Inhibitory effect of nano-fungicides and fungicides on radial mycelial growth of *Bipolaris sorokiniana* causing spot blotch of wheat under *in vitro* condition

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 at 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 at 0.2% followed by hexaconazole (78.96%) and folicure (75.92%) under *in vitro* condition.

Key word: nanofungicides , fungicides , mycelia growth ,Bipolaris sorokiniana , spot blotch

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 tons 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, Karnel 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, Procloraz, and Triadimenol) and dicarboxymides (*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 filing stages, have been proved to be cost effective". (Malik et al., 2008) 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 at 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 *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 $HgCl_2$ (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 the sterilized pieces on folds of sterilized blotter paper. Petri-plates were taken and sterilized at 165^{o}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 leaf 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^{o}C. The Petri-plates were observed daily to take notice of the presence of the mycelial 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 using the description of 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 by PDA medium using poisoned food technique against *B. sorokiniana* in the laboratory. The PDA medium incorporated with the nanofungicides at 100ppm was inoculated with *B. sorokiniana* and incubated at 25 ± 1^{o}C for 8 days. Three replications were used for each treatment. 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 at 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 center 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±1°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

3.1. 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.

3.2. Evaluation of Nanofungicides against *Bipolaris sorokiniana*

Data presented in Table (1) and illustrated by Fig. (1) reveal that all the tested nanofungicides caused inhibition for the mycelial growth of pathogen. Among the nano fungicides, the minimum radial growth of mycelium was found in silver nanofungicide at 100 ppm with 86.10 per cent inhibition followed by treatment Agritec nanofungicide @ 100 ppm (81.41%). While, minimum inhibition was observed in treatment silica nanofungicide at 100 ppm (75.51%). Among fungicides tested, Propiconazole at 0.2% was found most the effective one with significantly least mycelial growth of the test pathogen (15.52 mm) followed by Hexaconazole at 0.2% (18.73 mm). The highest mycelial growth inhibition (82.57%) of the test pathogen was occurred by Propiconazole at 0.2% followed by Hexaconazole at 0.2% (78.96%).

Azoxystrobin at 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 nano-fungicides and fungicides on radial mycelial growth of *Bipolaris sorokiniana*.

S. No.	Treatments details	Radial mycelial growth (mm) after (days)				Radial mycelial growth (mm)
		2	2	2	2	
T_1	Agritec nanofungicide at 100 ppm	11.43	12.44	14.78	16.55	81.41
T_2	Silver nanofungicide at 100 ppm	08.41	09.37	10.52	12.37	86.10
T_3	Selenium nanofungicide at 100 ppm	14.72	17.54	18.78	20.47	77.01
T_4	Silica nanofungicide at 100 ppm	14.28	16.20	19.44	21.80	75.51
T_5	Propiconazole at 0.2%	09.74	11.32	13.67	15.52	82.57
T_6	Folicure at 0.2%	13.86	16.35	19.98	21.44	75.92
T_7	Hexaconazole at 0.2%	12.23	13.73	14.70	18.73	78.96
T_8	Azoxystrobin at 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	-

