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Field efficacy of Fungicides, Biocontrol Agents, Botanicals and Plant Defence Activators against Maydis Leaf Blight of Maize caused by *Bipolaris maydis*

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ABSTRACT: Maydis leaf blight (MLB) of maize is one of the devastating foliar diseases which causes variable losses across the world. The experimental study was carried out to manage maydis leaf blight with fungicides, plant defense activators, botanicals and biocontrol agents in field conditions for two consecutive years during *rabi* 2020-21 and 2021-22 at Navsari Agricultural university, Gujarat. In the present study various treatments were evaluated in field and among the different fungicidal treatments, foliar spray with trifloxystrobin 25% + tebuconazole 50% WG at 0.05% was found more effective with least per cent disease incidence (PDI) (35.75 %) at 90 days after sowing (DAS) and higher grain yield (56 q/ha) which is at par with propiconazole 25 EC at 0.1 per cent (54.50 q/ha), azoxystrobin 25% EC + difenconazole 25% SC at 0.05 per cent 54.33 q/ha and azoxystrobin 23 SC at 0.1 per cent (52.33 q/ha). Among non-chemical methods, lowest PDI *i.e.*, 45.46 % at 90DAS was recorded when crop was sprayed with salicylic acid (0.75%) which was significantly lower followed by 46.67% in *Pseudomonas fluorescens* at 0.5% and also higher grain yield (Pooled) was recorded in case of salicylic acid at 0.75% (47.11 q/ha) followed by *P. fluorescens* at 0.5% (45.67 q/ha).

Keywords: Maydis leaf blight, Plant Defence activators, Field evaluation, fungicides, botanicals, biocontrol agents, *Trichoderma harzianum*, *Pseudomonas fluorescens*.

INTRODUCTION

Maize is a diverse crop that can be cultivated in a variety of farming systems, making a significant contribution to the country's nutritional food security. It is also a significant cereal crop that is grown in more than 150 nations throughout the world. Global maize area of about 201.98 million hectares stands with the production of 1162.35 million tons and average productivity is about 5.75 tons/ha (FAOSTAT, 2021). In India, Maize is grown in an area of about 9.86 million hectares and the majority of maize output is utilized for feed (63%), food (23%), starch industries (12%), seed, and other purposes (2%) (Malik *et al*., 2018).

The fungal infections that affect the leaves, stem, and ear are among the major maize diseases that threaten both human health and crop productivity of maize (Balint-Kurti and Johal 2009). The yearly global harvest has been projected to have lost 22.5% (19.5-41.1%) of its output owing to illnesses in the maize crop (Savary *et al*., 2019). MLB is a serious foliar disease to the sustainable production of maize in India, where tropical maize is grown (White, 1999). It is caused by the ascomycete fungus *Cochliobolus heterostrophus* (Drechs), and its imperfect stage is known as *Bipolaris maydis* Syn. *Helminthosporium maydis*. Maydis leaf blight has historical significance because of the massive epidemic losses it caused in Southern America in the years 1970–1971 (Ullstrup, 1972).

Fungicide usage was rare previously, but now usage of foliar fungicides increased against plant diseases of maize to reduce the losses caused by phytopathogens. Disease management through biological agents, botanical extracts and plant defence activators is also of greater importance to reduce the environmental damage caused by chemicals and also to diminish the fungicide resistance acquired by phytopathogens due to the continuous usage of synthetic chemicals with similar mode of action (Ons *et al*., 2020). Since durable sources of disease resistance are not yet available, crop losses must be prevented through both chemical and non-chemical (botanical, biological, and defensive) ways of management. As a result, it is necessary to determine the appropriate sources of management using biorational techniques and to assess their efficacy for management. In the current study, efforts were undertaken to identify the most efficient chemical and non-chemical control approaches for the treatment of MLB disease in the south Gujarat.

