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Prasoon Bharti
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Bihar, Samastipur, India.

Phool Chand
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Bihar, Samastipur, India.

Prince Kumar Gupta
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Bihar, Samastipur, India.

Corresponding Author:
Prasoon Bharti
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Bihar, Samastipur, India.

Effective chemical protection against Maydis Leaf Blight of Maize incited by *Helminthosporium maydis* under *the in-vitro* and *in-vivo* condition

Prasoon Bharti, Phool Chand and Prince Kumar Gupta

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Abstract
Maydis leaf blight, a serious devastating disease affecting maize crop throughout the country, is characterized by long, spindle-shaped elliptical and tan lesion which appears first on lower leaves caused by *Helminthosporium maydis*. In the present study, different fungicides were tested under *in-vitro* as well as *in-vivo* condition. The result revealed that among the fungicides used under *in vitro* at 50,100,150,200 and 250 ppm concentration, Propiconazole was effective against the pathogen followed by Mancozeb, Carbendazim, Chlorothalonil and COC over control respectively. While under field condition, One, two and three sprays of 4 fungicides along with seed treatment with SAAF @ 3gm/kg seed were evaluated and found that Propiconazole @ 0.1% showed a reduction in PDI (%) at all spray i.e. One (28.67%), two (31.00%) and three (34.75%) over control which showed PDI of 64.01%, 61.09% and 56.38% at all three sprays respectively. Based on antifungal efficiency, Chlorothalonil @ 0.1 % found less effective with 63.50%, 67.50% and 73.20% PDI at all three consecutive sprays.

Keywords: Fungicide, *Helminthosporium maydis*, Maydis leaf blight, maize

Introduction
Maize (*Zea mays* L.) also known as "queen of cereals" because of its highest genetic yield potential among the cereals. Maize domesticated in Mexico, about 10,000 years ago, has become an important cereal for food and nutritional security, animal feed and also in industrial use especially as an important raw material in food processing, poultry, dairy, meat and ethanol production. With its traditional uses, it becomes one of the fastest-growing cash crops, among cereals, in the world. In India, maize is the third most important food crop after rice and wheat, so the present area under this crop is about 9.47 (million ha) and production of 28.72 (MT) with 3032 kg/ha productivity. Andhra Pradesh is the leading state in maize production followed by Karnataka, Rajasthan and Maharashtra. Bihar has become pioneer state in maize with the production of (2.42 MT) in area of 0.67 (million ha) which contribute the highest productivity of 3623 kg/ha (Agricultural Statistics at glance, 2018) [1]. However, cultivation of this crop is seriously jeopardized, if the plants get infected with a large number of pathogenic fungi, bacteria as well as viruses. Different fungal diseases *viz;* smut, rust, anthracnose stalk rot, charcoal rot, curvularia leaf spot, downy mildews, brown spot, blended leaf and sheath blight, as well as maydis leaf blight which affect at all stage of the crop (Ashwani *et al.*, 2002) [3]. Maydis leaf blight (MLB) caused by *Helminthosporium maydis* an important foliar disease in almost all the maize growing regions in India which leads to potential losses even up to 60% under severe disease conditions. With the view of the above economic significance of maize and the losses caused by *H. maydis,* the core aim of this research was to evaluated effective chemical protection against Maydis Leaf Blight of Maize incited by *Helminthosporium maydis* under *in-vitro* and *in-vivo* condition.

Material and Methods
I. In vitro effect of fungicides on mycelial growth of H. maydis
A. Isolation and purification of *H. maydis*
Disease specimen of maize showing characteristic symptoms of MLB (Maydis leaf blight), were collected from the farm of Tirhut College of Agriculture (DRPCAU), Dholi,

Muzaffarpur, Bihar during 2016-2017. The plant showing characteristic symptoms of MLB were brought to the laboratory of Department of Plant Pathology, DRPCAU, Pusa and washed with running tap water to remove dust and dirt and then kept in the refrigerator for further study. For isolation of the pathogen, the infected portion of the crop (leaves) was cut into small bits of 2-3mm dimension. These bits were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 seconds followed by washing in 2 changes of sterilized water, then placed aseptically on PDA slants with the help of inoculating needle under aseptic condition. These were incubated at 28±2 °C. After 4 days of incubation, the fungus was transferred to sterilize Petri plates containing PDA medium and incubated in the same manner. After 6 days of incubation, a bit of hyphal growth from growing tips was transferred aseptically to fresh PDA slants. The fungus was brought into a pure culture by employing the single hyphal tip method (Singh, 1988) [15]. The fungus was identified following a mycological description (Ou, 1985) [11]. However, the culture is preserved by routinely transfer on PDA slants for further studies.

