

Original Research Article

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Survey on the Incidence of Post Flowering Stalk Rot of Maize in Telangana State during *kharif* – 2019

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ABSTRACT

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Maize (*Zea mays* L.) is one of the important cereal crops of the world and world's third leading cereal crop, after wheat and rice. Maize is affected by various biotic and abiotic stresses. Among the biotic stresses, fungal diseases are one of the major constraints in realizing the potential yields of this crop. So keeping in view the importance of maize diseases a survey was carried out during kharif-2019, in order to study the incidence of post flowering stalk rot in 13 villages of four major maize growing districts of Telangana state, viz. Karimnagar, Khammam, Warangal and Rangareddy. The incidence of wilt in different areas surveyed was ranged from 27.0 to 76.8 per cent. The maximum wilt incidence of 76.8 per cent was recorded in Thimmapur village of Karimnagar district, followed by 65.6 per cent at Gundlapalli village of Karimnagar ditrict. The minimum wilt incidence of 27.0 per cent was recorded at Narsimhapuram village of Khammam district. The pathogen was isolated from the infected stem showing typical vascular discoloration symptoms by tissue segment method on potato dextrose agar medium (PDA). The associated pathogens were isolated and identified on the basis of morphological characters, in which 12 isolates of *F. verticillioides* and 5 isolates of *Macrophomina phaseolina* were recovered. During study *F. verticillioides* pathogen alone noticed in more than 60% of samples collected.

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. It is cultivated in tropics, sub tropics and temperate regions under irrigated and rainfed conditions. Globally, maize is known as queen of cereals, because it has the highest genetic yield potential among the cereals. In most of the developing countries maize is consumed directly as food. Maize

occupies an important place as a source of human food (26%), animal feed (11%), poultry feed (48%), industrial products (12%) and seed (3%) in India. Maize is cultivated in an area of 9380.07 thousand hectares with an annual production of 28752.8 thousand tons in India. In Telangana State, the crop is grown in almost all districts in an area of 630 thousand hectares with a production of 2555.64 thousand tonns and productivity of 4057Kg's/hectare (INDIANSTAT, 2017-2018). The other important maize growing

states in India are Karnataka, Bihar, Rajasthan, Maharashtra, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, and Himachal Pradesh etc. Maize is affected by various biotic and abiotic stresses. Among the biotic stresses, fungal diseases are one of the major constraints in realizing the potential yields of this crop. Of the fungal diseases, post flowering stalk rots poses a major threat to the productivity of maize crop. Post flowering stalk rot is complex disease which occurs at post flowering stage of the crop in both *kharif* and *Rabi* season. In India, eight fungi and three bacteria were reported to cause stalk rots (Raju and Lal, 1976). Among all, *Fusarium* stalk rot (*Fusarium verticillioides*), Charcoal rot (*Macrophomina phaseolina*), Late wilt (*Cephalosporium maydis*) are more prevalent and destructive in India (Khokhar *et al.*, 2014). Among the stalk rots, *Fusarium* stalk rot caused by *F. verticillioides* was first reported from USA by Pammel (1914) as a serious root and stalk disease. Later, Valleau (1920) reported that *F. moniliforme* was a primary cause of root and stalk rot of maize. In India, the disease was first reported from Mount Abu, Rajasthan (Arya and Jain, 1964) and prevalent in most of the maize growing areas of country where water stress occurs at the flowering stage of the crop.

The disease becomes apparent when crop enters senescence phase and severity increases during grain filling stage. The rotting extends from the infected roots to the stalk and causes premature drying, stalk breakage and ear dropping and thus resulting in reduction of maize yields (Colbert *et al.*, 1987). The disease causes internal decay and discoloration of stalk tissues, directly reducing yield by blocking translocation of water and nutrients, thus resulting in death and lodging of the plant (Dodd, 1980). The fungus survives on crop residues in the soil or on the soil surface. Field observations revealed the difference in virulence with in

Fusarium verticillioides populations from different conventional maize growing areas indicating the emergence of new pathotypes. Hence, a survey was carried out in maize growing districts of state to determine the extent of disease severity and to trace the favourable conditions for disease occurrence.