MATERIAL AND METHODS

A field experiment was carried out to manage the maydis leaf blight of maize throughout the two consecutive *rabi* seasons of the year in 2020–2021 and 2021–2022 at College Farm, N.M. College of Agriculture, NAU, Navsari. The experiment was laid out in Randomized Block Design (RBD) with twelve treatments along with control and three replications were maintained for all the treatments.

Maize cultivar CM-202 was used which is susceptible for maydis leaf blight. Size of the plot was 4.2 m × 3.0 m and the spacing was 60 cm × 20 cm. Standard agronomical practices were followed to raise the crop. Treatments details are given in Table 1. First spray was at 40 days after sowing and second spraying was at 25 days after first spray. Sixty-five plants as net plot per treatment per replication were selected and recorded observations at 45, 60, 75 and 90 days after sowing and Per cent disease incidence was assessed by formula as mentioned by Wheeler (1969).

$$\text{PDI} = \frac{\text{Sum of total ratings}}{\text{Total no. of ratings}} \times \frac{100}{\text{Maximum disease grade}}$$

At the time of crop harvest, maize grain yield per plot in both of the years (2020–21 and 202–22) was recorded, and yield in q/ha was determined.

RESULTS AND DISCUSSION

Maydis leaf blight of maize was managed at variable level with the use of various chemical and non-chemical approaches and the grain yield obtained was also varied from each management practice.

A. Efficacy of fungicides on incidence of maydis leaf blight

All fungicides (Propiconazole 25 EC, Mancozeb 75 WP, Azoxystrobin 23 SC, Azoxystrobin 25 + Difenconazole 25 SC, Carbendazim 12 + Mancozeb 63 WP, Trifloxystrobin 25+ Tebuconazole 50 WG) were found to be significantly effective in lowering the PDI in both years, according to results shown in Table 2, Table 3, Fig. 1 and 2. According to the data in Table 2, foliar spray with Trifloxystrobin 25+ Tebuconazole 50 WG at 0.05% resulted in a minimal disease index percentage of 35.49% at 90 DAS during *rabi* 2020-21. In comparison to the untreated check, Trifloxystrobin 25+ Tebuconazole 50 WG at 0.05% had the lowest percent disease index (37.75%), followed by propiconazole (25 EC) at 0.1 percent (54.50 q/ha), azoxystrobin (25% EC) + difenconazole (25% SC) at 0.05 percent (54.33 q/ha), azoxystrobin (23 SC) at 0.1 per cent (52.33 q/ha), mancozeb (75 WP) at 0.2 per cent (50.00 q/ha) and carbendazim (12%) + mancozeb (63%) at 0.2 per cent (48.61 q/ha) during *rabi* 2021-22. The maximum disease control was achieved with Trifloxystrobin 25+ Tebuconazole 50 WG at 0.05% and propiconazole at 0.1% for *rabi* 2020–21 and 2021–22, respectively, out of all six tested fungicides. Fungicides were sprayed to maize plots, and this resulted in an increase in yield. Trifloxystrobin 25+ Tebuconazole 50 WG at 0.05%, or 57 and 55 q/ha in *rabi* 2020-21 and 2021-22, respectively, and propiconazole at 0.1%, or 55.67 and 53.33 q/ha in *rabi* 2020-21 and 2021-22, respectively, were the two treatments that produced the greatest increase in yield. Average disease intensity during both the years and also pooled is lower *i.e.*, 28.86 % in Trifloxystrobin 25 + Tebuconazole 50 WG at 0.05% compared to other chemical fungicides and in control (46.96%). In *in vitro* many of the systemic and combination fungicides showed cent per cent mycelial inhibition and also botanicals, bio agents and plant defence activators also showed significant inhibition in mycelial growth of *Bipolaris maydis* (Prasanna and John 2022 a and b).