B. Evaluation of fungicides against *H. maydis*

Efficacy of different fungicides (Table. 2) against *H. maydis* was studied by applying poisoned food technique (Sharvelle, 1961) [14]. Five fungicides were evaluated at 50, 100, 150, 200 and 250 ppm concentrations against the *H. maydis* to inhibit their mycelial growth. For preparing the fungicidal stock solution, 50ml of stock solution of 10000 ppm concentration of each fungicide was prepared in the distilled water. The required amount of this solution was added into 100 ml flask containing 100 ml of sterilized melted media to attain required concentrations of 50, 100, 150, 200 and 250 ppm. The medium was mixed well before plating. Twenty ml of poisoned medium was gently poured in each of the sterilized Petri plates (Gul *et al.*, 2015) [2]. Mycelial disc of 5 mm was taken from the periphery of ten-day-old culture and placed in the centre and incubated at 28±2 ^{0}C. Suitable checks were also maintained without the supplement of any fungicide and three replications were kept for each treatment. The diameter of the colony was measured in two directions and the average was worked out till fungus in control plate reach to 90mm. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947) [18].

Per cent inhibition = C-T/C × 100

Where,

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

II. Effect of fungicides against *H. maydis* under field condition

The field trial was conducted during *Kharif* season 2016 -17 at Tirhut College of Agriculture, Dholi and laid out in a randomized block design with three replications. This experiment was conducted to evaluate the efficacy of different fungicides on control of Maydis leaf blight of maize.

A. Artificial inoculation of fungus *H. maydis*

The pathogens are isolated from diseased leaf lesions by following standard lab technique placed in a moist chamber. Spore formed on the lesion surface after 2 to 3 days and then picked up with the help of fined needle under a microscope. Then the spores are placed in a droplet of sterile water and streaked gently across the acidified water agar in Petri plates. Spores start germinating after a few hours; it again transferred to hard medium (acidified PDA). The culture kept for incubation in B.O.D for 20-25 ºC. After two weeks of incubation, the culture transferred for multiplication in Petri plate containing acidified PDA. Petri dishes (approx 20 plates) of full-grown cultures are macerated with the help of warring blender for about 15-30 seconds, and they permeated using a layer of cheese or muslin cloth and made up to a total of four to five litres of suspension. This stock suspension is brought to the field and attenuated in a compressed air sprayer @ 1 litre/12 litre of water. Normally, the spray should be done into the whorls portion of the plants where it will be conserved for a longer period/enough to permitting the spore germination. Inoculation of fungus should be done twice a week for three consecutive weeks when plants attained the height of 30-45 cm, normally 120 Petri dishes of pure culture will be enough for 1000 plants (Meena and shekhar) [9]. Then four fungicides with different treatments are listed in (Table 2 and 4) were sprayed only after disease initiation. The first spray was given in initial appearance of disease followed by one more spray at 15 days interval.

B. The fungicidal spray solution preparation

The fungicidal spray solution preparation of required concentration as per treatment was prepared freshly at the site of the experiment. The quantity of spray materials needs for an average of crop gradually increase as crop age advanced. So spray solution was prepared by applying the formula given by Singh (2009) [16].

N= T x P/ a.i

Where,

N= Quantity of a formulated fungicide required
T= Total spray fluid required
P= Percentage strength required
a.i= Given% strength of a formulated fungicide.

C. Disease Incidence (DI)

The incidence of maydis leaf blight was visually assessed in all the plots at a weekly interval from the first appearance of disease for each treatment. For each plot, the number of infected maize plants was counted and expressed as a percentage of the total number of maize plants in that plot. The mean percentage disease incidence for each treatment was obtained from the three replications. The data was further statistically analyzed. Disease incidence was calculated by the following formula (Wheeler, 1969) [20].

