Biological Forum – An International Journal **15(9): 552-556(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of different Nanofungicides on Radial Mycelial Growth of *Bipolaris sorokiniana* causing Spot Blotch of Wheat

Bipin Verma*, Samir Kumar Biswas, Naimish Kumar and Gajendra Pratap
Department of Plant Pathology,
Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (Uttar Pradesh), India.

(Corresponding author: Bipin Verma)*
(Received: 28 June 2023; Revised: 29 July 2023; Accepted: 30 August 2023; Published: 15 September 2023)
(Published by Research Trend)

ABSTRACT: Wheat (*Triticum aestivum* L.) is the most important food grain crop in India after rice as well as recognized as a world's major cereal crop and staple food of many regions. Wheat crops face the threat of numerous pathogens, with *Bipolaris sorokiniana* emerging as a significant menace in wheat-growing regions characterized by hot and humid climates. In South Asia, the estimated average yield losses caused by this disease amount to 19.6 percent, while in India, the figure stands at 15.5 percent. Particularly in the eastern part of India, under severe conditions, yield losses can soar to as high as 100 percent. 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. Though, the disease can be managed with various nanofungicides under *in vitro* condition assessed for their efficacy against the disease. The effect of four nanofungicides *i.e.*, Agritecnanofungicide, Silver nanofungicide, Selenium nanofungicide and Silica nanofungicide against *B. sorokiniana* was evaluated at different concentrations at different concentrations (50, 75 and 100 ppm) by poison food technique. The *in vitro* experiment revealed a noticeable reduction in the radial mycelial growth of *B. sorokiniana* as the concentration of nanofungicides increased. Specifically, at 8 days after inoculation (DAI), the following nanofungicides at a concentration of 100 ppm exhibited the highest levels of mycelial growth inhibition when compared to the control: Silver nanofungicide (86.41%), Agritecnanofungicide (80.23%), Selenium nanofungicide (74.88%), and Silica nanofungicide (71.90%).

Keywords: Nanofungicide, Fungicide, Spot blotch, Radial mycelial growth, Growth inhibition.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a globally cultivated cereal crop and serves as a staple food in many regions around the world. It holds the top position among cereals worldwide, both in terms of cultivation area and production. In India, wheat ranks as the second most important food crop, following rice. The origins of wheat are believed to trace back to the southwestern region of Asia, with some of the earliest remnants of the crop discovered in Syria, Turkey, and Jordan (Feldman *et al.*, 2001). Wheat belongs to the Poaceae family (Graminae) and is typically a self-pollinating C3 plant. It is cultivated in various regions of India, including plains, plateaus, and hills, at altitudes ranging from sea level to approximately 3000 meters (Kumar *et al.*, 2014). In the 2022-23 season, wheat production in India reached an impressive 112.50 million tonnes (USDA report, 2022-23). The major wheat-producing states in India include Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, and Bihar (Rai *et al.*, 2012). The wheat crop is vulnerable to a range of pathogens, including parasitic fungi, viruses, bacteria, and nematodes, all of which have the potential to significantly reduce crop yields. One notable threat in hot and humid wheat-growing regions, as well as in areas practicing rice-wheat cultivation, is spot blotch of wheat, which is caused by the fungus *Bipolaris sorokiniana*. This disease was identified as a major concern in these regions as reported by Van Ginkel and Rajaram (1998). *Bipolaris sorokiniana*, the causative agent of spot blotch disease, is categorized as a hemibiotrophic phytopathogenic fungus. Typically, it manifests symptoms on the leaves, leaf sheaths, and stems of wheat plants. In cases of severe infection, symptoms may also extend to the wheat spikes, leading to the development of low-weight, shriveled grains with a distinctive black point at the embryonal end of the kernels, as described by Kiesling (1985). Foliar fungicides can be categorized into two main types: non-systemic and systemic. Among these, dithiocarbamates like Mancozeb, triazoles including Propiconazole, Tebuconazole, Flutriazol, Procloraz, and Triadimenol, as well as dicarboxymides such as Iprodione, have established themselves as effective options for controlling fungal diseases in plants. 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, nanoparticles (ENPs) are particles intentionally manufactured with a distinctive dimension that falls within the range of 1 to 100 nanometers in at least one of their dimensions.