According to Malik *et al*. (2018), validamycin at 0.1% and trifloxystrobin 25 WG + tebuconazole 50 WG had significant effects against Banded leaf sheath blight (BLSB), while propiconazole at 0.1%, carbendazim 12 WP + mancozeb 63 WP at 0.125%, and sarpagandha leaves at 10% had significant effects against the MLB pathogen. Additionally, Saxena (2002) found that propiconazole was more effective than the commonly used fungicide carbendazim at preventing banded leaf sheath disease in maize.

Hulagappa *et al*. (2013) also revealed that fungicides in the form of foliar applications was shown to be very efficient in managing MLB which increases grain and fodder yields, and lowering PDI. Tebuconazole 250 EC @ 0.1% and Difenconazole 25 EC @ 0.1% were shown to be less efficacious against MLB than two sprays of Propiconazole 25 EC @ 0.1% at 35 to 50 DAS. Additionally, this treatment enhanced grain and fodder yields while having the best disease control effectiveness. Bharti *et al*. (2020) also studied chemical protection against maydis leaf blight and found propiconazole was very effective in completely inhibiting the development of fungal mycelium in the laboratory and progress of the disease in the field followed by Mancozeb, Carbendazim, Chlorothalonil and Copper Oxy Chloride over control.

B. Efficacy of plant defence activators/botanicals/bioagents on incidence of maydis leaf blight

Foliar diseases that spread quickly and cause rapid epidemics can be efficiently managed by developing appropriate integrated disease management methods. The experimental results of evaluated plant defence activators/botanicals/bioagents against MLB were found significantly effective and showed in Table 2, 3, Fig. 1 and 2. Lowest per cent disease incidence (PDI) *i.e.*, 41.93% and 42.85% at 90DAS was recorded when crop was sprayed with salicylic acid (0.75%) which was significantly lower than all the treatment except chemical fungicides followed by 42.70 % and 43.47% in *Pseudomonas fluorescens* at 0.5% for *rabi* 2020-21 and 2021-22, respectively. Significantly higher grain yield (Pooled) was also recorded in case of salicylic acid at 0.75% (47.11 q/ha) followed by *P. fluorescens* at 0.5% (45.67 q/ha). Plots sprayed with salicylic acid at 0.75% and *P. fluorescens* 21.31 and 17.60% disease control over untreated plants for *rabi* pooled (2020-21 and 2021-22), respectively.

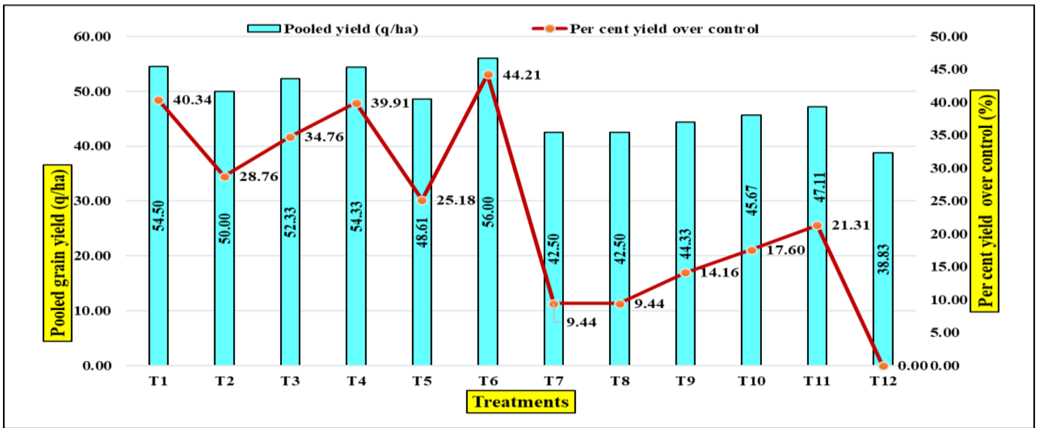
Plant defence activators such as Salicylic acid, Beta-amino butyroic acid, 2,4- Dichloro Iso nicotinic acid also provide antagonistic activity against MLB in *in vitro* but they can be more effective in the field as they produce antioxidant enzymes and give the plant defence response against many pathogens of the particular crop. To strengthen the integrated disease management strategy against MLB and other maize diseases, sarpagandha leaves extract, *Pseudomonas fluorescens*, and *Trichoderma viride* can be used safely in place of fungicides (Prasanna and John 2022b).

