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Efficacy of fungicides, aqueous plant extracts, bio-agents on *Bipolaris maydis in vitro*

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Abstract
Maize (*Zea mays* L.), commonly known as corn, is a highly adaptable crop that is widely grown across the world. It has been dubbed a "miracle crop" due to its versatility and ability to thrive under various agro-climatic conditions. However, this crop is susceptible to maydis leaf blight (MLB), a disease caused by the fungus *Bipolaris maydis* (Nisikado & Miyake) Shoemaker. MLB can cause a significant reduction in maize yields, ranging from 9.7 to 11.7% (Harlapur *et al.*, 2000), and under certain weather conditions, the disease has been associated with yield losses of up to 70% (Wang *et al*., 2001). To address this issue, a study was conducted in the laboratory of the Department of Plant Pathology at Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj (UP), to investigate the effectiveness of fungicides, aqueous plant extracts, and bio-agents against *Bipolaris maydis in vitro*. Two non –systemic and two combi- fungicides at 1000 ppm, 1500 ppm and 2000 ppm; four systemic fungicides at 500 ppm, 1000 ppm and 1500 ppm; nine different plant extracts and two bio-agents were evaluated against *B. maydis* Among the non-systemic and combi-fungicides, propiconazole 13.9% EC + difenoconazole 13.9% EC was the most effective at inhibiting the growth of *B. maydis*. It showed 80.33%, 88.44%, and 100% inhibition at 1000 ppm, 1500 ppm, and 2000 ppm concentrations, respectively. The next best was carbendazim 12% EC + mancozeb 63% EC, which showed 61.22%, 65.10%, and 78.22% inhibition, respectively. Among all systemic fungicides, propiconazole 25% EC was found to be the most effective, inhibiting 100% of *B. maydis* growth at all concentrations tested. Difenoconazole 25% EC was also very effective, inhibiting mycelial growth completely at 1000 ppm and 1500 ppm concentrations. The study showed that garlic clove extract is effective in inhibiting the growth of *B. maydis*, with a 76.11% reduction in mycelial growth at a concentration of 10%, and a 97.11% reduction at 15%. Neem leaf extract also demonstrated significant inhibition, with reductions of 62.33% and 73.78% at 10% and 15% concentrations, respectively. In terms of bio-agents, *Trichoderma viride* was the most effective in inhibiting mycelial growth, with a reduction of 73.44%, followed by *Pseudomonas fluorescens* (57.44%).

Keywords: Maize, *Trichoderma viride*, *Pseudomonas fluorescens* fungicides, plant extract, mycelial growth, maydis leaf blight, *B. maydis*

1. Introduction
Maize, also referred to as corn or by its scientific name *Zea mays* L., is an incredibly versatile crop that is widely cultivated around the world. Originally native to Mexico, it has been heralded as a "miracle crop" due to its adaptability to various agro-climatic conditions (Mangeldorf, 1974). Corn was introduced to India by the Portuguese in the 17th century and the word "corn" is derived from the Spanish word "Mahiz". With its high genetic yield potential, maize is often called the "queen of cereals".

Corn, also known as maize, holds the third position after rice and wheat as India's most important crop. Preliminary data suggests that it is cultivated on 9.89 million hectares of land (2020-2021), accounting for 81% of total cultivated land. Corn contributes nearly 9% of India's national food supply at current prices and ads over $100 billion to the agricultural GDP. Additionally, it creates employment opportunities for more than 100 million people in the agricultural sector as well as its downstream industrial and agricultural sectors.

Corn is cultivated across all states of India on 9.89 million hectares for various purposes such as grain, fodder, green corn cob, sweet corn, baby corn, and popcorn. It is a crop that does not discriminate. The domestic production stands at 31.99 quintals per hectare, resulting in a production volume of 31.65 million tonnes. Karnataka, with 1.68 million hectares, ranks first in the country in terms of area and recorded the highest production of 5.18 million tonnes, contributing 16.45% of the total national production. Its productivity is 3.09 tonnes per

hectare, followed by Madhya Pradesh, which ranks second in terms of area with 1.46 million hectares and recorded the highest production of 3.58 million tonnes, contributing 11.37% of the total national production. The cultivation of corn has become vital in the non-traditional region of Peninsular. Tamil Nadu ranks first in terms of productivity with 6.82 tonnes per hectare. Uttar Pradesh ranks ninth in both production (1.75 million tonnes) and productivity (2.33 tonnes per hectare) and shares 0.72 million hectares of the total cultivated area. (Source: Agriculture Statistics at a Glance, 2021). It is projected that India would require 121 million tonnes of corn production by 2050.