Abdel-Hafez *et al.* (2016) conducted experiment to understand the inhibitory effect of silver nanoparticles (AgNPs) at different concentrations (1, 5, and 10 ppm) was assessed *in vitro* and compared to the chemical fungicide Ridomil Gold Plus at a concentration of 2 gL^{-1}. Among the tested concentrations, 10 ppm of AgNPs demonstrated the highest inhibition rate (%) on the mycelial growth of three strains of *Alternaria solani*. Specifically, it yielded inhibition values of (88.9 ± 1.2, 87.8 ± 1.1, and 88.5 ± 1.0) for the respective strains, surpassing the performance of the chemical fungicide, which exhibited values of (61.4 ± 1.2, 60.9 ± 0.5, and 62.7 ± 1.3) for the same strains after an incubation period of 8 days. Lamsal *et al.* (2011) an evaluation was conduct to assess the impact of silver nanoparticles on the control of pepper anthracnose under various culture conditions. The results indicated that the application of a 100 ppm concentration of silver nanoparticles led to the highest level of inhibition in the growth of fungal hyphae and the germination of conidia when compared to the control group *in vitro*. Thirumurugan *et al.* (2011) investigated in the study of the effects of silver nanoparticles on phytopathogenic fungal growth, particularly on sclerotium-forming species such as *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *S. minor*, which play crucial roles in their survival and disease cycles, it was observed that silver nanoparticles exhibited a significant inhibitory effect on hyphal growth. This inhibition was observed to be dose-dependent, meaning that higher concentrations of silver nanoparticles led to greater inhibition of hyphal growth in these fungal species. In a study conducted by Ismail *et al*. (2016), the effectiveness of silver nanoparticles (AgNPs) against early blight disease caused by *Alternaria solani* on potatoes was investigated and concluded that the growth of *A. solani* was entirely inhibited when exposed to a concentration of 25 ppm of silver nanoparticles.

Park *et al*. (2006), investigated the effective concentration of nanosized silica-silver for suppressing the growth of various fungi. They discovered that several fungi, including *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, and *Rhizoctonia solani*, exhibited complete inhibition of growth when exposed to a concentration of 10 ppm of nanosized silica-silver.

MATERIALS AND METHODS

Experimental Site:

All experiments were carried out in the laboratory of the Department of Plant Pathology at C.S.A. University of Agriculture and Technology in Kanpur during the period from 2021 to 2023.

Isolation and Purification of the Pathogen. The collected leaves were taken, which were then thoroughly washed first with tap water and subsequently with distilled water to eliminate any dust particles. Diseased leaves that had been washed were subsequently chopped into small pieces, including some healthy sections. These chopped leaf fragments underwent surface sterilization using a 0.1% $HgCl_2$ (Mercuric Chloride) solution for 30 seconds under sterile conditions. They were then rinsed extensively, 3 to 4 times, with sterilized water to remove any remaining traces of Mercuric Chloride. The surface-sterilized portions were placed onto potato dextrose agar (PDA) medium in sterile petri plates and were then incubated at a temperature of 25±1°C. These petri plates were observed daily to monitor the presence of mycelial growth around the leaf fragments. After an incubation period of 48-72 hours, the cultures were purified using the hyphal tip method as described by Dhingra and Sinclair (1985). The resulting fungal cultures were maintained separately in agar slants or petri plates. Additionally, the pathogenicity of *B. sorokiniana* was confirmed by conducting Koch's postulates on healthy wheat plants.

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. Observation of size, colour, length, breadth and septum of conidia were taken into consideration with the help of standard text and photographs (Ellis, 1971).

Maintenance of the cultures. To maintain a pure culture, it was regularly sub-cultured onto potato dextrose agar slants at 30-day intervals. To preserve the culture for longer periods, the sealed end of the culture tubes was dipped in molten wax, creating an airtight seal. These sealed culture tubes were then stored in a refrigerator at a temperature of 7.5±1°C.

