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Variability of *Fusarium verticillioides* Isolates causing Maize Post Flowering Stalk Rot with Respect to Growth Parameters on Culture Media

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A B S T R A C T

Maize Post flowering stalk rot causing caused by *Fusarium verticillioides* is major disease and causing yield loss up to 34 per cent. Different liquid media supporting the growth of the different isolates of fungus was observed. Among the eight-liquid media tested for the dry mycelial weight and sporulation of six isolates of Fusarium stalk rot pathogen (*F. verticillioides*), Isolate FV-3 yielded highest dry mycelial weight of 787.66mg, followed by FV-2 (647.66mg), FV1(581.00mg), FV-5 (451.00mg), FV-6(421.00mg) and FV-7(277.66mg) in Takahashii's broth. Whereas, highest sporulation was seen in Takahashii's broth, Potato dextrose broth and Yeast extract broth by all the six isolates of Fusarium stalk rot pathogen.

Introduction

Maize or corn (*Zea mays* L.) is prominent cereal crop of the world. The main centre of origin of maize is considered to be the Central America and Mexico. Maize is one of the most important cereal crops with a wide adaptability under varied environmental conditions. Universally, maize is recognized as "queen of cereals" because of its immense genetic yield potential compared to other cereals (Anon., 2012). Maize is consumed as a staple food in many parts of the world. It is a third foremost crop of the world after rice and wheat (Sandhu *et al.*, 2007). The principal producer of maize is United States of America (USA) producing about 35 per cent of the overall world maize production. It is called mother grain of Americans and it is the major contributor of the US economy (Milind and Isha, 2013).

The major maize growing states of India are Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka and Jammu and Kashmir which all together accounts for over 95% of

the country's maize production (Milind and Isha, 2013). The important maize growing districts of the Karnataka state are Davanagere, Haveri, Belgaum, Bagalkot, Shivamogga, Bengaluru rural, Bellary, Bijapur, Chamarajanagar, Chitradurga, Gulbarga, Dharwad, Gadag, Kolar and Mysore. In the state, area under maize is increasing at a rapid pace because of the favourable environment, higher yield and easy cultivation practices of crop (Archana, 2017).

The cultivation of maize is frequently challenged by diseases that results in both quantitative and qualitative loss in grain yield in spite of its highest genetic yield potential. Maize succumb to many diseases, but Post-flowering stalk rots (*F. verticillioides, Macrophomina phaseolina, Cephalosporium maydis*), are the important limitations in achieving the potential yield of the crop. Fusarium stalk rot is reported to cause 10-42 per cent yield loss in Karnataka (Harlapur *et al.,* 2002), whereas All India Co-ordinated Research Project (Anon., 2014) on maize has estimated 38 per cent loss in total yield due to the disease.

In Karnataka, hitherto, there were few studies carried on comparative studies of different isolates of Fusarium talk rot pathogen, and hence it was thought valuable to initiate studies on Fusarium stalk rot pathogen. Hence, a preliminary studies with respect to assessment of cultural and physiological variability and sporulation of different isolates of Fusarium stalk rot pathogen was initiated as there are only scanty information available on these aspects.

Materials and Methods

The study was conducted to assess the growth variability of *F. verticillioides* isolates on different media during 2018 at Department of Plant Pathology, College of Agriculture, V.C. Farm, Mandya.

Growth and sporulation studies of *F. Verticillioides* isolates on different liquid media

Growth studies of *F. verticillioides* isolates on different media

The eight liquid media *viz.,* Potato dextrose broth (Potato 200 g + Dextrose 20 g + Distilled water 1000 ml), Takahashii's broth (Sucrose 10.0 g + Peptone 10.0 g + Sodium chloride 5.0 g + Yeast extract 5.0g + Distilled water 1000 ml) , Richard's synthetic broth (Potassium nitrate 10.0 g + Potassium monobasic phosphate 5.00 g + Magnesium sulphate 2.50 g + Ferric chloride 0.02 g + Sucrose 50.0 g + Distilled water 1000 ml), Sabouraud's dextrose broth (Peptone 10 g + Dextrose 40 g + Distilled water 1000 ml), Oat meal broth (Oat flakes 30.0 g + Distilled water 1000 ml), Corn meal broth (Corn meal infusion form 50 g + Dextrose 02 g + Distilled water 1000 ml), Yeast extract broth (Soluble starch 10.0 g + Yeast extract 1.0 g + Distilled water 1000 ml) and Nutrient broth (Beef extract 3.0 g + Peptone 5.0 g + NaCl 5.0 g + Distilled water 1000 ml) were prepared by adding the above mention constituents without addition of agar-agar. A 100 ml of each respective prepared broth was dispensed in 250 ml conical flasks which was plugged with non-absorbent cotton which was further covered with a brown paper and was incubated at 28 ± 1º C for eight days. Later, the broth was filtered using 12.5 cm diameter Whatman No 1 filter paper discs. The mycelial mat along with the filter paper was dried at 60ºC in a hot air oven. Finally, the weight of the harvested mycelia mat and sporulation were recorded. Average of dry mycelial weight of the three treatments was calculated to compare the effect of different liquid broth on mycelia dry weight of fungus.

