

RESEARCH ARTICLE

Studies on stalk rot of maize caused by *Fusarium moniliforme* Sheldon

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ABSTRACT

Stalk rot of maize caused by *Fusarium moniliforme* is one of the most damaging diseases of maize causing enormous losses. This disease has now achieved much importance in maize growing areas of Khandesh area of Maharashtra state. Therefore, the investigations were aimed at isolation, pathogenicity, identification and study of morphological characters of the pathogen along with *in vitro* evaluation of fungicides for controlling growth of the pathogen. The pathogenicity of the isolated *Fusarium* sp. was proved by sick soil method. The colonies of pathogen were circular, brilliant white, and compact with smooth margin. Macroconidia were slender, sickle shaped, pedicilate and scattered. Mostly they were septate and measured 43-46 x 3-3.5 μ m. Microconidia were in chain, white in colour and measured 5-12 x 2-4 μ m. Copper oxychloride was found most effective in retarding growth of *Fusarium moniliforme* which showed 100 per cent inhibition of fungal growth over control. It was followed by carbendazim (0.1%), thiram(0.2%), and thiophanate-methyl (0.1%) which showed 86.67, 79.52 and 71.90 per cent inhibition, respectively and these treatments found significantly different to each other.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops grown for food and fodder. In Indian Agriculture maize crop has occupied a prominent place, ranked fourth position in area among the cereals. The major portion of the area under maize in India is confined to the states of Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh and Punjab, while its cultivation in Maharashtra state is limited. The area under this crop is confined in the districts of Dhule, Pune, Solapur, Nasik, Aurangabad, Kolhapur, Chandrapur, Osmanabad, Satara and Sangli of Maharashtra state. The total area of maize has been estimated to be 8.26 million hectares in India during the year 2007-08 along with 15.09 metric ton production and 2331 kg/ha yield in India (Anonymous, 2008-2009).

The losses caused by the stalk rot disease are obvious on account of the death of the plants. On the other hand, the foliar diseases debilitate the affected plants resulting in

reduction of the size of the ear and grain. A conservative estimate of the losses caused by maize diseases are about 13 per cent revealing about 280 million rupees loss in india. Stalk rot is important disease of maize, which caused 10-15 per cent losses (Thind and Payak, 1985). Considering the seriousness of the disease, present studies were undertaken to isolate the pathogen, to prove the pathogenicity, to study the morphological characters and *in vitro* evaluation of different fungicides against the pathogen responsible for causing stalk rot of maize in Dhule region of Maharashtra.

MATERIAL AND METHODS

Isolation :

The stalk rot pathogen was isolated from infected stalks of maize collected from various locations of Dhule region of Maharashtra. In order to isolate the pathogen from stalk, samples were cut into small pieces, surface sterilized in 1:100

HgCl₂ for two minutes and then washed in sterile water 2-3 times to remove the residues of HgCl₂ and these pieces were transferred on sterilized Potato dextrose agar medium in Petriplate under aseptic conditions. The plates were incubated in BOD incubator at 27 ± 2°C. The isolates obtained from the pieces were transferred to PDA slants. In this way, pure culture of the pathogen was obtained and maintained in the laboratory for further study.

Pathogenicity test :

Pathogenicity of the isolated fungus was tested by soil inoculum technique (Sen and Kapoor, 1975).

For this purpose, Sand maize medium was used for mass multiplication of the fungal isolate in the laboratory. The medium was prepared by autoclaving 20 g maize grains, 80 g dry sand with 80 ml sterile water in 500 ml conical flasks and sterilized in an autoclave at 15 lbs psi for 30 minutes.

Sand maize medium was then inoculated with pure culture of isolate in aseptic conditions and incubated in an incubator at 27 ± 2°C for ten days. The flasks were shaken frequently to avoid clumping of grains and to facilitate early growth of fungus on the grains. The grains turned whitish due to mycelial growth of the fungal isolate.

