

Inhibitory effect of nanofungicides and fungicides on radial mycelia growth of *Bipolaris sorokiniana* causing spot blotch of wheat

Abstract

Bipolaris sorokiniana (sacc.) Shoem, causing spot blotch of wheat is most important disease of wheat in north eastern plains zone (NEPZ) representing warm and humid climate in India as well as in other South Asian countries. The disease is known to cause yield losses up to 50% as well as deterioration in seed quality. The present study is carried out to check inhibitory effect of nanofungicides and fungicides against *Bipolaris sorokiniana*. Among nanofungicides, silver nanofungicide @ 100 ppm was found highly effective and inhibit the growth by 86.10% followed by agritec nanofungicide (81.41%) and selenium nanofungicide (77.01%). Among fungicides, maximum inhibition (82.57%) was observed in propiconazole @ 0.2% followed by hexaconazole (78.96%) and folicure (75.92%) under *in vitro* condition.

1. Introduction

Wheat (*Triticum aestivum* L.) crop belongs to family Poaceae (Graminae), is one of the oldest cereal crops. It is the second important staple cereal food in India after rice and has played vital role in stabilizing the food grain production in the country. Generally, wheat is a self-pollinated and hexaploid plant. It provides edible grain which forms staple food for a large number of people across the world. Wheat is believed to have originated in South West part of Asia. Some of the earliest remains of the crop were found in Syria, Jordan and Turkey (Feldman, 2001).

The world's major wheat producing areas are Northern China, Northern India, Northern USA and adjoining areas of Canada, Europe, Russia, Latin America and Africa. In 2022-23, the total global production of wheat was 770 million tons. China, India and Russia are the three largest individual wheat producers in the world, accounting for about 41% of the world's total wheat production. In India, it is grown in plains, plateaus as well as hills at altitude ranging from mean to 3000 m above sea level. With a production of 112.50 million tonnes during 2022-23 (USDA 2022-23). It is utilized for bread, cakes, cookies, noodles, pestri-products, chapatti and morconi etc. Beside staple food for human being, wheat straw is a good source of feed for a large

population of cattle in our country. Wheat grain contains 60-68% starch, 8.0 to 15% protein, 1.5 to 2.0 fat, 2.0-2.5 cellulose and 1.5 to 2.0% minerals (Rathore, 2001).

The wheat cultivation in the warmer and humid region of North-Eastern plain zone has extended significantly after green revolution; however, many new diseases and pest problems have been encountered by this crop that created significant yield loss. Wheat crop is affected by many fungal diseases and likely to be exposed to various types of foliar diseases other than rust, powdery mildew, Karnal bunt and loose smut. Among these spot blotch emerged as number one problem in hot and humid wheat cultivating regions (Van Ginkel and Rajaram, 1998). The spot blotch pathogen was initially named as *Helminthosporium sorokinianum* Sacc. in Sorokin. Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia, germinating by one germ tube from each end (Bipolar germination) and renamed the spot blotch pathogen as *Bipolaris sorokiniana* (sacc.) Shoem syn. *Drechslera sorokiniana* (Sacc.).

It is most important disease of wheat in north eastern plains zone (NEPZ) representing warm and humid climate in India as well as in other South Asian countries. In recent years, spot blotch has caused serious damage on wheat crop in India particularly in Eastern and central India. The Problem of spot blotch is more prominent in the north eastern region and is being addressed through national programme. The disease is known to cause yield losses up to 50% as well as deterioration in seed quality (Malik et al. 2008).