Bipolaris maydis (Nisikado & Miyake) Shoemaker is a type of fungus that causes a disease called maydis leaf blight, also known as southern corn leaf blight. This fungal disease is a significant problem for maize cultivation in almost all regions of India. The pathogen has been categorized into three races: race "T," race "O," and race "C." Race "T" is of particular concern as it causes a highly virulent strain of the disease that historically caused a severe epidemic of leaf blight in the United States. This was due to the widespread use of Texas Male Sterile (TMS) lines, which are extremely susceptible to the disease (Misra, 1979; Sharma *et al*., 1978) [22]. Although there is a pathotype that resembles the Indian race T, the 'O' cultivar is the most widely grown worldwide, including in India. MLB is well-suited to hot and humid weather conditions. *Helminthosporium maydis* was first identified by Drechsler in the United States in 1925, but it was not until 1960 that Munjal and Kapoor identified it in India's Maldah District of West Bengal. Sharma *et al.* (1978) [22] also reported an outbreak of the disease in Rajasthan and Ludhiana.

Maydis leaf blight, caused by *Bipolaris maydis*, can lead to a reduction in maize yields by 9.7 to 11.7%, depending on weather conditions (Bera & Giri, 1979; Harlapur *et al*., 2000; Sharma *et al*., 2003; Kumar & Saxena, 2007) [2, 6, 23, 10]. However, some studies have reported even more substantial yield losses of up to 70% (Wang *et al*., 2001; Ali *et al*., 2012) [25,]. With this in mind, the current research project aimed to investigate the effectiveness of fungicides, aqueous plant extracts, and bio-agents on the *in vitro* mycelium growth of *Bipolaris maydis.*

2. Material and Methods

An *in vitro* study was conducted in the laboratory of the Department of Plant Pathology at Naini Agriculture Institute, SHUATS, Prayagraj, UP in 2021 to investigate the effectiveness of fungicides, aqueous botanical extracts, and bio- agents on the mycelial growth of *B. maydis.*

2.1 Efficacy of fungicides against *B. maydis in vitro*

The four systemic fungicides being assessed are propiconazole 25% EC, difenoconazole 25% EC, hexaconazole 5% EC, and carbendazim 50% WP, while the two non-systemic fungicides are mancozeb 75% WP and chlorothalonil 75% WP. Additionally, two combi fungicides are being tested, namely carbendazim 12% WP+ mancozeb 63% WP and propiconazole 13.9% + difenoconazole 13.9% EC. The effectiveness of these fungicides is being evaluated over a control to determine their efficacy against *B. maydis*, which causes MLB disease. The food poisoned techniques recommended by Sharvelle (1961) [21] and Nene and Thaplial (1779) are being used for this evaluation.

To prepare different concentrations of fungicide, namely 500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm, 0.05 mg, 0.1 mg, 0.15 mg, and 0.2 mg of fungicide were added to 100 ml of PDA medium, respectively. The mixture was then autoclaved at 121°C for 20 minutes. Each sterilized Petri dish was filled with approximately 20 ml of the poisoned medium, and a 5 mm mycelial disk was taken from the periphery of a ten-day-old culture and placed in the center of each dish. The plates were then incubated at a temperature of 27±1°C. Control plates were also prepared without any fungicide added. Five replicates were maintained for each treatment. Once the mycelium growth reached the periphery of the Petri plates, the diameter of the colony was measured in two directions, and the average was calculated. The percentage inhibition of mycelial growth was calculated using the formula provided by Vincent (1947) [24]. The data were analyzed statistically using a two-factor completely randomized block design.

