Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 2754-2762

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 2754-2762**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.329

Evaluation of Fungicides, Bioagents and Plant Extracts against *Exserohilum turcicum* Causing Turcicum Leaf Blight of Maize

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Keywords

Bioagents, *Exserohilum turcicum*, Fungicides, Maize, Plant etracts.

Article Info

Accepted:
23 June 2017
Available Online:
10 August 2017

A B S T R A C T

Twelve fungicides including systemic and non-systemic, five bioagents and eight plant extracts were evaluated *in vitro* against *Exserohilum turcicum*, the causal agent of Turcicum leaf blight of maize. All the test fungicides, bioagents and plant extracts showed fungistatic action and significantly inhibited mycelial growth of the test pathogen over untreated control. Among systemic fungicides, propiconazole was found best in inhibiting the mycelial growth of *E. turcicum* (96.51% mean inhibition), mancozeb among non-systemic fungicides was found best (95.23% mean inhibition), *Trichoderma harzianum* among bioagents was found best (76.38% inhibition) and among plant extracts, neem oil inhibited maximum mycelial growth of *E. turcicum* (62.33%). The best performing *in vitro* fungicides, bioagents and botanicals when tested in field showed that two foliar sprays with non-systemic fungicide, mancozeb 75 WP @ 0.25 per cent reduced the diseased intensity from 20.45 per cent in control to 5.69 per cent and increased the grain yield from 45.20 q/ ha in control to 52.50 q/ha; two foliar sprays with systemic fungicide, propiconazole 25 EC @ 0.1 per cent reduced the diseased intensity to 6.11 per cent and increased the grain yield to 52.25 q/ha; two foliar sprays with plant extract, neem oil @ 5 per cent reduced the diseased intensity to 10.90 per cent and increased the grain yield to 49.90 q/ha while seed treatment with bioagent, *Trichoderma harzianum* $2x10^8$ cfu/g @ 0.4 per cent followed by two foliar sprays with mancozeb 75 WP @ 0.25 per cent could further reduce the disease intensity to 5.40 per cent and increase grain yield to 53.60 q/ha.

Introduction

Turcicum leaf blight of maize (*Zea mays* L.) caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs, was first reported by Passerini in 1876 from Perma, Italy.

This disease is popularly known as Northern Corn Leaf Bight (NCLB) in the United States of America. The disease is favoured by mild temperature and high humidity (Ullstrup, 1970). Heavy dews, cool temperature and frequent rains are environmental conditions conducive for disease development (Jordan *et al.*, 1983). In India the disease was first reported by Butler *et al.*, (1920). Later the disease was reported from Kashmir (Koul, 1957) and Himachal Pradesh (Chenula and Hora, 1962). The disease is considered to be one of the most devastating diseases as its occurrence and incidence assumes greater significance resulting in reduction of grain yield by 28 to 91 per cent (Kachapur, 1998 and Harlapur *et al.*, 2000). Hence an attempt

was made to evaluate various fungicides, bioagents and plant extracts *in vitro* and *in vivo* to manage the disease.

Materials and Methods

Isolation of the pathogen

The fungus was isolated by tissue isolation technique. The diseased tissues along with some healthy leaf portion (1-2 mm^2) were cut with a sterilized razor blade at the margins of the diseased spots and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds. The segments were then rinsed thrice in sterilized distilled water to remove the traces of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar (PDA) medium (pH adjusted to 6.5 with N/10 HCl) in sterilized petriplates under aseptic conditions. The inoculated PDA plates were incubated at 25±1^oC for 7 days and sub cultured onto fresh PDA medium at the same temperature for 15 days.

Purification by single spore technique

Exserohilum turcicum being a sporulating fungus was purified by single spore technique (Johnston and Booth, 1983). Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing 8 to 10 spores per microscopic field at low power from 15 days old culture.

One ml of such suspension was spread uniformly on 2 per cent solidified water agar plates and incubated at 25±1^oC for 12 hours. The plates were examined under stereoscopic microscope, single spores were marked with a marker, allowed to germinate and finally picked up using cork borer and transferred aseptically to potato dextrose agar medium in sterilized petriplates for further growth in the incubator at 25±1^oC. The pure culture thus obtained, was used for further studies.