Disease incidence = (No. of diseased plant/ total no. of planned examined) × 100

D. Disease index (DX)

Observations on the severity of the disease were recorded on a 1-5 scale (Table. 1) (Payak and Sharma, 1983) [12]. Plants were selected randomly and assessed in each plot for disease rating and the per cent disease index was recorded. Per cent disease index was calculated by using the following formula [20].

Disease index = (The sum total of numerical ratings/ No. of plant examined ×Maximum grade) × 100

Table 1: The standard rating scale for maydis leaf blight severity

Scale	Infection type	Disease severity (%)
1.0	Very slight to slight infection, one or two too few scattered lesions on the lower leaves.	1-10 % Highly resistant (HR)
2.0	Light infection, a moderate number of lesions on lower leaves, few on middle leaves	11-25% Resistant
3.0	Moderate infection, abundant lesions on lower leaves, few on middle leaves	26-50% Moderately resistant
4.0	Heavy infection, lesions abundant on lower and middle leaves, extending to upper leaves	51-75% Susceptible
5.0	Very heavy infection, lesions abundant on almost all leaves, plants prematurely dried or killed by the disease	>75% Highly susceptible (HS)

Table 2: Fungicides details used against *Helminthosporium maydis* causal agent of maydis leaf blight of maize

Trade name	Chemical name	Formulation	Chemical group
Bavistin	Carbendazim	50% WP	Benzimidazole
Tilt	Propiconazole	25% WP	Phenylamide
Dithane M-45	Mancozeb	80% WP	Dithiocarbamate
Blitox-50	Copperoxychloride	50% WP	Copper compound
Kavach	Chlorothalonil	75% WP	Nitrate compound

Data analysis

Data were statistically analyzed using statistical analysis software (SAS) packages as well as completely randomized design (C.R.D). Critical differences were calculated at 5% level of significance for comparison of treatment mean. The Microsoft Excel (2010) computer software package was used to prepare all the graphs.

Result and Discussion

The effect of various fungicides on fungal colony diameter of cultivated *Helminthosporium maydis* on Potato Dextrose Agar (PDA) medium is presented in Table-3 and illustrated in Fig-1. All fungicides found effective in inhibiting fungal mycelial growth and after nine days the data was recorded under *in-vitro* condition. The result revealed that among the tested fungicides, Propiconazole was found highly effective with 100 per cent inhibition of mycelial growth of *H. maydis* at the concentrations (150, 200 and 250 ppm). Mancozeb showed 92.37 per cent inhibition at the concentration of 250 ppm at par with Propiconazole at 50 ppm concentration followed by Carbendazim which showed 88.74% inhibition at 200 ppm and 85.71% at 250 ppm respectively. Chlorothalonil @ 250 ppm showed 84.37% inhibition of fungal growth over control. Based on antifungal efficacy, Copper oxychloride found less effective in inhibiting of mycelial growth (70.635) at 50 ppm. 70.63 per cent at 50 ppm). These results are in understanding with the finding of Jha *et al.* (2004) [5] who evaluated fungicides *viz.*, thiram, emisan, indofil M-45, captaf and bavistin at various concentrations separately and in integrations against *H. maydis.* The effectiveness of the fungicides propiconazole, mancozeb, carbendazim, chlorthalonil against *H. maydis* has been reported by many scientists (Harlapur *et al.*, 2007 [4]; Sanjeev Kumar *et al.*, 2009 [13]; Khedeker *et al.*, 2012 [6]; Waghe *et al.*, 2015 [19]).

