Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1395-1399
Received: 12-06-2020
Accepted: 03-08-2020

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Physiological variability of *Fusarium verticillioides* causing post flowering stalk rot in maize

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DOI: https://doi.org/10.22271/phyto.2020.v9.i5t.12523

Abstract
Post flowering stalk rot of maize caused by *Fusarium verticillioides* is the major disease and the occurrence of variability in the pathogen is one of the key factors for difficulty in management of the disease. The effect of temperature regimes showed the maximum radial growth and highest sporulation was obtained at 30°C by all six isolates of *F. verticillioides*. At pH 7 maximum radial growth of all isolates was obtained except FV 1 and FV 7 isolates which produced maximum radial growth at pH 6. Highest sporulation of all six isolates was seen at pH 6. Exposure of pathogen to continuous dark (24 hours) condition yielded maximum radial growth in all isolates except in FV 6 isolate which produced maximum radial growth when it was exposed to continuous light (24 hours). Optimum sporulation of all the six isolates was seen under continuous dark condition followed by alternate cycles of 12 hours light and 12 hours darkness. Isolates FV 2 and FV 7 were produced maximum radial growth on soluble starch, whereas FV 3 and FV 6 isolates on sucrose. Fructose and maltose produced maximum radial growth of FV 1 and FV 5 isolate respectively. Optimum sporulation of all six isolates was obtained by supplying fructose and sucrose as carbon source.

Keywords: Fusarium stalk rot, *F. verticillioides;* physiological variability, maize

Introduction
Maize or corn (*Zea mays* L.) 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) [1]. 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) [20]. 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 countries maize production (Milind and Isha, 2013) [18].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, cultivational area under maize is increasing at a rapid pace because of the favourable environment, higher yield and easy cultivation practices of crop (Archana, 2017) [3]. Post flowering stalk rot caused by *F. verticillioides* is a serious stalk and root disease of maize and it was first identified in Asia (Lal and Singh, 1984) [16]. In India, Mount Abu, Rajasthan was the place at which the Fusarium stalk rot disease was first reported (Arya and Jain, 1964) [4]. The disease is mostly prevalent in areas with hot and dry climatic conditions (Doohan *et al.*, 2003) [10] particularly before or during pollination (Pascale *et al.*, 2002) [19]. The disease was found to cause an estimated 38 per cent loss in total yield on maize (Anon., 2014) [2]. In Karnataka, hitherto, there were very few studies carried out on physiological variability of maize post flowering stalk rot pathogen, and hence it was thought valuable to initiate studies in this direction. Keeping the above aspects in view, a preliminary study was conducted to assess the physiological variability to know the dynamics of the pathogen.

Material 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. Seven isolates collected from different regions of South Karnataka viz., Alur (FV 1), Belur (FV 2), Gouribidanur (FV 3), Hassan (FV 4), Haveri (FV 5), Mandya (FV 6)

and Malavalli (FV 7) was used for the studying the effect different temperature, p^H, light regimes and carbon sources on Takahashii's medium, as it was proved to be best in supporting the growth of the pathogen.

Effect of temperature and light on growth of *F. verticillioides*

Twenty ml of autoclaved Takahashii's medium was dispensed in to each sterilized Petri plate and was allowed to solidify. Seven days old, 5 mm mycelial disc of *F. verticillioides* was placed inversely at the centre of the medium. Each treatment was triplicated and incubated at different temperature *viz.,* 10º, 15º, 20º, 25º, 30º, 35º, 40º and 45ºC for eight days to take up observation on colony diameter and sporulation. Similarily, another experiment was conducted to determine the response of *F. verticillioides* to the different light exposures. All the six isolates were grown on Takahashii's medium were exposed to different light treatments *viz.,* continuous light, continuous dark and alternate cycles of 12 hours light and 12 hours dark. Each treatment was replicated three times and plates were incubated at 28 ± 1ºC for a period of eight successive days under continuous light (Fluorescent light of 40 watts), continuous dark, and alternate cycles of 12 hours light and 12 hours dark in an environmental chamber. Later, the observation on radial growth and sporulation were recorded eight days after inoculation. The difference in growth rate of the pathogen in different treatments was recorded and was statistically analysed.