Non-systemic and systemic foliar fungicides belonging to the dithiocarbamates (*viz*; Mancozeb) and Triazoles (*viz*. Propiconazole, Tebuconazole, Flutriazol, Prochloraz, and Triadimenol) and dicarboximides (*viz.*, Iprodione) are known to be effective. Foliar applications especially with systemic fungicides such as Tebuconazole, Epoxiconazole, Flutriazol, Cyproconazole, Flusilazole, Epoxiconazole and Metaconazole applied between heading and grain filling stages, have been proved to be cost effective. Duveiller *et al.* (2005) observed that Triazole group (*e.g.*-Tebuconazole and Propinazole) have especially proven to be very effective against spot blotch disease. Singh *et al.* (2008) proposed that three foliar application of Propiconazole @ 0.1% after appearance of the disease significantly reduced the disease and increased yield over several locations of India. However reported use of same chemicals may raise resistant strain among the pathogens. Therefore, use of Nano-fungicidal control of spot blotch disease cannot be overstated. Engineered nanoparticles (ENPs) plays a very important role

in disease management, (ENPs) are defined as intentionally produced particles that have a characteristic dimension between 1 and 100 nm in at least one dimension. There are various types of ENPs such as silver nanoparticles (AgNPs), silica nanoparticles (SiO₂-NPs), agritec nanoparticles and selenium nanoparticles have been found effective for management of several plant diseases (Barrena *et al.*, 2009; Lamsal *et al.*, 2011; Stampoulis *et al.*, 2009). Abdel-Hafez *et al.* (2016) conducted experiment to understand the inhibitory effect of AgNPs at different concentrations (1, 5 and 10 ppm) compared to the chemical fungicide (Ridomil gold plus 2 gL⁻¹) was analyzed *in vitro*. The results clearly showed that the AgNPs markedly exhibited higher antifungal activity. Lamsal *et al.*, (2011) evaluated the effect of silver nanoparticles against pepper anthracnose under *in vitro* condition. Silver nanoparticles were applied at various concentrations to determine antifungal activities. The application of 100 ppm concentration of silver nanoparticles produced maximum inhibition of the growth of fungal hyphae as well as conidial germination. Thirumurugan *et al.* (2011) investigated the effects of silver nanoparticles on the phytopathogenic fungal growth. Especially for sclerotium-forming species *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor*, due to their important roles in survival and disease cycle. They found that silver nanoparticles remarkably inhibit the hyphal growth in a dose-dependent manner. Ismail *et al.* (2016) studied the effectiveness of silver nanoparticles and selenium nanoparticles against the *Alternaria solani* caused early blight disease on Potato and they concluded that the growth of *A. solani* was completely inhibited at 25 ppm AgNPs. This study is designed and carried out for the documentation and evaluation of four of nanofungicides (*viz.* agritec, silver, selenium and silica) and fungicides (*viz.* propiconazole, hexaconazole, folicure and azoxystrobin) against radial mycelial growth of *B. sorokiniana* causing spot blotch *in vitro*.

2. Materials and methods

2.1 Isolation of pathogen

The diseased leaf samples, showing distinct symptoms were collected for isolation of the pathogen. The collected leaves were taken and washed thoroughly with tap water and after wards with distilled water to remove all dust particles. Washed diseased leaves were cut into small bits, with some healthy portions, with the help of sterilized scalpel and forceps. The cut leaf pieces were surface sterilized with 0.1 per cent HgCl₂ (Mercuric Chloride) solution for 30 seconds

under aseptic conditions and washed thoroughly 3 to 4 times with sterilized water to remove the traces of Mercuric Chloride. Excess moisture was removed by placing these in the folds of sterilized blotter paper. The pieces, thus sterilized were transferred in Petri dishes with the help of sterilized inoculation needle. Petri Plates were taken and sterilized at 165°C for 2 hrs in hot air oven. These plates were placed in laminar air flow chamber and prepared autoclave PDA was poured in the plates. After solidification of media, the surface sterilized leaves pieces were placed in to position in each plate with the help of sterilized forceps. The plates were finally sealed with Para film tape and were incubated at 25± 1°C. The Petri plates were observed daily to take notice of the presence of mycelia growth around bits.

2.2 Identification of the pathogen

The pathogen was identified on the basis of colony characteristics and morphological characters of somatic and reproductive structure of the fungus seen under microscope. Colony was dark grey to brownish black in colour with smooth to wavy margin. Conidiophores were dark brown to dark olivaceous, unbranched straight to geniculate. Conidia of *B. sorokiniana* were yellowish brown to light brown coloured, straight to curved, ellipsoidal to smooth, broadest in middle with rounded ends Morejon *et al.* (2006).

