International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(4): 682-686

Received: 01-05-2019
Accepted: 03-06-2019

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In vitro evaluation of different fungicides and bioagents against *E. turcicum* causing turcicum leaf blight disease of maize

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Abstract
Maize is *kharif* season crop and member of grassy family Poaceae. Northern corn leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs having worldwide importance devastating disease causes severe losses in yield. *In-vitro*, the effectively colony growth of fungus *E. turcicum* was inhibited through different fungicide at different concentration and different bioagent. Among the fungicides tested were capable of inhibiting the growth of *E. turcicum* at various concentrations; the hexaconazole 5 SC was the most effective fungicide with 96.17 per cent mean mycelial growth inhibition and significantly superior over rest of the treatments, while fenamidon 10% + mancozeb 50% WG found second best fungicide. Azoxystrobin 23 SC was found least effective fungicide with mean mycelial growth inhibition of 18.15, 32.22 and 58.89% at all the tested concentrations. Among different *Trichoderma* spp. tested against *E. turcicum*, maximum inhibition was recorded in *T. viride* (NAU isolate) (70.74%) which was statistically at par with *T. asperellum* (AAU isolate) (70.63%) with fungal colony diameter of 26.33 mm and 26.43 mm of *E. turcicum.* While, least inhibition was recorded in *T. harzianum* (JAU isolate) (54.37%) with fungal colony diameter of 41.07 mm of *E. turcicum* as compared to the control.

Keywords: Corn, northern corn leaf blight, fungicides, concentration, *in vitro*, mycelial growth inhibition and colony diameter

Introduction
Maize is *kharif* season crop, short-day and C_4 plant is member of grassy family Poaceae. Maize is suffer from different disease, among them northern leaf blight (Turcicum leaf blight) caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs having worldwide importance (Carlos, 1997) [1]. Now era fungicide is effective control and final remedy for management of any disease. Fungicide give immediate result for manage disease. Spraying of fungicide with recommended dose or optimum dose that give best result to manage the disease over other method. From 2008 to 2010 total near about 33 trials were published for the management corn diseases, which showed interest that the increased research data on corn fungicides (Wise and Mueller, 2011) [15]. Sakhi *et al.* (1991) [10] evaluated that fungicides under *in-vitro*, against *H. turcicum* showed that mycelial growth were completely inhibited by the propineb, chlorothalonil and pyrifenox at 10 and 40 ppm, while benomyl inhibited at 20 ppm. For Due to several side effect of chemicals used in plant disease management has diverted plant pathologists or mankind to find out the other alternative methods for plant disease control with antagonistic microorganism. *Trichoderma* is one of the best antagonist against several soil borne fungal disease management in different crop. An experiment was undertaken to determine the antagonistic effect of different isolates of *Trichoderma* spp. and evaluation of different fungicides against *E. turcicum* fungus causes of turcicum leaf blight disease of maize.

Materials and methods
In-vitro assessment of different nine fungicides (systemic, non-systemic and ready-mix) and seven bioagent were tested for relative efficacy was carried out at Department of Plant Pathology, Anand Agricultural University, Anand during *kharif* 2018 against *E. turcicum*. The pathogen was isolated from diseased leaves, collected from infected fields during *Kharif* 2018. Three to four surface-sterilized with sodium hypochlorite diseased leaves bits of 3 to 5 mm size were aseptically transferred to potato dextrose agar media and these bits are inoculated in petri plates and incubated at 25 ± 2 °C for eight to nine days. The cultures were purified by hyphal tip method (Rangaswami, 1972; Dasgupta, 1988) [9, 2].

***In-vitro* evaluation of fungicides by poisoned food technique**

The experiment was carried out in Completely Randomized Design (CRD) with 10 treatments and 3 replications including control as a treatment with three concentrations 250, 500 and 750 ppm of each treatment as described in Table 1. *In-vitro* tested for relative efficacy of fungicide against *E. turcicum* using poisoned food technique (Grove and Moore, 1962) [4].

