"STUDIES ON STALK ROT OF MAIZE CAUSED BY *Fusarium moniliforme* Sheldon"

By

Miss. Musmade Shaila Annasaheb

Reg.No.10/219

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

PLANT PATHOLOGY SECTION

COLLEGE OF AGRICULTURE, DHULE-424 004
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APPROVED BY

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PLANT PATHOLOGY SECTION
COLLEGE OF AGRICULTURE, DHULE-424 004
MAHARASHTRA, INDIA
2012

Dr. D.N. Padule
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Maharashtra, (India).

CERTIFICATE

This is to certify that, the thesis entitled, **"STUDIES ON STALK ROT OF MAIZE CAUSED BY *Fusarium moniliforme* Sheldon"**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** (AGRICULTURE) in **PLANT PATHOLOGY** embodies the results of piece of *bona fide* research work carried out by **MISS. MUSMADE SHAILA ANNASAHEB,** under my guidance and supervision and that, no part of the thesis has been submitted to any other University or Institute for degree or diploma.

The assistance and help received during the course of present investigations and sources of reference have been duly acknowledged.

Place : Dhule (D.N. Padule)
Dated : / /2012. Chairman and Research Guide

Dr. A.J. Patil
Associate Dean,
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Maharashtra, (India).

CERTIFICATE

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Place : Dhule (A.J.Patil)
Dated : / /2012. Associate Dean

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"All glories to Shree Gurudev Datt"

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Place: Dhule

Date: / /2012 (S.A. Musmade)

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ABBREVIATIONS

%	Percentage
/	Per
C.D.	Critical difference
cm	Centimeter
dia	Diameter
et al.	et alia (and associate)
etc.	et. cetra
Fig.	Figure
FSM	Fusarium Specification
g. /gm	gram (s)
ha	hectare
i.e.	idest (that is)
Kg	Kilogram
Lb. psi	Pound per square inch
mg	Milligram (s)
ml	Milli liter (s)
No	Number
oC	Degree celsius
PCNB	Pentachloro nitro benzene
ppm	Parts per million
qt	Quintal
S.E. (m) ±	Standard error of mean
Spp / sp	species (Singular and pleurals)
var	Variety
viz	Namely
PDC	Per cent Disease Control
PDI	Per cent Disease Incidence
@	At the rate
mm	millimeter
sig	significant
T	Treatment
µm	micro meter

ABSTRACT

STUDIES ON STALK ROT OF MAIZE CAUSED BY *Fusarium moniliforme* Sheldon

By

Miss. Musmade Shaila Annasaheb

A candidate for the degree
of
MASTER OF SCIENCE (AGRICULTURE)
in

PLANT PATHOLOGY

COLLEGE OF AGRICULTURE, DHULE

2012

Research Guide : Dr. D.N. Padule
Section : Plant Pathology Section, College of Agriculture Dhule

Maize (*Zea mays* L.) is one of the most important cereal crop grown for food and feed. Among several diseases, stalk rot caused by *Fusarium moniliforme* is the most damaging to the maize crop in field and causing enormous losses. This disease has now achieved much importance in maize growing areas of Khandesh. Therefore, the investigations were carried out with regards to isolation of pathogen, pathogenicity, identification of pathogen, morphological characters of the pathogen, and *in vitro* evaluation of fungicides for controlling the growth of pathogen.

The affected samples of maize stalk rot were collected from Dhule district of Maharashtra. The tissue isolations were made from affected samples which yielded *Fusarium* spp.

The pathogencity of the isolated *Fusarium* spp. was proved by sick soil method. The pathogen was found pathogenic. The pathogen has produced symptoms of yellowing of leaves, drooping of leaves and development of dark streaks on rind and basal portion and blackening of vascular bundles and formation of cavity in basal internode in maize.

The pathogen was identified as *Fusarium moniliforme* Sheldon with available literature and get confirmed from the Mycologist, Agharkar Research Institute, Pune.

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 µm. Microconidia were in chain, white in colour and measured 5-12 X2-4µm. Mycelium is white in colour.