2.3 Evaluation of Nanofungicides against *Bipolaris sorokiniana*

Efficacy of nanofungicides (*viz.* agritec, silver, selenium and silica) with suitable concentration was evaluated in PDA medium using poisoned food technique against *B. sorokiniana* in the laboratory. The nanofungicides with 100ppm were inoculated with *B. sorokiniana* was incorporated aseptically in autoclaved PDA at 0.2 per cent tested with three replications per treatment and incubated at 25 ± 1°C for 8 days. The mycelial growth was measured at 2, 4, 6 and 8 days of incubation. Petri plate without nanofungicides served as control.

2.4 Evaluation of fungicides against *Bipolaris sorokiniana* (*in-vitro*)

Efficacy of various fungicides (Propiconazole, Hexaconazole, Azoxystrobin and Folicure @ 0.20%) against mycelial growth of *B. sorokiniana* was studied under *in vitro* condition by Poisoned Food Technique (Sharvelle, 1961). 20 ml of poisoned PDA was poured aseptically in each Petri plate. The Petri plate were inoculated with 5 mm mycelial disc cut with sterilized cork borer from 7 days old culture and transferred aseptically to the centre of each Petri plate. A

suitable check was also maintained without adding any fungicide in PDA (control). Each treatment was replicated three times. Petri plates were incubated at $25\pm 1^{\circ}\text{C}$ for 8 days. After 8 days of incubation, observations were recorded by measuring radial growth of the colony at right angle. Mean colony diameter was taken to calculate per cent inhibition of radial growth by the formula given by Horsfall (1956).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

2.5 Statistical analysis

The data recorded on radial growth were statistically analyzed using completely randomized block design (Gomez and Gomez, 1984).

3. Results

All the tested nanofungicides caused inhibition of mycelial growth of pathogen. Among the nano fungicides, the minimum radial growth of mycelium was found in Silver nanofungicide @ 100 ppm with 86.10 per cent inhibition was observed in treatment and it was followed by treatment Agritec nanofungicide @ 100 ppm (81.41%) While minimum inhibition was observed in treatment Silica nanofungicide @ 100 ppm (75.51%). Among fungicides tested, Propiconazole @ 0.2% was found most effective with significantly least mycelial growth of the test pathogen (15.52 mm) followed by Hexaconazole @ 0.2% (18.73 mm) and highest mycelial growth inhibition (82.57%) of the test pathogen followed by Hexaconazole @ 0.2% (78.96%), respectively. Azoxystrobin @ 0.2% was found comparatively less effective with maximum mycelial growth (23.44 mm) and minimum mycelial growth inhibition (73.67%).

Table 1: Effect of nanofungicides and fungicides on radial mycelia growth of *Bipolaris sorokiniana*.

		Radial mycelial growth (mm)	Per cent
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S. No.	Treatments details	2 nd Day	4 th Day	6 th Day	8 th Day	inhibition over control
T ₁	Agritec nanofungicide 100 ppm	11.43	12.44	14.78	16.55	81.41
T ₂	Silver nanofungicide 100 ppm	08.41	09.37	10.52	12.37	86.10
T ₃	Selenium nanofungicide 100 ppm	14.72	17.54	18.78	20.47	77.01
T ₄	Silica nanofungicide 100 ppm	14.28	16.20	19.44	21.80	75.51
T ₅	Propiconazole @ 0.2%	09.74	11.32	13.67	15.52	82.57
T ₆	Folicure @ 0.2%	13.86	16.35	19.98	21.44	75.92
T ₇	Hexaconazole @ 0.2%	12.23	13.73	14.70	18.73	78.96
T ₈	Azoxystrobin @ 0.2%	14.70	17.48	20.57	23.44	73.67
T ₉	Control	24.65	45.50	62.33	89.05	-
	CD	1.308	1.352	1.547	1.538	-
	SE (m)	0.433	0.447	0.511	0.509	-

