# Taxonomic Studies on the genus *Colletotrichum* Corda and its Molecular Characterization

H. Thusith Rohana Wijesekara

Division of Plant Pathology
Indian Agricultural Research Institute
New Delhi – 110 012

2005

*Dedicated*

*To*

*My Beloved Parents*

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**By**

H. Thusith Rohana Wijesekara

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Submitted to the Faculty of Post-Graduate School,
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**in**

**PLANT PATHOLOGY**

**2005**

**Approved by:**

| | | |
|---|---|---|
| **1. Dr. D. K. Agarwal** | **(Chairman)** | 29/8/05 |
| **2. Dr. P. Bahadur** | **(Co-Chairman)** | 29/8/05 |
| **3. Dr. (Mrs.) R. Aggarwal** | **(Member)** | |
| **4. Dr. R. K. Sharma** | **(Member)** | 29/8/05 |
| **5. Dr. K. S. Jauhri** | **(Member)** | 29/8/05 |
| **6. Dr. T. R. Sharma** | **(Member)** | 30/8/05 |

**Dr. D. K. Agarwal**
***Principal Scientist***

**Division of Plant Pathology**
**Indian Agricultural Research Institute**
**New Delhi 110 012, India**

# CERTIFICATE

This is to certify that the thesis entitled **"Taxonomic Studies on the Genus *Colletotrichum* Corda and its Molecular Characterization"** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Plant Pathology,** is a faithful record of *bona fide* research work carried out by **Mr. HEWAGE THUSITH ROHANA WIJESEKARA (Roll No. 8830),** under my guidance and supervision. He has submitted no part of the thesis for any other degree or diploma.

I further certify that such help or sources of information that have been availed of this connection have been duly acknowledged.

Place: New Delhi

Dated: 29 August, 2005

**(Dr. D.K. Agarwal)**
Chairman
Advisory Committee

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**(H. T. Rohana Wijesekara)**

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# INTRODUCTION

*Colletotrichum* is an economically important genus of the family Melanconiaceae, order Melanconiales, class Deuteromycotina. The genus was established by Corda in 1831. The teleomorph stage of many *Colletotrichum* species is *Glomerella* and both stages are widely prevalent in hot and humid climates. Species of *Colletotrichum* are responsible for causing several economically important diseases on cereals, cucurbits, legumes, forage crops, fruits and vegetables. The fungus causes anthracnose, damping off, leaf spots, dieback, blight, crown and root rots (Agrios, 2004). This genus includes more than 900 species. In India, one hundred species of *Colletotrichum* and two species of its perfect stage, *Glomerella* (*G. cingulata, G. tucumensis*) have been recorded during the period of 1932 – 1992 (Ramakrishnan and Subramanian, 1952; Subramanian and Ramakrishnan, 1956; Tandon and Chandra, 1963; Tilak and Ramachandra, 1968; Sarbhoy *et al*., 1974, 1980, 1986 and 1996).

The anthracnose disease of strawberry is caused by a complex of species, i.e. *C. acutatum* Simmonds, *C. fragariae* Brooks, *C. gloeosporioides* (Penz.) Penz. and Sacc. and *C. dematium* (Pers.) Grove. Of these *C. acutatum* and *C. gloeosporioides* are ubiquitous and polyphagous (Dyko and Mordue, 1979). The symptoms may appear as sunken, dark lesions on petioles and stolons, which may then be girdled. The fungus often spreads to the crowns of young plants, which it rots, and then the plants die in the nursery or after they are transplanted. Other symptoms include bud rot, flower blight, black leaf spots throughout the leaf, irregular leaf spots on the leaf margins and tips (Agrios, 2004). The disease in nurseries can cause death of plants up to 80% and yield losses more than 50% in USA (Howard & Albergts, 1983; Wilson *et al.*, 1990). In the UK where *C. acutatum* alone has been recorded is considered a potential threat to the strawberry industry and detection of the disease results in the compulsory destruction of a crop (Cook & Popple, 1984). The often overlapping symptoms caused by the anthracnose fungus and the lack of distinguishable characters between *C. gloeosporioides* and *C. fragariae* have led to considerable uncertainty regarding the causal agent of various forms of strawberry anthracnose (Sreenivasaprasad *et al.*, 1992). Leaf spot and stem canker caused by *Colletotrichum gloeosporioides* on important forage legume *Stylosanthes* spp. is becoming a constraint in improving cattle production in tropical and sub tropical regions (Lenne & Sonda, 1978). Anthracnose disease is a major constraint to the production of yam (*Dioscorea* spp.) where most widely distributed species being particularly susceptible to the disease (Winch *et al.*, 1984). The epidemics that commence prior to or during tuber formation can result in yield losses in excess of 85%. In Nigeria anthracnose has been encountered throughout the yam production belt of the country. The disease is severe in the humid forest agro-ecological zones (Abang *et al.*, 2002). Resistant breeding is hampered by uncertainties regarding the variability of the yam anthracnose pathogen (Green *et al.*, 2000). Identification and differentiation of *Colletotrichum* species based on morphological characteristics have often been inadequate (Brown *et al.*, 1996) as some fungal isolates appeared similar to both *C. gloeosporioides* and *C. acutatum*. Isolates identified as *C. gloeosporioides* are highly variable in both morphology and virulence (Abang *et al.*, 2002).

Anthracnose of lupin is a world wide problem and yield loss up to 50% on narrow leaf lupin and up to 100% on white lupin has been experienced and is considered as most devastating disease of lupines (Yang and Sweetingham, 1998). The taxonomy of *Colletotrichum* isolates causing lupin anthracnose has been the subject of much debate (Talhinhas *et al.*, 2002).

The most serious disease of sugarcane in India is red rot caused by *Colletotrichum falcatum* Went. The disease destroyed thousands of cane fields of the dominant commercial variety Co 213 in the years 1938 – 41. Both the cultivator and the factory were badly affected so much so that the outputs of factories in the affected areas were reduced to one-third during 1938-39 and one-half in 1939-40 (Chona and Hingorani, 1950a). The perfect stage, *Glomerella tucumensis* of the red rot fungus has been obtained by Chona and Srivastava (1952) for the first time in India on sterilized leaf and leaf sheath pieces.

Most important anthracnose diseases caused by *Colletotrichum* species and its teleomorph stage (*Glomerella*) are on bean, cotton, soybean and sweet pea (Agrios, 2004). Fungus attacks all growing stages of plants and causes pre-emergence damping off. The fungus produces characteristic symptoms (dark brown sunken lesions with pink or white mass of conidia in the centre of the affected tissues. *G. cingulata* also causes cankers and die-back of woody plants such as camellia and privet (Agrios, 2004)

Nine species of Colletotrichum have been recorded on legumes in tropical and temperate regions. These include *C. capsici* (Sydow) Butler and Bisby, *C. coccodes* (Waller) Hughes, *C. crassipes* (Speg.) Arx, *C. dematium*

(Pres.) Grove, *C. destructivum* O'Gara, *C. gloeosporioides* (Penz.) Penz. and Sacc., *C. lindemuthianum* (Sacc. and Magn.) Br. and Cav., *C. trifolii* Bain and Essary and *C. truncatum* (Schw.) Andrus and Moore. (Lenne 1992). *Colletotrichum graminicola* (Cesati) Wilson causes the anthracnose disease of a variety of cereals and grasses (Wilson, 1914). Pre-harvest and post-harvest losses of many high value crops are substantial in the tropics because of various diseases caused by *Colletotrichum gloeosporioides* (Dodd *et al.*, 1992). *C. gloeosporioides* causes pre and post-harvest anthracnose diseases of avocados in many countries (Binyamini and Schiffmann, 1972; Sivanathan and Adikaram, 1989). Anthracnose lesion can develop in immature fruits on the tree following wounding caused by insect damage. The major disease problem is a post harvest ripe rot where infections remain quiescent as sub-cuticular hyphae (Prusky *et al.*, 1991). In *Citrus* spp. infection by *C. gloeosporioides* results in necrosis of flowers and abnormal young fruit fall which give persistent button (Fagan, 1979). Anthracnose of mango is severe on young leaves and if wet weather occurs during flowering, it prevents fruit setting. Infection occurs during fruit development up to harvest but remain quiescent until fruit ripens (Fitzell and Peak, 1984). In papaya *C. gloeosporioides* causes both chocolate spot and anthracnose. Infections are initiated during early fruit development and remain quiescent as an appressorium with infection peg or as sub-cuticular hyphae until fruit reaches the climacteric (Dickman and Alvarez, 1983). *C. gloeosporioides* is a major cause of foliar and pod diseases on cocoa in southern India (Chandra Mohan, 1978). Prasad and Varma (1966) observed perfect stage of *Colletotrichum gloeosporioides* Penz. on the leaves of *Saraca indica* L. in Bihar state of India as *Glomerella cingulata* (Stomen.) Spauld and Shrenk.

The species of *Colletotrichum* are extremely variable in morphology in culture (Simmonds, 1965; Sutton, 1980; 1992; Waller *et al.*, 1993; von Arx, 1957a; Hawksworth *et al.*, 1995; Sherriff *et al.*, 1994; Yang and Sweetingham, 1998; Bajaj *et al.*, 1964; Abang *et al.*, 2002; Smith and Black, 1990; Bonde *et al.*, 1991; Howard and Albregts, 1984; Mass and Howard, 1985; Cannon and Kirk, 2000). The red rot fungus, *C. falcatum*, is also highly mutable on artificial culture media (Lucas, 1942; Ramakrishnan, 1942; Chona and Hingorani, 1950b). The often-overlapping symptoms caused by the anthracnose fungi and the lack of distinguishable characters between *Colletotrichum* spp. has led to considerable uncertainty regarding the anthracnose fungi (Sreenivasaprasad *et al.*, 1992).

The concept of species in the genus Colletotrichum is not well established and nor universally accepted (Sutton, 1992). The taxonomy of *Colletotrichum* species has been developed through classical descriptive criteria such as the shape and size of conidia, setae and appressoria coupled with knowledge of the identity of the host plant. The lack of sufficient morphological characters and the ability of *Colletotrichum* species to infect wide range of plants has led to a large number of taxa being considered (Sutton, 1992). The criteria for creating a new species are based on indistinguishable morphological characters because they have been isolated from different host plants (Sherriff *et al.*, 1994). The host origin however has been deemed less important and the number of species was reduced to 11 by von Arx (1957a), and to 25 by Sutton (1980), though an increased reliance on host origin and specially, host specificity led this number to be increased to 37 by Sutton (1992).

The criteria used in differentiating *Colletotrichum* species is mainly based on straight / falcate shaped conidia. Most straight conidial forms fall into *Colletotrichum gloeosporioides* "species group" (von Arx 1957b, 1981), but individual species are recognized based on host specificity, which is considered highly specific (Sutton, 1992).

*Colletotrichum* isolates show extensive variation in culture. Hence, the identification of a species based on small distinction between sizes of conidia and their shape can be obscured by the variation within an isolate. Furthermore, the culture conditions used for morphological assessments, including the media, the age of the culture and the temperature used can not be standardized. Due to above constraints Sutton (1992) stated "no progress in the systematics and identity of isolates is likely to be made based on morphology alone."

Several alternative taxonomic criteria have been proposed and these include various biochemical tests and protein profiles but variation due to lack of standardization of methods hinder their use (Monte *et al.*, 1990). The most significant advances in taxonomy in general are coming from approaches based on analysis of DNA sequences. These approaches include analysis of Restriction Fragment Length Polymorphism (RFLPs), Amplified Fragment Length Polymorphism (AFLPs) and Random Amplified Polymorphic DNA (RAPDs) (Welsh & McCleland, 1990; William *et al.*, 1990). DNA polymorphism assay based on the amplification of random DNA fragments flanked by 10-mer of arbitrary nucleotide sequence (RAPD) is useful in detection of variation among individuals and has been used for solving taxonomic problems

(Crowhurst *et al.*, 1991; Hodson *et al.*, 1993; Sherriff *et al.*, 1994; Thakur *et al.*, 1998; Zambino & Szabo, 1993).

Considering the above facts the present study was initiated with the following objectives:-

1. Study the morphological and cultural characters of *Colletotrichum* isolates available in Indian Type Culture Collection (ITCC) and isolates from the fields of different places of India. Testing of pathogenicity of commonly available *Colletotrichum* species.

2. Use of RAPD markers for discrimination of commonly available *Colletotrichum* species.

The genus *Colletotrichum* consists of several other genera, which have been described as Deuteromycetous and there are about 11 genera synonymous. Some of these are taxonomic synonyms and some are nomenclatural synonyms (Sutton, 1992). There are about 900 species described in or assigned to *Colletotrichum*. During past 200 years different systems for identification of species have been practiced. Morphology of the fungi, host-pathogen relationships and host specificity have been used in above systems (Sutton, 1992).

Tode (1790) described the genus *Vermicularia* as a starting of the genus *Colletotrichum*. According to the rules of the Code in force (August 1981) the starting point for nomenclature of the group containing *Colletotrichum* was 1821. Fries (1821-1832) who was the authority for names published before 1821 placed *Vermicularia* as synonymy of *Sphaeria* Haller ex Fr. Fries (1825) accepted *Vermicularia* so the author citation for the generic name was 'Tode ex Fr.'. This means that the fungal name *Vermicularia* dated 1825, being a later homonym of *Vermicularia* published by Moench in 1802 for a genus in the Verbenaceae, was illegitimate (Sutton, 1992). Therefore, it couldn't be used for, or compete with, the later name *Colletotrichum* which was introduced by Corda (1837a). Corda (1837b) initially introduced it as *Colletothrichum* and later changed it to *Colletotrichum*. In August 1981 the starting point for fungal names was changed from 1821 to 1753. This had the effect of reinstating the fungus *Vermicularia* of 1790 over the phanerogamic *Vermicularia* of 1802. It is doubtful any species originally described by Tode belong to what we now know as *Colletotrichum* (Sutton, 1992). Setae are not associated with the fruiting bodies of *V. pseudosphaeria* Tode or *V. pubescens* Tode. Some species of *Colletotrichum,* such as *C. musae* (Berk. and M.A. Curtis) von Arx, do not possess conidiomatal setae (Sutton and Waterston, 1970). The globose superficial conidiomata of these *Vermicularia* species and *V. hispida* Tode are another indication that *Colletotrichum* is inappropriate (Sutton, 1992). Hughes (1958) doubted that anyone had actually validated the original species names in *Vermicularia.* Fries (1825) didn't accept any species in the genus *Vermicularia.* The problem was aggravated by the fact that all Tode's specimens have been destroyed (Stafleu and Cowan, 1986). This problem was not solved until the identities of the three original species described by Tode are verified in some way and a lectotype species selected. Due to this it cannot be determined whether *Vermicularia* is an earlier name for *Colletotrichum* or not and it remains something of a nomenclatural spectre hanging over workers in the group (Sutton, 1992). This can be overcome because conservation of later taxonomic generic synonyms over earlier names is allowed by the Code (Sutton, 1992). Hence, it would be desirable to preserve *Colletotrichum* in this way (Sutton, 1992).

Different taxonomists have different opinion about the generic limits of *Colletotrichum*, the genus has been described under several different names although Corda (1837a) has given clear illustrations and diagnostic characters (Sutton, 1992). So far there are 17 generic synonyms for *Colletotrichum* and two further names, which are doubtfully included (Sutton, 1980). There are several reasons for this confusion. The genus *Steirochaete* Braun and Caspari, was separated from *Colletotrichum* in repeatedly having catenate conidia, but Southworth (1890b) transferred the name of the type species to *Colletotrichum*. Later von Arx (1957a) considered it as a synonym of *C. gloeosporioides*. Similarly *Gloeosporiopsis* Speg. was separated by the supposed catenate conidia but conidiomata without setae (Sutton, 1992). Petrak and Sydow (1935) considered *Gloeosporiopsis* to be identical with *Colletotrichum*. Although *Colletotrichopsis* Bubak, and *Colletotrichum* are differentiated in having pycnidial wall with setae, von Arx (1957a) considered both as *Colletotrichum*. Although the anamorph of *Colletostroma* Petrak is *Caulochora baumgartneri* Petrak the latter was considered to be the same as *Glomerella cingulata* (Stonem.) Spauld and Schrenk by von Arx and Muller (1954). As a result *Colletostroma* became a synonym of *Colletotrichum*. Although above genera contained few species, *Vermicularia* and *Gloeosporium* have been described with many hundreds of species and their eventual relegation to synonymy with *Colletotrichum* have constituted major advances in the taxonomy of the genus (Sutton, 1992). Sutton (1966) and Nag Raj (1973) have demonstrated how developmental studies can identify taxonomic status of questionable species or genera.

The names *Vermicularia* and *Colletotrichum* were used interchangeably during the 19$^{th}$ and early 20$^{th}$ centuries for species we now accept as *Colletotrichum*. Wollenweber and Hochapfel (1949) placed the curved spored species in *Vermicularia* and the straight-spored species in *Colletotrichum*. But the type species of *Colletotrichum, C. lineolum* Corda, had curved conidia. Clements and Shear (1931) differentiated *Colletotrichum* and *Vermicularia* by marginal setae in former species while setae dispersed throughout the conidiomata in latter. Other mycologists separated these 2 species on the basis on *Vermicularia* being pycndial and *Colletotrichum* being acervular (Negru, 1960). Grove (1937) stated that setae in *Vermicularia* is essential while in *Colletotrichum* it is less important. Duke (1928) demonstrated that conidiomatal structure, form and presence of setae are highly variable and has no significance at generic level. This resulted in a large number of species having their names changed from *Vermicularia* to *Colletotrichum*.

There are about 750 species described in the genus *Gloeosporium* Desm. and Mont. (Montagne, 1849). This genus is closely related to *Colletotrichum* and separated based on the absence of setae. von Arx (1957b) showed that the type species of *Gloeosporium, G. castageni* Desm. and Mont., is a species of *Marssonina* Magnus, *M. castageni* (Desm. and Mont.) Magnus (Magnus 1906). The presence of setae in conidiomata is controlled genetically, because some *Colletotrichum* species (*C. musae* and *C. gossypii* Southw. var. *cephalosporioides* A.S. Costa), consistently fail to produce them (Sutton and Waterston, 1970). Environmental conditions especially relative humidity play an important role in setae production (Frost, 1964). Due to above reasons nearly all species of *Gloeosporium* have been assigned to other genera. The names of some of the species have been transferred to *Colletotrichum* as distinct species while majority have been reduced to synonymy with already existing species (von Arx, 1957b).

Cesati (1852) introduced genus *Dicladium* with a single species *Dicladium graminicola* Cesati. Westendrop (1861) placed the type species in *Vermicularia*. Later it was placed in *Steirochaete* Braun and Caspari by Saccardo (1886) thinking that conidia were catenate. Wilson (1914) finally placed it in *Colletotrichum*. But Vassiljevsky and Karakulin (1950) resurrected it based on the peculiar method of conidiomatal development. Minute hyphae similar to penetration peg grow towards epidermis and break the upper epidermal wall. Conidiomatal formation is initiated between upper epidermal wall and cuticle. In graminicolous substrata similar development occurs in *C. capsici* on *Ananas* and in *C. dematium* and *C. gloeosporioides*. In non-graminicolous substrata these 3 species produce epidermal to sub-epidermal conidiomata. Therefore, in the normal manner by producing an epidermal to sub-epidermal stroma, which ruptures, the epidermis and any other overlying host tissues, *Dicladium* was transferred to the genus *Colletotrichum* (Sutton, 1992).

The conidia with distinctive extension of the conidial apex into a filiform cellular appendage were named as *Ellisiella* by Saccardo (1880). This taxa was formed with one species *E. caudate* Peck. ex Sacc. but conidiomatal development, production of appressoria and setae in culture is identical with other graminicolous *Colletotrichum* species. Sutton (1966) suggested that it would be more correctly placed in *Colletotrichum* as *C. caudatum.* Nag Raj (1973) formally rectified this situation.

