Impact of Inoculation Methods and Fruit Maturity on Development of Fusarium Fruit Rot (Fusarium musae) Disease in Banana

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ABSTRACT
Background: Fusarium fruit rot symptoms appear at all stages of banana fruit once infection initiates and progress from the stylar end as dark brown to black small spots which eventually turns black rotten areas leading to rotten pulp which renders the fruit unmarketable. Very meagre research work has been done on Fusarium fruit rot disease of banana in India and hence the present investigation on Fusarium fruit rot diseases of banana was undertaken south Gujarat condition.

Methods: During the period 2018-2019 tested different inoculation methods were tested on the development of banana Fusarium fruit rot disease viz., stylar end pricking, rubbing, pricking at epicarp and without injury on banana fruit. Disease severity and incidence were recorded at 4th and 8th day after inoculation. In order to find out the most vulnerable stage of fruit for initiation of infection and development of Fusarium fruit rot disease, three stages of fruits were selected i.e., unripe, semi-ripe and ripe.

Conclusion: Among, the different methods with fruit injury, stylar end pricking method was found best for the infection and development of Fusarium fruit rot (34.14%) after 8 days of inoculation followed by pricking at epicarp (27.79%). Further it was observed that injury of fruit surface is essential for infection and development of Fusarium fruit rot in banana. However, the severity of Fusarium fruit rot revealed that the highest severity was recorded in ripe fruits (30.21%) as compared to semi ripe (30.08%) and unripe (14.70%) fruits.

Key words: Banana, Fruit maturity stages, Fusarium fruit rot, Fusarium musae, Inoculation methods, Post-harvest.

INTRODUCTION
Banana (Musa paradisiaca L.) is one of the most important commercial fruit crop grown all over the world in the tropical and subtropical areas. It is the second largest fruit crop which belongs to the family Musaceae and the order Zingiberales. It is indigenous to Indo-Malayan region. The banana fruit infecting pathogen Fusarium musae was originally known as a distinct population within Fusarium verticillioides. However, Van Hove et al., 2011 showed by multilocus phylogeny and mating experiments that this population represents a unique lineage with in the Fusarium fujikuroi species complex (FFSC) and consequently they installed the new species Fusarium musae being closely related to (i.e. sister species) but distinct from Fusarium verticillioides. An alternative hypothesis is that Fusarium musae is not only present on banana fruits, but also on other plant hosts or environmental sources. In a more recent survey performed laboratory testing 390 fungal isolates collected between July 2012 and July 2013 from 2 hospitals located in Brussels, one Fusarium musae strain was found among the 20 Fusarium isolates identified. This Fusarium musae strain was isolated from a blood sample of an immune-suppressed patient (Triest et al., 2016). Fusarium rot symptoms appear on all stages of banana fruit once infection initiates and progress from the stylar end as dark brown to black small spots which eventually turns black rotten areas leading to rotten pulp which renders the fruit unmarketable. The Fusarium musae is widely distributed throughout the tropics as a common pathogen and saprophyte on bananas. It is responsible for a black spot disease of leaves, crown rot disease of fruits and spotting of fruits. In a saprophytic capacity, the fungus occurs on pseudosterns, leaves, bracts and flower parts in plantation. Very meagre research work has been done on Fusarium fruit rot diseases of banana and their management in India, therefore to extend the shelf life of banana fruits and to reduce the losses caused by post-harvest diseases; it is felt worthwhile to carry out the investigations on Fusarium fruit rot diseases of banana and its management under South Gujarat condition. Therefore, the present investigation was undertaken to identify the most vulnerable stage of the fruit and most appropriate inoculation method with respect to fruit rot in banana.

MATERIALS AND METHODS
Identification of pathogen
Identification of the pathogen was carried out by studying
the Cultural and morphological characters. The microphotographs of mycelium and spore structure were taken with the help of digital camera. The pure culture was sent to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi – 110 012 for identification.