Effect of pH and carbon source on growth of *F. verticillioides*

To study the effect of Hydrogen-ion concentration (pH) on the mycelial growth and sporulation, all the six isolates of *F.verticillioides* were grown on Takahashii's medium with different pH levels *viz.,* 5.0, 6.0, 7.0, 8.0 and 9.0. The different pH levels of the media were adjusted by adding either 1N acid (HCl) or 1N base (NaOH) with the help of digital pH meter. Seven days old, 5 mm mycelial disc of the fungus was placed inversely at the centre of the medium. Each treatment was triplicated and incubated at 28 ± 1ºC for a period of eight consecutive days. To identify the ability in utilizing various carbon sources by the *F. verticilloides*, five different carbon sources *viz.,* sucrose, lactose, maltose, fructose and soluble starch were used for the study. On the basis of their molecular weights the amount of carbon source to be added was calculated, in order to add an equal amount of carbon or dextrose present in the basal medium. All the sugars were separately dissolved thoroughly in basal Takahashii's medium with a neutral pH and were autoclaved at 121.6ºC at 15 psi for 20 minutes. Seven-day old, 5 mm mycelial disc of six different isolates the fungus was placed inversely at the centre of the different carbon source contained solidified medium. The rate of growth of the pathogen in different treatments was taken and statistically analysed.

The sporulation of the all isolates at different physiological conditions was studied by using haemocytometer. The spore counting was grouped as (+) Poor sporulation (1-10 conidia); (++) Fair sporulation (11-25 conidia); (+++) Good sporulation (26-40 conidia); (++++) Very good sporulation (>40 conidia); No sporulation (-). The number of conidia per microscopic field under 10x considered for categorization.

Results and discussion

Effect of temperature regimes on the growth of Fusarium stalk rot pathogen (*F. verticillioides*)

Highest radial growth of 90 mm was recorded in the Isolate FV 5 and FV 6, which was followed by 89.33, 89, 88.67 and 83.33 mm radial growth from the isolates FV1, FV6, FV 2 and FV 4 respectively at 30^0 C. All isolates exhibited good sporulation at 30^0 C. However, growth drastically decreased at 15°C and 35°C with a poor and fair sporulation, respectively in all the isolates and 30°C was the most optimum and favourable temperature. The results are in line with the Farooq *et al.* (2005) [11], Siddique *et al.* (2012) [22] in case of *F. oxysporum* f. sp. *ciceri* which produced maximum colony diameter at 30°C. Desai *et al.* (2016) [9], in case of *F. udum* recorded optimum sporulation and mycelial growth at temperature ranging from 25-30°C (Table 1).

The isolate FV 7 of *F. verticillioides* revealed that fungus grew at the temperature ranging from 10-35°C. However, the radial growth of the fungus was severely reduced at 35°C and above and it started to decline below 25°C because these temperatures do not support the growth of the most mesophilic fungi. It was found that at 30°C the fungus attained maximum colony diameter of 90.00 mm with very good sporulation followed by 25°C (65.00 mm) with a good sporulation. The results are in agreement with Chaudhary *et al.* (2018) [7] in case of *F. udum* which produced maximum colony diameter and higher sporulation at 30°C. No mycelial growth was recorded by all isolates at 40°C and 45°C.

Effect of light regimes on the growth of Fusarium stalk rot pathogen (*F. verticillioides*)

Light is very crucial for fungi since it act as an important signal for fungi which influences many physiological process such as pigmentation, reproduction, conidial production, the circadian clock and various secondary metabolisms (Corrochano, 2007) [8].

