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# Pathogenecity of Fusarium Spp. Incitant of Sugarcane Pokkah Boeng Disease

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**Abstract**

Pokkah boeng is one of the recently emerging disease in all sugarcane growing countries incited by *Fusarium* species complex. This study has mainly focused on ascertaining the pathogenicity of *Fusarium* spp. on the basis Koch's postulates. A roving survey was conducted in a total of 15 villages from seven mandals of Vishakhapatnam district of Andhra Pradesh such as Anakapalle, Chodavaram, Kasimkota, Butchayyapeta, Cheedikada, Munagapaka during *Kharif* 2020. Highest percent incidence was noticed from Anakapalle region (40 %). The infected leaf samples showing typical symptoms of pokkah boeng were subjected to pathogen isolation and isolated around 20 *Fusarium* isolates. All the isolates were tested for pathogenicity using stem injection method and proved Koch's postulates. Of twenty, nineteen isolates (F 1, F 2, F 3, F 4, F 5, F 6, F 7, F 8, F 9, F 10, F 11, F 12, F 13, F 15, F 16, F 17, F 18, F 19 and F 20) showed reddish brown round to oval lesions on 3rd or 4th leaf from the top with shot holes. Discolouration of the affected area with reddish spots and stripes and the affected leaf collapsed at the point of infection. Whereas the F 14 isolate showed chlorotic area with reddish brown spots or stripes at the base of third and fourth leaf with twisted foliage at the point of infection. These isolates further characterised and confirmed through cultural, morphological and molecular studies as *Fusarium sacchari* (F1- F20, excluding F14) and *F. andiyazi* (F14).

**Key Words:** Pathogenecity**,** Pokkah boeng Disease, Sugarcane, *Fusarium sacchari*

## 1. Introduction

Sugarcane is the world's largest crop in terms of production and top most important cash crop. India placed in 2nd position among top five largest producer countries next to Brazil, which increasing the concern of researchers towards its protection against biotic and abiotic factors. Among the biotic factors affecting sugarcane, fungal pathogens are drastically affecting the crop economy by producing severe necrotic symptoms. (Vishwakarma *et al.*, 2013).

Pokkah boeng disease, considered as a minor foliar disease earlier, is now emerging as a major disease, causing substantial losses in cane weight, length, girth, total juice and total sugars (Singh *et al.*,

2006). In India, *Fusarium sacchari*, *F. proliferatum* and *F. moniliforme* var. *subglutinans* were found associated with Pokkah boeng disease.

Pokka boeng, recently well established, disease is dependent upon the environmental conditions, handling of the plants and quality of setts *e.g.*, exposing sugarcane plants to stress either from water stress, temperature, soil nutrition or pH. Hail damage can cause cane plants to be easily vulnerable to diseases due to the bruised stalks and broken leaves, giving the diseases access to the damaged setts. Some of the favourable conditions for disease development included drenched conditions of the soil, lack of cultural practices that result in the growth of weeds, constant cultivation of same variety in the field and existence of susceptible varieties in the surroundings. It is important for a farmer to prevent and control pests and diseases to avoid losses. *Fusarium* species complex can produce many kinds of toxic secondary metabolites known as mycotoxins, which can easily enter humans and animals through food and feed because of their resistance to milling, processing, and heating (Marasas *et al.*, 1991).

Pokkah boeng disease was differentiated into three categories *viz*., mild, moderate and severe on the basis of symptoms observed in the field given by AICRP on sugarcane, Technical Report-Plant Pathology (2020-21), SBI, Coimbatore.

**Mild** - Green plants with Pokkah boeng (curling/twisting of spindle leaves, tearing of leaves, whitish/chlorotic streaks on the leaves) at varying intensities.

**Moderate** - Yellowing of 3$^{rd}$ or 4$^{th}$ top leaf followed by complete yellowing of foliage and expression of top rot symptom.

**Severe** - Yellowing of leaves and discolouration (light coloured) of stalks + wilting symptom in opened stalks.

Under field conditions symptoms observed mostly on foliage as initial symptoms of the disease showing chlorotic or whitish areas at the base of the young leaves (3$^{rd}$ or 4$^{th}$ leaf). Frequent twisting, curling and bending of spindle leaves from the top portion, yellowing of foliage with shot holes at later stage with varying intensities indicating malformed or damaged top in highly susceptible varieties. Top rot symptoms in advanced stage of infection. The symptoms of knife-cut stage were observed in association with the acute (top rot) phase of the disease characterized by one or two or even more transverse cuts in the rind of the stalk /stem.

This study is mainly focussed on the testing pathogenicity of isolates obtained from diseased samples of sugarcane pokkah boeng disease.

