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Siddu Lakshmi Prasanna
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Priya John
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Corresponding Author
Siddu Lakshmi Prasanna
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Assessing the *In vitro* efficacy of fungicides against maydis leaf blight of maize caused by *Bipolaris maydis*

Siddu Lakshmi Prasanna and Priya John

Abstract
Maize is the most important cereal after rice and wheat. It is called as “Queen of the cereals” or “Miracle crop”. The present study was conducted to manage maydis leaf blight with new generation chemical molecules in *In vitro*. Five non-systemic fungicides, six systemic fungicides and six combination fungicides at three different concentrations were screened in *In vitro* for evaluating the efficacy against *B. maydis* which caused maydis leaf blight**.** Among non-systemic fungicides, at 1000ppm concentration chlorothalonil showed maximum inhibition in mycelial growth (88.16%) followed by copper oxychloride (82.42%) and least inhibition was found in captan (33.83%). Among systemic fungicides, at 500 ppm concentration, hexaconazole, propiconazole, difenconazole and kresoxim methyl showed cent per cent inhibition in mycelial growth followed by azoxystrobin (79.61%) and the least inhibition was observed in carbendazim (74.62%). Among combination fungicides, at 500 ppm concentration, trifloxystrobin + tebuconazole, captan + hexaconazole, pyraclostrobin + epoxiconazole and azoxystrobin + difenconazole showed cent per cent inhibition in mycelial growth followed by thiophanate methyl + pyraclostrobin (76.22%) and the least inhibition was observed in carbendazim + mancozeb (28.29%).

Keywords: Poisoned food technique, maydis leaf blight, *Bipolaris maydis*, *In vitro*, systemic fungicides

1. Introduction

Maize has become a staple food in many parts of the world, with the total production of maize surpassing that of wheat or rice. In addition to being consumed directly by humans (often in the form of masa), maize is also used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup (Foley, 2019) [2]. It is native of South America and belongs to the tribe maydeae of the grass family Poaceae. It is a dual-purpose crop, mainly utilized for human consumption and livestock/poultry feed. During the last few years, there has been a progressive escalation in its demand for the value-added products, like glucose, sorbitol, dextrose, starch-based products and oil. Maize also serves as a basic raw material as an ingredient to thousands of industrial products that include starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package, paper industries *etc*. (Rao *et al.*, 2014) [6].

Though maize is great source of food and protein it is attacked by various biotic and abiotic stresses which cause in less productivity. Among the biotic factors, foliar diseases are highly important constraints in tropical maize production since the airborne fungi account for the greatest losses due to the drastic reduction of photosynthetic leaf surface. Maydis leaf blight (MLB) is one of the foliar biotic stresses caused by *Bipolaris maydis* ((Nisikado and Miyake) Shoemaker), (Telomorph: *Cochliobolus heterostrophus*) is a serious fungal disease of maize throughout the world where maize is grown under warm and humid conditions (White, 1999) [6]. To manage the maydis leaf blight pathogen new generation fungicide molecules which are available in market with less toxicity and more effectiveness were tried. Hence, evaluation of fungicides to manage the maydis leaf blight of maize were very much useful with good grain quality of maize product. Therefore, *In vitro* evaluation of fungicides against *B. maydis* is crucial for the effective disease management.

2. Methodology

2.1. Isolation of Pathogen

Disease sample of maydis leaf blight of maize were collected from the maize field at Navsari Agricultural University, Gujarat. Pathogen was isolated from the lesions of disease affected plant parts and cultured on Potato Dextrose Agar media.

2.2. *In vitro* evaluation of different fungicides for the management of the maydis leaf blight of maize

The effect of fungicides on mycelial growth of *B. maydis* was tested by poisoned food technique. Non-systemic, systemic and combined fungicides at different concentrations were tested against maydis leaf blight of maize using PDA as a medium. The required quantity of each test fungicide was added in conical flask containing 100 ml molten PDA medium so as to get required concentration in ppm. The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicide and 20 ml was poured in sterilized Petri plates. On solidification of the medium, the plates were inoculated with five mm disc of mycelial bit taken from the periphery of seven days old culture with the help of cork borer. The inoculated Petri plates were incubated at 28±1 °C. Petri plates without fungicide were served as control. Colony diameter was measured when the control treatment with pathogen reached full growth. The per cent growth inhibition (PGI) of fungus over control was calculated by using the following formula (Vincent, 1947) [7].