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

Where
I = Mycelial growth inhibition in percent,
C = Mycelial growth in control (mm) and
T = Mycelial growth (mm) in treatment.

2.2 Efficacy of aqueous extract of botanicals *in vitro*

Nine aqueous extracts of botanicals including garlic, onion, neem, *eucalyptus*, *parthenium*, kaner, ginger, *lantana*, and turmeric were tested for their efficacy against *B. maydis* using the poison food technique. Extracts were prepared by washing and crushing 100 g of freshly harvested plant parts with 100 ml of sterile distilled water. The supernatant was filtered and centrifuged at 5000 rpm for 30 min., and 10% and 15% concentrations were prepared by adding 10 ml and 15 ml of supernatant in approximately 20 ml of sterilized molten PDA medium. The medium was poured into petri dishes and inoculated with 5 mm mycelial discs cut from a 10-day-old fungal culture. The inoculated Petri plates were kept in BOD incubator at 27±1°C. Once the mycelium growth reached the periphery of the Petri dishes, the diameter of the colony was measured in two directions, and the average was calculated. The percentage inhibition of mycelial growth was calculated using the formula provided by Vincent (1947) [24]. The data were analyzed statistically using a two-factor completely randomized block design.

2.3 Efficacy of bio- agents against *Bipolaris maydis in vitro*

In this experiment, two biological agents, *Trichoderma viride* and *Pseudomonas fluorescens*, were tested against *Bipolaris maydis*. The experiment involved pouring 20 milliliters of PDA medium into sterile Petri plates and allowing it to solidify. For the dual fungal culture technique, a 5 mm mycelial disc from a 7-day-old culture of *B. maydis* and *Trichoderma viride* were transferred aseptically to the Petri plates, leaving some space between the two discs. In the dual fungal and bacterial culture technique, a 5 mm disc of *B. maydis* from a 7-day-old culture was transferred aseptically to one corner of the Petri plates, and *Pseudomonas fluorescens* culture was streaked at other corners using an inoculating loop while leaving sufficient space. Eight replications were maintained for each treatment, and the Petri plates were incubated at a temperature of 27±1°C until the mycelium growth reached the periphery in the control plate. The

diameter of the colonies of the test pathogen was measured, and the percentage inhibition of mycelial growth was calculated using the formula provided by Vincent (1947) [24].

3. Results and Discussion

3.1. Efficacy of fungicides, plant extracts and bio- agents *in vitro* against *B. maydis*

The result on the efficacy of fungicides, plant extracts and bio agents tested against *B. maydis* causing maydis leaf blight disease are presented hereunder

3.1.1 Efficacy of fungicides under *in vito* agaist *B. maydis*

Investigation, comparing the effectiveness of various non-systemic and combi fungicides against *B. maydis*, propiconazole 13.9% EC + difenoconazole 13.9% EC showed the highest efficacy, inhibiting 80.33%, 88.44%, and 100% of mycelial growth at concentrations of 1000 ppm, 1500 ppm, and 2000 ppm, respectively. Following closely behind was carbendazim 12% WP + mancozeb 63%WP, which inhibited 61.22%, 65.77%, and 76.00% of mycelial growth at the same concentrations respectively. The least effective treatment was chlorothalonil 75% WP, which showed only 31.22%, 34.00%, and 45.96% inhibition at 1000ppm, 1500ppm, and 2000ppm, respectively, over to the control. Similar conclusion was made by Kumar *et al.* (2009a) [11]; Kumar *et al.* (2019) [9]; Bharti *et al.* (2020) [3]; for propiconazole and combination with it.

All concentrations of each non-systemic and combi fungicide showed a significant difference from each other over the control. In terms of interactions, all of the interactions were found to be significantly different from the control. However, mancozeb 75% WP at 1000 ppm and chlorothalonil 75% WP at 2000 ppm were found to be non-significant from each other. Additionally, at a concentration of 1000 ppm, propiconazole 13.9% EC + difenoconazole 13.9% EC and carbendazim 12% WP + mancozeb 63% were found to be non-significant from each other, as were carbendazim 12% WP and mancozeb 63% WP at a concentration of 2000ppm.

