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Full Length Research Paper

Morphological characterization and reaction of partial purified toxin of sugarcane red rot pathogen *Colletotrichum falcatum* collected from Southern India

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Sugarcane is an important cash crop and used as the chief source of sugar in India. Sugarcane production is challenged by various biotic and abiotic stress among the biotic factors, red rot disease caused by *Colletotrichum falcatum* is a major disease leading to severe reduction in sugarcane production. Morphological studies conducted under in vitro in Potato Dextrose Agar (PDA) showed characteristic variation in their cultural and colony characters which were collected from different places. Based on pathogenicity test the variety Coc671 showed more susceptiblilty to all the isolate. The variety Co 86249 resistant to all the isolates except Cf3 which showed intermediate reaction. Toxins from various isolates was extracted from culture filtrate grown in Czapek-Dox yeast (CDY) broth and extracted using ethyl acetate. The ethyl acetate fraction was injected to two different varieties. Among the crude toxins extracted from different isolates, the ethyl acetate fraction from Cf3 showed maximum reddening symptoms on 6th day with 92.3 (% infected area on cane stem) in 200 µl injected samples. Cf3 partially purified toxins when treated in leaves showed maximum reddening area of 70.20 % infected area on leaf in Coc671. Serial dilution of toxin to 10^{-5} showed symptoms in susceptible cultivar whereas no reaction was noticed below 10^{-1} dilution in resistant variety. Thus present study brings the morphological variations and virulence characters of *C. falcatum*.

Keywords: Pathogencity, Coc 671, Co 86249, toxin, Czapek–Dox yeast.

INTRODUCTION

Sugarcane is an economically important crop which is vegetatively propagated in the tropics and sub-tropics which is used as the chief source of sugar and ethanol production.

Sugarcane cultivation is challenged by several biotic and abiotic factors. The most important one is the red rot disease caused by *Colletotrichum falcatum* Went. (Perfect stage: *Glomerella tucumanensis* Speg.). Red rot infection in cane causes a loss of total weight to about 29.07% leading to 30.8% loss in sugar recovery (Hussnain and Afghan 2006) besides reducing the yield attributes it also reduces the juice qualities such as brix value, sucrose content, purity and commercial cane sugar.

The genus *Colletotrichum* has shown wide variations in morphology (Amarjit singh et al*.,* 2006; Prema et al., 2011). Similarly variations in mycelial growth, mycelial dry matter production, mycelial color, texture, topography, acervuli initiation, sporulation and spore germination, appressorium formation and shape of the appressorium were observed in *C. falcatum* (Viswanathan et al., 2003). Major pathotypes used in screening programme were distinguished at morphological, cultural, serological and pathogenicity level (Viswanathan et al., 2000; 2003). Alexander et al. (1985) and Beniwal et al. (1989), classified *C. falcatum* isolates in India into different pathotypes based on their differential reaction. Malathi et al. (2010), studied differential interaction of 9 pathotypes indicating that Cf92061 (tropical) and Cf7717 (sub tropical)

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were found to be highly virulent types, while Cf64 and Cf767 were least virulent types among all the pathotypes. The toxic metabolites produced by *Colletotrihum* plays a major role in the pathogencity and virulence on sugarcane
(Malathi et al., 2002). Several authors have evaluated phytotoxins as factors in the pathogenesis of *Colletotrichum* and also determined whether the toxin is host-specific (Alleyne, 2001; Yoshida et al., 2000). The pathogen *C. falcatum* produces a phytotoxin that reproduces some of the symptoms of the disease (Olufolaji and Bomgboye, 1986; Mohanraj et al., 2002). This phytotoxin also induces an accumulation of phytoalexins (anthocyanidin pigments) in treated canes similar to that caused by the pathogen (Viswanathan et al., 1996). Malathi et al. (2002), reported that *C. falcatum* is known to produce a phytotoxic metabolite identified as an anthroquinone compound. Therefore, the phyotoxin isolated from *Collectotrichum falcatum* was tested for its ability to produce lesion on sugarcane tissue similar to the pathogen.
Thus this research looks into morphological variation and virulence among different isolates of *Colletotrichum falcatum.*

MATERIALS AND METHODS

Collection and isolation

In order to find variations of *C. falcatum* among the different parts of Tamil Nadu, an extensive survey was conducted in major sugarcane growing areas of Tamil Nadu covering Tirunelveli, Sivagangai, Cuddalore, Villupuram districts and also two other state namely Pondicherry and Andhra Pradesh during 2011-2012. Infected canes were split open by sterilized knife and observed for reddish tissue and white transverse band. The red rot pathogen was isolated by tissue segment methods as described by Rangaswami (1958), after 5 days of incubation, the plates having red sporulation were purified by sub- culturing. All the isolates were further purified by single spore dilution technique (Riker and Riker, 1936). The fungus from the pure cultures obtained was examined microscopically in order to match it with the characters of the pathogen examined from the diseased samples. The pure cultures were maintained in Potato Dextrose Agar slants. Five known races of *C. falcatum* (Cf03, Cf04, Cf05,Cf06 and Cf08) were obtained from Sugarcane Breeding Institute, Coimbatore, India, a total of 30 isolates were used in the study as given in Table 1.

Morphological Characters

Morphological characters of the spores *viz.,* size, color and shape of the conidia were observed. Measurements of 100 spores were taken under the image analyzer 100 X magnifications. The mean values and the range were determined. In the pathogen, colony color, substrate color, margin of colony and topography were recorded through naked eye.

Pathogenicity Test

Seven month old sugarcane plants (Coc671, susceptible and Co86249, resistant) were inoculated with each isolate of *C. falcatum* by the standard plug method (Chona, 1954). Test pathogen *C. falcatum* was grown on PDA medium at room temperature (28±5ºc) for fifteen days. Cultures were flooded with sterile distilled water and washes were combined to obtain a spore suspension of $1x10^6$ conidia/ml. A bore hole was made in the 3rd internode from the base of the standing cane using the red rot inoculators and one ml of the spore suspension ($1x10^6$ conidia/ml) was injected in to the bore hole of each cane using hypodermic syringe needle having 16G size. The bore hole was sealed with modeling clay to protect the canes from oxidation and contamination, the disease severity was recorded 60 days after inoculation. The canes were split open longitudinally 60 days after inoculation and the disease intensity was assessed on a 0-9 scale based on drying of cane tops, lesion width, lesion transgression across the nodes and presence of prominence of white spots in the stalks (Srinivasan and Bhat, 1961).

Extraction of toxin from *C. falcatum*

To characterize all the isolates based on the banding pattern of their secondary metabolites on silica gel plates, mycelial disc were inoculated into 250 ml conical flask containing 100 ml Czapek–Dox yeast (CDY) broth. The cultures were incubated by shaking for15 days. Un-inoculated CDY served as the control. After incubation, 0.2 ml of 85 % phosphoric acid was added and the flasks were shaken vigorously. The contents were passed through two layers of cheesecloth to reduce fungal biomass. Ethyl acetate was used as solvent for extracting the phytotoxic fraction from the culture filtrates. The organic solvent was used at a ratio of 1:1 (v/v) (culture filtrate to organic solvent). The extraction process was repeated twice. Concentrated Na_2SO_4 was added to the combined extract from two extractions to remove water and incubated overnight, after which the solvent was evaporated in a rotary evaporator under reduced pressure then dissolved in 250 microlitre of ethyl acetate and stored at 20 °C (Abang et al., 2009).

High Performance Thin Layer Chromatography (Hptlc) Analysis

The ethyl acetate extracts of all isolates were separated by applying the extracts to a hptlc pre-coated silica gel glass

Table 1. Isolates used in the study.

Isolates	Cultivar	Location	District/State
Cf1	Co86032	Armalkulam	Tirunelveli
Cf2	Co86032	Ambai	Tirunelveli
Cf3	Coc671	Devakottai	Sivagangai
Cf4	Co6304	Nellikuppam	Cuddalore
Cf5	Co6304	Pennadam	Cuddalore
Cf6	Co0323	Nellikuppam	Cuddalore
Cf7	Co94012	Nellikuppam	Cuddalore
Cf8	Co87012	Nellikuppam	Cuddalore
Cf9	Co86032	Kallakurchi	Villupuram
Cf10	Co6304	Thiyagavalli	Cuddalore
Cf11	Co87012	Pennadam	Cuddalore
Cf12	Coc671	Ariyakudi	Sivagangai
Cf13	Coc671	Kallakurchi	Villupuram
Cf14	Coc671	Padamathur	Sivagangai
Cf15	Si94045	Pahour	Pondicherry
Cf16	91V83	Annakapalli	Andrapradesh
Cf17	81V48	Annakapalli	Andrapradesh
Cf18	Co86032	Sethiathope	Cuddalore
Cf19	Co7219	Annakapalli	Andrapradesh
Cf20	Co 6304	Pennadam	Cuddalore
Cf21	Si94045	Naduveerapattu	Cuddalore
Cf22	Si94045	Madapattu	Villupuram
Cf23	Co87012	Arasur	Villupuram
Cf24	Co87012	Tittagudi	Cuddalore
Cf25	Si 94025	Kandarkottai	Cuddalore
Cf03(R)			
Cf04(R)			
Cf05(R)	Sugarcane Breeding Institute, Coimbatore		
Cf06(R)			
Cf08(R)			

Cf- *Colletotrichum falcatum*
Cf(R)-*Colletotrichum falcatum* Race

plate. A mixture of n-hexane and ethyl acetate at a ratio of 3:1 (v/v) was used as solvent after tests with different solvent combinations and ratios showed that this solvent gave the best results. Compounds were detected under light at 254 and 366 nm. Rf values for observed bands were calculated using the formula:

Rf= distance travelled by the spot/distance travelled by the solvent.