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Colony diameter in control
T = Colony diameter in treated medium

3. Results

3.1. Effect of different non-systemic fungicides on growth inhibition of *B. maydis*

The relative efficacy of non-systemic fungicides *viz*., captan 50 WP, mancozeb 75 WP, copper oxychloride 50 WP, chlorothalonil 75 WP and thiram 75 WP were evaluated at 500, 750 and 1000 ppm concentration by poisoned food technique. The observation regarding per cent growth inhibition were presented in Table 1, Figure 1 and Photo 1.

Table 1: *In vitro* effect of non-systemic fungicides on growth inhibition of *B. maydis*

Sr. No.	Non-systemic fungicide	Per cent growth inhibition			
		500 ppm	750 ppm	1000 ppm	Mean
1.	Captan 50 WP	5.92*(14.08)**	20.30(26.77)	33.83(35.56)	20.01(26.57)
2.	Mancozeb 75 WP	26.78(31.16)	61.27(51.51)	70.95(57.39)	53.00(46.72)
3.	Copper oxychloride 50 WP	69.36(56.39)	74.06(59.38)	82.42(65.21)	75.28(60.18)
4.	Chlorothalonil 75 WP	77.91(61.96)	80.26(63.62)	88.15(69.87)	82.11(64.97)
5.	Thiram 75 WP	23.94(29.29)	41.05(39.84)	61.71(51.77)	42.23(40.53)
	S Em±	0.47	0.54	0.61	
	CD at 5%	1.46	1.67	1.89	
	CV%	1.40	1.96	2.74	

*Figures in outside parenthesis are original values, **Figures in parenthesis are Arc sine transformed values

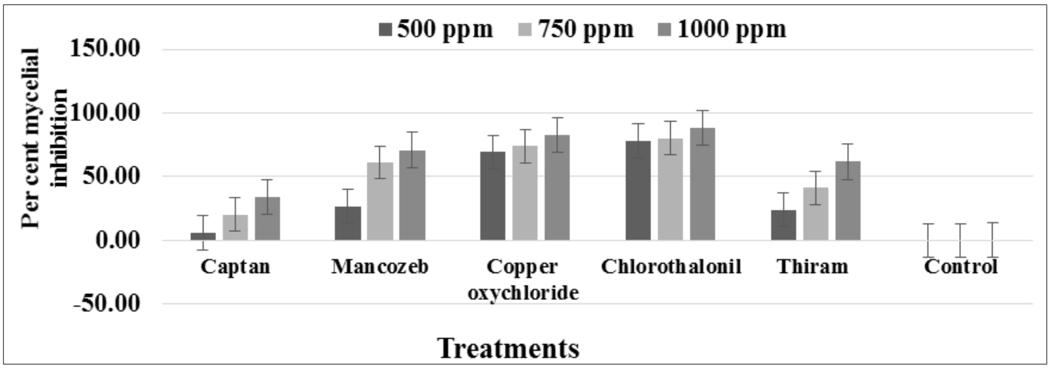


Fig 1: Effect of non-systemic fungicides against *B. maydis*

The results presented in Table 1 revealed that all the five non-systemic fungicides at different concentrations (500, 750 and 1000 ppm) found promising against *B. maydis*. The chlorothalonil fungicide found significantly highest per cent growth inhibition over control was recorded as (88.15%) at 1000 ppm concentration. Next best in order of merit was copper oxychloride (82.42%), mancozeb (70.95%) and thiram (61.71%) growth inhibition over control at 1000 ppm concentration. The five non-systemic fungicides greatly varied in their efficacy to inhibit the growth of fungus under study.

From the results it is interfered that there was very less mycelial growth of the pathogen in chlorothalonil fungicide at all the different concentrations. So, chlorothalonil proved the most effective fungicide for *B. maydis*. Next best fungicide in order of merit was copper oxychloride. The fungicide captan was found least effective at all concentration as compared to other fungicides.