Effect of different nanofungicides against *Bipolaris sorokiniana.* The effectiveness of Agritecnanofungicide, Silver nanofungicide, Selenium nanofungicide, and Silica nanofungicide at various concentrations (50, 75, and 100 ppm) was assessed in a laboratory setting using a poisoned food technique on PDA medium against the target pathogen. Each nanofungicide treatment was subjected to three replications. Before initiating the experiment, the fungus was grown on PDA medium for a period of 10 days. The preparation of the PDA medium involved melting it, and subsequently, the appropriate concentrations of nanofungicides were incorporated into the molten medium. Sterilized Petri plates were filled with 20 ml of the poisoned medium. Sterilized cork borers were used to cut 5 mm mycelial discs from a 7-day-old culture, and these discs were aseptically transferred to the center of each Petri plate for inoculation. Additionally, a control was established by maintaining a Petri plate with PDA medium without the addition of any nanofungicide. Petri plates were incubated at 25±1°C for 7 days. The radial growth of the fungus on the poisoned medium was measured on different days. All experiments were conducted in triplicate, and the percentage inhibition of mycelial growth over the control was calculated using the Horsfall formula (1956):

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

Where I = Percentage inhibition of mycelium
C = Colony diameter (mm) in the control
T = Colony diameter (mm) in the treatment

Treatments details: T_1-Agritec nanofungicide@ 50ppm; T_2-Agritec nanofungicide@ 75 ppm; T_3-Agritec nanofungicide@ 100 ppm; T_4-Silver nanofungicide@ 50 ppm; T_5-Silver nanofungicide@ 75 ppm; T_6-Silver nanofungicide@ 100 ppm; T_7-Selenium nanofungicide@ 50 ppm; T_8-Selenium nanofungicide@ 75 ppm; T_9-Selenium nanofungicide@ 100 ppm; T_{10}-Silica nanofungicide@ 50 ppm; T_{11}-Silica nanofungicide@ 75 ppm; T_{12}-Silica nanofungicide@ 100 ppm; T_{13}-Control.

Statistical analysis. Completely Randomized Design (CRD) used for the experiments will be conducted in laboratory in Petri plates. The data will be statistically analysis by means of critical difference (CD) at 5 per cent level of significance.

RESULTS AND DISCUSSION

Effect of nanofungicides against *Bipolaris sorokiniana*(*in vitro*). Different nanofungicides like Agriteck, Silver, Selenium and Silica with different concentrations (50ppm, 75ppm and 100ppm) were tested *in vitro* through food poison technique. The results demonstrated a significant suppression of B. sorokiniana mycelial growth across all four tested concentrations when compared to the control (refer to Table 1 and Figure 1). Among the treatments, the most pronounced inhibition of mycelial growth in comparison to the control occurred in T6, where Silver nanofungicide was applied at 100 ppm, resulting in an 86.41% reduction. This was followed by T3, employing Agritecknanofungicide at 100 ppm, with an 80.23% decrease, T9, utilizing Selenium nanofungicide at 100 ppm, with a 74.88% reduction, and T12, using Silica nanofungicide at 100 ppm, with a 71.90% reduction. These reductions corresponded to mycelial growth measurements of 12.23 mm, 17.66 mm, 22.44 mm, and 25.10 mm at 8 days after inoculation (DAI), respectively. Conversely, the lowest inhibition of mycelial growth, when compared to the control, was observed in the case of Agritecknanofungicide at 50 ppm, resulting in a 29.23% reduction, corresponding to mycelial growth of 63.23 mm at 8 DAI. Similar observation was reported previously by several workers (Abdel-Hafez *et al.*, 2016; Lamsal *et al.*, 2011; Thirumurugan *et al.*, 2011; Mohamed, 2015) found that silver nanofungicide effective against plant disease causing pathogens *i.e.*, *Alternaria solani*. pepper anthracnose, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. Park *et al.* (2006), they investigated the impact of nanosized silica-silver on inhibiting the growth of several fungi. Their findings revealed that *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinererea*, and *Rhizoctonia solani* exhibited complete growth inhibition when exposed to a concentration of 10 ppm of nanosized silica-silver. Silver nanoparticles, with an average diameter of 10 ± 5 nm, were utilized in an *in vitro* assay at concentrations of 50 ppm, 100 ppm, and 150 ppm. The outcomes of the study indicated that the application of silver nanoparticles at a concentration of 150 ppm exhibited strong antifungal activity against *Alternaria alternate* and *Alternaria citri* (Abdelmalek and Salaheldin 2016). Kim *et al.* (2012) conducted experiment to quantify the antifungal effects of Silver nanoparticles (AgNPs) were tested for their effectiveness against a range of plant pathogenic fungi. The study involved using concentrations of 10 ppm, 25 ppm, 50 ppm, and 100 ppm of AgNPs. Eighteen different plant pathogenic fungi were subjected to treatment with AgNPs on potato dextrose agar (PDA), malt extract agar, and corn meal agar plates. The findings from this research revealed that AgNPs possessed antifungal properties against these plant pathogens, albeit at varying degrees. Notably, the most significant inhibition of plant pathogenic fungi was observed when AgNPs were applied on potato dextrose agar (PDA) at a concentration of 100 ppm.