In vitro evaluation of fungicides, copper oxchloride was found most effective in checking the growth of the pathogen (*Fusarium moniliforme*) which showed absolutely no growth of the fungus and percent inhibition over control was 100(%). It was followed by carbendazim (0.1%), thiram(0.2%), and thiophanate-methyl (0.1%) which showed 86.67(%), 79.52(%), and 71.90(%), percent inhibition respectively, these treatments found significantly different to each other.

CANDIDATE'S DECLARATION

I hereby declare that, this thesis or part there of has not been submitted by me or other person to any other University or Institute for a Degree or Diploma

Place : Dhule (S.A. Musmade)

Dated : / /2012

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal food and feed crops grown in the world. It ranks next to wheat and paddy in total production in the world. It is now grown in all the continents except Antartica and under a more varied range of climate than any other cereal crops. Further, maize has the highest yield potential per hectare among the cereal crops.

It is cultivated in tropics, sub-tropics and temperate regions under irrigated to semi-arid conditions. In warm climate, green maize is available throughout the year in central and southern India. It can be grown through out the year. However, in the northern belt, it is mainly cultivated in the rainy season.

In Indian Agriculture maize crop occupies a prominent position. In terms of area, maize ranks fourth among the cereals in India. 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 it's 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/A).

The productivity of maize in Maharashtra State is 14.5 q/ha (Anonymous, 2008-09/B)

In the world, the area and production of maize is 132 million hecters and 570 metric tons, respectively (Anonymous 2008-09/B).

Each part of the maize plant is put to one or the other use and nothing goes as waste. Because of worldwide distribution and relatively lower price, maize has wider range of uses. It is used directly for human consumption, in food processing industries as livestock feed, in industrially non-food products such as starches, acids and alcohols. Recently, there has been interest in using maize for production of ethanol, a substitute for petroleum based fuels.

Nutritionally maize contains 60-80 per cent starch and 7-15 per cent protein. Opaque seeded types are more nutritious and contains higher percentage of amino acids. Yellow maize is the richest source of vitamin 'A'. Maize contains 1.2-5.7 per cent edible oil. This oil has quality to reduce human blood cholesterol. Maize is a major source for manufacture of starch, syrup, dextrose, oil, gelatin, lactic acid etc. Corn flour is used as thickening agent in the preparation of many edibles like soups, custard powder etc. Corn sugar (dextrose) is used in pharmaceutical formulations as sweetening agent in soft drinks etc. Corn gel on account of it's moisture retention character is used as bonding agent for ice-cream cones.

Maize is extensively used in livestock feeds viz., cattle, poultry and piggery both in the form of seeds and fodder. The

green fodder can be fed to milch cattle to boost the milk production.

Maize is consumed directly as breakfast foods like corn flakes, roasted green ears, rotties. The central rachis of maize cob is used in agriculture as a litter for poultry and as a soil conditioner. Based on the chemical properties the processed cobs find their use in the manufacture of furfurol, fermentable sugars, solvents, liquid fuels, charcoal gas and also in the manufacture of pulp, paper and hard boards. Thus, each part of maize plant has a value.

The maize crop is reported to be affected by about 60 diseases in the world, of which only a few are economically important. They are i) those affecting stalk ii) those affecting foliage and iii) small group affecting the ear, seed and seedlings. Indian maize, however, suffers from near about 25 major and minor diseases. The maize grain production is limited mainly due to seedling blights, stalk rot, leaf blights and ear rot which reduces the production appreciably.

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. The most important disease in India is the stalk rot which caused 10-15 per cent losses (Thind and Payak, 1985).

Thus, there is an urgent need for the incorporation of disease resistance in hybrids and composites. In the objective of a crop improvement programme the identification of resistant source to prevalent diseases has dominant role. This takes preference over other methods of disease control due to certain advantages and also economic conditions.

