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Research Paper

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Effect of Foliar Spray of Fungicides on Development of Leaf Blight Disease of Maize (*Zea mays* L.)

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A B S T R A C T

A large part of reclaimed wetland in the Eastern fringe of Kolkata is being used for maize farming. Fungicides are frequently being used by maize growers. Leaf blight is a common disease of maize in this area which is caused by *Exserohilum turcicum*. Inhibited growth response of the pathogen was noted *in-vitro* when fungicides namely Chlorothalonil and Mancozeb were added the basal medium. Field experiment showed that the foliar spray of those two fungicides significantly controlled the disease intensity of maize leaf blight. Mancozeb was found to be most effective at 100 and 1500 µg/ml for selected and recommended doses respectively in field condition.

Key words: Wetland, maize, Leaf blight disease, *Exserohilum turcicum* , Fungicide, Bioassay

The vast tract of reclaimed wetland located in the eastern fringe of Kolkata is of tremendous importance to the dwellers of the city. The area has been acted as a municipal waste recycling ground in the backyard of the city and immensely contributing to the harmonious development of the people living in this fringe. At the same time, this wetland is also being utilized for agricultural purposes. Maize is a very important crop regarding its nutrition (Nuss and Tanumihardjo 2010) and also very significant raw material in several industrial applications. Maize farming is a popular practice in this wetland area. Because of the reclaimed nature of the wetland soil, infection of the crop plants is quite probable. Maize is susceptible to several foliar fungal spot and blight diseases (Balint-Kurti and Johal 2009, White 1999). On the basis of the distribution of pathogenic organisms in the wetland area, *Exserohilum turcicum* is one of the major pathogens causing leaf blight disease of maize (Chakraborty and Purkayastha 1999). It causes serious problems in terms of leaf damage, and severe loss of grain yield (Wise 2011, Mueller and Wise 2013). Farmers are regularly applying fungicides to their corn fields for disease management and yield enhancement. A great risk is there involving residue-borne disease-driven yield loss in maize; it leads to greater interest in foliar fungicides (Wise and Mueller 2011). Chlorothalonil (tetrachloroisophthalonitrile) and Mancozeb (manganese ethylene bisdithiocarbamate with zinc salt) are popular fungicides which are frequently being used by crop growers. In this investigation, two aforesaid fungicides have been applied to study their individual effect on growth response of *E. turcicum* as well as on the development of leaf blight disease of maize.

MATERIALS AND METHODS

Growing of plants in the field

One plot (33 m^2 each) was selected in reclaimed wetland in the eastern fringe of Kolkata. Maize seeds (cv. MSF 56) were disinfected with 0.1% $HgCl_2$ solution for 5 minutes, washed thoroughly with sterile distilled water, soaked in water for overnight and then sown in rows (60- 75 cm apart) in the field (reclaimed wetland soil).

Isolation and identification of causal organism of leaf blight disease of maize

Infected maize leaves with characteristic spots (oblong to "cigar" shaped) were collected from the field. Small

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pieces of infected leaves were disinfected with 0.1% $HgCl_2$, washed thrice with sterile distilled water and transferred aseptically to potato-dextrose-agar (PDA) slants. After 12 days, the cultures were examined, described and identified as *Exserohilum turcicum.*

Fungal culture

The culture of *E. turcicum was* maintained at 4°C and also at room temperature (30-32°C). Subculturing was done at regular interval of time. To obtain spores in culture, Malt-dextrose-peptone-agar (agar -25g, malt extract -20g, dextrose -20g, peptone -1g, distilled water -1L) medium was used. Since the number of spores gradually decreased in culture after 4-5 subculturing, infected plants were also maintained in the field for obtaining sporulating culture by fresh isolation of organism.

Assessment of mycelial growth in liquid media

Basal medium was prepared, dispensed in flasks (50 ml / 250 ml flask), plugged with non-absorbent cotton and sterilized in autoclave. After inoculation, the flasks were incubated for a desired period (8 days). At the end of the incubation period, the mycelia were collected, dried at 60°C for 96h, cooled and weighed.