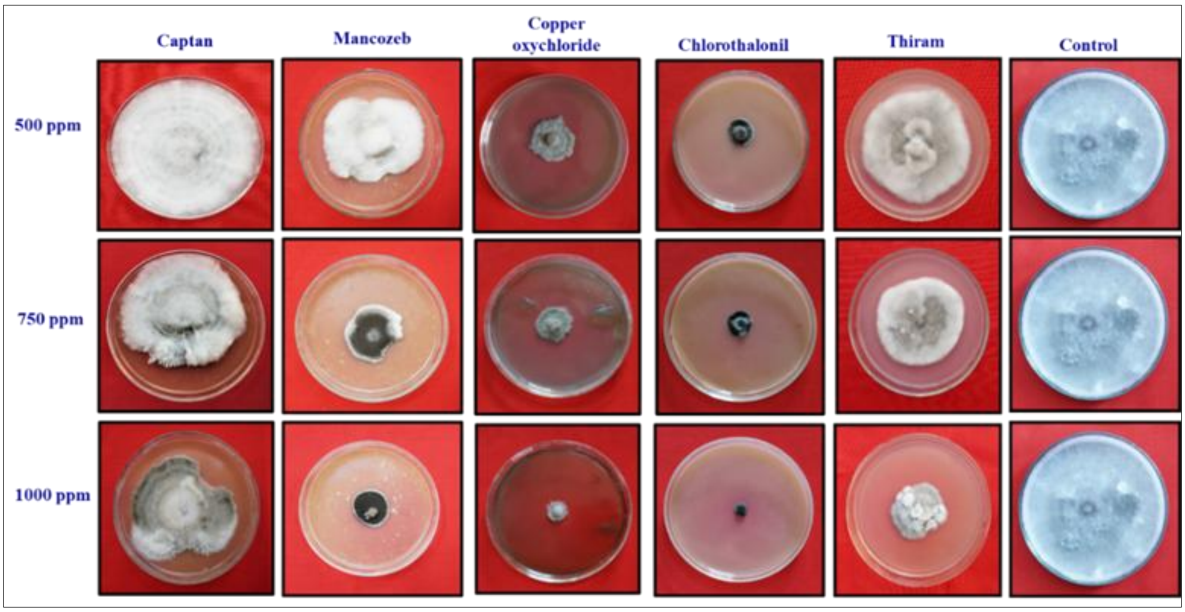


Photo 1: *In vitro* evaluation of non-systemic fungicides against *B. maydis*

3.2. Effect of different systemic fungicides on growth inhibition of *B. maydis*

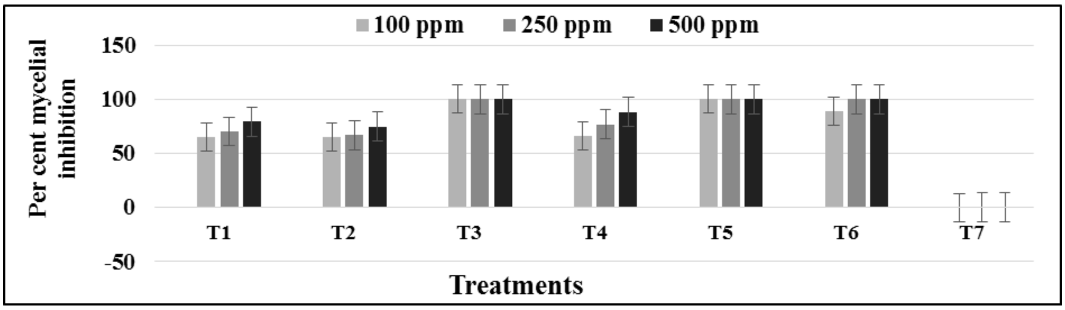
The relative efficacy of systemic fungicides *viz*., azoxystrobin 23 SC, carbendazim 50 WP, hexaconazole 5 SC, Kresoxim methyl 44.3 SC, propiconazole 25 EC and difenconazole 25 EC were evaluated at 100, 250 and 500 ppm concentration by Poison Food Technique. The observation on per cent growth inhibition were presented in Table 2, Figure 2 and Photo 2.

The results presented in Table 2 revealed that all the six systemic fungicides at different concentrations (100, 250 and 500 ppm) found promising against *B. maydis*. The triazole group fungicides hexaconazole and propiconazole found significantly highest per cent growth inhibition over control was recorded as cent per cent at all the three concentrations whereas, difenconazole was also showed cent per cent inhibition in 250 and 500 ppm concentrations but at 100 ppm there was 88.62 per cent growth inhibition over control. Next best in order of merit was kresoxim methyl (88.15%) growth inhibition over control at 500 ppm concentration. The six systemic fungicides greatly varied in their efficacy to inhibit the growth of fungus under study. From the results it is interfered that there was very less mycelial growth of the pathogen in hexaconazole, propiconazole and difenconazole fungicide at all the different concentrations. So, triazole group of chemicals proved the most effective fungicide against *B. maydis*. The fungicide carbendazim was found least effective at all concentration as compared to other fungicides.