## 2. Material and Methods

In this present investigation, Pokkah boeng disease of sugarcane incited by *Fusarium* spp. in Visakhapatnam district was thoroughly studied and proved Koch's postulates in order to provide scientific reference for pathogenicity and disease occurrence.

A roving survey was carried out during *kharif* 2020-2021 in the months of September-October to assess the incidence and severity of Pokkah boeng disease in major sugarcane growing mandals of Visakhapatnam district of Andhra Pradesh. The disease incidence in each cultivar was recorded by counting the number of infected plants out of the total number of plants assessed per cultivar and was expressed in percentage (Verma *et al.*, 2020).

### 2.1.Disease sample collection

The field visits in different mandals of Visakhapatnam district *viz.*, Munagapaka Chodavaram, Cheedikada, Anakapalle, Makavarapalem, Butchayyapeta and Kasimkota, were made between September and October months in the year 2020.

The samples of Pokkah boeng disease that were exhibiting clear cut symptoms *viz.*, chlorosis, knife cut and top rot stages were collected and diseased specimens were wrapped in clean polythene bags, labelled properly and brought to the laboratory for isolation of the causal organism for further studies. A total of 20 samples collected from different locations during the survey were kept inside the refrigerator till isolation.

### 2.2. Retrieving causal organism from the diseased sample

Leaf samples collected from survey were brought to the laboratory for further assessment of the causal organism of Pokkah boeng disease. Petriplates placed with exact fit blotting paper were made wet by sprinkling sterile distilled water. Inside that moistened plate, a glass slide having small bit of diseased specimen was placed and closed the lid. This was kept under room temperature for 48 hrs. Later the mycelial growth on the slide was observed under microscope and captured the photographs of fungal morphology from different isolates such as micro and macroconidia, mycelial branching, phialide arrangement and septation *etc.* for initial confirmation of the pathogen identity. All the samples collected during survey were brought to the laboratory for isolation of the pathogen. Infected plant parts were washed thoroughly in running water to remove dust particles and thereafter placed in between blotting paper to remove excess moisture, if any. The infected portion was critically observed for symptoms of disease.

The diseased parts of leaves and top rotted canes were cut with the help of sterilized blade and made into pieces (1 mm size) having half healthy and half diseased parts. These pieces were dipped in 0.1% mercuric chloride solution for about one minute followed by two to three washings with sterilized distilled water and placed on blotter paper to remove excess moisture. The sterilized and melted medium was poured aseptically in sterilized Petriplates at the rate of 15 to 20 ml media per plate, approximately. The diseased tissues of Pokkah boeng fungus were picked up, surface sterilized with 1% sodium hypochlorite for 1 min. and washed twice with distilled water and transferred aseptically to previously sterilized potato dextrose agar (PDA) plates in laminar flow cabinet. The inoculated Petriplates were incubated at 28 ± 1°C for 4 days in BOD incubator and observed for cultural and morphological characters for genus confirmation. The fungus was sub cultured on the PDA slants and allowed to grow at 28 ± 1°C temperature for one week and later the culture was stored in refrigerator at 4°C for further studies and was sub cultured periodically.

#### 2.2.1. Identification of Pathogen through Microscopic Study

All the fungal culture isolates were identified on the basis of their cultural and microscopic characters. Cultural characters of isolates were observed and recorded from the mycelial colonies of the pathogen grown on Potato Dextrose Agar medium (PDA). Microscopic study was done by slides prepared in cotton blue stain and examined under compound microscope.

### 2.3.Proving the pathogenicity of the isolates

Single node seedlings of Pokkah boeng susceptible sugarcane variety '2009A 107' were raised in portrays containing cocopeat and vermicompost (1:1) as potting mixture. Four seedlings of thirty day old were transplanted into cement pots of 80 kg capacity. To prove the pathogenicity, four seedlings in each pot were inoculated with 2 ml conidial suspension ($1x10^6$ conidia/ml) of each isolate 30 days after transplantation by stem injection method (Das and Patil, 2015). Observations on the type of symptoms developed after inoculation were recorded for each isolate from the first appearance of symptoms. The test pathogen was re-isolated from the infected plant parts to prove Koch's postulates for each isolate (Waraitch, 1982).

### 2.4.Cultural and molecular characterization of *Fusarium* isolates

Pathogenic isolates were cultured on potato dextrose agar and carnation leaf agar medium for cultural and molecular identification. Using modified CTAB method DNA of all the isolates were extracted, amplified with ITS 1 and $ universal primers later sequenced and phylogenetically analysed to check the relatedness of all the seqences from F1 to F20.

