Chapter 3

Morphological Characterization and Virulence Behaviour of *Colletotrichum falcatum* isolates

Introduction

Red rot is one of the most widespread sugarcane diseases in the country and has been a constraint for sugarcane productivity for the past 100 years. The disease is responsible for the elimination of many commercial varieties in India in the earlier decades. Epidemics of the disease have been very common ever since its occurrence in India. Currently the disease occurs in all the sugarcane growing states in India except Karnataka and Maharashtra states. Recently reports of red rot occurrence in CoC 671 in parts of Kolhapur and Solapur districts in Maharashtra were reported (Viswanathan *et al.*, 1997). In the recent years the break down of the the important commercial varieties (CoS 8436, CoSe 95422, CoSe 92423 and BO 138) of subtropical region to red rot was reported (Viswanathan and Rao, 2011). Severe damage to crop stand was found in these varieties due to disease epidemics in the states of Haryana, Uttar Pradesh and Bihar. The pathogen affects the economically valuable stalk tissues and even the limited infection can bring about drastic changes in the juice quality. The disease affected cane gives poor sugar recovery because of impaired sucrose metabolism. The red rot infection reduced total carbohydrates in the diseased canes and the reduction was more in highly susceptible varieties (Agnihotri, 1990, 1996). Moreover, the pathogen produces abundant quantities of acid invertases which break the sucrose into glucose and fructose which are consumed by the pathogen. Higher production of acid invertases in the highly susceptible varieties was recorded upon pathogen infection as compared to resistant varieties (De Silva *et al.*, 1977). Pathogen infections also results in increased levels of total soluble salts, acidity, reducing sugars and gum and simultaneously decrease in pH, sucrose and purity of cane juice in affected canes (Singh and Waraitch, 1977). Increased activity of the enzyme invertase or inhibition of normal synthesis of normal carbohydrates and/or to the inhibition to the utilization of these act as a substrate by the pathogen as indicated by degradation of sucrose and increase in the levels of reducing sugars. Similar studies conducted at Sugarcane Breeding Institute (SBI), Coimbatore revealed...
that pathogen infection has drastically reduced brix, sucrose percentage, purity and CCS per cent in the diseased canes. The affected canes recorded 25–75% reduced sucrose content than the healthy canes (Viswanathan and Samiyappan, 1999). Studies of Satyavir et al. (2002) in Haryana revealed that red rot infection reduces 7.1–32.5% in juice extraction, 7.4–38.7% in polarity, 0.5–8.3% in purity co-efficient, 7.8–39% in commercial cane sugar and increase of 19.2–40.95% in reducing sugars. During the milling process, mixing of juice from healthy and diseased canes result in spoilage of entire juice due to inversion of sucrose. Similarly ‘jaggery’ setting will also be affected if red rot affected canes are crushed with healthy ones. Usually ratoon crops suffer more than plant crops. Resistance to red rot in sugarcane varieties is not static; hence, practically all varieties under cultivation are susceptible. Once a resistant variety occupying large areas succumbs to the disease, extensive damage to cane cultivation occurs. The red rot fungus has exhibited a wide array of variation in cultural characters and virulence. Based on the variations in cultural characters and that of fruiting structures, different isolates have been characterized. The development of physiological races has been attributed to hybridization, mutation, conidial and hyphal fusion and heterokaryosis. The virulence pattern is altered by nutritional factors like certain specific amino acids increase the virulence of the fungus when supplemented in the medium. Detailed studies were conducted at SBI, Coimbatore on identifying variation in different C. falcatum pathotypes based on serological reaction and vegetative compatibility grouping and they gave a clear variation among the pathotypes (Viswanathan et al., 2003). Based on the cultural characters early workers established the existence of physiological races in C. falcatum into light and dark races. Among them the light race produced abundant conidia and proved more virulent than the dark race. Currently, the C. falcatum isolates are grouped based on their pathogenic reaction on a set of 14 host differentials at 12 sugarcane research centres in the country. Studies conducted so far revealed existence of 11 pathotypes (CF01–CF11), seven from subtropical region and four from tropical region (Viswanathan, 2010).