In Dhule region, the stalk rot disease of maize observed in severe form causing losses to the extent of 13 per cent (Anonymous, 2008-2009/A). As maize being a major crop in the Dhule district and also in the southern part of Maharashtra State, it was decided to undertake detailed investigations to find out the role of different pathogens responsible for causing stalk rot. As this complex disease is found to be caused by about 17 pathogenic organisms including mainly fungi, bacteria and nematodes in various proportion of agroclimatic zones of India (Reinking and Wollenweber, 1927; Hingorani M.K., 1964). As this being very important problem in Dhule region, it was decided to work on stalk rot of maize with following objectives

a. To isolate the pathogen causing stalk rot of maize in Dhule region.
b. To prove the pathogenicity of isolated pathogen.
c. To study the morphological characters of the pathogen associated with stalk rot of maize.
d. To evaluate the different fungicides against the pathogen *in-vitro.*

2. REVIEW OF LITERATURE

Several fungal and bacterial organisms have been cited as pathogens causing stem rot of maize. Many of these also were found as incitent of ear rot, kernel rot, root rot and seedling blight of corn. Stalk rot of maize is currently considered as an important disease. The organisms inciting stalk rot are damaging the growing plant leading into the stalk breaking and death of plant.

2.1 Pathogen associated with stalk rot of maize

The *Fusarium* causing the disease of corn was first described in 1904 in Nebraska by Sheldon as *Fusarium moniliforme;* since that time, this species has been known to be a parasite of corn.

Pammel *et al.* (1916) reported the *Fusarium* stalk rot disease of maize. The injured stalks were broken over at one of the lower nodes or at joints or near the soil. The pith, soft material or parenchyma in the corn stalk was destroyed and turned brownish or in some cases, reddish in colour. The fibre turned soft which were broken off easily. Such stalks were often barren or had only small ears. On the surface of stalk and in the nodes, there was an abundance of the mold, especially when there was much humidity.

Reinking and Wollenweber (1927) have compiled and described in detail about ' Tropical Fusaria '. In all forty-seven species have been reported of which *Fusarium semitectum, F. acuminatum,* Eillis and E.V. *F. moniliforme* Sheldon, *F. oxysporum*

schlecht, *F. solani* (Mart), Sacc. were predominant to cause stalk rot of maize.

Leonian (1932) from West Virginia University made 150 isolations from corn seedlings in which 110 proved to be *F. moniliforme,* 20 *F. culmorum* Wr. and the remaining consisted of various saprophytic forms. Only 20 strains of the foregoing 110 cultures of *F. moniliforme* proved to be pathogenic to corn.

Edward (1935) first reported physiological specialization and the perithecial state of *F. moniliforme* var. *subglutinans.* He described two distinct races which occurred on maize in New South Wales. Since then a considerable range of virulence has been reported.

Hingorani (1964) described stalk rot of maize caused by at least two bacterial species and a number of fungi such as *Pythium, Fusarium* and *Cephalosporium* have been reported.

Foley (1962) isolated *F. moniliforme* from kernels, roots, leaf sheaths, axillary buds and stalks of *Zea mays* L.

Alejandro Ortega (1974) reported close association between maize pathogen and insects. Maize ear and stalk rots caused by *Fusarium* sp. can become more prevalent when ear worm and stalk borer larvae were in abundance.

Erzhibev *et al.* (1980) observed in the Kabardino Balkariya, USSR, that maize was severely attacked by species of *Fusarium* and *Sclerotinia.* The *Fusarium* infection was more common, in the infected stem which became yellowish and leaves drooped prematurely.

Fischi (1983) observed the plant litter was the most important source of primary infection. *Fusarium* spp. were isolated from 38-44 per cent of the litter samples examined.

Windham and King (1983) isolated mycoflora from root of maize plant from 10 locations were *Trichoderma* spp., *F. moniliforme* and *F. oxysporum,* frequency of specific fungi and total mycoflora varied considerably among the locations. Invasion was not associated with a particular part of the root system and was commonly associated with symptomless stalk tissues.

Gunay (2002) reported that stalk rot can be caused by several different pathogen. Mostly fungi, which may work alone or in combination. *Fusarium moniliforme* is capable of infecting corn stalks.

Afolabi *et al.* (2008) noted in Nigeria, that *Fusarium moniliforme* had been implicated as the major stalk rot pathogen.

2.2 Economic losses due to stalk rot of maize

Pammel *et al.* (1916) expressed that it was difficult to estimate the losses due to *Fusarium* stalk rot disease of corn. However, in some fields the crop showed damage ranging from 5-50 per cent.

Wilcoxson (1962) observed that although one rotted internode reduce the yield of corn considerably, several inoculations per stalk caused even greater reduction in yield.

