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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(6): 399-406

www.thepharmajournal.com
Received: 11-04-2021
Accepted: 21-05-2021

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In vitro evaluation of fungicides, botanicals and bio-agents against the maydis leaf light disease of maize caused by *Helminthosporium maydis*

Chandan Kumar, Phool Chand, CS Choudhary, NS Akhtar and Bimla Rai

Abstract
Maize (*Zea mays* L.) is an important cereal crop belonging to the family, Graminae. It is the most versatile crop, adapted to different agro-ecological and climatic conditions. Maize is known as queen of cereals because of its high genetic yield potentiality. Maize is an essential human nutrient, element of animal feed and raw material for so many industrial products. Maydis leaf blight is a serious foliar fungal disease causes considerable losses to the maize crop. If infection occurs prior to silking and weather conditions are favourable, damage is most critical. The present investigation on "*In vitro* Evaluation of Fungicides, Botanicals and Bio-agents against Maydis Leaf Blight Disease of Maize Caused by *Helminthosporium maydis"* were undertaken for the management of Maydis leaf blight disease of maize. Nine fungicides (5 systemic and 4 non-systemic fungicides) were tested against Maydis leaf blight disease under laboratory condition with different concentrations *viz*., 50, 100 and 200 ppm. Ten botanicals were also tested *in vitro* at 5%, 10%, 15% and 20%. *In vitro* ten isolates of *Trichoderma* sp. from the native rhizosphere was also used for bio control experiment against the *Helminthosporium maydis*. Among the systemic fungicides, Propiconazole was found highly effective and inhibited cent per cent of mycelial growth of *H. maydis* at all the concentrations (50, 100 and 200 ppm). Carboxin showed 100 per cent inhibition at 100 and 200 ppm concentrations. After Propiconazole and Carboxin, Crabendazim showed 87.88 per cent inhibition at 200 ppm concentration. Amongst all the non-systemic fungicides evaluated Mancozeb was found to be most effective and significantly superior over all other treatments followed by Thiram and Chlorothalonil. Among Botanicals, Turmeric was found to be most effective and significantly superior over all other treatments at all the concentrations. Next to Turmeric, Garlic was found significantly effective at 5, 10 and 15 per cent concentrations, respectively. Neem was found effective at 20 per cent concentrations followed by Garlic, Ginger and Onion. All the isolates of *Trichoderma* reduced the mycelial growth of the test fungus. Maximum inhibition in mycelial growth (93.48%) was recorded in RT-6 isolate followed by RT-9 isolate (91.25%), RT-7 (89.36%) and minimum inhibition in mycelial growth (73.94%) was measured in RT-1 isolate.

Keywords: Maize, silking, maydis leaf blight, systemic fungicides, *Trichoderma* isolates, botanicals, mycelial growth, inhibition, propiconazole, Carboxin, mancozeb, concentrations, neem, turmeric, garlic

1. Introduction
Maize is the most versatile crop adapted to different agro-ecological and climatic conditions. Maize is an important cereal crop next to rice and wheat all over India and World. Maize is one of the largest (49.33t) consumable cereals in the world. Due to increasing demand, Maize production in India was 27.23 million tons from an area of 9.18 million hectare area with the productivity of 2965 kg per hectare during 2018-19 (Directorate of Economics & Statistics, DAC & FW 2019). Bihar has become pioneer state in maize with the production of (3.02 mt) in area of 0.68 (mha) which contribute the highest productivity of 4451 kg/ha (Directorate of Economics & Statistics, DAC & FW, MoA & FW, GoI, 2019. It is known as queen of cereals because of its high genetic yield potential.

