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VARIABILITY IN COLONY MORPHOLOGY AND VIRULENCE OF *CYLINDROCLADIUM QUINQUESEPTATUM* ISOLATES CAUSING LEAF AND SEEDLING BLIGHT OF *EUCALYPTUS*

Pooja Arya, P.S. Mohanty, Amit Pandey and N.S.K. Harsh
Forest Pathology Division, Forest Research Institute, Dehradun, India.

ABSTRACT
Paper, pulp and plywood industries of north Indian states *viz.* Uttarakhand, Haryana and Punjab heavily depend on *Eucalyptus* plantations for raw material. However, *Cylindrocladium* leaf and seedling blight cause large scale mortality in nurseries thus jeopardizing plantation programs. In the present study 71 isolates of the fungal pathogen *Cylindrocladium quinqueseptatum* collected from three north Indian states were screened for their relative virulence. Representative pathogen isolates were screened on different culture media for variability in cultural and micro-morphological traits.

KEYWORDS: *Cylindrocladium* leaf blight, pathogenicity, virulence, disease resistance, Eucalyptus

INTRODUCTION

Though *Eucalyptus* is grown in many parts of the country it has become commercially very important as raw material for the pulp, paper and plywood industries of northern India. However, large scale mortality due to leaf and seedling blight caused by *Cylindrocladium quinqueseptatum* Boedijn and Reitsma has attained epidemic proportion in *Eucalyptus* nurseries of Uttarakhand, Punjab and Haryana. In nurseries the initial symptom includes leaf necrosis leading to twig and stem blight, complete defoliation and outright killing of the seedlings. In subsequent years the disease severity increases which even lead to the mortality of complete nursery stock. *Cylindrocladium*, a soil borne pathogen, is known to cause foliage as well as root diseases of *Eucalyptus* in Kerala (Sharma *et al.* 1984). Different species of this pathogen has been reported to cause Eucalyptus diseases in south India. However, in northern part of India only one species, *C. quinqueseptatum* is encountered causing seedling blight in *Eucalyptus* nurseries. In plantations, leaf and twig blight leads to epicormic branching which hampers the tree growth. The disease is favored by high humidity and aggravates during monsoon period.

A wide range of pathogenic variation was observed among the various isolates of *C. quinqueseptatum* collected from different parts of Kerala (Sharma and Mohanan, 1991). Since sources of resistance in *Eucalyptus* to CLB (*Cylindrocladium* leaf blight) are not understood, for an effective and viable selection programme for developing *Eucalyptus* with a long lasting resistance, it is essential to assess the variation in pathogenicity of the population of *C. quinqueseptatum* and also to know whether different *C. quinqueseptatum* isolates possess general or specific virulence (Hadley *et al.*1979). Whether this variation among *C. quinqueseptatum* isolates is also reflected in their pathogenicity on *Eucalyptus* is not known.

Since this disease is causing large scale mortality of nursery seedlings in north India, in order to study the variability in the virulence, 71 *C. quinqueseptatum* isolates collected from different parts of Uttarakhand, Punjab and Haryana were studied. Some representative isolates were also studied for the differences in cultural and microscopic characters.

MATERIALS AND METHODS

Isolation of fungi:

Diseased leaves and twigs of Eucalyptus from nurseries and plantations were collected different parts of Uttarakhand, Haryana and Punjab. Four isolates of Uttar Pradesh and two from Orissa were also incorporated in the study for comparison. For isolation of pathogen, bits of 1x1cm cut across leaf lesions were surface sterilized by submerging in 0.1% $HgCl_2$ for thirty seconds and were rinsed in five changes of sterilized distilled water and placed on the Potato Dextrose Agar (PDA) medium. The plates were incubated at $25^0 \pm 1^0$C for four days. Emerging colonies were pure cultured and transferred to PDA plates and slants for further use. Total 71 isolates were isolated and studied for their relative virulence. Twelve isolates were studied on nine different media for their colony morphology and microscopic characters.

Determining taxonomic identity:

Single spore of 71 isolates were plated onto carnation leaf agar (CLA) (Crous and Wingfield, 1994) and incubated at 25^0C. The plates were examined after 7 days or until sporulation occurred (not later than 9 days) and only conidiophore on the carnation leaves were examined. For each isolate, mounts were prepared in cotton blue and measurement of at least 30 conidia, vesicles, stipes, branches and philades were made at 450X magnification with an optical microscope.

