurement varied from -200 to 200 mV and the data acquisition time is usually less than 1 min for zeta potential measurement and the laser light is divided into two beams as input light and reference light. Scattered light by sample particles and reference light modulated by the modulator interfere in the prism are detected and the detected signals are changed into digital signal to be calculated (Sridhar, 2012).

### Scanning electron microscopy (SEM)

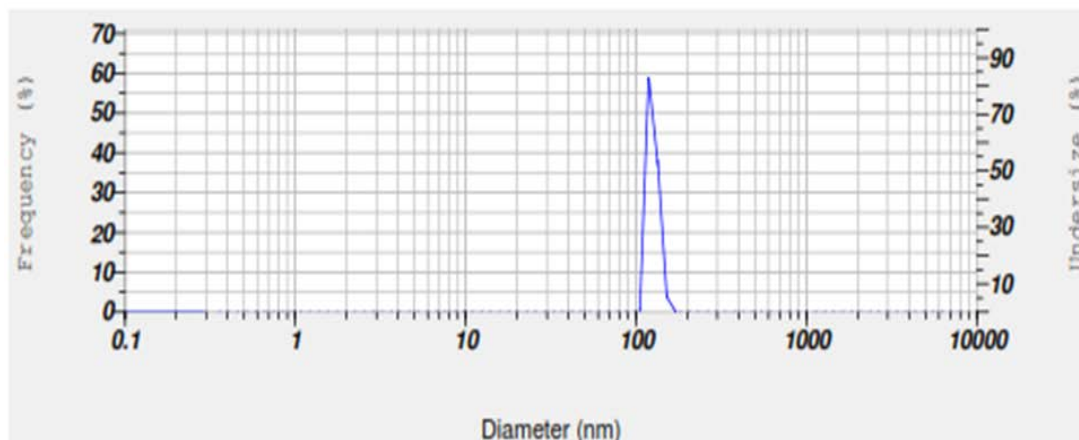
SEM (FEI QUANTA 250) was used to characterize the size and morphology of the nanoparticles. Sample of test nanoparticles (0.5 to 1.0 mg) was dusted on one side of the double sided adhesive carbon conducting tape, and then mounted on the 8 mm diameter aluminum stub. Sample surface were observed at different magnification and the images were recorded (Shyla, 2014).

### *In vitro* assay of antifungal activity of nanofungicides

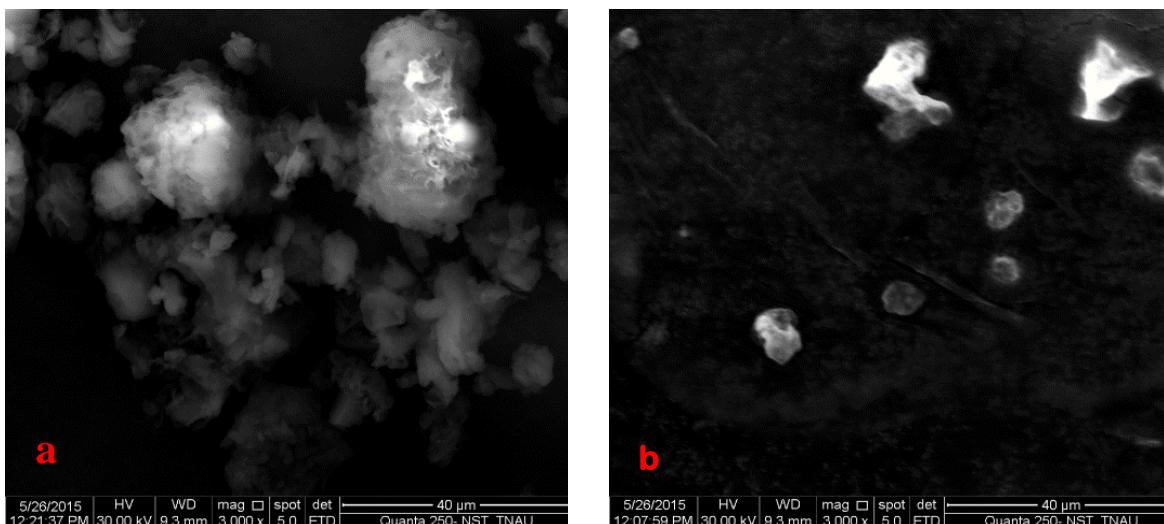
The antifungal activity of nanofungicides with active ingredient Trifloxystrobin 25% + Tebuconazole 50% (75 WG) were evaluated against *M. phaseolina* by using poisoned medium technique (Packia Lekshmi et al., 2012) using potato dextrose agar (PDA) medium amended with different concentrations (5, 10, 15, and 25 ppm). Non-amended medium served as a control. A nine millimeter disc of the actively growing *M. phaseolina* from a 7 days old culture was placed at the centre in each of the nanoparticles amended medium as well as in the untreated check. The mycelial growth of the pathogen was measured after five days of inoculation by incubating the Petri plates at 28±2°C. The percent inhibition of the mycelial growth over control was calculated to express the antifungal activity.