The mycelial growth of FV 1 isolate was highest (88.33 mm) under exposure to continuous dark, but to get very good sporulation it is necessary to expose target isolate to alternate cycles of 12 hours light and 12 hours dark condition. Exposure to continuous light yielded relatively less mycelial growth (80.66 mm) with good sporulation (Table 2). FV 2 isolate of *F. verticillioides* in terms of mycelial growth and sporulation disclosed that maximum colony diameter of 88.00 mm with good sporulation was recorded when target fungus was exposed to continuous dark period of 24 hours followed by exposure to alternate cycles of 12 hours light and 12 hours dark condition (85.33 mm) with a very good sporulation. However, minimum mycelial growth of 77.00 mm was recorded under continuous light with a good sporulation. Hence, exposure of FV 2 isolate to both continuous dark period and alternate cycles of 12 hours light and 12 hours dark condition will yield good mycelial production and good sporulation. The continuous dark condition was best for FV 3 isolate as it produced a highest mycelial growth of 90.00 mm with a very good sporulation. Next best light regime was exposure to continuous light condition (84.33 mm) with a good sporulation, whereas lowest mycelial growth (55.66 mm) with a fair sporulation was recorded under alternate cycles of 12 hours light and 12 hours dark condition. Exposure of FV 5 isolate to continuous 24 hours of dark gave a maximum mean radial growth (88.00 mm) accompanied

with very good sporulation. Good sporulation was recorded with relatively good mycelial growth of 72.66 mm when exposed to alternate cycles of 12 hours light and 12 hours dark condition while least mycelial growth and fair sporulation was seen when exposed to the continuous light period.

FV 6 isolate was better under continuous 24-hour light with mycelial growth of 85.33 mm with good sporulation followed by continuous 24-dark condition (84.66 mm) with fair sporulation. However, a very good sporulation was observed under alternate cycles of 12hrs light and 12hrs dark but with relatively low mycelial growth of 77.00 mm. Hence, exposure of FV 6 isolate to continuous 24-hour light yields good sporulation and maximum mycelial growth. The FV 7 isolate developed very good colonies with rich mycelial growth of 89.00 mm and good sporulation under continuous dark followed by continuous light (85.33 mm) with a fair sporulation. However, alternate cycles of 12 hours light and 12 hours dark produced relatively less mycelial growth (78.66 mm) with a poor sporulation. Therefore, exposure of FV 7 isolate to continuous dark condition yields maximum radial growth and sporulation. The results are in tune with results of Kausar *et al.* (2009) [15] who revealed that exposure of *F. solani* and *Lasiodiplodia theobromae* to continuous light yielded widest colony diameter. Similarly, Thammaiah and Somu (2015) [23] found that under alternate light and dark cycle, *F. oxysporum* f. sp. *cubense* showed highest sporulation. Benaouali *et al.* (2014) [5] observed that *F. oxysporum* f. sp. *radicis lycopersici* produces maximum radial growth under continuous dark conditions. Gheorghe *et al.* (2015) [12] who found that under permanent light of 24 hours resulted in highest mycelial growth and more sporulation in *F. oxysporum* f. sp. *glycines*.

Effect of Hydrogen-ion concentration (pH) on the growth of Fusarium stalk rot pathogen (*F. verticillioides*).

In general, any fungi will utilize and absorb the contents of substrate only if the reaction of the substrate is favourable and conducive for its growth and metabolism. Hence, pH of the substrate or artificial nutrient medium supplied is of great importance in order to get maximum radial growth under *in vitro* conditions. All the Six isolates were grown on Takahashii's medium with pH of medium adjusted to pH levels *viz.*, 5.0, 6.0, 7.0, 8.0 and 9.0 and incubated at 28 ± 1ºC for eight consecutive days. The data on mean radial growth and sporulation of different isolates was statistically analysed and presented in Table 3.

