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Integration of fungicides, bioagents and plant extracts for the management of Turcicum leaf blight of maize caused by *Exserohilum turcicum*

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Abstract
To manage turcicum leaf blight of maize, six systemic, three non-systemic, two combination fungicides and leaf extract of different plant species were evaluated against *Exserohilum turcicum* under *in vitro* conditions. Isolates of bioagents were also collected from different maize growing fields of Jammu division and tested under *in vitro* studies revealed that *Trichoderma harzianum* (bio agent), *Datura stramonium* (leaf extract), Propiconazole (systemic) and mancozeb (non-systemic) were most effective in inhibiting mycelial growth of *E. turcicum*. Under field studies, Propiconazole showed maximum disease inhibition and this treatment was at par with integrated treatment comprising of mancozeb (seed treatment) @ 0.25%, *Trichoderma harzianum* (s.t) followed by foliar sprays of *Datura stramonium* extract (50%) and mancozeb (f.s) which showed minimum disease incidence intensity (16.16%) coupled with maximum yield of 39.52 q/ha.

Keywords: *Exserohilum turcicum*, integrated disease management, fungicides, bioagents, plant extracts

Introduction
Maize (*Zea mays* L.) is a widely distributed crop grown throughout the world in tropical, sub-tropical and temperate regions under irrigated to semi-arid conditions. India is the 7th largest producer of maize. But as many as 18 foliar diseases are reported to occur on maize out of which Turcicum leaf blight caused by *Exserohilum turcicum* is the most serious disease. This disease is prevalent in almost all the maize growing areas. Severe losses in grain yield due to epiphytotics have been reported in several parts of India and these losses vary from 25 to 90 per cent depending upon the severity of the disease (Jha, 1993) [8]. Integrated approach is the novel idea to manage crop diseases as it involves minimum fungicidal load in nature (Khedekar *et al*., 2010) [9]. Attempts were therefore made to evaluate plant extracts, bio control agents and fungicides (showing promising results under *in vitro* conditions) both alone as well as in combination with each other under field conditions to develop a module for integrated disease management of Turcicum blight of maize.

Materials and methods
In vitro evaluation of fungicides against *E. turcicum*
Six systemic fungicides (azoxystrobin, carbendazim, propiconazole, hexaconazole, triadimefon and difenoconazole), three non-systemic fungicides (mancozeb, copper oxychloride, chlorothalonil and two combination fungicides (Mancozeb 64% + Metalaxyl 8% and carbendazim (12%) + mancozeb (63%) were assayed for their efficacy against *E. turcicum* under *in vitro* condition. The systemic fungicides were tested at 10, 25, 50 and 100 ppm concentration, whereas rest of the fungicides were tested at 50, 100, 250 and 500 ppm concentration using poisoned food technique (Nene and Thapliyal, 1993) [13].

In vitro evaluation of bio control agents against *E. turcicum*
Isolation of bio control agents
Bio control agents were isolated from the maize rhizosphere. Identity of the isolates of *Trichoderma* was established by taking standard references of Rifai (1969) [16]. *Pseudomonas fluorescence* and *Bacillus subtilis* were identified as per Migula (1895) [12] and Cohn (1872) [5]. Isolates of *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* were collected from different maize growing fields of Jammu division (Jammu, Kathua, Samba, Akhnoor and Udhampur).

King's B medium (KB) was used for isolation of *Pseudomonas fluorescens* and *Bacillus subtilis* whereas, Trichoderma selective medium (TSM) was used for isolation of *Trichoderma* spp. (Askew and Laing, 1993) [3]. The isolates showing fastest and vigorous growth were selected for evaluating their antagonistic activity against the test pathogen using dual culture technique (Morton and Stroube, 1955) [11]. Per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927) [19].