Table 1: List of fungicides evaluated against *E. turcicum* by poisoned food technique

Tr. No.	Fungicides	Concentrations (ppm)		
T_1	Tebuconazole 25.9 EC	250	500	750
T_2	Hexaconazole 5 SC	250	500	750
T_3	Pyraclostrobin 20 WG	250	500	750
T_4	Azoxystrobin 23 SC	250	500	750
T_5	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	250	500	750
T_6	Tebuconazole 10% + Sulphur 65% WG	250	500	750
T_7	Tebuconazole 50% + Trifloxystrobin 25% WG	250	500	750
T_8	Fenamidon 10% + Mancozeb 50 % WG	250	500	750
T_9	Carbendazim 12% + Mancozeb 63% WP	250	500	750
T_{10}	Untreated (Control)	-	-	-

The required quantity of each fungicide was incorporated aseptically in 100 ml of PDA media containing in 250 ml flasks to make various concentrations of fungicides. The medium was shaken well to give uniform dispersal of the fungicide and then 20 ml of PDA was poured aseptically in each of the Petri plates were allowed to solidify. After solidification, the plates were inoculated with mycelial discs of the tested fungus of 4 mm diameter of seven to eight days old pure culture of test fungus. The mycelium disc, which was placed in an inverted position to make a direct contact with the poisoned medium placed in the centre of the plates after this plates were incubated at 25 ± 2 ºC for seven days to eight days until the control plates is fully covered.

***In-vitro* evaluation of *Trichoderma* spp. by dual culture technique**

Evaluation accomplished with different isolates of *Trichoderma* spp. against the *E. turcicum* fungus with three replication by the Dual culture technique given by Dennis and Webster (1971) [3]. For this study, all isolates of *Trichoderma* spp. and *E. turcicum* fungus were cultured on potato dextrose agar media. Twenty milliliter of PDA was poured aseptically in each of the Petri plates were allowed to solidify. Mycelial disc of 4 mm in diameter of both *i.e.,* each antagonist and test fungus were placed on media in the same Petri plates approximately 7 cm away from each other for the antagonism study by Dual culture method. Treatment detail are as given in Table 2.

Table 2: Microbial antagonist tested against *E. turcicum* by dual culture technique

Tr. No.	Antagonists
T_1	*Trichoderma viride* Pers. Ex. Fr. (AAU isolate)
T_2	*Trichoderma viride* Pers. Ex. Fr. (JAU isolate)
T_3	*Trichoderma viride* Pers. Ex. Fr. (NAU isolate)
T_4	*Trichoderma harzianum* Rifai (AAU isolate)
T_5	*Trichoderma harzianum* Rifai (JAU isolate)
T_6	*Trichoderma harzianum* Rifai (NAU isolate)
T_7	*Trichoderma asperellum* Samuels, lieckfeldt & Nirenberg (AAU isolate)
T_8	Control (Test Pathogen only)

Observation on the radial growth (mm) was recorded from 24 hrs of incubation at 25±2 °C until the complete growth of test pathogen in control plates. The linear growths of the fungal colonies were measured from two different angles in millimeter (mm) and the mean values were calculated. Per cent growth inhibition (PGI) over control for both fungicide and bioagent evaluation was calculated by using formula given by Vincent (1947) [13].

Per cent growth inhibition (PGI) = $\frac{C-T}{C} \times 100$

Where,

- C = Average diameter of mycelial colony in control treatment (mm)
- T = Average diameter of mycelial colony in treated plate (mm)

Result and Discussion

***In-vitro* evaluation of fungicides by poisoned food technique**

Different nine fungicides (systemic, non-systemic and ready-mix) were tested for relative efficacy at three concentrations 250, 500 and 750 ppm using poisoned food technique. The data on per cent mycelial growth inhibition are presented in Table 3 and Plate 1.