### Ultra microscopic changes on *M. phaseolina* induced by nanofungicides

Structural abnormality, hyphal lysis and inhibition of sclerotial formation induced by Trifloxystrobin 25% + Tebuconazole 50% (75 WG) nanofungicides were examined under SEM (Model FEI Quanta 250) at various resolutions (2500 to 6000X). The stub of SEM fixed with double-side adhesive carbon tape was gently placed over the mycelia mat of Petri dishes having hampered growth and removed immediately upon visual confirming for the presence of hyphae, then was fixed in the appropriate location of the SEM and observed for hyphal characters under low vacuum condition.



**Figure 1.** Particle size of ball milled nano fungicide.



**Figure 2.** SEM image: (a) Commercially available fungicide at 3000x; (b) After ball milled nano formed fungicide at 3000x.

## RESULTS AND DISCUSSION

### Characterization of nanofungicide Trifloxystrobin 25% + Tebuconazole 50% (75 WG)

The surface morphology of the nanofungicides synthesized and examined under particle size analyzer (PSA) revealed that the particle size range from 108 to 130 nm diameter (Figure 1). But the size of traditionally available Trifloxystrobin 25% + Tebuconazole 50% (75 WG) was of 1.3 to 3.0  $\mu\text{m}$  diameter.

To confirm the results of PSA, the same nanoformulated Trifloxystrobin 25% + Tebuconazole 50% (75 WG) fungicide was characterized under SEM. Irregular shaped nanoparticles was observed and it ranged from 108 to 130 nm (Figure 2).

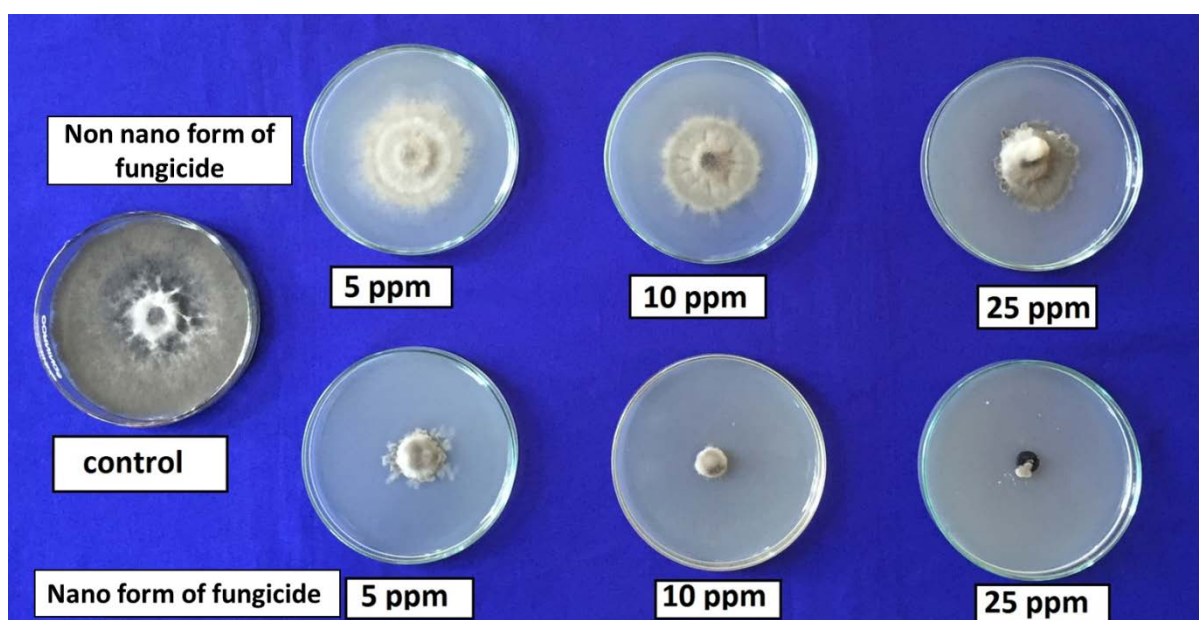
### Antifungal activity of nanofungicide

The poisoned medium technique was employed to determine inhibitory efficacy of different concentrations of nanoform of Trifloxystrobin 25% + Tebuconazole 50% (75 WG) as compared to commercial fungicide against *M. phaseolina* (Table 1). Among the different concentrations, the maximum inhibition of the mycelial growth was obtained with 15 ppm concentration which accounted for 99.6% reduction of mycelial growth over control in *M. phaseolina*. This was followed by complete inhibition of mycelial growth at 25 ppm which accounted for 100% reduction of mycelial growth over control (Figure 3).