Table 2: *In vitro* effect of systemic fungicides on growth inhibition of *B. maydis*

Sr. No.	Systemic fungicides	Per cent growth inhibition			
		100 ppm	250 ppm	500 ppm	Mean
1.	Azoxystrobin 23 SC	65.41*(53.97)**	70.30(56.97)	79.6(63.15)	71.77(58.03)
2.	Carbendazim 50 WP	64.66(53.52)	66.91(54.88)	74.62(59.75)	68.73(56.05)
3.	Hexaconazole 5 SC	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
4.	Kresoxim methyl 44.3 SC	66.54(54.65)	76.69(61.13)	88.15(69.87)	77.13(61.88)
5.	Propiconazole 25 EC	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
6.	Difenconazole 25 EC	88.62(70.29)	100.00(90.00)	100.00(90.00)	96.20(83.43)
	SEm ±	0.49	0.39	0.19	
	CD at 5%	1.49	1.17	0.59	
	CV%	3.12	2.84	1.82	

Figures in outside parenthesis are original values, **Figures in parenthesis are Arc sine transformed values



T1. Azoxystrobin, T2. Carbendazim, T3. Hexaconazole, T4. Kresoxim methyl, T5. Propiconazole, T6. Difenconazole, T7. Control.

Fig 2: Effect of systemic fungicides against *B. maydis*

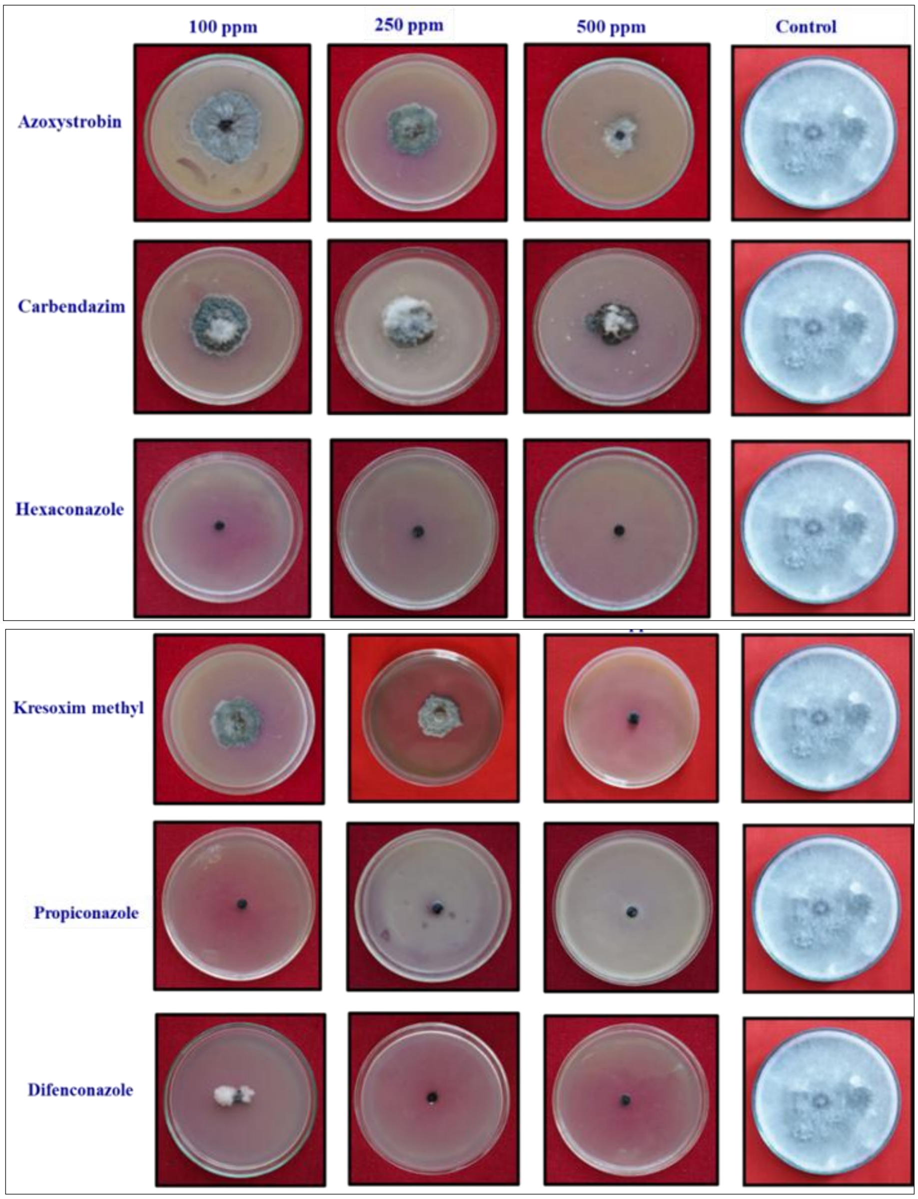


Photo 2: *In vitro* evaluation of systemic fungicides against *B. maydis*

3.3. Effect of different combination fungicides on growth inhibition of *B. maydis*

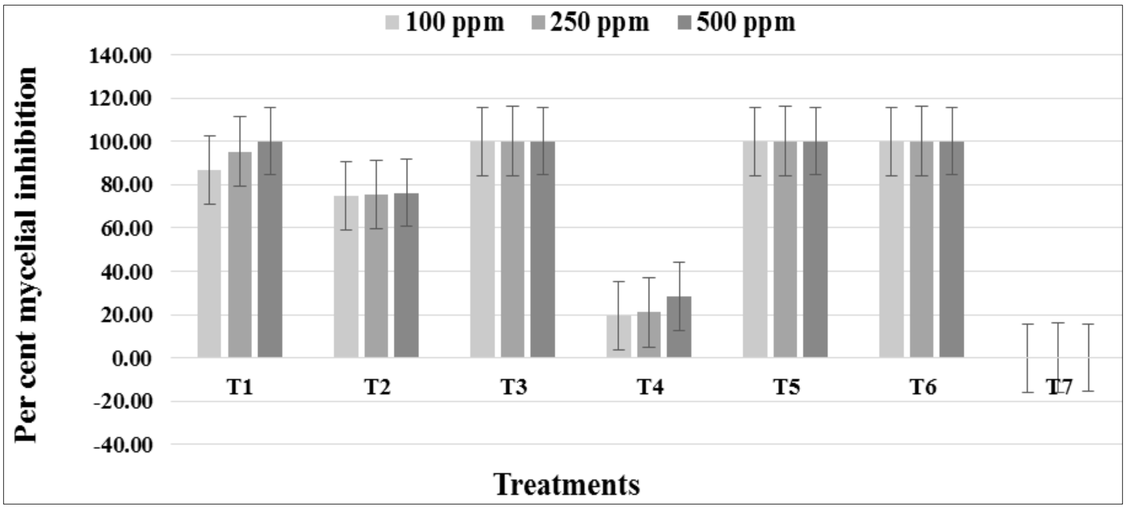
The relative efficacy of combination fungicides *viz*., azoxystrobin 18.2% EC + difenconazole 11.4% SC, thiophanate methyl (450 g/L) + pyraclostrobin (50 g/L), trifloxystrobin 25% + tebuconazole 50% WG, carbendazim 12% WP + mancozeb 63% WP, captan 70% WP + hexaconazole 5% WP and pyraclostrobin 13.3% + epoxiconazole 5% SE were evaluated at 100, 250, 500, and 1000 ppm concentration by poisoned food technique. The observation regarding per cent growth inhibition were presented in Table 3, Figure 3 and Photo 3.