In vitro evaluation of fungicides

Twelve fungicides consisting of six systemic viz., carbendazim 50 WP (Bavistin), propiconazole 25 EC (Tilt), difenconazole 25 EC (Score), tridemorph 75 EC (Calaxin), thiophanate methyl 70 WP (Topsin-M) and hexaconazole 5 EC (Anvil); four non-systemic viz., mancozeb 75 WP (Indofil M 45), copper oxychloride 50 WP (Connect), captan 50 WP (Merimain) and chlorothalonil 75 WP (Kavach) and two combi-products viz., captan 70 per cent + hexaconazole 5 per cent (Taqat 75 WP) and carbendazim 12 per cent + mancozeb 63 Per cent (Saaf 75 WP) were assayed for their efficacy against *E. turcicum* under *in vitro* condition. The systemic fungicides were tested at 100, 250 and 500 ppm concentrations, non-systemic fungicides were tested at 500, 1000 and 1500 ppm concentrations, whereas, one combi-product, carbendazim 12 per cent + mancozeb 63 per cent (Saaf 75 WP) was tested at same concentration as of non-systemic fungicides, i.e., 500, 1000 and 1500 ppm, while as other combi-product, captan 70% + hexaconazole 5% (Taqat 75 WP) was tested at same concentration as used for systemic fungicides, i.e., 100, 250 and 500 ppm. Poisoned food technique was adopted for *in vitro* testing of fungicides (Nene and Thapliyal, 1979). The calculated amount of fungicides were thoroughly mixed in the medium before pouring into petriplates so as to get desired concentration of active ingredient of each fungicide separately. Twenty ml of fungicide amended medium was poured in each of 90 mm sterilized petriplates and allowed to solidify. The plates were inoculated centrally with 5 mm disc of ten days old young sporulating culture of *Exserohilum turcicum*. Control plates without fungicides were also maintained. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The inoculated petriplates were incubated at

25±1°C in the laboratory. The colony diameters were measured when the fungus touched the periphery in control plates. Per cent inhibition of growth was calculated by using formula given by Vincent (1927) as:

$$I = \frac{C-T}{C} \times 100$$

Where,

I=Per cent Inhibition
C=Colony diameter in control;
T=Colony diameter in treatment

In vitro evaluation of bioagents

The fungal bio-control agents, viz., *Trichoderma viride* Pers., *Trichoderma harzianum* Rifai, *Aspergillus niger* Van Tiegh, *Pseudomonas fluorescens* (Trevisan) Migula and *Bacillus subtilis* (Ehrenberg) Cohn, were evaluated against *Exserohilum turcicum* for their antagonistic activity by using dual culture method as described below. Culture discs (5 mm) of each fungal antagonist and the pathogen were taken from the margin of the actively growing cultures and transferred to potato dextrose agar (PDA) medium contained in 90 mm petriplates on opposite side, approximately at 10 mm from the wall of the plate. Similarly, bacteria were streaked on the opposite side of the pathogen.

A check having the test pathogen only was kept for comparison. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The petriplates were subsequently incubated at 25±1°C till mycelial inhibition was observed. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded as the per cent growth inhibition of the test pathogen over control, calculated according to the formula given by Vincent (1927).

In vitro evaluation of plant extracts

Eight plant extracts, viz., onion clove, garlic bulb, mint leaf, datura leaf, datura seed, datura root, artimesia leaf and neem oil were tested against the growth of Turcicum leaf blight of maize pathogen, *E. turcicum* under *in vitro* conditions by poisoned food technique (Nene and Thapliyal, 1979). The fresh leaves and other parts of healthy plants were collected and washed with sterile distilled water and air dried. Ten grams of plant tissue was ground using pestle and mortar by adding equal amount (10 ml) of sterilized distilled water (1:1w/v). The extract was filtered through muslin cloth and centrifuged at 2000 rpm for 30 minutes at ambient temperature (26 ±2°C). This supernatant was used as standard plant extract solution (100%). The plant extracts were tested at 10 per cent concentration and besides plant extracts, commercial neem formulation, Nimbicidin (neem oil) was tested at 5 per cent concentration and this was achieved by incorporating 10 ml supernatant of each plant extract and 5 ml of Nimbicidin into 90 ml and 95 ml of PDA media in conical flasks, respectively, and such conical flasks [containing PDA (food) and plant extract (poison) known as poisoned food technique] were sterilized by autoclaving at 15 psi for 20 minutes (Sood and Dohroo, 2003; Verma and Dohroo, 2003; Dutta and Kahla, 2011).