Exploitation of nanofungicides in future may pave the way for effective management of seed borne and soil borne pathogens like *M. phaseolina*; thereby may reduce

**Table 1.** Effect of nano form of Trifloxystrobin 25% + Tebuconazole 50% (75 WG) fungicide on the mycelial growth of *M. phaseolina* *in vitro*.

Nanofungicide (concentration, ppm)	Mycelial growth inhibition over control (%)*	
	Fungicide (without ball milling)	Nano fungicide (ball milled)
5	26.7 <sup>f</sup>	73.7 <sup>d</sup>
10	40.0 <sup>e</sup>	84.1 <sup>c</sup>
15	60.4 <sup>d</sup>	99.6 <sup>b</sup>
25	76.3 <sup>c</sup>	100.0 <sup>a</sup>
50	87.8 <sup>b</sup>	100.0 <sup>a</sup>
100	100.0 <sup>a</sup>	100.0 <sup>b</sup>
Uninoculated control	0.0 <sup>g</sup>	0.3 <sup>e</sup>



**Figure 3.** Inhibition zones caused by nanofungicide and conventional micro sized fungicide against *M. phaseolina*

the bulk pesticide use in the environment. Exploitation of nanoparticles as antifungal agents is relatively as being reported by recent workers (Kumar, 2011; Sridhar, 2012). Nanoparticles interactions with fungal pathogen are dependent on the size and shape of the nanoparticles (Pal et al., 2007). Metal nanoparticles are an obvious choice due to their effective antimicrobial effects (Duncan, 2011). The results of the present study clearly revealed that the maximum level of inhibition zone was observed with the increasing concentration of nanoparticles. Which was comparatively superior in its antifungal activity against non nanoform of Trifloxystrobin 25% + Tebuconazole 50% (75 WG).

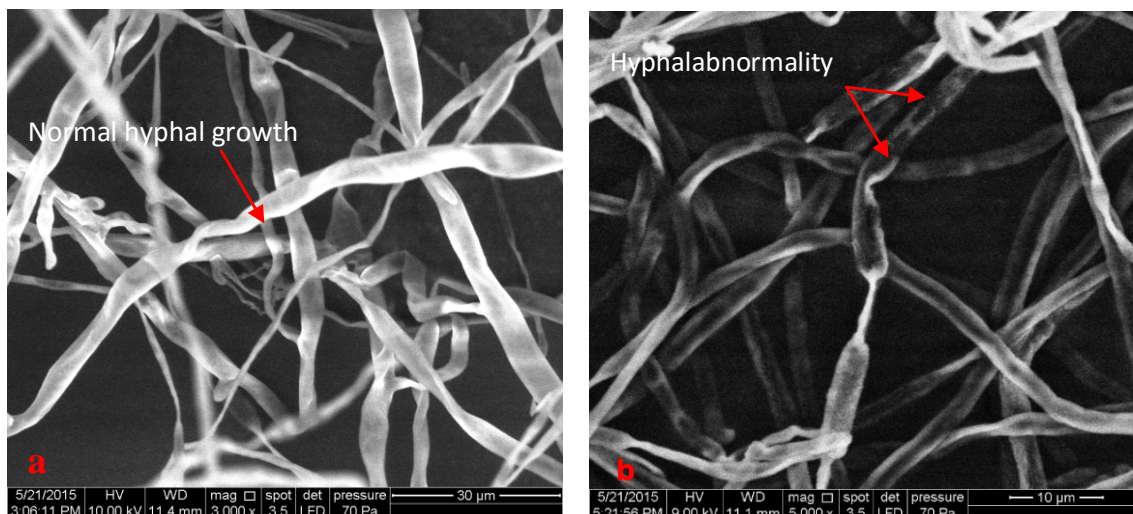
Nanoparticles are highly antimicrobial and antioxidant to several species of bacteria, fungi and viruses. Antimicrobial property of nanoparticles may be due to penetration of the cell wall and modulation of the cellular

level signaling by dephosphorylating putative key peptide substrates, which are critical for cell viability and cell division (Shrivastava et al., 2007). Nanoparticles are believed to inactivate microbial enzymes, facilitating production of reactive oxygen species that leads to microbial cell death (Allahverdiyev et al., 2011).