Under field condition, the data presented in (Table 4 and fig. 3) revealed that among one spray of all tested fungicides T_1- ST with SAAF (Carbendazim+ Mancozeb) @ 3.0gm/kg seed + one spray with Propiconazole @ 0.1% and T_4- ST + one spray with Mancozeb @ 0.2% were found most effective in reducing the maydis leaf blight (PDI of 28.67% and 33.87%, respectively, as compared to control i.e. 79.67% PDI. While T_2- ST+ one spray with Chlorothalonil @ 0.1% was found least effective in reducing disease in comparison to control. Among two sprays, T_5- ST + two sprays with Propiconazole @ 0.1% found most effective with least PDI 31.00 per cent and two sprays with Chlorothalonil shows its least efficacy against the disease. While three sprays did not show much increase in yield and decrease in PDI as compared to one and two sprays of fungicides. The result revealed that, statistically significant differences among the treatments for PDI and grain yield. Foliar sprays of fungicides were found more effective against MLB and resulted in decreased PDI and increased grain yield. These results are in agreement with the finding of Kumar *et al.* (1977) [8] who evaluated eight fungicides and found that dithane M-45, unizeb and dithane-Z-78 significantly reduced the maize leaf blight severity by 55, 47.4 and 44.43 per cent, respectively, and increased grain yield by 8.54, 10.12 and 9.90 per cent. Vaibhav *et al.* (2011) [17] also reported that Propiconazole 25 EC was highly effective and it ensured minimum disease intensity (21.40%) and highest yield (29.37 q/ha) followed by chlorothalonil (27.93% disease intensity and 27.60 q/ha yield). The similar results were recorded by Kommedahl and Lang (1973) [7], Nasir *et al.*, (2012) [10, 19].

Benefit: Cost Ratio (BCR)

The total cost incurred for application of fungicides including the cost of fungicides and labours were calculated. Additional benefit due to increased yield in each treatment over control was worked out and the benefit-cost ratio was calculated using additional benefits and total costs. It is calculated by the following formula:

B: C = Gross return/Total cost of cultivation

Effect of fungicides on BCR

Overall efficacies of various fungicidal treatments were finally assessed and compared based on benefits realized in monitory terms and the data about these economical parameters are presented in table 5. The result showed that the increased yield and added benefit over control (Rs. /ha) varied in respect of the average yield obtained in various treatments. All treatments proved profitable over control. The highest benefit-cost ratio i.e. 5.04:1 was recorded in one spray of Propiconazole followed by Mancozeb (4.02:1) in two sprays and three sprays with Mancozeb showed 2.92:1. The least benefit-cost ratio was in all sprays with Chlorothalonil.

Table 3: Inhibitory effect of different fungicides on the growth of *Helminthosporium maydis* (at 9 days)

Fungicides	*Percent inhibition of radial growth (at 9 days)									
	Concentration (ppm)									
	50		100		150		200		250	
	G	I	G	I	G	I	G	I	G	I
Propiconazole	6.70	92.55	4.46	95.04	0	100	0	100	0	100
Carbendazim	24.96	72.26	18.33	79.63	15.30	83.00	12.86	85.71	10.13	88.74
Mancozeb	16.66	81.48	14.90	83.44	12.26	86.37	10.26	88.60	6.86	92.37
Chlorothalonil	21.46	76.15	19.96	77.82	17.63	80.41	15.96	82.26	14.06	84.37
COC	26.43	70.63	24.96	72.26	22.00	75.55	20.86	76.82	18.46	79.48
Check	90.00	-	90.00	-	90.00	-	90.00	-	90.00	-
	Fungicide (A)			Concentration (B)				Interaction (A×B)		
CD at 5%	0.57			0.55				1.27		
SE(m)±	0.20			0.18				0.45		
CV (%)	4.16									