Stalk rot of corn caused extensive yield reduction in Illionis annually even though partially resistant hybrids were in general use (Britton, 1963).

Kruger (1983) observed that in heavily infected plants with root and stalk rot of maize, the 1000 grain weight was reduced to a relative value of 83 % and the yield to 80 % compared with 100 % in healthy plants. The moisture content of the grain was reduced by 1-2 per cent.

Bohra *et al.* (2005) reported that susceptibility of stalk rot of maize increased with the plant age up to 60 days after sowing, sixty days old plants found most susceptible. Stalk rot reduced the total grain yield of variety VL-42 and Sartaj by 14.2% and 5.4%, respectively.

2.3 Pathogenicity of the isolate responsible for stalk rot of maize

Reddy and Holbert (1924), Koehler *et al.* (1925), Hooker (1956), Rane (1967) and Bausch *et al.* (1982) used seedling inoculation of maize for pathogenicity. The surface sterilized seeds were sown in sterilized soil. About 0.25 ml of spore suspension was injected into the stem of one month old seedling, 2-3 cm above the soil surface. Seedling injected with sterile water served as control. The stem was covered with moist cotton wool at the point of inoculation to prevent drying.

Koehler *et al.* (1925), Luttrell and Garren (1952), Hingorani (1964), Rane (1967) and Khalil *et al.* (1980) followed soil inoculation method for pathogenicity test. The 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.

Smith *et al.* (1938) used a technology for inoculating stalks by puncturing internodes with a steel needle and injecting the inoculum into the puncture.

Milevoj and Rihtar (1983) showed that the pathogenicity of all isolates of *F. moniliforme* from maize in Slovenia decreased in agar culture regardless of the method of maintenance and reduction in pathogenicity was greatest in highly pathogenic isolates.

2.4 Morphological characters of the pathogen

Ito and Kimura (1931) described that the conidia of *Gibberella fujikur*oi germinated rapidly in water in five to six hours at 25˚C.

Martyn (1932) Studied the morphological characters of *Fusarium moniliforme* and reported that the three septate macrospores measured 26.4 - 49.5 × 3.3-4.9 µ, the non septate microspores 6.6-13.2 × 3.3 – 4.9 µ.

Wollenweber and Reinking (1935) described the morphology of fungus *F. moniliforme* var. *anthophilum.* They observed that the initial growth of fungus was rather filmy, colourless and rapid, cultures from typically dark violet but occasionally paler, lilac, vinaceous or even cream. They also observed that the aerial mycelium was generally dense and delicately floccose to felted, white vinaceous to felted with a powdery appearance due to the formation of microconidia. Microconidia are fusiform to clavate with a slightly flattened base and measured 5 – 12 × 1.5 – 2.5 µ.

Bilay (1955) reported that the strains of *F. moniliforme* var. *anthophilum* were considered to be synonyms of *F. moniliforme* var. *subglutinans.*

2.5 Evaluation of fungicides against the pathogen responsible for stalk rot of maize

Younts and Musgrave (1958) found that chloride level in the plant appears to be the determining factor in retarding stalk rot disease. They also observed that broadcasting potassium chloride at high rate resulted in less stalk rot probably because of higher chloride level in the plant.

Britton (1963) evaluated seven applications of two protective fungicides (Thiram and $MgCl_2$) applied to the basal portion of corn stalks at weekly intervals beginning from one week before silking failed to reduce the amount of stalk rot in comparison with unsprayed plants. The inability of control the disease by above ground fungicide treatment during the period though most favourable for infection and disease development indicated that little, if any, infection occurred through the basal stalk nodes in this experiment.

Payak (1983) suggested that the stalk rot of maize could be controlled by soil drenching with captan (150 g/100 lit. water), when the crop was 5-7 week old.

Borhra *et al.* (2001) evaluated the different six fungicides i.e. carbendazim, triadimefon, iprobenfos, captan, thiram, and mancozeb fungicides were used at 50,100,200,400, and 800 ppm concentration against stalk rot of maize *In vitro.*

The results of *in vitro* study showed that all treatments significantly inhibited the growth of *Fusarium moniliforme*.