## 2.1. Identification of *Colletotrichum* species

The species identification in *Colletotrichum* slightly differs from those, which have evolved for other groups of deuteromycetes. It has taken much longer time for archic 19$^{th}$ century taxonomic practices to be replaced by modern ideas and current procedures for identification of *Colletotrichum* species and it lag someway behind those in Deuteromycete genera (Sutton, 1992).

The problem with micro fungi is that there are a number of difficulties, mainly of historic nature, which need to be overcome before much sense can be emerge about species concept. When a micro fungus found on a host, which has no record of that host-fungus relationship, earlier practice was to interpret it as a new fungal species (Sutton, 1992). Now these ideas are rejected by most of mycologists and plant pathologists. But species are occasionally described in *Colletotrichum* and many other genera belonging to both the ascomycetes and deuteromycetes purely on the basis of occurrence on a different host substratum. This practice has become a barrier to future progress of the taxonomy of *Colletotrichum*. The studies carried out on *Colletotrichum* have been based on the characters of the fungi on natural substrata because the behaviour in culture cannot be rationalized and there is no standardization of the methods used. Presently new taxa are described below at the species level such as variety, forma and forma-specialis. This lead to significant slowing down in the number of new taxa described in the genus (Table 1).

**Table 1. Number of new species described in *Colletotrichum*.**

| Decade | Number of new taxa | Percent sub specific taxa |
|---|---|---|
| 1991 – 2000 | --- | ---- |
| 1981 – 1990 | 8 | 88 |
| 1971 – 1980 | 20 | 75 |
| 1961 – 1970 | 95 | 9 |
| 1951 – 1960 | 79 | 15 |
| 1941 – 1950 | 17 | 6 |
| 1931 – 1940 | 77 | 8 |
| 1921 – 1930 | 95 | 6 |

Source - Sutton, 1992.

The morphological characters derived from growth of isolates in culture have rarely been standardized, especially between workers and inherent phenotypic plasticity of individual isolate which further complicates the confusion in identification of *Colletotrichum* species (Sutton, 1992). It has been difficult to rationalize behaviours in culture with those on natural substrata, especially when some taxa, such as *C. gloeosporioides, C. dematium, C acutatum* and *C. graminicola* which are having wide host ranges. This lead to adopt very broad, unreliable and non-predictive concepts about *Colletotrichum* species and their identification. A combination of classical criteria such as conidial shape and size, presence, absence and morphology of setae, presence of sclerotia and appressoria, and symptom expression on hosts are being used in species identification in *Colletotrichum* (Sutton, 1992). The confusion in the identification of *Colletotrichum* species is further aggravated when characters of the teliomorphs are added.

In a large genus like *Colletotrichum,* to reduce the number of accepted taxa, there need to be a degree of systematic catharsis. (Sutton, 1992). The number of species, accepted was reduced to 11 by von Arx (1957a) (i.e. *C. gloeosporioides* (Penz.) Penz and Sacc., *C. crassipes* (Speg.) von Arx, *C. lini* (Westerd.) Tochinai, *C. destructum* O'Gara, *C. fuscum* Laub., *C. fusarioides* (Ell. and Kellerm.) O'Gara, *C. phyllochoroides* (Ell. and Everh.) von Arx, *C. paludosum* (Ell. and Galw) von Arx, *C. atramentarium* (Berk. and Broome) Taubenh., *C. graminicola* (Ces.) Wilson and *C. dematium* (Pres. : Fr.) Grove). The taxa *C. lindemuthianum* (Sacc. and Magn.) Briosi and Cav., *C. orbiculare* (Berk. And Mont.) von Arx, *C. musae* (Berk. And Curt.) von Arx, *C. malvarum* (A.Br. and Casp.) Southw., *C. gnaphalii* Syd., *C. psoraleae* (Peck) von Arx, *C.*

**Table 2. Seed borne *Colletotrichum* species and their synonyms**

| Species | Synonyms |
|---|---|
| *C. dematium* (Pers. ex Fr.) Grove | *C. capsici* (Syd.) Butler and Bisby |
| | *C. circinans* Berk. |
| | *C. curvatum* Briant et Martyr |
| | *C. glycines* Hori |
| | *C. hibisci* Poll. |
| | *C. indicum* Dastur |
| | *C. lineola* (Cda.) Grove |
| | *C. multisetorum* Ibragivon |
| | *C. spinaciae* Ellis and Halst. |
| | *C. truncatum* Schw. |
| *C. graminicola* (Ces.) Wilson | *C. falcatum* Went |
| | *C. phaseolorum* Tak. |
| *C. lini* (Westend) Tochinai | *C. linicola* Pethybr. |
| *C. gloeosporioides* Penz. | *C. antirrhini* Stewart |
| | *C. camelliae* Massee |
| | *C. coffeanum* Noack |
| | *C. higginsianum* Sacc. |
| | *C. melongenae* Libik |
| | *C. phomoides* Chester |
| | *C. pisi* Pat. |
| | *C. tabacum* Bőning |
| | *C. violae-tricoloris* Smith |

Source: Kulshrestha *et al.*, (1976)

**Table 3. List of accepted taxa (species)**

| Name of the species | Shape/size of conidia | Shape/ size of appressoria |
|---|---|---|
| ***C. acutatum*** Simmonds ex Simmonds | straight, fusiform, abruptly tapered at each end, sometimes slightly medianly constricted, 8.5 - 16.5 x 2.5 -4 µm. | clavate, ovate to obovate to slightly irregular with margins entire or slightly lobed, 8.5 - 10 x 4.5 - 6 µm. |
| ***C. capsici*** (H. Syd.) E. Butl. and Bisby | falcate, fusiform, gradually tapered towards each end, 18 - 23 x 3.5 - 4 µm. | clavate to ovate, margin entire, becoming complex and forming long irregular chains, 9 - 14 x 6.5 - 11.5 µm. |
| ***C. caricae*** Stev. and Hall | straight, cylindrical, not tapered at either end, 9 - 20 x 3.5 - 6 µm. | ovate to pyriform, apparently not becoming complex |
| ***C. caudatum*** (Sacc.) Peck. | falcate, fusiform, 18.5 - 24 x 3.5 - 4 µm, the apex prolonged into an unbranched, cellular appendage 10 - 16 µm long | clavate or ovate, margin entire, 10 - 14 x 9 - 10 µm. |

Table 3. contd....

| Name of the species | Shape/size of conidia | Shape/size of appressoria |
|---|---|---|
| ***C. circinans*** (Berk.) Voglino | falcate, fusiform, gradually tapered to each end, 19 - 21 x 3.5 µm | clavate to irregular, margin entire, 10 - 14.5 x 6 - 6.5 µm |
| ***C. coccodes*** (Wallr.) S. Hughes | straight constricted in the middle and abruptly tapered to each end, 16 - 24 x 3 - 4 µm | long clavate, ovate, elliptical, margin entire but sometimes irregularly lobed, 8.5 - 16 x 4 - 11.5 µm |
| ***C. coffeanum*** Noack | Conidia formed from hyphae, not in conidiomata, straight, cylindrical, not tapered at either end, 12.5 - 19 x 4 µm. | clavate, long clavate to ovate, margin entire or slightly irregular, 8 - 9.5 x 5.5 - 6.5 µm, often becoming complex. |
| ***C. corchori*** Ikata and Tanaka | falcate, fusiform, gradually tapered to each end, 14.5 - 19.5 x 3.5 - 4 µm. | clavate, ovate, edge entire, 6.5 - 11 x 5.5 - 8.5 µm, sometimes becoming complex. |
| ***C. crassipes*** (Speg.) von Arx | straight, cylindrical, apex obtuse, base truncate, 14 - 28 x 5 - 7 µm. | long clavate to ovate, with crenate or deeply lobed, margins, 10.5 - 14 x 7 - 9.5 µm, often becoming complex |
| ***C. curvatum*** Briant and Martyn | fusiform, tapered gradually to acute end, 16 - 22 x 3.5 µm. | appressoria not mentioned |
| ***C. dematium*** (Pres.: Fr.) Grove | falcate, fusiform, gradually tapered to each end, 18 - 26 x 2 - 3 µm. | clavate, ovate to irregular, margins entire or slightly irregularly lobed, 7.5 - 18 x 4 -12.5 µm |
| ***C. destructivum*** O'Gara (Teleomorph: ***G. glycines*** Lehman and Wolf) | straight or slightly curved, abruptly tapered to an obtuse apex and truncate base 10 - 22 x 4 - 6 µm | irregularly clavate, ovate, 6 - 16 x 6 - 10 µm |

Table 3. contd....

| Name of the species | Shape/size of conidia | Shape/size of appressoria |
|---|---|---|
| ***C. falcatum*** went (Teleomorph: ***G. tucumensis*** (Speg.) von Arx and Müller | falcate (but no strongly), fusiform, tapered gradually to the base and more abruptly to the apex, 15.5- 26.5 x 4-5µm. | clavate to ovate, edges entire, 12.5 - 14.5 x 9.5 - 12 µm |
| ***C. fragariae*** Brooks | cylindrical, apex obtuse, tapered to the truncate base, 12.5 - 16.5 x 4.5 – 5 µm | clavate to ovate |
| ***C. fusarioides*** (Ell. and Kellerm.) O'Gara (Teleomorph: ***G. fusarioides*** Edgert.) | cylindrical to irregular, straight to slightly curved, slightly attenuated towards the base, obtuse at the apex, 18 – 32 x 4.5 – 6 µm | no details on appressoria |
| ***C. fuscum*** Laub. | cylindrical to ellipsoid, straight or slightly curved, apex obtuse, base slightly attenuated and truncate, 15-21 x 3.5-4 µm | Ovate, 4 – 7 µm |
| ***C. gloeosporioides*** (Penz.) Penz. and Sacc. (Teleomorph: ***G. cingulata*** (Stonem.) Spauld. and von Schrenk) | Straight, cylindrical, apex obtuse, base truncate, 12 - 17 x 3.5 – 6 µm | Clavate, ovate, obovate, sometimes lobed, 6 - 20 x 4 - 12 µm |
| ***C. gloeosporioides*** var. ***minus*** Simmonds (Teleomorph: ***G. cingulata*** var. ***minor*** Wollenw.) | straight, cylindrical, apex obtuse, tapered towards the truncate base, 11 - 17.5 x 3 - 5 µm | ovate to irregular and sometimes lobed, 8 - 9 x 6 - 6.5 µm |
| ***C. gnaphalii*** H. Syd. | smaller conidia, 8-15 x 4-6 µm | data not available |

Table 3. contd...

| Name of the species | Shape/size of conidia | Shape/size of appressoria |
|---|---|---|
| ***C. graminicola*** (Ces.) Wilson (Teleomorph: ***G. graminicola*** Politis) | falcate, fusiform, gradually tapered to the apex and base, 23.5 - 29 x 3.5 - 5 μm | edge very irregular, 17.5 - 20 (-30) x 12.5 - 14 μm. |
| ***C. helichrysi*** (Wint.) von Arx | straight, cylindrical, 15 - 19 x 4.5 - 6 μm. | no appressoria formation |
| ***C. higginsianum*** Sacc. | straight or slightly curved, fusiform, abruptly tapered to each end, 16.5 - 19 x 4 μm | no data on appressoria |
| ***C. liliacearum*** Ferr. (=***C. lilii*** Plakidas ex Boerema and Hamers) | falcate, fusiform, tapered gradually to each end, 13 - 23 x 3.5 - 5 μm | not described |
| ***C. lindemuthianum*** (Sacc and Magnus) Briosi and Cav. (Teleomorph: ***G. lindemuthiana*** Shear) (Syn. ***G. cingulata*** f.sp. ***phaseoli***) | straight, cylindrical to ellipsoid, both ends obtuse, 9.5 - 11.5 x 3.5 - 4.5 μm | clavate, ovate, edge regular, 8 x 6 - 7 μm |
| ***C. linicola*** Pethybr. and Lafferty | slightly curved or even straight, fusiform, abruptly tapered to each end, 16 - 19 x 3 - 4.5 μm | long clavate to irregular, margin entire to irregular or crenate, 10 - 13.5 x 6.5 - 13.5 μm |
| ***C. malvarum*** (Braun and Casp.) Southw. | straight, or slightly irregular, cylindrical to ellipsoid, apex obtuse, base truncate, 12 - 24 x 4 - 6 μm | not described |
| ***C. musae*** (Berk. and M.A. Curtis) von Arx (Teleomorph: ***G. musarum*** Petch) | straight, cylindrical, obtuse at the apex, truncate at the base, 12 - 17 x 4.5 - 5.5 μm | irregular in shape and often with large or deep lobes, 9 - 13 x 9 - 11.5 μm |
| ***C. nigrum*** Ell. and Halst. | straight, cylindrical to ellipsoid, apex obtuse, base truncate, 15 - 21 x 3.5 -6.5 μm | not described |

Table 3. contd....

| Name of the species | Shape/size of conidia | Shape/size of appressoria |
|---|---|---|
| ***C. nymphaeae*** (Pass.) van der Aa | ellipsoid to cylindrical or slightly curved, somewhat attenuated toward the base, 12 - 23 x 4 - 5.5 µm. | not described |
| ***C. orbiculare*** (Berk. and Mont.) von Arx | straight or very slightly curved, clavate, apex obtuse, tapered towards the base, 10 -15 x 4.5 - 6 µm | ovate, clavate to irregular, unlobed, often becoming complex, 6.5 - 16 x 5.5 - 10 µm |
| ***C. paludosum*** (Ell. and Galw.) von Arx | cylindrical to ellipsoid, 18 - 28 x 6 - 8 µm. | not described |
| ***C. phyllachoroides*** (Ell. and Everh.) von Arx | cylindrical with both ends obtuse or irregularly curved and then tapered towards each end, 18 - 24 x 4 - 6.5 µm | not described |
| ***C. psoraleae*** (Peck.) von Arx | cylindrical to ellipsoid, both ends tapered, 16 - 22 x 3.5 - 5.5 µm | not described |
| ***C. spinaciae*** Ell. and Halst. | falcate but not strongly so, fusiform, tapered gradually to the obtuse apex and truncate base, 21 - 28 x 3.5 - 5.5 µm | clavate to ovate, brown, 6 - 7 x 3.5 - 4 µm |
| ***C. sublineolum*** Henn. (Teleomorph: ***G. cingulata*** var. ***sorghicola***. Saccas) | falcate, fusiform, gradually tapered towards each end, 18.5 - 27.5 x 3 - 4.5 µm | edge tending towards the irregular, 11.5 - 15 x 8.5 - 9 µm. |

Table 3. contd....

| Name of the species | Shape/size of conidia | Shape/size of appressoria |
|---|---|---|
| ***C. trichellum*** (Fr.: Fr.) Duke | falcate, not strongly curved, fusiform, tapered abruptly towards each end, 14 - 24 x 4 - 6 µm. | edges crenate and lobed, usually simple, occasionally complex, 12 - 18 x 8.5 - 12 µm. |
| ***C. trifolii*** Bain and Essary | straight, cylindrical, apex obtuse, base truncate, 6 - 18 x 4 - 8 µm | clavate to ovate, sometimes irregularly lobed, 6 - 12 x 6 - 8 µm |
| ***C. truncatum*** (Schwein.) Andrus and Moore | falcate but not strongly so, fusiform, tapered gradually towards the obtuse apex but abruptly towards the truncate base, 12 - 16 x 4 - 6 µm | clavate to irregular, 6 - 12 x 6 - 12 µm |
| ***C. typhae*** H.C. Greene | straight, cylindrical to long clavate, sometimes slightly constricted in the middle, obtuse at the apex, attenuated towards the base, 11.5 - 21 x 3.5 - 5 µm | ovate to clavate, 8 - 11 x 5 - 9 µm, sometimes tending to become complex |

Source: Sutton, 1992.

## 2.4. Species proposed by Kulshrestha *et al.*, (1976)

The description of the *Colletotrichum* species proposed by Kulshrestha *et al.,* (1976) has been based on the relative size of the setae in relation to the conidial mass in acervuli on seeds under stereo-binocular microscope, and the shape of conidia under compound microscope.

1. ***Colletotrichum dematium*** (Fr.) Grove, *J. Bot. Lond.* **56**: 341 (1918)

Acervuli single or in groups. Setae numerous, blackish brown to dark black, longer than the conidial mass. Conidial mass white to dull white, pale orange or bright orange. Mycelium mostly absent on the seed surface, when present fine, shiny or whitish pink. Conidia hyaline, fusoid, ends rounded or slightly tapering 15-27 x 2-5 µm. Setae numerous, trichiform, brown to blackish brown, 0-9 septate, 48-468 x 2-7 µm.

2. ***Colletotrichum graminicola*** (Ces.) Wilson, *Phytopatholoy* **4**: 110 (1914).

Acervuli single or in groups, sometimes coalescing and covering the whole seed. Conidial mass dull white to dull orange or pinkish bright orange. Blackish brown sclerotia mostly present. Mycelium mostly absent, when present white to whitish orange. On some seeds profuse mycelium bearing conidiophores and conidia present. Conidia formed in false head on the tip of conidiophores as in *Fusarium moniliforme.* Conidia hyaline, falcate, one celled, both ends pointed, 15-31 x 3-5 µm. Setae numerous, brownish black to dark black, longer than the conidial mass, 0-4 septate, 30- 270 x 3-10 µm.

3. ***Colletotrichum gloeosporioides*** Penz., *Atti. R. 1st Ven. Sci. Lett. Art.* Ser. **6**, **2**: 670 (1884).

Acervuli single or in groups, sometimes appearing like pycnidial bodies emerging from ruptured, grayish black spots. Setae absent or inconspicuous, smaller than the conidial mass. Conidial mass, dull white to dull orange, or bright orange.

Mycelium mostly absent, when present white, shiny. At high moisture levels conidia are formed in false heads. Conidia hyaline, one-celled, straight, oval, oblong or cylindrical, ends rounded, 6-15 x 3-5 µm. Setae few, blackish brown, 0-2 septate, trichiform, blunt at tips 30-90 x 2-4 µm.

4. ***Colletotrichum lindemuthianum*** (Sacc. *et* Magn.) Bri. *et* Cav., *Funghi Parass.* 50 (1889).

Acervuli mostly in groups, coalescing and covering the seed, rarely single. Setae few, longer than the conidial mass. Conidial mass orange to bright orange. Mycelium, white and scanty. Conidia hyaline, oblong to dumbbell shaped, one celled, straight, ends rounded, 9-15 x 3-4 µm. Setae trichiform, light brown to dark brown, 2-5 septate, 60-120 x 2-4 µm.

5. ***Colletotrichum lini*** (Westend) Tochinai, *Sci. Proc. R. Dublin Soc.* **15**: 368 (1918).

Acervuli mostly in groups, rarely single. Setae numerous, but usually one or two are emerging from or near the center of the conidial mass, larger than the conidial mass. Conidial mass orange to salmon orange. Mycelium scanty, whitish gray, to brownish grey. Conidia hyaline, one celled, allantoid, both ends slightly curved and rounded, 11-21 x 3-4 µm. Setae filiform, straw coloured to brown, 2-8 septate, 90-150 x 3-4 µm.

6. ***Colletotrichum acutatum*** Simmonds ex Simmonds, *Qld. J. agric. Anim. Res.* **25**: 178A (1968).

Acervuli mostly single, rarely in groups. Setae numerous, brownish black, minute, smaller than conidial mass. Conidial mass bright orange. Mycelium absent.

Conidia hyaline, one celled, cylindro-fusoid, straight, both ends pointed, 6-12 x 2-3 µm. Setae trichiform, brownish black, 1-4 septate, 30-90 x 3-6 µm.