In vitro* evaluation of leaf extracts against *E. turcicum

Leaf extract of different plant species *viz*. *Datura stramonium* (datura), *Calotropis gigantea* (aak), *Lantana camara* (paanch phooli), *Phyllanthus emblica* (amla), *Cannabis sativa* (bhang), *Aleo vera* (gwar patha), *Azadirachta indica* (neem) was used for evaluating their efficacy against *E. turcicum.* Leaf extract was prepared by macerating leaf tissues in distilled water on weight/volume ratio (1:1 w/v) in mortar and pestle for 5 minutes and homogenized in an electric blender. The homogenate was filtered out through double layer of muslin cloth and then passed through Whatman filter paper No. 1. And further through sintered glass filter. The resultant extracts were considered as 100 per cent concentration. These leaf extracts were evaluated at three concentrations (10, 20 and 30%) against *E. turcicum* using poisoned food technique (Nene and Thapliyal, 1993) [13].

Mass multiplication of bio agent *Trichoderma harzianum*

For mass multiplication of *T. harzianum*, sorghum grains were soaked in water (1:5 W/V) for two hours in a container. Extra water was decanted and grains were shade dried so as to maintain 65-70% moisture. Autoclavable grain filled bags were sterilized in an autoclave at 15 psi for 20 minutes. After cooling of bags spore suspension (2ml per bag) of *T. harzianum* prepared by adding 20ml sterile water to one week old culture of fungus was added under aseptic condition. The inoculated bags were incubated at 28±2^{0}C for 15 days. The colonized sorghum gains were then shade dried at 30 °C and ground to powder using a laboratory blender (Prakash *et al*., 1999) [15]. The powder was used as inoculum for maize seed treatment.

Seed treatment with *Pseudomonas fluorescens*

The *Pseudomonas fluorescens* was isolated from soil on Kings B Medium and mixed with talc powder in the ratio of 1:2. The powder was used as inoculum for maize seed treatment.

Integrated disease management of Turcicum blight of maize

Bio control agent (*Trichoderma harzianum* and *Pseudomonas fluorescens*), leaf extract (*Datura stramonium*) and fungicides (Propiconazole, Hexaconazole, Triadimefon, Difenconazole, Mancozeb, Mancozeb + Carbendazim, Mancozeb + Metalyxl) found most effective under *in vitro* conditions were tested individually as well as in integration for their efficacy in management of Turcicum blight in the field. Compatibility of bio control agent, plant extract and fungicide were taken into consideration before formulating the treatments. For evaluation of different treatments experiments were conducted at Research Farm, of SKUAST-J, Chatha. Variety C-8 was laid out in plots with three rows of 4m length each with row-to-row distance of 75cm and plant to plant distance of 20 cm. The crop was raised as per recommended package of practices (Anonymous, 2012) [2]. *Trichoderma harzianum* and *Pseudomonas fluorescens* was applied as seed treatment while all the other treatments were given as sprays and applied at 65, 75 and 85 days after sowing. First spray was scheduled after the appearance of disease and subsequent two sprays were given at 10 days interval.

Results and Discussion

Plant diseases can be kept under check with repeated chemical sprays Khedekar *et al*., 2010, [9] but cost involved is high and moreover the excessive use of fungicides pose a threat due to pollution and health hazards which thereby demand for a safer and eco-friendly approach to manage this disease. Results presented in Table 1. Revealed that all the fungicides significantly inhibited the mycelial growth of *E. turcicum*. With the increase in concentration of fungicides, mycelial inhibition of the target fungus also increased and maximum inhibition was obtained at highest concentration (100 ppm) and Propiconazole and Hexaconazole were the most effective fungicide.

Efficacy (*in vitro*) of different contact and combination fungicides at concentration of 50, 100, 250 and 500 ppm assessed using poisoned food is presented in Table 2. The data revealed that all the test fungicides at various concentrations significantly inhibited the mycelial growth of *E. turcicum*. Mancozeb proved to be the most effective fungicide exhibiting maximum mean mycelial growth inhibition 71.67, 83.89, 94.44 and 94.44 per cent at 50, 100, 250 and 500 ppm respectively.