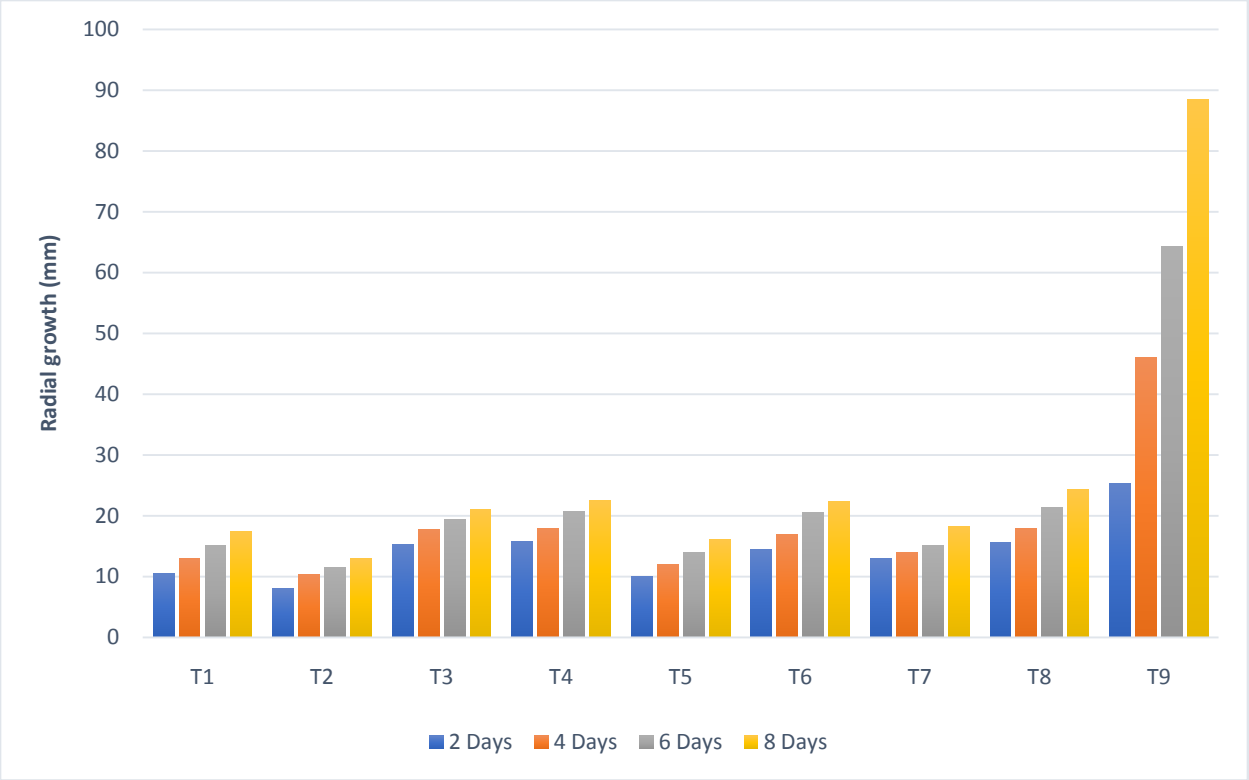
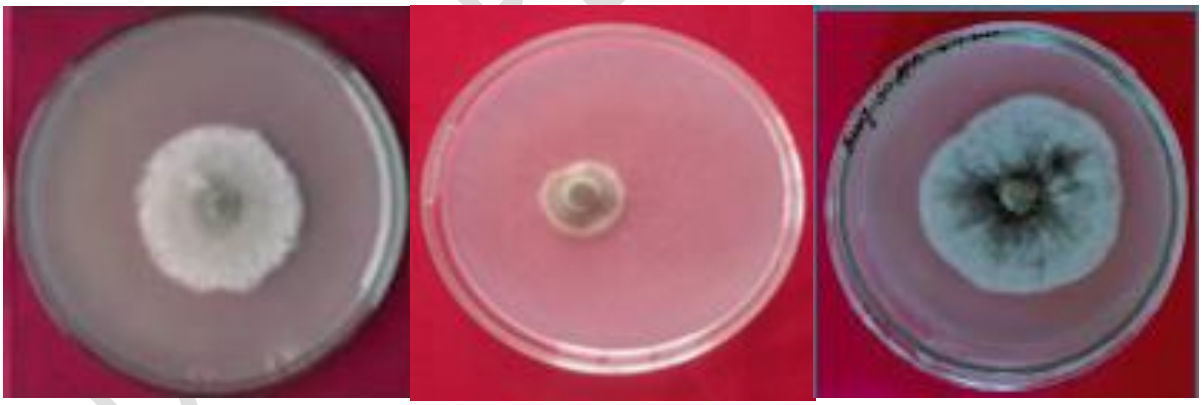


Fig.1: Effect of nanofungicides and fungicides on radial mycelia growth of *Bipolaris sorokiniana* at different days interval (*in-vitro*).