Sugarcane is responsible for 75% of the global sugar production in the countries including India, Cuba, Brazil, Australia, United States of America, West Indies, Mexico, China, South Africa, Fiji, etc (Paterson et al., 2000). Uttar Pradesh is a major sugarcane cultivating area in India with high potential of red rot threat because of maximum area under water logging conditions and favorable environmental condition for the spread of red rot, but not much study in the past has been done on evolution of newly available red rot isolates from the field’s canes. Since, the regular severe incidence of red rot disease has recorded in
many important commercial cultivars of sugarcane in different parts of Uttar Pradesh, India, the present study was undertaken with the objectives to study the morphological and virulence behavior of the newly collected red rot isolates, so that a thorough potential control strategy could be planned to manage the red rot spread in red rot affected areas of Uttar Pradesh, India.

**Review of literature**

**Pathogen**

The pathogen has both anamorphic and telomorphic stages. But anamorphic stage is (*Colletotrichum falcatum* Went., Family: Melanconiaceae, Class: Coelomycetes) infecting the standing cane is the most important. Occurrence of the teleomorphic stage was first reported by Chona and Srivastava (1952). This sexual phase of the fungus *Glomerella tucumanensis* was reported to occur on the leaf lamina, mid ribs and leaf sheaths of dry foliage in nature (Chona and Bajaj, 1953; Mishra, 1957). It was also recorded in culture plates (Chona *et al.*., 1964).

Went (1893) first observed red rot and described the causal organism as *Colletotrichum falcatum*. The sexual stage of *C. falcatum* was later reported by Spegazzini (1896) in Argentina who named it *Physalospora tucumanensis*. Later, the red rot causal organism was reclassified by Von Arx and Muller (1954) and included in the genus *Glomerella* as *G. tucumanensis*. The fungus produces falcate conidia, either in specialized fruiting structures, acervuli, or on the hyphal tips. The spore masses produced in the acervuli are in a mucilaginous matrix having a pinkish appearance. Conidia measure 16-40 μm in length and 4-8 μm in width. Septate setae present in the acervuli vary in number and size between isolates. In general, they are bulbous at the base tapering towards the tip, measure 100-200 x 4 μm. Conidia germinate and produce appresoria. Sometimes appresoria are produced on hyphal tips. These appresoria are smooth but thick-walled and cinnamon-buff in color. The prethecia, when produced are completely embedded in leaf tissues except for the protruding ostioles. They are 100-200 μm in width and 85-250 μm in height containing clavate asci and paraphyses. Ascospores are hyaline, straight to fusoid, single celled and 18-22 x 7-8 μm in size. Infection of the disease is caused other by spores or ascospores. The pathogen makes its entry into the host tissues through any sort of injury by insects or borers or natural growth cracks etc. After the entry, the infection thread develops normal hyphae which grows within
the host tissues for some time and then emerge out through the cells to the outer surface and develops acervuli.

**Conidial characteristics**

Abbott (1938) reported that length of conidia ranged from 16 to 40 µm, while Went (1893) reported 25 µm. Chona and Srivastava (1960) studied 32 isolates and reported that the length of the conidia being a minimum of 10 µm and with maximum of 36 µm. Various reports are available on the variation of conidial morphology and dimension. Two types are conidia, one with 28.9 x 3.1 µm and other with 30.1 x 5.6 µm (Sharma, 1970), conditional size of 17.1 to 25.8 x 4.5 to 6.8 µm (Pandey and Sakal, 1974), a new biotype with size ranging from 33.0 to 37.4 µm x 4.4 to 4.95 µm (Gupta et al., 1980), varying length of 23 to 30 µm (Khirbat et al., 1980), sickle shaped or falcate conidia measuring 16-40 x 5-7 µm in size with oil globule in the middle (Agnihotri, 1983) and 15-42.6 x 3.7-8.6 µm (Jothi, 1989) were reported. Khirbat et al. (1986) reported that among the five isolates one isolate RH3 was distinctly different from the other four isolates by its growth rate and longer conidial length.