Southern Corn Leaf Blight (SCLB) or Maydis Leaf Blight (MLB) caused by *Helminthosporium maydis* (Syn. *Bipolaris maydis* (Nisik.) Shoemaker), (teleomorph: *Cochliobolus heterostrophus*) is a serious fungal disease of maize throughout the world where maize is grown under warm, humid conditions (White, 1999) [47]. Three races of *Cochliobolus heterostrophus* known as O, T and C which have been described by (Smith *et al.*, 1970) [11]. Currently predominantly form of *Cochliobolus heterostrophus* is race O, which can cause yield losses of up to 40 per cent (Fischer *et al.*, 1976, Gregory *et al.*, 1978; Byrnes *et al.*, 1989) [11, 13, 5]. In 1970's an epidemic was caused by race T in maize with Texas male sterile cytoplasm in most maize growing regions of the USA but maize having normal cytoplasm was resistant to the pathogen. The earlier record of race T from India is from Non-maize hosts from

Delhi and Pusa. The prevalence of the disease is in warm humid tropical to temperate region, where the temperature ranges between 20-30^{0}C during cropping period (Singh and Srivastava, 2012) [37]. As the pathogen is able to overwinter in infected crop debris, management of crop debris between growing seasons can be helpful in reducing the initial amount of inoculums.

Keeping in view of the above facts the study has been proposed to work out on the topic "*In vitro* Evaluation of Fungicides, Botanicals and Bio-agents against Maydis Leaf Blight Disease of Maize Caused by *Helminthosporium maydis"*at RPCAU, Pusa, Samastipur, Bihar.

2. Materials and Methods

2.1 *In vitro* evaluation of fungicides against *Helminthosporium maydis*: *In vitro*, efficacy of different fungicides against *H. maydis* was studied by poisoned food technique (Sharvelle, 1961) [35]. Five systemic and four non-systemic fungicides were tested against *H. maydis* on the potato dextrose agar media using poison food technique under *in vitro* condition. The systemic and non-systemic fungicides were tested at 50, 100 and 200 ppm concentrations. Information about fungicides formulation and active ingredient is presented in Table 1.

Poisoned food technique

Ten ml stock solution of 10,000 ppm concentration of each fungicide was prepared in the distilled water in test tube. Required amount of the solution was added into 50 ml flask which contains 50 ml of the sterilized melted PDA, to get the final required concentrations of 50, 100 and 200 ppm for systemic as well as non-systemic fungicides.

Table 1: List of the systemic and non-systemic fungicides evaluated

Sl. No.	Trade name	Common name	Active ing- redients (%)	Mode of action	Formulation
Systemic fungicides					
1.	Vitavax	Carboxin	70	Contact and Systemic	WS
2.	Bavistin	Carbendazim	50	Systemic	WP
3.	Saaf	Carbendazim+ Mancozeb	75	Systemic	WP
4.	Tilt	Propiconazole	25	Systemic	EC
5.	Contaf	Hexaconazole	50	Systemic	WP
Non-Systemic fungicides					
6.	Thiram	Thiram	75	Contact	WP
7	Dithane M-45	Mancozeb	75	Contact	WP
8	Blitox 50	Copper Oxychloride	50	Contact	WP
9	Kavach	Chlorothalonil	75	Contact	WP

Before plating, the medium was mixed thoroughly. Each media toxicated with fungicide was poured in three petriplates. Non toxicated media was poured into petriplates and kept as a unreated or check. After solidification of media a 2 mm mycelial disc of 9 days old culture of the test pathogen was cut with sterile cork borer and placed in centre of the petriplate. The petriplates were incubated at 27 ± 1 ºC. After 9 days of incubation the radial growth was measured. The per cent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula (Vincent, 1947) [46]:

Per cent inhibition = X-Y/X ×100

Where,

X = colony diameter in control

Y = colony diameter in treated medium

2.2 *In vitro* evaluation of botanicals against *Helminthosporium maydis*

2.2.1 Preparation of botanical extracts

The leaf and bulb extracts of Neem, Garlic, Ginger, Bhang, Eucalyptus, Onion, Castor, Haldi, Bael, Ocimum, were prepared by cold water extraction method described by Shekhawat and Prasad (1971) [36]. The plant material were collected and firstly washed in tap water and then in distilled water. Hundred grams of fresh sample was chopped and crushed in a pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extracts were filtered through two layers of muslin cloth and through Whatmann No. 1 filter paper too. Finally filtrate thus obtained was used as stock solution.

The appropriate amount of plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. For bioassay, double strength concentrations of botanicals were prepared by dissolving 10, 20, 30 and 40 ml of plant extract in 90, 80, 70 and 60 ml of sterilized distilled water, respectively to get the final concentrations of 5, 10, 15 and 20 per cent. The extracts were tested against *H. maydis* on the culture media using poison food technique under *in vitro* condition. Details about the botanicals and part used are given in Table 2.