Table 1: Mycelial growth inhibition of *Bipolaris maydis* in different concentration of non-systemic and combi -fungicides *in vitro*

Treatment		Per cent inhibition in mycelium growth (mm)*						
No.	Non systemic and combi Fungicides	Concentration						
		1000 ppm		1500 ppm		2000 ppm		Conc. mean
		G	I	G	I	G	I	
T_1	Mancozeb 75% WP	49.89	44.57	41.20	54.22	22.20	75.33	37.76
T_2	Chlorothalonil 75%WP	61.90	31.22	59.40	34.00	48.64	45.96	56.67
T_3	Carbendazim 12% + Mancozeb 63% WP	34.90	61.22	31.41	65.10	19.60	78.22	28.64
T_4	Propiconazole 13.9% EC + Difenoconazole 13.9% EC	17.70	80.33	10.40	88.44	0.00	100.00	9.37
T_0	Control	90.00	-	90.00		90.00		90.00
Treatment means		50.88		46.48		36.09		
		Treatment (A)		Concentration (B)		Interaction (AxB)		
CD (5%)		1.32		1.03		2.30		
SE. M (±)		0.47		0.36		0.81		

G=Mycelium growth (diameter), I= Per cent inhibition, *Average of five replications

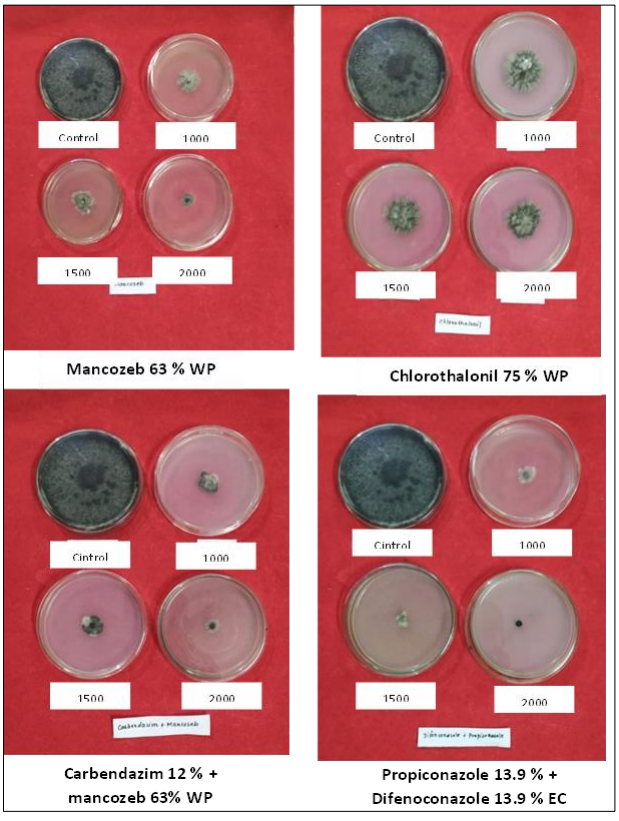

Fig 1: Mycelial growth inhibition of *Bipolaris maydis* in different concentration of non-systemic and combi fungicides *in vitro*

Among the systemic fungicides tested, the highest inhibition of mycelial growth was observed with propiconazole 25% EC, which completely inhibited mycelial growth at all concentrations *viz*. 500 ppm, 1000 ppm and 2000 ppm. Difenoconazole 25% EC showed 85.56%, 100%, and 100% inhibition, while Hexaconazole 5% EC exhibited 81.56%, 85.24%, and 100% inhibition, respectively. The least inhibition was observed with carbendazim 25% EC, which showed 60.11%, 83.51%, and 84.56% inhibition at 500 ppm, 1000 ppm, and 1500 ppm, respectively, over the control. Similar conclusion was made by Kumar *et al.* (2009a) [11]; Kumar *et al.* (2019) [9]; Bharti *et al.* (2020) [3]; for propiconazole and combination with it.