Isolate FV2 showed maximum radial growth of 90 mm and very good sporulationat pH 6 and 7. Isolates FV1 and FV5 were recorded maximum radial growth of 90 mm at pH 7 with good sporulation. However, Isolate FV 1 had very good sporulation at pH 5, but with less mycelial growth (68.00 mm). Isolate FV3 and FV 7 yielded 90 mm and 86.66 mm radial growth at pH 6 with very good and good sporulation respectively. The findings are in agreement with the results of Hossain *et al*. (2015) [14] who reported that pH 6 was best to obtain widest mycelial mat and more spore production of *F. moniliforme*. The results are in agreement with Cha *et al.* (2007) [6] in *F. oxysporum* and Siddique *et al.* (2012) [22] in *F. oxysporum* f. sp. *phaseoli* where they observed pH 7 and pH 6 were ideal to get maximum mycelial growth and sporulation. Sekar *et al.* (2017) [21] reported pH 7 was ideal to support maximum mycelial growth of *F. graminearium*. Similarly, Thaware *et al.* (2016) [24] confirmed that pH 6 was best to obtain excellent sporulation of *F. oxysporum* f. sp. *ciceri.* There was a significant decline in mycelial growth and sporulation occurred at pH 5 and pH 9 with fair and nil sporulation, in all isolates. Chaudhary *et al.* (2018) [7] reported pH 6 was ideal to get highest sporulation and maximum radial mycelial growth of *F. udum*.

Effect of different carbon sources on the growth of Fusarium stalk rot pathogen (*F. verticillioides*)

All the living organisms need carbon as it is an indispensable substance to carry out all the necessary metabolic activities of the fungi. Carbon is the main structural element of fungi as nearly half of the dry mycelial weight of fungi is made of carbon compound (Lilly and Barnett, 1951) [17]. With respect to effect of different carbon sources on radial growth and sporulation of FV 1 isolate, maltose was the best carbon source in producing maximum colony diameter (89.33 mm), whereas for very good sporulation, the FV 2 isolate showed better response on soluble starch as it produced maximum radial growth (82.33 mm), but fructose was best to obtain very good sporulation. However, sucrose produced relatively good mycelial growth (68.66 mm) with good sporulation compared to lactose (65.66 mm) and maltose (64.00 mm) with fair and good sporulation, respectively (Table 4).

Isolate FV 3 revealed sucrose as an excellent carbon source to achieve maximum radial growth (83.83 mm), whereas fructose and sucrose were best to obtain very good sporulation. Maltose (79.66 mm) and lactose (71.66 mm) produced relatively less radial growth with good and poor sporulation, respectively. Least mycelial growth (63.66 mm) with a fair sporulation was observed when soluble starch was supplied as carbon source. FV 5 isolate fructose was the best carbon source to attain highest mycelial growth (89.66 mm) with very good sporulation. Next best carbon source was maltose (83.16 mm) with very good sporulation followed by sucrose (81.00 mm) and lactose (77.33 mm) with a good and fair sporulation, respectively. Least mycelial growth (75.00 mm) and poor sporulation were obtained by soluble starch.

Maximum radial growth of (90.00 mm) was recorded in FV 6 isolate with sucrose and very good sporulation, followed by fructose (88.66 mm), maltose (81.33 mm) and lactose (77.83 mm) with good, fair and poor sporulation, respectively. The FV 7 isolate produced maximum mycelial growth (76.66 mm), but with poor sporulation on soluble starch followed by fructose (71.33 mm) and sucrose (70.66 mm) with good and fair sporulation, respectively. The findings are opposite to the findings of Goswami and Islam (2017) [13] where desirable carbon source for *F. oxysporum* f. sp. *lycopersici* was sucrose to obtain maximum radial growth and highest sporulation. Similar results were reported by Siddique *et al.* (2012) [22] in *F. oxysporum* f. sp. *phaseoli* which produced maximum colony diameter when supplied with sucrose. Farooq *et al.* (2005) [11] reported glucose as the best carbon source to obtain maximum radial mycelial growth (90 mm) of *Fusarium oxysporum* f. sp. *ciceri.*

Table 1: Effect of temperature regimes on growth of *F. verticillioides* isolates

