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DETERMINING THE IMPACT OF FUNGICIDES IN-VITRO CONDITION AGAINST ANTHRACNOSE DISEASE CAUSED BY *COLLETOTRICHUM LINDEMUTHIANUM* IN FRENCH BEAN

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ABSTRACT:
Anthracnose of French bean caused by *Colletotrichum lindemuthianum* L. is considered to be most devastating disease which occurred all over the world. The present investigation at Department of Plant Pathology, Bihar Agricultural university, Sabour Bhagalpur. Isolation of the *Colletotrichum lindemuthianum* was done by tissue isolation technique and maintained on PDA media. Pathogenicity of *C. lindemuthianum* on French bean plant was proved by seed soaking and sprays inoculation technique. Symptoms were developed after 7 days on Local variety of French bean. The fungus produces brownish-white, fluffy circular growth at the centre and greyish black at the periphery and reverse side of the colonies produced yellowish pigmentation on PDA media. Mycelium of *C. lindemuthianum* was filamentous, dense, septet and greyish black in colour. Conidia were hyaline, single celled, uninucleated, cylindrical with rounded conidia. The fungus *C. lindemuthianum* produced symptoms on all the aerial parts of the plant. In vitro evaluation of fungicides indicated that among six systemic fungicides, Propiconazole 25% EC (T3) showed cent per cent mycelial inhibition @ 50ppm and Carbendazim 50WP (T1) showed cent per cent mycelial inhibition @ 100ppm. While Tebuconazol 50% + Trifloxystrobin 25% 75WP (T2) showed 100% inhibition @ 150ppm. Thiophanate methyl 75WP (T6) showed 100 percent inhibition @ 300ppm while remaining fungicides Metalaxyl-M 4% + Mancozeb (64%) 64 WP (T4) and Azoxystrobin 11 % + Tebuconazol 18.3 w/w SC, (T5) showed 100% inhibition @ 600ppm.

KEYWORDS: Anthracnose, French bean, Colletotrichum lindemuthianum, In-vitro, Fungicides.

1. INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is an very important legume vegetable grown throughout the world also in India. French bean crops belongs to family Leguminaceae and is originated from central America and north America (Poonacha et al., 2020). It is popularly called as Bush bean, Common bean, dwarf bean, haricot bean, kidney bean, snap bean and navy bean (Raghupati et.al., 2020). It is rich source of protein, vitamins and minerals. It is used as vegetable (fresh beans), shelled green beans and dried seeds (Rajmash) as pulse. The dried bean seeds are marketed under the name of Rajma. They are rich source of protein and closely compared with meat.

French bean is grown in numerous countries, including Latin America, Canada, United States of America, India, Nepal, Bangladesh, and nearly all European countries (Bisht and Savita, 2020). French bean is grown both in Kharif and Rabi seasons in India. In India, french bean is being grown in 2.28 lakh ha area with 22.77 lakh MT production and 9.98 MT/ha productivity. Major growing states in India are Andhra Pradesh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka Odissa and Uttar Pradesh. (Anon., 2019; Maibam et al., 2015). French bean have four majorly cultivated species of Phaseolus, viz., *P. vulgaris, P. lunatus, P. coccineus and P. acutifolius* var. *latifolius* (Hema and Rana, 2020). Though the crops predominantly self- pollinated and belongs to Fabaceae family with 11 pairs of chromosomes (2n=2X=22) (Rathnapriya and Mani ckavasagan, 2020). Because of its importance as a legumineous vegetable cultivated in India, French bean is also known as "poor man's meat" and "super food".

French bean is grown in numerous countries, including Latin America, Canada, United States of America, India, Nepal, Bangladesh, and nearly all European countries (Bisht and Savita, 2020). The annual French bean production in worldwide is about 12 millon tonnes in which Brazil is the major contributor. In India during 2019-20, the area of bean cultivars was 221 thousand ha, with production of 2226 thousand Mt. The area and production in Bihar during 2017-18 was 5.50 thousand ha with annual production of 55.48 thousand MTt. It is often cultivated in the hilly region during the Kharif season and as a spring crop in the lower hill and Tarai regions. It is grown during the Rabi season in Maharashtra's north-eastern plain and hill tract.