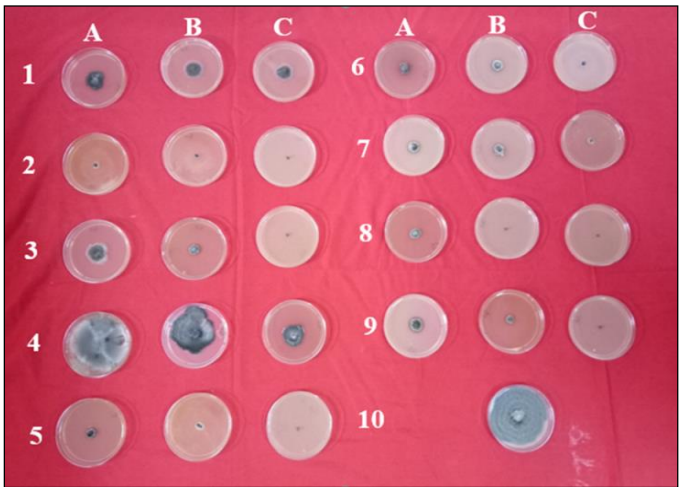
Data presented in Table 3 revealed that hexaconazole 5 SC significantly minimum mycelium growth was recorded at all three concentration 250, 500 and 750 ppm concentration were 2.33, 3.67 and 4.67 mm, respectively followed by fenamidon 10% + mancozeb 50% WG @ 750 and 500 ppm with 3.00 and 3.67 mm mycelium growth recorded. Next better in order was azoxystrobin 18.2% + difenoconazole 11.4% SC and pyraclostrobin 20 WG with 4.33 mm mycelium growth followed by carbendazim 12% + mancozeb 63% WP with 5.33 mm mycelium growth @ 750 ppm concentration of

fungicides. In treatment of azoxystrobin 23 SC maximum mycelium growth 73.67, 61.00 and 37.00 mm observed in all concentration @ 250, 500 and 750 ppm of fungicides, respectively. The untreated control was recorded the highest mycelial growth of 90.00 mm.

Data presented in Table 3 revealed that hexaconazole 5 SC was the most effective fungicide with 96.17 per cent mean mycelial growth inhibition and significantly superior over rest of the treatments, while fenamidon 10% + mancozeb 50% WG found second best fungicide with 92.66 per cent mean mycelial growth inhibition. This was followed by azoxystrobin 18.2% + difenoconazole 11.4% SC with 89.99 per cent mean mycelial growth inhibition, which is also statistically at par with carbendazim 12% + mancozeb 63% WP recorded 88.4 per cent mean mycelial growth inhibition. Among remaining fungicides tebuconazole 50% + trifloxystrobin 25% WG, tebuconazole 10% + sulphur 65% WG, pyraclostrobin 20 WG and tebuconazole 25.9 EC was moderately effective fungicide with mean mycelial growth inhibition 85.49, 85.45, 80.58 and 73.56 per cent, respectively. Azoxystrobin 23 SC was the least effective fungicide with 35.63 per cent mean mycelial growth inhibition. The effect of different concentration of fungicides indicated that the increase in fungicidal concentration result was increased in mycelial growth inhibition of test pathogen *E. turcicum*.

Among different fungicides at different concentration, hexaconazole 5 SC recorded the maximum mycelial growth inhibition 97.41 per cent and found significantly superior over rest of the treatments while it was found equally effective with fenamidon 10% + mancozeb 50% WG with 96.67 per cent mean mycelial inhibition at 750 ppm concentration. Hexaconazole 5 SC and fenamidon 10% + mancozeb 50% WG found next best effective fungicide with mean mycelial growth inhibition of 95.93 per cent at 500 ppm concentration and found at par with azoxystrobin 18.2% + difenoconazole 11.4% SC of 95.19 per cent and hexaconazole 5 SC with 94.81 per cent at 750 and 250 ppm concentration, respectively.

Azoxystrobin 23 SC found least effective fungicide with mean mycelial growth inhibition 18.15, 32.22 and 58.89 per cent at 250, 500 and 750 ppm concentrations, respectively. In case of concentration, mean maximum growth inhibition 90.05 per cent was recorded at 750 ppm concentration and lowest was 74.59 per cent at 250 ppm.



A = 250 ppm B = 500 ppm C = 750 ppm

Plate 1: *In-vitro* efficacy of various fungicides against *E. turcicum* under poisoned food technique

Table 3: *In vitro* evaluation of different fungicides against *E. turcicum* by poisoned food technique