The composition of basal medium was as follows:

Dextrose - 20g	Asparagine - 2 g
KH_2PO_4 - 1 g	$MgSO_4,7H_2O$ - 0.5 g
Distilled water - 1 L	pH - 5.6

Preparation of fungicide solution

Two fungicides namely Chlorothalonil (tetrachloroisophthalonitrile 75%) and Mancozeb (manganese ethylene bisdithiocarbamate with zinc salt 80 %) were used. Sterile distilled water was added for this purpose. Stock solutions were prepared on the basis of formulations and dilutions were made accordingly. A thin paste was made first which was mixed well with required volume of water.

Bioassay of fungicides

The basal medium (above mentioned) supplemented with 1.5% $CaCO_3$ was autoclaved for 20 min at 1 atm. pressure (Steinberg 1935) to remove the trace element contaminants. After standing overnight, the clear solution was taken and supplemented separately with different conc. (1, 10, 50 and 100 µg/ml) of fungicides. Flasks were inoculated with the test organism i.e. *E. turcicum* (one agar block with 4-day-old mycelia / flask) and incubated for 8 days at room temperature (30 ± 1°C). At the end of the incubation period, the mycelia were collected, dried at 60°C for 96h, cooled and weighed. Control set (basal medium without any fungicide) was maintained in each case.

Foliar spray with fungicides (Chlorothalonil and Mancozeb)

Fungicide solutions for both Cholorothalonil and Mancozeb were prepared separately as already described. For Cholorothalonil, 100 and 1000 µg / ml concentrations were applied as selected and recommended doses respectively. These doses differed in case of Mancozeb which were 100 and 1500 µg/ml for selected and recommended doses respectively. Each concentration was applied as foliar spray in inoculated and non- inoculated plants. Foliar spray was carried out twice at an interval of 48h before inoculation (first spray on 56-day old plants and second spray on 58-day old plants). Approximately 50 to 100 ml of fungicide solution was used per plant.

Inoculation technique and assessment of disease intensity

Disinfected maize seeds were sown in rows (60-75 cm apart) and plants in the rows were spaced at 20-25 cm in the reclaimed wetland soil. Two leaves of each of ten treated and ten untreated plants (60-day-old) were inoculated at random with spore suspension (1×10^6 spores/ml) of *E. turcicum* by automizer and covered with moist polythene bags for 48h and then removed.

Disease intensity was measured after 7,14, 21 and 28 days following inoculation (Chakraborty and Purkayastha 1999). The spots were graded into 4 size groups according to the length of the spot viz. < 2 mm; <5 mm; 5-10 mm and >10 mm with respective values of 0.01, 0.1, 0.2 and 0.4. The total number of spots of each size group was multiplied by respective value. The total score was divided by the total number of leaves inoculated. Disease index was calculated as follows:

$$\frac{\text{D.I.}}{\text{Leaf}} = \frac{\text{Total score}}{\text{No. of leaves inoculated}}$$

Control leaves were sprayed with sterile distilled water instead of spore suspension.

RESULTS AND DISCUSSION

Bioassay of fungicides

Sterilized basal medium was taken, trace metal contaminants were removed and was supplemented separately with different conc. (1, 10, 50, 100 µg/ml) of Cholorothalonil and Mancozeb. Flasks were inoculated with the test organism i.e. *E. turcicum* following the procedure as stated in Materials and Methods. Control (basal medium without fungicides) was maintained in each case.