Identification of virulent isolates with crude toxins produced by *C. falcatum*

Two sugarcane varieties used for the bioassay throughout the study were: Coc671 (Susceptible variety) and (Co 86249) Resistance Variety. The crude extract were injecting at different volumes (50, 100 and 200 µl) at the middle of 10cm long sugarcane stem of each variety. The stems were observed for emergence and degree of disease development. Un-inoculated liquid medium was used for the control.

Serial dilutions (10^{-1} to 10^{-6}) of the toxin were prepared with sterile distilled water. Distilled water was used for the control. Each toxin dilution was used to screen the two sugarcane varieties, to determine the degree of resistance/susceptibility of the varieties towards the red-rot disease. The treatment was carried out by aseptically injecting different dilutions of crude toxin solution into one cut end of 10 cm long sugarcane stem of each variety. The stems were wrapped with aluminum foil and incubated for 5 days before they were observed for disease development. To determine the effect

Table 2. Conidial characteristics of different isolates of *C. falcatum.*

Isolates	Length (µm) *	Width (µm) *	Color	Shape
Cf1	35.0 abc	11.0 abc	Hyaline	Falcate
Cf2	32.7 b-e	11.5 a	Hyaline	Falcate
Cf3	37.0 a	10.5 bcd	Hyaline	Falcate
Cf4	32.0 cde	10.0 d	Hyaline	Falcate
Cf5	33.7 a-e	10.5 bcd	Hyaline	Falcate
Cf6	33.0 b-e	10.0 d	Hyaline	Falcate
Cf7	34.0 a-d	10.5 bcd	Hyaline	Falcate
Cf8	32.7 b-e	10.2 cd	Hyaline	Falcate
Cf9	32.7 b-e	10.0 d	Hyaline	Falcate
Cf10	30.3 e	10.5 bcd	Hyaline	Falcate
Cf11	36.7 a	11.5 a	Hyaline	Falcate
Cf12	37.0 a	11.0 abc	Hyaline	Falcate
Cf13	33.0 b-e	11.4 ab	Hyaline	Falcate
Cf14	34.0 a-d	10.5 bcd	Hyaline	Falcate
Cf15	35.0 abc	10.0 d	Hyaline	Falcate
Cf16	35.7 ab	10.5 bcd	Hyaline	Falcate
Cf17	32.0 cde	10.8 a-d	Hyaline	Falcate
Cf18	32.7 b-e	10.5 bcd	Hyaline	Falcate
Cf19	30.7 de	10.4 cd	Hyaline	Falcate
Cf20	34.0 a-d	10.4 cd	Hyaline	Falcate
Cf21	32.7 b-e	11.0 abc	Hyaline	Falcate
Cf22	31.0 de	10.5 bcd	Hyaline	Falcate
Cf23	30.3 e	11.5 a	Hyaline	Falcate
Cf24	33.7 a-e	10.0 d	Hyaline	Falcate
Cf25	31.7 cde	10.5 bcd	Hyaline	Falcate
Cf03(R)	30.7 de	11.0 abc	Hyaline	Falcate
Cf04(R)	32.0 cde	10.5 bcd	Hyaline	Falcate
Cf05(R)	31.0 de	10.5 bcd	Hyaline	Falcate
Cf06(R)	30.7 de	11.5 a	Hyaline	Falcate
Cf08(R)	33.0 b-e	11.0 abc	Hyaline	Falcate

*Mean of ten conidia
In a column, means followed by a common letters are not significantly different at the 5 % level by DMRT
Cf- *Colletotrichum falcatum*
Cf(R)-*Colletotrichum falcatum* Race

of the toxin on the leaves, matured leaves were detached and cut into pieces each into 10 cm long. The mid-veins were injected as described for the stem. The lesion size was measured in centimeter and then it is converted to percent infected area.

STATISTICAL ANALYSES

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

The morphological characteristics of different isolates of *C. falcatum* on PDA medium were studied, significant variations were observed with respect to conidial dimensions among the isolates. The isolates of *C. falcatum* produced hyaline falcate shaped conidia. The length of conidia ranged from 30.0-37.0µm. Highest length

Table 3. Cultural characters of different isolates of *C. falcatum.*

Isolate	Colony color	Substrate color	Margin	Topography	*Colony diameter (mm)	Sporulation
Cf1	White	Black	Smooth	Mycelium flat growth	89.00 ^{ab}	++
Cf2	White	black	Smooth	Raised fluffy growth	89.61 ^{ab}	++
Cf3	Whitish orange	Black	Smooth	Mycelium flat growth	89.10 ^{ab}	+++
Cf4	Greyish White	Dark grey	Smooth	Raised fluffy growth	74.00 ^{c}	++
Cf5	White	black	Smooth	Raised fluffy growth	89.00 ^{ab}	+++
Cf6	Greyish White	black	Smooth	Raised fluffy growth	81.06 ^{bc}	++
Cf7	White	Dark grey	Smooth	Raised fluffy growth	86.03 ^{ab}	+++
Cf8	Light Grey	Black	Irregular	Mycelium flat growth	86.40 ^{ab}	+++
Cf9	Whitish orange	Pinkish black	Smooth	Raised fluffy growth	88.45^{ab}	++
Cf10	Whitish orange	Pinkish black	Smooth	Raised fluffy growth	87.25 ^{ab}	+++
Cf11	White	Pinkish black	Smooth	Raised fluffy growth	60.50 ^{d}	++
Cf12	White	Pinkish black	Smooth	Raised fluffy growth	85.50 ^{ab}	++
Cf13	White	Black	Smooth	Mycelium flat growth	88.75 ^{ab}	+
Cf14	Grey	black	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf15	Whitish orange	Pinkish black	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf16	Grey	black	Smooth	Raised fluffy growth	86.55 ^{ab}	+++
Cf17	Greyish white	Dark grey	Smooth	Raised fluffy growth	89.00 ^{ab}	+++
Cf18	White	Black	Smooth	Raised fluffy growth	89.50 ^{ab}	++
Cf19	Black	Black	Smooth	Raised fluffy growth	88.25 ^{ab}	+++
Cf20	Black	Black	Smooth	Raised fluffy growth	89.00 ^{ab}	+++
Cf21	Greyish white	Black	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf22	Greyish white	Black	Irregular	Raised fluffy growth	90.00 ^{a}	+++
Cf23	Grey	Grey	Smooth	Raised fluffy growth	85.50 ^{ab}	++
Cf24	White	White	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf25	Grey	Blackish grey	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf03(R)	Grey	Blackish white	Smooth	Raised fluffy growth	90.00 ^{a}	++
Cf04(R)	Greyish white	Black	Smooth	Raised fluffy growth	90.00 ^{a}	+++
Cf05(R)	White	white	Irregular	Raised fluffy growth	90.00 ^{a}	++
Cf06(R)	Greyish white	Black	Smooth	Raised fluffy growth	87.50 ^{ab}	+++
Cf08(R)	Greyish White	Black	Irregular	Raised fluffy growth	90.00 ^{a}	+++