The *C. falcatus* isolates showed marked variation in conidial germination. On germination some isolates produced short, long and branched promycelia, in which length ranged from 21-230 µm. Certain isolates did not produce promycelia and virulent than dark types. Conditional termination was a maximum of 90.3 per cent in a light isolate and minimum of 22.6 per cent in dark isolate. Germination in light types ranged from 22.6 to 63.3 per cent whereas, it was from 35.0 to 90.3 in light isolates. However, Chona and Srivastava (1960) results were contrast to the finding. They identified unipolar, bipolar, horizontal or from the middle region germination. Jothi (1989) found four types of germination with the 30 isolates tested. Variation in promycelium length was also observed. She found appressoria were lobed, globose, triangular or irregular. Singh and Singh (1989) also reported similar findings.

**Cultural variation**

Abbott (1935) described for the first time cultural variation in *C. falcatus*. He grouped large number of isolates from USA, representing various sugarcane belts into two groups readily distinguished by the color and texture of the mycelial growth. The colony turf of one group was dark grey in color and had a velvety surface, while that of the other varied from almost white to light ashy grey in color and was cottony or sub floccose in texture. The dark types fruit sparingly, acervuli generally being absent or limited to the periphery of the colony. Although, there are exception.
Srinivasan (1962) commented after his extensive studies on cultural variation in the fungus as the range of variation in the conidial state of the fungus appears to be considerable. In the cultures at one end are the rare isolates, which are moniliaceous in morphology, producing their conidial indefinitely from vegetative hyphae and at the other extreme are those that produce strong tuberculoid stromata subtending the acervuli. In between are those, which are most common and develop the typical melanconiaceous acervuli which may or may not bear setae. Another observation he made was all the wild cultures possessed light colored mycelium and relatively good sporulation and virulence on first isolation and none of the isolates belonged to dark type. However, many of the isolates soon changed into non-sporulating avirulent dark type while others retained their original character as light isolates. This happened irrespective of whether the isolates were carried through successive cultural transfers or passed through host. He concluded existence of two distinct types of isolates, viz. those that are relatively stable in their morphological and pathogenic character and those that are unstable and are soon replaced by the dark type. Dark type variants were more frequent in the foliar parts of the lesions and relatively less so in the advancing margin of the lesion. Loss of sporulation in dark-type variant sectors in the plates was observed. Dark type variants were more frequent in the older parts of the lesions and relatively less so in the advancing margin of the lesion. Loss of sporulation in dark-type variants was irreversible even when they were passed through the host. He reported that the light-colored, highly virulent ones generally prevalent in the red rot epiphytotic areas of North India such as Eastern UP, Northern Bihar and pockets of Punjab, and the dark-colored, poorly sporulating and relatively a virulent strains generally prevalent more particularly in areas in South India where red rot is not serious. He further classified four different morphological types based on the spore-bearing structure, and occurred of setae in the acervuli. He described the most frequent type as aerial mycelium floccose, white in young cultures, sporulation in one two weeks; acervuli appearing black at first, surround by setae, later developing salmon orange, slimy, conidial masses, conidia falcate, 14.0-32.0 x 4.6-9.0 µm.