Table 1: Effect of different nanofungicides on radial mycelial growth (mm) of *Bipolaris sorokiniana.*

Treatments	Name of nanofungicides	Concentration	Radial mycelial growth (mm)				Per cent inhibition over control
			2nd Day	4th Day	6th Day	8th Day	
T1	Agritecnanofungicide	**50 ppm**	16.33	26.40	40.50	63.23	29.23
T2		**75 ppm**	14.33	24.10	37.45	51.53	42.18
T3		**100 ppm**	10.33	13.00	16.10	17.66	80.23
T4	Silver nanofungicide	**50 ppm**	14.66	27.33	38.33	59.66	33.22
T5		**75 ppm**	12.50	22.66	35.33	45.90	48.63
T6		**100 ppm**	08.30	09.36	10.46	12.23	86.41
T7	Selenium nanofungicide	**50 ppm**	15.56	29.33	40.66	61.10	31.61
T8		**75 ppm**	14.66	26.33	38.66	57.66	35.67
T9		**100 ppm**	14.28	16.20	19.44	22.44	74.88
T10	Silica nanofungicide	**50 ppm**	15.33	26.40	40.50	59.10	33.85
T11		**75 ppm**	13.10	22.33	38.66	51.66	42.18
T12		**100 ppm**	12.76	18.66	23.33	25.10	71.90
T13	Control		17.10	30.15	63.20	89.35	-
	CD		1.360	1.774	2.141	1.909	-
	SE (m)		0..465	0.607	0.732	0.653	-