*Mean of three replications, G= Mycelial Growth (mm); I= Inhibition Per cent

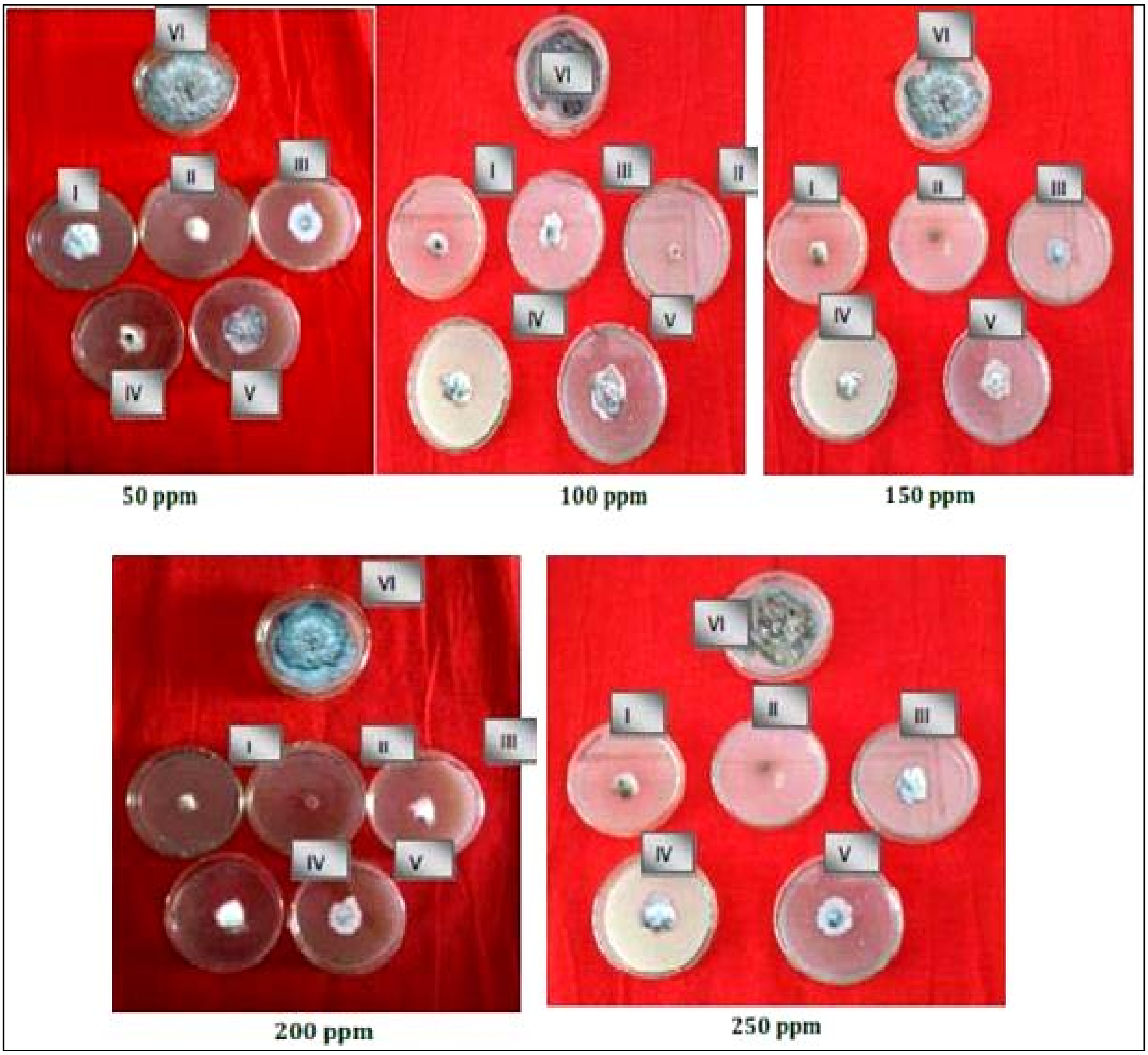

Fig 1: *In vitro* inhibition of fungicides at different concentrations against *H. maydis*

I. Mancozeb II. Propiconazole III. Carbendazim IV. Chlorothalonil V. Copper Oxychloride VI. Control

Table 4: Effect of fungicides on the incidence of maydis leaf blight and grain yield of maize