Chona and Hingorani (1950) study the cultural condition under which dark types arise from light types and have shown that this was an irreversible variation. Chattopadhyay and Sarkar (1960) studied C. falcatum isolates in West Bengal and divided them in to distinct morphological group. Chona and Srivastava (1960) reported that isolates of C. falcatum were usually unstable in cultural and there was no correlation between growth rate and morphology.
Physiological behavior and virulence

Sharma (1970) reported cultural character of 3 isolates based on the colour of the mycelium as light and dark. Panday and Sakal (1974) observed great variability and apparent ability of red rot flora produced new and possible new virulent strain from time to time. Satyanarayan and Rao (1995) reported that all the three major pathotypes viz. Cf 119, Cf 997 and Cf 671 have completely covered by 11 days after inoculation in 100 mm Petri plates. They found no appreciable difference either in cultural characteristics or in size of conidia of the three pathotypes. Jothi (1989) on the basis of cultural character viz. color, texture and sporulation, divided 30 isolates on to 5 groups. The isolates showed tremendous variation in their redial growth on oatmeal agar, growth in liquid medium and sporulation. All the isolates were able to produce acervuli in cultural and the diameter ranged from 0.639 to 1.54 mm, setae were found in all the isolates and in some cases it was rare. Number of state ranged from 3 to 20 per acervulus and length ranged from 90 µm to 220 µm. Conidiophores length ranged from 120 µm to 330 µm. Variation in sporulation by the isolates at different temperature regimes was found. There were also variations in the response of the isolates towards different fungicides.

Chona (1956) reported that a light colored, highly sporulating type of red rot fungus predominated in the isolation made from red rot affected canes from endemic areas of UP and Bihar, whereas all the previous isolates prior to 1938 were of a dark type with spares sporulation. The new, light type isolate was much more virulent than old date type. By the virtue of its profuse production of spores is naturally well suited for quick and widespread dissemination. In this change of red rot flora in the epidemic areas resulting in the predominance of a highly sporing, highly virulent strain, combined with secondary infection was found an explanation for the failure important commercial cane varieties. A great deal of different in the virulence of various isolates was observed but they do not appear to exist any highly specialized physiologic forms like those of the wheat rust fungus, with definite specificity between an isolate and a particular cane variety or set of varieties. Most important feature of the red rot epidemic, as the development and predominance of a new more virulent strain may results in the failure of any commercial variety and may thus upset the varietal set up of a tract which takes quite a few years to build up.

Collection of isolates over several years by various workers indicated appearance of several new strains with marked physiologic different as judged by their virulence on different cultivars (Kiritikar et al., 1964; Pandey and Sakal, 1974; Anon, 1990). Kiritikar et al.
(1964) studied 3 isolates for its comparative virulence on 25 varieties and found that in nature some intermediate types in between well-known dark and light colored strains had risen which showed greater virulence. Alexander and Rao (1972) conducted a study with the same set of genotypes using the same isolates by inoculating simultaneously at Coimbatore and Karnal and they attributed the deference to environmental factors. Kar et al. (1965) concluded that the virulence is not necessarily connected with morphology. Fontennot et al., (1973) studied the relative pathogenicity of isolates of C. falcum from sugarcane seed pieces from 15 locations and reported that they varied in pathogenicity to 3 sugarcane varieties leaf and midrib isolates and the Budhraja (1975) explained the differences between leaf and midrib isolates and the isolates collected from sugarcane stalk tissues. Differences in virulence in different pathotypes of red rot fungus from varieties CoLK 8001, Co 8529 and Co 1148 was reported from Shahjahanpur, Pusa and Seorahi, respectively (Anon, 1990). Jothi (1989) studies virulence pattern of the 6 isolates on 16 sugarcane verities whose reaction to C. falcum is known. The results confirmed that the virulence of an isolate is not necessarily connected with the morphology of the isolate .The varieties behaved different to the isolates.