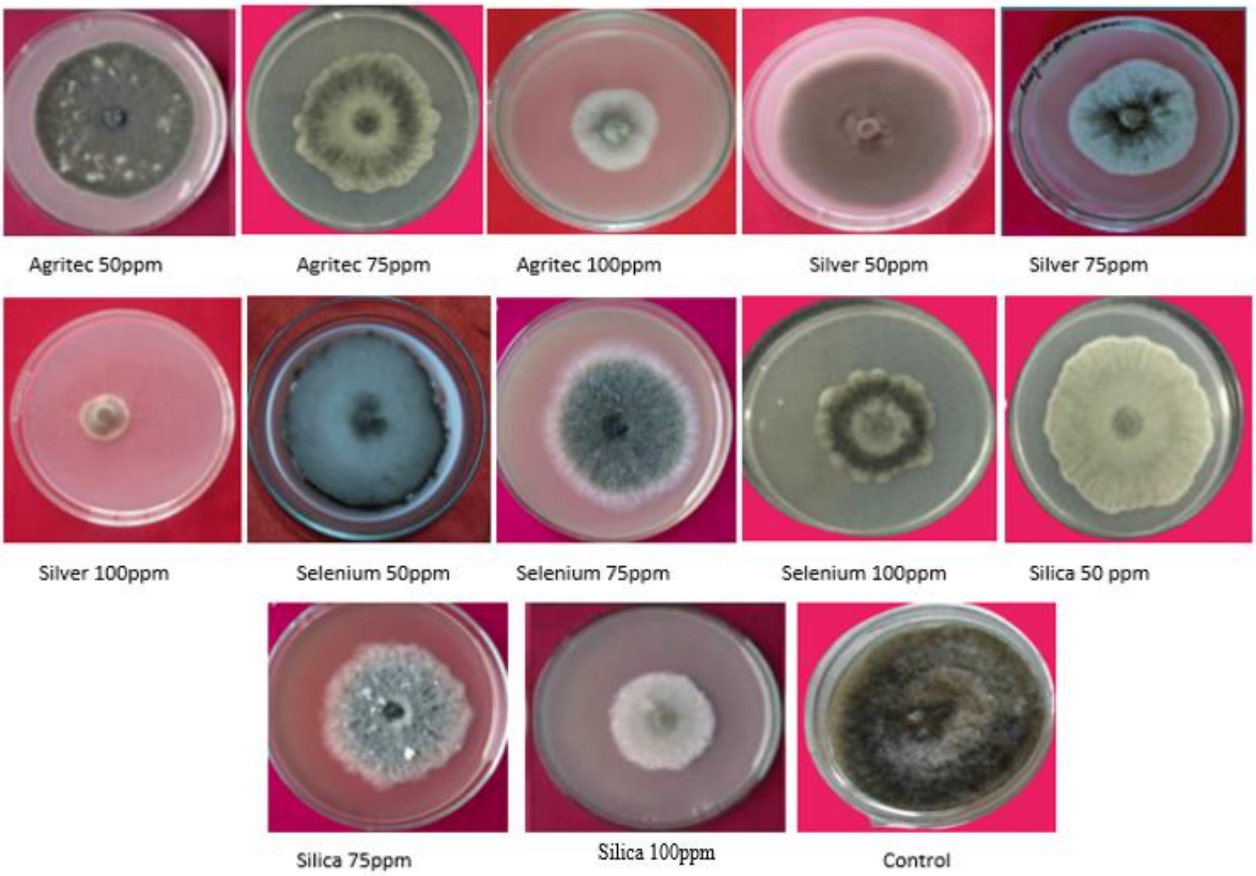

Plate 1: Effect of different concentration of nanofungicides on radial mycelial growth (mm) of *Bipolaris sorokiniana.*

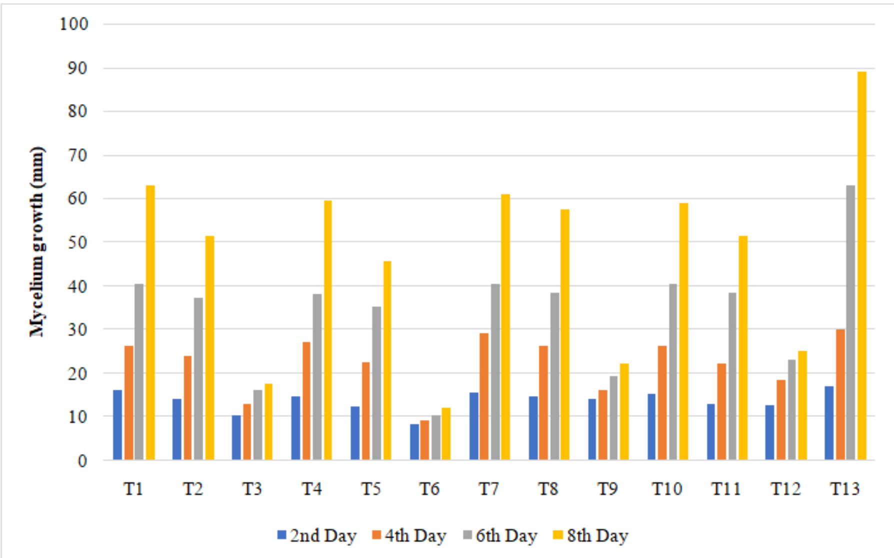

Fig. 1. Effect of different concentration of nanofungicides on radial mycelial growth (mm) of *Bipolaris sorokiniana*.

CONCLUSIONS

Wheat crop is attacked by a number of pathogens among them *Bipolaris sorokiniana* emerged as a major threat in hot and humid wheat growing regions. For management systemic and non-systemic fungicides are used but use of same chemicals may raise resistant strain among the pathogens. Therefore, use of nanofungicidal control of spot blotch disease cannot be overstated. Nanofungicides are a safe and effective alternative to chemical fungicides in mycelial growth inhibition of *B. sorokiniana* and are regarded as the best option in this scenario. Nanofungicides like Agriteck, Silver, Selenium and Silica were used to treat pathogens that cause plant diseases. It was observed that the higher concentrations of nanofungicides considerably slowed the radial mycelial growth of *B. sorokiniana* under *in vitro* conditions. At 8 DAI, Silver nanofungicide @100 ppm (86.41%), Agritecnanofungicide @100 ppm (80.23%), Selenium nanofungicide @100 ppm (74.88%) and Silica nanofungicide @ 100 ppm (71.90%) had the highest inhibition of mycelial growth compared to the control Therefore, using these nanofungicides as antifungal agents has the potential to decrease the environmental pollution and toxicity associated with chemical fungicides.

Acknowledgement. The authors express their heartfelt gratitude to the Head of the Department (HOD) of Plant Pathology CSAUAT, Kanpur, for not only granting them access to essential resources but also for offering valuable guidance throughout the course of their experiments.
Conflicts of Interest. None.