Media preparation for single spore isolation:

Cylindrocladium quinqueseptatum does not sporulate in potato dextrose agar (PDA) and rarely sporulate on other media. A new minimal medium was derived by substituting dextrose with leaf extract of *Eucalyptus*. The composition of the medium used for sporulating cultures of all the collected isolates of *C. quinqueseptatum* was $MgSO_4$ (1gm), $K2PO_4$ (1gm), $NaNO_3$ (2 gm), Eucalyptus leaf extract (50 ml), agar (15gm) and double distilled water (950 ml).

Preparation of *Eucalyptus* leaf extract:

Healthy leaves of *Eucalyptus* were collected aseptically and washed with 70% ethanol. Leaves were then ground with mortar and pestle (500gm of leaves with 750ml of double distilled water) and boiled thoroughly. The extract was filtered through sterile muslin cloth.

Single spore isolation:

Single spore isolation of each isolate was done by the method described by Choi *et al*. (1999). The pure colonies of isolates originating from single spore were maintained on PDA slants for further studies.

Virulence tests:

Single spore cultures were grown in 20 ml potato dextrose broth (PDB) in tubes by incubating them at $25^0 \pm 1^0$C for 20 days. The tubes were centrifuged and mycelium settled at the bottom was discarded. The fresh leafy twigs of *Eucalyptus sp*. collected from the Central Nursery, Forest Research Institute, Dehradun were cut under sterilized conditions, placed in the culture tubes containing supernatant, incubated for 45 hours in growth chambers and compared with the twigs placed in supernatant of uninoculated PDB. The symptoms were recorded at the interval of 15, 30 and 45 hours. After 45 hrs leaves of the twigs placed in control culture tubes started becoming flaccid and observation beyond 45 hrs were not recorded. The experiment was conducted in triplicate for all the isolates including control. Visual observations of the symptoms were categorized as least virulent (+ and ++), moderately virulent (+++ and ++++) and highly virulent (+++++) based on the observations *viz*. tips and sides of the leaves becoming flaccid (+), half leaf portion showing wilt (++), complete wilting of leaves (+++), complete wilting and drooping of leaves (++++), complete foliage wilting accompanied by blighting with drooping of apical portion of the twig (+++++).

Cultural characteristics

Cultural characters and colony diameter of different isolates of the pathogen were studied on various media as described by Booth (1971) and Barnett and Hunter (1978). Cultural characters of twelve isolates of *C. quinqueseptatum* from different geographical regions were studied on nine different media *viz*. malt extract agar (MEA), lima bean agar (LBA), potato dextrose agar (PDA), corn meal agar (CMA), soya bean agar (SBA), oat meal agar (OMA), yeast malt agar (YMA), czapex dox agar (CDA), glucose yeast extract agar (GYEA) and casein hydrolysate agar (CHA) manufactured by Hi-Media (Mumbai, India) using three replicates of flat bottom assay Petri dishes (7 cm diameter), each containing 15 ml of the medium as described by Sharma *et al.*(1992). From the margins of actively growing cultures, 3 mm dia discs were placed in the centre of the Petri dishes and incubated at $25^0 \pm 1^0$C. Colony diameter, colony colour, pigmentation, mycelial morphology, and formation of conidia, microsclerotia and chlamydospores were recorded after 7 days and 14 days of incubation. Since no conidia, microsclerotia and chlamydospore formation was recorded in 7 days, the data after 14 days have only been given in Table 2. All the observations were taken under 450X magnification. Colony growth data was subjected to ANOVA.

RESULTS

Determining taxonomic identity:

The identification of pathogen was done by the key described by Crous and Wingfield, (1994) and it was confirmed that all the isolates under study were *C. quinqueseptatum.*

TABLE1: Colony growth of *Cylindrocladium quinqueseptatum* isolates in different media after fourteenth days of incubation