Atri *et al.* (2022) investigated the field efficacy of biocontrol agents, plant extracts, and elicitors for the management of Maydis Leaf Blight (MLB), which infected maize crops under field conditions for three seasons (2018–2020) and found that MLB was least severe (20.17%), with disease control of 53.46 percent compared to untreated control (43.33%). In plots treated with two chitosan foliar sprays at 0.05% at the commencement of disease and second spray at 0.25% 10 days later, there was a 47.94 and 42.90% decrease in maize maydis leaf blight, followed by *Murraya koenigii* (3.0%) and mancozeb (0.25%). In a field study by Kumar *et al.* (2009), some plant extracts were tested for their ability to manage maydis leaf blight and plots sprayed with garlic clove extract (17.0 and 18.0 q/ha), tulsi leaf extract (13.5 and 14.5 q/ha), and neem leaf extract (16.0 and 15.3 q/ha) had significantly higher grain yields than unsprayed plots.

Since maydis leaf blight is a foliar disease that spreads quickly due to favorable weather circumstances and lack of contribution by *Trichoderma* spp. to maize yield enhancement suggested that the antagonistic feature of the bioagent may not have offered prompt plant response to the yield enhancement. According to the current research, the use of chemical fungicides, plant defense activators, biocontrol agents, and botanicals results in a significantly higher grain yield when compared to untreated controls. However, of the five fungicides tested, propiconazole 25 EC at 0.1% and trifloxystrobin 25% + tebuconazole 50% WG at 0.05% provided the best control of maydis leaf blight and significantly higher grain yield.

Table 1: Chemicals, botanicals, bioagents, plant defence activators used against MLB and grain yield.

Tr.	Treatments	Concentration (%)	Grain yield (q/ha)			Per cent yield over control
			2020-21	2021-22	Pooled	
T1	Propiconazole 25% EC	0.1	55.67	53.33	54.50	40.34
T2	Mancozeb 75% WP	0.2	50.33	49.67	50.00	28.76
T3	Azoxystrobin 23 % SC	0.1	53.33	51.33	52.33	34.76
T4	Azoxystrobin25% EC+ Difenconazole25% SC	0.05	54.67	54.00	54.33	39.91
T5	Carbendazim 12% + mancozeb 63% WP	0.1	49.33	47.89	48.61	25.18
T6	Trifloxystrobin 25% + Tebuconazole 50% WG	0.05	57.00	55.00	56.00	44.21
T7	*Allium sativum* L. (Garlic clove extract)	10	43.33	41.67	42.50	9.44
T8	*Azadirachta indica* Juss. (Neem leaf extract)	10	42.33	42.67	42.50	9.44
T9	*Trichoderma harzianum* 1×10^8cfu/ml	0.5	45.00	43.67	44.33	14.16
T10	*Pseudomonas fluorescens* 1×10^8cfu/ml	0.5	46.33	45.00	45.67	17.60
T11	Salicylic acid	0.75	47.89	46.33	47.11	21.31
T12	Control	-	40.33	37.33	38.83	0.00
	S.E.m±		2.35	2.59	1.65	
	C.D. at 5 %		6.93	7.64	4.88	
	C.V.		8.33	9.47	5.95	



(T1). Propiconazole 25 EC at 0.1% (T2). Mancozeb 75 WP at 0.2% (T3). Azoxystrobin 23 SC at 0.1% (T4). Azoxystrobin25% EC+ Difenconazole25% SC at 0.05% (T5). Carbendazim 12% + Mancozeb 63% WP at 0.1% (T6). Trifloxystrobin 25% + Tebuconazole 50% WG at 0.05% (T7). Garlic clove extract at 10% (T8). Neem leaf extract at 10% (T9). *Trichoderma harzianum* 1×10^8 cfu/ml at 0.5% (T10). *Pseudomonas fluorescens* 1×10^8 cfu/ml at 0.5% (T11). Salicylic acid at 0.75% (T12). Control

Fig. 1. Effect of biocontrol agents, plant activators, botanicals and fungicides on maize grain yield.