Table 3: *In vitro* effect of combination fungicides on growth inhibition of *B. maydis*

Sr. No.	Combination fungicide	Per cent growth inhibition			
		100 ppm	250 ppm	500 ppm	Mean
1.	Azoxystrobin 18.2% EC + Difenconazole 11.4% SC	86.75*(68.65)**	95.30(77.47)	100.00(90.00)	94.01(75.84)
2.	Thiophanate methyl (450g/L) + Pyraclostrobin (50g/L)	74.81(59.87)	75.28(60.18)	76.22(60.81)	75.43(60.28)
3.	Trifloxystrobin 25% + Tebuconazole 50% WG	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
4.	Carbendazim 12% WP + Mancozeb 63% WP	19.27(26.03)	21.05(27.30)	28.29(32.13)	22.87(28.56)
5.	Captan 70% WP + Hexaconazole 5% WP	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
6.	Pyraclostrobin 13.3% + Epoxiconazole 5% SE	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
	SEm ±	0.37	0.37	0.34	
	CD at 5%	1.13	1.13	1.03	
	CV%	2.33	2.45	2.35	

*Figures in outside parenthesis are original values, **Figures in parenthesis are Arc sine transformed values

The results presented in Table 3 revealed that all the six combination fungicides at different concentrations (100, 250 and 500 ppm) found promising against *B. maydis*. The fungicides which contain triazole group such as trifloxystrobin 25% + tebuconazole 50% WG, captan 70% WP + hexaconazole 5% WP and pyraclostrobin 13.3% + epoxiconazole 5% SE found significantly highest per cent growth inhibition over control was recorded as cent per cent at all the three concentrations whereas, azoxystrobin 18.2% EC + difenconazole 11.4% SC was also showed cent per cent inhibition at 500 ppm concentrations but at 100 ppm there was 86.75 per cent growth inhibition over control. Next best in order of merit was thiophanate methyl (450 g/L) + pyraclostrobin (50 g/L) (76.22%) growth inhibition over control at 500 ppm concentration. The six combination fungicides greatly varied in their efficacy to inhibit the growth of fungus under study.

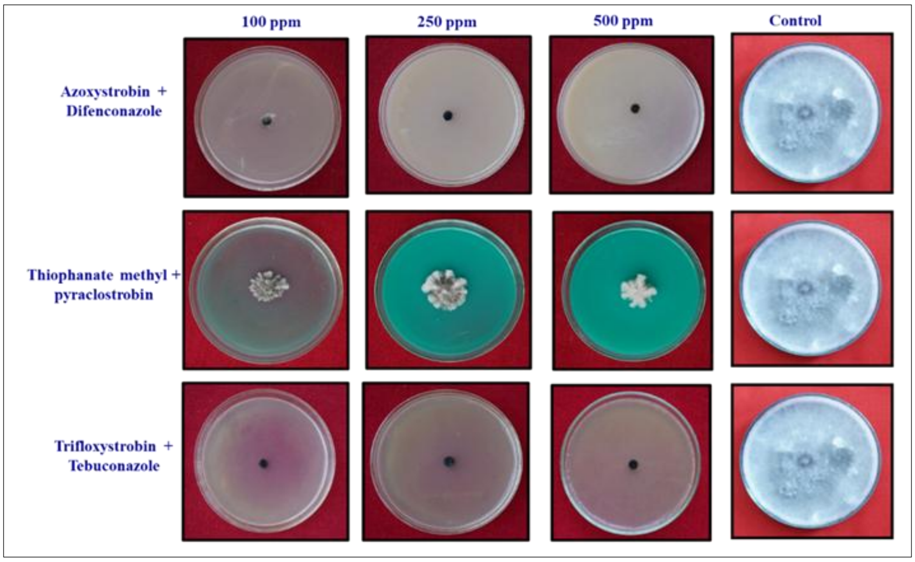


T1. Azoxystrobin + Difenconazole, T2. Thiophanate methyl+ Pyraclostrobin, T3. Trifloxystrobin+ Tebuconazole, T4. Carbendazim+ Mancozeb, T5. Captan + Hexaconazole, T6. Pyraclostrobin + Epoxiconazole, T7. Control

Fig 3: Effect of combination fungicides against *B. maydis*

From the results it is interfered that there was very less mycelial growth of the pathogen in trifloxystrobin 25% + tebuconazole 50% WG, captan 70% WP + hexaconazole 5% WP, pyraclostrobin 13.3% + epoxiconazole 5% SE and azoxystrobin 18.2% EC + difenconazole 11.4% SC fungicide at all the different concentrations. So, combination fungicides which contain triazole group of chemicals proved the most effective fungicide against *B. maydis*. The fungicide carbendazim 12% WP + mancozeb 63% WP was found least effective at all concentration as compared to other fungicides.