Table 2: List of botanicals used in study

Sl. No.	Common name	English name	Botanical name	Family	Parts
1	Neem	Neem	*Azdirachta indica*	Meliaceae	Leaves
2	Lahsun	Garlic	*Allium sativum*	Alliaceae	Bulb
3	Adarak	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
4	Safeda	Eucalyptus	*Eucalyptus globules*	Myrtaceae	Leaves
5	Pyaj	Onion	*Allium cepa*	Alliaceae	Leaves
6	Arandi	Castor	*Ricinus communis*	Euphorbiaceae	Leaves
7	Haldi	Turmeric	*Curcuma longa*	Zigiberaceae	Rhizome
8	Tulsi	Ocimum	*Ocimum sanctum*	Lamiaceae	Leaves
9	Bael	Wood apple	*Aegle marmelos*	Rutaceae	Leaves
10	Bhang	Bhang	*Cannabis sativa*	Cannabaceae	Leaves

2.2.2 Bioassay procedure

Poisoned food technique (plant extract amended PDA medium) was used to screen different plant extracts *in vitro* (Nene and Thapliyal, 1973) [28], different concentrations (5, 10, 15, and 20%) of plant extracts were incorporated in double strength concentration PDA medium for inoculation of the test pathogen in sterilized petriplates. The 2 mm disc of test pathogen grown on PDA medium was placed at the centre of petriplates containing different concentration of the poisoned medium and incubated at 27±1 ºC. Three replications were maintained and radial growth was taken when maximum growth occurred in the control plates. The inhibition per cent of radial growth over the control which was calculated by using the following formula (Vincent, 1947) [46].

Per cent inhibition = X-Y/X ×100

Where,

X= colony diameter in check

Y = colony diameter on amended medium

2.3 *In vitro* evaluation of bio-control agent against *Helminthosporium maydis*

2.3.1 Isolation of bio control agent, *Trichoderma* species

The soil samples collected from different native rhizospheres of TCA, Dholi and RPCAU, Pusa farm and were used for isolation of *Trichoderma* species. Isolation was done with the help of serial dilution technique on *Trichoderma* selective medium. Morphologically distinct colonies were picked on the basis of their morphology and purified on PDA following sub culturing.

2.3.2 Identification of *Trichoderma* isolates

After the isolation of all isolates, it was examined under a microscope for the identification and confirmatory of *Trichoderma* species. Macroscopic visualization showed rapid growth rate and colonies are wooly becoming compact in time; and the surface colony color was white and scattered greenish patches become visible as the conidia are formed. After then, growth observed in those plates was taken for microscopic study, colony characteristics and morphology of each *Trichoderma* isolate was observed. Examination of the shape, size, arrangement and development of conidiophores (repeatedly branched conidiophores) or phialides (irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask - shaped phialides) or conidia (unicellular, round or ellipsoidal, green in color, smooth walled or rough conidia) provided a tentative identification of *Trichoderma* species. Identification details are given in table 3.

In vitro ten isolates of *Trichoderma* spp. *viz*; RT-1, RT-2, RT-3, RT-4, RT-5, RT-6, RT-7, RT-8, RT-9, RT-10 were used for experiments. Both bio-control agents and test fungus were dual cultured on PDA in order to get fresh and active growth of fungus. These bio-control agents were isolated from native rhizosphere of TCA farm and RPCAU, Pusa farm.

Table 3: Identification of *Trichoderma* species

Sl. No.	Isolates of *Trichoderma*	Identification of *Trichoderma* Species
1.	RT-1	*Trichoderma aureoviridae*
2.	RT-2	*Trichoderma harzianum*
3.	RT-3	*Trichoderma harzianum*
4.	RT-4	*Trichoderma harzianum*
5.	RT-5	*Trichoderma koningii*
6.	RT-6	*Trichoderma viridae*
7.	RT-7	*Trichoderma viridae*
8.	RT8	*Trichoderma viridae*
9.	RT-9	*Trichoderma viridae*
10.	RT-10	*Trichoderma koningii*

2.3.3 Dual culture test

Bio-agents were tested against the pathogen for their efficacy through dual culture technique. The bio-agents and the test fungus were inoculated side by side on a single petriplate containing solidified PDA medium. Three replications were maintained for each treatment with one check by maintaining the pathogen separately. Inoculated plates were incubated at 27 ± 1 ºC for twelve days. The diameter of the colony of both bio-agents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula described by (Vincent, 1947) [46].