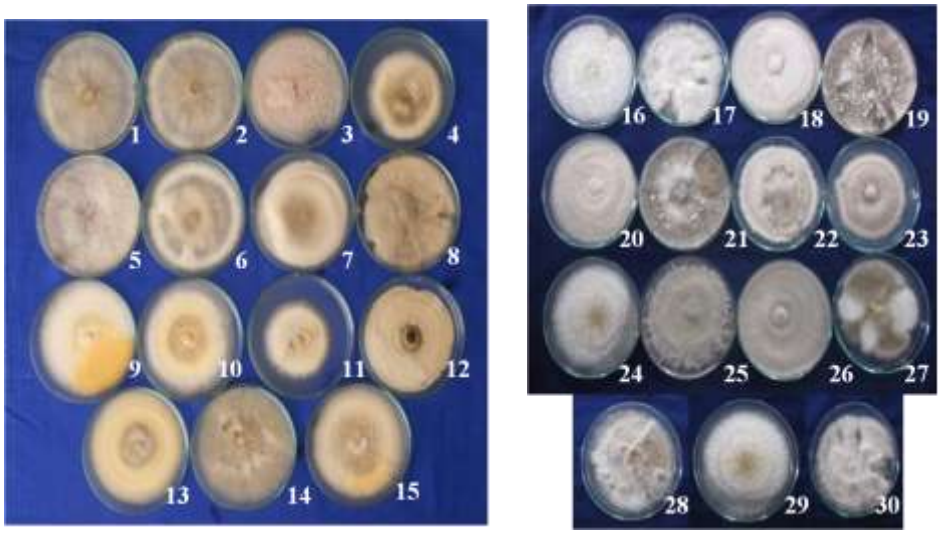
+Poorsporulation : 1-10 spores / microscopic field (100X); ++ Medium sporulation : 11-50 spores/ microscopic field (100X), +++ Good sporulation : More than 100 spores/ microscopic field (100X)* Mean of three replications,In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT.
Cf- *Colletotrichum falcatum,* Cf(R)- *Colletotrichum falcatum* Race.

Table 4. Pathogenicity of different isolates of *C. falcatum.*

Isolates	CoC671	Co 86249
Cf1	S	R
Cf2	S	R
Cf3	S	I
Cf4	S	R
Cf5	S	R
Cf6	S	R
Cf7	S	R
Cf8	S	R
Cf9	S	R
Cf10	S	R
Cf11	S	R
Cf12	S	R
Cf13	S	R
Cf14	S	R
Cf15	S	R
Cf16	S	R
Cf17	S	R
Cf18	S	R
Cf19	S	R
Cf20	S	R
Cf21	S	R
Cf22	S	R
Cf23	S	R
Cf24	S	R
Cf25	S	R
Cf03(R)	S	R
Cf04(R)	S	R
Cf05(R)	S	R
Cf06(R)	S	R
Cf08(R)	S	R

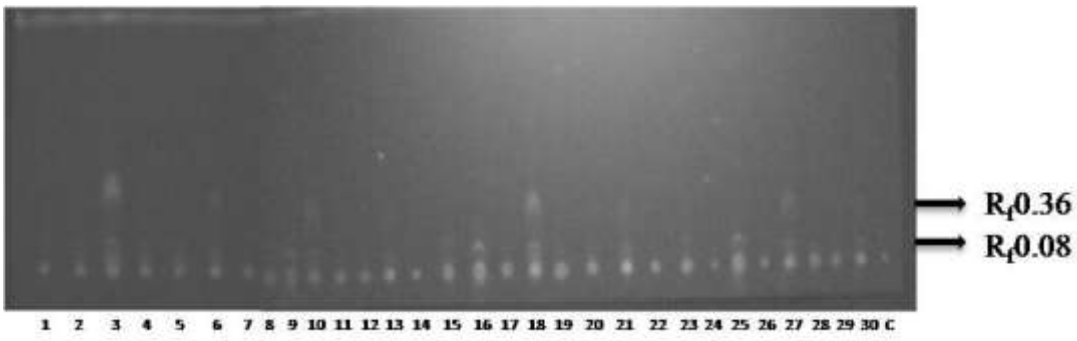
R (Resistant) - Lesion width laterally restricted, nodal transgression up to two nodes, white spots, rind infection, sporulation over the rind and yellowing/drying of top absent.
S (Susceptible) - Lesion width laterally spreading, nodal transgression more than 2 nodes, white spots progressive or restricted. In case of progressive white spots rind infection, sporulation over the rind and yellowing/drying of top absent/present.
I(Intermediate) - Lesion width laterally restricted or spreading; nodal transgression more than 2 nodes; white spots absent or present (restricted type), rind, infection, sporulation over the rind and yellowing/drying of top absent.
Cf- *Colletotrichum falcatum*
Cf(R)-*Colletotrichum falcatum* Race

Figure 1. Colony Characters of *C. falcatum* isolates.



1. Cf1	7. Cf7	13.Cf13	19. Cf19	25.Cf25
2. Cf2	8. Cf8	14. Cf14	20. Cf20	26.Cf03(R)
3. Cf3	9. Cf9	15.Cf15	21. Cf21	27.Cf04(R)
4. Cf4	10.Cf10	16.Cf16	22. Cf22	28.Cf05(R)
5. Cf5	11. Cf11	17. Cf17	23. Cf23	29.Cf06(R)
6. Cf6	12. Cf12	18. Cf18	24.Cf24	30.Cf08(R)

Figure 2. Secondary metabolite profile of various isolates.

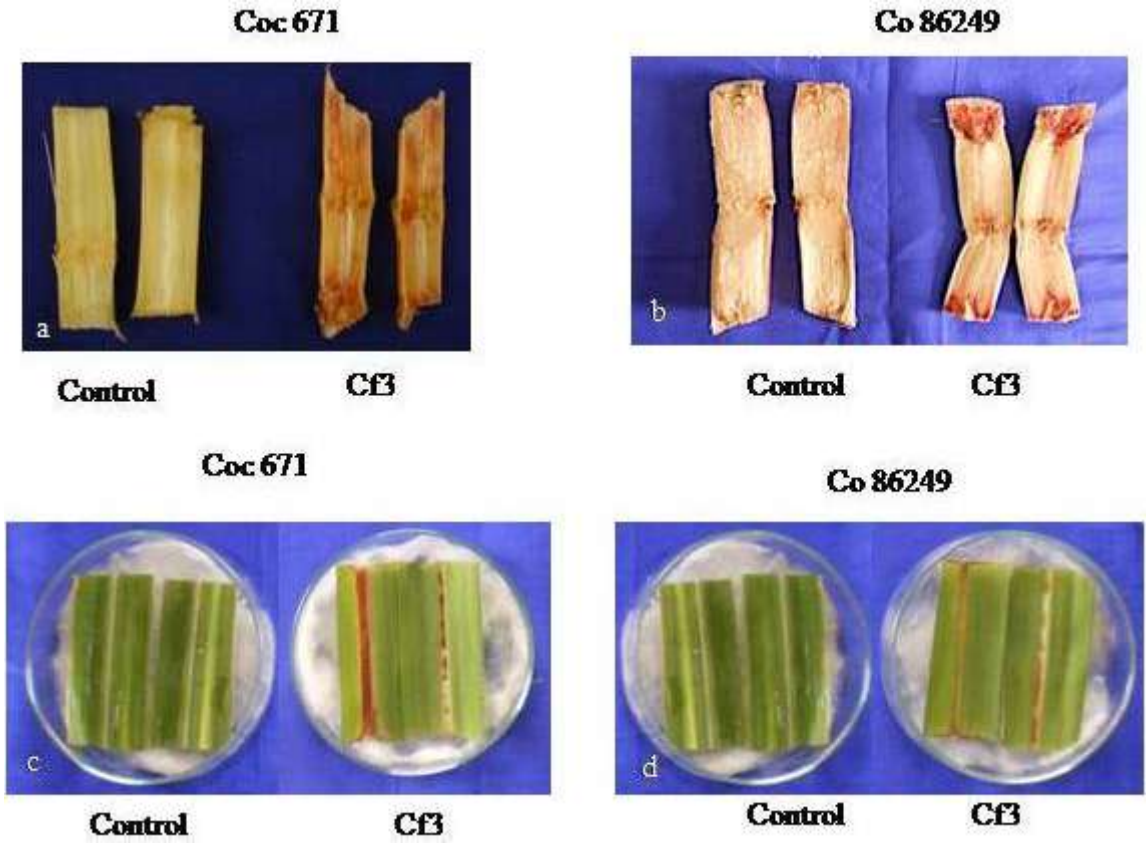


1. Cf1	7. Cf7	13.Cf13	19. Cf19	25.Cf25
2. Cf2	8. Cf8	14. Cf14	20. Cf20	26.Cf03(R)
3. Cf3	9. Cf9	15.Cf15	21. Cf21	27.Cf04(R)
4. Cf4	10.Cf10	16.Cf16	22. Cf22	28.Cf05(R)
5. Cf5	11. Cf11	17. Cf17	23. Cf23	29.Cf06(R)
6. Cf6	12. Cf12	18. Cf18	24.Cf24	30.Cf08(R)

C- Ethyl acetate

of conidia was observed in Cf3 and Cf12 isolate (37 μm) followed by Cf11 isolate (36.5 μm) and shortest conidia was recorded in C10 and C23 isolates (30 μm). Width of the conidia ranged from 10 to 11.5 μm. Conidia were falcate/sickle shaped with a round apical end tapering towards the base (Table 2).