Trivedi *et al.* (2002) evaluated efficacy of different fungicides i.e. carbebdazim, mancozeb, copper oxychloride, thiophinate-methyl, captan, thiram, and metalaxyl at 100,250,500 and 1000 ppm by poisoned food technique against *Fusarium moniliforme* causing stalk rot of maize. All fungicides tested inhibited the growth of pathogenic fungi.

Muhammad *et al.* (2011) in poisoned food technique, efficacy of topsin-M (0.25%), derosal (0.25%) and daconil (0.25%) were found effective with less mycelial growth of *Fusarium moniliforme.*

Raju and Lal (1977) showed that captan and thiram were effective against *F. moniliforme*.

Singh and Goswami (2003) reported that emisan 6 exhibited maximum inhibition (83.73%) of radial growth followed by carbendazim (79.96%), benomyl (78.91%) and thiram (73.23%) against wilt of sugarcane caused by *Fusarium moniliforme* Sheldon *in vitro* condition.

Honmane (2007) found that the fungicides viz., carbendazim (0.1%), propiconazole (0.05%), hexaconazole (0.05%) and copper hydroxide (0.2%) were most effective against *Fusarium moniliforme* as completely inhibited the mycelial growth of test fungus causing wilt of anthurium.

3. MATERIAL AND METHODS

The present investigations "Studies on stalk rot of maize caused by *Fusarium moniliforme* Sheldon" was conducted in Plant Pathology Section, College of Agriculture, Dhule. The details of materials used and methods adopted during the course of this investigation are included in this chapter.

3.1 Material

The following materials were used during the present investigations.

3.1.1 Diseased samples

The samples of diseased stalks of maize were collected from the fields of Satana, Dhule, Phagne, Shirpur and Shindkheda.

3.1.2 Seeds of maize

Seeds of maize were obtained from All India Co-ordinated Maize Improvement Project, Kolhapur, Rahuri. The seeds of local variety and hybrid were collected and used in experimentation.

3.1.3 Fungicides

Following fungicides were used for experimental purposes as per recommended concentrations.

3.1.3.1 Carbendazim (Bavistin)

a.	Chemical Name	:	2(methoxy carbonyl amino)benzimidazole
b.	Active ingredient	:	50 %WP
c.	Concentration used	:	0.1 %
d.	Manufacturer	:	BASF, India Ltd., Mumbai

3.1.3.2 Mancozeb (Dithane M-45 / Indofil M-45)

a. Chemical Name : Manganese ethylene bisdithiocarbamate zinc sulphate
b. Active ingredient : 75 % WP
c. Concentration used : 0.25 %
d. Manufacturer : Indofil Chemical Ltd., Mumbai

3.1.3.3 Captan (Captaf)

a. Chemical Name : N-trichloromethyl thio-4) tetra cyclohexane-1, 2-dicarboximide
b. Active ingredient : 75 % WP
c. Concentration used : 0.25 %
d. Manufacturer : Rallis India Ltd., Mumbai

3.1.3.4 Chlorothalonil (Kavach)

a. Chemical Name : Alkalnapthalene sulphate
b. Active ingredient : 75 % WP
c. Concentration used : 0.25 %
d. Manufacturer : M/S Syngenta India Ltd.

3.1.3.5 Copper-oxychloride (Blitox)

a. Chemical Name : Copper oxycloride
b. Active ingredient : 50 % WP
c. Concentration used : 0.25 %
d. Manufacturer : Rallis India Ltd., Mumbai.

3.1.3.6 Thiophanate methyl (Topsin M-70)

a. Chemical Name : 1, 2, bis (3-methoxy carbamy 1-2 thiourlido) benzyne
b. Active ingredient : 70 % WP
c. Concentration used : 0.1 %
d. Manufacturer : Motilal Pesticide India Ltd., New Delhi

3.1.3.7 Thiram (Thiride)

a. Chemical Name : Tetramethyl thiram disulphide
b. Active ingredient : 75 % WP
c. Concentration used : 0.2 %
d. Manufacturer : The Alkali and Chemical Corporation of India Ltd., Calcutta

3.1.3.8 Hexaconazole

a. Chemical Name : Tetramethyl thiram disulphide
b. Active ingredient : 5 % SC
c. Concentration used : 0.1 %
d. Manufacturer : Rallis India Ltd., Mumbai.