This species is similar to *C. gloeosporioides* in having setae smaller than the conidial mass but different in having straight, cylindro-fusoid, small conidia pointed at both end.

## 2.5. Taxonomy and Molecular Biology

In recent years there has been vast progress in the development of molecular biological techniques. These have been increasingly applied to the study of taxonomic and phylogenetic relationships among fungi (Yoder and Turgeon, 1985). These methods include restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), analysis of random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphism (AFLP) (Welsh and McClelland, 1990; Williams *et al.,* 1990; Foster *et al.,* 1993). The ability of the polymerase chain reaction to amplify specific fragment of DNA has been the basis for many PCR based methods, in a great variety of areas, from gene cloning and evolutionary studies to pathogenicity and forensic testing (Kocher and White, 1989; Dallapiccola *et al.,* 1991; Kwok and Sninsky, 1989; von Beroldingen *et al.*, 1989). One of the first uses of PCR in fungal studies was the amplification of ribosomal DNA sequences and the determination of their evolutionary relationships (White *et al.,* 1989). Smith and Black (1990) utilized PCR techniques to study wild populations of basidiomycetes, revealing hitherto unsuspected facets of fungal ecology.

## 2.6. Polymerase chain reaction (PCR)

The polymerase chain reaction method allows specific and very sensitive detection and production of DNA fragments from nucleic acids obtained from wide variety of sources. The mechanism of PCR is simple and DNA sequence to be amplified (template), two single stranded oligo nucleotide pieces (primers), DNA polymerase, deoxyribonucleotide triphosphates (dNTPs), buffer and salts are heated and cooled to required to temperatures to drive the reaction. This process consist of three steps; denaturation of template DNA, annealing of primers to the template and extension of the annealed primer to synthesize daughter strand of the template DNA.

The double stranded template DNA is first denatured by incubation at high temperature and remain single stranded until temperature is lowered sufficiently to allow annealing. The two primers anneal to their complementary sites, which is determined by the template sequence. The primers must anneal to the opposite DNA strands of the template for successful amplification. The 3' ends of the primers should face each other when they are properly aligned on the template DNA. The 3' end of the primer is the site of addition of dNTPs for the synthesis of daughter strand.

The third step is DNA polymerase mediated and extension of daughter strand occurs from 5' to 3' of the template DNA. The resultant product will be shorter than the template DNA and get hybridized as the templates. After the second cycle the shorter fragments exceed that of template and more shorter fragments of same size will be produced. Repeating the cycles ultimately result detectable amount of fragments.

## 2.7. Analysis of PCR products

A simple agarose gel provides sufficient resolution for separation of the fragments produced in PCR. Fragments are detected under UV light after the gels are stained with ethidium bromide. For the analysis of reverse transcriptase PCR (RT-PCR) products bands are separated in 5% polyacrylamide gel and staining with silver is suggested (Sarkar *et al.*, 1992). Agarose gels of 1-2% give good separation of DNA fragment of 200-800 base pairs, while pieces larger than 800 base pairs 0.7-1.0% agarose gels are used (Foster *et al.*, 1993).

## 2.8. RAPD analysis

Instead of two primers a single primer of 10 nucleotides in length with unspecific sequence is used in RAPD reaction (Williams *et al.,* 1990). The primer finds homology in the template DNA and initiate extension. Since the primer sequence is random, the annealing temperature used is kept low usually 35-37$^0$C to ensure that the primer will anneal to the template. After 30-45 cycles the products are run on an agarose gel and is viewed over a UV light following staining with ethidium bromide.

## 2.9. RFLP analysis

The interested DNA is digested with a six base recognizing restriction enzyme and the resulting fragments are separated on a 0.7-1.0% agarose gel (Botstein *et al.,* 1980). The bands are viewed by staining with ethidium bromide (Buddie *et al.,* 1999: Abang *et al.*, 2002). The sizes of the fragments are compared with DNA size standards.

Instead of ethidium bromide staining, radioactive probes can be hybridized to the DNA fragments after they are transferred on to a membrane. The hybridized probes are detected exposing the membranes to X ray film (Wang *et al.*, 2000; Fabre *et al.,* 1995; Freeman *et al.,* 1996; Sreenivasaprasad *et al.*, 1993).

## 2.10. Amplified Fragment Length Polymorphism (AFLP)

AFLP is a tool that allows differentiation between individuals, genotypes and strains and the assessment of genetic diversity and phylogeny. Vos *et al.*, (1995) developed it for genetic mapping in plants. The AFLP markers generated are neutral and are generated from a large number of independent loci from different parts of the genome.

AFLP analysis involves selective amplification of fragments from restriction enzyme digests of genomic DNA. Genomic DNA is digested simultaneously with a heaxnucleotide cutter and a tetranucleotide cutter. The digested fragments are ligated with *Mse* 1 adapter and a biotinylated *Eco* R1 adapter. Fragments with *Eco* R1 adapters will be subjected to PCR with primers designed to match each of the restriction enzyme adapter sequences with the addition of an arbitrary 2 base extensions at the 3' end. PCR is performed under stringent conditions, which leads to the amplification of manageable number of fragments. These can be resolved in denaturing polyacrylamide gels.

**Table 4. The utility of different molecular methods.**

| Method | Utility |
|---|---|
| PCR | Usable at any taxonomic rank |
| RFLP | Usable at any taxonomic rank |
| AFLP | Usable at any taxonomic rank |
| RAPD | Usable at species level. |

Out of above methods RAPD is an easy technique to perform and the knowledge of the primers and the sequences are not needed. Furthermore, a single primer for both forward and reverse directions is utilized.

Cooke *et al.*, (1996) used RAPD to separate group I *Phytophthora* species and were able to get separation between *P. iranica, P. pseudosugae* and *P. cactorum*. They further observed that within *P. cactorum* collar rot isolates from apple clustered from strawberry crown rot isolates. Thompson and Latorre (1999) performed RAPD analysis of 29 isolate of *Botrytis cinerea* Pres. ex Fr. isolated from table grapes and other crops in Chile. They found that none of the 29 primers tested differentiated the isolates based on host, or geographical region. The DNA profiles generated with primers OPA4 and OPA11 distinguished isolates of *Botrytis cinerea* from other epiphyte fungi found on table grape. Further obtained three fragments one high intensity 1.2kb fragment, a 1.10 kb fragment and a 0.7 kb fragment with primers OPA4 and OPA11 regardless of the host origin. These fragments were obtained when there is only one spore of *B. cinerea* in the test samples.

Megnegneau *et al.,* (1993) used RAPD for assessing the variability among the black strains of *Aspergilli* and related species. They found eight main groups as assigned by RFLP were also distinguishable by RAPD pattern. They further concluded that RAPD is a quick and reliable tool for establishing the amount of genetic variability in closely related species.

Hodge *et al.*, (1995) used RAPD to compare 38 isolates of *Zoopthora radicans* from different hosts around the world to investigate whether the applied fungus has established in the field. They found that aphid-infecting isolates are distinct from those infecting Ciccadelids and Lepidoptera and were able to identify 10 groups of the fungus based on RAPD patterns. Similarly, studies conducted on the origin of an out break of *Zoophthora phytomoni* on leaf hoppers by Hajek *et al.*, (1996) has revealed that there are two distinct groups based on RAPD patterns.

Sorghum infecting *Colletotrichum graminicola* (Ces.) Wilson collected from different places of Andra Pradesh and Maharashtra when subjected to RAPD analysis showed very high genetic variability among isolates (Latha *et al.,* 2002). Further a very high degree of genetic variability was also observed among the isolates from a single lesion in RAPD profile. The morphological characters of the isolates from a single lesion also have showed high variability.

Therefore, in the present studies detailed PCR-RAPD techniques have been used to reassess and clarify the species concept in *Colletotrichum.*

# MATERIALS AND METHODS

Investigations to study the variability in the genus *Colletotrichum* causing common diseases on different crops of India were conducted. The specimens available in the Herbarium Cryptogamme Indiae Orientalis (HCIO) at IARI, New Delhi were examined besides collections made individually from different isolations. Fifteen *Colletotrichum* isolates comprising of five species were taken for this study. In the present investigations the variability of the *Colletotrichum* species was examined based on, cultural and morphological characters of the fungus. Random Amplified Polymorphic DNA (RAPD) markers were also evaluated for differentiation of the species. The various methods employed and materials used are described below under different headings:-

## 3.1. Isolation of *Colletotrichum*

Fifteen *Colletotrichum* isolates comprising of five species *C. capsici, C. dematium, C. falcatum, C. gloeosporioides,* and *C. lindemuthianum* (Table 5) were obtained from Indian Type Culture Collection (ITCC), IARI, New Delhi. Another ten isolates of the fungus were isolated from the diseased samples (bean, bell pepper, soybean and sugarcane) collected from different places of India (Karnal, Pantnagar and Solan) (Table 6). The collected diseased samples were kept in polythene bags and stored in a refrigerator until isolation of the fungus in the laboratory. The symptoms occurring on the diseased samples were recorded while sampling. Small pieces from the edge of the affected lesion were cut and surface sterilized in 0.25% sodium hypochlorite solution for 1 – 3 minutes depending the thickness of the material. Surface sterilized pieces were washed thrice with sterile distilled water and dried on sterilized blotter sheets and then plated on potato dextrose agar (PDA) plates. Plates were incubated in an incubator (Therm, Kuhner, Switzerland) with 12 hours light and 12 hours dark period at 23 ±2 $^{0}$C. Cultures were examined and purified.

**Table 5. Species of *Colletotrichum* and their isolates obtained from the Indian Type Culture Collection (ITCC).**

| Species | ITCC number | Host Plant | Location |
|---|---|---|---|
| *C. capsici* | 4764 | Lal mirich | New Delhi |
| | 4871 | Gomphrena | Sikkim |
| | 5008 | Melia leaf | New Delhi |
| | 5107 | Bottle brush | New Delhi |
| | 5227 | Lemon leaf | New Delhi |
| *C. dematium* | 4970 | *Passiflora foetida* | Culcatta |
| | 5306 | Ashwagandha leaf | Lucknow |
| *C. falcatum* | 4800 | Sugar cane | Daurala |
| | 4803 | Sugar cane | Daurala |
| | 4893 | Sugarcane | Navasari |
| *C. gloeosporioides* | 4573 | Egg plant | Solan |
| | 5132 | Grape leaves | Pune |
| | 5213 | Cattleya | Sikkim |
| | 5255 | Cashew nut | Dapoli, |
| *C. lindemuthianum* | 4765 | French Bean | New Delhi |

Pure cultures of *Colletotrichum* were obtained by sub-culturing from the advancing edge of the colony and transferred to PDA tubes as well as in perti-dishes. Another set of cultures (on PDA) was maintained under sterile distilled water. Spore suspensions of the fungal isolates were made in sterilized distilled water and a loopful of spore suspension was streaked on PDA plate for obtaining single spore colonies. These plates were observed under the light microscope to locate a single germinating spore. An agar piece containing the spore was removed with the help of cork-borer and placed in the centre of fresh PDA plates.

**Table 6. Species of *Colletotrichum* and their isolates collected from fields.**

| Location (from India) | Host plant | Number (ITCC) | Species |
|---|---|---|---|
| Pantnagar | Sugar cane | 20 (6085-05) | *C. falcatum* |
| | Sugarcane | 23 (6086-05) | *C. falcatum* |
| | Soybean | CdP (6088-05) | *C. dematium* |
| Karnal, Haryana | Sugarcane | CfK (6087-05) | *C. falcatum* |
| Solan, Himachal Pradesh | Bell Pepper | BP1 (6082-05) | *C. gloeosporioides* |
| | Bell Pepper | BP2 (6083-05) | *C. gloeosporioides* |
| | Bell Pepper | BP3 (6084-05) | *C. gloeosporioides* |
| | Bean | SB1 (6089-05) | *C. lindemuthianum* |
| | Bean | SB2 (6090-05) | *C. lindemuthianum* |
| | Bean | SB3 (6091-05) | *C. lindemuthianum* |

CdP- *Colletotrichum dematium* from Pantnagar, CfK- *C. falcatum* from Karnal, BP1- bell pepper isolate 1, BP2- bellpepper isolate 2, BP3- bellpepper isolate 3, SB1- Solan bean isolate 1, SB2- Solan bean isolate 2, SB3- Solan bean isolate 3.

The single spore culture obtained was used for extracting total genomic DNA and for other morphological studies. A duplicate set of single spore culture was also maintained. To prevent bacterial growth and contamination, streptomycin sulphate was added into the medium at the rate of 50 µg/ml.

### 3.2. Growth rate assay

PDA and Modified Mathur's Medium (MMM) as suggested by Tu, (1985) were used for growth rate assay. The following composition of the MMM was used:-

yeast extract 1.0 g, bacto peptone 1.0 g, sucrose 10.0 g, $MgSO_4.7H_2O$ 250 mg, $KH_2PO_4$ 270 mg, agar 12.0 g, distilled water 1.0 l and amphicillin 25 mg.

The plates containing the media was inoculated in the center with 0.5 cm diameter agar disc of the actively growing edge of the fungal colony and were kept for incubation at 23 ±2 $^0$C. These plates were observed every alternate day to record the colony diameter until the colony covered the whole 9 cm diameter of the plate. This experiment was carried out in three replications.

For the preparation of liquid medium (broth) agar was not added to the medium. The flasks containing media were sterilized at 121$^0$C for 30 minutes. Fifteen ml of the medium was transferred to sterilized petriplates under aseptic conditions in triplicate and kept upside down after solidification of the medium to facilitate evaporation and to prevent condensation of water vapour on the lid of petri dish and medium.

## 3.3. Colony Characteristics

The colour of colony, its texture, growth characteristics and outline were recorded for each isolate. The colour of colonies was assessed by matching with the Roberts Ridgeway's "Colour Standards and Colour Nomenclature".

## 3.4. Morphotaxonomic Characters

For morphotaxonomic studies a small portion of the soprulating mycelium was mounted in a drop of glycerol-cotton blue with the help of inoculating needle and covered with a cover slip. The slide was luke-warmed over a spirit lamp flame to expel the air bubbles and to fix the specimen. Edges of the cover slip were sealed with colourless nail polish to prevent drying of the specimen. These slides were examined under light microscope to study the shape and size of conidia, setae, appressoria, sclerotia and chlamydospores of the fungus. Camera Lucida drawings of the same were made. The dimensions were determined by ocular micrometer after calibrating with stage micrometer. At least 25 measurements per isolate were taken and the mean, standard deviations were also calculated for each isolate. Microphotographs of the fungi were taken with the help of an Olympus SC 35 Type 12 camera attached to the Olympus BS 50 stereomicroscope. The isolates were identified up to species level. Identified cultures were deposited in the ITCC, New Delhi.

### 3.5. Pathogenicity Test

Pathogenicity tests of the fungus were carried out (i) on young seedlings grown in between wet blotting papers or cotton wool (ii) on stem cuttings, fruits after making a fissure on the stem or fruits and inoculating with mycelium along with small piece of agar.

In chilli, the fruits were surface sterilized with 0.25% sodium hypochlorite solution for 5 minutes and washed with sterile distilled water to remove excess hyphochlorite. A small cut was made with a sterile needle on the fruit surface and a small piece of agar with mycelium was inserted in to subsurface. Control fruits were kept uninoculated. Both the inoculated and control fruits were kept in moist polythene bags at 23 ±2°C until the symptoms of the disease developed. Similarly brinjal fruits were also inoculated with *C. gloeosporioides* cultures. Brinjal seedlings grown in plastic pots were sprayed with spore suspension of the fungus and covered with wet polythene bags for two weeks to see the effect. The bags were sprayed with water to avoid dryness.

In sugar cane, cuttings with 3 – 4 nodes were surface sterilized with 70% alcohol and 1 cm diameter hole was made on the cuttings just above the node up to the pith with a sterile cork-borer. An agar piece with actively growing mycelium of the fungus of same diameter was inserted through the hole into the pith region. This was covered with the same piece of bark removed while cutting. The wound was covered with a Para film to prevent the growth of the saprophytes. Wet pieces of cotton wool were placed on both ends of the cane cutting and covered with Para film to prevent its drying. In case of control, the same method was used without using the inoculum. Sugarcane pieces were kept in labeled polythene bags and incubated for two weeks in BOD incubator at 23±2$^0$C. As soon as the cuttings rooted, they were transplanted in to clay pots containing loam soil. The pots were watered thrice a week. These cuttings were uprooted when they showed wilting symptoms and were cleaned, by washing with water to remove soil and split opened to observe the rate of growth of infection in the pith. Reisolation of the fungus was carried out to confirm pathogenicity of the isolate.

In case of lemon, the leaves were inoculated with spore suspension ($10^6$ spores/ml) of the fungus after damaging the cuticle layer with the help of needle for testing pathogenicity. The leaves were washed with sterile distilled water and drops of spore suspension was placed on scratched leaf surface. These leaves were covered with wet polythene bags and incubated at room temperature. The bags were wetted daily to maintain the relative humidity until symptoms appeared.

Bean pods were surface sterilized with 0.25% sodium hypochlorite solution for 5 minutes and washed with sterile water. Spore suspension ($10^6$ spores/ml) drops of *C. lindemuthianum* were applied at several places on the pod after making slight wounds with the help of sterile needle. Inoculated pods were kept in moist polythene bags after drying the pods in the open. Proper control was maintained in sterile distilled water without inoculations.

Germinating soybean seedlings were placed on wet cotton wool in a petri plate and a piece of agar with mycelium of *C. dematium* was placed in contact with them. Seedlings were observed for symptoms.

## 3.6. Random Amplified Polymorphic DNA Analysis

### 3.6.1. Isolation of DNA

One hundred ml of modified Mathur's medium (MMM) in 250 ml flasks were inoculated with five, 0.5cm diameter agar disks with each isolate of *Colletotrichum* and incubated at 23 ±2 $^0$C in BOD incubator for one week. Mycelial growth was harvested on Whatman filter paper discs (No. 1) under sterile conditions by filtration using steam-sterilized funnels. Excess moisture was dried with sterile blotting paper and the mycelium was wrapped in sterile aluminium foil. These were stored in a deep freezer at –20$^0$C until used for DNA extraction.

The frozen mycelium was ground to a fine powder in a sterile pre-chilled pestle and mortar using liquid nitrogen. DNA extraction was carried out by cetyltrimethylamoniumbromide (CTAB) method as described by Sambrook, *et al.*, (1982) with modifications. The powder was transferred into a sterile centrifuge tube and 10 ml of pre-warmed at (65 $^0$C) total nucleic acid extraction buffer (100 mM Tris HCl (pH 8.0), 1.4 M NaCl, 50 mM EDTA (pH 8.0) and 2% CTAB) was added, mixed well and incubated in a water-bath at 65 $^0$C by gentle stirring for an hour. An equal volume of chloroform: isoamyl alcohol (24:1) was mixed gently to denature proteins, for about 10 minutes and centrifuged at 10,000 rpm in room temperature for 20 minutes. The aqueous phase was transferred to a freshly sterilized tube and the total nucleic acid was precipitated with 0.6 volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2) with gentle mixing for 5 minutes at room temperature. The nucleic acid was pelleted by centrifuging tubes at 10000 rpm for 10 minutes at 25$^0$C. The supernatant was decanted and the pellet was washed with 70% ethanol by shaking gently and centrifuging at 8000 rpm for 5 minutes to remove the salts. After decantation of ethanol, the pellet was dried at room temperature and dissolved in 100-200 µl of sterile distilled water depending upon the size of the pellet. Dissolved nucleic acid was transferred into 1.5 ml sterile eppendorf tubes and stored at –20 $^0$C for further use.