Sporulation studies of *F. verticillioides* isolates on different media

Sporulation was estimated by dissolving the eight days old 5 mm disc of pathogen culture in 10 ml of distilled water and shaken well to obtain the spores in solution. The spores were counted with the help of compound microscope under 10x magnification. The results were expressed as very good, good, fair, poor and no sporulation on the basis of the following scale as mentioned in Table 1.

Results and Discussion

Growth variability of *F. verticillioides* isolates on liquid media

The Takahashii's broth was most favourable for growth of *F. verticillioides* FV 1 isolate with maximum mean dry mycelial weight of 581.00 mg after eight days of inoculation followed by Richard's synthetic broth and Oat meal broth which showed growth of 459.33 mg and 301.66 mg, respectively whereas minimum mean dry mycelial weight was shown on Nutrient broth (3.33 mg). Almost, similar result was noticed by Khilare and Ahmed (2012) with maximum dry mycelial weight of *F. oxysporum* f. sp. *ciceri* in Richard's synthetic broth (Table 2).

The FV 2 isolate's growth in terms of the mean dry mycelial weight ranged from 9.33 mg (Nutrient broth) to 647.66 mg (Takahashii's broth). However, significantly highest mean dry mycelial weight (647. 66 mg) was recorded on Takahashii's broth eight days after inoculation followed by Potato dextrose broth (624.33 mg). Sekar *et al.,* (2017) found *F. graminearum* produced highest mycelial growth on Potato dextrose broth.

Out of eight different liquid media tested, highest mean dry mycelial weight (787.66 mg) of FV 3 isolate was recorded on Takahashii's broth eight days after inoculation. The next best broth was Potato dextrose broth which yielded 577.66 mg of mean dry mycelial weight followed by Oat meal broth (302.66 mg). Least mean dry mycelial weight was observed on Nutrient broth. Chopada *et al.,* (2014) found *F. oxysporum* f. sp. *lycopersici* produced highest mycelia growth on Potato dextrose broth.

For FV 5 isolate Takahashii's medium was the best medium for getting maximum mean dry mycelia weight of 451.00 mg eight days after inoculation followed by Richard's synthetic broth (312.66 mg) and Potato dextrose broth (245.33 mg) while no mycelial growth was observed on Nutrient broth. The results obtained from Chaudhary *et al.,* (2018) showed *F. udum* yielded maximum dry mycelia weight on Richard's broth.

The results of cultural studies of FV 6 isolate revealed that out of eight different liquid media tested, the mean dry mycelia weight was maximum (421.00 mg) on Takahashii's broth followed by Oat meal broth (310. 66 mg) and Yeast extract broth (179.33 mg) and minimum mean dry mycelia weight was seen on Nutrient broth (2.00 mg). The findings of Gupta *et al.,* (2010) revealed *F. oxysporum* f. sp. *psidii* and *F. solani* produced highest dry mycelia weight on malt extract broth.

Out of eight different liquid media tested for growth of FV 7 isolate, the mean dry mycelial weight was highest (277.66 mg) on Takahashii's broth eight days after inoculation followed by Potato dextrose broth (107.33 mm) and Oat meal broth (90.66 mg) and least mean dry mycelia weight was seen on Nutrient broth (8.00 mg) and Sabouraud's dextrose broth (9.00 mg). Pradeep *et al.,* (2013) found *F. moniliforme* KUMBF1201 produced highest dry mycelia weight on Potato dextrose broth.

Table.1 Details of expression of sporulation of Fusarium stalk rot pathogen

Sporulation	Representation	No of conidia/10x microscopic field
Very good	++++	>40
Good	+++	26-40
fair	++	11-25
Poor	+	1-10
No	-	0

