Research Article

Effect of Different Media, pH and Temperature on Growth and Sporulation of *Fusarium verticillioides* causing Crown Rot of Guava

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Abstract

Laboratory studies were conducted to study the effect of different culture media, pH and temperature on mycelia growth, sporulation and dry mycelia weight of *Fusarium verticillioides.* The fungus grew the best on PDA and Richard's agar media among seven culture media were tested. The most suitable pH level for growth of fungus was 6.0 and 6.5 with excellent sporulation. Growth of *F. verticillioides* was maximum at 27^0C after seven days of inoculation, which was reduced drastically below 18^0C and above 30^0C.

Keywords: Media, pH, Temperature, *F. verticillioides*, Crown rot, Guava

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Introduction

Guava (*Psidium guajava*) belonging to family Myrtaceae are well-known tropical fruits and are included among the "super fruits" due to its nutritive value. The medicinal properties of guava fruit, leaves, and other parts of the plant are also well-known in the traditional medicine [1]. Its fruit comprises high amounts of vitamins A, B1, B2, dietary minerals, lutein, zeaxanthine, lycopene, folic acid, potassium, copper, and manganese [2]. However, guava fruit ripens rapidly and are perishable due to their climacteric nature. Fruit ripening in guava is characterized by loss of green color, softening, shrinkage, loss of brightness and rot development [3].

In guava fruits, fungi are the causal agents of anthracnose, light blight, stem rot, and crown rot. Crown rot of guava caused by *Fusarium verticillioides* is gaining importance in Madhya Pradesh state of India owing to introduction of Thai variety of guava. Infection results in the appearance of discolored, malformed, or necrotic areas on the fruits. During infection, the pathogens grow, multiply, establish contact within the plant tissues, and procure nutrients from them. Guava fruits infected with fungal disease had a significantly lower amount of carbohydrates, crude fiber, ash, fat, protein, calcium, iron, and phosphorus.

Environmental factors such as temperature, water activity and pH have a great influence on fungal development [4]. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influence on the growth of pathogen [5-7]. Present work depicts the role of different pH, media and temperature to understand ecological survival of pathogen which will be helpful in management strategy in the field.

Material and Methods

Isolate of *Fusarium verticillioides* was recovered from diseased guava plants from research farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya-Jabalpur. The fallen fruits are badly affected. The skin of the fruit below the whitish cottony growth becomes a little soft, turns light pink to dark. Infected fruit portions of guava fruit was cut into small fragments (1-2 cm) and surface-sterilized in 0.1% mercuric chloride for 30 sec. They were then washed three times in sterile distilled water, plated on potato dextrose agar (PDA) medium containing streptomycin sulphate and incubated at room temperature (28±2°C) for seven days. Based on cultural and morphological characters the fungus was identified as *Fusarium verticilloides* which was confirmed by Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012. (I.D. No. 9949.15). Studies of the following physiological aspects of *Fusarium verticilloides* isolates were conducted in laboratory.

Effect of culture media

Following seven culture media were used to find out the most suitable one for the mycelial growth and sporulation. Each culture medium was prepared in 1 liter of water and autoclaved at 121.6ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 90 mm Petri dishes for solidification.

1. Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g).
2. Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-agar 20g).
3. Czapeks Dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g, Sucrose 30g, Agar-agar 20g).
4. Asthana & Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen Phosphate 1.75g, Magnesium sulphate 0.75g, Agar- agar 20g).
5. Ashby's agar medium (Mannitol 20g, Di potassium phosphate 0.2g, Magnesium sulphate 0.2g, Sodium chloride 0.2g, Potassium sulphate 0.1g, Calcium carbonate 5g, Agar-agar 15g, final pH (at 25°C) 7.4±0.2).
6. Browns agar (BA) medium (Dextrose 2g, Tri basic potassium phosphate 1.25g, Magnesium sulphate 0.75g, Agar-agar 20g).
7. Coon's agar (CA) medium (Sucrose 7.2 g, Dextrose 3.60g, Magnesium sulphate 1.23g, Potassium nitrate 2.02g, Potassium di- phosphate2.72g, Agar- agar 15g).