Evaluation of fungicides *in vitro* is a handy tool to screen a large number of fungicides. In the present study, the laboratory evaluation of fungicides by poison food technique revealed that all the evaluated fungicides inhibited the mycelial growth of *E. turcicum* even at their lowest dose. It was observed that with the increase in the concentration of fungicide, there was a significant decrease in the respective mycelial growth and accordingly more inhibition was observed at high concentratins than at lower concentrations. Kumar *et al*. (2021) [10] reported that amongst the systemic fungicides, Propiconazole was found highly effective and inhibited 100 per cent of mycelial growth of *H. maydis* at all the concentrations and amongst all the nonsystemic fungicides evaluated Mancozeb was found to be most effective and significantly superior over all other treatments followed by Thiram and Chlorothalonil. The effectiveness of fungicides mancozeb, carboxin and propiconazole against *E. turcicum* has also been observed by other authors (Bowen and Pederson, 1988 [4]; Singh and Gupta, 2000 [17] and Patil, 2000 [14]).

The results presented in Table 3. showed that the *Trichoderma harzianum* (Th_2) was the most effective antagonist exhibiting 69.44 percent mycelial inhibition of the pathogen followed by *Pseudomonas fluorescens* (Pf_3) (52.59%). Our results are in agreement with (Harlapur *et al*., 2007) [7] who reported the effectivity *T. harzianum* against *E. turcicum* probably due to competition and / or antibiosis. Plant metabolites and plant-based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Verma and Dubey, 1990) [18]. Plants produce an enormous array of secondary metabolites, and it is

commonly accepted that a significant part of this chemical diversity serves to protect plants against microbial pathogens (Dixon, 2001) [6]. Therefore, plant extracts have assumed tactical significance in the present-day strategy of developing ecologically safe method of plant disease management Leaf extracts of plants at different concentrations *viz.* 10, 20 and 30 per cent were evaluated for their efficacy in mycelial inhibition of *E. turcicum*. The results presented in Table 4 revealed that all leaf extracts inhibited the mycelial growth of *E. turcicum*. *Datura stramonium* extract at 10, 20 and 30 per cent was most effective inhibiting 57.41, 73.17 and 76.57 per cent of the mycelial growth of *E. turcicum* respectively. Harlapur *et al*. (2007) [7] evaluated under *in vitro* conditions, thirteen botanicals against Turcicum leaf blight of maize and observed significant reduction in growth of *E. turcicum*.

The treatments found best under *in vitro* conditions *viz. Trichoderma harzianum* (Th_2) (bio control agent), *Datura stramonium* (plant extract) and fungicides were evaluated singly and in combination were tested under field conditions for management of turcicum blight of maize. The results of integrated disease management trials depicted in Table 5 revealed that all the treatments significantly reduced the disease as compared to control. However, the magnitude of reduction varied among treatments.

A perusal of the pooled data indicated that treatment comprising of seed treatment with *Trichoderma harzianum* (Th_2) and Mancozeb followed by 1 foliar spray of *Datura stramonium* and 2 foliar sprays of Mancozeb exhibiting disease intensity (16.16%) which was significantly at par with three foliar sprays of Propiconazole (15.25%). In present study, all the treatments significantly reduced the disease as compared to control. However, the magnitude of reduction varied among treatments. This suggested that integration of plant extract and bio agents with fungicides give better efficacy than fungicide alone.

Table 1: *In vitro* evaluation of systemic fungicides against *Exserohilum turcicum* causing Turcicum leaf blight of maize

Fungicide	Conc. (ppm)	Radial Growth (cm)	Inhibition (%)
Azoxystrobin	10	5.17	42.59 (40.72) *
	25	4.97	44.81 (42.00)
	50	3.17	64.81 (53.60)
	100	2.42	73.15 (58.77)
Hexaconazole	10	2.30	74.44 (59.62)
	25	1.08	87.96 (69.70)
	50	0.50	94.44 (76.33)
	100	0.50	94.44 (76.33)
Propiconazole	10	1.67	81.48 (64.50)
	25	1.05	88.33 (70.00)
	50	0.50	94.44 (76.33)
	100	0.50	94.44 (76.33)
Difenoconazole	10	2.17	75.93 (60.60)
	25	1.30	85.56 (67.64)
	50	1.09	87.89 (69.61)
	100	0.86	90.48 (72.03)
Carbendazim	10	5.60	37.81 (37.93)
	25	4.75	47.22 (43.39)
	50	3.85	57.22 (49.13)
	100	2.85	68.33 (55.73)
Triadimefon	10	3.88	56.89 (48.94)
	25	2.80	68.89 (56.09)
	50	1.92	78.70 (62.49)
	100	1.47	83.70 (66.23)
Control	-	9.00	-
Factor	SEm±	C.D at 1%	
Fungicide	0.04	0.10	
Concentration	0.23	0.08	
Fungicide×Conc.	0.07	0.20	

