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Prevalence, cultural and morphological characterization of *Fusarium verticillioide* sassociated with ear rot of maize

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ABSTRACT

A roving survey was carried out in Ballari, Davanagere, Haveri, Koppal, Mandya, Mysuru, Raichur, and Shivamogga districts of Karnataka during rabi 2020-21 to determine the prevalence of ear rot of maize incited by Fusarium verticillioides. According to the survey data, Shivamogga had the highest disease incidence (36.10 %), followed by Ballari (28.34 %), while Raichur had the lowest (17.98 %). In eight maize-growing districts, the average percent incidence of Fusarium ear rot was 25.26. Twenty-four isolates of F. verticillioides were collected in different districts that varied in terms of cultural and morphological characteristics. All of the isolates had feathery mycelial growth with either a serrated or smooth edge, with colony diameters ranging from 68.33 mm to 89.33 mm and colony colors ranging from white to purple. The isolates generated septate, hyaline mycelia with verticillate branching ranging in width from 2.10 µm to 5.97 µm. Few elongated with blunt ends thin walled two to three-celled hyaline macroconidia ranging in size from 22.43 × 4.38 µm to 8.70 × 3.66 µm and plentiful single-celled oblong microconidia ranging in size from 7.78 × 2.75 µm to 4.49 × 2.30 µm formed in chains or cluster. Only Fv 9 isolate (Raichur) produced chlamydospore-like structures. The information generated during the survey will be useful in identifying endemic and risk-free areas of maize ear rot across districts. The pathogen isolates have variability concerning their cultural and morphological characters which depict the geographical distribution of the pathogen.

Keywords: *Disease incidence, ear rot, Fusarium verticillioides, maize, microconidia, mycelia, percent infection, starburst.*

INTRODUCTION

Maize popularly known as “corn” belongs to the family Poaceae of the tribe Maydae has been originated in Central Mexico 7000 years ago. It is the most versatile crop with wider adaptability in varied agroecological regions and has the highest genetic yield potential among food grain crops, hence earning the title "Queen of Cereals" [1]. Globally maize occupies an area of 193.7 mha with a production of 1161.86 mt accompanied with a productivity of 5.75 t/ha. In India maize is cultivated in an area of 10.1 mha with 33.6 mt production by recording a productivity of 3.3 t/ha [2]. Maize grain is rich in starch (72 %), followed by protein (10 %) and fat (4 %), which supplies an energy density of 365 kcal/100g. It has myriad end uses as a portion of human food (17 %), feed (61 %), and industrial products (22 %).

Ear rot of maize caused by *Fusarium verticillioides* is the newly emerging disease due to erratic and heavy rainfall that coincides with high relative humidity in the maize-growing countries and is reported to cause 10-30 percent yield loss. Symptoms typically transpire on random kernels or groups of kernels or physically injured kernels and appear tan or brown in colour, in case where fungal growth is visible on the ear, infected kernels appear whitish to pink colored and white streaks radiating from the point of silk attachment to the cap of the kernel known as the “starburst” symptom. Along with the contamination of grain with mycotoxins, it also has an impact on the seed's physical, physiological, and phytosanitary characteristics, as well as its nutritional quality [3].

F. verticillioides (Sacc.) Nirenberg (telomorph: *Gibberella moniliformis* Wineland) is both a saprophyte and parasite on maize. It can be found as a systemic endophyte in the symptomless biotrophic state or as a hemibiotrophic pathogen depending on the environmental conditions. It is widely distributed throughout the world and is notably connected to crops including sorghum, maize, rice, sugarcane, wheat, bananas, and asparagus[4].

In this view, the current inquiry was carried out to map the endemic and risk-free areas of *Fusarium* ear rot of maize across eight districts of Karnataka. Additionally, the cultural and morphological features of the *F. verticillioides* were investigated to know the variability of the isolates collected from the different geographical areas.

MATERIAL AND METHODS

Collection of samples: A roving survey was taken up to know the incidence and severity of *Fusarium* ear rot of maize in eight districts of Karnataka during *rabi* 2020-21. In each district, three taluks were selected and in each taluk, two villages were covered to collect five samples randomly from the field or market or godown to assess the percent infection of cob and percent infection of grain per cob was calculated by the formula[5].