Figure 3. Identificiton of virulent isolate with crude toxin



a. Reaction of Cf3 crude toxin in susceptible variety Coc 671 showing redding symptoms on 6th day after inoculation in stem
b. Reaction of Cf3 crude toxin in resistant variety Co 86249 showing redding symptoms on 6th day after inoculation in stem
c. Reaction of Cf3 crude toxin in susceptible variety Coc 671 showing redding symptoms on 6th day after inoculation in leaves
d. Reaction of Cf3 crude toxin in resistant variety Co 86249 showing redding symptoms on 6th day after inoculation in leaves

Different colony colors *viz.*, grey, light grey, Black, whitish orange and white were observed. Red rot isolates *viz.,*Cf1,Cf2, Cf5,Cf7, Cf11, Cf12, Cf13, Cf18, Cf24 and Cf05(R) isolates exhibited White, while other isolates *viz.,*Cf14, Cf16, Cf23,Cf25 and Cf03(R) were grey. The isolates Cf19 and Cf20 showed Black mycelia. Isolates of others grayish white and whitish orange. All the isolates showed variation regarding substrate color, margin, topography, colony diameter and sporulation (Figure 1; Table 3).

Pathogenicity

Among the 30 isolates tested against the two varieties, Coc671 showed highly susceptible to all the isolates and whereas Co 86249 showed resistant reaction against all the isolates of *C. falcutm* except Cf3, which showed intermediate reaction, isolate Cf3 was highly virulent which was later tested for the correlation of virulence and toxin produced (Table 4).

High Performance Thin Layer Chromatography (Hptlc) Analysis

Attempts were made to differentiate *C. falcatum* isolates based on their secondary metabolite profile. Ethyl acetate extracts prepared for each of 3 isolates with known morphological and virulence characteristics were chromatographed on hptlc plates. A single band (Rf = 0.36) was identified for Cf3, Cf6, Cf10, Cf18, Cf04(R) isolates when visualized under UV light at a wavelength of 366 nm. The band was conspicuously absent in control and also a more complex pattern of bands was observed with Rf = 0.08 in various isolates (Figure 2).

Identification of virulent Isolates with crude toxins produced by *C. falcatum*

In order to test the virulent nature of *C. falcatum* isolates, crude extract was subjected to two varieties in stems and

Table 5. Screening of toxins produced different isolates of *C. falcatum* against Susceptible and Resistant varieties (Stem injection).

Isolates	Coc 671 (% infected area on cane stem)*												Co 86249(% infected area on cane stem)*											
	2 DAI				4 DAI				6 DAI				2 DAI				4 DAI				6 DAI			
	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl
Cf1	-	-	-	0.00 ^{j} (0.28)	-	-	0.00^{h} (0.28)	0.00^{l} (0.28)	-	-	31.5 ^{i} (34.1)	42.5 ^{j} (40.6)	-	-	-	-	-	-	-	-	-	-	-	0.00 ^{o} (0.28)
Cf2	-	-	-	0.00 ^{j} (0.28)	-	-	0.00^{h} (0.28)	0.00^{l} (0.28)	-	-	30.5 ^{i} (33.5)	40.4^{j} (39.4)	-	-	-	-	-	-	-	-	-	-	-	0.00 ^{o} (0.28)
Cf3	-	-	-	17.2^{a} (24.4)	-	-	56.4^{a} (48.6)	71.8^{a} (57.9)	-	-	88.5^{a} (70.4)	92.3^{a} (74.4)	-	-	-	-	-	-	-	-	-	-	-	30.6^{a} (33.5)
Cf4	-	-	-	12.5^{ghi} (20.7)	-	-	32.5^{fg} (34.7)	52.5^{ijk} (46.4)	-	-	70.2 $^{c-g}$ (56.9)	73.3 ^{i} (58.9)	-	-	-	-	-	-	-	-	-	-	-	20.2^{mn} (26.7)
Cf5	-	-	-	12.2^{ghi} (20.4)	-	-	34.3 ^{f} (35.8)	54.4^{ijk} (47.5)	-	-	72.1 $^{b-g}$ (58.1)	80.5^{e-i} (63.8)	-	-	-	-	-	-	-	-	-	-	-	20.2^{mn} (26.7)
Cf6	-	-	-	11.4^{i} (19.7)	-	-	30.4^{g} (33.4)	48.3^{k} (44.0)	-	-	64.5 ^{gh} (53.4)	72.8^{i} (58.5)	-	-	-	-	-	-	-	-	-	-	-	23.6 $^{g-j}$ (29.0)
Cf7	-	-	-	12.1 ^{hi} (20.3)	-	-	32.7^{fg} (34.8)	50.1^{jk} (45.0)	-	-	66.7 $^{e-h}$ (54.7)	74.5^{hi} (59.7)	-	-	-	-	-	-	-	-	-	-	-	22.6^{ijk} (28.3)
Cf8	-	-	-	12.8^{fgh} (20.9)	-	-	38.5^{de} (38.3)	53.5^{g-j} (47.0)	-	-	68.9 $^{d-g}$ (56.1)	76.2^{ghi} (60.8)	-	-	-	-	-	-	-	-	-	-	-	21.7 $^{j-m}$ (27.7)
Cf9	-	-	-	12.6^{fgh} (20.7)	-	-	38.1^{e} (38.5)	54.9^{e-j} (47.8)	-	-	72.6 $^{b-g}$ (58.4)	78.4^{f-i} (62.3)	-	-	-	-	-	-	-	-	-	-	-	23.9 ^{ghi} (29.2)
Cf10	-	-	-	11.9^{hi} (20.1)	-	-	32.1^{fg} (34.5)	50.6^{jk} (45.3)	-	-	65.3 ^{fgh} (53.9)	74.5^{hi} (59.7)	-	-	-	-	-	-	-	-	-	-	-	24.0 $^{f-l}$ (29.3)
Cf11	-	-	-	12.8^{fgh} (20.9)	-	-	38.2^{e} (38.1)	54.1^{e-j} (47.3)	-	-	72.3 $^{b-g}$ (58.2)	81.5^{d-i} (64.6)	-	-	-	-	-	-	-	-	-	-	-	23.0 ^{hij} (28.6)
Cf12	-	-	-	16.7 ^{ab} (24.1)	-	-	41.5^{b-e} (40.1)	57.8^{b-h} (49.4)	-	-	77.3 $^{b-e}$ (61.6)	87.4^{a-e} (69.4)	-	-	-	-	-	-	-	-	-	-	-	27.4 ^{bc} (31.5)
Cf13	-	-	-	16.2^{ab} (23.7)	-	-	40.3^{b-e} (39.4)	56.4 $^{b-i}$ 48.6)	-	-	74.2 $^{b-g}$ (59.5)	88.7 $^{a-d}$ (70.6)	-	-	-	-	-	-	-	-	-	-	-	28.3 ^{b} (32.1)
Cf14	-	-	-	16.5^{ab} (23.9)	-	-	42.5^{bc} (40.6)	58.1^{b-g} (49.6)	-	-	75.7 $^{b-f}$ (60.5)	89.^{6abc} (71.4)	-	-	-	-	-	-	-	-	-	-	-	27.9 ^{b} (31.8)

Table 5. Cont.