Comparison of tropical and sub-tropical pathotypes for their virulence on a set of sugarcane clones/cultivars indicated that pathogen isolated originated from tropical India were more virulent than the sub-tropical ones. The pathotype from isolated from CoC 671 was found to be the most virulent among all pathotypes tested and that from Co 1148 was the most virulent one (Padmanaban et al., 1996). Further, Pathotypes isolated from CoC 671, CoC 8001, CoC 85061, CoC 86062, CoC 91061 and CoC 92601 which have succumbed to red rot in their virulence. Vishwanathan et al. (1997) also found a gradual reduction in red rot resistance year after year from 1985 to 1995 in the progenies developed in pre-zonal variant trial at the Institute. Higher susceptibility of most the clones tested in the later years was from due to use of more aggressive pathotypes such as Cf 671 and Cf 92061. Though Cf 1148 pathotype from subtropics was prevalent for over 20 years back the variety was not withdrawn from cultivation since its virulence was comparatively less. On the other hand higher aggressiveness of pathotypes Cf671 and Cf92061 on the cultivars CoC 671 and CoC 92601 caused extensive loss to sugarcane cultivation in many parts of Tamil Nadu, Andhra Pradesh, parts of Karnataka and Gujarat. The results were in contrast to the earlier work at Sugarcane Breeding Institute (SBI) which (Alexander et al., 1986). This study clearly established that acquisition of higher virulence in the pathotypes led to server epidemics of the disease and loss of sugarcane varieties.
Materials and Methods

Isolation and Maintenance

Ten *Colletotrichum falcatum* isolates prevalent in subtropical (northwestern zone) India, were collected from sugarcane growing areas of Eastern, Central and Western regions of Uttar Pradesh during the survey in the session 2007-2009. Red rot infected canes were directly collected from the fields, surface sterilized and the internodal tissues were cut into small pieces of 0.2 to 0.3 cm. These were gently placed on Oat Meal Agar (OMA) in Petri Plates under aseptic conditions and maintained at room temperature (30±5°C) until the mycelial growth initiated. These cultures were further purified by regular transfer it to the fresh OMA medium and incubated at 30±5°C until sporulation.

Morphological Characteristics of Red Rot isolates

Morphological characteristics of different red rot isolates in terms of mycelial growth, texture, color and conidial growth were recorded. Cultures of individual isolates of red-rot were regularly observed under the microscope for the production of conidia and acervuli. Their size, shape and germination behavior was also recorded.

Virulence behavior of red rot isolates

Freshly sporulating 2-3 weeks old culture of six isolates of red rot pathogens were used for the purpose of inoculation in a set of susceptible varieties for grading their virulence behavior as method described by Srinivasan and Bhatt (1961). Conidial suspension at spore strength of 1x10^6 spores per ml solution was prepared. About 25 stalks of each variety were inoculated by plug method of inoculation. Inoculation was done in the middle of the third exposed internode from the bottom and two drops of the spore suspension were injected with large syringe after making a puncture with the cork borer. The inoculated canes were slight open longitudinally after 60 days at inoculation. The varieties were evaluated on 0-9 scale for grading resistant to red-rot pathogen (Singh and Singh, 1989).

Results

Morphological Characteristics of Red Rot isolates

The ten isolates of red rot were cultured *in vitro* for their cultural characteristics viz. color, texture and sporulation. The tested isolates showed tremendous variation in their radial growth on oat meal agar medium and sporulation. Nearly all the isolates were able to produce conidia in culture with a diameter ranged from 27µm to 30µm (Table and Plate 3.1).
Cultural characteristics of ten major *C. falcatum* isolates of Uttar Pradesh revealed a clear cut variation in mycelial growth among the tested isolates. The mycelia showed different patterns as concentric rings, fluffy growth, moderately slant and vertical growth, uneven marsh growth with dark color mycelium at centre with white, muddy, dull white coloured mycelium at outer side (Plate 3.1). Isolates Cf-Kushinagar (variety CoSe 95422) recorded highest mycelial dry weight and Cf-17 (variety CoSe 95422) recorded the least. Regarding other characteristics, Cf-1B (variety CoS 8436), Cf-Kushinagar (variety CoSe 95422) and Cf- 20 (CoSe 92423) were producing thicker matty mycelium and they were darker as compared to other pathotypes. Among the pathotypes Cf-20, Cf-19, Cf-18 and Cf-1B were fast in initiating conidia production and these isolates recorded higher conidial germination. The reported length of conidia being a minimum of 27µm x 7.6 and with maximum of 30µm x 7.8 (Table 3.1). Typical conidia are produced singly on the conidiophores, each being cut- off at maturity as another starts to develops. They are hyaline one celled falcate or sickle shaped, sometimes fusoid, usually with one end rounded and the
other slightly pointed (Plate 3.2, a, b). Acervuli, resting structure appeared in 15-20 days culture of the red rot isolate Cf-2B (Plate 3.2c).