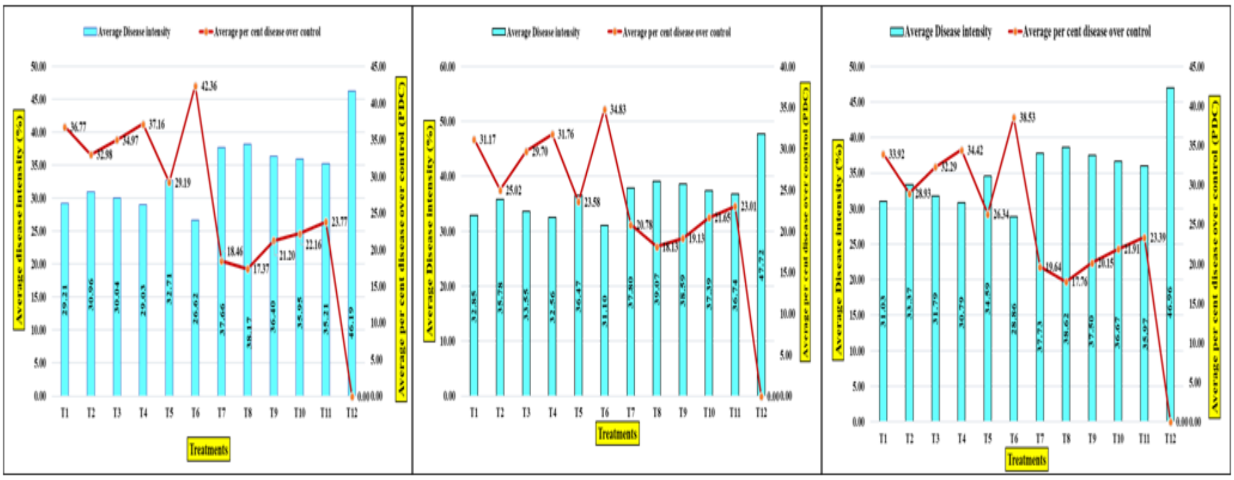
Table 2: Per cent disease incidence of maydis leaf blight in maize cv. CM-202.