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$$\text{Per cent infection of cob} = \frac{\text{Number of infected cobs}}{\text{Total number of cobs observed}} \times 100$$

$$\text{Per cent infection of grain per cob} = \frac{\text{Number of infected grains}}{\text{Total number of grains observed}} \times 100$$

Fungal isolation and maintenance: The isolation of *F. verticillioides* was carried out by following the standard tissue isolation method where the infected surface sterilized kernels were placed on potato dextrose agar medium containing 100 ppm streptomycin sulfate in Petri plates. The plates were incubated for seven days at 25 ± 2 °C. The hyphal tip technique was used to obtain the pure culture of the isolates. The pure culture of the pathogen was inoculated into the agar slants and stored at 4 °C. Twenty-four isolates were obtained and were designated as Fv 1 to Fv 24.

Cultural characterization: The isolates were cultured by inoculating a 5 mm culture disc which was taken from seven days old culture on onto the PDA medium at the center of each Petri dish and incubated at 25 ± 2 °C. The colony diameter of the fungal isolate was calculated by taking the average diameter measured on two axes at seven days post-inoculation with three replications. Cultural characteristics such as colony color, pigmentation, mycelial type, growth pattern, and colony margin were recorded for each isolate at seven days post inoculation along with mean mycelial growth.

Morphological characterization: The morphological characteristics of *F. verticillioides* isolates such as width of hyphae, length, and width of microconidia and macroconidia, their shape, number of septa, and presence or absence of chlamydospores-like structures were recorded under high power objective at 40x microscopic field. Spore dimensions were estimated for five spores of each isolate and their mean was calculated and further subjected to statistical analysis.

RESULTS

Disease incidence of *Fusarium* ear rot: The survey on *Fusarium* ear rot incidence in eight districts of Karnataka revealed that percent disease incidence varied widely among eight districts (Figure 1). The maximum per cent incidence of *Fusarium* ear rot was noticed in Kowthi village of Shivamogga district which accounts for 45.14 followed by Marakadadoddi of Mandya with per cent incidence of 41.87. The minimum incidence (11.79 %) was noticed in Askihal village of Raichur district. Among 24 taluks, the maximum incidence of ear rot (40.93 %) was recorded in Sagara followed by Malavalli with 37.54, whereas the minimum disease incidence (12.64 %) was noticed in Raichur. The disease scenario of eight districts exhibited that incidence was maximum in Shivamogga (36.10 %) followed by Ballari (28.34 %) and the minimum was found in Raichur (17.98 %). The average percent incidence of ear rot in eight maize-growing districts was 25.26.

Symptomatology: The major symptoms of ear rot in maize included tan or brown discoloration of the kernels, further white mycelial growth on the cob was noticed, as a result, the infected kernel appeared whitish to pink or salmon colored with the progress of the disease severity peculiar “starbursts” symptom which includes white streaks radiating from the point of silk attachment to the cap of the kernel were also observed (Figure 2). The symptom was scattered randomly on the kernel or group of kernels or physically injured kernels. This disease also affected the seed quality and quantity due to the contamination of fumonisin.

Cultural and morphological characteristics of *F. verticillioides:* All 24 isolates showed variation concerning the colony mean diameter. The maximum growth of 89.33 mm was recorded in Fv 6 isolate followed by Fv 5 and Fv 7 which showed 89.00 mm and 88.67 mm mean colony diameter, respectively, and are on par with each other. Fv 20 showed a minimum colony diameter of 68.33 mm (Figure 3). They have feathery mycelial growth patterns and the sectors were absent in all isolates except Fv 8. The colony color varied among the 24 isolates from whitish growth of the mycelium was seen in most of the isolates to the pinkish white color of the colony, which was noticed by the Fv 9 and Fv 10 isolates, whereas Fv 2 showed light pink color and purplish white color was exhibited by Fv 8 isolate. They showed differences with respect to pigmentation such as creamish white to purple. The variability found in the colony color of different isolates of *F. verticillioides* was associated with the pigment contained in the hyphal cell wall.