Treatments	Treatment details	Mean PDI	(%) Disease control over check	Mean Grain yield (q/ha.)	% increase over check
T_1	ST with SAAF (Carbendazim+Mancizeb) @ 3.0 gm/kg seed+ one spray with Propiconazole @0.1%	28.67 (32.33)	64.01	38.87	52.19
T_2	ST + one spray with Chlorothalonil @0.1%	63.50 (52.80)	20.30	28.24	10.57
T_3	ST + one spray with Carbendazim @0.1%	44.64 (41.92)	43.97	29.33	14.84
T_4	ST + one spray with Mancozeb @0.2%	33.87 (35.59)	57.49	29.94	17.23
T_5	ST + two spray with Propiconazole @0.1%	31.00 (34.04)	61.09	43.47	70.20
T_6	ST + two spray with Chlorothalonil @0.1%	67.50 (55.24)	15.28	30.58	19.73
T_7	ST +two spray with Carbendazim @0.1%	52.23 (47.98)	34.44	32.15	25.88
T_8	ST +two spray with Mancozeb @0.2%	44.80 (41.82)	43.77	37.48	46.75
T_9	ST +three spray with Propiconazole @0.1%	34.75 (36.32)	56.38	45.75	79.13
T_{10}	ST + three spray with Chlorothalonil @0.1%	73.20 (68.89)	8.12	32.57	27.53
T_{11}	ST +three spray with Carbendazim @0.1%	66.45 (54.62)	16.59	33.52	31.25
T_{12}	ST +three spray with Mancozeb @0.2%	53.20 (46.83)	33.22	38.50	50.74
T_{13}	Control	79.67 (63.45)	-	25.54	-
	SE(m)±	3.23		1.89	
	CD at 5%	9.48		5.57	
	CV (%)	10.80		9.60	

(Figures within the parenthesis are angular transformed values)

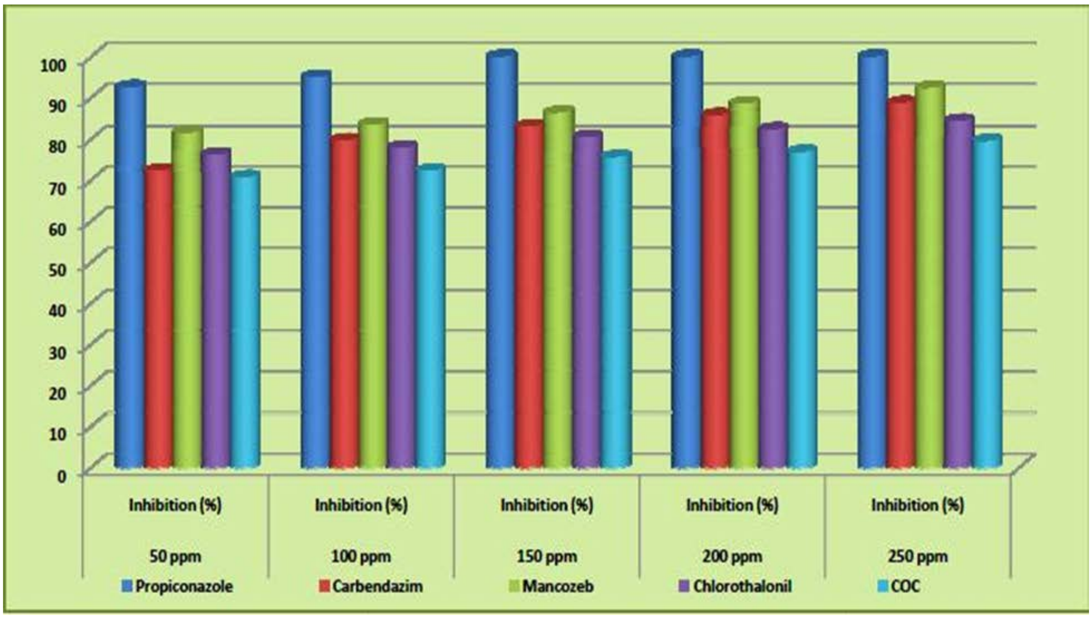

Fig 2: Inhibitory effect of different fungicides on the growth of *Helminthosporium maydis* at 9 day