Materials and Methods

Survey, collection and isolation of stalk rot infected samples

An intensive roving survey was conducted during *Kharif* 2019 to assess the wilt incidence in different maize growing areas of Telangana viz., Karimnagar (Jammikunta, Veenavanka, Huzurabad, Thimmapur, Gundlpalli), Khammam (Allipuram, Thanikella, Narsimhapura, Chintakani), Warangal (Arepally, Oglapur), Rangareddy (Rajendranagar) districts. At each location, the disease incidence recorded by counting the number of wilted plants out of total number of plants in a 4 X 4m area at all the four corners as well as centre of the field (Ramesha and Naik, 2017). Per cent disease incidence was calculated by using the formula

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Maize plants showing typical symptoms were collected from different locations and used for isolation of the pathogen (Plate. 1). These samples were first washed with tap water followed by sterile distilled water. Diseased portions with some healthy portion were cut into small bits of 3-5 mm size, surface sterilized by dipping them in sodium hypochlorite (1%) solution for one minute and then 3-4 bits were transferred aseptically to petri plates containing potato dextrose agar (PDA) medium and were incubated at 25 ± 1°C in an incubator (Aneja, 2003).

Single spore isolation

The pathogen isolates from the infected tissue were further purified by single spore isolation method as described by Ho and Ko (1997). Spores were harvested from ten-day old culture with the help of an inoculation needle and suspended in one ml of sterile distilled water in a culture tube.

The suspension was vortexed for making a uniform suspension. Concentration of the spores was adjusted by addition of sterile distilled water. One ml of the spore suspension was transferred to petri plates containing 15 ml of 2 per cent water agar medium.

The plates were incubated at $25 \pm 1^\circ\text{C}$ for five hours. A well isolated single spore which initiated germination was located by observing the plate in an inverted position under a compound microscope.

Correspondingly a circle was marked around the spore over the plate and a circular disc of the medium containing the spore was picked up with the help of a sterile needle and transferred aseptically to petri plates containing PDA medium and incubated at $25 \pm 1^\circ\text{C}$ for further studies. Infected stalks collected were air dried under shade and kept for further studies.

Results and Discussion

A roving survey was conducted during *Kharif*, 2019 in different major maize growing areas of Telangana to assess the incidence of post flowering stalk rot and to isolate the associated pathogens. Survey was carried out in 13 villages of four major maize growing districts of Telangana and information on PDI, GPS, irrigated/unirrigated and soil type was recorded (Table 1 & Fig. 1). The incidence of wilt in different

areas surveyed was ranged from 27.0 to 76.8 per cent. The maximum wilt incidence of 76.8 per cent was recorded in Thimmapur village of Karimnagar district, followed by 65.6 per cent at Gundlapalli village of Karimnagar district. The minimum wilt incidence of 27.0 per cent was recorded at Narsimhapuram village of Khammam district.

During survey it was observed that, wilt incidence was comparatively more prevalent in red soils with low soil moisture holding capacity and high soil temperature under rainfed conditions. Over all the survey reports revealed that PFSR incidence varied from field to field or location to location and it may be due to wide genetic diversity in corn hybrids, environmental conditions prevailed at a particular location, cropping pattern and management practices followed by the farmers.

Another important reason could be water stress at critical stages of crop growth especially during flowering because nutrient uptake and water stress leads to severe wilt incidence.

This results are in agreement with the Usharani and Menakasivam, (2019) who reported that increase or decrease trend in the incidence may be due to intense cultivation and continuous availability of host, pathogen and agronomic practices followed by the farmer. Rameshaand Krishna Naik, (2017) also found that maximum disease incidence was noticed in Chikkaballapura district because of favourable conditions prevailing during flowering compared to other districts.

Khokhar *et al.*, (2014) also reported that the late sown crop suffered more because of heavy inoculum built up in the soil and moisture stress during flowering period and vice versa.

Table.1 Survey on the incidence of PFSR of maize in different maize growing districts of Telangana during *kharif*-2019