REFERENCES

Abdel-Hafez, S. I., Nafady, N. A., Abdel-Rahim, I. R., Shaltout, A. M., Daro`s, J. A. and Mohamed, M. A. (2016). Assessment of protein silver nanopar-ticles toxicity against pathogenic *Alternaria solani*. *Biotech*., *6*, 199

Abdelmalek, G. A. M and Salaheldin, T. A. (2016). Silver Nanoparticles as a Potent Fungicide for Citrus Phytopathogenic Fungi. *J Nanomed Res*., *3*(5), 65.

Ellis, M. B. (1971). Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, pp608.

Feldman, M., Bonjean, A. P. and Angus, W. J. (2001). Origin of cultivated wheat, *The world wheat book, a history of wheat breeding* Paris, France, Lavoisier Publishing, 3-56.

Dhingra, O. D. and Sinclair, J. B. (1985). Plant Path. Meth., CRC Press, Inc

Horsfall, J. G. (1956). Principles of fungicidal action. *Cronical Botanica* (U.S.A.) public, 207.

Ismail, A. W. A., Nagwa, M. S., Rawhia, A. A., Rasha, M. F. and Ahmed I. E. (2016). Evaluation of *in vitro* Antifungal Activity of Silver and Selenium Nanoparticles against *Alternaria solani* Caused Early Blight Disease on Potato. *BBJ*., *12*(3), 1-11.

Kiesling R. L. (1985). The diseases of barley. In: Rasmusson DC (eds.) Barley agronmonogr 16. ASA and SSSA, Madison, WI, 269-308.

Kim, S. W., Jin, H. J., Kabir, L., Yun, S. K., Ji, S. M. and Youn, S. Li. (2012). Antifungal Effects of Silver Nanoparticles (AgNPs) against Various Plant Pathogenic Fungi. *Mycobiology 40*(1), 53-58.

Kumar, A., Singh, R., Singh, G., Sharma, R. K., Saharan, M. S., Chhokar, R. S., Tyagi, B. S., Sendhil, R., Chand, R. and Sharma, I. (2014) Wheat cultivation in India (Pocket Guide). Directorate of Wheat Research, Karnal, Extension Bulletin, 52: pp. 34.

Lamsal, K., Sang, W. K., Jin, J., Yun, K., Kyong, S. and Youn, S. L. (2011). Application of Silver Nanoparticles for the Control of *Colletotrichum* Species *invitro* and Pepper Anthracnose Disease in Field. *Mycobiology*, *39*(3), 194-199.

Mohamed, M. A. (2015). One-Step Functionalization of Silver Nanoparticles Using the Orsellinic Acid Compound Isolated From the Endophytic Fungus Epicoccum Nigrum: Characterization and Antifungal Activity. *International Journal of Nanomaterials and Chemistry*, *1*, 103-110.

Park, H. J., Kim, S. H., Kim, H. J. and Choi, S. H. (2006). A new composition of nanosized silica-silver for control of various plant diseases. *J. Plant Pathol.*, *22*, 295 302.

Rai. M., Agarwal, D. K., Sharma, R. P., Verma, S. A., Kumar, A. T., Pradhan, S., Bharti, V. K. and Gupta, K. B. (2012) Field crop. In: Hand book of agriculture. 6thedn.Directorate of knowledge management in agriculture, ICAR, pp.964-1087.

Thirumurugan, G., Veni, V. S., Ramachandran, S., Rao, J. V. and Dhanaraju, M. D. (2011). Superior wound healing effect of topically delivered silver nanoparticle formulation using eco-friendly potato plant pathogenic fungus: synthesis and characterization. *J Biomed. Nanotechnol*, *7*(5), 659-666.

USDA, World Agricultural Production (2023) Office of Global Analysis, FAS.

Van Ginkel, M. and Rajaram, S. (1998). Breeding for resistance to spot blotch in wheat Global perspective. *Helminthosporium* Blight of Wheat: Spot Blotch and Tan Spot. Mexico, DF: CIMMYT, 162–169.

How to cite this article: Bipin Verma, Samir Kumar Biswas, Naimish Kumar and Gajendra Pratap (2023). Effect of different Nanofungicides on Radial Mycelial Growth of *Bipolaris sorokiniana* causing Spot Blotch of Wheat. *Biological Forum – An International Journal, 15*(9): 552-556.