Based on the results, all treatments showed a significant difference from the control. At a concentration of 500 ppm, all treatments showed significant differences from each other. At 1000 ppm, there was a significant difference between hexaconazole 5% EC and carbendazim 50% WP, but no significant difference between propiconazole 25% EC and difenoconazole 25% EC. At 1500 ppm, carbendazim 50% WP was significantly different from the other treatments, while there was no significant difference between propiconazole 25% EC, difenoconazole 25% EC, and hexaconazole 5% EC.

In terms of concentrations, hexaconazole 5% EC and carbendazim 50% WP were significantly different at all concentrations, while propiconazole 25% EC was not significantly different at any concentration. Difenoconazole 25% EC was significant at 500 ppm but not at 1000 ppm or 1500 ppm.

In terms of interactions, all interactions were significantly different from the control. However, hexaconazole 5% EC and carbendazim 50% WP were significantly different from each other at 500 ppm, and carbendazim 50% WP was significantly different from the other treatments at 1000 ppm and 1500 ppm. Propiconazole 25% EC was not significantly different from difenoconazole 25% EC or hexaconazole 5% EC at any concentration, and difenoconazole 25% EC was not significantly different from propiconazole 25% EC or hexaconazole 5% EC at 1000 ppm and 1500 ppm, while hexaconazole 5% EC was not significantly different from propiconazole 25% EC or difenoconazole 25% EC at 1500 ppm.

Table 2: Mycelial growth inhibition of *Bipolaris maydis* at different concentration of systemic fungicides

Treatment No.	Treatment name	Per cent inhibition in mycelial growth in mm*						
		Concentration						
		500 ppm		1000 ppm		1500 ppm		Conc. mean
		G	I	G	I	G	I	
T_1	Propiconazole 25% EC	0.00	100.00	0.00	100.00	0.00	100.00	0.00
T_2	Difenoconazole 25% EC	13.00	85.56	0.00	100.00	0.00	100.00	4.33
T_3	Hexaconazole 5% EC	16.60	81.56	13.28	85.24	0.00	100.00	9.96
T_4	Carbendazim 50% WP	35.90	60.11	14.84	83.51	11.50	87.22	20.31
T_0	Control	90.00		90.00		90.00		90.00
Treatment means		31.10		23.62		20.04		
		Treatment (A)		Concentration (B)		Interaction (AxB)		
CD (5%)		0.55		0.43		0.96		
S.E m (±)		0.19		0.15		0.34		

G=Mycelium growth (diameter), I= Per cent inhibition, *Average of five replications

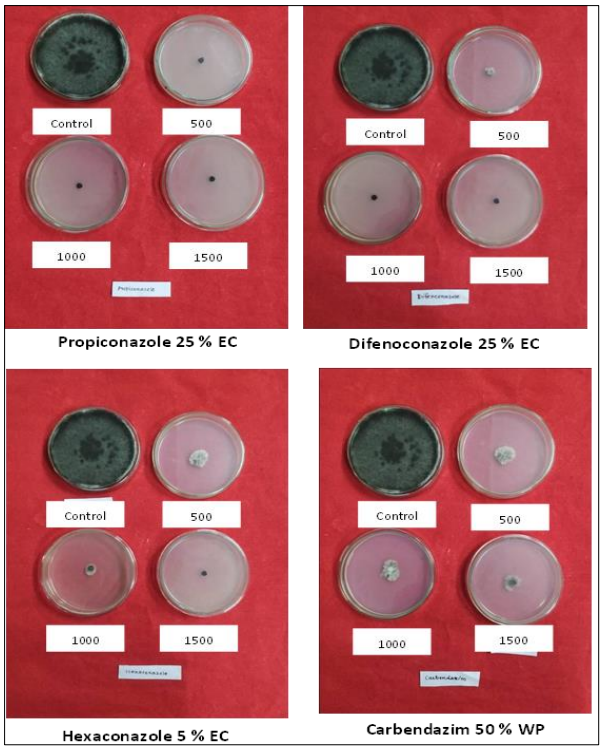

Fig 2: Mycelial growth inhibition of *Bipolaris maydis* at different concentration of systemic fungicides *in vitro*