Cf15	-	-	-	13.1^{fgh} (21.2)	-	-	39.6^{b-e} (38.9)	53.7^{f-j} (47.1)	-	-	70.4^{b-g} (57.0)	80.5^{e-i} (63.8)	-	-	-	-	-	-	-	-	-	-	-	20.7^{k-n} (27.0)
Cf16	-	-	-	13.4^{efg} (21.4)	-	-	40.8^{b-e} (39.6)	55.1^{d-j} (47.9)	-	-	72.5^{b-g} (58.4)	83.9^{bg} (66.4)	-	-	-	-	-	-	-	-	-	-	-	20.6^{k-n} (26.9)
Cf17	-	-	-	13.8^{ef} (21.8)	-	-	40.9^{b-e} (39.7)	55.3^{c-j} (48.0)	-	-	72.9^{b-g} (58.6)	84.0^{bg} (66.5)	-	-	-	-	-	-	-	-	-	-	-	20.1^{mn} (26.6)
Cf18	-	-	-	0.00^{j} (0.28)	-	-	0.00^{h} (0.28	0.00^{l} (0.28)	-	-	30.3 i (33.3)	40.2^{j} (39.3)	-	-	-	-	-	-	-	-	-	-	-	19.1^{n} (25.9)
Cf19	-	-	-	12.5^{ghi} (20.7)	-	-	33.3^{fg} (35.2)	52.5^{ijk} (46.4)	-	-	73.5^{b-g} (59.0)	82.3^{ch} (65.2)	-	-	-	-	-	-	-	-	-	-	-	20.5^{lmn} (26.9)
Cf20	-	-	-	12.8^{fgh} (20.9)	-	-	33.9^{f} (35.6)	53.4^{g-j} (46.9)	-	-	71.4^{b-e} (57.6)	80.8^{e-i} (64.0)	-	-	-	-	-	-	-	-	-	-	-	19.6^{n} (26.2)
Cf21	-	-	-	16.1^{ab} (23.6)	-	-	40.1^{b-e} (39.2)	58.5^{b-g} (49.9)	-	-	76.7^{b-e} (61.1)	85.4^{b-f} (67.6)	-	-	-	-	-	-	-	-	-	-	-	25.3^{c-g} (30.1)
Cf22	-	-	-	16.2^{bc} (23.7)	-	-	40.4^{b-e} (39.4)	58.8^{b-f} (50.0)	-	-	78.3^{bcd} (62.2)	86.1^{a-f} (68.2)	-	-	-	-	-	-	-	-	-	-	-	26.1^{b-f} (30.7)
Cf23	-	-	-	16.6^{bc} (24.0)	-	-	41.9^{bcd} (40.3)	59.1^{b-e} (50.2)	-	-	80.6^{b} (63.9)	89.9^{ab} (71.7)	-	-	-	-	-	-	-	-	-	-	-	24.3^{e-l} (29.5)
Cf24	-	-	-	14.5^{de} (22.3)	-	-	39.4^{cde} (38.8)	52.8^{h-k} (46.6)	-	-	70.5^{b-g} (57.1)	80.4^{e-i} (63.7)	-	-	-	-	-	-	-	-	-	-	-	22.$^{4\ i-l}$ (28.2)
Cf25				16.3^{ab} (23.8)			42.6^{bc} (40.7)	60.3^{bc} (50.9)			78.1^{bcd} (62.1)	84.8^{b-f} (67.1)		-	-	-	-	-	-	-	-	-	-	22.9^{ij} (28.5)
Cf03(R)	-	-	-	15.3^{cd} (23.0)	-	-	42.1^{bcd} (40.4)	60.1^{bcd} (50.8)	-	-	78.2^{bcd} (62.2)	85.4^{b-f} (67.6)	-	-	-	-	-	-	-	-	-	-	-	25.1^{d-h} (30.0)
Cf04(R)	-	-	-	15.7^{bc} (23.3)	-	-	42.9^{bc} (40.9)	60.7^{b} (51.1)	-	-	79.$^{bcd)}$ (62.2)	86.4^{a-f} (68.5)	-	-	-	-	-	-	-	-	-	-	-	26.4^{b-e} (30.9)
Cf05(R)	-	-	-	15.6^{cd} (23.2)	-	-	42.7^{bc} (40.8)	60.4^{bc} (51.0)	-	-	79.0^{bcd} (62.8)	86.0^{a-f} (68.1)	-	-	-	-	-	-	-	-	-	-	-	26.2^{b-e} (30.7)
Cf06(R)	-	-	-	15.4^{cd} (23.1)	-	-	42.5^{bc} (40.6)	60.3^{bc} (50.9)	-	-	80.1^{bc} (62.7)	87.0^{ae} (69.0)	-	-	-	-	-	-	-	-	-	-	-	25.2^{c-g} (30.1)
Cf08(R)	-	-	-	15.8^{bc} (23.4)	-	-	43.2^{b} (41.0)	61.3^{b} (51.5)	-	-	81.8^{bc} (63.5)	88.6^{a-d} (70.5)	-	-	-	-	-	-	-	-	-	-	-	27.1^{bcd} (31.3)

*Mean of three replications
In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine transformed values
DAI- Days After Inoculation
Cf- *Colletotrichum falcatum*
Cf(R)- *Colletotrichum falcatum* Race

Table 6. Screening of toxins produced different isolates of *C. falcatum* against Susceptible and Resistant varieties (Leaf injection).

Isolates	Coc 671 (% infected area on cane stem)*												Co 86249(% infected area on cane stem)*											
	2 DAI				4 DAI				6 DAI				2 DAI				4 DAI				6 DAI			
	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl	C	50 µl	100 µl	200 µl
Cf1	-	-	-	0.00 ^{k} (0.28)	-	-	0.00^{l} (0.28)	0.00^{m} (0.28)	-	-	21.2 ^{m} (27.4)	32.4 ^{n} (34.6)	-	-	-	-	-	-	-	-	-	-	-	0.00 ^{o} (0.28)
Cf2	-	-	-	0.00 ^{k} (0.28)	-	-	0.00^{l} (0.28)	0.00^{m} (0.28)	-	-	24.7 ^{l} (29.7)	34.7^{m} (39.4)	-	-	-	-	-	-	-	-	-	-	-	0.00 ^{o} (0.28)
Cf3	-	-	-	12.2^{a} (20.4)	-	-	54.3^{a} (47.4)	61.9^{a} (51.8)	-	-	65.1^{a} (53.8)	70.2^{a} (56.9)	-	-	-	-	-	-	-	-	-	-	-	28.5^{a} (32.2)
Cf4	-	-	-	7.5^{gh} (15.8)	-	-	28.4^{jk} (32.1)	32.5^{kl} (34.7)	-	-	33.6 ^{jk} (35.4)	37.3 ^{m} (37.6)	-	-	-	-	-	-	-	-	-	-	-	14.1^{k} (22.0)
Cf5	-	-	-	7.2^{gh} (15.5)	-	-	29.4^{ij} (32.8)	34.4^{k} (35.9)	-	-	60.5^{b} (51.0)	66.2^{abi} (54.4)	-	-	-	-	-	-	-	-	-	-	-	14.2^{k} (22.1)
Cf6	-	-	-	6.2^{i} (14.4)	-	-	25.7^{k} (30.4)	38.6^{j} (38.4)	-	-	35.5 ^{jk} (36.5)	42.6^{l} (40.7)	-	-	-	-	-	-	-	-	-	-	-	15.7^{hij} (23.3)
Cf7	-	-	-	7.1 ^{gh} (15.4)	-	-	29.8^{ij} (33.0)	30.6^{l} (33.5)	-	-	32.5 ^{k} (34.7)	44.5^{kl} (41.8)	-	-	-	-	-	-	-	-	-	-	-	14.4 ^{jk} (22.2)
Cf8	-	-	-	7.8^{g} (16.2)	-	-	33.7^{gh} (35.4)	43.7^{gh} (41.3)	-	-	37.3 ^{ij} (37.6)	46.6^{jkl} (43.0)	-	-	-	-	-	-	-	-	-	-	-	13.5 ^{kl} (21.5)
Cf9	-	-	-	0.0^{k} (0.28)	-	-	33.9^{fgh} (35.6)	44.8^{fgh} (42.0)	-	-	39.3 ^{hi} (38.8)	48.5^{ijk} (44.1)	-	-	-	-	-	-	-	-	-	-	-	14.9 ^{ijk} (22.7)
Cf10	-	-	-	5.5^{j} (13.5)	-	-	28.3^{jk} (32.5)	38.6^{j} (38.4)	-	-	35.2 ^{jk} (36.3)	48.5^{ijk} (44.1)	-	-	-	-	-	-	-	-	-	-	-	16.0 ^{hi} (23.5)
Cf11	-	-	-	5.8^{ij} (13.9)	-	-	33.4^{gh} (35.3)	44.5^{fgh} (41.8)	-	-	40.2 ^{hi} (39.3)	51.8^{ghi} (46.0)	-	-	-	-	-	-	-	-	-	-	-	17.0 ^{gh} (24.3)
Cf12	-	-	-	11.1 ^{bcd} (19.4)	-	-	41.4^{c} (40.0)	52.5^{cd} (46.4)	-	-	53.3 ^{c} (46.8)	64.5^{b} (53.4)	-	-	-	-	-	-	-	-	-	-	-	19.4 ^{f} (26.2)
Cf13	-	-	-	11.3^{bcd} (19.6)	-	-	30.3^{ij} (33.3)	46.4 $^{e-h}$ (42.9)	-	-	43.4 ^{fgh} (41.2)	55.7 $^{d-g}$ (48.2)	-	-	-	-	-	-	-	-	-	-	-	17.7 ^{g} (24.8)
Cf14	-	-	-	11.7^{ab} (20.0)	-	-	31.4^{hij} (34.0)	42.5^{hi} (40.6)	-	-	41.8 ^{gh} (40.2)	52.7^{f-i} (46.5)	-	-	-	-	-	-	-	-	-	-	-	22.4 ^{cd} (28.2)
Cf15	-	-	-	9.2^{f} (17.6)	-	-	31.8 ^{hi} (34.3)	42.6^{hi} (40.7)	-	-	41.5 ^{gh} (40.1)	52.9^{f-ii} (46.6)	-	-	-	-	-	-	-	-	-	-	-	16.7 ^{gh} (24.1)
Cf16	-	-	-	7.4^{gh} (15.7)	-	-	36.9 ^{def} (37.4)	47.8^{efg} (43.7)	-	-	46.5 ^{def} (42.9)	57.7^{def} (49.4)	-	-	-	-	-	-	-	-	-	-	-	16.9 ^{gh} (24.2)

Table 6. Cont.