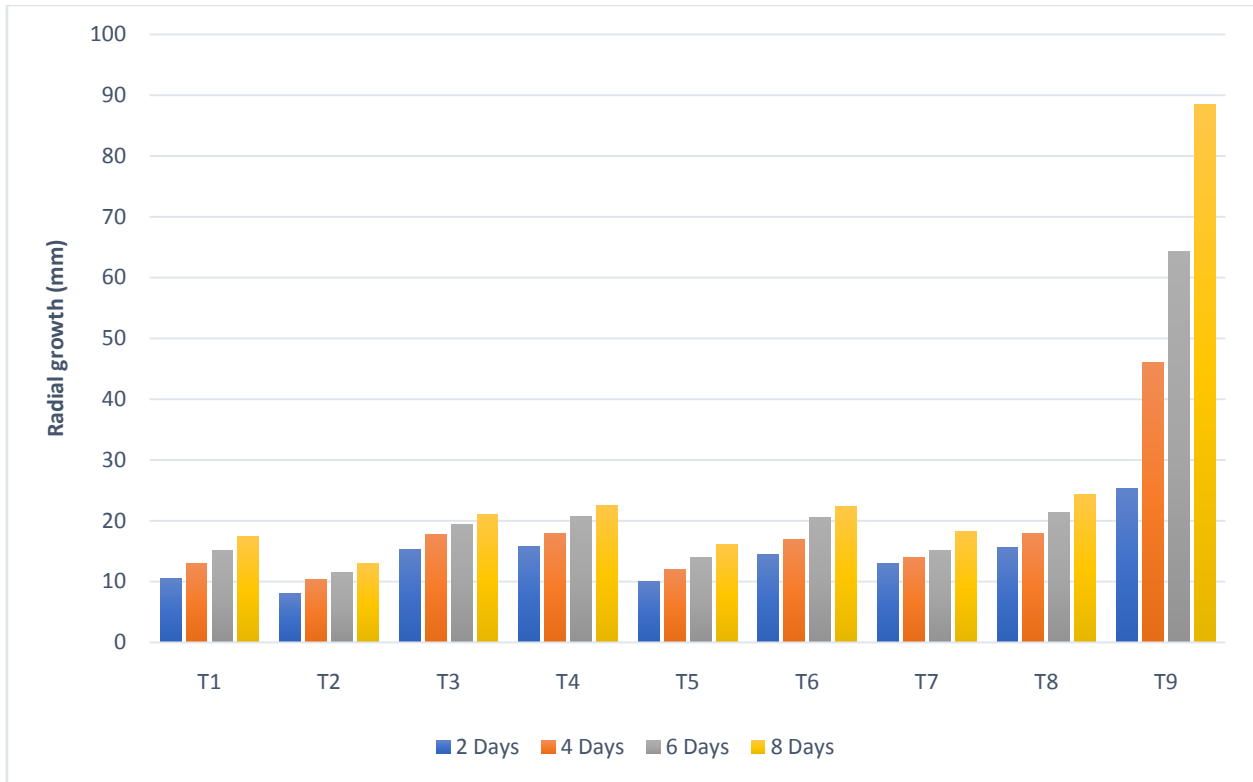
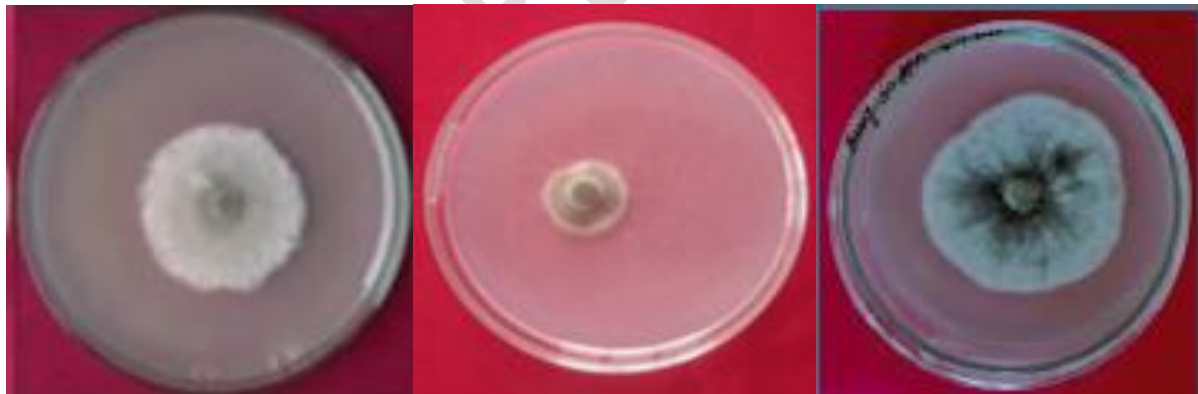