Percent inhibition= A-B/A × 100

Where,

A= Diameter of fungal growth in check plate

B= Diameter of fungal growth in treatment

3. Results

3.1 *In vitro* evaluation of fungicides against *Helminthosporium maydis*

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 ppm). Carboxin showed 100 per cent inhibition at 100 and 200 ppm concentrations. After Propiconazole and Carboxin, Crabendazim showed 87.88 per cent inhibition at 200 ppm concentration followed by Carbendazim+Mancozeb (73.04% at 200 ppm and 70.04% at 100 ppm). The least inhibition of mycelial growth among the systemic fungicides was observed in Hexaconazole (35.93%) at 50 ppm concentration. Among the three concentrations assessed, 200 ppm was found significantly superior over 100 and 50 ppm concentration in inhibition of growth of the fungus. The results related to effect of different systemic fungicides in inhibiting the pathogen are presented n table 4. Amongst all the non-systemic fungicides evaluated Mancozeb was found to be most effective and significantly superior over all other treatments followed by Thiram and Chlorothalonil has been shown in table 5.

3.2 *In vitro* evaluation of botanicals against *Helminthosporium maydis*

As plant extracts are cost effective means of management so an effort was made to find out the efficacy of different plant extracts against *H. maydis*. Observations on diameter of mycelial growth of fungus were recorded. The per cent

inhibition of mycelial growth of the fungus was calculated and presented in Table 6 and Fig. 3. From the data it can be summarized that, Turmeric was found to be most effective and significantly superior over all other treatments at all the concentrations. Next to Turmeric, Garlic was found significantly effective at 5, 10 and 15 per cent concentrations, respectively. Neem was found effective at 20 per cent concentrations followed by Garlic, Ginger and Onion. No inhibition was shown by wood apple at 5%, 10% and 15% concentrations. Among the four concentrations assessed, 20 per cent was found significantly superior over 15, 10 and 5 per cent concentration in inhibiting the growth of the fungus.

Table 4: Mycelial inhibition of *Helminthosporium maydis* by different systemic fungicides

Fungicides	Per cent inhibition of radial growth*					
	Concentration					
	50 ppm		100 ppm		200 ppm	
	G	I	G	I	G	I
Propiconazole	0.00	100.00	0.00	100.00	0.00	100.00
Carboxin	4.13	95.41	0.00	100.00	0.00	100.00
Carbendazim	30.63	65.96	22.50	75.00	10.90	87.88
Mancozeb + Carbendazim	41.83	53.52	26.96	70.04	24.26	73.04
Hexaconazole	57.66	35.93	47.90	46.77	42.60	52.66
Check	90.00	0.00	90.00	0.00	90.00	0.00
Mean	37.37	-	31.22	-	27.96	-
	Fungicide (F)		Concentration (C)		Interaction (FxC)	
S.Em +	0.53		0.37		0.92	
CD at 5%	1.54		1.08		2.66	