Isolates	Diameter growth (cm) of different isolates on different media									Mean
	CDA	PDA	CHA	LBA	GYEA	OMA	YMA	CMA	SBA	
FCQ305	7.00	5.50	3.53	5.50	5.47	7.00	7.00	6.47	3.77	5.69[b]
FCQ165	7.00	5.50	4.81	5.00	5.10	7.00	6.20	6.13	5.00	5.76[b]
FCQ234	5.63	5.50	3.61	4.87	5.17	7.00	6.03	5.46	3.67	5.21
FCQ235	6.46	5.57	3.81	4.77	5.07	7.00	6.46	6.03	4.16	5.49 [b]
FCQ227	4.33	4.43	2.81	3.43	4.13	6.10	4.16	5.53	4.07	4.33
FCQ248	4.26	4.67	3.61	2.71	4.23	6.16	4.03	5.83	5.03	4.51
FCQ180	3.33	5.00	3.40	4.10	5.10	6.20	6.03	5.81	3.56	4.73
FCQ106	5.43	3.56	3.20	4.77	4.10	6.53	6.43	6.16	3.53	4.86
FCQ174	7.00	6.96	4.10	3.91	5.73	7.00	7.00	6.53	4.36	5.85[b]
FCQ141	7.00	6.23	4.43	5.50	5.56	7.00	7.00	7.00	4.67	6.04[a]
FCQ119	7.00	6.50	6.40	6.00	6.10	6.50	7.00	7.00	4.71	6.35[a]
FCQ232	5.41	3.61	3.21	4.76	4.06	6.50	5.36	6.16	3.71	4.73
Mean	5.82[b]	5.25	3.91	4.61	4.99	6.67[a]	6.07[b]	6.18[b]	4.17	
CD (0.05)	Isolates=0.041			Media=0.036			Isolate× Media=0.124			

Cultural study

In different growth media significant differences were observed in the colony growth of different isolates (Table 1). Growth behavior of 12 isolates on nine different media showed significant difference in color, morphology and pigmentation along with the production of conidia, microsclerotia and chlamydospores (Table 2). For vegetative growth OMA was the best medium where all the isolates showed maximum radial growth in 7 days. All the isolates in GYEA, OMA and CDA showed chlamydospore formation in 14 days old culture. However, no chlamydospores were formed in any of the isolate in any media after 7 days of incubation. Microsclerotia were observed after 14 days of incubation in CMA where 11 out of 12 isolates showed microsclerotia formation, however, in other media formation of microsclerotia was variable and no uniform trend were observed. All the isolates in CMA showed dark brown pigmentation after 14 days of incubation whereas in CDA the pigmentation color ranged between brown to dark brown. No specific trend was observed in other media. Colony color after seven days was mostly white, however, in some cases, mostly in CMA and SBA, whitish brown to dark brown colonies were also observed. One of the isolate FCQ 119 showed creamish (in GYEA) and pinkish white (in CHA) colony. However, there was high variability in colony color after 14 days of incubation and no clear trend was observed. In general isolates tend to attain darker shades.

Virulence testing

Supernatant of all the isolates showed varying degree of wilting symptoms in twigs except for five isolates within fifteen hours of incubation. After thirty hours of incubation all the isolates showed wilt symptoms. Results obtained after 45 hours of incubation were evaluated to differentiate the isolates on the basis of their relative virulence. As shown in Table 3 from Uttarakhand, Haryana and Punjab, 34, 21 and 10 isolates were examined for their virulence, respectively. Isolates from Uttar Pradesh (4) and Orissa (2) were also incorporated in the study for comparison. Out of the total 71 isolates 46% isolates were highly virulent. Results showed that all the isolates (11.2%) categorized as least virulent were from Uttarakhand (8.4%) and Haryana (2.8%). Uttarakhand had 29.4% isolates categorized as most virulent, Haryana 57.1% and Punjab 60%. None of the isolate of Punjab was categorized as least virulent. The isolates from Uttar Pradesh and Orissa which were incorporated for comparison revealed that all the isolates from Orissa were highly virulent, however, Uttar Pradesh isolates ranged between moderate to high virulence. However, unfortunately since we had only 4 isolates from Uttar Pradesh and 2 from Orissa conclusive comparison with them was not possible.