Agritec at 100ppm Silver at 100ppm Selenium at 100ppm

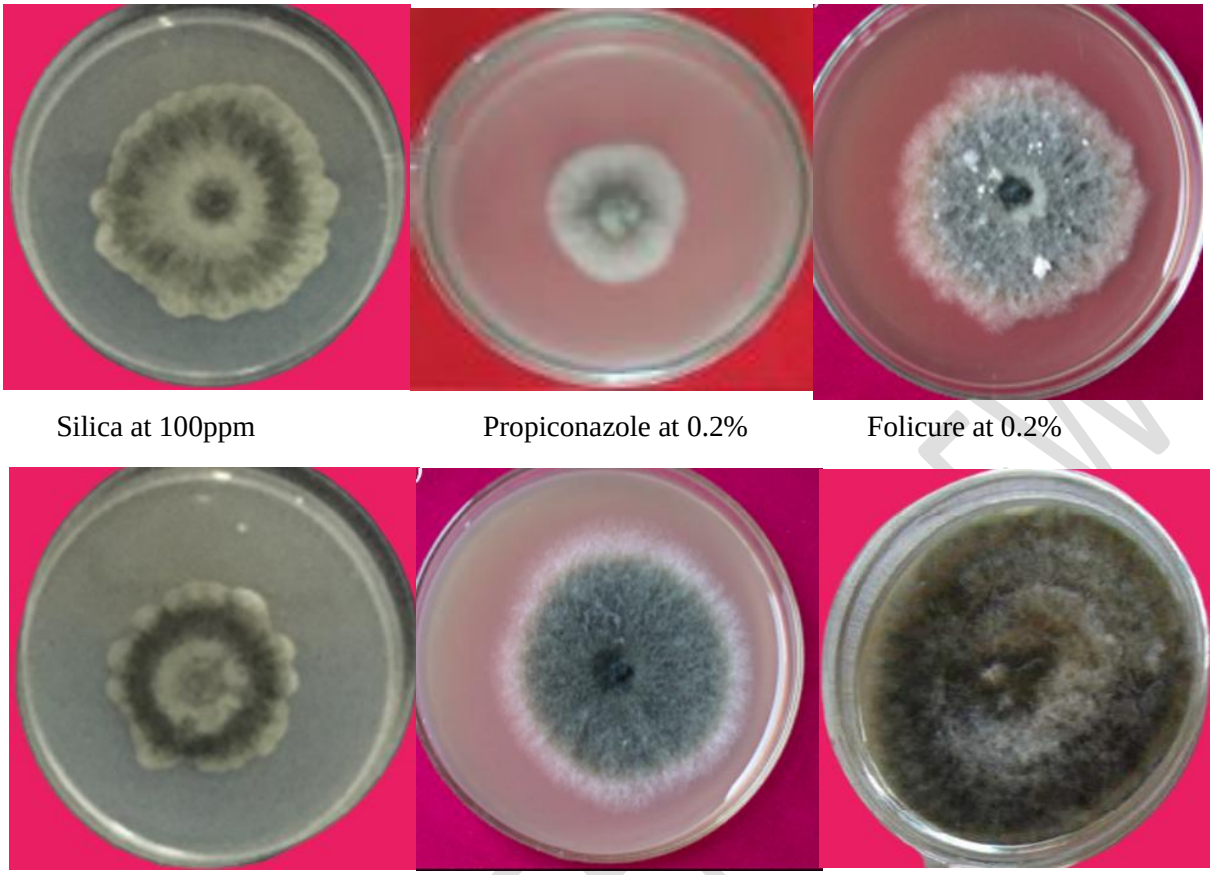


Fig. 2. The growth of the tested pathogens on different nanofungicides and fungicides *in vitro.*

4. Discussion

The efficacy of the test concentration (100 ppm) of nano-fungicide Agriteck, silver, selenium and silica as well as the four fungicides *i.e.*, Propiconazole, Folicure , Hexaconazole and Azoxystrobin at 0.2% against *B. sorokiniana* showed that among the nano-fungicide, the minimum radial growth of mycelium was found in silver nano-fungicide at 100 ppm with 86.10 per cent inhibition followed by treatment Agritec nano-fungicide (81.41%) and among fungicides tested, Propiconazole at 0.2% was found the most effective with significantly least mycelial growth of the test pathogen (15.52 mm) followed by Hexaconazole at 0.2% (18.73 mm). The highest mycelial growth inhibition (82.57%) of the test pathogen followed by Hexaconazole at 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), inhibitory effect of AgNPs at

different concentrations (1, 5 and 10 ppm) compared to the chemical fungicide Ridomil gold plus at 2 gL^{-1} *in vitro*. The results clearly showed that the AgNPs markedly exhibited higher antifungal activity. The mycelial growth of pathogens *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 the pathogen *A. solani* mycelial growth, being 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 of 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 nanoparticles strongly inhibited the fungal growth and sclerotial germination growth. Nel *et al.* (2003), also, suggested that nanometer-sized silvers possess different properties might come from morphological, structural and physiological changes. Hasan *et al.* (2012) reported that "Propiconazole, Hexaconazole and Difenoconazole + 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 evaluated various nanofungicides (agritec, silver, selenium and silica nanofungicide at 100ppm) and fungicides (Propiconazole, Hexaconazole, Azoxystrobin and Folicure at 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.

6. References

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