Twenty ml of PDA containing plant extract was poured into sterilized petriplate under aseptic conditions (in laminar air flow) and allowed to solidify. PDA plates containing the plant extracts were inoculated aseptically in the center with 5 mm disc of ten days old young sporulating culture of *Exserohilum turcicum*. Control plates without any plant extracts were also maintained. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The inoculated

petriplates were incubated at 25±1°C in the laboratory. The colony diameters were measured when the fungus touched the periphery in control plates. Per cent inhibition of growth was calculated by using formula given by Vincent (1927).

In vivo evaluation of fungicides, bioagents and plant extracts

Field experiments were conducted during kharif 2012 and 2013 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar campus Srinagar to manage the disease using the best performing fungicides (mancozeb and propiconazole), bioagent (*Trichoderma harzianum*) and plant extract (neem oil) identified *in vitro*, which were integrated under field conditions as seed treatment and foliar sprays. The experiment was laid in randomized block design using Turcicum leaf blight susceptible variety of maize (C 15) and cultivated as per recommended package of practice. Artificial epiphytotic conditions were created twice at 25 and 30 DAS with two different methods of inoculation, first with spore suspension having spore load of 5 x 10^4 spores per ml sprayed with atomizer on foliage of maize and second with whorl drop method inoculation of plants done with *E.turcicum* multiplied on Sorghum grains. The inoculations were done in the evening time and light water was sprayed after both inoculations to create high relative humidity for infection. The disease intensity was recorded at silk drying stage using 1-5 disease rating scale (James, 1971). The data on yield was recorded and analyzed statistically.

Results and Discussion

Evaluation of fungicides

Among the systemic fungicides, under *in vitro* conditions, the maximum inhibition of mycelial growth (100%) was observed with propiconazole, followed by difenconazole (84.75%) and the least inhibition was noticed with thiophanate methyl (70.35%) at 500 ppm concentration (Table 1). At 250 and 100 ppm concentrations, similar trend was noticed. Further when the same fungicide, propiconazole 25 EC at 0.1% was assessed under field conditions against Turcicum leaf blight of maize as two foliar sprays at 40 and 50 DAS, the disease intensity was reduced from 20.45 per cent recorded in control to 6.11 per cent and grain yield was increased from 45.20 q/ha recorded in control to 52.25 q/ha. These results are in agreement with Bowen and Pederson (1988) who studied the effect of propiconazole on *E. turcicum* in laboratory and field and found that three sprays of propiconazole at weekly intervals was effective in reducing Turcicum leaf blight in maize.

Among non-systemic fungicides, under *in vitro* conditions, the maximum inhibition of mycelial growth (100%) was observed with mancozeb and least inhibition was recorded with captan (35.15%) at 1500 ppm concentration (Table 2). The combi-product, mancozeb + carbendazim also showed maximum inhibition (100%) at 1500 ppm while at lower concentrations, i.e., 1000 and 500 ppm, it was not as effective as mancozeb alone. Further mancozeb 75 WP at 0.25% was assessed under field conditions against Turcicum leaf blight of maize as two foliar sprays at 40 and 50 DAS, the disease intensity was reduced from 20.45 per cent recorded in control to 5.69 per cent and grain yield was increased from 45.20 q/ha recorded in control to 52.50 q/ha. These results are in agreement with Kumar *et al.*, (1977) who evaluated eight fungicides and found that mancozeb, Unizeb and Dithane Z-78 significantly reduced the maize leaf blight severity by 55, 47.4 and 44.33 per cent, respectively and increased the grain yield by 8.54, 10.12 and 9.9 per cent,

respectively. Other investigations also indicated that mancozeb was most effective in controlling Turcicum leaf blight of maize (Pandurangegowda, 1987 and Begum *et al.*, 1993). Reddy *et al.*, (2013) evaluated seven fungicides *in vitro* against *E. turcicum* causing leaf blight of maize and found treatment mancozeb @ 0.25 per cent and combination treatment of mancozeb and carbendazim, i.e., Saff @ 0.25 per cent recorded the lowest per cent disease index (PDI) reducing the disease by 73.0 and 72.1 per cent, respectively

Table.1 *In vitro* efficacy of various systemic fungicides in inhibiting the mycelial growth of *Exserohilum turcicum*