### 3.6.2. Purification of DNA

A stock solution of Ribonuclease-A (RNase-A) (10 mg/ml in 10 mM Tris-HCl pH 8.0 and 15 mM sodium chloride) was prepared, boiled for 10 minutes to destroy Deoxyribonuclease (DNase) and allowed to cool slowly at room temperature. Two to 4µl of RNase A stock solution was added into each eppendorf tube depending upon the volume of extract and was incubated at 37$^0$C for one hour to destroy RNA to get pure DNA. The concentration and the purity of DNA were assessed by measuring the ultra violet absorbance at 260 nm and 280 nm in a spectrophotometer.

### 3.6.3. Spectrophotometric Measurements

Spectrophotometer (Model UVICON 931 Spectrophotometer, Kontron Instruments, Italy) was calibrated at 260 and 280 nm using sterile distilled water in curvettes. Two µl of DNA was properly mixed with 1 ml of sterile distilled water and optical density (OD) at both 260 and 280 were recorded. The concentration of the DNA was estimated by employing the following formula :

$$\text{Amount of DNA } (\mu g/\mu l) = \frac{(OD_{260} \times 50 \times \text{dilution factor})}{1000}$$

The quality of the DNA was judged from the ratio of OD values obtained at 260 and 280. A value of OD260/OD280 ratio, around 1.9 (1.85 – 1.95) indicated best quality DNA. Part of the isolated DNA was diluted with sterile double distilled water (SDW) to a final concentration of 100ng/ µl (working solution). This was used for standardization and for further PCR. These solutions were stored at $-20^0$C until further use.

3.6.4. Optimization of Polymerase Chain Reaction

Polymerase Chain Reaction was performed using short cycling parameters based on Williams *et al.*, (1990). Random primers having double stranded 10 mer oligonucleotide sequences in kits of 20 sequences supplied as lyophilized salts (Operon Technologies, Inc., USA) were used in PCR. The powder was suspended in 1 ml of Tris-EDTA buffer (pH 8.0) to give working solution with a concentration of 33 µg/ml. These were sub divided into 250 µl aliquots and stored at $-20^0$C. Twenty-nine oligonucleotides from sets D and N were used as single primers for the amplification (Table 7).

Taq DNA polymerase, 10X concentrated buffer, 2.5 mM Magnesium chloride solution and 10 mM dNTP mixture were obtained from Genei India Limited.

Varied concentrations of Taq, PCR buffer, template DNA, Primer and $MgCl_2$ salt were used in optimization of PCR.

**Table 7. Primers used for RAPD analysis of *Colletotrichum* species.**

| Code | Sequence (5' to 3') | Code | Sequence (5' to 3') |
|---|---|---|---|
| OPD-01* | ACCGCGAAGG | OPN-05* | ACTGAACGCC |
| OPD-02* | GGACCCAACC | OPN-06* | GAGACGCACA |
| OPD-03 | GTCGCCGTCA | OPN-08* | ACCTCAGCTC |
| OPD-04* | TCTGGTGAGG | OPN-09 | TGCCGGCTTG |
| OPD-05* | TGAGCGGACA | OPN-10* | ACAACTGGGG |
| OPD-06* | ACCTGAACGG | OPN-11* | TCGCCGCAAA |
| OPD-07 | TTGGCACGGG | OPN-12* | CACAGACACC |
| OPD-08* | GTGTGCCCCA | OPN-13 | AGCGTCACTC |
| OPD-09 | CTCTGGAGAC | OPN-14 | TCGTGCGGGT |
| OPD-10* | GGTCTACACC | OPN-15 | CAGCGACTGT |
| OPD-11 | AGCGCCATTG | OPN-16 | AAGCGACCTG |
| OPD-13 | GGGGTGACGA | OPN-17 | CATTGGGGAG |
| OPN-01* | CTCACGTTGG | OPN-18 | GGTGAGGTCA |
| OPN-02* | ACCAGGGGCA | OPN-19 | GTCCGTACTG |
| OPN-03* | GGTACTCCCC | | |

* primers selected for analysis after preliminary screening.

### 3.6.5. Amplification Reaction

The procedure described by Williams *et al.*, (1990) with some modifications was used to carryout PCR. The reaction mixture was prepared in sterile 0.5 ml microfuge tube and its volume was adjusted with sterilized double distilled water. Twenty-three µl of the mixture were dispensed into sterile 0.2 ml PCR tubes containing 2 µl of DNA working solution. These tubes were mixed properly, and centrifuged for few seconds to bring ingredients to the bottom of the tube. The amplification reaction was performed in a Bio-RAD Gene Cycler™ (Bio-RAD, USA) thermocycler. Dispensing of solutions were performed using sterile disposable micropipette tips. Each reaction tube consisted of 2.5 µl of 1X buffer (10 mM Tris-HCl pH 8.3 and 50 mM KCl), 5 mM $MgCl_2$, 0.5 units of Taq DNA polymerase, 10 mM each of dATP, dCTP, dGTP and dTTP, 1µM of 10 mer primer and approximately 25 ng of template DNA. A control reaction was set with all ingredients other than template DNA to check for any contamination.

The reaction cycles used were initial denaturation step for 2 minutes at $94^0$C followed by one-cycle with 3 steps, denaturation at $94^0$C for 5 seconds, primer annealing at $37^0$C for 37 seconds, primer extension $72^0$C for 1 minute. Then another 35 cycles of $94^0$C for 5 seconds, $37^0$C for 15 seconds, $72^0$C for 1 minute and final extension at $72^0$C for 7 minutes. PCR reaction products were stored in $-20^0$C deep freezer until agarose gel electrophoresis.

### 3.6.6. Agarose Gel Electrophoresis and Analysis of PCR Products

Amplified products were analyzed by electrophoresis on 0.7% agarose gel containing 0.2 µg/ml of ethidium bromide in 1X TE buffer (pH 8.0) along with a standard molecular marker (λ DNA double digested with *Eco*RI and *Hind*III). The electrophoresis was carried out at constant voltage of 65V for three hours and visualized under ultraviolet transilluminator and photographed.

Each amplification product was considered as an RAPD marker. Gels were scored on the basis of the presence (1) or absence (0) of each band for all isolates. Jacquard's similarity coefficient values for each pair wise comparison between isolates were calculated (Jacquard, 1908) and a similarity coefficient matrix was constructed. This matrix was subjected to unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using average linkage procedure. All the numerical taxonomic analysis was conducted using software NTSYS-PC, version 1.80.

# RESULTS AND DISCUSSION

Taxonomy is important for sound beginning of the applied aspects of any pathogenic organism, and without having knowledge of correct identity of an organism, all studies are misleading.

The present investigations were undertaken to collect and identify *Colletotrichum* species and to study their variability by light microscopy as well as to differentiate different species by polymerase chain reaction (PCR) based random amplification of polymorphic DNA using random primers (RAPD). Results obtained are presented herein.

Five species of *Colletotrichum* i.e. *C. capsici, C. dematium, C. falcatum, C. gloeosporioides and C. lindemuthianum* comprising of five, two, three, four and one isolates respectively were obtained from Indian Type Culture Collection (ITCC). Further a total of ten isolates comprising of four species *C. dematium* (one isolate), *C. falcatum* (three isolates), *C. gloeosporioides* (three isolates) and *C. lindemuthianum* (three isolates) were collected from the fields. Two isolates of *C. falcatum* and one isolate of *C. dematium* from Pantnagar, Uttranchal, one of *C. falcatum* isolate from Karnal, Haryana and three *C. gloeosporioides* and three *C. lindemuthianum* isolates from Solan, Uttranchal during April 2003 to July 2004. These isolates were identified up to the species level.

These *Colletotrichum* species were identified based on the characters of their fruiting bodies, size and shape of conidia, appressoria and setae. Identified cultures were deposited in the ITCC, New Delhi. Detailed morphotaxonomical descriptions of the five species is as under:

## 4.1 Growth Rate

Growth rate of different isolates are given in the Table 8a and 8b. Isolates were found to be fast growing on PDA than on modified Mathur's medium (MMM) (Tu, 1985). The growth was significant at 5% level (CD= 0.3166). *C. lindemuthianum* isolates grew faster on PDA. Sporulation of all isolates were poor in MMM irrespective of their geographical location and host plants. Further, the thickness of colony mat was also found lower in MMM. Although Tu, (1985) obtained good growth and sporulation of *C. lindemuthianum* in MMM, results obtained clearly revealed that PDA is superior to MMM for the growth of different species.

## 4.2 Colony Characteristics

The colony colour of each isolate was found to be dependant upon the colony itself (Table 9). The colour of *C. capsici* isolates varied from whitish cream to blackish gray. The colonies of *C. capsici* on PDA appeared as thin mat of mycelium with out aerial growth. The colonies of the isolate No. 5008 (ITCC) appeared as granular due to the production of large number of acervuli in the culture. Its sporulation also was found to be very high. Colonies of isolate No. 5227 (ITCC) were thin but showed radial growth pattern and also sporulated abundantly. All *C. capsici* isolates didn't produce sclerotia in the culture.

Colonies of *C. dematium* were cream buff to light gray olive in colour and appeared as loose to compact mat of mycelium with little aerial growth (Table 9). Colonies of isolate CdP (ITCC No. 6088-05) were darker in colour and with no aerial growth. Isolate CdP sporulated profusely, produced appressoria abundantly and produced sclerotia in concentric rings. These sclerotia readily germinated when plated on fresh medium. Colonies of isolate No. 5306 (ITCC) were thick and loose, with little aerial growth and its sporulation was found to be moderate but produced few appressoria in culture (Table 9).

*C. falcatum* colonies were creamy gray to black brown in colour and appeared as compact mat of mycelium without aerial growth. Generally sporulation in *C. falcatum* isolates was found to be poor and they didn't produce acervuli in culture (Table 9). Colonies of isolate No 20 (ITCC No. 6085-05) and appeared as thin leathery mat of mycelium, creamy gray in colour and produced both appressoria and conidia moderately. Isolate CfK (ITCC No. 6087-05) produced gray colonies with thin mat of mycelium, without aerial growth and its sporulation and appressoria production was found to be moderate. Isolate No. 23 (ITCC No. 6086-05) produced smoky gray colonies with thin leathery mat of mycelium with radial growth and this isolate produced few conidia as well as appressoria.

*Colletotrichum gloeosporioides* colonies were sparse to wooly with little aerial growth and whitish cream to gray in colour (Table 9). Isolate No. 4573 (ITCC) produced whitish cream to neutral gray colonies with thin mat of mycelium with little aerial growth and sporulated profusely but production of appressoria was moderate. In isolate No. 5132, 5213, 5255 (ITCC) sporulation was moderate while appressoria production was low. Colonies of isolate BP1 (ITCC No. 6082-05) were whitish gray to dark purplish gray, wooly with little aerial growth. This isolate produced very less conidia while sclerotia and appressoria production were found to be abundant. Isolate BP2 (ITCC No. 6083-05) also produced whitish gray to dark purplish colonies but sparse colonies were submerged with coarse granular appearance due to the production of sclerotia. This isolate produced conidia, appresoria and sclerotia moderately. As in the case of other two bell pepper isolates of *C. gloeosporioides,* isolate BP3 (ITCC No. 6084-05) also produced whitish gray to dark purplish gray colonies but consisted of loose mat of mycelium with some aerial growth, appressoria and conidia production was found to be moderate while produced less sclerotia.

Colonies of *C. lindemuthianum* were whitish gray to deep olive gray in colour, sparse to wooly with some aerial growth and none of the isolates produced sclerotia (Table 9). Sporulation of all 4 isolates (ITCC No. 4765, 6089-05 (SB1), 6090-05 (SB2), 6091-05 (SB3) were found to be moderate. In isolates SB1 – SB3 (ITCC No. 6089-05, 6090-05, 6091-05) appressoria production was found to be moderate while isolate No. 4765 (ITCC) produced appressoria abundantly in culture and its colonies were whitish gray, sparse to wooly with little aerial growth.

### 4.3 Pathogenicity Test

Species of *Colletotrichum* are able to infect wide range of plant species. Therefore, to study the pathogenicity of the isolates of *Colletotrichum capsici, C. dematium, C. falcatum, C. gloeosporioides* and *C. lindemuthianum* under laboratory conditions, an experiment was designed to evaluate the commonly available crops, using bean (*Phaseolus vulgaris* L.), brinjal (*Solanum melongena* L.), chilli (*Capsicum annum* L.), soybean (*Glycine soja* L.) and the host plants from which the particular isolate was obtained. The results of pathogenicity tests obtained for different *Colletotrichum* species are given below.

**Table 8a: Growth rate (cm/day) of *Colletotrichum* isolates obtained from ITCC on PDA and Modified Mathur's Medium (MMM).**

| Species | *C. capsici* | | | | | *C. dematium* | | *C. falcatum* | | | *C. gloeosporioides* | | | | *C. lindemuthianum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate No. | 4764 | 4871 | 5008 | 5107 | 5227 | 4970 | 5306 | 4800 | 4803 | 4893 | 4573 | 5132 | 5213 | 5255 | 4765 |
| PDA* | 1.05 | 1.10 | 0.91 | 1.0 | 1.01 | 0.89 | 1.1 | 1.23 | 1.13 | 1.13 | 0.87 | 1.01 | 105 | 1.38 | 1.01 |
| MMM | 0.61 | 0.71 | 0.58 | 0.74 | 0.68 | 0.65 | 0.67 | 0.61 | 0.71 | 0.64 | 0.70 | 0.74 | 0.82 | 0.79 | 0.74 |

* growth rate on PDA significant at 5% level, CD=0.3166

**Table 8b: Growth rate (cm/day) of *Colletotrichum* isolates collected from the field on PDA and Modified Mathur's Medium (MMM).**

| Species | *C. dematium* | *C. falcatum* | | | *C. gloeosporioides* | | | *C. lindemuthianum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Isolate No. | CdP | 20 | 23 | CfK | BP1 | BP2 | BP3 | SB1 | SB2 | SB3 |
| PDA * | 0.76 | 0.96 | 1.13 | 1.05 | 1.05 | 0.64 | 1.00 | 0.91 | 0.82 | 0.93 |
| MMM | 0.69 | 0.95 | 0.82 | 0.57 | 0.90 | 0.50 | 0.89 | 0.90 | 0.88 | 0.90 |

growth rate significant at 5% level. CD= 0.3116

**ITCC No. of isolates:** CdP- 6088-05, 20- 6085-05, 23- 6086-05, CfK- 6087-05, BP1- 6082-05, BP2- 6083-05, BP3- 6084-05, SB1- 6089-05, SB2- 6090-05, SB3- 6091-05.

**Host plants of isolates:** 4764- lal mirich, 4871- *gomphrena*, 5008- *Melia* leaf, 5107- bottle brush, 5227- lemon leaf, 4970- *Passiflora foetida*, 5306- asheagandha leaf, 4800, 4803 and 4893- sugarcane, 4573- egg plant, 5132- grape leaves, 5213- cattleya, 5255- cashew nut, 4765- French bean, CdP- soybean, 20, 23 and CfK- sugarcane, BP1, BP2 and BP3- bell pepper, SB1, SB2 and SB3- French bean.

**Table 9. Colony colour, appearance, sporulation and production of appressoria and sclerotia of *Colletotrichum* isolates**

| Species/Isolate | Colony colour on PDA | Appearance on PDA | Sporulation | Appressoria | Sclerotia |
|---|---|---|---|---|---|
| ***C. capsici*** | **Whitish cream – Blackish gray** | **Thin mat, no aerial growth** | | | |
| 4764 | Carbon gray – Gray | Thin mat, no aerial growth | +++ | +++ | - |
| 4871 | Whitish cream | Thin mat, no aerial growth | ++ | ++ | - |
| 5008 | Gray | Thin mat with granular appearance | +++ | + | - |
| 5107 | Cream – Light brown | Thin mat, no aerial growth | ++ | +++ | - |
| 5227 | Gray – Light brown | Thin mat, radial growth | +++ | ++ | - |
| ***C. dematium*** | **Cream buff – Light gray olive** | **Loose- compact mat, little aerial growth** | | | |
| 4970 | Cream – Light brown | Compact mat, no aerial growth | ++ | ++ | - |
| 5306 | Light gray olive | Thick loose mat, little aerial growth | ++ | + | - |
| 6088-05 (CdP) | Olive gray – Dark gray | Thin mat, no aerial growth | +++ | +++ | +++ |
| ***C. falcatum*** | **Creamy gray - Black brown** | **Compact mat, no aerial growth** | | | |
| 4800 | Creamy gray – Black brown | Thin mat, no aerial growth | + | ++ | - |
| 4803 | Gray | Compact mat, no aerial growth | + | + | - |
| 4893 | Gray | Thin mat, no aerial growth | + | + | - |
| 6085-05 (20) | Creamy gray | Thin leathery mat, no aerial growth | ++ | ++ | - |
| 6086-05 (23) | Smoky gray | Thin leathery mat, radial growth | + | - | - |
| 6087-05 (CfK) | Gray | Thin mat, no aerial growth | ++ | ++ | - |
| ***C. gloeosporioides*** | **Whitish cream – Gray** | **Sparse – wooly, little aerial growth** | | | |
| 4573 | Whitish cream – Neutral gray | Thin mat, little aerial growth | +++ | ++ | - |
| 5132 | Whitish gray | Wooly sparse colony, little aerial growth | ++ | + | - |
| 5213 | Pale pinkish gray – Gray | Loose mat, little aerial growth | ++ | + | - |
| 5255 | Whitish gray | Sparse colony, little aerial growth. | ++ | + | - |
| 6082-05 (BP1) | Whitish gray – Dark purplish gray | Wooly, little aerial growth | + | +++ | +++ |
| 6083-05 (BP2) | Whitish gray – Dark purplish gray | Submerged colony, coarse granular appearance | ++ | ++ | ++ |
| 6084-05 (BP3) | Whitish gray – Dark purplish gray | Loose mat some aerial growth | ++ | ++ | + |
| ***C. lindemuthianum*** | **Whitish gray – Deep olive gray** | **Sparse – wooly, little aerial growth** | | | |
| 4765 | Whitish gray | Wooly- sparse, little aerial growth | ++ | +++ | - |
| 6089-05 (SB1) | Olive gray | Wooly, some aerial growth | ++ | ++ | - |
| 6090-05 (SB2) | Deep olive gray | Wooly, some aerial growth | ++ | ++ | - |
| 6091-05 (SB3) | Olive gray | Wooly, some aerial growth | ++ | ++ | - |

**(+) poor, (++) moderate, (+++) abundant. (Generalized character for the species are indicated in bold)**

***Colletotrichum capsici***

Surface sterilized chilli seeds were kept in between blotting papers, wetted with sterile water. After seven days, the seeds germinated and the germinated seedlings were inoculated by spraying with spore suspension ($10^6$ spores/ml) of *C. capsici* isolates (ITCC No, 4764, 4871, 5008, 5107 and 5227). Blotting papers were kept moist consistently to prevent drying of seedlings. *C. capsici* isolates 4764 and 5008 produced damping off in the inoculated seedlings seven days after inoculation and a large number of acervuli were also produced on dead seedlings of chilli (Plate 1a).

In another experiment, surface sterilized chilli fruits were inoculated with a small piece of agar containing actively growing mycelium of *C. capsici* isolates (ITCC No, 4764, 4871, 5008, 5107 and 5227). The inoculated fruits were kept in sterile polythene bags wetted with sterile water to prevent drying and incubated at 25±2 $^0$C until the symptoms developed. Characteristic anthracnose lesions were noticed on fruits inoculated with isolate No. 4764 after ten days of inoculation (Plate 1b).

The experiment was further carried out on soybean seedlings by inoculating *C. capsici* isolates by placing an agar piece with actively growing mycelium, in contact with seedlings. Infection was observed in the cotyledons, roots and stem base resulting in death of seedlings in isolate No. 5008 (ITCC) two weeks after inoculation (Plate 2a). A large number of acervuli with brownish black setae were also observed on dead seedlings three weeks after inoculation (Plate 2b).