Tr. No.	Fungicides	Colony diameter of pathogen (mm)*			Growth inhibition (%)			Mean growth inhibition (%)
		250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	
T_1	Tebuconazole 25.9 EC	27.67	23.00	21.00	56.37 (69.27)	59.67 (74.44)	61.15 (76.67)	59.06 (73.56)
T_2	Hexaconazole 5 SC	4.67	3.67	2.33	76.90 (94.81)	78.43 (95.93)	80.82 (97.41)	78.71 (96.17)
T_3	Pyraclostrobin 20 WG	29.33	24.00	4.33	55.22 (67.41)	58.95 (73.33)	77.38 (95.19)	63.85 (80.58)
T_4	Azoxystrobin 23 SC	73.67	61.00	37.00	25.22 (18.15)	34.60 (32.22)	50.15 (58.89)	36.65 (35.63)
T_5	Azoxystrobin 18.2% + difenoconazole 11.4% SC	14.67	9.67	4.33	66.25 (83.70)	70.95 (89.26)	77.49 (95.19)	71.56 (89.99)
T_6	Tebuconazole 10% + sulphur 65% WG	17.00	14.67	9.33	64.28 (81.11)	66.23 (83.70)	71.28 (89.63)	67.26 (85.05)
T_7	Tebuconazole 50% + trifloxystrobin 25% WG	16.67	13.00	10.00	64.56 (81.48)	67.71 (85.56)	70.58 (88.89)	67.62 (85.49)
T_8	Fenamidon 10% + mancozeb 50% WG	16.33	3.67	3.00	64.84 (81.85)	78.43 (95.93)	79.62 (96.67)	74.29 (92.66)
T_9	Carbendazim 12% + mancozeb 63% WP	17.33	11.33	5.33	64.02 (80.07)	69.28 (87.41)	76.04 (94.07)	69.78 (88.05)
T_{10}	Untreated (Control)	90.00	90.00	90.00				
	Concentration mean				59.73 (74.59)	64.91 (82.02)	71.61n (90.05)	
					Fungicide (F)	Conc. (C)	F x C	
	S.Em. ±	0.77	0.65	0.67	0.41	0.24	0.72	
	CD at 5%	2.26	1.92	1.99	1.17	0.68	2.03	
	CV %	4.32	4.43	6.26	1.89			
	*Mean of three repetition Figures in parentheses are re-transformed value of arc sine							

***In-vitro* evaluation of *Trichoderma* spp.**

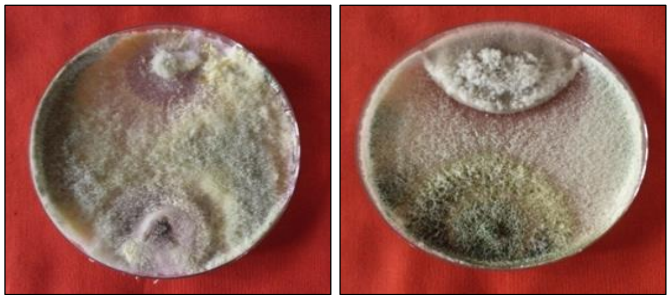
Percent growth inhibition (PGI) of tested fungus by different *Trichoderma* spp. over control were calculated and statistically analysed results presented in Table 4 and Plate 2 revealed that, all the antagonists tested against *E.* turcicum were effective in inhibiting the growth of the pathogen. Out of seven antagonists tested *Trichoderma* spp, maximum inhibition was recorded in *T. viride* (NAU isolate) (70.74%) which was statistically at par with *T. asperellum* (AAU isolate) (70.63%) with fungal colony diameter of 26.33 and 26.43 mm of *E. turcicum* whereas, *T. harzianum* (NAU isolate) (66.30%) which was statistically at par with *T. viride* (AAU isolate) (64.07%) was next best antagonist and *T. harzianum* (AAU isolate) (61.67%) which was statistically at par with *T. viride* (JAU isolate) (61.11%) were moderately effective to inhibit fungal growth with fungal colony diameter of 30.33, 32.33, 34.50 and 35.00 mm of *E. turcicum*, respectively. Least inhibition was recorded in *T. harzianum* (JAU isolate) (54.37%) with fungal colony diameter of 41.07 mm of *E. turcicum*.