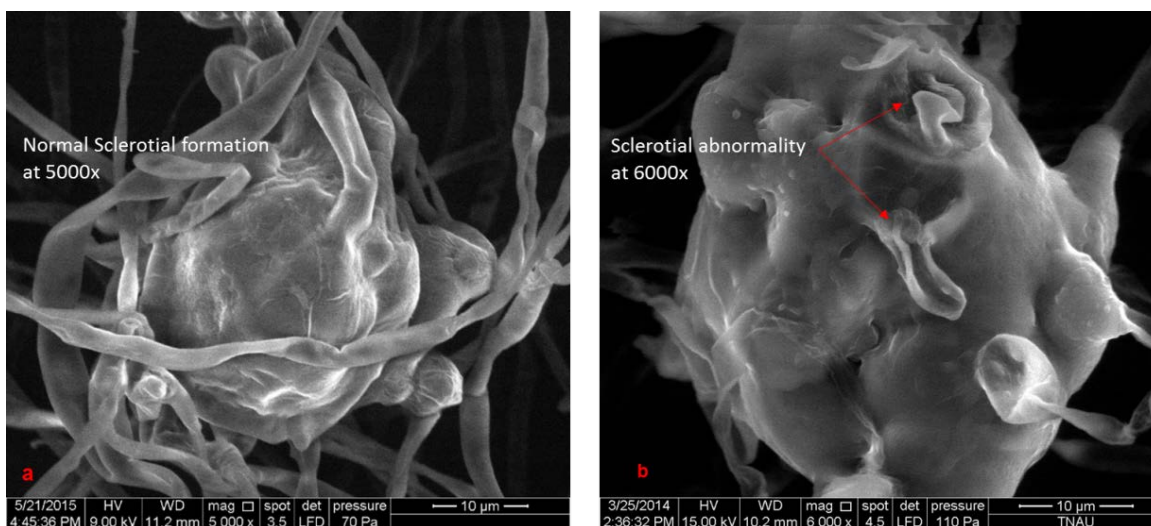
### Morphological modification of mycelia

The mycelial fragment from nanoparticles treated and untreated plates were examined under SEM at various resolutions. Hyphal filament was smooth walled and equal in thickness throughout the length with blunt tips and found to bear sclerotial bodies (Figures 4a and 5a). However, in nanoparticles treated plates, hyphae were found broken and sclerotial formation either lacking or





**Figure 4.** SEM image of *M. phaseolina* surface ultrastructure in (a) control normal hyphal formation at 3000x and (b) hyphal abnormality at 5000x.



**Figure 5.** SEM image of *M. phaseolina* surface ultrastructure in NPs treated: (a) control normal sclerotial formation at 5000x; (b) sclerotial abnormality at 6000x magnification.

abnormal, if formed. In addition, the cell surface of hyphae was observed to be crinkled in *M. phaseolina* (Figures 4b and 5b).

The metallic nanoparticles are most promising as they have remarkable antibacterial properties due to their large surface area to volume ratio (Gong et al., 2007; Rai et al., 2009). These results lead us to consider that nanofungicide may be the most effective for controlling *M. phaseolina*. Size reductions involve the increase of contact surface, which is an important condition for the effects of nanofungicide. Another reason for considering nanofungicide as superior is its broad antimicrobial

activity (Spacciapoli, 2001).

## Conclusion

The present investigation is an offshoot of the main research on exploring the possibilities of nanofungicide for increasing the seed qualities especially in vegetables where the maintenance of seed viability is difficult. In the present work, it was demonstrated that, nanoformulation of Trifloxystrobin 25% + Tebuconazole 50% (75 WG) has significant fungicidal property against the fungi *M.*

*phaseolina*. Thus, it can be effectively explored against the soil borne pathogen *M. phaseolina* to protect various crop seeds, instead of using the commercially available synthetic fungicides, which shows higher toxicity to humans. Moreover, this report opens up for further research; field experiments are to be carried out in order to recommend the nanofungicide against the disease and the area of mode of action of nanocomposites against phytopathogenic fungi.

### Conflict of Interests

The authors have not declared any conflict of interests.

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