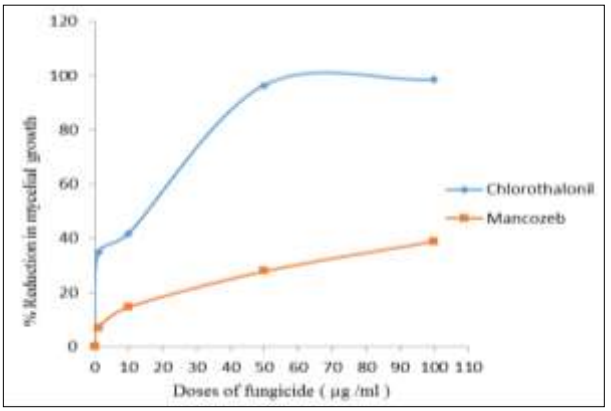
Table 1 Effect of fungicides (Chlorothalonil and Mancozeb) on mycelial growth of *E. turcicum*

Concentration (µg/ml)	Mycelial dry wt. (mg) with S.E.	
	Chlorothalonil treatment	Mancozeb treatment
Control (Basal medium)	159.16 ± 4.69	170.30 ± 3.42
1	103.81 ± 0.63	158.56 ± 4.29
10	92.77 ± 0.52	145.46 ± 0.74
50	5.72 ± 0.82	122.91 ± 0.79
100	2.54 ± 1.48	104.07 ± 1.30

Average of 5 replicates / treatment; Initial pH - 5.6
Incubation time - 8 days; Temperature - 30 ± 1° C

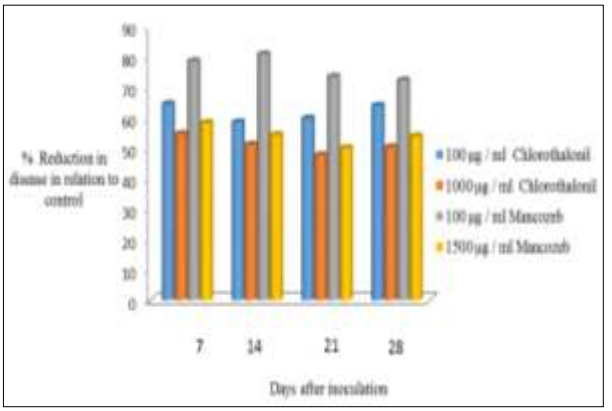
Results in (Table 1, Fig 1) show that mycelial growth was decreased with increasing concentration of fungicides in both cases. Chlorothalonil was found to be more effective. It causes about 98% growth inhibition at 100 µg/ml level whereas 39% reduction in mycelial growth was observed in

case of Mancozeb treatment at same concentration. In another research, similar fungistatic response of fungicides was noted in case of *E. turcicum* grown in vitro. In that experiment, Chlorothalonil and Mancozeb in addition to other fungicides were used and showed 100% inhibition in fungal growth (Wathaneeyawech *et al*. 2015).



Average of 5 replicates / treatment
Initial pH - 5.6
Incubation time - 8 days
Temperature - 30 ± 1°C

Fig 1 Effect of fungicides (Chlorothalonil and Mancozeb) on percent reduction of mycelial growth in E. *turcicum*



Age of the plant - 60 days (at the time of inoculation)
Age of culture - 15 days ; 20 leaves / treatment (foliar spray)
Temperature - 24.4-32°C
Relative humidity: Maximum - 98.5%; Minimum - 47%

Fig 2 Effect of foliar spray of fungicides (Chlorothalonil and Mancozeb) on percent reduction in disease intensity in relation to control in maize

Effect of foliar spray of fungicides (Cholorothalonil and Mancozeb) on development of leaf blight disease of maize

Two leaves of each of ten treated and untreated plants were inoculated and disease intensity was measured following the steps as already described in Materials and Methods. Results are summarized in (Table 2, Fig 2).