DISCUSSION

It is concluded that on OMA the vegetative growth of *Cylindrocladium quinqueseptatum* was maximum followed by CMA, YMA and CDA. Least vegetative growth was observed in CHA. Sharma *et al.* (1992) also reported variation in cultural characters of south Indian isolates of *C. quinqueseptatum*. It was noted that all the 5 isolates which did not show any wilt symptom in first 15 hours of incubation belonged to Uttarakhand. The percentage of virulent strains in Uttarakhand was less than half as compared to Punjab and Haryana. However, since these states are neighbours, there is great likelihood of introduction and dissemination of highly virulent strains from Punjab and Haryana to Uttarakhand as there is continuous flow of planting materials and propagules between these states apart from air mediated conidial transmission. Though it cannot be proved from the present study but the pathogen might have naturalized first in Punjab and Haryana region, and there is likelihood that the highly virulent strains present in Uttarakhand might have reached on account of the exchange of planting materials and nursery propagules. Climatic conditions in Haryana and Punjab appears to favour the pathogen as these are warmer as compared to Uttarakhand. Though Uttarakhand is more humid probably favouring the luxuriant high vegetative growth. However, it seems that due to selection pressure on account of high temperature and low humidity in Punjab and Haryana, as a survival strategy isolates might have become more virulent. It is also an area of investigation that the difference in virulence may have bearing on the response of the efficacy of chemical fungicides and their doses, which should be worked out before recommending them for disease management. Though we have not conducted vegetative growth of all the isolates in different media, it is evident from the results that the isolates showing high growth rates were least virulent and the slow growing isolates were found to be highly virulent. FCQ 119 and 141 which showed high growth rates were least virulent. The slowest growing isolates e.g. FCQ 227, 248 and 180 showed high virulence. Moderately growing isolates e.g. FCQ 174 and 165 showed least to medium virulence.

CONCLUSION

From this study it can be concluded that since Punjab and Haryana have most virulent isolates, the planting material grown in these states may be avoided for raising nurseries and plantations of Eucalyptus in Uttarakhand, though more conclusive studies are needed in this direction along with the strain-wise screening of fungicides. There appears to be correlation between the vegetative growth of pathogen isolates with its virulence as highly virulent isolates showed lesser growth rates.

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TABLE 2: Colony and micro morphology of different isolates of *Cylindrocladium quinqueseptatum* in different culture media.

Media	Isolate	Color		Pigmentation		Morphology		Conidia	Microsclerotia	Chlamydospores
		7 d	15 d	7 d	15 d	7 d	15 d	15 d	15 d	15 d
PDA	FCQ-305	W	W	-	-	C	C	-	-	-
	FCQ-165	W	W	B	B	C	C	-	+	+
	FCQ-234	W	W	-	B	C	C	-	+	+
	FCQ-235	W	W	B	B	C	C	-	+	+
	FCQ-227	W	G	-	LB	C	F	-	-	-
	FCQ-248	W	Y	B	LB	C	C	-	+	+
	FCQ-180	W	CB	-	LB	C	C	-	+	+
	FCQ-106	W	W	B	B	C	C	-	+	+
	FCQ-174	W	W	B	B	C	F	+	-	-
	FCQ-141	W	W	-	-	C	C	-	+	-
	FCQ-119	WB	WB	B	DB	C	C	-	+	-
	FCQ-232	W	W	B	B	C	C	+	+	+
CHA	FCQ-305	W	W	-	-	C	C	+	-	-
	FCQ-165	W	W	-	-	O	O	+	+	+
	FCQ-234	W	W	-	-	C	C	+	-	-
	FCQ-235	W	W	B	B	O	O	-	+	+
	FCQ-227	W	WB	-	LB	C	C	+	-	+
	FCQ-248	W	W	-	-	O	O	-	+	+
	FCQ-180	W	W	-	-	F	F	+	+	+
	FCQ-106	W	W	B	B	O	O	+	+	+
	FCQ-174	W	W	-	-	C	C	+	-	+
	FCQ-141	W	W	-	-	O	O	-	+	+
	FCQ-119	PW	PW	-	-	F	F	-	+	+
	FCQ-232	W	W	B	B	O	O	-	+	+
LBA	FCQ-305	W	W	-	-	C	C	-	-	-
	FCQ-165	W	W	B	B	SO	SO	-	+	+
	FCQ-234	W	W	B	B	SO	SO	-	-	-
	FCQ-235	W	W	B	B	O	O	-	+	+
	FCQ-227	W	DB	LB	RP	F	OF	-	-	+
	FCQ-248	W	WB	DB	DB	O	OF	-	-	+
	FCQ-180	W	WB	B	DB	F	F	-	-	+
	FCQ-106	W	W	B	B	O	O	-	+	+
	FCQ-174	W	W	-	-	F	OF	-	-	+
	FCQ- 141	W	W	-	-	O	OF	-	-	+
	FCQ-119	W	W	-	-	F	F	-	-	+
	FCQ- 232	W	W	B	B	O	O	+	+	+
GYEA	FCQ-305	W	W	B	B	C	C	+	+	+
	FCQ-165	W	W	B	B	SO	SO	+	+	+
	FCQ-234	W	W	B	B	SO	SO	+	-	+
	FCQ-235	W	W	B	B	C	C	+	+	+
	FCQ-227	W	DB	LB	LB	C	F	+	+	+
	FCQ-248	W	W	B	B	SO	SO	+	-	+
	FCQ-180	W	W	-	-	O	O	+	-	+
	FCQ-106	W	W	B	B	C	C	+	+	+
	FCQ-174	W	W	-	-	C	F	+	+	+
	FCQ-141	W	W	-	-	SO	SO	-	-	+
	FCQ-119	C	C	-	-	O	O	+	-	+
	FCQ-232	W	W	B	B	C	C	+	+	+
OMA	FCQ-305	W	W	B	DB	C	C	-	-	+