Fungicide	Per cent growth inhibition at different concentrations (ppm)			
	C_1	C_2	C_3	Mean
	100	250	500	
Carbendazim 50 WP	33.25 (35.21)	57.47 (49.29)	78.24 (62.16)	56.32 (48.63)
Propiconazole 25 EC	90.27 (71.82)	99.26 (85.06)	100.00 (90.00)	96.51 (79.23)
Difenconazole 25 EC	56.74 (48.87)	70.62 (57.17)	84.75 (67.01)	70.70 (57.22)
Tridemorph 75 EC	55.71 (48.27)	68.54 (55.88)	82.61 (65.35)	68.95 (56.13)
Thiophenate methyl 70 WP	43.30 (41.14)	56.27 (48.60)	70.35 (57.00)	56.64 (48.81)
Hexaconazole 5 EC	53.51 (47.01)	66.47 (54.61)	80.67 (63.91)	66.88 (54.86)
Captan (70%) + Hexaconazole (5%)	54.51 (47.58)	67.34 (55.14)	81.33 (54.41)	67.73 (55.37)
Mean	55.32 (48.04)	69.42 (56.42)	82.56 (65.31)	

Figures in the parenthesis are arc sine transformed values

$CD_{(p \leq 0.05)}$

Fungicide : 0.26

Concentration : 0.17

Fungicide x Concentration : 0.45

Table.2 *In vitro* efficacy of various non-systemic fungicides in inhibiting the mycelial growth *Exserohilum turcicum*

Fungicide	Per cent growth inhibition at different concentrations (ppm)			
	C_1	C_2	C_3	Mean
	500	1000	1500	
Mancozeb 75 WP	89.12 (70.74)	96.57 (79.32)	100.00 (90.00)	95.23 (77.38)
Copper oxychloride 50 WP	41.94 (40.36)	45.44 (42.38)	51.75 (46.00)	46.36 (42.91)
Captan 50 WP	29.61 (32.96)	33.67 (35.46)	35.15 (36.36)	32.81 (34.94)
Chlorothalonil 50 WP	61.80 (51.82)	66.22 (54.46)	71.74 (57.88)	66.58 (54.68)
Mancozeb (63%) + Carbendazim (12%)	88.16 (69.87)	95.53 (77.79)	100.00 (90.00)	94.56 (76.51)
Mean	62.12 (52.01)	67.48 (55.23)	71.72 (57.87)	

-Figures in the parenthesis are arc sine transformed values

$CD_{(p \leq 0.05)}$

Fungicide : 0.26

Concentration : 0.20

Fungicide x Concentration : 0.46

Table.3 *In vitro* efficacy of various bio-agents on mycelial growth inhibition of *Exserohilum turcicum*

S. No.	Bio-agent	Per cent inhibition in mycelial growth
1.	*Trichoderma viride* Pers (Fr.)	62.61 (52.31)
2.	*Trichoderma harzianum* Rifai	76.38 (60.92)
3.	*Aspergillus niger* Van Teigh	42.52 (40.70)
4.	*Pseudomonos fluorescens* Migula	37.95 (38.03)
5.	*Bacillus subtilis* (Ehrenberg) Cohn	46.41 (42.94)
	S.Em±	0.23
	CD (p≤0.05)	0.95

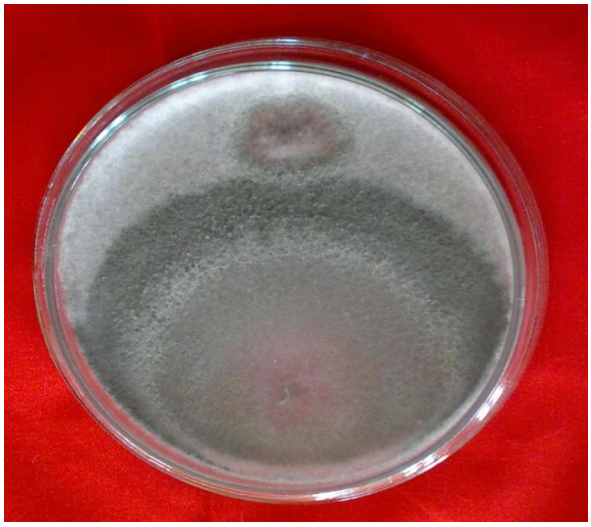
-Figures in parenthesis indicate arc sine values

Table.4 *In vitro* efficacy of various plant extracts on mycelial growth inhibition of *Exserohilum turcicum*

S. No.	Plant species	Part used	Conc. (%)	Per cent inhibition of mycelial growth
1.	*Allium cepa* L.	Bulb	10	25.33 (30.21)
2.	*Allium sativum* L.	Clove	10	36.67 (37.26)
3.	*Mentha spicata*	Leaf	10	20.00 (26.56)
4.	*Datura stramonium*	Leaf	10	41.33 (40.00)
5.	*Datura stramonium*	Seed	10	23.67 (29.11)
6.	*Datura stramonium*	Root	10	22.33 (28.19)
7.	*Artimesia annua*	Leaf	10	40.00 (39.23)
8.	*Azadirachta indica*	Oil	5	62.33 (52.13)
	S.E±			0.07
	CD ($p \leq 0.05$)			0.34