* Figures in the parenthesis indicate arc sine values

Table 2: *In vitro* evaluation of non-systemic and combination fungicides against *Exserohilum turcicum* causing Turcicum leaf blight of maize

Fungicide	Conc. (ppm)	Radial Growth (cm)	Inhibition (%)
Mancozeb	50	2.55	71.67 (57.84) *
	100	1.45	83.89 (66.32)
	250	0.50	94.44 (76.33)
	500	0.50	94.44 (76.33)
Copper oxychloride	50	5.08	43.52 (41.24)
	100	4.75	47.22 (43.39)
	250	2.48	72.41 (58.29)
	500	2.13	76.30 (60.84)
Chlorothalonil	50	4.00	55.56 (48.20)
	100	2.92	67.59 (55.28)
	250	2.30	74.44 (59.64)

	500	1.60	82.22 (65.04)
Metalyxl+Mancozeb	50	3.15	65.00 (53.71)
	100	1.97	78.11 (62.09)
	250	0.50	94.44 (76.33)
	500	0.50	94.44 (76.33)
Mancozeb+Carbendazim	50	2.72	69.78 (56.64)
	100	1.63	81.93 (64.82)
	250	0.50	94.44 (76.33)
	500	0.50	94.44 (76.33)
Control	-	9.00	-
Factor	SEm±	C.D at 1%	
Fungicide	0.07	0.21	
Concentration	0.06	0.17	
Fungicide×Concentration Concentration	0.15	0.42	

*Figures in the parenthesis indicate arc sine values

Table 3: Effect of bio control agents on the growth of *Exserohilum turcicum* using dual culture method

Sl. No.	Bio control agent	Mycelial growth(cm)	Per cent inhibition in mycelial growth
1	*Trichoderma harzianum* (*Th*$_2$)	2.75	69.44 (56.46)
2	*Trichoderma viride* (*Tv*$_5$)	4.42	50.93 (45.53)
3	*Pseudomonas fluorescens* (*Ps*$_3$)	4.45	50.56 (44.94)
4	*Pseudomonas fluorescens* (*Ps*$_4$)	4.27	52.59 (46.47)
5	*Bacillus subtilis* (*Bs*$_4$)	5.62	37.59 (37.80)
	Control	9.00	-
	S.Em±	0.31	
	C.D. at 1%	0.96	

Figures in the parenthesis indicate arc sine values

Table 4: *In vitro* evaluation of leaf extracts against *Exserohilum turcicum*.

Leaf Extract	Conc. (%)	Radial growth(cm)	% Inhibition over control
Datura stramonium	10	3.83	57.41 (11.28)
(Datura)	20	2.42	73.17 (9.69)
	30	2.11	76.57 (8.35)
Lantana camara	10	7.63	15.24 (16.03)
(Panch Phooli)	20	5.52	38.67 (13.58)
	30	2.99	66.78 (9.95)
Phyllanthus emblica	10	6.63	26.31 (14.92)
(Amla)	20	5.94	33.98 (14.10)
	30	4.98	44.63 (12.89)
Cannabis sativa	10	6.86	23.76 (15.18)
(Bangh)	20	5.75	36.11 (13.85)
	30	4.49	50.17 (12.22)
Calotropis gigantea	10	7.51	16.52 (15.90)
(Aak)	20	5.14	42.93 (13.09)
	30	3.22	64.22 (10.33)
Aleo barbadensis	10	6.67	25.89 (14.96)
(Aleo vera)	20	5.75	36.11 (13.87)
	30	5.06	43.78 (12.99)
Azadirachta indica	10	6.21	31.00 (14.42)
(Neem)	20	3.41	62.09(10.64)
	30	2.65	70.61(9.36)
Control		9.00	
Factors	SE(m)±	C.D at 1%	
Leaf extract (A)	0.077	0.218	
Conc. (B)	0.047	0.133	
Leaf Extract×Conc. (A×B)	0.133	0.377	