Fig.1: Effect of nanofungicides and fungicides on radial mycelia growth of *Bipolaris sorokiniana* at different days interval (*in-vitro*)



Agritec 100ppm

Silver 100ppm

Selenium 100ppm

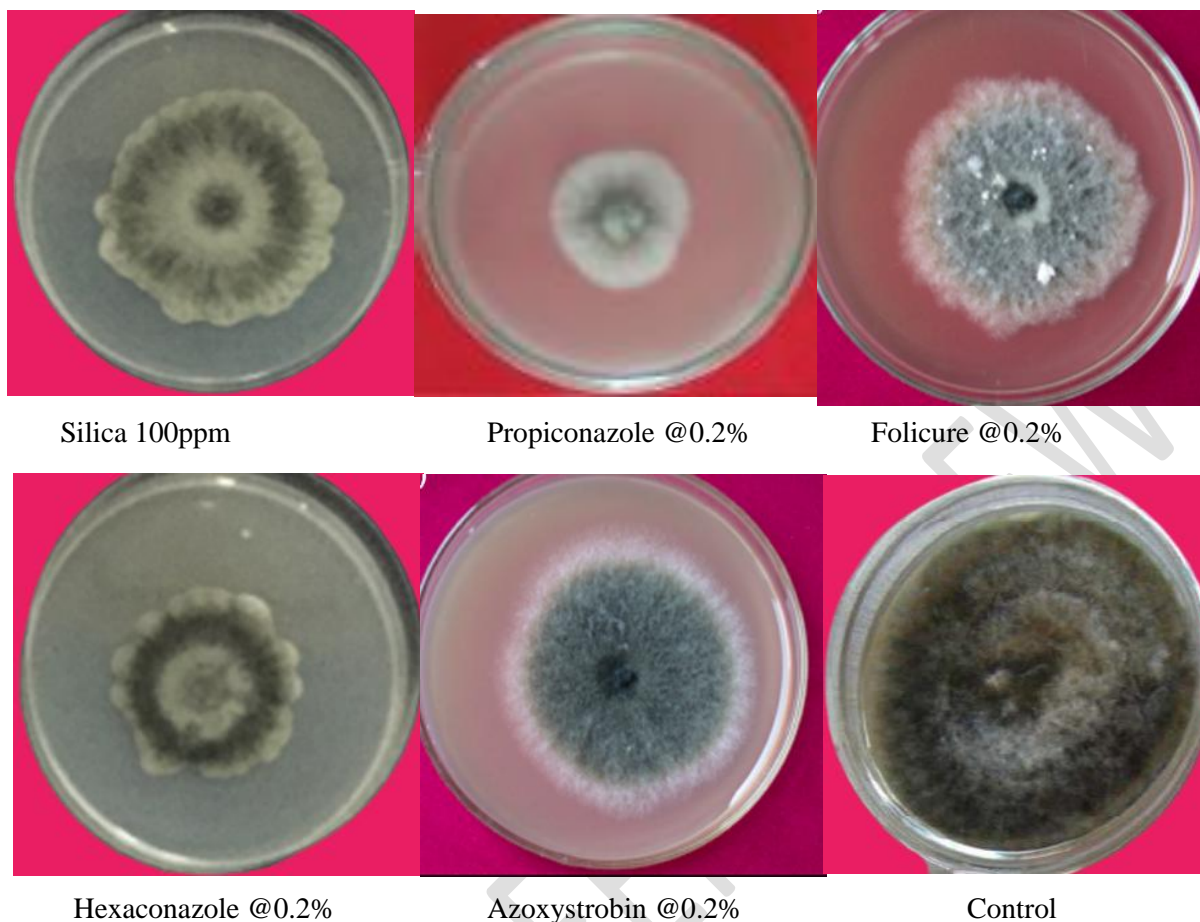


Plate 1. **Pathogens in different** Petri plates

4. Discussion

The efficacy of the suitable concentration of nanofungicide (Agriteck 100 ppm, Silver 100 ppm, Selenium 100 ppm and Silica 100 ppm) and four fungicides (Propiconazole @ 0.2%, Folicure @ 0.2%, Hexaconazole @ 0.2% and Azoxystrobin @ 0.2%) against *B. sorokiniana* showed that among the nanofungicide, the minimum radial growth of mycelium was found in Silver nanofungicide @ 100 ppm with 86.10 per cent inhibition was observed in treatment and it was followed by treatment Agritec nanofungicide (81.41%) and among fungicides tested, Propiconazole @ 0.2% was found most effective with significantly least mycelial growth of the test pathogen (15.52 mm) followed by Hexaconazole @ 0.2% (18.73 mm) and highest mycelial growth inhibition (82.57%) of the test pathogen followed by Hexaconazole @ 0.2% (78.96%), respectively. Among the treatments, Azoxystrobin @ 0.2% was found comparatively less effective with maximum mycelial growth (23.44 mm) and minimum mycelial growth inhibition

(73.67%). Abdel-Hafez *et al.* (2016) also found the inhibitory effect of AgNPs at different concentrations (1, 5 and 10 ppm) compared to the chemical fungicide (Ridomil gold plus 2 gL⁻¹) was analyzed *in vitro*. The results clearly showed that the AgNPs markedly exhibited higher antifungal activity. The mycelia growth of pathogenic *Alternaria solani* F11 (KT721909), *Alternaria solani* F12 (KT721910) and *Alternaria solani* F14 (KT721911) was inhibited to various extents by AgNPs. A concentration of 10 ppm induced the highest levels of inhibition rate (%) of the three strains of pathogenic *A. solani* mycelial growth with values (88.9 ± 1.2, 87.8 ± 1.1 and 88.5 ± 1.0), respectively, compared to the chemical fungicide which showed values (61.4 ± 1.2, 60.9 ± 0.5 and 62.7 ± 1.3), respectively, after incubation period 8 days. The antifungal activity of silver nanoparticles was evaluated against sclerotium-forming phytopathogens, *R. solani*, *S. sclerotiorum*, and *S. minor* during a study conducted by Min *et al.