Plate 1. Symptoms produced by *Colletotrichum capsici*

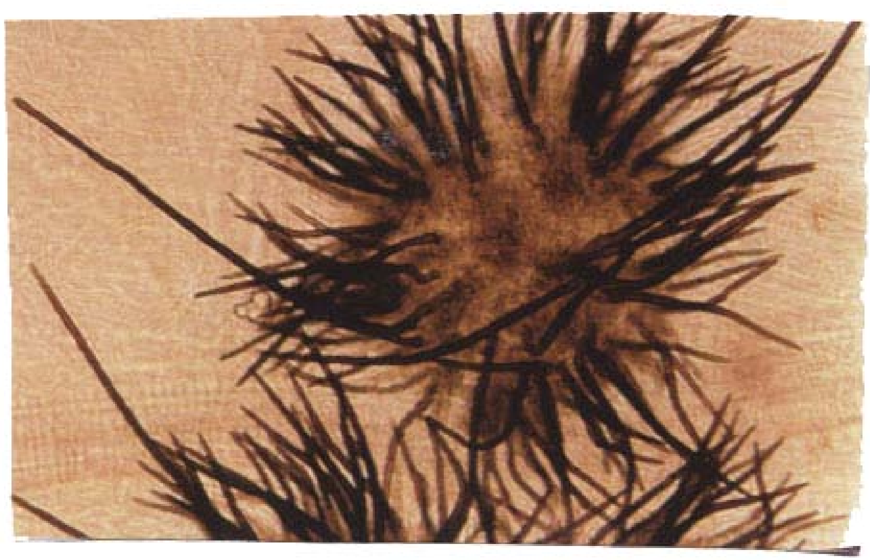
(a) Damping off symptoms on chilli seedlings (ITCC No. 4764)

1 2

(b) Anthracnose symptoms produced on chilli fruits (ITCC No. 4764). 1. inoculated, 2. control

**Plate 2. Anthracnose lesions produced by *Colletotrichum capsici***


(a) Isolate No. 5008 (ITCC) on soybean seedlings. 1. control, 2. inoculated



(b) Acervuli of isolate No. 5008 (ITCC) on soybean seedling (x 200)

No symptoms developed in French bean pods and brinjal fruits when inoculated.

Another experiment was conducted to examine the virulence of isolate No. 5227 (ITCC) on lemon leaves. Detached lemon leaves were surface sterilized with 70% alcohol and a drop of conidial suspension of *C. gloeosporioides* isolate No. 5227 (ITCC) (at concentration $10^6$ conidia /ml) was placed on the surface of the leaf. Anthracnose symptoms were observed, under humid conditions, two weeks after inoculation of the leaves.

***Colletotrichum dematium***

Different isolates of *C. dematium* (ITCC No. 4970, 5306, 6088-05 (CdP)) were inoculated as per the methods described above on various hosts viz. chilli fruits, bean pods, brinjal fruits and soybean seedlings. Inoculated pods, fruits and seedlings tested didn't produce any symptoms of anthracnose.

***C. falcatum***

Various isolates of *C. falcatum* (ITCC No. 4800, 4803, 4893, (20), 23 and 6087-05 (CfK)) were inoculated into bean pods, brinjal fruits, chilli fruits and soybean seedlings as per the method described above but none of the isolates produced disease symptoms on their respective hosts. Same number of isolates, were also inoculated into the pith region of sugarcane cuttings by making a hole with the help of cork borer and placing the actively growing mycelium of the fungus. Typical red rot disease symptoms were observed in the pith of the inoculated cuttings, and the shoots withered within three month after inoculation (Plate 3). The inoculated fungus was reisolated.

Plate 3. Red rot symptoms in sugarcane cuttings inoculated with *Colletotrichum falcatum*


from left- isolates No. 4800, 4803 (ITCC), CfK and control


top- isolate No. 23 (ITCC No. 6086-05), bottom isolate No. 20 (ITCC No. 6085-05)

### *C. gloeosporioides*

*C. gloeosporioides* isolates ITCC No. 4573, 5132, 5213, 5255, 6082-05 (BP1), 6083-05 (BP2) and 6084-05 (BP3) were inoculated into bean pods, brinjal fruits, chilli fruits and soybean seedlings as described earlier and incubated under humid conditions at 25$^0$C. All the isolates tested with above crops didn't produce any symptom even after three weeks of incubation.

Bell pepper fruits were surface sterilized with 70% alcohol and a small piece of agar with actively growing mycelium of *C. gloeosporioides* isolates Nos. 6082-05 (BP1), 6083-05 (BP2) and 6084-05 (BP3) was placed on the fruit and was incubated at 25$^0$C under high humidity (>90%). Isolate BP1 (6082-05) and BP2 (6083-05) produced typical anthracnose symptoms on the fruits two weeks after inoculation (Plate 4).

### *C. lindemuthianum*

*C. lindemuthianum* isolates (ITCC No. 4765, 6089-05 (SB1), 6090-05 (SB2) and 6091-05 (SB3)) were also inoculated into bean pods, brinjal fruits, chilli fruits and soybean seedlings as per the method described above. Anthracnose lesions were observed only in bean pods ten days after inoculation when kept at 25$^0$C under humid conditions (Plate 5). Pinkish white conidia developed in the lesions on bean pods.

In another experiment, spore suspension (10$^6$ spores/ml) of *C. lindemuthianum* isolate No. 4765 was applied into the pots having previously grown 7days old bean seedlings. No anthracnose symptoms were observed, even after one month of incubation, under glass house conditions.

Plate 4. Anthracnose lesions on bell pepper fruits inoculated with *Colletotrichum gloeosporioides*

Isolate BP1 (ITCC NO. 6082-05)

Isolate BP2 (ITCC No. 6083-05)

Plate 5. Anthracnose lesions on bean pods inoculated with *Colletotrichum lindemuthianum* isolate No. 4765 (ITCC).

## 4.4 Morphotaxonomic characters

***Colletotrichum capsici*** *(Syd.) Butler & Bisby, Imp. Counc. Agric. Res. India Sci. Monogr.* **1**: 152, 1931.

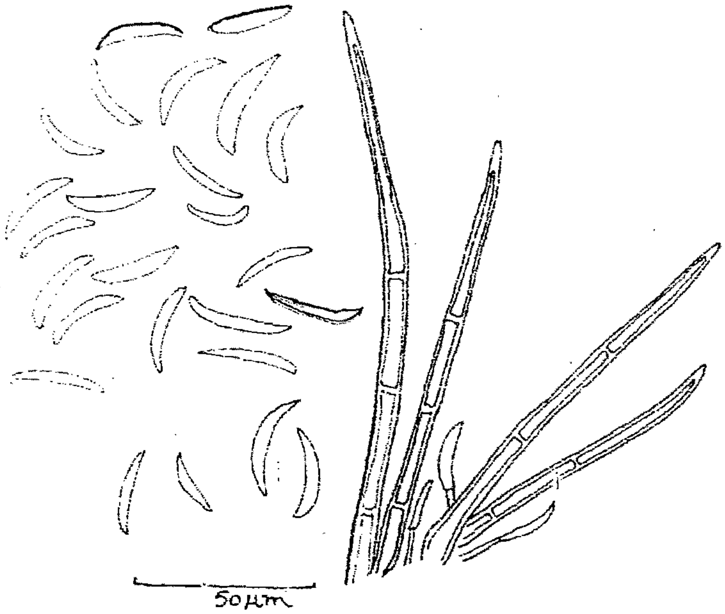
Colonies on PDA, whitish cream to blackish gray (Ridgeway plate XLVII), with thin mat of mycelium without aerial growth. Reverse side brownish gray to black. Colonies with entire or wavy margins. Hyphae, initially hyaline, turning dark at maturity. Acervuli brown to black, setae longer than conidial mass. Conidial mass gray to light brown. Conidia falcate to fusiform, apices acute, 13.41 – 31.71 x 1.22 – 6.1 µm. Appressoria clavate to ovate, margins entire, 6.10 – 18.29 x 3.66 – 12.20 µm. Appressoria sometimes become complex by forming short branched chains. Setae brown to dark brown, tips blunt to round, 2 – 4 septate, 58.93 – 144. 34 x 5.36 – 6.43 µm (Plate 6 and 7).

**Teleomorph**: not observed.

Butler and Bisby (1931) described *C. capsici* in India, which is considered as a synonym of *C. dematium* (Pres. ex Fr.) Grove. *J. Bot.* Lond. **56**: 341, 1918.

Although some differences in range of conidial dimensions 13.41 – 31.71 x 1.22 – 6.1 µm and appressorial dimensions 6.1 – 18.29 x 3.66 – 12.2 µm were observed as compared to original descriptions as given by Sutton, (1992) but no differences in other characters were observed.

**Plate 6. Camera Lucida drawings of conidia, appressoria and setae of *C. capsici***

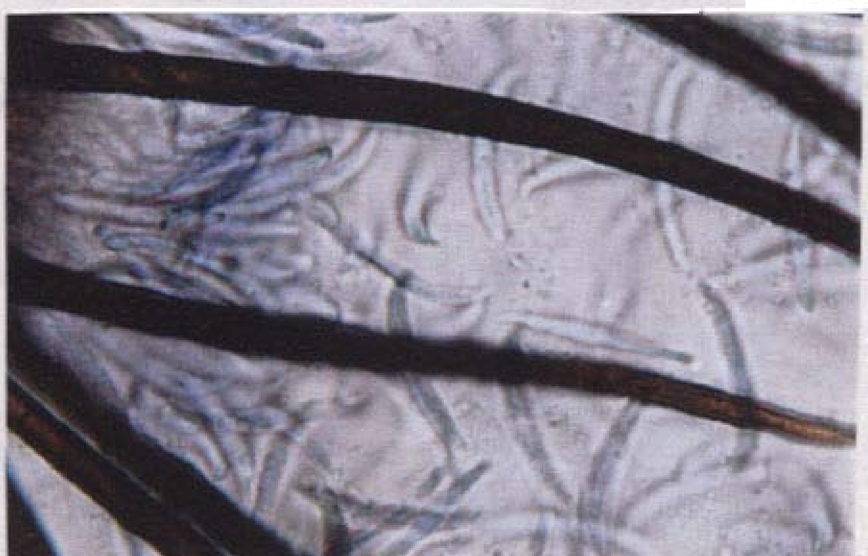



**Isolate 5008**

**Isolate 4764**

## Plate 7. Microphotograph of *Colletotrichum capsici*

(a) Acervulus with conidia and setae (x 200)



(b) Conidia and setae (x 800)

**Cultures Examined**:

ITCC Nos. 4764 on Lal mirich, New Delhi; 4871 on *Gomphrena* Sikkim; 5008 from *Melia* leaf, New Delhi; 5107 on Bottle brush, New Delhi; 5227 on Lemon leaf, New Delhi.

**Table 10. Analysis of variance for conidial size of *C. capsici* isolates grown on PDA.**

| | | Mean squares | |
|---|---|---|---|
| Source | df | Length (µm) | Width (µm) |
| Isolates | 2 | 138.951 *** | 7.00 *** |
| Error | 67 | 8.02 | 0.726 |

*** Significant at the *P*=0.01 level. CD= 0.3393 (length) CD= 0.2236 (width)

**Table 11. Analysis of variance for apperssorial size of *C. capsici* isolates grown on PDA.**

| | | Mean squares | |
|---|---|---|---|
| Source | df | Length (µm) | Width (µm) |
| Isolates | 2 | 34.764 * | 10.1004 * |
| Error | 33 | 10.085 | 2.8294 |

* Significant at the *P*=0.05 level. CD =0.1728 (length), CD= 0.1779 (width)

Isolate No. 5227 (ITCC) of *C. capsici* had radial growth and produced thinnest conidia, 17.07 – 25.61 x 1.22 – 4.88 µm, with a mean of 20.67 x 2.85 µm.

Sutton (1992) has recorded conidia, 18 – 23 x 3.5 – 4.0 µm, appressoria 9.0 – 14.0 x 6.5 – 11.5 µm. The conidial and appressorial dimensions observed by Arora *et al.*, (1990) were 18.0 – 30.0 x 3.0 – 4.5 µm and 4.5 – 23.5 x 4.0 – 10.0 µm respectively. The present study revealed that the conidial dimensions are more than those recorded by earlier workers. Analysis of variance for conidial and appressorial dimensions also significant at the *P*=0.01 and P=0.05 level respectively (Table 10 and 11).

Therefore, the description of the fungus is amended for the range of conidial and appressorial dimensions as under:

Conidia 13.41 – 31.71 x 1.22 – 6.1 µm

Appressoria 6.10 – 18.29 x 3.66 – 12.2 µm

***Colletotrichum dematium*** (Pers. ex Fr.) Grove, *J. Bot.* Lond. **56:** 341, 1918.

Colonies on PDA whitish cream to blackish gray (Ridgeway plate XLVII), with thin mat of mycelium having little or no aerial growth, and with entire or wavy margins. Hyphae initially hyaline, turning brown, at maturity. Brownish inflated short chlamydospores, produced intercalary or terminally. Acervuli single or in groups, setae numerous, blackish brown (Ridgeway plate XLV) to dark black in colour, conidial masses yellowish brown to gray. Conidia 10.98 – 32.96 x 2.38 – 6.10 µm, (average 21.97 x 4.24 µm) single celled, hyaline, falcate to fusiform, apices acute or slightly tapering. Appressoria abundant, brown, circular to clavate, edges usually entire, 7.32 – 17.07 x 6.10 – 12.20 µm (average 10.36 x 9.15 µm). Sometimes appressoria become complex and form long branched chains. Some isolates produced black, round to irregular sclerotia in culture. Setae, brown to blackish brown, tips blunt, 0 – 5 septate, 54.88 – 162.19 x 6.10 – 8.54 µm (average 86.94 x 7.32µm) (Plates 8 and 9).

**Plate 8. Camera Lucida drawing of conidia, appressoria, sclerotia and setae of *C. dematium*.**

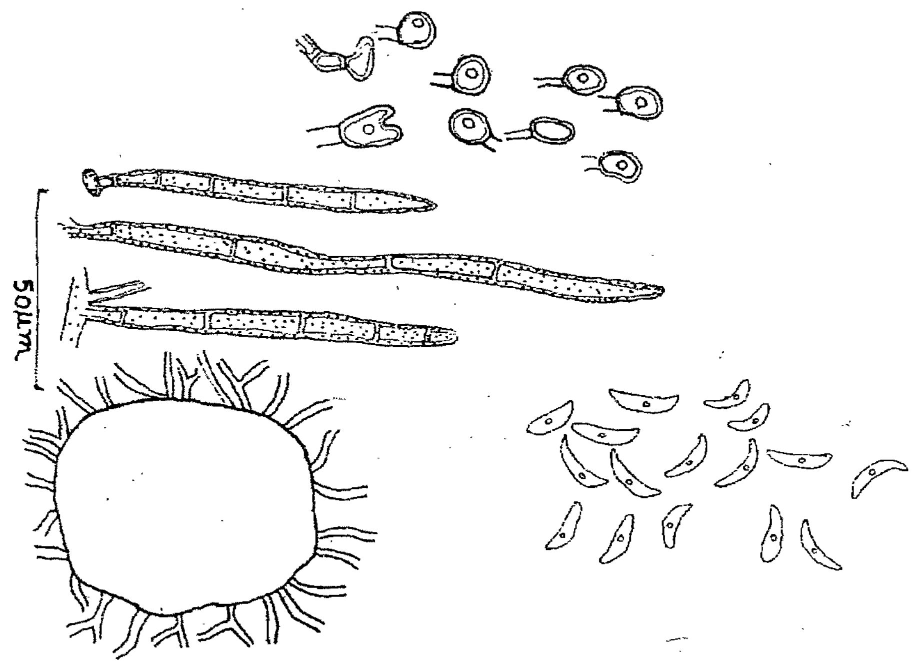
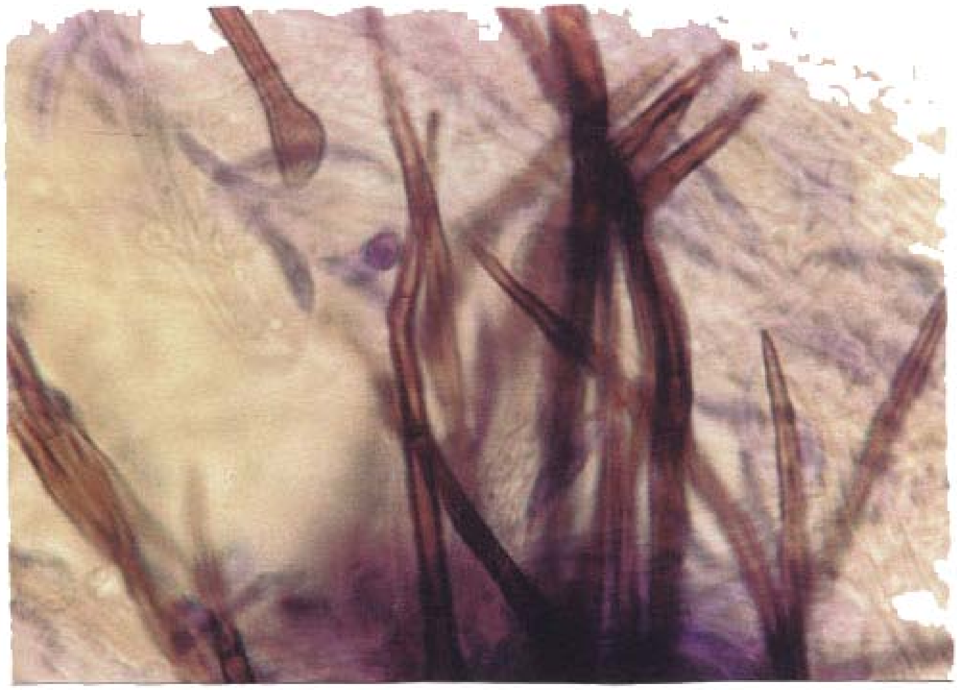
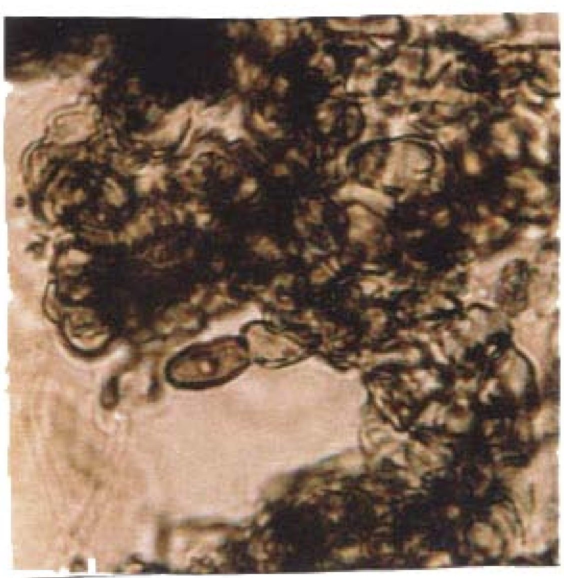


Plate 9. Microphotograph of *Colletotrichum dematium.*



(A) Setae (x 800)



(B) Appressoria (x 800)

(C) Conidia (x 800)

**Teleomorph**: not observed.

Noble and Richardson (1968) proposed the following species as synonyms of *C. dematium*:

*Colletotrichum dematium* (Pres. ex Fr.) Grove *J. Bot.* Lond. **56**: 341, 1918

=*C. capsici* (Syd.) Butler & Bisby *Imp. Counc. Agric. Res. India Sci.Monogr.* **1**: 152, 1931.