Cf17	-	-	-	7.8 g (16.2)	-	-	38.4^{cde} (38.2)	47.8^{efg} (43.7)	-	-	48.8 d (44.3)	57.0^{def} (49.0)	-	-	-	-	-	-	-	-	-	-	-	16.3 ^{ghi} (23.8)
Cf18	-	-	-	0.00 ^{k} (0.28)	-	-	0.00^{l} (0.28	0.00^{m} (0.28)	-	-	22.3 ^{lm} (28.1)	34.2^{mn} (35.7)	-	-	-	-	-	-	-	-	-	-	-	12.3^{l} (20.5)
Cf19	-	-	-	5.5^{j} (13.5)	-	-	28.8^{ij} (32.4)	38.9^{ij} (38.5)	-	-	39.8 ^{hi} (39.1)	50.2^{hij} (45.1)	-	-	-	-	-	-	-	-	-	-	-	15.7 ^{hij} (23.3)
Cf20	-	-	-	5.8^{ij} (13.9)	-	-	29.9^{ij} (33.1)	38.4^{j} (38.2)	-	-	40.4 ^{hi} (39.4)	48.8^{ijk} (44.3)	-	-	-	-	-	-	-	-	-	-	-	16.3 ^{ghi} (23.8)
Cf21	-	-	-	11.1^{bcd} (19.4)	-	-	36.2 ^{efg} (36.9)	48.2^{ef} (43.9)	-	-	46.8 ^{def} (43.1)	58.6^{cde} (49.9)	-	-	-	-	-	-	-	-	-	-	-	21.7 ^{cde} (27.7)
Cf22	-	-	-	11.3^{bcd} (19.6)	-	-	34.2^{fgh} (35.7)	45.5^{e-f} (42.4)	-	-	44.5 ^{efg} (41.8)	55.1^{d-h} (47.9)	-	-	-	-	-	-	-	-	-	-	-	20.8 ^{def} (27.1)
Cf23	-	-	-	11.7^{ab} (20.0)	-	-	37.6^{de} (37.8)	49.2^{de} (44.5)	-	-	47.6 ^{de} (43.6)	59.8^{cd} (50.6)	-	-	-	-	-	-	-	-	-	-	-	20.3 ^{ef} (26.7)
Cf24	-	-	-	9.2^{f} (17.6)	-	-	31.7^{hi} (34.2)	42.6^{hi} (40.7)	-	-	41.8 ^{gh} (40.2)	53.5^{e-i} (47.0)	-	-	-	-	-	-	-	-	-	-	-	21.5 ^{cde} (27.6)
Cf25				11.4^{abc} (19.7)			38.5^{cde} (38.3)	48.0^{ef} (44.1)			48.1 ^{de} (43.9)	58.9^{cd} (50.1)	-	-	-	-	-	-	-	-	-	-	-	22.9 ^{bc} (28.5)
Cf03(R)	-	-	-	9.7^{f} (18.1)	-	-	37.4^{de} (37.6)	52.5^{cd} (46.4)	-	-	47.7 ^{de} (43.6)	62.8^{bc} (52.4)	-	-	-	-	-	-	-	-	-	-	-	22.1 $^{e-l}$ (28.0)
Cf04(R)	-	-	-	9.9^{ef} (18.3)	-	-	39.9^{cd} (39.1)	54.3 ^{bc} (47.4)	-	-	49.5 ^{d} (44.7)	64.9^{b} (53.6)	-	-	-	-	-	-	-	-	-	-	-	22.4^{cd} (28.2)
Cf05(R)	-	-	-	10.6^{cde} (18.9)	-	-	45.7^{b} (42.5)	56.6^{b} (48.7)	-	-	55.8 ^{c} (48.3)	66.3^{ab} (54.5)	-	-	-	-	-	-	-	-	-	-	-	22.2^{cd} (28.1)
Cf06(R)	-	-	-	10.5^{de} (18.9)	-	-	45.5^{b} (42.4)	56.9^{b} (48.9)	-	-	55.1 ^{c} (47.9)	66.7^{ab} (54.7)	-	-	-	-	-	-	-	-	-	-	-	22.1^{cd} (28.0)
Cf08(R)	-	-	-	10.9^{bcd} (19.2)	-	-	45.2^{b} (42.2)	56.3^{bc} (48.6)	-	-	55.5 ^{c} (48.1)	66.4^{ab} (54.5)	-	-	-	-	-	-	-	-	-	-	-	24.5^{b} (29.6)

*Mean of three replications
In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine transformed values
DAI- Days After Inoculation
Cf- *Colletotrichum falcatum*
Cf(R)- *Colletotrichum falcatum* Race

Table 7. Screening of different toxins dilution of *C. falcatum* against Susceptible and Resistant varieties (Stem injection).

Isolates	Coc 671 (% infected area on stem) Serial dilution of the toxin							Co 86249 (% infected area on stem) Serial dilution of the toxin						
	C	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	C	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Cf1	-	44.5^{h} (41.8)	38.7^{j} (38.4)	30.4^{l} (33.4)	22.5^{m} (28.3)	5.6^{rs} (13.6)	-	-	0.00 ^{m} (0.28)	-	-	-	-	-
Cf2	-	42.1^{h} (40.4)	36.4^{j} (37.1)	29.5^{l} (32.8)	19.9^{m} (26.4)	4.3^{s} (11.9)	-	-	0.00 ^{m} (0.28)	-	-	-	-	-
Cf3	-	93.1^{a} (75.4)	86.3^{a} (68.4)	80.4^{a} (63.7)	71.2^{a} (57.5)	50.5^{ab} (45.2)	-	-	32.1^{a} (34.5)	-	-	-	-	-
Cf4	-	74.9^{fg} (59.9)	68.2^{hi} (55.6)	60.3^{jk} (50.9)	51.5^{jkl} (45.8)	20.5^{q} (26.9)	-	-	21.2^{jkl} (27.4)	-	-	-	-	-
Cf5	-	81.4^{c-f} (64.5)	76.4^{b-g} (60.9)	68.4^{f-i} (55.8)	59.1^{d-h} (50.2)	28.4^{op} (32.1)	-	-	22.5^{g-k} (28.3)	-	-	-	-	-
Cf6	-	71.4^{g} (57.6)	66.9^{i} (54.8)	58.3^{k} (49.7)	47.9^{l} (43.7)	27.3^{p} (31.4)	-	-	24.5^{d-g} (29.6)	-	-	-	-	-
Cf7	-	76.4^{fg} (60.9)	70.2^{f-i} (56.9)	63.5^{h-k} (52.8)	54.7^{g-k} (47.7)	34.1^{k-n} (35.7)	-	-	22.7^{g-j} (28.4)	-	-	-	-	-
Cf8	-	76.4^{fg} (60.9)	70.5 $^{f-i}$ (57.1)	62.8^{ijk} (52.4)	53.0^{i-l} (46.7)	32.3^{mn} (34.6)	-	-	22.0^{h-l} (27.9)	-	-	-	-	-
Cf9	-	79.4^{efg} (63.0)	72.6^{e-i} (58.4)	64.3^{h-k} (53.3)	55.5^{f-k} (48.1)	36.2^{i-l} (36.9)	-	-	24.0^{e-h} (29.3)	-	-	-	-	-
Cf10	-	75.5^{fg} (60.3)	69.2^{ghi} (56.3)	60.2^{jk} (50.8)	50.1^{kl} (45.0)	31.1^{no} (33.8)	-	-	23.6^{f-l} (29.0)	-	-	-	-	-
Cf11	-	82.0^{c-f} (64.9)	76.4^{b-g} (60.9)	67.5^{f-i} (55.2)	56.4^{e-j} (48.6)	35.3^{j-m} (36.4)	-	-	28.0^{bc} (31.9)	-	-	-	-	-
Cf12	-	88.5^{a-d} (70.4)	82.4^{abc} (65.2)	75.3^{a-e} (60.2)	66.3^{abc} (54.5)	45.2^{cd} (42.2)	-	-	29.1^{b} (32.6)	-	-	-	-	-
Cf13	-	88.9^{abc} (70.7)	83.1^{ab} (65.8)	77.4^{a-d} (61.6)	67.8^{ab} (55.4)	46.3^{c} (42.8)	-	-	28.8^{b} (32.4)	-	-	-	-	-
Cf14	-	89.8^{ab} (71.6)	83.6^{ab} (66.2)	77.7^{abc} (61.8)	70.0^{a} (56.8)	51.4^{a} (45.8)	-	-	21.2^{jkl} (27.4)	-	-	-	-	-
Cf15	-	82.5^{b-f} (65.3)	76.8^{b-g} (61.2)	69.2^{e-i} (56.3)	58.4^{d-i} (49.8)	37.2^{h-k} (37.5)	-	-	21.4^{jkl} (27.5)	-	-	-	-	-
Cf16	-	83.5^{b-f} (66.1)	77.3^{b-f} (61.6)	70.1^{e-h} (56.8)	60.3^{def} (50.9)	39.9^{fgh} (39.1)	-	-	21.0^{jkl} (27.2)	-	-	-	-	-

Table 7. Cont.