Both biotic and abiotic constraints are responsible for reducing the production of common beans. Among the biotic factors such as fungi, bacteria, virus, nematode and phytoplasma cause significant yield loss in French bean. Several

scientists reported several fungal disease in French bean such as Anthracnose, Rust, White mold, Damping off, Powdery mildew, Fusarium wilt, Root rot and Common bean blight which causes significant damage to the French bean crop.
Colletotrichum lindemuthianum (Sacc. & Magn.) Bri. & Cav.is a destructive fungal seed borne pathogen causing anthracnose of french bean. The pathogen occurs globally and develops relatively well in cool and humid conditions (Leitich et al., 2016). The pathogen affects all the above ground portion viz., leaves, petioles, pods and also seeds. The pathogen is highly viable, more than 100 pathogenic variants and races were reported (Katungi et al., 2009).The disease could be devastating causing upto 100 per cent yield loss (Mohammed, 2013).
Anthracnose is the most devastating of these diseases, causing full defoliation, a fall in yield, and a decrease in bean quality, despite the fact that it occurs all over the world, including both tropical and subtropical climates (Devi and Narayanswamy, 2017). This disease is transmitted by both seed and soil (Singh, 2018). Due to this seed born nature and pathogenic variability this fungus generates difficulties in breeding for resistance in cultivars *Colletotrichum lindemuthianum* is the anamorph or imperfect stage of the anthracnose pathogen and acervulus is produced that erupt through the epidermis. The acervulus has short conidiophores capped with conidia. *Glomerella lindemuthianum* is a sexual form, teleomorph, or perfect stage that forms asci or perithecia in laboratory produced culture, a characteristic of ascomycetes. (Padder et al., 2017). Teleomorph phases are uncommon in field conditions. Bean anthracnose is a common recurrence in India, with a wide spectrum of pathogenic and genetic diversity. (Sharma et. al., 2007). Anthracnose disease is caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) describe results heavy loss of crop in worldwide. The losses are more in the regions with prevailing high humidity and moderate temperatures 13 to 27 0C. Anthracnose is susceptible bean cultivars under ideal disease circumstances may result in an epidemic with a 100% yield loss. In the view of the above facts and losses caused by Anthracnose disease in French bean crop the present work have been formulated.
Fungicides are still a key player in plant disease management. In vitro evaluation of fungicides against the pathogen reveals the effectiveness of fungicide in disease management. Timely use of correct fungicides in proper dose significantly reduces the disease severity and yield losses.

2. MATERIALS AND METHODS

The present investigation was undertaken at Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India.

2.1 Collection of disease samples

A large number of disease sample of French bean plants carrying the characteristics symptoms of anthracnose disease was Collected from College farm and field, the specimens/ symptom was collected in paper bag and labeled properly and brought to the laboratory and critically examined and the causal organism was isolated.

2.2. Preparation of Culture Media (PDA)

Potato infusion (300g), Agar-agar (20g) Dextrose (20g) and water (1liter) these ingredients were taken. Firstly, 300g peeled potatoes were boiled in 2 liter of water for 30 minutes until the potato became soft. The water was filtered with muslin cloth and used for further media preparation. Then 20g of agar and 20g dextrose was weighed with the help of electric balance and were poured in a 2000 ml capacity media bottle. The volume was made up to 1000 ml by adding the potato starch extracted water. The ingredients were thoroughly mixed using an electric stirrer and then autoclaved at 121 ^{0}C for 20 minutes under 15 pounds per square inch (PSI) pressure. After autoclaving, the media was allowed to cool. The Luke-warm media was poured in sterilized petri dishes and allowed to solidify.

2.3 Culture methodology

To isolate, purify, multiply, and maintain a pure culture of *Colletotrichum lindemuthianum* in vitro cultural we have to first confirm identify and detailed studies on the morphological feature of the pathogen, the organism was cultivated in potato dextrose agar (PDA) and potato dextrose broth (PDB) medium were utilized as the basic media.