3.1.2 Efficacy of aqueous extract of botanicals *in vitro* against *B. maydis*

Out of all the botanicals tested against *B. maydis*, the garlic clove extract exhibited the maximum inhibition of mycelium growth with a 76.11% inhibition rate at a 10% concentration, and a 97.11% inhibition rate at a 15% concentration. Following this, the neem leaf extract showed inhibition rates of 62.33% at a 10% concentration, and 73.78% at a 15% concentration. On the other hand, the *parthenium* leaf extract showed the least inhibition with rates of 16.56% at a 10% concentration and 29.55% at a 15% concentration, in comparison to the control. The findings were in accordance with previous findings of Kumar *et al.* (2010) [14]; Kumar *et al.* (2009b) [12]; Khamari *et al.* (2015) [7] for garlic clove extracts.

Based on the results presented in Table 3 and Figures 3 and 4, it can be observed that all of the treatments show a significant difference from the control at both 10% and 15% concentrations. At a 10% concentration, garlic clove extract, neem leaf extract, *eucalyptus* leaf extract; *parthenium* leaf extract, kaner leaf extract, and *lantana* leaf extract are all significantly different from each other. However, onion bulb extract, ginger rhizome extract, and turmeric rhizome extract are not significantly different from each other. At a 15% concentration, garlic clove extract; neem leaf extract, *parthenium* leaf extract, and turmeric rhizome extract are significantly different from each other, while onion bulb extract, *eucalyptus* leaf extract, and kaner leaf extract are not significantly different from each other. Additionally, ginger rhizome extract and lantana leaf extract are not significantly different from each other at 15% concentration.

Both concentrations (10% and 15%) are significant in each treatment. Regarding the interaction of aqueous extracts of botanicals and concentrations, at a 15% concentration, garlic clove extract and turmeric rhizome extract are significant from each other. At a 10% concentration, neem leaf extract, *parthenium* leaf extract, and *lantana* leaf extract are significant from each other. However, garlic clove extract at a 10% concentration and neem leaf extract at a 15% concentration are not significantly different from each other. Onion bulb extract, ginger rhizome extract, and *lantana* leaf extract at 15% concentration are not significantly different from each other. Additionally, turmeric rhizome extract at a 10% concentration, ginger rhizome extract at a 15% concentration, and *lantana* leaf extract at a 15% concentration are not significantly different from each other. Onion leaf extract, ginger rhizome extract, and turmeric rhizome extract at a 10% concentration, as well as *eucalyptus* leaf extract and kaner leaf extract at a 15% concentration, are not significantly different from each other.

Table 3: Mycelial growth inhibition of *Bipolaris maydis* at different concentration of aqueous extracts of botanicals *in vitro*

No.	Botanicals	Botanical name	Plant Parts used	Per cent inhibition in mycelium growth (mm)*				
				Concentrations				Conc. mean
				10%		15%		
				G	I	G	I	
T_1	Garlic	*Allium sativum* (L.)	Cloves	21.50	76.11	2.60	97.11	12.05
T_2	Onion	*Allium cepa* (L.)	Bulb	51.70	42.56	45.60	49.33	48.65
T_3	Neem	*Azadirachta indica* (A. Juss.)	Leaves	33.90	62.33	23.60	73.78	28.75
T_4	*Eucalyptus*	*Eucalyptus globulus* (L.)	Leaves	60.80	32.44	53.06	41.04	56.93
T_5	*Parthenium*	*Parthenium hysterophorus (L.)*	Leaves	75.10	16.56	63.40	29.55	69.25
T_6	Kaner	*Cascabela thevetia* (L.)	Leaves	65.60	27.11	51.40	42.89	58.50
T_7	Ginger	*Zingiber officinale (Roscoe)*	Rhizome	52.80	41.33	47.94	53.27	50.37
T_8	*Lantana*	*Lantana camara* (L).	Leaves	56.70	37.00	48.30	46.33	52.50
T_9	Turmeric	*Curcuma longa (L.)*	Rhizome	50.76	43.60	37.80	58.00	44.28
T_0	Control			90.00		90.00		90.00
Treatment means				55.89		46.37		
			Treatment (A)	Concentration (B)		Interaction (A x B)		
CD (5%)			2.08	0.93		2.95		
S.E m (±)			0.74	0.33		1.05		