Table.2 Growth of isolates of *F. verticillioides* in different liquid media

Sl. No.	Liquid media	Dry mycelial weight (mg)					
		Isolates					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	Potato dextrose broth	78.66	624.33	577.66	245.33	165.66	107.33
2	Takahashii's broth	581.00	647.66	787.66	451.00	421.00	277.66
3	Richard's synthetic broth	459.33	186.66	112.00	312.67	52.33	49.00
4	Sabouraud's dextrose broth	49.33	30.66	210.33	40.00	21.33	9.00
5	Oat meal broth	301.66	170.66	302.66	181.66	310.66	90.66
6	Corn meal broth	80.00	220.33	22.33	51.00	30.00	9.33
7	Yeast extract broth	181.00	119.33	139.33	129.33	179.33	87.33
8	Nutrient broth	3.33	9.33	7.66	0.00	2.00	8.00
F		**	**	**	**	**	**
S. Em ±		**0.90**	**1.25**	**1.17**	**0.79**	**0.83**	**1.13**
CD @ 1%		**3.73**	**5.17**	**4.86**	**3.30**	**3.44**	**4.67**

** Significant at 1% level

Table.3 Sporulation of isolates of *F. verticillioides* in different liquid media

Sl. No.	Liquid media	Sporulation					
		Isolates					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	Potato dextrose broth	++++	++++	++++	++++	++++	++++
2	Takahashii's broth	++++	++++	++++	++++	+++	++++
3	Richard's synthetic broth	++	+	+++	+++	-	+
4	Sabouraud's dextrose broth	++	++	++	++	+	-
5	Oat meal broth	+++	++	++	++	+++	++
6	Corn meal broth	++	+	+	+	+	+
7	Yeast extract broth	+++	+++	+++	++++	++++	+++
8	Nutrient broth	+	-	-	-	-	-

Poor sporulation (+) (1-10 conidia); Fair sporulation (++) (11-25 conidia); Good sporulation (+++) (26-40 conidia); Very good sporulation (++++) (>40 conidia); No sporulation (-); Number of conidia per microscopic field under 10x considered for categorization

Sporulation variability of *F. verticillioides* isolates on liquid media

All the *F. verticillioides* isolates apart from FV 1, sporulated in seven liquid media tested except in Nutrient broth where as FV 1, sporulated in all the media including Nutrient broth (Table 3).

FV 1 isolate recorded very good sporulation in Takahashii's broth and Potato dextrose broth and poor sporulation was recorded in Nutrient broth and similar results was noticed in case of FV 2 and FV 3 isolates as well. The results obtained are in confirmation to the findings of Rana *et al.*, (2017) where *F. oxysporum* f. sp. *cubense* attained very good sporulation in Potato dextrose broth and also the results are in tune with the Thaware *et al.*, (2016) where they found *F. oxysporum* f. sp. *ciceri* showed excellent sporulation in Potato dextrose broth. Whereas, Chaudhary *et al.*, (2018) found *F. udum* produced highest sporulation in Richard's broth medium.

The sporulation of FV 5 isolate was observed in all the tested liquid media and very good sporulation was recorded in Takahashii's broth, Potato dextrose broth and Yeast extract broth and poor sporulation in Corn meal broth. The findings are in agreement with Chandel and Sharma (2010) and Thaware *et al.,* (2016) in *F. oxysporum* f. sp. *dianthi* and *F. oxysporum* f. sp. *ciceri*, respectively. The sporulation of FV 6 isolate was very good in Yeast extract broth while poor sporulation was observed in Sabouraud's dextrose broth and Corn meal broth. However, no sporulation was seen in Richard's synthetic broth and Nutrient broth. *F. oxysporum* f. sp. *dianthi* showed highest sporulation in Potato dextrose broth (Chandel and Sharma, 2010).

Sporulation of FV 7 isolate was very good in Potato dextrose broth and Takahashii's broth and poor sporulation was recorded in Richard's synthetic broth and Corn meal broth. However, no sporulation was recorded in Sabouraud's dextrose broth and Nutrient broth. The results obtained are in confirmation with the Thaware *et al.,* (2016) where they found *F. oxysporum* f. sp. *ciceri* shown excellent sporulation in Potato dextrose broth.

The findings in the current investigation, clearly reveals the occurrence of variability among the *F. verticillioides* isolates with regard to their growth parameters on different culture media. Most of the isolates exhibited better mycelia growth on Takahashii's broth followed by Potato dextrose broth and least or no growth was noticed on Nutrient broth. Whereas, very good sporulation of all the isolates was noticed in Potato dextrose broth and Takahashii's broth followed by Yeast extract broth and no sporulation noticed on Nutrient broth.

In conclusion the variation was noticed among all the isolates of *F. verticillioides* in present study with regard to growth in terms of dry mycelia weight and amount of sporulation on different liquid culture media tested. Majority of the isolates showed better mycelia growth and sporulation in Takahashii's broth and Potato dextrose broth with variations. The present findings also deciphered that these two media are better for obtaining rich mycelia growth of *F. verticillioides* and could aide in molecular studies. Further, the variation among the isolates needs to be authenticated by virulence and molecular studies.

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