Tr.	Treatments	45 DAS			60 DAS			75 DAS			90 DAS			Pooled Yield (q/ha)
		2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	
T1	Propiconazole 25% EC	27.50** (21.33)*	29.63** (24.44)*	28.58 (22.89)	31.19 (26.83)	34.08 (31.41)	32.65 (29.12)	33.64 (30.70)	37.06 (36.33)	35.37 (33.52)	38.03 (37.96)	38.76 (39.20)	38.39 (38.58)	54.50[a]
T2	Mancozeb 75% WP	28.50 (22.78)	31.37 (27.11)	29.96 (24.94)	32.59 (29.03)	35.27 (33.35)	33.95 (31.19)	34.67 (32.37)	39.03 (39.66)	36.87 (36.01)	39.03 (39.67)	40.97 (43.00)	40.00 (41.33)	50.00[b]
T3	Azoxystrobin 23 % SC	28.50 (22.78)	31.16 (26.78)	29.85 (24.78)	31.69 (27.60)	33.76 (30.89)	32.73 (29.24)	33.97 (31.23)	37.30 (36.74)	35.65 (33.98)	38.38 (38.55)	39.10 (39.78)	38.74 (39.17)	52.33[a]
T4	Azoxystrobin25% EC+Difenconazole25% SC	27.74 (21.67)	30.43 (25.67)	29.10 (23.67)	31.3 (27.00)	33.57 (30.59)	32.45 (28.80)	33.41 (30.33)	36.75 (35.81)	35.10 (33.07)	37.52 (37.10)	38.16 (38.18)	37.84 (37.64)	54.33[a]
T5	Carbendazim 12% + mancozeb 63% WP	29.77 (24.66)	31.30 (27.00)	30.54 (25.83)	32.79 (29.33)	35.88 (34.36)	34.35 (31.85)	36.37 (35.18)	39.33 (40.18)	37.86 (37.68)	40.2 (41.66)	41.74 (44.33)	40.97 (43.00)	48.61[b]
T6	Trifloxystrobin 25% + Tebuconazole 50% WG	27.03 (20.66)	29.55 (24.33)	28.31 (22.50)	29.33 (24.00)	32.08 (28.22)	30.72 (26.11)	32.01 (28.11)	35.71 (34.07)	33.88 (31.09)	35.49 (33.72)	37.92 (37.77)	36.71 (35.75)	56.00[a]
T7	*Allium sativum* L. (Garlic clove extract)	30.07 (25.11)	32.01 (28.11)	31.05 (26.61)	36.80 (35.89)	35.73 (34.11)	36.26 (35.00)	40.22 (41.70)	40.22 (41.7)	40.22 (41.70)	43.82 (47.96)	43.44 (47.29)	43.63 (47.62)	42.50[c]
T8	*Azadirachta indica* Juss. (Neem leaf extract)	30.37 (25.57)	32.30 (28.57)	31.34 (27.07)	37.13 (36.44)	37.13 (36.45)	37.13 (36.45)	40.39 (42.00)	40.54 (42.26)	40.47 (42.13)	44.23 (48.67)	44.42 (49.00)	44.33 (48.83)	42.50[c]
T9	*Trichoderma harzianum* 1×10^8cfu/ml	28.91 (23.38)	33.02 (29.71)	31.01 (26.54)	36.56 (35.48)	35.75 (34.15)	36.16 (34.82)	39.27 (40.07)	40.30 (41.85)	39.79 (40.96)	43.08 (46.66)	44.23 (48.66)	43.66 (47.66)	44.33[c]
T10	*Pseudomonas fluorescens* 1×10^8cfu/ml	28.68 (23.04)	32.01 (28.11)	30.38 (25.58)	36.40 (35.22)	35.12 (33.11)	35.76 (34.16)	38.97 (39.55)	39.81 (40.99)	39.39 (40.27)	42.70 (46.00)	43.47 (47.33)	43.08 (46.67)	45.67[b]
T11	Salicylic acid	29.25 (23.89)	31.87 (27.89)	30.58 (25.89)	35.59 (33.88)	34.99 (32.89)	35.29 (33.38)	38.29 (38.41)	39.18 (39.93)	38.74 (39.17)	41.93 (44.67)	42.85 (46.26)	42.39 (45.46)	47.11[b]
T12	Control	32.44 (28.77)	33.27 (30.11)	32.86 (29.44)	42.19 (45.11)	43.53 (47.44)	42.86 (46.27)	46.20 (52.11)	47.22 (53.89)	46.71 (53.00)	50.05 (58.77)	50.44 (59.44)	50.24 (59.11)	38.83[d]
	S.E.m±	1.09	1.21	0.88	1.64	1.86	1.23	1.73	2.03	1.09	2.08	1.87	1.53	1.65
	C.D. at 5 %	3.22	3.57	2.61	4.83	5.50	3.62	5.12	6.00	3.21	6.15	5.53	4.51	4.88
	C.V.	8.00	7.66	6.00	8.82	9.51	6.44	8.16	8.74	4.89	8.30	7.20	5.98	5.95

*Figure in parenthesis is original value, **Figures outside parenthesis is arcsine transform value

Table 3: Average disease intensity and average per cent disease over control of maydis leaf blight in maize cv. CM-202.