F. verticillioides had hyaline, septate mycelia with verticillate branching whose width ranged from 2.10 µm to 5.97 µm (Figure 4). Twenty-four isolates differed with sporulation ranging from 7.9×10^6 to 2.0×10^6/ml. *F. verticillioides* produced few hyaline, two to three-celled macroconidia which were elongated with blunt end shapes whose average size ranged from 22.43 × 4.38 µm to 8.70 × 3.66 µm. The hyaline, single-celled, oblong-shaped, abundant microconidia were formed in chains or clusters on V-shaped monophialides pairs to give a “rabbit ear” appearance. They had mean size ranging from 7.78 × 2.75 µm to 4.49 × 2.30 µm. Chlamydospores-like structures were observed only in Fv 9 isolate (Shiggaon, Haveri) and the remaining 23 isolates did not have such structures. These structures may indirectly indicate the fumonisin production capability, as well as the virulence of F. verticillioides, isolates.

DISCUSSION

Ear rot caused by *F. verticillioides* is newly emerging disease of maize that results in 10-30 percent reduction in yield across the globe. This investigation found that the average per cent incidence of ear rot in eight maize-growing districts was 25.26. The roving survey conducted during *Kharif* 2017 in major maize-growing districts of Karnataka has assessed the maximum disease incidence of *Fusarium* ear rot (47. 20 %) in Shivamogga [6]. The disease incidence of *F. verticillioides*-caused maize stalk rot ranged from 18 to 45 per cent in 10 districts of Karnataka. The information gathered during the survey revealed that the maximum incidence of *Fusarium* ear rot in Shivamogga was attributed to monocropping, leaving the crop residues especially damaged cobs in the field till the next season crop, inadequate storage facilities, favorable weather including high humid conditions and 25 to 27 °C temperatures along with continued rainfall towards the end of the crop period creates optimal condition for ear rot infection. Raichur had the lowest disease occurrence rates might be due to the crop was produced there in a dispersed manner and under extremely dry conditions that included temperatures above 30 °C and reduced relative humidity (60-70 %) that were unfavourable for fungal growth.

Fusarium ear rot, often known as "pink ear rot," is characterized by mycelia that range in color from white to pink or salmon and typically grow on the ear tip, but they can also appear in other parts of the ear or even dispersed throughout it. The affected kernels became tan or brown colored with whitish to pink mycelial filaments and in severe cases white streaks radiating from the point of silk attachment to the cap of the kernel known

as the "starburst" symptom was also noticed [8].

The indetailed studies on cultural features of *F. verticillioides* showed that the 24 isolates showed colony mean diameter ranging from 68.33 mm to 89.33 mm. The isolates of *F. verticillioides* divided into slow, medium, and fast growing with average colony growth of 63.7 to 74.8 mm, 75.7 to 79.5 mm, and 80.2 to 85 mm, respectively [9]. Even in another work, 20 isolates of *F. verticillioides* were classified into good, moderate, and poor growers with average colony growth > 80 mm, 70-80 mm, and 60-70 mm, respectively [6]. The 30 isolates of *F. verticillioides* are grouped into category-I (60-70 mm) and category-II (70-80 mm) [8]. The feathery mycelial colony color varied from white to purple, which is associated with the pigment contained in the hyphal cell wall. The 56 isolates of *F. verticillioides* showed considerable variability with respect to colony color and pigmentation varied from white and light pink to purple and dark purple colonies [9]. The *F. verticillioides* namely Fv SC-01 to Fv SC-04 differed in colony color (cottony pinkish white, purple violet, dirty white, and pinkish white) and pigmentation (pink, pink, violet, and brown) [10].

The morphological features of *F. verticillioides* include hyaline, septate mycelia with verticillate branching with width ranging from 2.10 µm to 5.97 µm as well as these are also differed with sporulation capacity ranging from 7.9 × 10^6 to 2.0 × 10^6/ml. *Fusarium* spp. infecting onion have hyaline, septate, and branched mycelia whose width ranged from 3.1-3.6 µm [11]. The pathogen produces few hyaline, two to three-celled macroconidia which were elongated with blunt end shapes, and the enormous amount of hyaline, single-celled, oblong-shaped microconidia and also chlamydospores-like structures are seen in local isolate. *F. verticillioides* forms microconidia oval to club-shaped with size 3.32-12.43 × 1.03-4.94 µm and macroconidia were rarely observed with apical cell curved and tapered and basal cell notched and measured 24.73- 35.94 × 4.61-7.22 µm and chlamydospores were totally absent[7].

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Conflict of interest: The authors confirm that they have no conflict of interest.

Future scope of the study: Future studies should concentrate on understanding the linkage between chlamydospore-like structures with fumonisin toxin production. As these cultural and morphological characters acts as a basement for identification of the pathogen, more concern should be placed on development of an intigrated management stratergy starting from pre-harvest up to it reaches to the consumer's hand.