3.1.4 Chemicals

The chemicals required during the various experimentations were sodium hypochlorite, $HgCl_2$, dextrose, agar-agar and others.

3.1.5 Glasswares

The different types of glasswares were used in the experimental work. The common materials were Petriplates, test tubes, conical flasks, measuring cylinder, glass rods, slides, cover slips etc.

3.1.6 Equipments

The common laboratory equipments used were autoclave, laminar air flow, incubator, refrigerator, research microscope, electron top pan balance, stereo binocular microscope, cork borer etc.

3.1.7 Culture media

The common laboratory medium i.e. potato dextrose agar (PDA) was used for these studies.

3.1.8 Miscellaneous materials

The miscellaneous material required during the experimentation were inoculation needle, spirit lamp, cotton, marking pen, mercuric chloride, scale, cork borer, soil and compost mixture etc.

3.2 Methods

3.2.1 Isolation

The stalk rot pathogen was isolated from infected stalks of maize collected from various locations. In order to isolate the pathogen from stalk, samples were cut into small pieces, surface sterilized in 1:100 $HgCl_2$ for two minutes and then washed in sterile water 2-3 times to remove residues of $HgCl_2$ 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 $\pm$ 2^0C. The fungi obtained from the pieces were transferred to PDA slants. In this way pure culture of the pathogen was obtained.

3.2.2 Maintenance of isolates

The isolates were periodically sub-cultured and maintained on potato dextrose agar slants for further studies.

3.2.3 Pathogenicity test of the isolate

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 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 at 15 lbs psi for 30 minutes.

The sand maize media were then inoculated with pure culture of isolate in aseptic conditions and incubated in incubator at 27 $\pm$ 2^0C for ten days, The flasks were shaken 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 isolate.

Two weeks old growth of pathogen in sand maize medium was used as inoculum to the soil at the rate of 50 gm/kg of soil. The soil mixture contained soil + compost in proportion of 3:1. The inoculated soil was kept for 15 days for fungus multiplication, and then used for filling earthen pots for pathogenicity.

Two earthen pots of 9 inch diameter were taken and sterilized with 5% $CuSo_4$ solution. Out of that one pot was filled with sick soil and another pot was filled with sterilized soil. In each of the pot five seeds of maize sterilized with 0.1% $HgCl_2$ solution was sown and pots were watered regularly. After germination of seedling they were observed for the incidence of stalk rot.

3.2.4 Reisolation

The reisolation was made from the rotted stalks of maize observed in pathogenicity test. The fungus obtained in reisolation was pure and found identical in all respects with the original fungus culture.

3.2.5 Identification of isolate

The isolated culture found pathogenic was preserved in pure form and identified upto species level with the help of published literature. (Booth 1971) and confirmed from mycologist Agharkar Research Institute (M.A.C.S), Pune.

3.2.6 Morphology of fungus

A slide culture technique was mainly employed for this purpose. PDA was prepared, sterilized and poured in petriplates. After solidification, cubes were prepared with the help of sterilized cork borer. Sterilized slide was kept in the sterilized petriplate and the 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 incubator at 27 $\pm$ 1^0C. After 3-4 days, there was sufficient growth of mycelium on the slide and coverslip. The slides was then removed from the petriplate and after taking out the medium (cube) without disturbing the mycelium. Permanent slide was prepared. The observations in respect of colour of mycelium, length & breadth of microconidia, macroconidia & septation were measured. The measurements were recorded with the help of stage and ocular micrometer at M.P.K.V. Rahuri in the Seed Pathology laboratory.

3.2.7 Effect of fungicides on the growth of *Fusarium moniliforme* Sheldon

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 dia.) made with sterilized cork borer from 7 days old culture. Control sets were run simultaneously with normal PDA. These plates were incubated in BOD incubator at 28 $\pm$ 2^0C. Radial growth was measured (mm) on 7^{th} days after inoculation and percent growth inhibition was calculated on the basis of three replications.

The per cent of growth inhibition was calculated by using 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 in control plate in mm.

T = Growth in treated plate in mm

3.2.8 Statistical analysis

The data obtained from different experiments were computed statistically by using the standard statistical method (Panse and Sukhatme, 1978) for it's statistical analysis.