2.4 Isolation of Pathogen

The diseased sample were washed using distilled water, and then dried using blotter paper and cut with sterilized sharp blade into small bits, keeping half diseased and half healthy portion intact and were surface sterilized with 1 % sodium hypochlorite in a petriplate followed by three sequential washes with distilled water in three separate petriplates store remove the traces of sodium hypochlorite under laminar airflow cabin. The washed sample bits were blot dried and inoculated on a cooled sterilized PDA by keeping two bits on each Petri plates and incubated in BOD incubator at 27 ±1 ^{0}C. After a week from incubation, pure culture of the test pathogen was obtained and the test pathogen was multiplied by sub-culturing, purified, and then maintained separately on agar slant using hyphal tip techniques and stored in refrigerator for further studies. Later pathogenicity test was also performed for confirmation.

2.5 Re-isolation

The micro-organism was reisolated from the infected inoculated plants and showing the characteristic symptoms of root-rot and then brought into pure form according to the procedures described earlier.

2.6 In vitro Efficacy of Fungicides

The effectiveness of fungicides (alone) or combi product of fungicides against *C. lindemuthianum* was evaluated in vitro. The poisoned food technique (Nene and Thapliyal, 1993) was used to analyze Five concentrations of the

fungicides (Recommended, above, and below the recommended doses), using PDA medium as the primary culture medium. Based on the active ingredient, the required quantity of the test fungicides was calculated. To attain the desired concentration, each test fungicide was thoroughly mixed individually with autoclaved and chilled PDA medium in sterile glass conical flasks (250 ml). After being independently mixed with the test fungicides, this PDA medium was aseptically poured (20 ml/plate) into sterile glass Petri plates (90 mm), where it was then left to harden at room temperature, these all activity was being down in laminar air flow. For each of the test fungicides, three replications were kept at the desired concentrations. After being incubated at 27 ±1 0C, each plate was infected with a 5 mm pure culture disc made from a culture of the test fungus that had been grown for seven days. Untreated control was performed using petri plates containing plain PDA without fungicides and infected with test fungal discs.
Aseptically pouring this PDA media (20 ml/plate) into sterile glass Petri plates (90 mm) after it had been independently altered with the test fungicides, and allowing it to harden at room temperature. For each of the test fungicides at the desired concentrations, three replications each concentration were taken. All of the plates were inoculated with a 5 mm pure culture disc made from an active, seven-day-old culture of the test fungus, and they were then incubated at a temperature of 27± 1 0C. Untreated controls included petri plates with plain PDA without fungicides that were inoculated with test-fungus discs.

Table 1: Details of Experiment

Design	**:**	**CRD**
Treatments	:	Six
Replications	:	Six
Doses / Concentration	:	50, 100, 150, 300, 600, 900 ppm

Table 1A: List of fungicides used for in vitro evaluation

S. No.	**Common Name**	**Trade Name**
1	Carbendazim 50WP	Bavistin by Crystal Crop Protection Ltd.
2	Tebuconazol 50% + Trifloxystrobin 25% 75WP	Nativo by Bayer CropScience
3	Propiconazol 25EC	Tilt by Syngenta
4	Metalaxyl-M 4% + Mancozeb (64%) 64WP	Ridomil Gold by Syngenta
5	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	Spectrum by Dhanuka,
6	Thiophanate Methyl 70% WP	Roko by Biostadt India Limited

Table 2: Treatments Details of Fungicides

S.No.	**Treatment No.**	**Chemical Name**	**Active ingredient**	**Dose in ppm**
1	T1	Carbendazim	50 WP	50, 100, 150, 300, 600, 900
2	T2	Tebuconazol 50% + Trifloxystrobin 25%	75WP	50, 100, 150, 300, 600, 900
3	T3	Propiconazol	25EC	50, 100, 150, 300, 600, 900
4	T4	Metalaxyl-M 4% + Mancozeb (64%)	64WP	50, 100, 150, 300, 600, 900
5	T5	Azoxystrobin 11 % + Tebuconazol 18.3	w/w SC	50, 100, 150, 300, 600, 900
6	T6	Thiophanate Methyl	70WP	50, 100, 150, 300, 600, 900

Every 24 hours, the test pathogen's colony mycelial growth was monitored until the untreated control plates had fully covered the plate. Applying the below formula, the percent mycelial inhibition of the test pathogen was determined.

Percent inhibition = $\frac{C-T}{C} X\ 100$

Where,
C = Growth of the test fungus in control
Plate = Growth of test fungus in treated plate.