Effect of pH

There were seven different pH level ranging from 5.0 to 8.0 with a difference of 0.5 were prepared by using pH meter and by using either N/10 HCl or NaOH before autoclaving the PDA medium. For each pH value, three replications were maintained. The Petri plates containing sterilized medium was inoculated with 5mm mycelium disc and incubated at 28±1ºC. At the interval of 24hrs, the linear growth was measured till 7 days. The range of sporulation test ranges on various pH was recorded after seven days. Sporulation was calculated with the help of haemocytometer.

Effect of temperature

The experiments were conducted to find out, the most suitable temperature for mycelial growth and sporulation of *Fusarium verticillioides*. The sterilized poured petriplates with PDA were inoculated with 5 mm disc of the test pathogen of seven days old culture. The petriplates were incubated at 15, 18, 21, 24, 27, 30 and 33ºC temperature. Three replications were maintained for each treatment and observation for mycelial growth was recorded after seven days. Sporulation was recorded at seven days after inoculation with the help of haemocytometer.

Results and Discussion

A total of seven media were used for studying the growth of *Fusarium verticillioides*. The results are given in **Table 1**. The radial growth was maximum on Potato dextrose agar (80.6 mm) which was significantly superior over all other medium. This was followed by Richards's agar (76.3 mm), Czapek's Dox agar (74.0 mm) Asthana and Hawker's agar (69.0 mm) and Coon's agar (64.3 mm). No radial growth was obtained in Asbhy's agar and Browns agar media. The test fungus sporulates only in five medium tried but excellent sporulation were observed in PDA and Richard's agar medium. No sporulation was observed in Asbhy's agar and Browns agar media. Maximum dry weight (374.90 mg) of *Fusarium verticillioides* was recorded on Potato dextrose broth medium followed by Richard's broth medium which yielded 359.83 mg dry mycelial weight. The test fungus sporulates only in five medium but excellent sporulation was not observed in any medium. Good sporulation was recorded in Potato dextrose broth medium and Richard's broth. No sporulation was observed in Asbhy's agar and Browns agar media.

Growth characters of test pathogen studied on different solid media indicated that Potato dextrose agar supported maximum growth of fungal colony. The colour and the physical aspect of the mycelium varied from one medium to another. The mycelium was reddish white fluffy with regular margin on Potato dextrose agar and Coon's agar medium while white appressed with regular margin on Asthana and Hawker's agar and Richards's agar media. White fluffy growth with regular margin was observed on Czapek's dox agar. The test fungus supported excellent sporulation on Potato dextrose agar and Richards's agar medium where as fair sporulation was recorded in Coon's medium and Asthana and Hawker's agar medium. Poor sporulation was observed in case of Czapek's Dox agar medium, No sporulation was recorded in Ashby's agar and Browns agar medium. Gangadhara, *et al.,* (2010) [8] reported that *F. oxysporum* f. sp. *vanillae* isolates showed best growth on Richard's agar and Potato dextrose agar media. Khan *et al.,* (2011) [9] studied effect of media on *F. oxysporum* f. sp. *ciceri* and found that PDA was best for the growth of isolates. Recently, Singh *et al.,* (2016) [10] studied effect of different solid media and liquid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Chaudhary *et al.,* (2018) [7] reported maximum growth, dry weight and excellent sporulation of the *Fusarium udum* on PDA and Richard's agar media. There results very much support the present result. The present study also indicated that Potato dextrose agar and Richard's agar are best medium for growth and sporulation of *F. verticillioides*.

Table 1 Effect of solid and liquid media on radial growth and sporulation of *Fusarium verticillioides*

S. No.	Name of the medium	Solid Media			Liquid Media	
		Colony diameter (mm) after 168 hrs*	Growth characters	Sporulation	Dry mycelial weight (mg) after 21 day*	Sporulation
1	Potato dextrose agar	80.6	Reddish white fluffy growth with regular margin	++++	374.9	+++
2	Richard's agar	76.3	White appressed growth with regular margin	++++	359.8	+++
3	Czapek's Dox agar	74.0	White fluffy growth with regular margin	+	255.1	++
4	Asthana and Hawker's agar	69.0	White appressed growth with regular margin	++	284.7	++
5	Coon's medium	64.3	Reddish white fluffy growth with regular margin	++	294.7	++
6	Ashby's agar	--	No growth	-	0.00	-
7	Browns medium	--	No growth	-	0.00	-
	SE(m)	0.57				
	CD (0.05)	1.77			1.951	