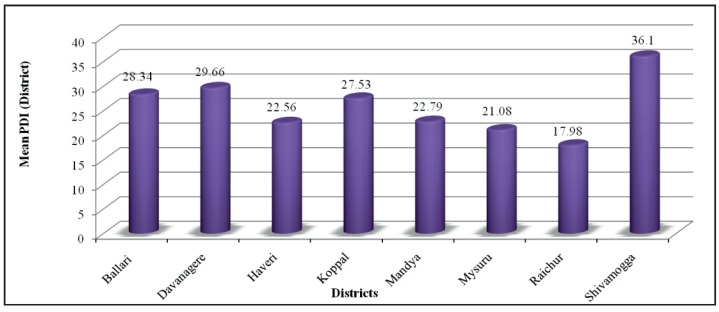


Figure 1. Status of ear rot of maize in different districts of Karnataka

Figure 2. Symptoms of ear rot of maize caused by F. verticillioides

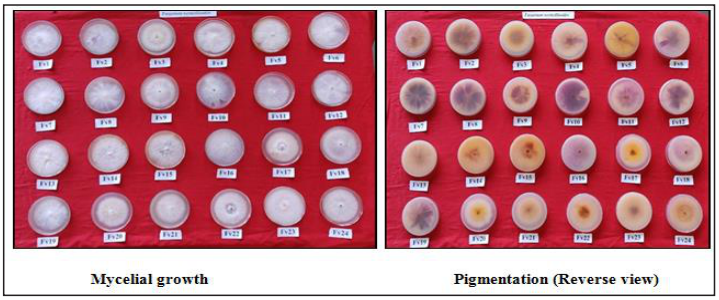
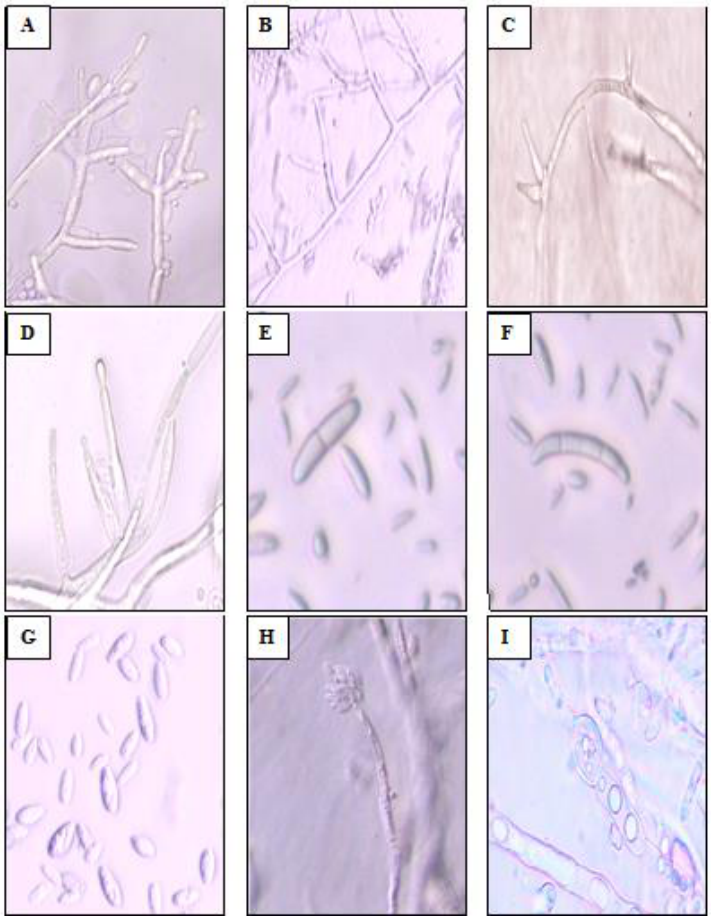


Figure 3. Cultural characteristics of 24 isolates of F. verticillioides



A: Septate hyaline mycelia with verticillate branching B: Conidiophore C and D: : V-shaped monophialides pair (rabbit ear appearance) E and F: Macroconidia with one and two septa G and H: Single celled free and cluster micronidia I: Intercalary chlamydospore like structure

Figure 4. Morphological characteristics of F. verticillioides.

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