Sr. No.	Treatments	*Rabi* 2020-21		*Rabi* 2020-21		*Rabi* Pooled	
		Average disease intensity (%)	Average per cent disease over control (%)	Average disease intensity (%)	Average per cent disease over control (%)	Average disease intensity (%)	Average per cent disease over control (%)
1.	**Propiconazole 25 EC**	29.21	36.77	32.85	31.17	31.03	33.92
2.	**Mancozeb 75 WP**	30.96	32.98	35.78	25.02	33.37	28.93
3.	**Azoxystrobin 23 SC**	30.04	34.97	33.55	29.70	31.79	32.29
4.	**Azoxystrobin25% EC+ Difenconazole25% SC**	29.03	37.16	32.56	31.76	30.79	34.42
5.	**Carbendazim 12% + mancozeb 63% WP**	32.71	29.19	36.47	23.58	34.59	26.34
6.	**Trifloxystrobin 25% + Tebuconazole 50% WG**	**26.62**	**42.36**	**31.10**	**34.83**	**28.86**	**38.53**
7.	***Allium sativum* L. (Garlic clove extract)**	37.66	18.46	37.80	20.78	37.73	19.64
8.	***Azadirachta indica* J. (Neem leaf extract)**	38.17	17.37	39.07	18.13	38.62	17.76
9.	***Trichoderma harzianum* 1×10^8 cfu/ml**	36.40	21.20	38.59	19.13	37.50	20.15
10.	***Pseudomonas fluorescens* 1×10^8 cfu/ml**	35.95	22.16	37.39	21.65	36.67	21.91
11.	**Salicylic acid**	35.21	23.77	36.74	23.01	35.97	23.39
12.	**Control (Untreated)**	46.19	-	47.72	-	46.96	-



Rabi 2020-21 *Rabi* 2021-22 Pooled

(T1). Propiconazole 25 EC at 0.1% (T2). Mancozeb 75 WP at 0.2% (T3). Azoxystrobin 23 SC at 0.1% (T4). Azoxystrobin25% EC+ Difenconazole25% SC at 0.05% (T5). Carbendazim 12% + Mancozeb 63% WP at 0.1% (T6). Trifloxystrobin 25% + Tebuconazole 50% WG at 0.05% (T7). Garlic clove extract at 10% (T8). Neem leaf extract at 10% (T9). *Trichoderma harzianum* 1×10^8 cfu/ml at 0.5% (T10). *Pseudomonas fluorescens* 1×10^8 cfu/ml at 0.5% (T11). Salicylic acid at 0.75% (T12). Control

Fig. 2. Effect of biocontrol agents, plant activators, botanicals and fungicides on maydis leaf blight disease intensity.

CONCLUSIONS

According to the findings from the present study, two sprays, first at disease initiation (40 DAS) and second at 25 days after the first spray with trifloxystrobin (25%) + tebuconazole (50%) at 0.05 per cent recorded significantly lowest disease intensity (28.66 %) with the highest yield (56.00 q/ha) and found to be the most effective for the management of maydis leaf blight disease and increasing grain yield over control. It also concludes that trifloxystrobin (25%) + tebuconazole (50%) at 0.05 per cent was found effective and also other chemical treatments such as propiconazole (25% EC) at 0.1 per cent, azoxystrobin (25% EC) + difenconazole (25% SC) at 0.05 per cent, azoxystrobin (23% SC) at 0.1 per cent, mancozeb (75% WP) at 0.2 per cent which can be used as alternate option because all these treatments were found at par with each other along with integration of non-chemical approaches reduces the environmental hazards and production cost incurred on the farmers.

FUTURE SCOPE

Biocontrol agents, Botanicals and Plant defense activators has reduced the disease incidence upto certain level but it is less compared to fungicides. But, the integration of these biological agents helps the farmers to reduce the chemicals into the environment and also cost incurred on production can be reduced in long term. Further, the studies on plant defense activators in non-chemical approaches on various crop plants can be studied and its impact of the environment.

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Conflict of Interest. None.

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