## 3. Result and Discussion

### 3.1. Isolation of pathogen

Using standard tissue isolation method, plant samples showing clear cut symptoms of pokkah boeng disease were subjected to pathogen isolation. A total of twenty isolates recovered from the infected leaf samples were subjected to pathogenicity test and the results are discussed in detail further (Anuradha *et al.*, 2019).

### 3.2.Pathogenicity test

All the *Fusarium* isolates were tested for their pathogenicity on sugarcane using stem injection method using conidial suspension. In this method the potting mixture of cocopeat and vermicompost (1:1) filled in portrays and single node seedlings of sugarcane variety, 2009A 107 were raised. Thirty-day old seedlings were transplanted into cement pots (four seedlings in each pot).

A month after transplanting, 2 ml of conidial suspension ($1x10^6$ conidia/ml) of each isolate was injected into the stem. Both (inoculated and uninoculated) the pots were watered adequately and maintained for further studies.

Symptoms of Pokkah boeng of all inoculated *Fusarium* isolates started appearing on the young leaves at four to five days after inoculation. The symptoms include production of chlorotic area on the base of $3^{rd}$ or $4^{th}$ leaf from the top with reddish brown round to oval spots or stripes in the affected area. The progress of symptoms on the foliage led to twisting of the foliage at the point of inoculation followed by drying of foliage or cutting of foliage at the affected area. The different symptoms produced by *Fusarium* species obtained in the study were presented in the Table 1 and Figure 1.

**Table 1. Pathogenicity of *Fusarium* sacchari and *F. andiyazi* causing Pokkah boeng disease of sugarcane**

| Test isolates | Incubation period | Pokkah boeng disease symptoms |
|---|---|---|
| F 1, F 2, F 3, F 4, F 5, F 6, F 7, F 8, F 9, F 10, F 11, F 12, F 13, F 15, F 16, F 17, F 18, F 1 9, F 20. | 3-5 days | Reddish brown round to oval lesions on 3rd or 4th leaf from the top with shot holes. Discolouration of the affected area with reddish spots and stripes and the affected leaf collapsed at the point of infection |
| F 14 | 5 days | Chlorotic area with reddish brown spots or stripes at the base of third and fourth leaf with twisted foliage at the point of infection. |