*Mean of three replications, G= Mycelial Growth; I= Per cent inhibition

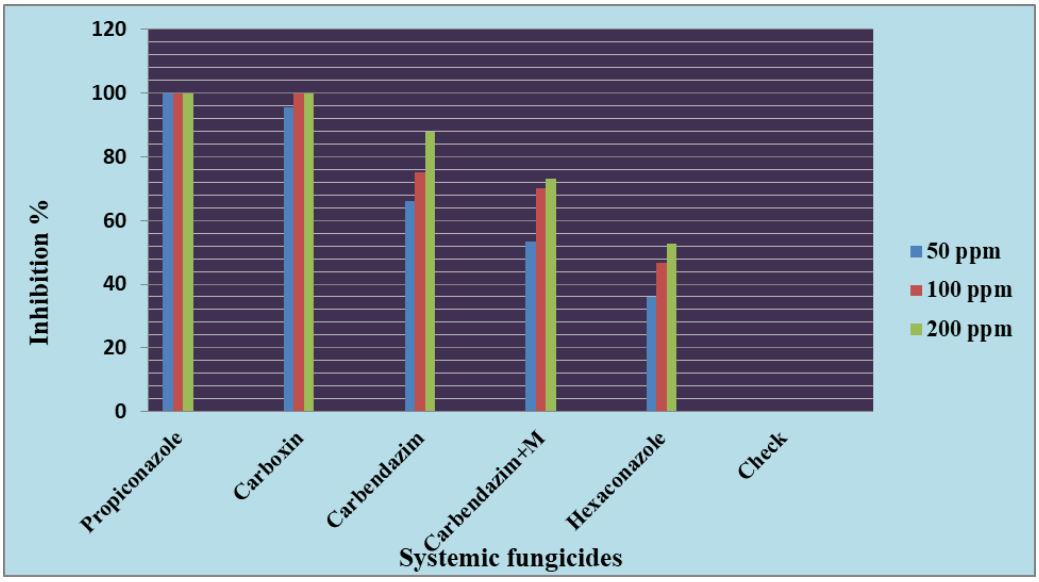

Fig 1: Mycelial inhibition of *Helminthosporium maydis* by different systemic fungicide

Table 5: Mycelial inhibition of *Helminthosporium maydis* by different non-systemic fungicides

Fungicides	Per cent inhibition of radial growth*					
	Concentration					
	50 ppm		100 ppm		200 ppm	
	G	I	G	I	G	I
Thiram	20.93	76.74	9.73	89.18	0.00	100.00
Mancozeb	18.20	79.77	6.36	92.93	3.86	95.71
Chlorothalonil	25.03	72.18	8.90	90.11	5.56	93.82
Copper Oxychloride	55.56	38.26	40.30	55.22	23.00	74.44
Check	90.00	0.00	90.00	0.00	90.00	0.00
Mean	41.94	-	31.06	-	24.48	-
	Fungicide(F)		Concentration(C)		Interaction(FxC)	
S.Em +	0.53		0.41		0.92	
CD at 5%	1.54		1.20		2.68	

*Mean of three replications, G= Mycelial growth; I= Per cent inhibition

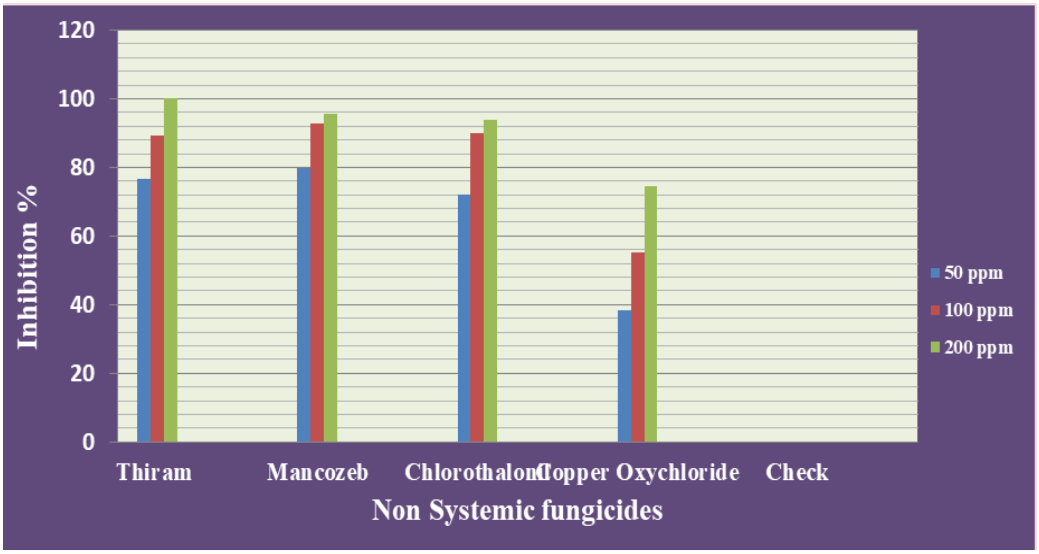

Fig 2: Mycelial inhibition of *Helminthosporium maydis* by different non-systemic fungicides