S.No	District	Mandal	Place of collection	Latitude & longitude	Soil type	Rainfed / Irrigated	*Wilt incidence (%)
1	Warangal	Shyampet	Arepally	18°50.1024'' N 79°37'20.892''E	Red soils	Rainfed	46.8 (43.13)**
2	Warangal	Atmakur	Oglapur	18°2'59.244'' N 79°39'53.53''E	Red soils	Rainfed	37.8 (37.86)
3	Rangareddy	Rajendranagar	Rajendranagar	17°19'33.15'' N 78°23'49.0416'' E	Black soils	Irrigated	49.2 (44.52)
4	Karimnagar	Jammikunta	Jammikunta	18°17'28.107'' N 79°27'50.74''E	Red soils	Rainfed	63.8(53.00)
5	Karimnagar	veenavanka	Veenavanka	18°21'7.96'' N 79°24'46.17''E	Red soils	Rainfed	59.6 (50.52)
6	Karimnagar	Huzurabad	Huzurabad	18°22'25.32'' N 79°50'46.33''E	Red soils	Irrigated	56.8 (48.89)
7	Karimnagar	Thimmapur	Thimmapur	18°16'22'' N 79°14'40''E	Red soils	Rainfed	76.8 (60.65)
8	Karimnagar	Medipalli	Gundlapalli	18°17'47'' N 79°6'33''E	Red soils	Irrigated	65.6 (54.07)
9	Khammam	Konijerla	Allipuram	17°13'25'' N 80°9'11''E	Red soils	Rainfed	28.4 (32.14)
10	Khammam	Konijerla	Thanikella	17°14'24.81'' N 80°15'22.87'' E	Black soils	Irrigated	32.0 (34.42)
11	Khammam	Thallada	Narsimhapuram	17°8'4'' N 80°11'5'' E	Black soils	Irrigated	27.0 (31.27)
12	Khammam	Chintakani	Chintakani	17°13'57.06'' N 80°12'5.2524'' E	Black soils	Irrigated	30.4 (33.43)
13	Khammam	Chintakani	Raghavapuram	17°8'4'' N 80°11'54'' E	Black soils	Irrigated	27.8 (31.79)
				CD (P = 0.05)			4.35
				SE(m)±			1.52
				C. V.			7.38

Table.2 Collection, isolation and identification of different pathogens associated with PFSR of maize in Telangana during *kharif* -2019

S. No.	Place of collection	District	Name of the isolate	Code allotted
1	Arepally	Warangal	<i>Fusarium verticillioides</i>	F-ISO-1
2	Oglapur	Warangal	<i>Fusarium verticillioides</i>	F-ISO-2
3	Rajendranagar	Rangareddy	<i>Fusarium verticillioides</i> + <i>Macrophomina phaseolina</i>	F-ISO-3+ M-ISO-1
4	Jammikunta	Karimnagar	<i>Fusarium verticillioides</i> + <i>Macrophomina phaseolina</i>	F-ISO-4+ M-ISO-2
5	Veenavanka	Karimnagar	<i>Fusarium verticillioides</i>	F-ISO-5
6	Huzurabad	Karimnagar	<i>Fusarium verticillioides</i>	F-ISO-6
7	Thimmapur	Karimnagar	<i>Fusarium verticillioides</i> + <i>Macrophomina phaseolina</i>	F-ISO-7 + M-ISO-3
8	Gundlapalli	Karimnagar	<i>Fusarium verticillioides</i>	F-ISO-8
9	Allipuram	Khammam	<i>Fusarium verticillioides</i>	F-ISO-9
10	Tanikella	Khammam	<i>Fusarium verticillioides</i>	F-ISO-10
11	Narasimhapuram	Khammam	<i>Fusarium verticillioides</i>	F-ISO-11
12	Chintakani	Khammam	<i>Fusarium verticillioides</i> + <i>Macrophomina phaseolina</i>	F-ISO-12+ M-ISO-4
13	Raghavapuram	Khammam	<i>Macrophomina phaseolina</i>	M-ISO-5

Fig.1 Survey on the incidence of post flowering stalk rot of maize in different maize growing areas of Telangana