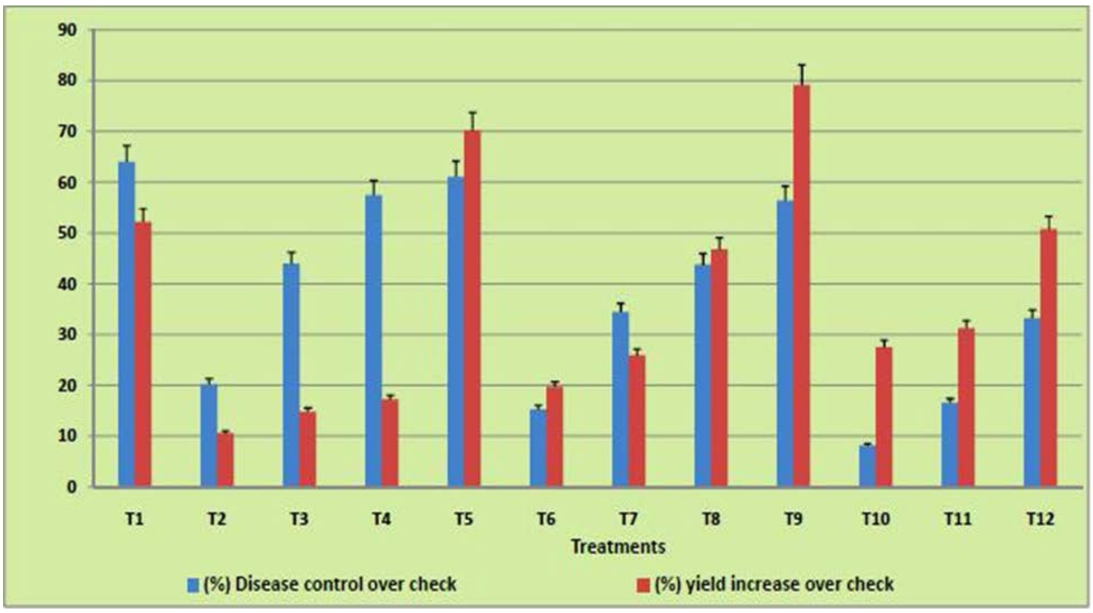

Fig 3: Effect of fungicides on the incidence of maydis leaf blight and grain yield of maize

Table 5: B: C ratio of different treatments against maydis leaf blight of maize

Treatment	Mean Yield (q/ha	Added benefit over control* (Rs./ha)	Cost of fungicide+ spraying machine (Rs/day) + labour cost (Rs/ha.)	Net profit (Rs./ha)	Benefit: cost ratio
ST with SAAF (carbendazim+Mancozeb) @ 3.0 gm/kg seed+ one spray with Propiconazole @0.1%	35.87	10,330	2,046	8,284	5.04:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ one spray with Chlorothalonil @0.1%	28.24	2,700	1,946	754	1.38:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ one spray with Carbendazim @ 0.1%	29.33	3,790	1,386	2,404	2.73:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ one spray with Mancozeb @ 0.2%	29.94	4,400	1,506	2,894	2.92:1
ST with SAAF (carbendazim+Mancozeb) @ 3.0 gm/kg seed+ two spray with Propiconazole @0.1%	39.47	13,930	4,046	9,854	3.22:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ two spray with Chlorothalonil @0.1%	30.58	5,040	3,846	1,194	1.31:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ two spray with Carbendazim @ 0.1%	32.15	6,610	2,726	3,884	2.42:1
ST with SAAF (Carbendazim+Mancozeb) @3.0 gm/kg seed+ two spray with Mancozeb @ 0.2%	37.48	11,940	2,966	8,974	4.02:1
ST with SAAF (Carbendazim+Mancozeb) @ 3.0gm/kg seed+ three spray with Propiconazole @0.1%	41.75	16,210	6,046	10,164	2.68:1
ST with SAAF (Carbendazim+Mancozeb) @3.0gm/kg seed+ three spray with Chlorothalonil @0.1%	32.57	7,030	5,746	1,284	1.22:1
ST with SAAF (Carbendazim+Mancozeb) @3.0gm/kg seed+ three spray with Carbendazim @ 0.1%	33.52	7,980	4,066	3,914	1.96:1
ST with SAAF (Carbendazim+Mancozeb) @3.0gm/kg seed+ three spray with Mancozeb @ 0.2%	38.50	12,960	4,426	8,534	2.92:1
Control	25.54	-	-	-	-

Conclusion

All tested fungicides significantly reduced the maydis leaf blight disease of maize caused by *Helminthosporium maydis* under both in vitro and in vivo condition. Among all the fungicides, Propiconazole was found quite effective in cent per cent inhibition of fungus mycelial growth laboratory and disease progress in the field followed by Mancozeb, Carbendazim, Chlorothalonil and COC over control. Despite management, Propiconazole gives the highest benefit-cost ratio followed by Mancozeb while the least benefit-cost ratio was recorded with Chlorothalonil.

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