G=Mycelium growth (diameter), I= Per cent inhibition, *Average of five replications

Fig 3: Mycelial growth inhibition of *Bipolaris maydis* at 10% concentration of aqueous extracts of botanicals

Fig 4: Mycelial growth inhibition of *Bipolaris maydis* at 15% concentration of aqueous extracts of botanicals

3.3 Efficacy of bio agents *in vitro* against *B.maydis*

In a laboratory experiment, the effectiveness of two bio-agents, namely *Trichoderma viride* and *Pseudomonas fluorescens*, was individually tested against the growth of mycelium of *B. maydis*. The results showed that *T. viride* exhibited the highest level of inhibition of mycelial growth, with a percentage of 74.33%, followed by *P. fluorescens*, which inhibited growth by 57.44% compared to the control. Statistical analysis using DMRT at a significance level of 5% indicated that both treatments were significantly different from the control, and there was also a significant difference between T_1 and T_2. The findings were in accordance with previous findings of Jha *et al.* (2005) [16]; Kumar *et al.* (2009c) [13] for *T. viride* (table 4 and fig. 5).

Table 4: Mycelial growth inhibition of *Bipolaris maydis* by *Trichoderma viride* and *P. fluorescens*

Treatment No.	Treatment name	Per cent inhibition in mycelium growth (mm)*	
		Growth in diameter	per cent inhibition
T_1	*Trichoderma viride*	27.00	70.00
T_2	*Pseudomonas fluorescens*	38.13	57.63
T_0	Control	90.00	
Mean		51.71	
CD (5%)		6.53	
S.Em (±)		0.38	

*Average of eight replications

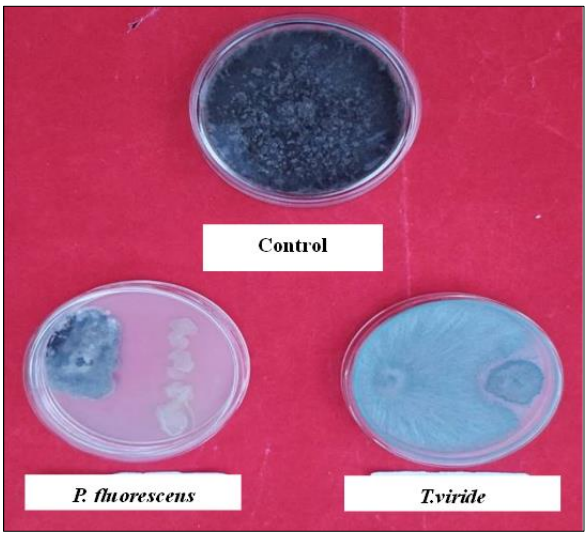

Fig 5: Mycelial growth inhibition of *Bipolaris maydis* by *Trichoderma viride* and *P. fluorescens*

4. Conclusion

To summarize the current research, various treatments consisting of fungicides, botanicals, and bio-control agents were evaluated against the mycelium growth of *B. maydis*. In *in vitro* evaluations of non-systemic and combi- fungicides at 1000 ppm, 1500 ppm, and 2000 ppm, the maximum mycelial growth inhibition was observed in Propiconazole 13.9% EC + Difenoconazole 13.9% EC. *In- vitro* evaluations of systemic fungicides at 500 ppm, 1000 ppm, and 1500 ppm, the maximum mycelial growth inhibition was observed in Propiconazole 25% EC. *In -vitro* evaluations of botanicals at 10% and 15% concentrations, the maximum mycelial growth inhibition was observed in garlic and *in vitro* evaluations of bio-agents, the maximum mycelial growth inhibition was observed in *Trichoderma viride*. Therefore, a combination of these fungicides, botanicals, and bio-controls can be recommended for managing maydis leaf blight disease *in situ*.

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