Two weeks old growth of pathogen in Sand maize medium was used as inoculum to the soil at the rate of 50 g/kg of previously sterilized soil. The soil mixture contained soil + compost in proportion of 3:1. The inoculated soil was kept at room temperature for 15 days for fungus multiplication, and then used for filling earthen pots for pathogenicity. After germination of seedling, they were observed for the incidence of stalk rot.

Morphology of fungus :

A slide culture technique was mainly employed for this purpose. For this PDA was prepared, sterilized and poured in sterilized Petriplates aseptically. After solidification of the medium, cubes were prepared with the help of sterilized cork borer. Sterilized glass slide was kept in the sterilized Petriplate and then PDA cube was aseptically placed on the slide. The inoculum was placed on the cube and sterilized coverslip was kept on it.

The Petriplates were then placed for incubation in an incubator at 27 ± 2°C. After 3-4 days of incubation, there was sufficient growth of mycelium on the slide and coverslip. The slides were then removed from the Petriplate and after taking out the medium (cube) without disturbing the mycelium. Permanent slide was prepared. The observations recorded in respect of colour of the mycelium, length and breadth of microconidia, macroconidia and septation. The measurements were recorded with the help of stage and ocular micrometers.

Effect of fungicides on the growth of *Fusarium moniliforme*:

The effect of fungicides on the radial growth of *Fusarium*

moniliforme at recommended concentration was studied by the 'Poisoned Food Technique' (Horsefall, 1956).

Sterilized PDA incorporated with different fungicides with recommended concentration poured into Petriplates. These Petriplates were centrally inoculated with mycelial disc (5 mm diameter) made with the help of sterilized cork borer from 7 day old culture. Control sets were run simultaneously with normal PDA. These plates were incubated in BOD incubator at 28 ± 2°C. Radial growth was measured (mm) on 7th days after inoculation and per cent growth inhibition was calculated on the basis of three replications.

The per cent growth inhibition was calculated by using the following formula suggested by Vincent (1947) and Horsefall (1956):

$$\text{Inhibition percentage (I)} = \frac{C - T}{C} \times 100$$

where,

- I = Inhibition percentage
C = Growth of the fungus in control plate (mm)
T = Growth of the fungus in treated plate (mm)

RESULTS AND DISCUSSION

The results of the present study alongwith relevant discussion have been presented as under :

Isolation of pathogen :

The tissue isolations were made from the samples collected from various areas showing typical symptoms of stalk rot and wilting, which yielded the growth of the fungal pathogen. The growth of fungus from infected tissue was distinctly visible after three to four days of incubation in Petriplates containing the Potato dextrose agar medium. A pure culture was obtained from hyphal tip method on PDA Petriplates, transferred and maintained on PDA slants for further studies. These results are in agreements with Reinking and Foley (1962) and Hingorani (1964). They reported that *Fusarium moniliforme* is associated with stalk rot of maize.

Pathogenicity of the isolate :

Pathogenicity test of the isolate was carried out by soil inoculation method. The seeds of maize were sown in two pots, one pot with sick soil and another pot without sick soil *i.e.* sterilized soil, which served as a control.

The symptoms of stalk rot was observed regularly. The typical symptoms of the stalk rot was noticed in three weeks from sowing in sick soil pot *i.e.* yellowing and drooping of leaves. After 6th week from sowing, the disease symptoms were very prominent on seedling. On the 6th week, the brown streaks were observed on stalk and subsequently converted into black colour. On splitting, pith of the infected stalk was found rotted along with hollow cavities. The black bundle phase was seen in the infected seedling. The seedling grown

in sterilized soil had not showed any disease symptoms which was served as control.

The results of present investigation are in agreements with the results obtained by Koehler *et al.* (1925), Luttrell and Garren (1952), Hingorani (1964), Rane (1967) and Khalil *et al.* (1980). They proved the pathogenicity of *Fusarium moniliforme* by soil inoculation method. Inoculum was prepared by growing the fungus for 15 days on sterilized sand-maize-meal-medium having 80.00 gm sieved fine river sand, 20.00 gm maize meal and 80.00 ml water as a components.