=*C. circinans* (Berk.) Voglino, *Ann. Rep. Accad. Agr.* Torino. **49**: 175, 1907.

=*C. curvatum* Briant and Martyn, *Trop. Agric.* **6**: 9, 1929.

=*C. spinaciae* Ellis & Halst., *J. Mycol.* **6**: 34, 1890.

=*C. truncatum* (Schwein.) Andrus and Moore, *Phytopatholoy.* **25**: 121, 1935.

Although some differences in conidial size, 10.98 – 32.96 x 2.38 – 6.1 µm and appressorial size, 7.32 – 17.07 x 6.10 – 12.2 µm were observed as compared to original description, however, no differences in other taxonomical characters were observed.

**Cultures examined**:

ITCC No. 4790 on *Passiflora foetida*, Calcutta and 5306 on ashwagandha leaf, Lucknow and isolate 6088-05 (CdP) on *Glycine max,* Pantnagar, Uttranchal, 30 April, 2003, Coll. H.T.R. Wijesekara.

Isolate CdP (ITCC No. 6088-05) produced black acervuli and sclerotia, which were found scattered throughout the colony. Sclerotia measured 70.93 X 53.49 µm in size, round to irregular in shape.

**Table 12. Analysis of variance for conidial size of *C. dematium* isolates grown on PDA.**

| Source | df | Mean squares | |
|---|---|---|---|
| | | Length (µm) | Width (µm) |
| Isolate | 2 | 343.681 *** | 0.561 |
| Error | 54 | 12.75 | 0.715 |

*** Significant at the *P*=0.01 level. CD= 0.4996 (length), CD=0.282 (width)

*C. dematium* is the falcate spore-bearing group of species as accepted by von Arx (1957a) but Sutton (1962) suggested synonyms with some inaccuracies. He later on distinguished, *C. dematium* from *C. capsici* in having narrower conidia and cultures, which often developed sclerotia. Kulshrestha *et al.*, (1976) reported dimensions of conidia and setae, 15.0 – 27.0 x 2.0 – 5.0 µm and 48.0 – 468.0 x 2.0 – 8.0 µm respectively. Sutton (1992) reported conidial dimensions of 18.0 – 26.0 x 2.0 – 3.0 µm. In the present study, conidial and setae dimensions ranged from 10.98 – 32.96 x 2.38 – 6.1 µm and 54.88 – 162.19 x 6.1 – 8.54 µm respectively. Statistical analysis of conidial length has shown that values obtained in this study significantly higher at P=0.01 level (Table 12). Since the results of present study revealed broader range for conidial dimensions, the description of the fungus is amended for conidia as under:

Conidia 10.98 – 32.96 x 2.0 – 6.1 µm

***Colletotrichum falcatum*** Went, *Arch. Java Suikerindustrie,* **1**: 265, 1893

Colonies formed of compact mat of mycelium, grayish white to blackish-brown (Ridgeway plate XLV) with sparse aerial growth. Reverse of the plate light brown to black. Hyphae, initially hyaline, turning brown with maturity.

Some isolates didn't produce distinct black acervuli and were without abundant sporulation. Conidia, single celled, hyaline, falcate to fusiform, apices obtuse to pointed, 9.76 – 26.83 x 2.44 – 4.88 µm. Sclerotia absent. Setae not observed. Apperssoria sparse or abundant, brown, clavate or circular with entire or irregular margins, 8.54 – 20.73 x 6.04 – 20.73 µm (Plates 10 and 11).

**Teleomorph**: not observed.

von Arx (1957a) included most of the *Colletotrichum* species described on Gramineae as synonyms of *Colletotrichum graminicola*. Noble and Richardson (1968) considered the following species as synonyms of *Colletotrichum graminicola*.

*Colletotrichum graminicola* (Ces.) Wilson. *Phytopathology,* **4**: 110, 1914

=*Colletotrichum falcatum* Went, *Arch. Java Suikerindustrie,* **1**: 265, 1893

**Cultures examined:**

ITCC isolates 4800, 4803 on sugarcane, Daurala, 4893 on sugarcane, Navasari and 6085-05 (20) on sugarcane, Pantnagar, Uttranchal. 30 April, 2003, Coll. H.T.R. Wijesekara; 6086-05 (23) on sugarcane, Pantnagar, Uttranchal. 30 April, 2003, Coll. H.T.R. Wijesekara; 6087-05 (CfK) on sugarcane, Karnal, Haryana. 3 September 2003. Coll. H.T.R. Wijesekara.

Isolate 20 did not produce acervuli and supported poor sporulation (Table 9). Appressoria were not detected in isolate 23. Black acervuli of isolate CfK were in concentric rings on PDA plates.

**Plate 10. Camera Lucida drawing of conidia and appressoria of *C. falcatum***

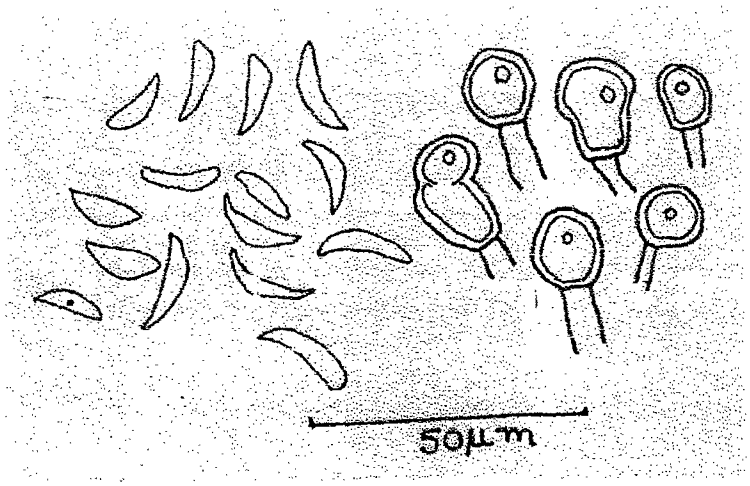
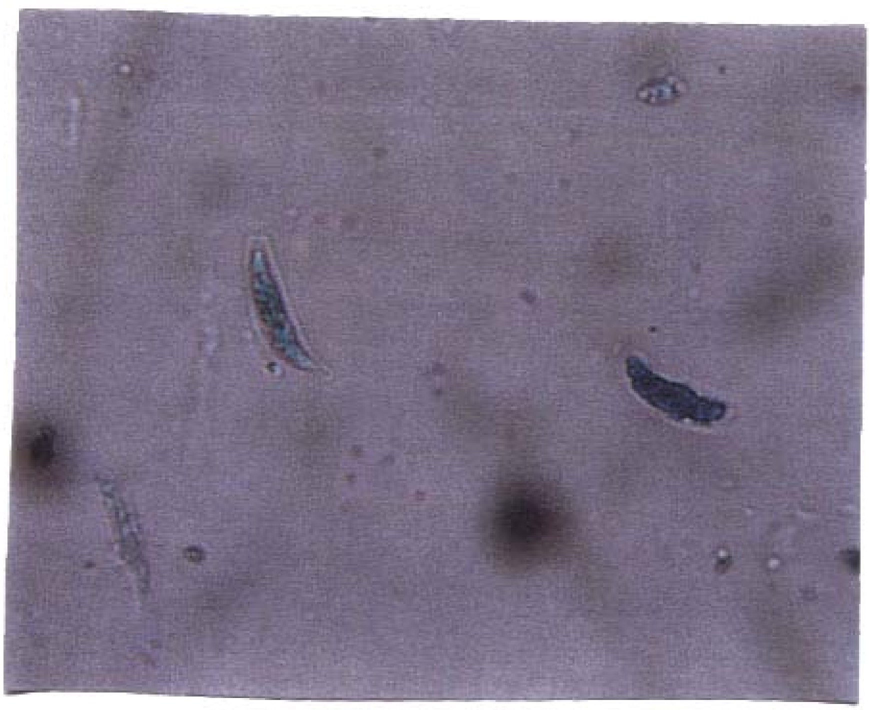


Plate 11. Microphotograph of conidia of *Colletotrichum falcatum*.



(x 800)

**Table 13. Analysis of variance for conidial size of *C. falcatum* isolates grown on PDA.**

| Source | df | Mean square | |
|---|---|---|---|
| | | Length (μm) | Width (μm) |
| Isolate | 3 | 196.119*** | 5.4812 *** |
| Error | 82 | 16.791 | 0.5714 |

*** Significant at the *P*= 0.01 level. CD= 0.2994 (length), CD= 0.2598 (width)

**Table 14. Analysis of variance for appressorial size of *C. falcatum* isolates grown on PDA**

| Source | df | Mean square | |
|---|---|---|---|
| | | Length (μm) | Width (μm) |
| Isolate | 3 | 27.489** | 33.595*** |
| Error | 32 | 7.297 | 6.173 |

*** Significant at the *P*= 0.01 level. ** Significant at the *P*= 0.025 level
CD= 0.261 (length), CD= 0.451 (width)

Kulshrestha *et al.,* (1976) have observed conidial dimensions of15.0 – 31.0 x 3.0 – 5.0 (mean 23.0 x 4.0) μm while Sutton (1992) has reported conidial and appressorial dimensions of 15.5 – 29.0 x 4 – 5 (mean 19.25 x 4.5) μm and 12.5 – 20.0 x 9.5 – 14.0 (mean 16.25 x 11.25) μm respectively. In the present study conidial and appressorial dimensions observed were, 9.76 – 26.83 x 2.44 – 4.88 (mean 18.30 x 3.66) μm and 8.54 – 20.73 x 6.04 – 20.73 (mean 14.64 x 13.39) μm respectively. This indicated that, the range for the conidial and appressorial dimensions are more than that observed by previous workers. Statistical analysis of appressorial and conidial sizes revealed that values obtained in this study are significantly different at *P*=0.01 level for conidia and at *P*=0.025 level for appressorial length and at *P*=0.01 level for appressorial width (Table 13 1nd 14).

Therefore, the limits of the dimensions of conidia and appressoria of *C. falcatum* have been expanded as below and the description of the fungus amended.

Conidia 9.76 – 26.83 x 2.44 – 5.0 µm.

Appressoria 8.54 – 20,73 x 6.04 – 20.73 µm

***Colletotrichum gloeosporioides*** (Penzig) Penzig and Sacc., *Fungi Agrum.* **2**: 6, 1882

Colonies with sparse mycelium to wooly, with little aerial growth, at first creamy white, turning to gray black with age. Colony characteristics were highly variable in nature. Conidia variable, in shape and size; i.e. single celled, hyaline, straight, cylindrical, ellipsoidal or slightly curved. Conidial apices round or obtuse with narrow truncate base. Conidia, 8.54 – 21.95 x 2.44 – 7.32 µm. Appressoria brown, clavate or irregular, sometimes becoming complex, 7.32 – 14.63 x 7.32 – 10.98 µm. Setae were not observed in any of the cultures studied. Sclerotia were observed only in isolate BP1 (Plates 12 and 13).

**Teleomorph**: not observed.

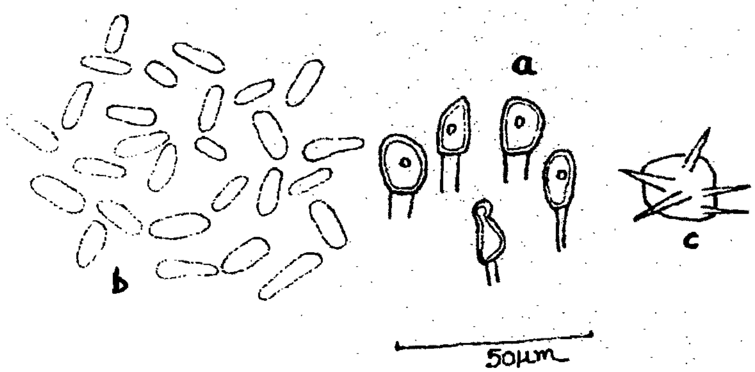
In culture *C. gloeosporioides* shows extensive variation, therefore, a large number of synonyms were proposed, by different authors. Noble and Richardson (1968) considered the following as synonyms:

*Colletotrichum gloeosporioides* (Penz.) Penzig and Sacc., *Fung. Agrum.* **2**: 6, 1882.

=*C. coffeanum* Noack, *Zeitschr. Pflanzenkr.* **11**: 202, 1901.

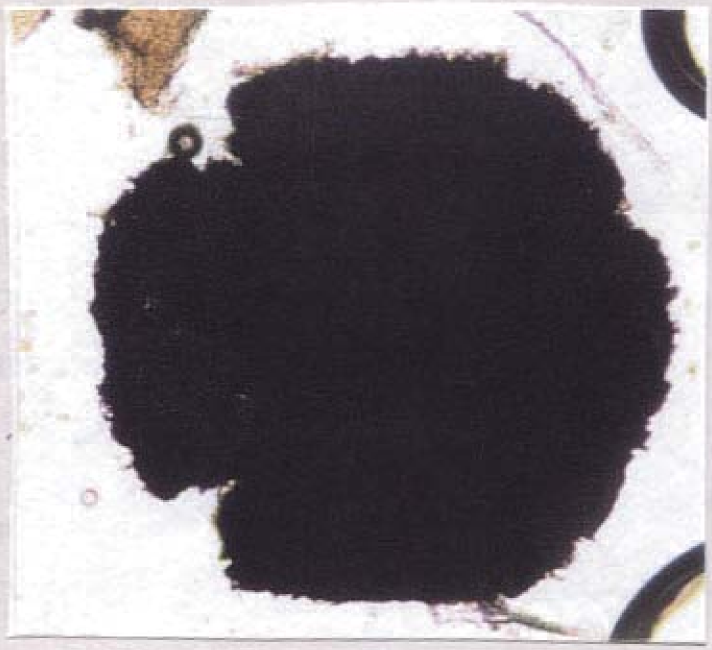
=*C. higginsianum* Sacc., *J. Agric. Res.* **10**: 161, 1917.

**Plate 12. Camera Lucida drawings of *Colletotrichum gloeosporioides*.**

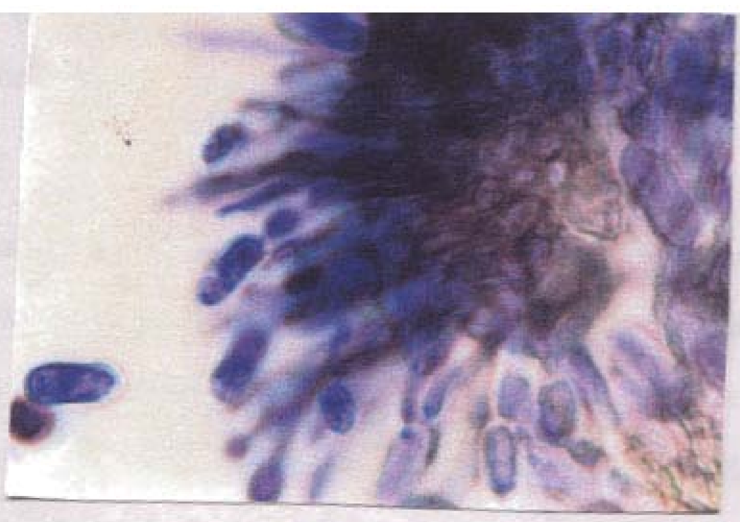



**(a) appressoria, (b) conidia, (c) sclerotium**

Plate 13. Microphotograph of *Colletotrichum gloeosporioides*



(a) Sclerotium (x 200)



(b) acervulus with conidia (x 800)

**Cultures examined**

ITCC isolates 4573 on egg plant, Solan; 5132 from grape leaves, Pune; 5213 on cattleya, Sikkim; 5255 on cashew nut, Dapoli; and 6082-05 (BP1) on *Capsicum annum* L. var. *annum*, Solan, Himachal Pradesh, India, 10 July 2004, Coll. S.K. Gupta: 6083-05 (BP2) on *Capsicum annum* L. var. *annum*, Solan, Himachal Pradesh, India, 10 July 2004, Coll. S.K. Gupta: 6084-05 (BP3) on *Capsicum annum* L. var. *annum*, Solan, Himachal Pradesh, India, 10 July 2004, Coll. H.T.R. Wijesekara:

**Table 15. Analysis of variance for conidial size of *C. gloeosporioides* isolates grown on PDA.**

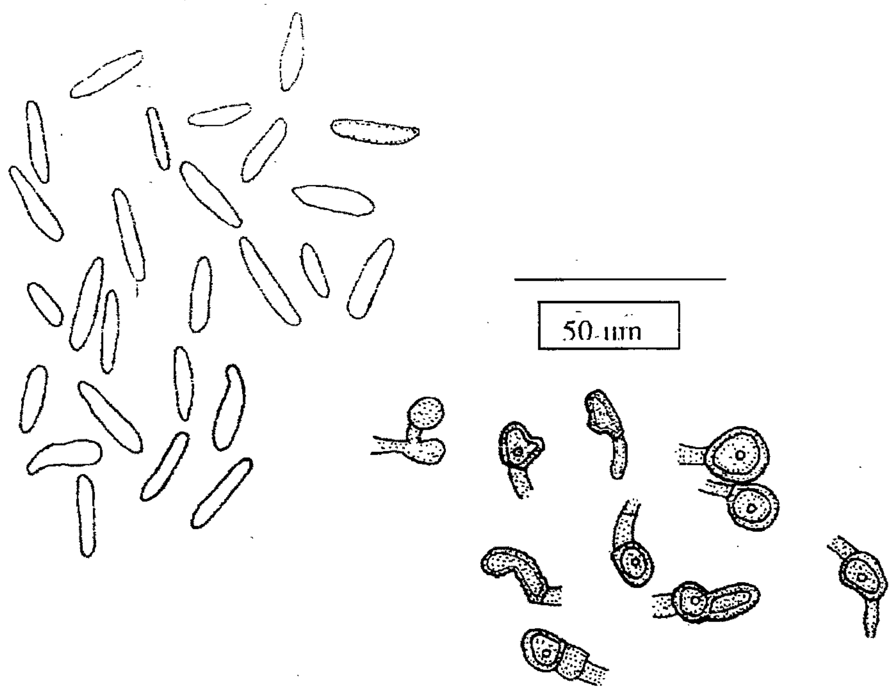
| | | Mean square | |
|---|---|---|---|
| Source | df | Length (µm) | Width (µm) |
| Isolate | 2 | 20.348* | 17.946*** |
| Error | 57 | 6.1198 | 0.7313 |

*** Significant at the *P*= 0.01 level. * Significant at the *P*= 0.05 level
CD= 0,1045 (length), CD= 0.4627 (width)

Longest conidia (Average 16.24 µm) were observed in isolate BP2. Widest conidia were observed in isolate BP3 (Average size 5.55 µm) (Plate 14).

Kulshrestha *et al.*, (1976) observed conidial measurements of *C. gloeosporioides* in the range of 6.0 – 15.0 x 3.0 – 5.0 µm, while Sutton (1992) described this range to be 12.0 – 17.0 x 3.5 – 6.0 µm. Present study revealed, that the conidial dimensions ranged from 8.54 – 21.95 x 2.44 – 7.32 µm. Statistical analysis of conidial dimensions indicated that the values obtained are statistically significant at *P*=0.05 level and *P*= 0.01 level for length and width

**Plate 14. Camera Lucida drawing of *C. gloeosporioides* isolate BP2.**




**Conidia and appressoria**

respectively (Table 15). Hence, amendment to the conidial dimensions is suggested.