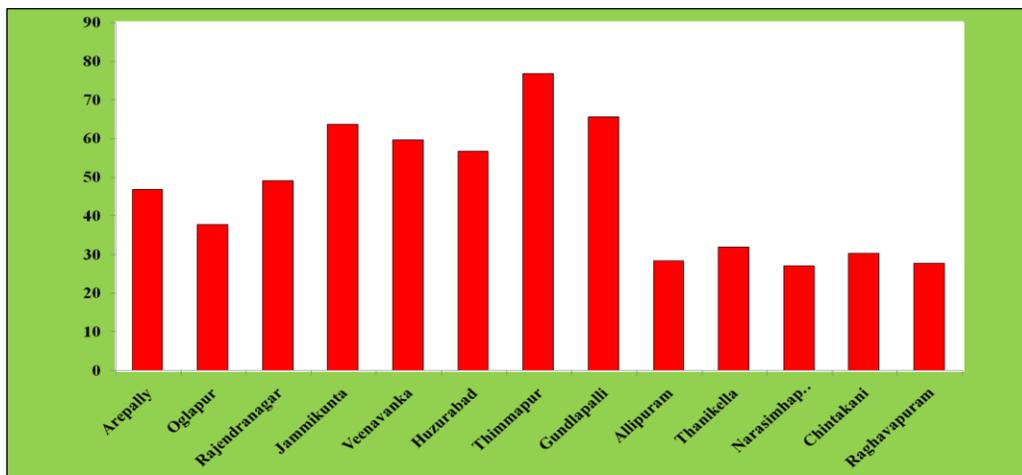


Plate.1 Symptoms of post flowering stalk rot of maize



(a) Wilt infected stem in healthy field

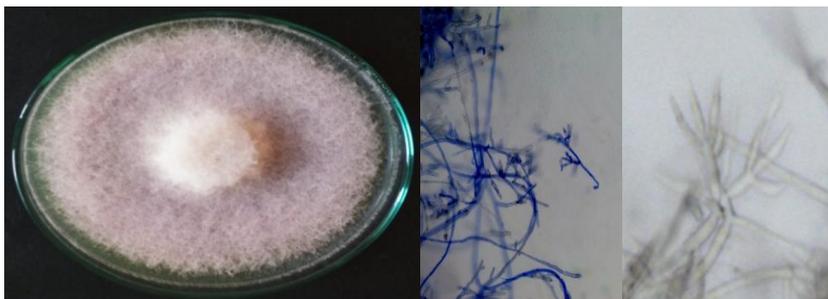
(b) Complete wilt infected field



c) Brownish discolouration of vascular in wilt affected stem

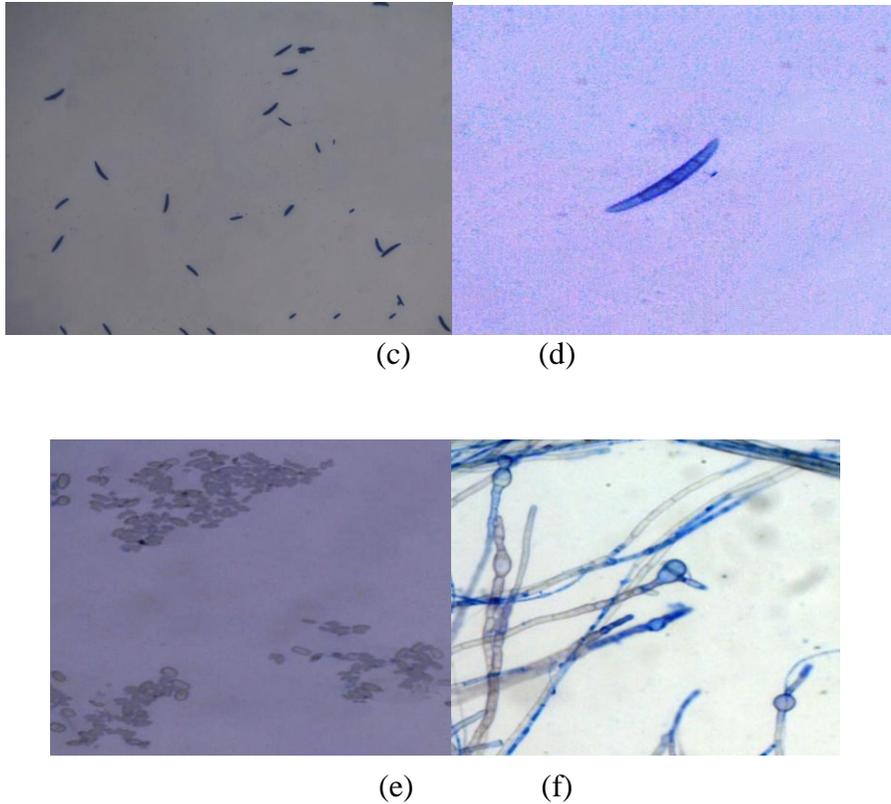
d) Black sclerotial bodies inside the stem tissue

Plate.2 Morphological characters of *Fusarium verticillioides* (F-ISO-7)



(a)

(b)



Symptomology

Typically two types of symptoms were observed in the field during the survey they are