Plate 3.2: Conidia and acervuli of the *C. falcatum* isolates (a) conidia of Cf–Kushinagar CoS 95422 (10x) (b) enlarged view of conidia (c) Acervuli of Cf-2B (40x)

**Test of virulence of red rot isolates**

In the virulence test, screening of six red rot isolates on a set of ten susceptible varieties (CoS 453, Co 312, Bo17, Bo 70, CoS770, CoJ 64, CoS 1158, CoS 443, CoS 510, UP 01) indicated that the isolate Cf-Basti proved more virulent followed by Cf-Kushinagar and Cf-2B (Bareilly). The virulency behavior observed in tested isolates was found in the order Cf-Basti > Cf-Kushinagar > Cf-2B > Cf-20 > Cf-18 > Cf-17 (Table 3.2).

Our results suggested that the isolates Cf-Basti was the most virulent one and responsible for knocking down the commercial varieties of sugarcane in the affected area. Further studies are required to know the other factors responsible to make the isolate more virulent in the particular area from which they originally belong and the epidemiological data.

**DISCUSSION**

During the survey of the sugarcane field of the different regions of the Uttar Pradesh incidence of the disease was high and it was up to 100% in many fields. The most common symptoms observed in red rot affected fields was discoloration and yellowing of the young crown leaves followed by drying of entire stalks in the affected field. The affected canes from
the different varieties and locations were brought to the laboratory for the further morphological studies. The tested isolates showed tremendous variation in their radial growth on oat meal agar medium and sporulation. Nearly all the isolates were able to produce conidia in culture with a diameter ranged from 27µm to 30µm. In the virulency test Cf Basti was found more virulent in compare to the other selected isolates in the present study.

Earlier, Chona (1954) reported that a light colored, highly sporulating type of red rot fungus predominated in the isolation made from red rot affected canes from endemic areas of UP and Bihar, where as all the previous isolates prior to 1938 are of a dark type with spares sporulation. The new, light type isolate is much more virulent than old date type. By the virtue of its profuse production of spores it is naturally well suited for quick and widespread dissemination. In this change of red rot flora in the epidemic areas resulting in the predominance of a highly sporulating, highly virulent strain, combined with secondary infection was found an explanation for the failure important commercial cane varieties. Most important feature of the red rot epidemic, as the development and predominance of a new more virulent strain may results in the failure of any commercial variety and may thus upset the varietal set up of a tract which takes quite a few years to build up. Despite concerted effort to establish a uniform classification system for C. falcatum pathotypes, several constraints still exist. For instance, the environment, the nature of inoculums, timing of inoculums etc. which may vary considerably from one center to another. In addition, the subjectivity during disease evaluation may lead to mis classification of the same strain by different pathologists.