RESULTS

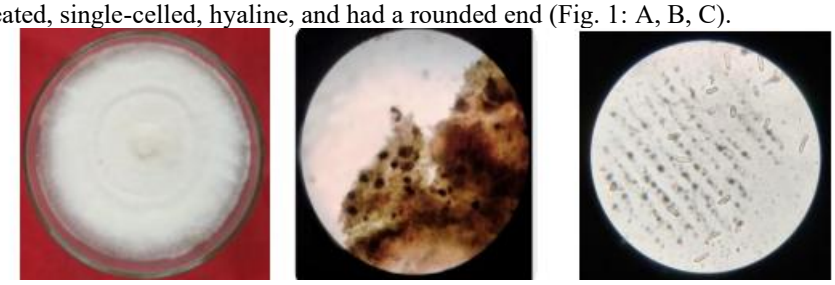
3.1 Cultural Character

The fungus induces greyish black in the periphery and brownish-white, fluffy circular growth in the centre. Only the core of the colonies' reverse side's golden pigmentation and remaining black were formed. The fungus initially generated white- colour mycelial colonies, which gradually turned grey and eventually produced greyish-black colour colonies.
In seven days, *C. lindemthiuanum* mycelium completely covered a 90 mm Petri plate (Fig. 1 (A)). On PDA media at 27 ±1 °C, the diameter of mycelial development was 28 mm on the 2nd day, 63 mm on the fourth day, and 90 mm on the

seven day. The fungus initially developed mycelium that was white in colour, which began to become grey on the eight day and eventually turned greyish-black after two weeks.
Concentric rings were occasionally, though infrequently, seen in several of the culture plates. But none of the culture plates contained any acervulus. After three weeks, the fungus began to produce conidia.

3.2 Morphological Characteristics

Coletotrichum lindemuthianum has a filamentous, septet, thick, and greyish-black mycelium. The conidia were cylindrical, uninucleated, single-celled, hyaline, and had a rounded end (Fig. 1: A, B, C).



A B C

Fig. 1: A - Full Growth Culture, B - Acervuli, C - Conidia

3.3 In vitro efficacy of fungicides on mycelia growth of *C. lindemuthianum*

The present study for the evaluation of the inhibitory effect of different fungicides against anthracnose disease of French bean revealed that fungicides at the concentration 50ppm T3 - Propiconazol 25% EC showed the maximum mycelial inhibition (100%) followed by T1 - Carbendazim 50WP showed (92.42%), T2 - Tebuconazol 50% + Trifloxystrobin 25% 75WP showed mycelial inhibition (81.81%), T5 - Azoxystrobin 11 % + Tebuconazol 18.3 w/w SC showed mycelial inhibition (78.97%), T4 - Metalaxyl-M 4% + Mancozeb (64%) 64WP showed mycelial inhibition (49.05%) and the least mycelial inhibition Per cent found in T6 Thiophanate methyl 70WP showed (27.84%).
On increasing concentration of fungicides @100 ppm the T3 - Propiconazol 25% EC and T1 - Carbendazim 50WP showed the maximum mycelial inhibition of 100% followed by T2 - Tebuconazol 50% + Trifloxystrobin 25% 75WP showed (91.09%), T5- Azoxystrobin 11 % + Tebuconazol 18.3 W/W SC showed (80.68%), T4- Metalaxyl-M 4%+ Mancozeb (64%) 64WP showed (51.13%) and the least mycelial inhibition Perecent found to be showed by T6 - Thiophanate methyl 70WP showed (34.84%) inhibition.
On increasing the concentration of fungicides @150ppm of fungicides I found that the T3 - Propiconazol 25EC , T1 - Carbendazim 50WP and T2 - Tebuconazol 50% + Trifloxystrobin 25% 75WP showed 100% the maximum mycelial inhibition, followed by T5 - Azoxystrobin 11% + Tebuconazol 18.3 W/W Sc showed (82.09%), T4 - Metalaxyl-M 4% + Mancozeb 64% 64WP (55.30%) and the least inhibition Per cent of mycelia was found T6 -Thiophanate methyl 70WP showed (43.18%) inhibition.

Table 3: In vitro efficacy of systemic fungicides @ 50, 100, 150, 300, 600 ppm concentration.