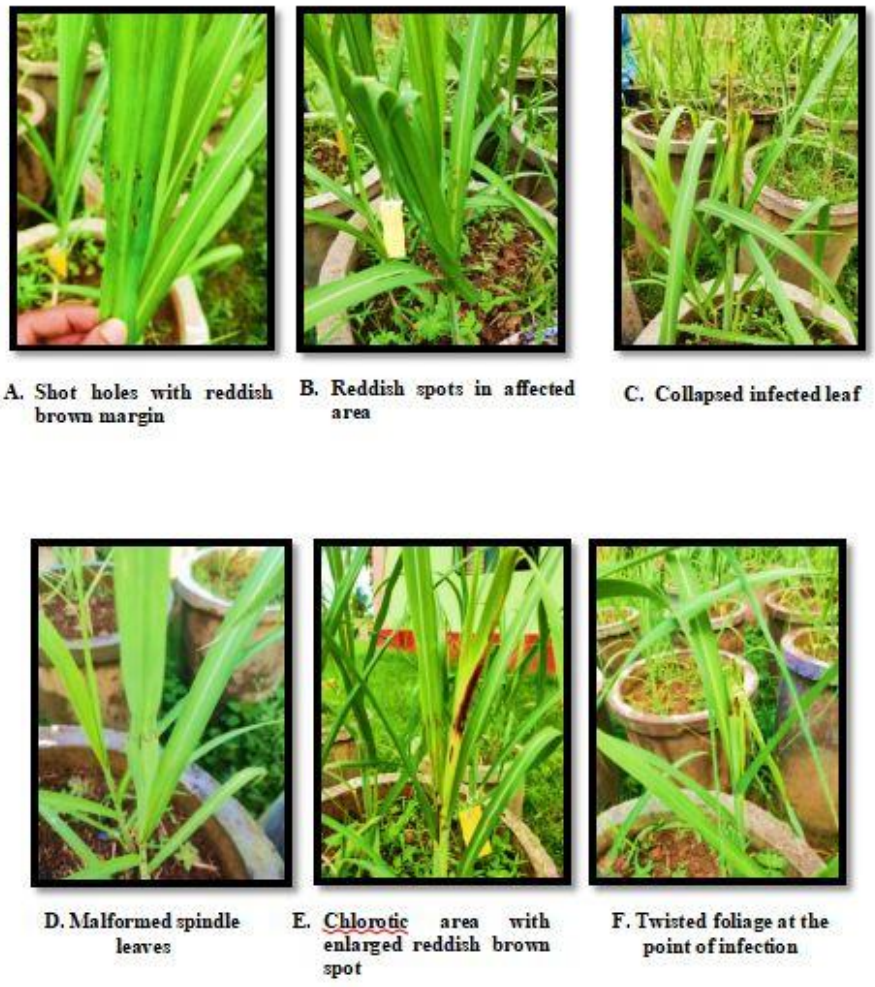


**Figure 1. Pathogenicity of *Fusarium* sacchari and *F. andiyazi* causing Pokkah boeng disease of sugarcane**

The test pathogen was re-isolated aseptically on PDA plates from artificially Pokkah boeng affected sugarcane plant, studied and compared its cultural and morphological characteristics with the original culture of *Fusarium* isolates obtained from naturally Pokkah boeng diseased sugarcane plants.

### 3.3.Cultural and molecular characterization of *Fusarium* isolates

The isolates showing pathogenicity were further characterised via cultural and molecular studies and confirmed as *Fusarium sacchari* (F1 to F20 except F14), and *F. andiyazi* (F14) Figure 2. Similar results were observed by Sharma and Kumar (2015) while studying variability in *Fusarium moniliforme*, the causal agent of Pokkah boeng.

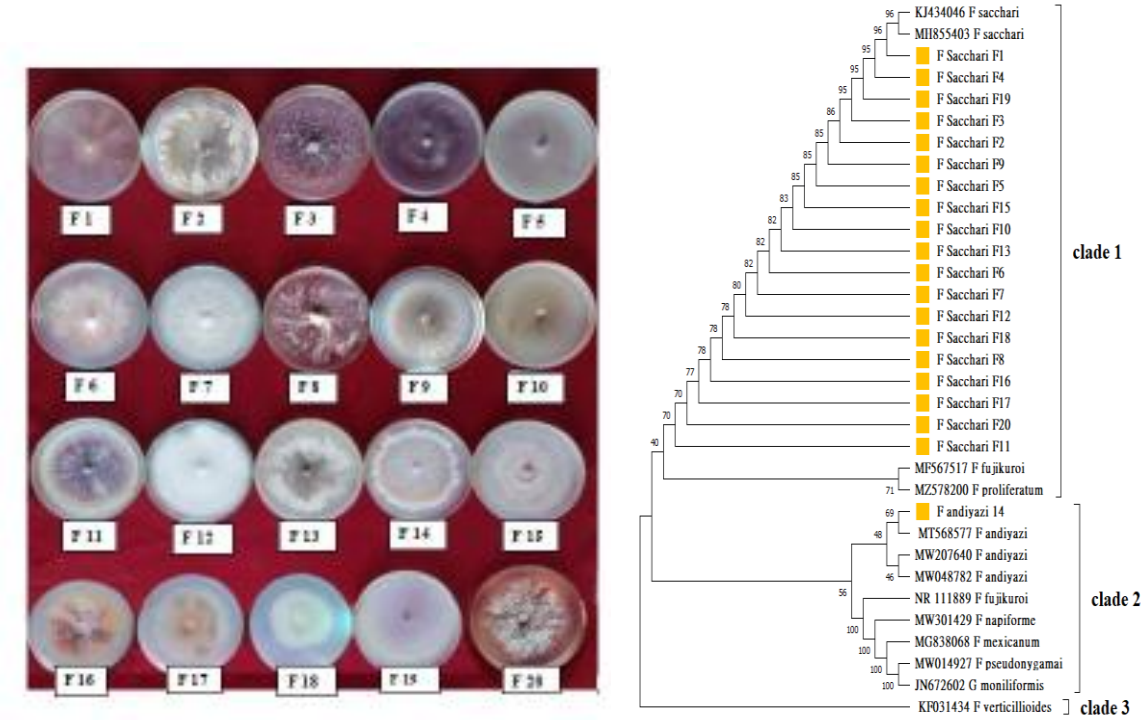


**Figure 2. Cultural and molecular characterization of *Fuarium* isolates**

### Conclusion

This extensive study on pathogenicity of *Fusarium* isolates provided needful information about the identity of causal agent of pokkah boeng disease of sugarcane. The susceptible variety 2009A 107 was clearly showed distinguishable symptoms produced by different isolates of *Fusarium* spp. thereby indicating the variability among the isolates in terms of pathogenicity possessed among them. This study reporting the peculiar nature o *F. sacchari* and *F. andiyazi* with respect to symptom production on the same cultivar. Later this pathogen identification was also supported by cultural and molecular characterization which is confidently concluding role of pathogen in causing the disease.

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