Morphological characters :

Morphological characters of the fungal pathogen under study were recorded from eight days old culture grown on Potato dextrose agar medium. Mean length, breadth, septation of conidia was measured with ocular micrometer.

The colonies were circular, compact with smooth margin and white in colour. Microconidia in chains, or in fall heads formed in white to isabella colour, mycelium spindle to ovoid in shape and measured 5 to 12 x 2.0 to 4 µm. Macroconidia were slender, sickle shaped, pedicellate, scattered or in sporodochia or pinnotes, brownish white to orange cinnamon, mostly three septate, sclerotia blue, stroma violet, chlamydospore absent, macroconidia measured 43 to 46 x 3.0 to 3.5 µm.

These results are in close agreements with Martyn (1932) who reported the morphological characters of *Fusarium moniliforme* i.e. three septate macroconidia measured 26.4-49.5 x 3.3-4.9 µm. The non-septate microconidia measured 6.6-13.2 x 3.3-4.9 µm.

Effect of fungicides on growth of *F. moniliforme* :

The results in respect of effect of fungicides on growth of *Fusarium moniliforme* are presented in Table 1. All the chemical treatments were significantly superior over control

in checking the growth of *F. moniliforme*. Among all the treatments copper oxychloride (0.25%) was found significantly most effective in checking the growth and showed absolutely no growth of the fungus with 100 per cent inhibition. It was followed by carbendazim (0.1%), thiram (0.2%), and thiophanate-methyl (0.1%) and which showed 9.3, 14.3 and 19.6 mm mycelial growth, respectively as against 70 mm in the control treatment. These treatments showed 86.67%, 79.52%, and 71.90%, per cent inhibition, respectively. These treatments were found significantly different from each other. Next to these treatments, chlorothalonil (0.25%) and hexaconazole (0.1%) were at par with each other and showed 35 and 35.6 mm mycelial growth with and 50% and 49.04% per cent growth inhibition, respectively. Captan (0.25%) and mancozeb (0.25%) were found least effective in checking the growth of *Fusarium moniliforme*, which showed 46 mm mean colony diameter in each of the fungicides with 34.29 per cent inhibition over control.

These results are more or less in close agreements with the result achieved by Bohra *et al.* (2001) who showed that carbendazim and thiram @ 50, 100, 200, 400 and 800 ppm showed significant inhibition of the growth against *Fusarium moniliforme* *in vitro*. Trivedi *et al.* (2002) reported that carbendazim, thiophanale methyl and thiram @ 100, 250, 500 and 1000 ppm inhibited the growth of *Fusarium moniliforme*. Muhammad and Javed (2011) showed that Topsin M (0.25%) i.e. thiophanate-methyl (0.1%) was effective against *Fusarium moniliforme*. Raju and Lal (1977) showed that thiram was effective against *Fusarium moniliforme*. Singh and Goswami (2003) reported that maximum inhibition of *Fusarium moniliforme* recorded in carbendazim (79.96%) and thiram (73.23%). Honmane (2007) found that carbendazim (0.1%) was most effective against *Fusarium moniliforme* and showed complete inhibition of fungus.

Table 1 : Bio-efficacy of different fungicides on mycelial growth of *Fusarium moniliforme*, the pathogen causing stalk rot of maize

Sr. No.	Treatments	Concentration %	Mean colony diameter*	Per cent growth inhibition over control
1.	Captan	0.25	46.0	34.29
2.	Carbendazim	0.1	9.3	86.67
3.	Chlorothalonil	0.25	35.0	50.00
4.	Copperoxychloride	0.25	0.0	100.00
5.	Hexaconazole	0.1	35.6	49.04
6.	Mancozeb	0.25	46.0	34.29
7.	Thiophanate methyl	0.1	19.6	71.90
8.	Thiram	0.2	14.3	79.52
9.	Control	-	70.0	-
	S. E m ±		0.59	
	C.D at 5%		1.75	
	CV		3.32	

* Mean of three replications

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