4. EXPERIMENTAL RESULTS

The results of the different experiments are presented as under.

4.1 Collection of disease samples

The stalk rot disease of maize caused by *Fusarium moniliforme* was noticed in severe form in Khandesh region of Maharashtra State. The leaves of affected plant showed slight pale colour or yellowish tinge, drooping of leaves. The maize plants were affected at collar region and development of dark streaks on rind and basal portion and blackening of vascular bundles and formation of cavity in basal internode was observed. Such samples were collected from various places of Khandesh region i.e. Satana, Dhule, Phagne, Shirpur and Shindkheda.

4.2 Isolation, Pathogenicity and Reisolation of pathogen

4.2.1 Isolation of pathogen

The tissue isolations were made from infected stalks collected from various areas showing typical symptoms of rot, wilting, yielded the growth of the fungal pathogen. The growth of fungus from infected tissue was distinctly visible after three to four days in petriplates containing the potato dextrose agar medium. A pure culture was obtained from hyphal tip method on PDA petriplates, transferred and maintained on potato dextrose agar slants for further studies (Plate-1).

4.2.2 Pathogenicity of the isolate

Pathogenicity test of isolate was carried out by soil inoculation method. The seeds of maize were sown in two pots, one pot containing 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 (Plate-3). After 6th week from sowing the disease symptoms was very prominent on seedling. On the 6th week the brown streaks were observed on stalk and subsequently converted into black colour. On spliting the infected stalk the pith was found rotted and in the same seedling showed hollow cavities .The black bundle phase was seen in the infected seedling. The seedling grown in sterilized soil did not show any disease symptoms which was served as control (Plate- 4).

4.2.3 Reisolation of the isolate

Reisolation was made from artificially inoculated plants of maize. The reisolated fungal culture resembled in all respects with the original culture used for inoculation (Plate-5).

4.3 Identification of culture

The pure culture obtained from infected maize stalks was identified upto species level on the basis of morphological characters and spore measurement and with the help of available literature.

As well as the isolate was confirmed as *Fusarium moniliforme* Sheldon from the Mycologist, Division of Plant Science, Agharkar Research Institute (M.A.C.S.), Pune (Plate- 6).

4.4 Morphological characters

Morphological characters of 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 to12 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 to46 x 3.0 to 3.5 µm (Plate-6).

4.5 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 and showed in Fig-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 percent 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%, percent inhibition, respectively. These treatments were found significantly different from each other.

Next to these treatments chlorothalonil (0.25%), hexaconazole (0.1%) were significantly at par with each other and showed 35 and 35.6 mm mycelial growth with and 50% and 49.04% percent 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.

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*	Percent 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 replication**

5. DISCUSSION

Maize (*Zea mays* L.) is one of the most important cereal food and fodder crop in India. 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. Studies on isolation and pathogenicity of pathogen, Morphological characteristic of the pathogen, and evaluation of different fungicides *in vitro* against the pathogen were carried out. The results obtained from the investigations are discussed here under.

5.1 Pathogen associated with stalk rot of maize

The isolations were made from infected stalk showing typical symptoms of stalk rot of maize. All the isolations were yielded *Fusarium moniliforme*. It indicated that the *Fusarium moniliforme* is strongly associated with stalk rot of maize. These results are in agreements with Sheldon (1904) in Nebraska , who reported the *Fusarium moniliforme* causing stalk rot disease to the maize.

These results are also in agreements with Reinking and Wollenweber (1927), Leonian (1932), Edward (1935), Hingorani (1964), Foley (1962). They reported that *Fusarium moniliforme* is associated with stalk rot of maize.

5.2 Pathogenicity Test

During the present investigations pathogenicity of the *Fusarium moniliforme* was tested by soil inoculation method. It has been found that *Fusarium moniliforme* was proved to be pathogenic and responsible for causing stalk rot of maize. 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.

5.3 Morphological characters of pathogen

During the present investigations it has been observed that colonies of *Fusarium moniliforme* were circular, compact with smooth margin; microconidia in chains, or in fall heads formed in white to isabella colour, spindle to ovoid in shape and measured 5-12 x 2-4 µm. Macroconidia were slender, sickle shape, pedicellate, scattered, brownish white to orange cinnamon, mostly three septate, measured 43-46 x 3.0-3.5 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 x3.3-4.9 µm. The non-septate microconidia measured 6.6-13.2 x 3.3-4.9 µm.