Treatment	**% Inhibition mycelial growth**				
	50ppm	**100ppm**	**150ppm**	**300ppm**	**600ppm**
T1 - Carbendazim 50WP	92.42	100	100	100	100
T2- Tebuconazol 50% + Trifloxystrobin 25%	81.81	91.09	100	100	100
T3-Propiconazol 25EC	100	100	100	100	100
Metalaxyl-M 4% + Mancozeb (64%)	49.05	51.13	55.30	76.13	100
T5-Azoxystrobin 11% + Tebuconazol 18.3 w/w SC	78.97	80.68	82.09	84.09	100
T6-Thiophanate methyl 70% wp	27.84	34.84	43.18	100	100
T7-Control	0	0	0	0	0
CV	1.73	1.16	1.75	0.82	0
SEM	0.71	0.51	0.81	0.44	0
CD at 5%	2.20	1.58	2.49	1.91	

**Mean of five observations.*
The treatment are compared using Duncan multiple range test (DMRT)

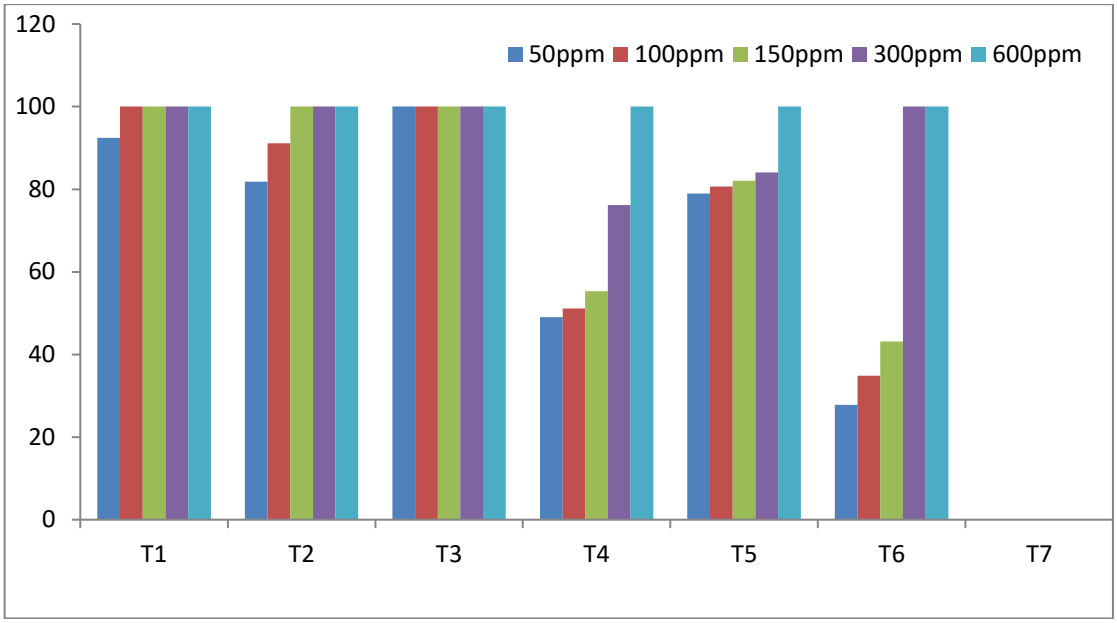


Fig.2 In vitro efficacy of fungicides on mycelia growth of C . lindemuthianum

	50ppm	100ppm	150ppm	300ppm	600ppm
Tebuconazo l50% + Triflostrobin 25% WP					
Metalaxy l4% + Mancozeb 64% (64WP)					
Thiophanate methyl 70WP					
Azoxystrobin11 % +Tebuconazole 18.3% w/w SC					
Propiconazole 25EC					

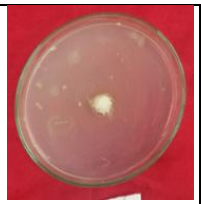
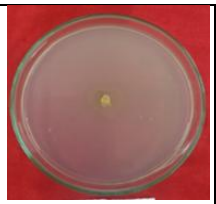
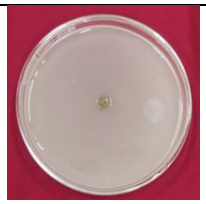
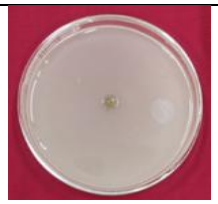
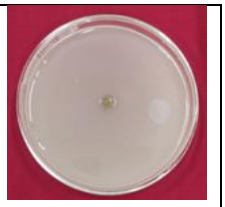
Carbendazim 50WP					