Conidia 6.00 – 21.95 x 2.44 – 7.32 µm

***Colletotrichum lindemuthianum*** (Sacc. et Magn.) Bri. *et* Cav., *Fungi Parass.* 50, 1889.

Colonies whitish gray to deep olive gray. Mycelium with sparse to wooly, with some aerial growth, reverse side black in colour. Acervuli produced three months after inoculation on PDA slants. Some isolates didn't produce distinct acervuli in culture. Conidial mass whitish in colour. Conidia hyaline, single celled, straight, cylindrical, dumb-bell shaped, oblong with rounded ends, 4.88 – 24.39 x 1.22 – 7.31 µm. Appressoria sparse, pale to dark brown, clavate, circular and sometimes forming chains, 8.54 – 30.49 x 6.1 – 20.73 µm. Setae trichiform, light brown to dark brown, 113.41 – 117.07 x 4.88 – 7.32 µm, 0 – 3 septate, few per acervuli. Sclerotia were not observed (Plate 15 and 16).

**Teleomorph**: not produced.

**Cultures examined**:

ITCC isolate 4765 on French bean, New Delhi and isolates 6089-05 (SB1) on French bean pods, Solan, Himachal Pradesh, 10 July 2004, Coll. H.T.R. Wijesekara; 6090-05 (SB2) on French bean pods, Solan, Himachal Pradesh, 10 July, 2004, Coll. H.T.R. Wijesekara; 6091-05 (SB3) on French bean pods of, Solan, Himachal Pradesh, 10 July, 2004, Coll. H.T.R. Wijesekara.

**Plate 15. Camera Lucida drawings of conidia, appressoria and setae of *C. lindemuthianum*.**

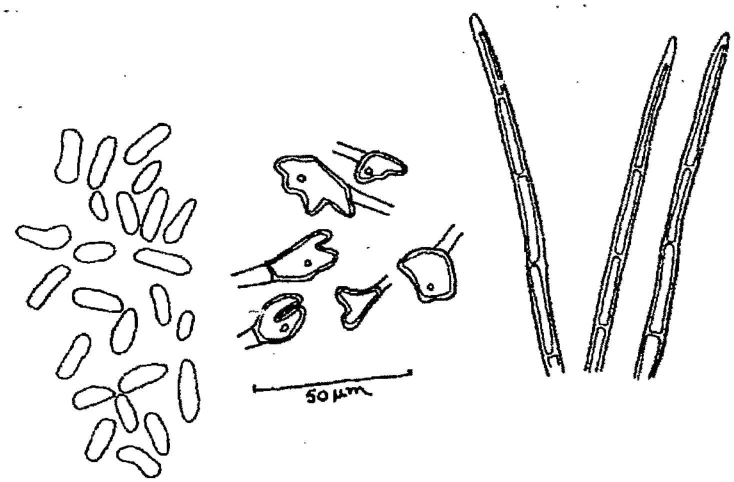
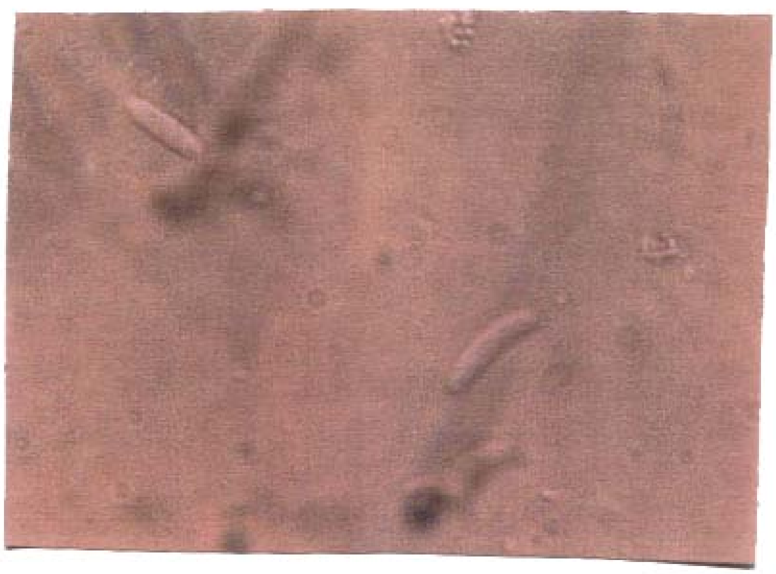


Plate 16. Microphotograph of *Colletotrichum lindemuthianum*

(A) Conidia (x800)

(B) Appressoria (x800)



(C) Conida of isolate SB3 (ITCC No. 6091-05) (x800)

Smallest conidia and appressoria, 8.54 – 17.07 x 1.22 – 4.88 µm with a mean of 11.76 x 3.61 µm and 8.54 – 18.29 x 6.1 – 12.2 µm with a mean of 11.81 x 8.65 µm respectively, were observed in isolate SB2. Dark brown intercalary and terminal chlamydospores were abundant in isolate SB3 with oblong or circular, dimensions of 8.54 – 17.07 x 8.54 – 12.2 µm.

**Table 16. Analysis of variance for conidial size of *C. lindemuthianum* isolates grown on PDA.**

| Source | df | Mean square | |
|---|---|---|---|
| | | Length (µm) | Width (µm) |
| Isolate | 3 | 57.995*** | 87.43*** |
| Error | 83 | 7.973 | 1.183 |

*** Significant at the *P*= 0.01 level. CD= 0.2082 (length), CD= 0.7276 (width).

**Table 17. Analysis of variance for appressorial size of *C. lindemuthianum* isolates grown on PDA.**

| Source | df | Mean square | |
|---|---|---|---|
| | | Length (µm) | Width (µm) |
| Isolate | 3 | 93.290 *** | 55.99*** |
| Error | 46 | 10.164 | 4.213 |

*** Significant at the *P*= 0.01 level. CD= 0.3745 (length), CD= 0.4643 (width).

Kulshrestha *et al.*, (1976) have observed conidial and setae dimensions of 9.0 – 15.0 x 3.0 – 4.0 (mean 7.0 x 3.5) µm and 60.0 – 120.0 x 2.0 – 4.0 (mean 90.0 x 3.0) µm respectively. The dimensions of conidia and appressoria reported by Sutton (1992) were 9.5 – 11.5 x 3.5 – 4.5 (mean 10.5 x 4.0) µm and 8.0 x 6.0 – 7.0 (mean 8 x 6.5) µm respectively. The values for conidia observed in the present study 4.88 – 24.39 x 1.22 – 7.31 (mean 14.64 x 4.27) µm is larger than that observed by these workers. The appressorial dimensions observed in this study 8.54 – 30.49 x 12.2 – 20.73 (mean 19.52 x 16.47) µm is also higher than that reported by Sutton (1992), and width of the setae, 4.88 –

7.32 (mean 6.10) µm is also towards the higher side as reported by Kulshrestha *et al.*, (1976). Statistical analysis of appressorial and conidial dimensions revealed that present values are significantly different at P= 0.01 level for both the structures (Table 16 and 17).

Besides these results a revision in the dimensions of conidia and appressoria are proposed and the description of the fungus is amended.

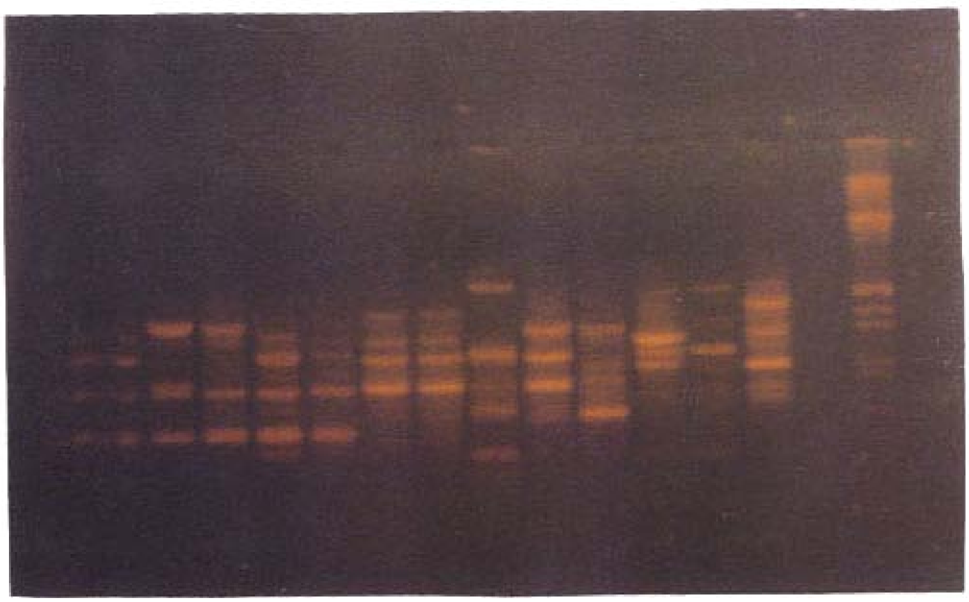
Conidia 4.88 – 24.39 x 1.22 – 7.31 µm

Appressoria 8.0 – 30.49 x 6.0 – 20.73 µm

**4.5 Molecular Analysis**

Twenty isolates of different species of *Colletotrichum* viz *C. capsici* (4 isolates), *C. dematium* (3 isolates), *C. falcatum* (6 isolates), *C. gloeosporioides* (6 isolates) and *C. lindemuthianum* (1 isolate) were chosen for this study. These isolates comprised 14 of ITCC and 6 collected from fields at different places like Pantnagar, Karnal and Solan (Table 5 & 6). Molecular diversity was assessed over the entire genome using 16 different primers from OPD and OPN series. These were selected based on the primary assessment with 14 ITCC isolates. Each primer tested produced a specific pattern displaying 1 – 12 bands per isolate. The size of the amplified products ranged from 200 bp to 2500 bp (Plate 17). For a given primer, it is assumed that fragments amplified in different isolates and displaying the same molecular weight were identical. The polymorphic bands produced correspond to RAPD markers. They are either shared by other isolates or are specific to one isolate. The results obtained are discussed below species wise. Isolates formed two main clusters with all the 16 primers tested and there were 57% similarity between these two

Plate 17. RAPD profiles of five *Colletotrichum* species obtained from ITCC with primer OPN1.



Lanes: 1-4 *C. capsici*, 5-6 *C. dematium*, 7-9 *C. falcatum*, 10-13 *C. gloeosporioides*, 14 *C. lindemuthianum*, M- 1Kb DNA ladder.

ITCC Nos. of isolates in lanes: 1- 4764, 2- 4781, 3- 5008, 4- 5107, 5- 4970, 6- 5306, 7- 4800, 8- 4803, 9- 4893, 10- 4573, 11- 5132, 12-5213, 13- 5255, 14- 4765 clusters (Fig. 1). Sugarcane isolate 20 and bell pepper isolate BP2 formed a small cluster sharing 61.76% similarity. Among the other isolates clustered together, 4 isolates (23, CfK, CdP and BP1) obtained from Pantnagar, Karnal and Solan shared 61.76% similarity with other isolates (4764, 4871, 4970, 5107, 5306, 5008, 4765, 4800, 4803, 4573, 5132, 5213, 5255 and 4893). In this sub-cluster BP1 isolate formed a separate cluster with 63% similarity to other isolates within sub cluster. *C. falcatum* isolate (CfK), and *C. dematium* isolate (CdP) shared 68.12% similarity with *C. falcatum* isolate 23. Isolates 23 from Pantnagar, CfK from Karnal, BP1 from Solan and CdP from Pantnagar clustered together with 66.04% similarity to other 14 isolates obtained from places like New Delhi, Sikkim, Calcutta, Lucknow, Durala, Navasari, Solan, Pune and Dapoli.

With all the primers tested, the falcate shaped conidia bearing species *C. capsici* and *C. dematium* formed a distinct sub cluster, with exception of *C. dematium* isolate CdP being clustered with *C. falcatum* isolates 23 and CfK. *C. falcatum* isolates 4800 and 4803 were identical in RAPD profile and grouped separately with 67.6%. Similarity. *C. gloeosporioides* isolates obtained from ITCC grouped together in a distinct sub cluster with exception that bell pepper isolates BP1 and BP2 from Solan clustered with falcate shaped conidia bearing isolates CfK and CdP and isolate 20 respectively. There was no relationship among the isolates with respect to geographical origin of the isolate.

***C. capsici***

The total numbers of bands obtained for C. capsici isolates viz. 4764, 4871, 5107, 5008 were 143 and out of these 112 were polymorphic. The number of polymorphic bands produced, per primer were 4 – 14. The primer

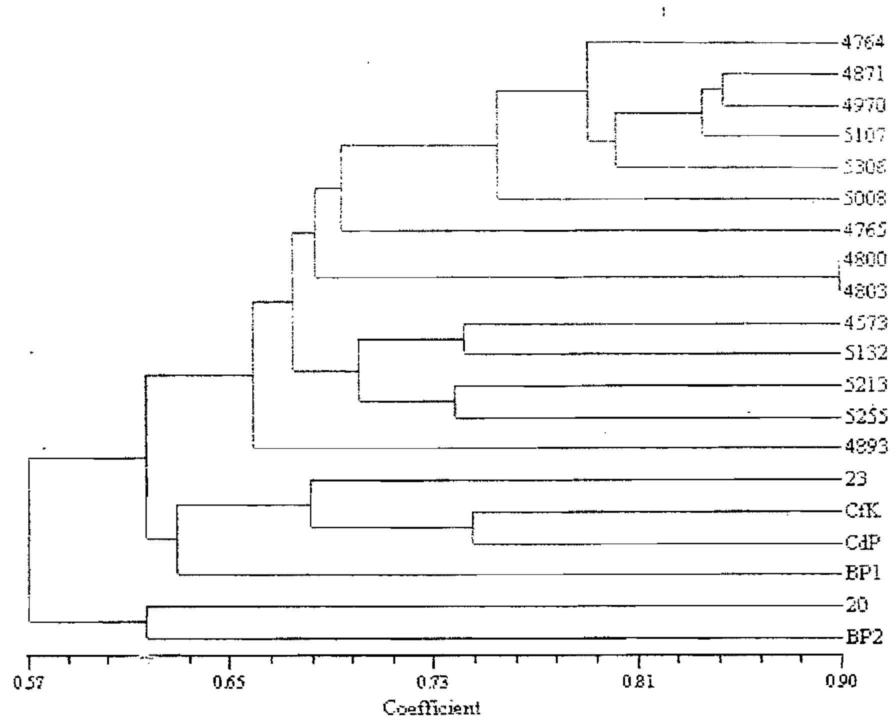



**Fig. 1. Dendrogram of combined analysis of data set obtained with 16 RAPD primers from all the *Colletotrichum* isolates. Scale indicate similarity coefficient.**

**ITCC Nos. of species:**
**4764, 4871, 5008, 5107 – *C. capsici*,**
**4970, 5306, 6088-05 (CdP) – *C. dematium*,**
**4800, 4803, 4893, 6085-05 (20), 6086-05 (23), 6087-05 (CfK) – *C. falcatum*,**
**4573, 5132, 5213, 5255, 6082-05 (BP1), 6083-05 (BP2) – *C. gloeosporioides*,**
**4765 – *C. lindemuthianum*.**

**Sources of isolates:**
**4764 – lal mirich, New Delhi,**
**5008 – *Melia* leaf, New Delhi,**
**4970 – *Passiflora foetida*, Culcatta,**
**CdP – soybean, Pantnagar,**
**4893 – sugarcane, Navasari,**
**CfK – sugarcane, Karnal,**
**5132 – grape leaves, Pune,**
**5255 – cashew nut, Dapoli,**
**4871 – *Gomphrena*, Sikkim,**
**5107 – bottlebrush, New Delhi,**
**5306 – ashwagandha leaf, Lucknow,**
**4800, 4803 – sugarcane, Daurala,**
**20, 23 – sugarcane, Pantnagar,**
**4573 – eggplant, Solan,**
**5213 – cattleya, Sikkim,**
**BP1, BP2 – bell pepper, Solan.**

OPD10 produced maximum number (14) of bands and all of them were polymorphic (Table 18). The data obtained from RAPD analysis were subjected to UPGMA analysis and a dendrogram was prepared using the similarity coefficient of RAPD marker (Fig. 1). The dendrogram showed the genetic variability of isolates tested.

*C. capsici* isolates viz. 4764, 4871, 5107, 5008 were grouped and formed a distinct cluster, apart from other species with the sixteen primers tested. Isolate 5008 from *Melia* leaves, was distantly related and shared 76.4% similarity with isolates 4764, 4871, and 5107. Isolate 4764 was separated from isolates 4871 and 5107 with 80% similarity while isolate 5107, shared 84.4% similarity with isolate 4871.

**Table 18. Polymorphic, monomorphic, total number of bands produced and percent polymorphism for *C. capsici* isolates with respect to different primers used.**

| Primer | Total bands | Polymorphic Bands | Monomorphic Bands | Percent Polymorphism |
|---|---|---|---|---|
| OPN 1 | 9 | 6 | 3 | 66.67 |
| OPN 2 | 6 | 6 | 0 | 100.00 |
| OPN 3 | 7 | 4 | 3 | 57.14 |
| OPN 5 | 5 | 5 | 0 | 100.00 |
| OPN 6 | 4 | 4 | 0 | 100.00 |
| OPN 8 | 9 | 6 | 3 | 66.67 |
| OPN 10 | 10 | 10 | 0 | 100.00 |
| OPN 11 | 11 | 7 | 4 | 63.64 |
| OPN 12 | 12 | 7 | 5 | 58.33 |
| OPD 1 | 10 | 6 | 4 | 60.00 |
| OPD 2 | 10 | 8 | 2 | 80.00 |
| OPD 4 | 6 | 6 | 0 | 100.00 |
| OPD 5 | 11 | 6 | 5 | 54.55 |
| OPD 6 | 11 | 11 | 0 | 100.00 |
| OPD 8 | 8 | 6 | 2 | 75.00 |
| OPD 10 | 14 | 14 | 0 | 100.00 |

### *C. dematium*

The total number of bands obtained for *C. dematium* isolates viz. 4970, 5306 and CdP were 152, and out of these 136 was polymorphic. The numbers of polymorphic bands, produced per primer were 5 – 13. The primers OPD5 produced the maximum number of 15 bands and 11 of them were polymorphic (Table 19). The dendrogram showed the genetic variability of isolates tested (Fig. 1).

The isolates 4970 and 5306 clustered with *C. capsici* isolates while other isolate CdP clustered with *C. falcatum* isolate CfK from Karnal. There was 80.4% relatedness between isolate 4970 and 5306 whereas isolate CdP was found to be 39.8% distant from the other two isolates 4970 and 5306 from Culcatta and Lucknow.

**Table 19. Polymorphic, monomorphic, total number of bands produced and percent polymorphism for *C. dematium* isolates with respect to different primers used.**

| Primer | Total bands | Polymorphic Bands | Monomorphic Bands | Percent Polymorphism |
|---|---|---|---|---|
| OPN 1 | 8 | 8 | 0 | 100.00 |
| OPN 2 | 6 | 6 | 0 | 100.00 |
| OPN 3 | 5 | 5 | 0 | 100.00 |
| OPN 5 | 5 | 5 | 0 | 100.00 |
| OPN 6 | 11 | 10 | 1 | 90.91 |
| OPN 8 | 9 | 9 | 0 | 100.00 |
| OPN 10 | 6 | 6 | 0 | 100.00 |
| OPN 11 | 11 | 9 | 2 | 81.82 |
| OPN 12 | 10 | 7 | 3 | 70.00 |
| OPD 1 | 14 | 13 | 1 | 92.86 |
| OPD 2 | 11 | 10 | 1 | 90.91 |
| OPD 4 | 9 | 9 | 0 | 100.00 |
| OPD 5 | 15 | 11 | 4 | 73.33 |
| OPD 6 | 11 | 10 | 1 | 90.91 |
| OPD 8 | 9 | 7 | 2 | 77.78 |
| OPD 10 | 12 | 11 | 1 | 91.67 |

### *C. falcatum*

A total of 246 bands were obtained for *C. falcatum* with the 16 primers tested (Table 7) and all of them were found to be polymorphic. The number of polymorphic bands, produced per primer was 8 – 24. The primers OPD4 produced the maximum number of 24 bands (Table 20). The dendrogram showed the genetic variability of isolates tested (Fig. 1). Isolate 4800 and 4803 were 100% related with each other, where as isolate 4893 grouped in the same main cluster with 65% genetic relatedness to former two isolates. Isolate 20 formed a separate cluster with bell pepper isolate BP2 of *C. gloeosporioides* showing only 60.2% relatedness to all other isolates.