Collection of isolates over several years by various workers indicated appearance of several new strains with marked physiologic different as judged by their virulence on different cultivars (Anon, 1990; Kirtikar et al., 1964; Pandey and Sakul, 1974; Viswanathan, 2005). Kirtikar et al. (1964) studied 3 isolates for its comparative virulence on 25 varieties and found that in nature some intermediate types in between well-known dark and light colored strains had risen which showed greater virulence. Alexander and Rao (1972) conducted a study with the same set of genotypes using the same isolates by inoculating simultaneously at Coimbatore and Karnal and they attributed the difference to environmental factors (Duttamajumder, 2008). They concluded that the virulence is not necessary associated with morphology. Fontennot et al. (1973) studied the relative pathogenicity of isolates of C. falcatum from sugarcane seed pieces from 15 locations and reported that they varied in pathogenicity to 3 sugarcane varieties leaf and midrib isolates and the Agnihotri and
Budhraja (1975) explained the differences between leaf and midrib isolates and the isolates collected from sugarcane stalk tissues. Differences in virulence in different pathotypes of red rot fungus from varieties CoLK 8001, Co 8529 and Co 1148 was reported from Shajahanpur, Pusa and Seorahi, respectively (Anon., 1990). Jothi (1989) studied virulence pattern of the 6 isolates on 16 sugarcane varieties whose reaction to *C. falcatum* is known. The results confirmed that the virulence of an isolate is not necessarily connected with the morphology of the isolate. The varieties behaved different to the isolates.

According to the Padmanabhan *et al.* (1996) comparison of tropical and sub-tropical pathotypes for their virulence on a set of sugarcane clones/cultivars indicated that pathogen originated from tropical India were more virulent than the sub-tropical ones. The pathotype from isolated from CoC 671 was found to be the most virulent among all pathotypes tested and that from Co 1148 was the most virulent one (Padmanabhan *et al*., 1996). Studies of Vishwanathan *et al.* (1997) also found a gradual reduction in red rot resistance year after year from 1985 to 1995 in the progenies developed in pre-zonal variant trial at the Institute. Higher susceptibility of most the clones tested in the later years was due to use of more aggressive pathotypes such as CoC 671 and CoC 92061. Though CoC 1148 pathotype from subtropics was prevalent for over 20 years back, the variety was not withdrawn from cultivation since its virulence was comparatively less. This study clearly established that acquisition of higher virulence in the pathotypes led to server epidemics of the disease and loss of sugarcane varieties. In the present study the currently epidemic of red rot disease may because of the appearance of new higher virulence pathotype of red rot pathogen.

Characterization of *Colletotrichum* species has relied on a number of criteria, including morphology, optimal growth temperature, vegetative compatibility, binomial sensitivity and recently, molecular methods. Studies on vegetative compatibility offer another approach to determining genetic relatedness in anamorphic populations of *Colletotrichum* species. Studies with respect to vegetative compatibility grouping has little attempted so far in *C. falcatum* and studies in this line of work in combination with sero-grouping may give information on the relation among the different pathotypes collected from a same variety location (Srinivasion and Bhat, 1961). In present study isolates Cf-Kushinagar (variety CoSe 95422) recorded highest mycelial dry weight and Cf-17 (variety CoSe 95422) recorded the least. Regarding other characteristics, Cf-1B (variety Cos 8436), Cf-Kushinagar (variety CoSe 95422) and Cf-20 (CoSe 92423) were producing thicker matty mycelium and they were darker as compared to other pathotypes. Among the
pathotypes Cf-20, Cf-19, Cf-18 and Cf-1B was fast in initiating conidia production and these pathotype recorded higher conidial germination.

The point which needs careful further investigation are: how the new strains arise in nature; how these get spread over large areas so quickly; how can these be detected quickly and what in it that makes a strain more virulent; or a variety susceptible. In 1957, Abbott pointed out that the success achieved in detecting susceptible or resistant varieties will depend on the adequate sample and will depend on the pathologist’s experience with the disease in a given area. Various workers have established the constantly changing nature of the pathogenic flora. Hence emphasis must be given on the constant monitoring of changes and exercising proper care in releasing sugarcane varieties which may be fairly resistant to red rot. This would be possible only if the inoculum used in resistant test is representative of the prevailing flora. Information on variation in differential host interaction, vegetative compatibility grouping, serotypic variation and molecular variation need to be combined to draw clear picture on the existing *C. falcatum* variation. Scientific advancements in molecular biology and bioinformatics would immensely help in the future to resolve variation in the red rot fungal pathogen.