Table 4: *In-vitro* efficacy of different bioagents against *E. turcicum* by dual culture method

Sr. No	Antagonist	Colony diameter of pathogen (mm)*	Per cent inhibition over control*
T_1	*Trichoderma viride* Pers. Ex. Fr. (AAU isolate)	32.33	53.23 (64.07)
T_2	*Trichoderma viride* Pers. Ex. Fr. (JAU isolate)	35.00	51.45 (61.11)
T_3	*Trichoderma viride* Pers. Ex. Fr. (NAU isolate)	26.33	57.29 (70.74)
T_4	*Trichoderma harzianum* Rifai (AAU isolate)	34.50	51.78 (61.67)
T_5	*Trichoderma harzianum* Rifai (JAU isolate)	41.07	47.53 (54.37)
T_6	*Trichoderma harzianum* Rifai (NAU isolate)	30.33	54.55 (66.30)
T_7	*Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg (AAU isolate)	26.43	57.21 (70.63)
T_8	Control (Test pathogen only)	90.00	-
	S.Em. ±	0.84	0.59
	C.D. at 5%	2.51	1.78
	C.V. %	3.67	1.91

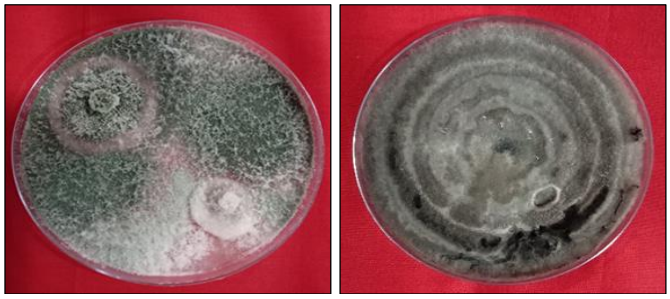
*Mean of three repetition
Figures in parentheses are re-transformed value of arc sine

T_1. *T. viride* (AAU isolate) T_2. *T. viride* (JAU isolate)



T_3. *T. viride* (NAU isolate) T_4. *T. harzianum* (AAU isolate)

T_5. *T. harzianum* (JAU isolate) T_6. *T. harzianum* (NAU isolate)



T_7. *T. asperellum* (AAU isolate) Control (Test Pathogen only)

Plate 2: *In-vitro* antagonism study of different *Trichoderma* spp. against *E. turcicum* under dual culture technique

It is evident from these studies that among all the antagonists evaluated by dual culture method *T. viride* (NAU isolate) and *T. asperellum* (AAU isolate) consistently showed strong antagonistic property against *E. turcicum* as compared to the other antagonists tested hence considered as potential antagonists.

Consonant denouement with Manu *et al.* (2017) [7] was observed that among the systemic fungicides, tebuconazole completely inhibit the pathogen growth at all the concentrations tested. In contact fungicides, propineb was highly effective with 83.89 per cent inhibition of *E. turcicum* at 500 ppm and among combi products, only carbendazim 12% + mancozeb 63% at 500 ppm exhibited complete inhibition of the mycelial growth of *E. turcicum*. Maximum inhibition of mycelial growth (98.65%) was noticed in *T. harzianum* statistically at par with (98.34%) in *T. viride*. The consummation recorded are in close agreement with those obtained by Wani *et al.* (2017) [14] studied on twelve fungicides including systemic and non-systemic and bioagent *in-vitro*. Among systemic fungicides, propiconazole was found best in inhibiting the mycelial growth of *E. turcicum* (96.51% inhibition) and among non-systemic fungicides, mancozeb was found best (95.23% inhibition). Among bioagents *T. harzianum* was found to be superior over all treatments with 76.38 per cent mycelial inhibition followed by *T. viride* was 62.61 per cent mycelial inhibition.

The present results congruent with those obtained by Patil (2000) [8] evaluated systemic and non-systemic fungicides propiconazole, difenoconazole, hexaconazole and tridemorph showed cent per cent inhibition followed by mancozeb.

These results were in consonant with Khedekar *et al.* (2012) [6] who reported that *Trichoderma harzianum* was effective in inhibiting the mycelial growth. These results are further supported by the denouement of Singh and Singh (2014) [12] and Harlapur (2005) [5] also revealed that *T. harzianum* caused significantly maximum inhibition (65.17%) followed by *T. viride* (56.95%) on mycelial growth of *E. turcicum* fungus by dual culture technique. Singh and Dutta (2017) [11] also exhibited that the *T. harzianum* showed (54.14%) mycelial inhibition followed by *T. viride* (53.88%).

Acknowledgment
The financial support from Main Maize Research station, Godhra, AAU, Anand and Directorate of Research and Dean PG, AAU, Anand is gratefully acknowledged.

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