*Average of 3 replications

Table 2 Effect of various pH on radial growth and sporulation of *Fusarium verticillioides*

S. No.	pH	Colony diameter (mm) after 120 hrs*	Colony diameter (mm) after 168 hrs*	Sporulation
1	5.0	56.6	64.00	++
2	5.5	59.3	67.6	+++
3	6.0	71.0	86.3	+++
4	6.5	62.3	87.3	++++
5	7.0	60.3	84.6	+++
6	7.5	58.0	76.0	+++
7	8.0	56.6	65.3	+++
SE(m)		0.43	0.42	
CD (0.05)		1.33	1.28	

*Average of 3 replications

Growth and sporulation of the test fungus was obtained at all the pH levels tested but it was maximum at pH 6.5 (87.3 mm) after 168 hrs of inoculation and result is presented in **Table 2**. pH 6. (86.3 mm) and pH 7.0 (84.6 mm) were also found favorable. Growth of the test fungus decreased by increasing or decreasing the pH level from the 6.5 level. The foremost acidic and alkaline pH is not suitable for the growth and sporulation of pathogen. Excellent sporulation was observed at pH 6.5. Good sporulation was recorded at pH 5.5, 6.0, 7.0, 7.5 and 8 while fair sporulation was observed at pH 5.0. The results of the present study are in agreement with those achieved by Khilare and Ahmed, (2012) [11] reported most suitable pH level for growth of *Fusarium oxysporum* f. sp. *ciceri* was 6.0 and 6.5. [5] reported that pH level 6.0 is the optimum pH for the growth as well as sporulation of the fungus. Further increases in the pH level showed retarding effect on growth and sporulation. Singh *et al.*, (2016) [10] reported maximum growth of the *Fusarium oxyspon* f. sp. *lentis* at pH 6.5. Chaudhary *et al.*, (2018) [7] reported most suitable pH level for growth of fungus was 6.0 and 6.5 with excellent sporulation. Kushwaha *et al.*, (2019) [12] reported most suitable pH level for growth of *Sclerotium rolfsii* was 6.5. There results very much support the present result in which the most suitable pH level for growth and sporulation of the test fungus was 6.5.

Growth of *F. verticillioides* was studied from 15 to 33ºC temperature and result is presented in **Table 3**. It was seen that there was quite a large variation in the growth of these isolate at different temperature after seven days. The maximum mycelial growth was recorded at 27ºC (85.33mm) followed by 30ºC (70.67mm), 24ºC (66.33mm) and 33ºC (56.66mm), while Least mycelial growth was recorded at 15 and 18ºC. Temperatures from 24 to 30ºC were most favorable for the growth of target pathogen. Excellent sporulation was observed at temperature 27ºC good sporulation was recorded at temperature 18ºC, 21ºC, 24ºC and 30ºC while fair sporulation was and 15ºC and 33ºC. Khilare and Ahmed (2012) [11] reported most suitable temperatures level for growth of *Fusarium oxysporum* f. sp. *ciceri* was 30ºC after seven days of inoculation, which was reduced drastically below 15ºC and above 35ºC. Desai *et al.*, (2016)

[13] reported maximum growth of the *Fusarium udum* at 28ºC while Chaudhary *et al.,* (2018) [7] reported that growth of *F. udum* was maximum at 30ºC after seven days of inoculation. Kushwaha *et al.,* (2019) [12] reported maximum growth of *Sclerotium rolfsii* at 30°C. There results very much support the present result in which the most suitable temperature level for growth and sporulation of the test fungus was 27ºC.

Table 3 Effect of different temperature on radial growth and sporulation of *Fusarium verticillioides*

S. No.	Temp. ^{o}C	Colony diameter (mm) after 120 hrs*	Colony diameter (mm) after 168 hrs*	Sporulation
1	15	7.33	11.66	++
2	18	20.33	27.33	+++
3	21	39.67	49.67	+++
4	24	59.33	66.33	+++
5	27	76.33	85.33	++++
6	30	68.67	70.67	+++
7	33	46.66	56.66	++
SE(m)		0.57	0.90	
CD (0.05)		1.76	2.78	

*Average of 3 replications

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Publication History
Received 01.07.2019
Revised 22.12.2019
Accepted 22.12.2019
Online 30.12.2019