Cf17	-	85.5^{b-e} (67.7)	79.3^{b-e} (63.0)	71.3^{d-g} (57.6)	61.6^{cde} (51.7)	40.1^{fgh} (39.2)	-	-	21.5^{i-l} (27.6)	-	-	-	-	-
Cf18	-	41.7^{h} (40.2)	37.1^{j} (37.5)	32.5^{l} (34.7)	23.5^{m} (28.9)	5.9^{r} (14.0)	-	-	22.1^{h-l} (28.0)	-	-	-	-	-
Cf19	-	83.6^{b-f} (66.2)	77.3^{b-f} (61.6)	70.1^{e-h} (56.8)	60.4^{def} (51.0)	38.5^{g-j} (38.3)	-	-	20.1^{l} (26.6)	-	-	-	-	-
Cf20	-	80.5^{d-g} (63.8)	74.1^{d-i} (59.4)	66.5^{g-j} (54.6)	55.3^{f-k} (48.0)	33.5^{lmn} (35.3)	-	-	20.5^{kl} (26.9)	-	-	-	-	-
Cf21	-	86.1^{b-e} (68.2)	80.2^{a-d} (63.6)	73.1^{c-g} (58.7)	62.4^{cd} (52.1)	40.3^{fgh} (39.4)	-	-	25.0^{def} (29.9)	-	-	-	-	-
Cf22	-	90.1^{ab} (71.9)	86.4^{a} (68.5)	79.1^{ab} (62.8)	69.3^{a} (56.3)	47.4^{bc} (43.5)	-	-	26.5^{cd} (30.9)	-	-	-	-	-
Cf23	-	81.8^{c-f} (64.8)	75.5^{c-h} (60.3)	67.2^{f-i} (55.0)	54.3^{h-k} (47.4)	32.5^{mn} (34.7)	-	-	24.0^{e-h} (29.3)	-	-	-	-	-
Cf24	-	85.2^{b-e} (67.5)	79.9^{a-e} (63.4)	70.2^{e-h} (56.9)	60.5^{def} (51.0)	39.8^{fgh} (39.1)	-	-	23.0^{f-j} (28.6)	-	-	-	-	-
Cf25		86.3^{b-e} (68.4)	80.3^{a-d} (63.7)	72.1^{c-g} (58.1)	60.4^{def} (51.0)	39.0^{ghi} (38.6)			24.4^{d-g} (29.5)					
Cf03(R)	-	85.6^{b-e} (67.8)	79.2^{b-e} (62.9)	71.2^{d-g} (57.5)	59.9^{d-g} (50.7)	38.3^{g-j} (38.2)	-	-	24.7^{d-g} (29.7)	-	-	-	-	-
Cf04(R)	-	87.0^{a-e} (69.0)	81.2^{a-d} (64.3)	73.4^{b-f} (58.9)	63.3^{bcd} (52.7)	41.4^{efg} (40.0)	-	-	26.0^{cde} (30.6)	-	-	-	-	-
Cf05(R)	-	87.5^{a-e} (69.4)	80.1^{a-e} (63.5)	72.9^{c-g} (58.6)	62.4^{cd} (52.1)	42.6^{def} (40.7)	-	-	26.6^{cd} (31.0)	-	-	-	-	-
Cf06(R)	-	88.0^{a-d} (69.9)	81,3^{a-d} (64.4)	73.0^{c-g} (58.7)	63.0^{bcd} (52.5)	44.2^{cde} (41.6)	-	-	26.0^{cde} (30.6)	-	-	-	-	-
Cf08(R)	-	89.0^{abc} (70.8)	81.9^{abc} (64.9)	73.5^{b-f} (59.0)	63.5^{bcd} (52.8)	44.7^{cde} (41.9)	-	-	28.9^{b} (32.5)	-	-	-	-	-

*Mean of three replications
In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine transformed values
Cf- *Colletotrichum falcatum*
Cf(R)- *Colletotrichum falcatum* Race

Table 8. Screening of different toxins dilution of *C. falcatum* against Susceptible and Resistant varieties (Leaf injection).

	Coc 671 (% infected area on leaf)							Co 86249 (% infected area on leaf)						
	Serial dilution of the toxin							Serial dilution of the toxin						
	C	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	C	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Cf1	-	30.5 ^{k} (33.5)	21.6 ^{k} (27.7)	10.2^{n} (18.6)	5.9^{n} (14.0)	-	-	-	0.00 ^{0} (0.28)	-	-	-	-	-
Cf2	-	32.0^{jk} (34.4)	22.9^{jk} (28.6)	10.9^{n} (19.2)	6.2 ^{n} (14.4)	-	-	-	0.00 ^{o} (0.28)	-	-	-	-	-
Cf3	-	68.3 ^{a} (55.7)	57.8 ^{a} (49.5)	35.3^{a} (36.4)	25.7^{a} (30.4)	-	-	-	26.8^{a} (31.1)	-	-	-	-	-
Cf4	-	35.2 ^{j} (36.3)	25.7^{j} (30.5)	14.3^{m} (22.2)	8.6 ^{m} (17.0)	-	-	-	11.4 ^{n} (19.7)	-	-	-	-	-
Cf5	-	63.3 ^{b} (52.7)	54.9^{ab} (47.8)	32.5^{b} (34.7)	21.4^{cde} (27.5)	-	-	-	12.1^{mn} (20.3)	-	-	-	-	-
Cf6	-	39.7 ^{l} (39.0)	29.1 i (32.6)	17.2^{l} (24.4)	10.2^{l} (18.6)	-	-	-	13.5^{kl} (21.5)	-	-	-	-	-
Cf7	-	40.5 ^{l} (39.5)	31.4i (34.1)	19.5^{k} (26.2)	12.3 ^{k} (20.5)	-	-	-	12.5^{lmn} (20.7)	-	-	-	-	-
Cf8	-	41.4 ^{l} (40.0)	32.2 i (34.6)	18.9^{kl} (25.7)	12.0 ^{k} (20.2)	-	-	-	11.7^{mn} (20.0)	-	-	-	-	-
Cf9	-	46.8 ^{h} (43.1)	37.3^{gh} (37.6)	23.6^{hij} (29.0)	16.6 ^{ij} (24.0)	-	-	-	11.8^{mn} (20.3)	-	-	-	-	-
Cf10	-	45.9 ^{h} (42.6)	36.4^{gh} (37.1)	22.4^{j} (28.2)	16.0^{j} (23.5)	-	-	-	14.5^{ijk} (22.3)	-	-	-	-	-
Cf11	-	49.9 ^{fgh} (44.9)	38.2^{gh} (38.2)	24.5 $^{f-j}$ (29.6)	18.6^{gh} (25.5)	-	-	-	15.4 ^{ij} (23.1)	-	-	-	-	-
Cf12	-	61.5 ^{bc} (51.6)	50.2^{cd} (45.1)	29.8^{cd} (33.0)	20.4^{def} (26.8)	-	-	-	17.3 ^{h} (24.5)	-	-	-	-	-
Cf13	-	50.6 ^{fgh} (45.3)	40.1^{fg} (39.3)	25.9^{fgh} (30.5)	19.0^{fgh} (25.8)	-	-	-	15.8 ^{i}(23.4)	-	-	-	-	-
Cf14	-	48.9 ^{fgh} (44.3)	37.2^{gh} (37.6)	23.2^{ij} (28.7)	17.0 ^{ij} (24.3)	-	-	-	20.7 ^{cd} (27.0)	-	-	-	-	-
Cf15	-	48.7 ^{fgh} (44.2)	38.1^{gh} (38.1)	23.9^{g-j} (29.2)	18.0^{hi} (25.0)	-	-	-	14.8 ^{ijk} (22.6)	-	-	-	-	-

Table 8. Cont.