Impact of inoculation methods on development of Fusarium fruit rot disease

Healthy, uniform semi-ripe fruits of banana cv. Grande Naine were collected from the fruit market, Navsari and brought to the laboratory in paper bags. The fruits were washed with tap water, then surface sterilized by dipping in 0.1 per cent NaOCl solution for one minute followed by three washings with distilled sterile water. The experiment was conducted in completely randomized design (CRD) with five replications. The fruits were injured by the following methods.

Styler-end pricking
The fruits were injured at styler-end with sterilized pins fixed on cork and dipped in spor suspension (10^6 cfu/ml) of the test pathogen.

Rubbing
The fruits were rubbed with each other by shaking manually for five minutes in crate and then the fruits were dipped in spor suspension (10^6cfu/ml) of the test pathogen.

Pin pricking on epicarp
The fruits were injured by pin pricking with sterilized pins fixed on cork of epicarp of fruit upto 2mm depth. The fruits were dipped in spor suspension (10^6 cfu/ml) of the test pathogen for 15min.

Without injury (Control)
The fruits without injury were dipped in spor suspension (10^6 cfu/ml) of the test pathogen for 15min. The inoculated and uninoculated fruits were placed in sterilized polythene bags. One fruit was accommodated in one bag. A piece of sterilized moist absorbent cotton swab was placed inside the bag and the mouth of the bags was loosely tied with rubber bands. The bagged fruits were kept at 27±2°C temperature. The observations on disease incidence and severity were recorded on 4th and 8th day with the help of assessment key.

Assessment key used for severity of Fusarium fruit rot disease of banana

| Scale | Per cent infection |
|-------|--------------------|
| 0     | 0%                 |
| 1     | 1-10%              |
| 2     | 11-20%             |
| 3     | 21-40%             |
| 4     | 41-50%             |
| 5     | > 60%              |

Severity (%) = \[
\frac{\text{Area of infected fruits}}{\text{Total area of fruit tissue}} \times 100
\]

RESULTS AND DISCUSSION

Identification of the pathogen

After purification of the pathogen, the cultural and morphological characters were studied for identification of the pathogen. The pure culture of the pathogen obtained was sent for identification to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi and was identified as Fusarium musae (ID. No. 11,229.19). Thus, the causal agent of fusarium fruit rot of banana in south Gujarat was confirmed as Fusarium musae.

Cultural and morphological characteristics of Fusarium musae

The fungi Fusarium musae produced white to light pink mycelial growth with abundant sporulation on PDA covering the entire Petri plate (90 mm) after 8 days of incubation at room temperature (27±2°C). In initial stage the fungal growth was white and rapidly becoming light pink. (Plate 1). The pathogen produced circular, fluffy and initially white mycelial growth and further showing light pink colour on PDA medium. The mycelium was hyaline and septate with slightly bulged compartments. Conidiophore arises singly or in small groups directly from immersed septate and it was 11-60μm x 2-4μm long. Microconidia abundant, borne in chains or false heads, hyaline, claviform or ellipsoidal, often truncated, aseptate or rarely one septate 5-17.5 x 1.5-4μm. Sporodochia absent. Macroconidia absent. Rare hyaline pseudochlamydospores single and globose, at the end of hyphae, 12–14μm (Plate 1). The cultural and morphological characters of the fungus were found similar to that of Fusarium musae causing fusarium fruit rot disease of banana.
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banana earlier reported by Van Hove et al. (2011). Thus, the causal agent of Fusarium fruit rot disease of banana in South Gujarat was confirmed as Fusarium musae.