In the present study the most virulent red rot isolates Cf- Basti and Cf-Kushinagar which are causing epidemic in the areas would alarm us to investigate on molecular approaches of characterization to know whether they are entirely new isolate / pathotypes / strains of red rot. So that significant initiatives should be taken well in advance towards developing resistant / tolerant genotypes through effective resistant breeding crossing programmes.
Table 3.1: Study of morphological characteristics of various isolates of *C. falcatum*

<table>
<thead>
<tr>
<th>Red rot isolates</th>
<th>Mycelial growth 7th day/15th day</th>
<th>Texture and color</th>
<th>Conidia size and shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cf-06</td>
<td>5.5mm/full plate</td>
<td>Dull white mild cottony growth, slow growing with very thin mycelial matt with even margin</td>
<td>Absent</td>
</tr>
</tbody>
</table>
| Cf-1B            | 7.0mm/full plate                 | Uneven cottony fluffy white growth with uneven margins Thick mycelial matt equally distributed with even outer margin. | Length 30.0µm  
Width 7.8 µm |
| Cf-20            | 7.5mm/full plate                 | Fast growing cotton arranged in concentric rings at outer circle equal distribution of mycelial growth. | Length 28.5m  
Width 7.5 µm |
| Cf-19            | 6.8mm/full plate                 | Slow growing cottony growth, highly over growth at centre | Length 28.8m  
Width 7.8 µm |
| Cf-Kushinagar    | 6.6mm/full plate                 | Cottony growth, vertically concentrated in centre followed by surface unequal growth | Length 27.0µm  
Width 6.6 µm |
| Cf-18            | 6.6mm/full plate                 | Fast growing massive cottony growth mycelial thickness observed erratically | Length 28.8µm  
Width 7.8 µm |
| Cf-17            | 5.5mm/full plate                 | Moderately growing thick mycelial matt even distribution dense at centre Massive cottony growth unequal distribution | Length 28.2 µm  
Width 7.2 µm |
| Cf-2B            | 7mm/full plate                   | Thick mycelial matt equally distributed with even outer margin | Length 29.9µm  
Width 7.8 µm |
| Cf-Basti         | 5.9mm/full plate                 | Massive cottony growth unequal distribution | Absent |
| Cf-khalilabad    | 6.5mm/full plate                 | Slow growing cottony growth highly over growth at centre | Absent |
Table 3.2: Virulence of red rot isolates in Uttar Pradesh (0-9 scale)

<table>
<thead>
<tr>
<th>Sugarcane variety</th>
<th>Cf-20</th>
<th>Cf-2B</th>
<th>Cf-17</th>
<th>Cf-18</th>
<th>Cf-Kushinagar</th>
<th>Cf-Basti</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoS 453</td>
<td>3.0(R)</td>
<td>4.0(S)</td>
<td>5.0(S)</td>
<td>3.0(R)</td>
<td>6.0(S)</td>
<td>4.0(S)</td>
</tr>
<tr>
<td>Co 312</td>
<td>6.0(S)</td>
<td>5.0(S)</td>
<td>5.0(S)</td>
<td>3.0(R)</td>
<td>4.0(S)</td>
<td>6.0(S)</td>
</tr>
<tr>
<td>Bo17</td>
<td>5.0(S)</td>
<td>6.0(S)</td>
<td>4.0(S)</td>
<td>2.0(R)</td>
<td>6.0(S)</td>
<td>11.0(S)</td>
</tr>
<tr>
<td>B0 70</td>
<td>5.0(S)</td>
<td>6.0(S)</td>
<td>5.0(S)</td>
<td>2.0(R)</td>
<td>6.0(S)</td>
<td>4.0(S)</td>
</tr>
<tr>
<td>CoS770</td>
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<tr>
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<td>2.0(R)</td>
<td>5.0(S)</td>
</tr>
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R=Resistant; S=Susceptible

References


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