Cf16	-	53.7 ^{def} (47.1)	42.6 ^{f} (40.7)	26.5^{ef} (30.9)	19.9^{efg} (26.4)	-	-	-	15.0^{ilj} (22.7)	-	-	-	-	-
Cf17	-	53.0 ^{efg} (46.7)	42.0 ^{f} (40.4)	26.0^{fg} (30.6)	20.1^{efg} (26.6)	-	-	-	14.9 ^{ij} (22.7)	-	-	-	-	-
Cf18	-	31.6 ^{jk} (34.1)	20.1 ^{k} (26.6)	10.0^{n} (18.4)	5.4 ^{n} (13.4)	-	-	-	14.3 ^{jk} (22.2)	-	-	-	-	-
Cf19	-	48.5 ^{gh} (44.1)	37.2^{gh} (37.6)	24.3^{f-j} (29.5)	18.0^{hi} (25.0)	-	-	-	15.7^{ij} (23.3)	-	-	-	-	-
Cf20	-	46.1 ^{h} (42.7)	35.8 ^{h} (36.7)	22.4 (28.2)	16.6 ^{ij} (24.0)	-	-	-	13.0^{lm} (21.1)	-	-	-	-	-
Cf21	-	56.7 ^{de} (48.8)	46.2 ^{e} (42.8)	28.5^{de} (32.2)	20.0^{efg} (26.5)	-	-	-	14.5^{ijk} (22.3)	-	-	-	-	-
Cf22	-	57.8 ^{cd} (49.4)	46.4 ^{e} (42.9)	28.9^{d} (32.5)	20.6^{def} (26.9)	-	-	-	18.9 ^{fg} (25.7)	-	-	-	-	-
Cf23	-	50.1 ^{fgh} (45.0)	40.0 ^{fg} (39.2)	25.0^{f-i} (30.0)	19.0^{fgh} (25.8)	-	-	-	18.4^{gh} (25.3)	-	-	-	-	-
Cf24	-	58.1 ^{cd} (49.6)	47.3^{de} (43.5)	30.0^{cd} (33.2)	21.5^{cde} (27.6)	-	-	-	20.5^{cde} (26.9)	-	-	-	-	-
Cf25		62.0^{bc} (51.9)	51.9^{bc} (46.1)	32.5^{b} (34.7)	22.6^{bc} (28.3)				19.1^{efg} (25.9)					
Cf03(R)	-	64.1 ^{ab} (53.2)	54.2^{ab} (47.4)	33.5^{ab} (35.3)	23.0^{bc} (28.6)	-	-	-	22.0^{bc} (27.9)	-	-	-	-	-
Cf04(R)	-	65.4^{ab} (53.9)	54.8^{ab} (47.8)	33.8^{ab} (35.5)	23.8 ^{b} (29.1)	-	-	-	20.3^{def} (27.0)	-	-	-	-	-
Cf05(R)	-	65.7 ^{ab} (54.1)	54.9^{ab} (47.8)	34.1^{ab} (35.7)	24.0^{b} (29.3)	-	-	-	20.7^{cd} (28.3)	-	-	-	-	-
Cf06(R)	-	64.8^{ab} (53.9)	52.2 ^{bc} (46.3)	31.9^{bc} (34.3)	22.0^{cd} (27.9)	-	-	-	20.6^{cde} (26.9)	-	-	-	-	-
Cf08(R)	-	64.9 ^{ab} (53.6)	52.4 ^{bc} (46.4)	32.0^{bc} (34.4)	21.9^{cd} (27.9)	-	-	-	22.5 ^{b} (28.3)	-	-	-	-	-

*Mean of three replications
In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine transformed values
Cf- *Colletotrichum falcatum*
Cf(R)- *Colletotrichum falcatum* Race

leaves (Figure 3). In Coc671, there was no reddening symptom induced by up to 100 µl in crude extract after 2 days of inoculation, but there were noticeable reaction with 200 µl reaction, expect Cf1 and Cf2. Six days after inoculation, all isolates showed reddening reaction with 100 µl of crude extract. Maximum reddening symptoms were observed on the 6th day with 92.3 (% infected area on cane stem) in 200 µl. In resistant variety Co 86249, there were no symptoms till 4th day. On sixth day reddening symptom was noticed in 200 µl crude extract. Similar results were noticed in leaves with maximum reddening area of (70.20 and 14.1, % infected area on leaf) in Coc671 and Co 86249 respectively (Table 5 and 6; Figure 3).

The results of screening of the sugarcane with serially diluted toxin are presented in Table 7 and 8. In the stem treatment with Coc671, toxin dilution of 10^{-1} to 10^{-5} showed reddening symptom. In 10^{-5} dilutions maximum % infected area was noticed in Cf3 (50.5%) and least was noticed in Cf18 (5.9%). In Co 86249, only 10^{-1} diluted toxin showed reddening symptom. Among isolates tested toxins from Cf3 showed maximum per cent infected area (32.1 %) and the isolates Cf1 and Cf2 did not show any reaction. Similarly, leaf treatment with Coc671, the various diluted toxin of Cf3 at 10^{-5} showed maximum percent infected area on leaves (17.3 %). Among the diluted toxins of thirty isolates tested against Co86249 leaves, only 10^{-1} diluted toxin showed reddening symptom. Toxin from the isolates Cf3 showed maximum per cent infected area (26.8 %) whereas toxins from Cf1 and Cf2 did not show any symptom.

DISCUSSION

Red rot caused by the fungus *C. falcatum* is dreadful and seed transmissible stalk disease which spreads from place to place through the infected sugarcanes (Ramesh Sundar et al., 2009; Malathi et al., 2010). Various measures have been developed to effectively control the disease but it being unsuccessful, since the pathogen adopts to improved fungicide and develops new races which break the resistant. In the present study, to understand the genetic population structure of *C. falcatum* isolates and their relation with the races prevalent in Tamil Nadu. Twenty five isolates were collected from various districts of Tamil Nadu and five races were obtained from Sugarcane Breeding Institute, Coimbatore. Morphological variation revealed that there exists a wide variation among the isolates which is the basic method for characterization of different isolates. Similar results have been obtained by Malathi et al. (2011), in a study with large number of isolates the growth of isolates has direct correlation with pathogencity and it revealed that the tropical isolates were light colored, fast growing and highly sporulating types. Prema et al. (2011), observed wide variation in *C. musae* isolates with respect to cultural and morphological characters, the isolates produced blackish white, light pink and dark orange colored colonies.

Studies on pathogenic variability during 1993-2000 revealed the existence of four new pathotypes *viz.,* Cf 07, Cf 08, Cf 09, and Cf 10. But all the pathotypes except Cf 09 were avirulent on CoS 767 (Sathyavir et al., 2001). In the current study the isolate Cf3 showed highly virulent, since it produced symptom in resistant variety Co 86249. The pathotype reaction of Cf 997 in Andhra Pradesh was studied by Nageswararao and Patro (2005) and concluded that there exist four pathotypes when they screened 12 host differentials.

Several evidence now a day are emerging suggesting that the genomes of plant pathogenic fungi are rich in genes that are likely to be involved in the synthesis of secondary metabolites, such as nonribosomal peptide synthases and polyketide syntheses, whereas saprophytes appear to be deficient in such genes (Yoder & Turgeon, 2001). This secondary metabolite production by the pathogenic strains boost and aid in the pathogenesis process for successful establishment of disease in susceptible host.

In the present study of stem and leaf bioassay, toxin from Cf3 which is isolated from the susceptible Coc671 showed maximum percent infection of 92.3 % on 6^{th} day, the same isolate shows some kind of disease reaction in the reported resistant variety Co 86249. Similar results were observed in leaves treated with the crude toxin. A successful pathogen requires potential warfare tools in the form of toxin and hydrolytic enzymes for penetration and infection in the host. Correlation between disease expression and production of secondary metabolites viz., toxin, enzymes and melanin by the pathogen has been established. This was confirmed by inoculating partially purified toxin from isolated from susceptible sugarcane cv. Coc671 have been compared for this purpose (Mohanraj et al*.,* 2003). In the present study, the serially diluted toxin tested showed positively up to 10^{-5} with maximum 50.5 % infected area on stem treated with the crude dilution of Cf3. Similarly Olufolaji (2000)*,* conducted bioassay with crude and partially purified toxins in stem and leaf and reported that CP70/1133 and LSI-803 showed more resistant attributes towards the *C. falcatum.* Thus from the current study we concluded that the *C. falcatum* isolates vary among themselves with respect to the cultivar and places from which they were isolated. Cf 3 isolate was highly virulent because could able to produce more amount of phytotoxin.

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