	FCQ-165	W	W	B	DB	C	C	-	-	+
	FCQ-234	W	W	B	DB	C	C	-	-	+
	FCQ-235	W	W	B	DB	C	C	-	-	+
	FCQ-227	W	WB	-	-	C	O	-	+	+
	FCQ-248	W	W	B	DB	C	C	-	+	+
	FCQ-180	W	WB	B	DB	C	F	-	+	+
	FCQ-106	W	WB	B	DB	C	C	-	-	+
	FCQ-174	W	W	-	-	C	O	+	+	+
	FCQ-141	W	W	B	DB	C	C	-	+	+
	FCQ-119	W	W	DB	DB	C	F	+	+	+
	FCQ-232	W	W	DB	DB	C	C	+	-	+
YMA	FCQ-305	W	W	B	DB	C	C	-	-	+
	FCQ-165	W	W	B	DB	C	C	-	-	+
	FCQ-234	W	W	B	DB	C	C	-	-	+
	FCQ-235	W	W	B	DB	C	C	-	-	+
	FCQ-227	W	WB	-	-	C	C	-	+	+
	FCQ-248	W	WB	B	DB	C	C	-	-	+
	FCQ-180	W	PW	B	RB	C	C	-	+	+
	FCQ-106	W	W	B	DB	C	C	-	-	+
	FCQ-174	W	W	RP	RP	C	C	+	+	+
	FCQ-141	W	W	RP	RP	C	C	+	-	+
	FCQ-119	W	W	RP	RP	C	C	+	+	+
	FCQ-232	W	W	B	DB	C	C	+	-	-
CDA	FCQ-305	W	W	B	DB	C	C	+	-	+
	FCQ-165	W	W	B	DB	C	C	+	-	+
	FCQ-234	W	W	B	B	S	S	+	-	+
	FCQ-235	W	W	B	DB	C	C	+	-	+
	FCQ-227	W	WB	B	DB	C	C	+	+	+
	FCQ-248	W	W	B	DB	C	C	+	-	+
	FCQ-180	W	W	B	B	S	S	+	-	+
	FCQ-106	W	W	B	DB	C	C	-	-	+
	FCQ-174	W	DB	DB	DB	C	C	+	+	+
	FCQ-141	W	WB	DB	DB	C	C	+	-	+
	FCQ-119	W	WB	B	B	S	S	-	-	+
	FCQ-232	W	WB	B	DB	C	C	+	-	+
CMA	FCQ-305	W	W	B	DB	S	S	-	-	+
	FCQ-165	B	B	B	DB	S	S	-	+	+
	FCQ-234	WB	WB	B	DB	SO	SO	-	+	-
	FCQ-235	B	B	B	DB	S	S	-	+	+
	FCQ-227	W	WB	LB	DB	SO	SO	-	+	+
	FCQ-248	B	B	B	DB	S	S	-	+	+
	FCQ-180	WB	WB	B	DB	O	O	-	+	+
	FCQ-106	B	B	B	DB	S	S	-	+	+
	FCQ-174	W	DB	LB	DB	SO	SO	+	+	+
	FCQ-141	B	DB	B	DB	S	S	+	+	+
	FCQ-119	WB	DB	B	DB	O	O	-	+	+
	FCQ-232	B	B	B	DB	S	S	+	+	+
SBA	FCQ-305	W	W	B	B	CW	CW	-	-	-
	FCQ-165	W	W	B	B	CW	CW	-	-	-
	FCQ-234	B	B	B	B	CW	CW	-	-	+
	FCQ-235	WB	WB	B	B	SO	SO	-	-	+
	FCQ-227	W	WB	LB	B	GC	GC	-	-	+
	FCQ-248	W	W	-	-	O	O	-	-	+
	FCQ-180	B	WB	B	LB	O	O	-	-	+
	FCQ-106	WB	WB	B	B	SO	SO	-	-	+
	FCQ-174	W	WB	LB	B	GC	GC	+	-	+
	FCQ-141	W	W	-	-	O	O	-	-	+
	FCQ-119	B	WB	B	LB	O	O	-	-	+
	FCQ-232	WB	WB	B	B	SO	SO	+	-	-