These results were in agreement with the findings of Kumar *et al*., (2009c) who screened efficacy of propiconazole (Tilt), mancozeb (Dithane M-45), copper oxychloride (Blitox 50), thiophanate methyl (Roko), carbendazim (Bavistin) and carbendazim + mancozeb (Companion) at 250, 500 and 1000 ppm inhibited the growth of H. maydis by poisoned food technique in *In vitro*. Similarly, Harlapur *et al*., (2007) reported that mancozeb and carboxin powder were effective against E. turcicum. Waghe *et al*. (2015) who tested efficacy of different fungicides *viz*., SAAF, mancozeb, chlorothalonil, propiconazole and azoxystrobin in *In vitro* against sunflower leaf blight pathogen by poisoned food technique and observed maximum inhibition was recorded with SAAF (90.36%) followed by mancozeb (88.88%).



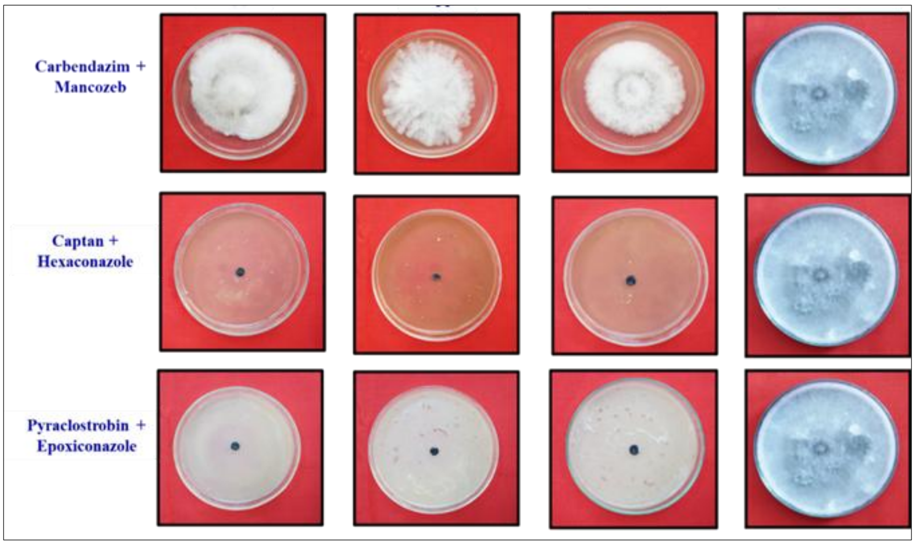


Photo 3: *In vitro* evaluation of combination fungicides against *B. maydis*

Bharti *et al*. (2020) also screened efficacy of five fungicides namely mancozeb, propiconazole, carbendazim, chlorothalonil and copper oxychloride at 50, 100, 150, 200 and 250 ppm concentrations against H. maydis and found propiconazole highly effective with cent per cent inhibition of mycelial growth of H. maydis at the concentrations (150, 200 and 250 ppm) followed by mancozeb at 250 ppm concentration showed 92.37 per cent inhibition which was at par with propiconazole at 50 ppm concentration. The results were also in agreement with Kumar *et al*. (2021) who tested different fungicides against H. maydis in *In vitro* through poisoned food technique. Amongst the systemic fungicides, propiconazole was found highly effective and inhibited 100 per cent of mycelial growth of H. maydis at all the concentrations (50, 100 and 200 pm). Carboxin showed 100 per cent inhibition at 100 and 200 ppm concentrations. Amongst all the non-systemic fungicides evaluated mancozeb was found to be most effective.

4. Conclusion

The studies of *In vitro* evaluation of fungicides against *B. maydis* revealed that chlorothalonil at 1000ppm concentration showed maximum inhibition in mycelial growth (88.16%) followed by copper oxychloride (82.42%) and least inhibition was showed in captan (33.83%). In systemic fungicides, hexaconazole, propiconazole, difenconazole and kresoxim methyl at 500 ppm concentration, showed cent per cent inhibition in mycelial growth followed by azoxystrobin (79.61%) and the least inhibition was observed in carbendazim (74.62%). Among combination fungicides, trifloxystrobin + tebuconazole, captan + hexaconazole, pyraclostrobin + epoxiconazole and azoxystrobin + difenconazole at 500 ppm concentration, showed cent per cent inhibition in mycelial growth followed by thiophanate methyl + pyraclostrobin (76.22%) and the least inhibition was observed in carbendazim + mancozeb (28.29%).

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