Table 2 Effect of foliar spray of fungicides (Chlorothalonil and Mancozeb) on development of leaf blight disease of maize

Treatment		Conc (µg/ml)	Disease Index / Leaf (days after inoculation)			
			7 days	14 days	21 days	28 days
Untreated	Non-inoculated	0	0	0	0	0
	Inoculated	0	34.48±2.75	65.36±0.97	82.32±1.73	92.86±2.34
Treated	Non-inoculated	100*	0	0	0	0
(Chlotothalonil)	Inoculated	100	22.18±0.42	38.14±3.85	49.06±2.14	59.24±1.42
	Non -inoculated	1000**	0	0	0	0
	Inoculated	1000	18.80±3.22	33.36±1.35	39.16±0.76	46.58±4.24
Treated	Non -inoculated	100*	0	0	0	0
(Mancozeb)	Inoculated	100	27.02±2.18	52.70±0.78	60.34±3.52	66.98±2.26
	Non -inoculated	1500**	0	0	0	0
	Inoculated	1500	20.04±0.64	35.40±1.36	41.04±3.46	49.92±0.74

*Selected dose
Age of the plant - 60 days (at the time of inoculation)
20 leaves / treatment (foliar spray)
Relative humidity: Maximum - 98.5%

**Recommended dose
Age of culture - 15 days
Temperature - 24.4 - 32°C
Minimum - 47%

Results indicate that foliar spray of fungicides could effectively control leaf blight disease in maize grown in wetland soil. Leaf blight is a major threat in maize field. Present investigation involves control of leaf blight of maize in the backdrop of hot, humid wetland environment. Addition of both fungicides (Cholorothalonil and Mancozeb) separately reduced the mycelial growth of *Exerohilum turcicum* in-vitro. Chlorothalonil was more effective as it showed nearly 98% decrease in growth than Mancozeb which achieved 39% reduction at the concentration of 100 µg/ml (Fig 1). The same compounds i.e. Chlorothalonil and Mancozeb when sprayed to the leaves of maize plant in field and the disease assessment was carried out, it showed 50% and 54% reduction respectively at their recommended doses (Fig 2). In the field trial, Mancozeb was found to be little more efficient in controlling disease intensity. The development of this disease is largely dependent on different environmental factors like soil factor, humidity, temperature which is not yet thoroughly investigated. Even the role of different biotic factors is also not ruled out (Yehouda and Yigel 1995). In-vitro condition exhibited more sensitivity of the pathogen

towards Cholorothalonil whereas the reverse response i.e. Mancozeb was found to be more effective in field condition. This could be due to the photo sensitivity of organochlorine nature of Chlorothalonil which lost its efficacy in intense light (Wallace *et al*. 2010, Monadjemi *et al*. 2011) in the field after foliar spray. On the contrary, Mancozeb being an inorganic compound, retained its stability as a foliar spray. Kolkata wetland is reclaimed because of dumping of municipal wastes; the soil may contain different components and is thoroughly heterogeneous in nature. Microorganisms present in rhizosphere and phyllosphere region are continuously interacting with the plant interfering disease development.

Efficacy of fungicides to manage foliar diseases in corn has been evaluated by several researchers (Shelby *et al*. 2018, Mallowa *et al*. 2015, Wise and Mueller 2011), including corn leaf blight (Blandino *et al*. 2012). It was observed that spraying fungicides prior to inoculation of pathogen could reduce disease intensity (Sommat 2000, Abebe and Singburaudom 2006, Wathaneeyawech *et al*. 2015). A number of factors like environmental conditions, stage of development, susceptibility of the cultivar, presence of inoculum and disease severity are important to make foliar spray of fungicide successful for disease management. The results of present work clearly indicate that spraying fungicides (Chlorothalonil and Mancozeb) before inoculation could successively control leaf blight disease of maize in wetland environment.

Present investigation concludes that fungicides (Chlorothalonil and Mancozeb) could inhibit mycelial growth of *Exserohilum turcicum* significantly when added in basal medium in laboratory. To control leaf blight disease of maize crops grown in Kolkata wetland environment, foliar spray of those fungicides before the appearance of disease symptoms is strongly recommended. In addition to this foliar spray of fungicides, biological and mechanical measures such as natural predators, crop rotations, hybrid seed selection and tillage practices should be taken into considerations (not investigated in this study) to maximize the outcome of disease management.

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