Table 5: Integrated Disease management of Turcicum leaf blight of maize under field condition

Treatment		PDI 95DAS 1st year	% disease inhibition over control	PDI 95DAS 2nd year	% disease inhibition over control	Pooled	% disease inhibition over control	Grain yield (q/ha) 1st year	2nd year	Pooled
T1	Propiconazole (f. s)[3*] (0.1%)	14.25 (22.17)*	68.43	16.26 (23.77)	66.08	15.25 (22.98)	66.81	39.86	38.83	39.34
T2	Hexaconazole (f. s)[3] (0.1%)	17.68 (24.85)	60.69	20.17 (26.67)	57.77	18.92 (25.77)	58.82	37.79	36.81	37.30
T3	Triadimefon (f. s)[3] (0.1%)	23.14 (28.74)	48.34	26.40 (30.90)	44.54	24.77 (29.83)	46.09	36.92	35.96	36.44
T4	Difenoconazole (f. s)[3] (0.1%)	21.16 (27.37)	52.82	24.13 (29.41)	49.36	22.64 (28.40)	50.72	36.31	35.36	35.83
T5	Mancozeb (f. s)[3] (0.25%)	18.23 (25.26)	59.45	20.79 (27.12)	56.45	19.51 (26.20)	57.55	38.35	37.35	37.85
T6	Mancozeb+Carbendazim (f.s)[3] (0.25%)	18.61 (25.55)	58.57	21.24 (27.43)	55.50	19.93 (26.50)	56.64	37.89	36.91	37.40
T7	Mancozeb+metalyxyl (f. s)[3] (0.25%)	22.93 (28.59)	48.83	26.16 (30.74)	45.06	24.54 (29.68)	46.59	36.94	35.98	36.46
T8	*Trichoderma harzianum* (Th_2) (s.t) $1x10^7$	35.56 (36.59)	20.27	40.57 (39.55)	14.44	38.07 (38.08)	17.16	31.09	30.28	30.69
T9	Datura (f. s)[1] (50%)	36.33 (37.05)	18.54	41.44 (40.06)	12.59	38.89 (38.56)	15.38	31.00	30.19	30.59
T10	Mancozeb (S.t) + THR (Th_2) (s.t)	25.09 (30.05)	43.94	28.62 (32.33)	39.83	26.85 (31.20)	41.56	35.41	34.49	34.95
T11	Mancozeb+Carbendazim (f.s)[1] +THR(Th_2) (s.t)	22.01 (27.97)	50.90	25.11 (30.06)	47.27	23.56 (29.03)	48.73	36.88	35.92	36.40
T12	*Trichoderma harzianum* (Th_2) (s.t)+Datura (F.s)[1]	34.29 (35.83)	23.14	39.12 (38.70)	17.53	36.70 (37.27)	20.12	32.39	31.54	31.96
T13	Mancozeb (S.t)+*Trichoderma harzianum* (Th_2) (s.t)+Datura (f.s)[1]	26.65 (31.07)	40.42	30.40 (33.45)	36.04	28.52 (32.27)	37.93	34.70	33.80	34.25
T14	Mancozeb (S.t)+*Trichoderma harzianum* (Th_2) (s.t) + Datura (f.s)[1] + mancozeb(f.s)[2]	15.09 (22.85)	66.54	17.22 (24.50)	64.03	16.16 (23.69)	64.84	39.52	38.49	39.01
T15	Mancozeb (S.t)+*Pseudomonas fluorescens* (Ps_3) (s.t) + Datura (f.s)[1] + mancozeb(f.s)[2]	16.67 (24.08)	62.56	18.16 (25.19)	61.66	17.42 (24.66)	62.08	38.31	37.76	38.04
T16	Control	44.53 (41.84)	-	47.37 (43.47)	-	45.95 (42.66)	-	30.17	29.38	29.77
	SE(m)±	0.33		0.31		0.31		0.03	0.02	0.022
	CD at 0.05%	0.96		0.90		0.91		0.08	0.05	0.065

*Figures in the parenthesis indicate arc sine value

*Figure in superscripts revealed no. of spray seed treatment (s.t), foliar spray (f.s)

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