Impact of inoculation methods on the development of Fusarium fruit rot disease

Tested different inoculation methods on the development of banana Fusarium fruit rot disease viz., stylar end pricking, rubbing, pricking on epicarp and without injury with Fusarium musae on banana fruit and recorded disease severity and incidence at 4th and 8th days after inoculation (Table 1). On 4th day after inoculation significantly highest Fusarium fruit rot severity was recorded (11.45%) in stylar end pricking method followed by pricking on epicarp (9.35%). Whereas, rubbing method (7.06%) found least effective in causing Fusarium fruit rot infection. Similar trends were recorded on 8th day after inoculation in which significantly highest Fusarium fruit rot severity (34.14%) was recorded in stylar end pricking method followed by pricking on epicarp (27.79%). Whereas, rubbing method (14.31%) was found least effective in causing Fusarium fruit rot infection. Fusarium fruit rot symptoms did not observe on fruits when inoculated without any injury. The results clearly indicated that the injury on fruit was essential for infection and further development of Fusarium fruit rot as Fusarium musae is a weak pathogen. Pin-pricking on stylar end, rubbing and pricking on epicarp exhibited 100 per cent disease incidence. Results similar to the present findings were reported by Zoier et al. (2017) inoculated different pathogens viz., Colletotrichum musae, Fusarium moniliforme, Thielaviopsis paradoxa, Fusarium roseum and Fusarium solani by small scratch, then inoculated by spore suspension of each fungal isolates (1×10^6 spores/ml) from 7 days old cultures. Singh (2011) tested different inoculation methods of Fusarium moniliforme on banana fruit by stem-end pricking, stylar-end pricking, pricking on epicarp, rubbing and without injury. Among all, stem end pricking method was found most effective for development of Fusarium rot.

Impact of fruit maturity on development of banana Fusarium fruit rot disease

In order to find out the most vulnerable stage of fruit for initiation of infection and development of Fusarium fruit rot disease, three stages of fruits were selected i.e., unripe, semi-ripe and ripe. The results presented in Table 2 revealed that maximum Fusarium fruit rot severity was recorded in ripe fruits as compared to semi-ripe and unripe fruits. On 4th day after inoculation significantly highest Fusarium fruit rot severity was recorded in ripe fruits (16.23%). While in semi ripe fruits it was (15.29%). Lowest per cent speckle severity was recorded in unripe fruits (8.03%). Similar trends were recorded at 8th day after inoculation, significantly highest Fusarium fruit rot severity was recorded (30.21%). While in semi ripe fruits it was (30.08%). Lowest per cent speckle severity was recorded in unripe fruit (14.70%). It is clear from the result that as the fruit

Table 1: Effect of different inoculation methods on severity and incidence of Fusarium fruit rot of banana.

| Inoculation Methods     | 4th day  | 8th day  | Disease incidence (%) |
|-------------------------|----------|----------|-----------------------|
|                         | Fusarium fruit rot severity (%) |            |                       |
| Stylar end pricking     | 19.77**  (11.45)*| 35.73**(34.14)*| 100                    |
| Rubbing                 | 15.39 (07.06)| 22.16 (14.31)| 100                    |
| Pricking on Epicarp     | 17.79 (09.35)| 31.79 (27.79)| 100                    |
| Without injury (Control)| 0.00 (0.00)| 0.00 (0.00)| 0.00                   |
| SEm ±                   | 0.36      | 0.79      |                       |
| CD at 5%                | 1.07      | 2.38      |                       |
| CV %                    | 6.01      | 7.93      |                       |

*Figure in parenthesis is original value and **outside is arcsine transform value; DAI: Day after incubation.
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Table 2: Impact of fruit maturity stages on the development of *Fusarium* fruit rot disease of banana.

| Fruit ripening stage | 4th day | 8th day |
|----------------------|---------|---------|
| Unripe fruits        | 16.43** (08.03)* | 22.51** (14.70)* |
| Semi-ripe fruits     | 23.00 (15.29) | 33.25 (30.08) |
| Ripe fruits          | 23.75 (16.23) | 33.33 (30.21) |
| Without injury (Control) | 0.00 (0.00) | 0.00 (0.00) |

SEM ± 0.39 0.54
CD at 5 % 1.18 1.62
CV % 5.58 5.43

*Figure in parenthesis is original value and **outside is arcsine transform value. DAI: Day after incubation.

It was observed that injury to fruit surface is essential for infection and development of *Fusarium* fruit rot in banana. The studies on impact of fruit maturity in relation to severity of *Fusarium* fruit rot revealed that the highest *Fusarium* fruit rot severity was recorded in ripe fruits (30.21%) as compared to semi ripe (30.08%) and unripe (14.70%) fruits after 8th day of inoculation.

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