S. No.	Temperature (º C)	Radial growth (mm) and sporulation					
		Isolates					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	10	3.16	4.33	3.00	3.00	2.00	4.00
		-	-	-	-	-	-
2	15	25.00	30.00	64.66	31.50	28.50	34.00
		+	+	+	+	-	+
3	20	35.00	35.00	77.00	49.33	56.33	37.50
		+++	++	++	+	+	+
4	25	60.00	37.50	83.33	82.00	72.33	65.00
		++++	++++	+++	+++	++	+++
5	30	89.33	88.67	90.00	90.00	89.00	90.00
		++++	+++	++++	++++	+++	++++
6	35	26.50	28.50	42.00	26.50	21.00	4.33
		++	+	++	++	+	+
7	40	1.66	0.00	0.00	0.00	1.33	0.00
		-	-	-	-	-	-
8	45	0.00	0.00	0.00	0.00	0.00	0.00
		-	-	-	-	-	-
F		**	**	**	**	**	**
S. Em ±		1.25	1.27	0.66	0.95	0.79	1.40
CD @ 1%		5.19	5.27	2.75	3.93	3.29	5.81

** Significant at 1% level

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

Table 2: Effect of light regimes on growth of *F. verticillioides* isolates

S. No.	Light regimes	Isolates					
		Radial growth (mm) and Sporulation					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	Complete light (24hrs)	80.66 +++	77.00 +++	84.33 +++	66.66 ++	85.33 +++	85.33 ++
2	Complete dark (24hrs)	88.33 ++	88.00 +++	90.00 ++++	88.00 ++++	84.66 ++	89.00 +++
3	Alternate cycles of (12hrs light and 12hrs dark)	75.33 ++++	85.33 ++++	55.66 ++	72.66 +++	77.00 ++++	78.66 +
F		**	**	**	**	**	**
S. Em ±		0.57	0.69	0.43	0.54	1.58	0.54
CD @ 1%		3.02	3.63	2.25	2.85	8.32	2.85

** Significant at 1% level

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

Table 3: Effect pH levels on growth of *F. verticillioides* isolates

S. No.	pH	Radial growth (mm) and sporulation					
		Isolates					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	5	68.00	67.33	51.00	50.00	56.33	44.00
		++++	++	+++	++	+++	+
2	6	86.00	89.00	90.00	82.00	80.00	86.66
		+++	++++	++++	+++	++++	+++
3	7	90.00	89.33	86.00	90.00	89.33	80.66
		++	+++	+++	++	++	++
4	8	78.00	84.33	80.83	87.66	87.67	77.33
		-	+	+	+	+	+
5	9	64.33	74.33	76.00	69.33	63.00	51.66
		-	-	-	-	-	-
F		**	**	**	**	**	**
S. Em ±		0.59	0.53	0.71	0.21	0.63	1.16
CD @ 1%		2.67	2.40	3.18	0.94	2.83	5.21

** Significant at 1% level

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

Table 4: Effect of carbon sources on growth of *F. verticillioides* isolates

S. No.	Carbon sources	Radial growth (mm) and sporulation					
		Isolates					
		FV 1	FV 2	FV 3	FV 5	FV 6	FV7
1	Sucrose	80.66	68.66	83.83	81.00	90.00	70.66
		+++	+++	++++	+++	++++	++
2	Fructose	84.66	80.66	78.33	89.66	88.66	71.33
		++++	++++	++++	++++	+++	+++
3	Lactose	77.56	65.66	71.66	77.33	77.83	66.33
		++	++	+	++	+	+
4	Maltose	89.33	64.00	79.66	83.16	81.33	67.66
		++++	+++	+++	++++	++	++
5	Soluble starch	71.66	82.33	63.66	75.00	29.33	76.66
		+	+	++	+	+	+
F		**	**	**	**	**	**
S. Em ±		0.43	1.01	0.67	0.38	0.45	0.66
CD @ 1%		1.95	4.53	3.04	1.73	2.03	2.98

** Significant at 1% level

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

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