Fig. 3: In vitro efficacy of fungicides on mycelia growth of C. lindemuthianum

On increasing concentration of fungicides up to 300ppm the T3 - Propiconazol 25% EC , T1 - Carbendazim 50WP, T2 - Tebuconazol 50% + Trifloxystrobin 25% 75WP and T6 - Thiophanate methyl 70WP showed the maximum mycelia linhibition of 100% followed by T5 - Azoxystrobin 11 % + Tebuconazol 18.3 W/W SC showed (84.09%) inhibition and the least inhibition Per cent found in Metalaxyl-M 4% + Mancozeb (64%) 64WP showed (76.13%) inhibition of mycelia. In vitro evaluation of fungicides in dicate that among six fungicides Propiconazol (T3), 25EC showed cent per cent mycelia inhibition @50ppm, 100ppm, 150ppm, 300ppm and 600ppm and Carbendazim 50 WP (T1) cent per cent mycelia inhibition @ 100ppm, 150ppm, 300ppm and 600ppm. While Tebuconazol 50% + Trifloxystrobin 25% 75WP (T2) showrd 100% mycelia inhibition @150ppm, 300ppm and 600ppm, Thiophanate methyl 70WP (T6) showed 100% mycelia inhibition @300ppm and 600ppm, while reaming fungicides Metalaxyl-M 4% + Mancozeb (64%) 64WP (T4) and Azoxystrobin 11% + Tebuconazol 18.3 (T5) W/W SC showed 100% inhibition @600ppm. All funghicides were showed 100% mycelia inhibition against @ 600 ppm. (Table 3, Fig. 2 and Fig. 3)

4. DISCUSSION

Total six fungicides viz., Carbendazim 50WP (T1), Tebuconazol 50% + Trifloxystrobin 25% 75WP (T2), Propiconazol 25EC (T3), Metalaxyl-M 4%+Mancozeb (64%) 64WP (T4), Azoxystrobin 11 % + Tebuconazol 18.3 w/w SC (T5), and Thiophanate Methyl 70%WP (T6) at five different concentrations (50, 100, 150, 300, 600ppm) were tested in vitro against *Colletotrichum lindemuthianum*, applying poisoned food technique (Nene and Thapliyal, 1993) and using basal media as Potato Dextrose Agar (PDA). The effect of the fungicides on colony diameter, growth and inhibition of *C. lindemuthianum* was recorded. Similar result obtained by Jyotika et al., (2023) for Carbendazim. same result for propiconazol by Badgujar et al., (2017), Chacko and Gokulapalan (2014), similar result was also obtain by Devi and Narayanaswamy et al., (2016) for Thiophanate Methyl 70%WP. Poonacha et al., (2020) Similar research were also found by (Rajesha G. et al., 2014) for Propiconazole and Carbendazim at 100 ppm.

5. CONCLUSION

One of the most damaging diseases of French bean (*Phaseolus vulgaris*) is anthracnose, which is caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara. The current investigations were carried out at Bihar agricultural university, Sabour taking into account the economic significance of the crop and the disease. In our present study by In vitro evaluation of fungicides indicate that among six fungicides Propiconazol (T3), 25EC showed cent percent mycelia inhibition @ 50ppm and Carbendazim 50 WP (T1) @100 ppm shows cent Perecent inhibition. While Tebuconazol 50% + Trifloxystrobin 25% 75WP (T2), show100% inhibition @ 150ppm, Thiophanate methyl 70% WP showed 100 Perecent inhibition @ 300ppm, while reaming fungicides Metalaxyl-M 4%+ Mancozeb (64%) WP and Azoxystrobin 11 % + Tebuconazol 18.3 W/W SC, showed 100% inhibition @ 600ppm. All funghicides were showes 100% inhibition against @ 600 ppm. This study will help the farmer for selection and quantification of correct combination of fungicides to manage the disease.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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