Table 6: Mycelial inhibition of *Helminthosporium maydis* by different botanicals

Botanicals	Per cent inhibition of radial growth*							
	Concentration							
	5%		10%		15%		20%	
	G	I	G	I	G	I	G	I
Turmeric	14.00	84.44	10.66	88.15	9.36	89.60	5.90	93.44
Garlic	24.90	72.33	24.26	73.04	22.36	75.15	17.96	80.04
Ginger	30.96	65.60	28.50	68.33	25.43	71.74	21.20	76.44
Onion	40.56	54.93	34.26	61.93	33.26	63.04	30.96	65.6
Neem	34.60	61.55	31.33	65.18	27.80	69.11	15.03	83.3
Eucalyptus	47.56	47.15	47.16	47.60	45.66	49.26	44.90	50.11
Ocimum	60.83	32.41	54.46	39.48	50.00	44.44	39.86	55.71
Bhang	90.00	0.00	90.00	0.00	90.00	0.00	68.00	24.44
Castor	90.00	0.00	74.46	17.26	70.00	22.22	64.40	28.44
Wood Apple	90.00	0.00	90.00	0.00	90.00	0.00	75.66	15.93
Check	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
Mean	54.39		51.14		53.91		44.53	
	Botanical (B)		Concentration (C)		Interaction (BxC)			
SEm+-	0.64		0.38		1.28			
CD at 5%	1.81		1.09		3.61			

*****Mean of three replications, G= Mycelial Growth; I= Per cent inhibition

3.3 *In vitro* evaluation of bio-agents against *Helminthosporium maydis*

Use of bio agents for controlling plant diseases is an old practice in India. In the last two decades great emphasis has been given to antagonistic organism to assess their potentiality for control of plant diseases, particularly as one of the component of an integrated disease management programme. In the present investigation, All the species reduced the mycelial growth of the test fungus. Maximum inhibition in mycelial growth (93.48%) was recorded in RT-6 isolate followed by RT-9 isolate (91.25%), RT-7 (89.36%) and minimum inhibition in mycelial growth (73.94%) was measured in RT-1 isolate (Table 7 and Fig. 3). The results thus obtained indicate a need of *in vitro* testing of more isolates of *Trichoderma* sp. against the pathogen, which could lead to better eco-friendly management of diseases in future.