Type 1

The stalk rot affected maize plants exhibited wilting appearance in batches at flowering. Showed symptoms of premature drying of lower leaves resulting in wilting or drying of the entire plant. The pith got dissolved, softened, stalk lost its strength and such stalks were straw colored and lodged. When we split open the stalks affected with stalk rot, light brown discoloration of the pith and some pink color pigmentation was observed. Pith region disintegrated but vascular bundles remain intact. Lodging was observed in severely affected plants. Infection of stalk was observed at 3rd intermodal regions from the stem to soil interface. These symptoms are in accordance with reports of earlier workers

Borah *et al.*, (2016) and Kenganal *et al.*, (2017) who reported the softening and discoloration of the stalks near the first three internodes from the bottom are due to *Fusarium verticillioides* stalk rot of maize. The similar symptoms also described by Hooda *et al.*, (2018) as a result of *Fusarium* stalk rot in maize.

Type 2

The Affected plant appeared with grayish streak on stalk. When stem cut open numerous minute black microsclerotia were observed on the shredded vascular bundles and on the inside of the stem, giving the interior parts of the stem a charred appearance. The crown region of the affected plant becomes dark in colour. Shredding of root bark and disintegration of root system were noticed. These symptoms are similar with reports of Smith, 1969 who confirmed that the disease is caused by soil borne fungus *Macrophomina phaseolina* (Tassi) Goid and

also in accordance with the description of Hooda *et al.*, (2018).

Isolation and identification of the fungus

The pathogens responsible for stalk rot of maize was isolated from affected plants showing typical symptoms on potato dextrose agar. Out of thirteen stalk rot samples used for the isolation of associated pathogens, from eight samples recovered only *F. verticillioides* and from four samples recovered both *F. verticillioides* and *M. phaseolina* indicating the complex nature of stalk rots, where as in one sample collected from Raghavapuram village with typical type two symptoms of charcoal rot yielded *M. phaseolin*. Dharanendra Swamy *et al.*, (2019) isolated 132 *Fusarium verticillioides* isolates and 18 isolates of *Macrophomina phaseolina* from survey conducted on PFSR of maize in Karnataka.

The isolates were allotted with the code of F-ISO for *Fusarium* isolates and M-ISO for *Macrophomina* isolates. The isolates were named as F-ISO-1 and F-ISO-2 collected from Warangal; F-ISO-3 and M-ISO-1 collected from Ranga Reddy; F-ISO-4, F-ISO-5, F-ISO-6, F-ISO-7, F-ISO-8, M-ISO-2 and M-ISO-3 from karimnagar and F-ISO-9, F-ISO-10, F-ISO-11, F-ISO-12, F-ISO-13, M-ISO-4 and M-ISO-5 from Khammam district (Table 2). Identification of these isolates was made based on morphological and cultural characters. The fungal colony of *Fusarium* isolates on PDA were initially white, floccose which turned purple to dark brown after 7 days of incubation at 28±2°C (Plate 2). Cultures developed pigmentation like pink, light purple, dark violet which varied with age.

Microconidia were hyaline, oval to club shaped with a flattened base and measured 5.12-6.41 X 2.04-3.18 (L×W). They were

formed from monophialides and were found in long chains. Macroconidia observed were sickle shaped, hyaline with apical cell curved and tapered, and basal cell notched. They were typically 4-6 celled with 3-5 septa and measured 20.01-31.12x2.01-3.21 (L×W) (Plate 1). Thaware *et al.*, (2017) also reported 8 isolates of *Fusarium oxysporum* f.sp. *cicero* with similar characteristics. Dharanendra Swamy *et al.* (2019) also confirmed these results in Karnataka. Based on the findings of cultural and morphological characters the pathogen was identified as *F. verticillioides*.

The mycelia growth was observed 24-48 h after inoculation on PDA medium in *Macrophomina* isolates. Within 6-7 days, the colonies became fluffy, carbonaceous, brown to black in colour covering the entire plate. With time numerous sclerotia developed throughout the colony. Mycelia were hyaline to grey or brown in colour, septate, branched, dendroid and frequently ran parallel to each other with right angled hyphae. Initially the hyphae was thin and became thicker with age. Sclerotia were black, varied from spherical to irregular in shape and measured 75 to 120 mm in diameter. Based on cultural and morphological characters the pathogen was identified as *M. phaseolina*. The morphology of the pathogen observed was in accordance with the description given for maize stalk rot pathogen *M. phaseolin* by Abhay *et al.*, (2020) and Iqbal *et al.*, (2018).

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