- = absent
+ = present

Colony colors: W=White, B=Brown, WB=White Brown, DB=Dark Brown, G=Greyish, Y=Yellowish, CB=Creamish Brown, PW=Pinkish white, C= Creamish

Morphology: C=Cottony, O=Oppressed, F=floccose, S= Sparsy, SO=Sparsy Oppressed, CW=Cottony Wrinkled, OF= Oppressed floccose, GC=Granular cottony

Pigmentation: B=Brown, WB=White Brown, DB=Dark Brown, RP=Redish Purple, LB=Light Brown

TABLE 3: Relative virulence of different isolates of *Cylindrocladium quinqueseptatum* on Eucalyptus twigs.

Isolate	Incubation Time (in hrs)			States	Isolate	Incubation Time (in hrs)			States
	15	30	45			15	30	45	
Control	-	-	-	Uttarakand	FCQ-225	++	+++	+++++	Punjab
FCQ-115	+	+++	+++		FCQ-227	++	+++	+++++	
FCQ-136	+++	+++	+++++		FCQ-228	++	++++	+++++	
FCQ-137	+++	+++	++++		FCQ-229	+++	++++	+++++	
FCQ-152	+	+++	+++++		FCQ-230	++	+++	++++	
FCQ-159	++	++	++		FCQ-232	++	++++	++++	
FCQ-165	+	+++	++++		FCQ-233	++	++	+++	
FCQ-174	-	++	++		FCQ-234	++	+++	++++	
FCQ-175	-	++	++		FCQ-235	++	++++	+++++	
FCQ-176	-	++	++		FCQ-236	+	++++	+++++	
FCQ-177	-	++	++		FCQ-119	+	++	++	Haryana
FCQ-178	-	++	++		FCQ-138	+	++++	+++++	
FCQ-180	++	+++	+++++		FCQ-139	++	++++	+++++	
FCQ-181	++	+++	+++++		FCQ-140	++	+++	++++	
FCQ-182	+	++++	+++++		FCQ-141	+	++	++	
FCQ-183	++	++++	++++		FCQ-142	++	+++	++++	
FCQ-190	++	+++	+++++		FCQ-143	++	++	++++	
FCQ-209	+	+++	+++		FCQ-144	++	++++	+++++	
FCQ-210	+	+++	+++		FCQ-145	+	+++++	+++++	
FCQ-211	+	+++	+++		FCQ-201	++	++	+++	
FCQ-221	++	++++	+++++		FCQ-205	++	++++	++++	
FCQ-222	+	++++	+++++		FCQ-237	++	++++	+++++	
FCQ-238	++	++++	+++++		FCQ-247	++	++++	+++++	
FCQ-268	++	++++	++++		FCQ-248	+	+++	+++++	
FCQ-269	++	++++	++++		FCQ-249	+	++++	+++++	
FCQ-270	++	++++	++++		FCQ-260	++	++++	++++	
FCQ-280	+	+++	++++		FCQ-264	++	++++	++++	
FCQ-281	+++	+++	+++++		FCQ-265	++	+++	+++++	
FCQ-282	+	+++	++++		FCQ-310	+++	++++	+++++	
FCQ-283	+	+++	++++		FCQ-311	+++	+++++	+++++	
FCQ-284	+	++++	++++		FCQ-312	++++	+++++	+++++	
FCQ-285	++	++++	++++		FCQ-212	++	+++	++++	Uttar Pradesh
FCQ-288	++	+++	++++		FCQ-106	++	+++	++++	
FCQ-289	+	++	++++		FCQ-313	+	++++	+++++	
FCQ-294	++	++++	++++		FCQ-309	++	++++	+++++	
					FCQ-305	++	++++	+++++	Orissa
					FCQ-306	++	++++	+++++	

+ and ++= Least virulent; +++ and ++++ =Moderately virulent; +++++ = Highly virulent.
+ =Tips and sides of the leaves started becoming flaccid
++ =Half leaf portion showing wilt.
+++ =Complete wilting of leaves
++++ =Complete wilting and drooping of leaves
+++++=Complete foliage wilting accompanied by blighting, wilting and drooping of apical portion of twig