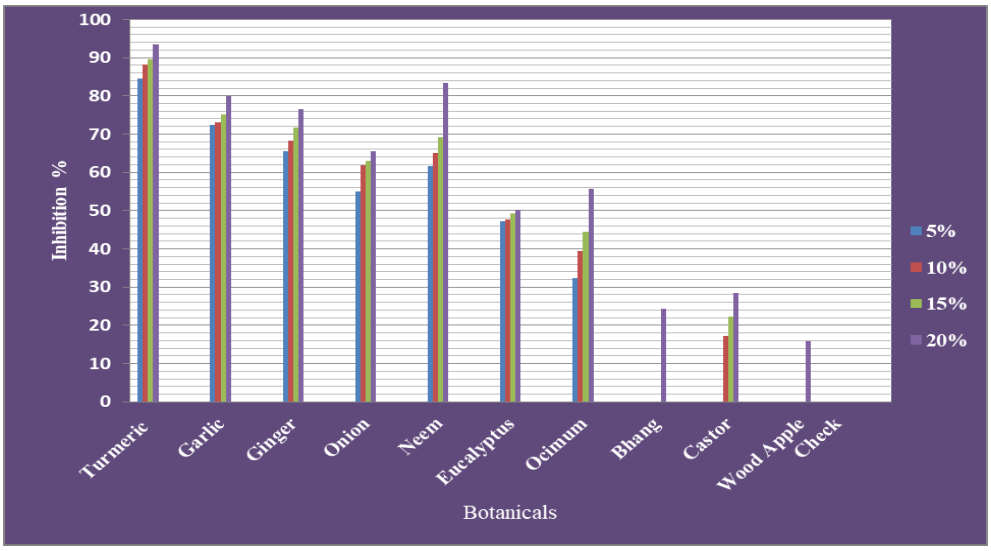

Fig 3: Mycelial inhibition of *Helminthosporium maydis* by different botanicals

4. Discussion

4.1 *In vitro* evaluation of fungicides against *Helminthosporium maydis*

In the absence of resistant cultivars, use of fungicides to control the disease is in practice, as it gives relief from the pathogen after the appearance of the disease. *In vitro* evaluation of fungicides provides preliminary information regarding the efficacy of fungicides against pathogen within a shortest period of time and therefore, serves as a guide for field testing. Hence, nine fungicides were screened under laboratory conditions. Among five screened 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 ppm). Carboxin showed 100 per cent inhibition at 100 and 200 ppm concentrations. After Propiconazole and Carboxin, Carbendazim showed maximum inhibition at 200 ppm concentration. The least inhibition of mycelial growth among the systemic fungicides was observed in Hexaconazole at 50 ppm concentration.

Table 7: Mycelial inhibition of *Helminthosporium maydis* by different isolates of *Trichoderma* sp.

Sl. No.	Isolates of *Trichoderma* sp.	Colony diameter(mm)*	Inhibition (%)
1	RT-1	23.45	73.94
2	RT-2	17.56	80.48
3	RT-3	14.84	83.51
4	RT-4	15.52	82.77
5	RT-5	15.10	83.22
6	RT-6	5.86	93.48
7	RT-7	9.57	89.36
8	RT-8	13.42	85.08
9	RT-9	7.87	91.25
10	RT-10	11.23	87.52
11	Check	90.00	-
	Mean	20.40	
	S.Em +	0.58	
	CD at 5%	1.72	

*Mean of three replications.

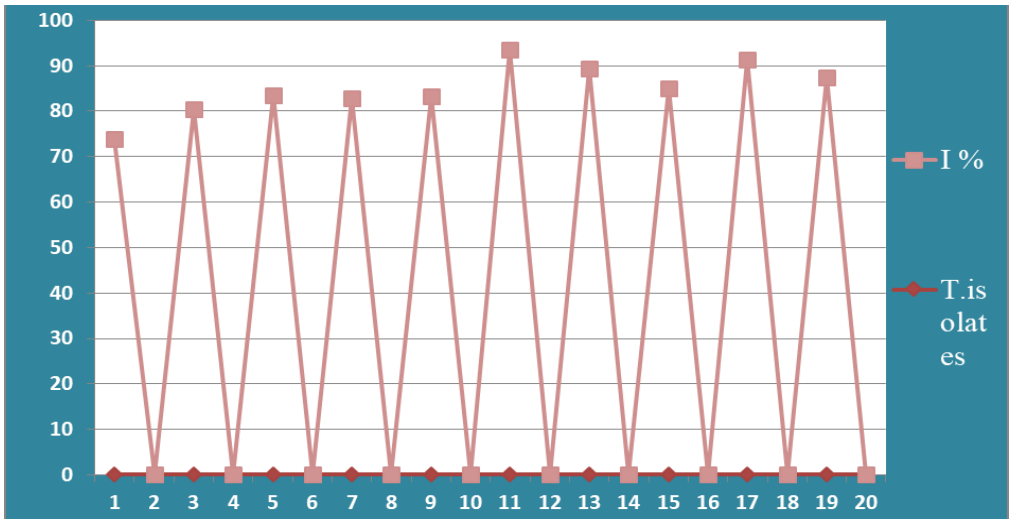

Fig 4: Mycelial inhibition of *Helminthosporium maydis* by different species of *Trichoderma* sp.