* (2009). They found that the that the nanoparticles strongly inhibited the fungal growth and sclerotial germination growth. Nel *et al.* (2003) It is also suggested that nanometer-sized silvers possess different properties, which might come from morphological, structural and physiological changes. Hasan *et al.* (2012) reported that Propiconazole, Hexaconazole and Difenconazole + Propiconazole fungicide inhibited the mycelial growth of *B. sorokiniana* under *in vitro* conditions at different concentrations (100, 200, 300, 400 and 500 ppm). Naresh *et al.* (2009) reported that azoxystrobin, carboxin, propiconazole, tubuconazole, mancozeb and thiram were highly effective in controlling *B. sorokiniana*. Duveiller and Dubin, (2002) found that triazole group tebuconazole and propiconazole was most effective in inhibition of mycelial growth *B. sorokiniana* of wheat under *in vitro* condition.

5. Conclusion

The present study identified various nanofungicides (agritec, silver, selenium and silica nanofungicide @ 100ppm) and fungicides (Propiconazole, Hexaconazole, Azoxystrobin and Folicure @ 0.20%) against the pathogen *B. sorokiniana*. Silver nanofungicide was found highly effective followed by agriteck nanofungicide. In fungicides, maximum per cent inhibition was observed in propiconazole followed by hexaconazole and folicure. All the nanofungicides and fungicides evaluated significantly control the disease. So, they can be used to manage the disease under natural field condition. Nanofungicides may be applied widely and safely instead of using the commercially available synthetic fungicides, which show higher toxicity to humans.

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