-Figures in parenthesis indicate arc sine values

Plate.1 *In vitro* evaluation of *T. harzianum* against *Exserohilum turcicum* by dual culture



Trichoderma harzianum* inhibiting *Exserohilum turcicum

Control (*Exserhilum turcicum*)

Evaluation of bioagents

It is apparent from the data presented in table 3 that all the antagonistic fungi and bacteria inhibited the growth of *E. turcicum* ranging from 37.95 to 76.38 per cent. *T. harzianum* was found to be superior over all treatments with 76.38 per cent mycelial inhibition (Plate-1) followed by *T. viride* (62.61%), *B. subtilis* (46.41%) and *A. niger* (42.52) while *P. fluorescens* was found to be least effective (37.95%). Further when most effective *in vitro* bioagent, *T. harzianum* was assessed and integrated with best *in vitro* fungicide under field conditions against Turcicum leaf blight of maize as seed treatment with *T. harzianum* $2x10^8$ cfu/g @ 0.4 per cent and two foliar sprays with mancozeb 75 WP @ 0.25 per cent the disease intensity was further reduced to 5.40 per cent and grain yield was further increased to 53.60 q/ha. The antagonism of *T. harzianum* and *T. viride* observed in the present studies are in tune with the findings of various workers (Biles and Hill, 1988; Mehmood *et al.*, 1995). Harlapur *et al.*, (2007) reported that maximum mean per cent inhibition of mycelial growth of *E. turcicum* was recorded in *T. harzianum* (65.17%) followed by *T. viride* (56.95%).

Evaluation of plant extracts

The data on effect of plant extracts on the growth of *E. turcicum* are presented in table 4. The data revealed that significant reduction in the mycelial growth of *E. turcicum* was observed in respect of all the plant extracts tested. Among these, neem (*Azadirachta indica*) oil @ 5 per cent concentration caused maximum inhibition of mycelial growth (62.33%) followed by leaf extract of *Datura stramonium* @ 10 per cent (41.33%), leaf extract of *Artimisia annulla* @ 10 per cent (40.0%), clove extract of garlic (*Allium sativum*) @ 10 per cent (36.67%), bulb extract of onion (*Allium cepa*) @10 per cent (25.33%) and seed extract of *Datura stromonium* @10 per cent (23.67%), while leaf extract of mint (*Mentha spicata*) @ 10 per cent and root extract of *Datura stramonium* @ 10 per cent were least effective (20.0% and 22.33% inhibition, respectively). Further when the most effective plant extract, i.e., neem oil (Nimbicibin) @ 5 per cent was assessed under field conditions against Turcicum leaf blight of maize as two foliar sprays at 40 and 50 DAS, the disease intensity was reduced from 20.45 per cent recorded in control to 10.90 per cent and grain yield was increased from 45.20 q/ha recorded in control to 49.90 q/ha. Similar results on antifungal activity of aqueous extracts of different plants has been reported by various workers (Shivapuri *et al.,* 1997; Singh *et al.,* 1998; Meena *et al.,* 2003). Harlapur *et al.,* (2007) reported that, neem seed kernal extract (NSKE) @ 5 per cent was highly effective with significantly maximum inhibition of the pathogen, onion bulb extract was also fairly effective in inhibiting the growth of *E. turcicum*. The inhibitory action of NSKE may be due to azadirchitin present in seed kernel which retards the growth and activation of the pathogen. The effectiveness of onion bulb extract may be due to the presence of antifungal compounds such as cycloallin and carbohydrate propenyl sulphuric acid. Khedkar *et al.,* (2012) studied the efficacy of phyto extract on *E. turcicum* by poisoned food technique under *in vitro* conditions and found that Nimbicidin @ 5 per cent concentration was superior to all other treatments with the maximum mycelial inhibition of 68.85 per cent followed by negunda (38.88%) and neem seed kernel extract (37.01%).

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How to cite this article:

Wani, T.A., Mushtaq Ahmad and Anwar, A. 2017. Evaluation of Fungicides, Bioagents and Plant Extracts against *Exserohilum turcicum* causing Turcicum Leaf Blight of Maize. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 2754-2762. doi: https://doi.org/10.20546/ijcmas.2017.608.329