These results were in agreement with the finding of Sanjeev Kumar *et al*., (2009c) [23] 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 vitro*. Similarly, Harlapur *et al*., (2007) [15] reported that Mancozeb and Carboxin powder were effective against *E. Turcicum.* Similar results were also reported by Meli and Kulkarni (1994) and

Jha *et al*., (2004) [24, 16]. The effectiveness of the systemic fungicides Tilt, Vitavax, Bavistin and Saaf against *H. maydis* has been reported by several authors (Cassini *et al*., 1973; Khatri, 1989; Sakhi *et al*., 1991; Tiwari *et al*., 2003; Kumar *et al*., 2009a) [6, 21, 32, 42, 23]. Similar studies were also conducted in case of *H. turcicum* by Singh and Gupta, 2000; Patil, 2000 and Debasis and Kaiser, 2003 [37, 29, 9].

Amongst all the non-systemic fungicides evaluated Mancozeb was found to be most effective and significantly superior over all other treatments followed by Thiram and Chlorothalonil. Efficacy of Mancozeb against the pathogen has been reported earlier by Miller (1972); Comstock *et al*., (1974); Jha *et al*., (2004b) and Naz *et al*., (2013) [25, 8, 18, 27]. Among the three concentrations assessed, 200 ppm was found significantly superior over 100 and 50 ppm concentration in inhibition of growth of the fungus. These data are in accordance with Jones *et al*., (1987); Cassini *et al*., (1973); Phelp and Soto (1993); Debasis and Kaiser (2003); Kumar *et al*., (2009a) and Yamashita *et al*., (2010) [19, 6, 30, 9, 23, 48].

4.2 *In vitro* evaluation of botanicals against *Helminthosporium maydis*

As plant extracts are cost effective means of management so an effort was made to find out the efficacy of different plant extracts against *H. maydis.* Turmeric was found to be most effective and significantly superior over all other treatments at all the concentrations. Next to Turmeric, Garlic was found significantly effective at 5, 10 and 15 per cent concentrations, respectively. Neem was found effective at 20 per cent concentrations followed by Garlic, Ginger and Onion. Among the four concentrations assessed, 20 per cent was found significantly superior over 15, 10 and 5 per cent concentration in inhibiting the growth of the fungus (Adeyemo and Abiala, 2015). Wood apple was not found effective at all the concentrations. Such type of similar results on antifungal activity of aqueous extracts of different plants have been reported by various workers (Garcia and Padilla, 1994; Jha *et al*., 2004a; Kumar *et al*., 2009c; and Begum *et al*., 2011; Bisht *et al*., 2013) [12, 17, 23, 3, 4].

The fungicidal property of leaf extracts of Garlic and Onion has already been investigated by Shekhawat and Prasada (1971), Misra and Dixit (1978) and Tariq and Magee (1990) [36, 26, 41]. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal compounds *viz*., Azadirachtin in *Azardiachta indica* (Verma *et al*., 2002) [45], Eucalyptol in *Eucalyptus globules* (Joseph *et al*., 2008) [20]. Garlic also possess fungicidal property might be due to the presence of allicin and diallyl sulphide. Higher plants have proved to be the useful source of fungitoxic substances as proven in previous studies by Faweet and Spencer (1970); Tripathi *et al*., (1978); Cimanga *et al*., (2002) and Gurjar *et al*., (2012) [10, 43, 7, 14].

4.3 *In vitro* evaluation of bio-agents against *Helminthosporium maydis*

Use of bio agents for controlling plant diseases is an old practice in India. In the last two decades great emphasis has been given to antagonistic organism to assess their potentiality for control of plant diseases, particularly as one of the component of an integrated disease management programme. All the species reduced the mycelial growth of the test fungus. Maximum inhibition in mycelial growth (93.48%) was recorded in RT-6 isolate followed by RT-9 isolate (91.25%), RT-7 (89.36%) and minimum inhibition in mycelial growth (73.94%) was measured in RT-1 isolate. The difference in per cent inhibition in mycelial growth indicates the difference in their efficacy against the pathogen. This may be due to the mechanism of antibiosis of pathogens which has been reported by several workers (Sivan and Chet, 1989; Upadhyay and Mukhopadhyay, 1986; Sharma and Doharoo, 1991; Bisht *et al*., 2013; Purohit *et al*., 2013; Singh and Singh, 2014) [40, 44, 34, 4, 31, 39]. The results thus obtained indicate a need of *in vitro* testing of more and more isolates of *Trichoderma* sp. against the pathogen, which would lead to better eco-friendly management of diseases in future.

5. References

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