5.4 Evaluation of fungicides against pathogen responsible for stalk rot of maize

During the present investigations copper oxychloride (0.25%) was found most effective in checking the growth of *Fusarium moniliforme*. This fungicide shows absolutely no growth of fungus and showed cent percent inhibition of fungal growth.

These results corborate with the results obtained by Trivedi *et al.* (2002) and Honmane (2007). Trivedi et al. (2002) showed that copper oxychloride @ 100,250, 500 and 1000 ppm inhibited the growth of *Fusarium moniliforme*. However Honmane (2007) reported that copper oxychloride (0.25%) was found most effective against *Fusarium moniliforme* in which this fungicide showed complete inhibition of mycelial growth.

Next to copper oxychloride carbendazim (0.1%), thiram (0.2%) and thiophanate-methyl (0.1%) were found effective in checking the growth of *Fusarium moniliforme*. These fungicides showed 86.67%, 79.52%, and 71.90% inhibition of the fungus respectively.

These results are more or less in close agreements with the result achieved by Bohra *et al.* (2001), Trivedi *et al.* (2002), Muhammad *et al.* (2011), Raju and Lal (1977), Singh and Goswami (2003) and Honmane (2007).

Bohra *et al.* (2001) showed that carbendazim, 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 inhibit the growth of

Fusarium moniliforme. Muhammad *et al.* (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.

5. SUMMARY AND CONCLUSIONS

Summary

Maize (*Zea mays* L.) is one of the most important cereal food and fodder crop grown in the world. 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. The most important disease of maize crop in India is the stalk rot which caused 10-15 per cent losses.

Looking to the seriousness of this disease which incurred huge losses, it was decided to study the pathogen in detail and hence, the systemic studies on various aspects were undertaken and summarized below:

The isolation was made up from stem, collar region of wilted, rotted maize plant yielded pathogen *Fusarium moniliforme.*

The pathogenicity test of *Fusarium moniliforme* was carried out by sowing seeds in pot with sick soil culture technique, there by proved the Koch's postulates.

The pathogen was identified as *Fusarium moniliforme* with the available literature and get confirmed from the Mycologist, Division of Plant Science, Agharkar Research Institute (M.A.C.S.), Pune.

The colonies of *Fusarium moniliforme* was circular, compact with smooth margin with white in colour. Microconidia

were in chains, or in fall heads formed in white to isabella colour, mycelium, spindle to ovoid in shape and measured 5-12 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 43-46 x 3.0-3.5 µm.

In vitro evaluation of different eight fungicides, copper-oxychloride (0.25%) was found very effective in checking the growth and absolutely showed no growth of the fungus and showed 100 percent inhibition over control. It was followed by carbendazim (0.1%), thiram (0.2) and thiophanate-methyl (0.1%) these treatments found significantly different from each other.

The results obtained during the present investigations were concluded as below:

Conclusions

1. The fungal pathogen *Fusarium moniliforme* was found to be associated with stalk rot disease of maize in the Khandesh region of Maharashtra State.
2. Due to infection of *F. moniliforme* the affected plant showed slight pale colour or yellowish tinge, drooping of leaves. The plants were affected at collar region and development of dark streaks on rind and basal portion and blackening of vascular bundles and formation of cavity in basal internode in maize.
3. Colonies of fungus were circular, compact with smooth margin and colour of mycelium was white to isabella.

4. Microconidia were spindle to ovoid in shape and measured 5-12 x 2-4 µm and macroconidia were slender, sickle shape, pedicilate, scattered, brownish white to orange cinnamon, mostly three septate and were measured 43-46 x 3.0-3.5 µm.
5. Among all the fungicides screened copper-oxchloride (0.25%) showed complete inhibition of the mycelial growth of *Fusarium moniliforme,* while carbendazim (0.1%), thiram (0.2%) and thiophanate-methyl (0.1%) were found effective in checking the growth of fungus.

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*** originals not seen**

9. VITA

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