### *C. gloeosporioides*

A total of 281 bands were obtained for *C gloeosporioides* isolates viz. 4573, 5132, 5213, 5255, BP1 and BP2 with the 16 primers tested and all of them were found to be polymorphic in nature (Table 20). The number of polymorphic bands, produced per primer was 8 – 21. The primer OPN12 produced maximum number of 21 bands (Table 20). The dendrogram showed the genetic variability of isolates tested (Fig. 1).

Isolates 4573, 5132, 5213 and 5255 formed a separate cluster with 74.43% and 74.04% relatedness among first (4573 and 5132) and second (5213 and 5255) isolate pairs respectively. Isolate BP2 was grouped with *C. falcatum* isolate 20 forming a separate sub cluster distantly relating (61.76% relatedness) to other isolates 4573, 5132, 5213, 5255 and BP1. Isolate BP1 formed a solitary cluster which is 61.76% related to the sub cluster of ITCC isolate No. 4573, 5132, 5213, 5255 and BP2. Isolate BP1had 63% similarity with *C. falcatum* isolates 23, CfK and *C. dematium* isolate CdP.

**Table 20. Polymorphic and total number of bands produced for *C. falcatum* and *C. gloeosporioides* isolates with respect to different primers used.**

| Primer | *C. falcatum** | | *C. gloeosporioides** | |
|---|---|---|---|---|
| | Total bands | Polymorphic Bands | Total bands | Polymorphic Bands |
| OPN 1 | 11 | 11 | 11 | 11 |
| OPN 2 | 12 | 12 | 10 | 10 |
| OPN 3 | 8 | 8 | 8 | 8 |
| OPN 5 | 13 | 13 | 17 | 17 |
| OPN 6 | 18 | 18 | 19 | 19 |
| OPN 8 | 10 | 10 | 13 | 13 |
| OPN 10 | 11 | 11 | 8 | 8 |
| OPN 11 | 21 | 21 | 20 | 20 |
| OPN 12 | 19 | 19 | 21 | 21 |
| OPD 1 | 21 | 21 | 17 | 17 |
| OPD 2 | 15 | 15 | 15 | 15 |
| OPD 4 | 24 | 24 | 18 | 18 |
| OPD 5 | 18 | 18 | 17 | 17 |
| OPD 6 | 15 | 15 | 16 | 16 |
| OPD 8 | 12 | 12 | 13 | 13 |
| OPD 10 | 18 | 18 | 14 | 14 |

* all bands were polymorphic

***C. lindemuthianum***

Isolate 4765 produced a total of 81 bands with 16 primers tested. It formed a solitary group in the main sub cluster with *C. capsici* isolates 4764, 4871, 5008, 5107, and *C. dematium* isolates 4970 and 5306 (Fig. 1). Isolate 4765 shared 70% similarity with above 6 isolates.

In RAPD profiles primer OPN3 gave good seperation of isolates into 4 different groups according to the conidial shape (Plate 18, Fig. 2). *C. capsici* and *C. dematium* isolates formed a distinct sub cluster, except CdP isolate on soybean from Pantnagar. The genetic variability of the isolates was indicated by the fact that no two isolates were identical. *C. gloeosporioides* isolates 4573, BP1, 5132, 5213 and 5255 formed a distinct group in the dendrogram indicating their similarity with each other (Fig. 2). But *C. gloeosporioides* isolate BP2 on bell pepper from Solan formed a distant solitary cluster (50% similarity) away from all other isolates tested. *C. falcatum* isolates 23, CfK, 20, 4800 and 4803 formed another group with the primer OPN3 (Fig. 2). Isolates 4800 and 4893 were identical and had 58.1% similarity to all other *C. falcatum* isolates. With this primer *C. lindemuthianum* isolate 4765 was grouped with *C. falcatum* isolates 4800, 4803 and 4893 with 63,3% similarity. Therefore, from the present studies it can be concluded that OPN3 primer is useful as a marker to differentiate *Colletotrichum* species like *C. falcatum* and *C. gloeosporioides.* However, species *C. capsici and C. dematium* could not be differentiated based on RAPD profile of OPN3.

The primer OPN11 gave separation of all the species except *C. capsici* and *C. dematium* but by using this primer also the *C. capsici* and *C. dematium* isolates were clustered together (Fig. 3 and Plate 19). *C. falcatum* isolates 4800 and 4803, formed separate group while isolates 23 and CfK were grouped in a separate sub cluster. Isolates 4893 and 20, formed solitary groups apart from other *C. falcatum* isolates. *C. gloeosporioides* isolates 5132, 4573 and 5255 were grouped together in the dendrogram, while isolate 5213 was grouped with *C. lindemuthianum* isolate 4765. Bell pepper isolates BP1 and

**Plate 18. RAPD profiles of five *Colletotrichum* species obtained with primer OPN3.**

**Lanes: M- 1 Kb DNA ladder, 1-4 *C. capsici*, 5-6 *C. dematium*, 7-9 *C. falcatum*, 10-13 *C. gloeosporioides*, 14 *C. lindemuthianum*, 15-17 *C. falcatum*, 18 *C. dematium*, 19-20 *C. gloeosporioides***

**ITCC Nos. of isolates in lanes: 1- 4764, 2- 4781, 3- 5008, 4- 5107, 5- 4970, 6- 5306, 7- 4800, 8- 4803, 9- 4893, 10- 4573, 11- 5132, 12-5213, 13- 5255, 14- 4765, 15- 6085-05 (20), 16- 6086-05 (23), 17- 6087-05 (CfK), 18- 6088-05 (CdP), 19- 6082-05 (BP1), 20- 6083-05 (BP2).**

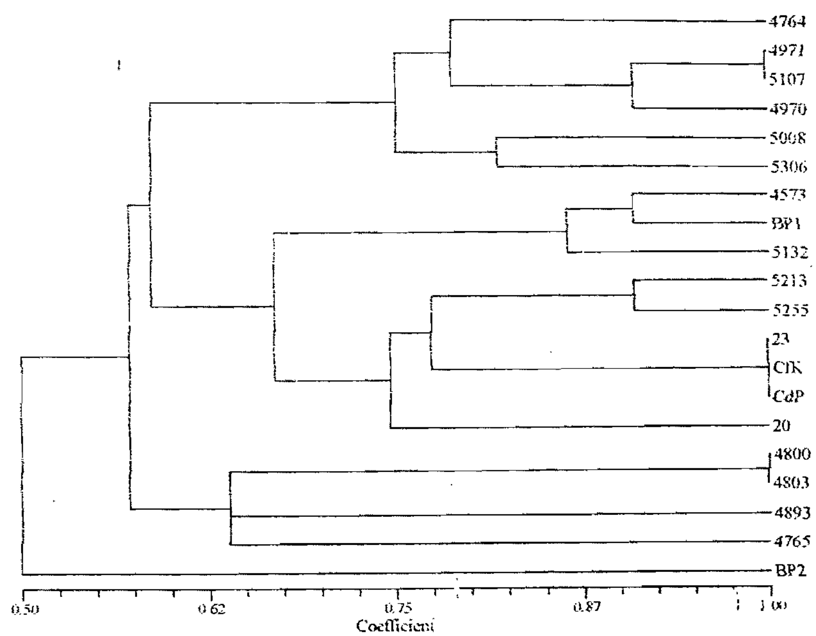
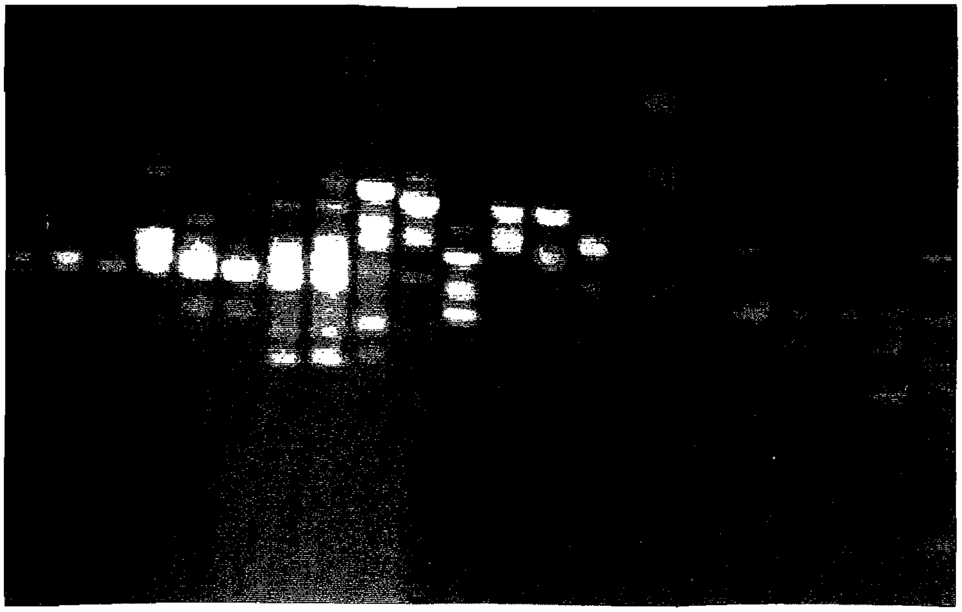



**Fig. 2. Dendrogram from RAPD analysis of *Colletotrichum* isolates with Primer OPN3. Scale is similarity coefficient.**

**ITCC Nos. of species:**
**4764, 4871, 5008, 5107 – *C. capsici.***
**4970, 5306, 6088-05 (CdP) – *C. dematium*,**
**4800, 4803, 4893, 6085-05 (20), 6086-05 (23), 6087-05 (CfK) – *C. falcatum*,**
**4573, 5132, 5213, 5255, 6082-05 (BP1), 6083-05 (BP2) – *C. gloeosporioides*,**
**4765 – *C. lindemuthianum*.**

**Sources of isolates:**
**4764 – lal mirich, New Delhi,**
**5008 – *Melia* leaf, New Delhi,**
**4970 – *Passiflora foetida*, Culcatta,**
**CdP – soybean, Pantnagar,**
**4893 – sugarcane, Navasari,**
**CfK – sugarcane, Karnal,**
**5132 – grape leaves, Pune,**
**5255 – cashew nut, Dapoli,**
**4871 – *Gomphrena*, Sikkim,**
**5107 – bottlebrush, New Delhi,**
**5306 – ashwagandha leaf, Lucknow,**
**4800, 4803 – sugarcane, Daurala,**
**20, 23 – sugarcane, Pantnagar,**
**4573 – eggplant, Solan,**
**5213 – cattleya, Sikkim,**
**BP1, BP2 – bell pepper, Solan.**

Plate 19. RAPD profiles of five *Colletotrichum* species obtained with primer OPN11.



Lanes: 1-4 *C. capsici*, 5-6 *C. dematium*, 7-9 *C. falcatum*, 10-13 *C. gloeosporioides*, 14 *C. lindemuthianum*, M- 1Kb DNA ladder, 15- *C. dematium*, 16-18 *C. falcatum*, 19-20 *C. gloeosporioides*

ITCC Nos. of isolates in lanes: 1- 4764, 2- 4781, 3- 5008, 4- 5107, 5- 4970, 6- 5306, 7- 4800, 8- 4803, 9- 4893, 10- 4573, 11- 5132, 12-5213, 13- 5255, 14- 4765, 15- CdP (ITCC No. 6088-05), 16- 20 (ITCC No. 6085-05), 17- 23 (ITCC No. 6086-05), 18- CfK (ITCC No. 6087-05), 19- BP1 (ITCC No. 6082-05), 20- BP2 (ITCC No. 6083-05)

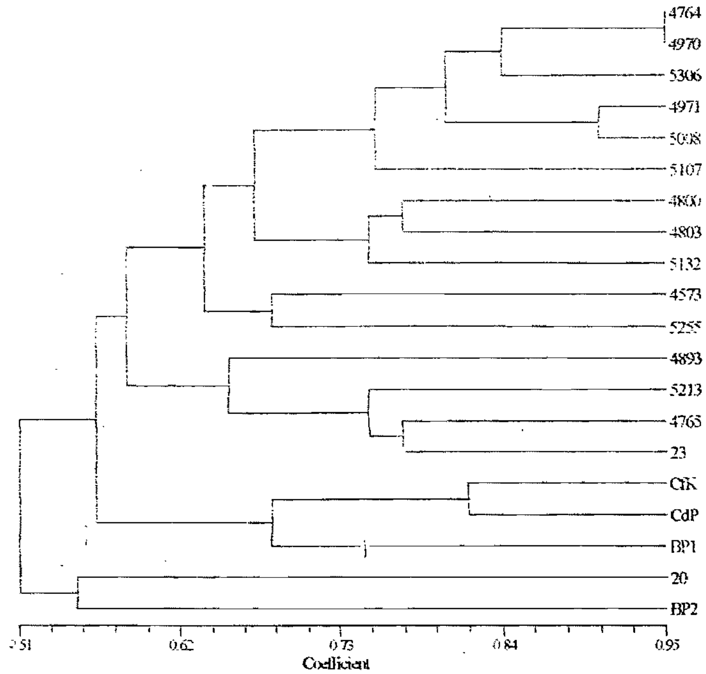



**Fig. 3. Dendrogram from RAPD analysis of *Colletotrichum* isolates with primer OPN11. Scale is the similarity coefficient.**

**ITCC Nos. of species:**
**4764, 4871, 5008, 5107 – *C. capsici.***
**4970, 5306, 6088-05 (CdP) – *C. dematium,***
**4800, 4803, 4893, 6085-05 (20), 6086-05 (23), 6087-05 (CfK) – *C. falcatum,***
**4573, 5132, 5213, 5255, 6082-05 (BP1), 6083-05 (BP2) – *C. gloeosporioides,***
**4765 – *C. lindemuthianum.***

**Sources of isolates:**
**4764 – lal mirich, New Delhi,**
**5008 – *Melia* leaf, New Delhi,**
**4970 – *Passiflora foetida*, Culcatta,**
**CdP – soybean, Pantnagar,**
**4893 – sugarcane, Navasari,**
**CfK – sugarcane, Karnal,**
**5132 – grape leaves, Pune,**
**5255 – cashew nut, Dapoli,**
**4871 – *Gomphrena*, Sikkim,**
**5107 – bottlebrush, New Delhi,**
**5306 – ashwagandha leaf, Lucknow,**
**4800, 4803 – sugarcane, Daurala,**
**20, 23 – sugarcane, Pantnagar,**
**4573 – eggplant, Solan,**
**5213 – cattleya, Sikkim,**
**BP1, BP2 – bell pepper, Solan.**

BP2 were grouped separately. Dendrogram showed that 4 main groups were formed with primer OPN11 with respect to *Colletotrichum* species (Fig. 3: Plate 19). This primer can also be used to differentiate *C. capsici* and *C. dematium* species from other *Colletotrichum* species.

Three major types of RAPD profiles were obtained among the five species of *Colletotrichum* under study, *C. capsici, C. dematium, C. falcatum, C. gloeosporioides* and *C. lindemuthianum.* Of the four isolates of *C. capsici* (4764, 4871, 5107 and 5008) none of the isolates produced same RAPD profile, this may be due to the fact that 4 species have been isolated from different host plants. Several workers (Latha *et al.,* 2002; Mathur *et al.,* 1997; Rao *et al.*, 1998) have shown that *Colletotrichum* isolates exhibit variable colony characters, but genetically similar when isolated from a single lesion (Latha *et al.,* 2002). Two characterized isolates of *C. dematium* 4790 and 5306 grouped together with *C. capsici* isolates forming a separate sub cluster with the exception that isolate CdP collected from Pantnagar on soybean grouped with *C. falcatum* isolates 23 and CfK. Kulshrestha *et al.*, (1976) and von Arx, (1957a) accepted *C. capsici* as a synonym of *C. dematium*. The results of the present study also confirmed that both species are closely related and thereby confirm the earlier results. Although, isolates 4800 and 4803 on sugarcane from Daurala were identical in RAPD profile, differed in colony characters and growth rate. Similar results were obtained by Latha *et al.*, (2002) for *C. graminicola* on sorghum. Isolate 4893 from Navasari on sugarcane formed a separate sub cluster, and it may be due to evolvement in different geographical location. Isolates 20, 23 and CfK were more distant to above three isolates and clustered with *C. gloeosporioides,* bell pepper isolates BP1, BP2 and C.

*dematium* isolate CdP, irrespective of the geographical region from which they were isolated. Two isolates 20 and 23 from Pantnagar sugarcane clustered in different sub groups and it revealed that there are several pathotypes/ variants of *C. falcatum* existing in Pantnagar area. Several workers (Latha *et al.*, 2002; Saha *et al.*, 2002) have observed similar RAPD profiles for different *Colletotrichum* species in their investigations. Although *C. gloeosporioides* isolates 4573, 5132, 5213 and 5255 obtained from different crop plants formed a distinct sub cluster, other isolates from Solan, BP1, BP2 were grouped into different sub clusters irrespective of the geographical region and host plant. Isolates BP1 and BP2 were distantly related with 61.76% similarity only although both were isolated from bell pepper in Solan area. *C. falcatum* isolates 4800 and 4803 showed dissimilarity in cultural characters, their RAPD patterns were similar. But in the case of bell pepper isolates, colour of the colonies was similar while appearance of the colony, production of sclerotia, growth rates and RAPD profiles were very different. Isolate BP1 had higher growth rate compared to BP2 and formed a single sub cluster. Isolate BP1 was a poor sporulator and produced sclerotia abundantly, while isolate BP2 produced sclerotia as well as conidia. The conidia of isolate BP2 were smallest among the bell pepper infecting *C. gloeosporioides* isolates. The above differences also may be contributing to the difference in RAPD profiles.

*C. lindemuthianum* isolate 4765 was grouped as a separate sub cluster in the main cluster with falcate conidia bearing *C. capsici and, C. dematium*. In RAPD banding pattern it was different from that of straight conidia bearing *C. gloeosporioides* isolates. Although colony colour was more or less similar to that of *C. gloeosporioides* isolates, appearance was different from those of *C.*

*gloeosporioides* isolates. Tu (1985) has obtained good growth and sporulation of *C. lindemuthianum* in modified Mathur's medium, present study revealed that it is inferior to PDA.

Out of 16 primers tested OPN3 gave good separation of *Colletotrichum* species but unable to distinguish between *C. capsici* and *C. dematium*. Primer OPN11 also gave good separation of species especially *C. capsici* together with *C. dematium* from other species. This primer also revealed the close relationship between *C. capsici* and *C. dematium* as stated by Kulshrestha *et al.*, (1976), von Arx (1957a) and Sutton 1980, 1992).

Out of five *Colletotrichum* species tested *C. capsici* isolates infected many plant species viz. chilli, soybean. ITCC isolate 5008 from *C. capsici* group was able to infect chilli and soybean seedlings although it was isolated from *Melia* leaves. This isolate seemed to be more virulent with wide host range. *C. lindemuthianum* isolates tested remained confined to French bean and didn't infect other legumes such as soybean. Isolates of *C. dematium* species also did not infect the crops tested like bean, brinjal, chilli and soybean. This could be due to the differences in the variety, which was used for pathogenicity test or due to the fact that the isolate lost its pathogenic ability on long-term maintenance in artificial medium. In some cases, young plants showed resistance while adult plants became susceptible to diseases (Pastor-Corrales and Frederiksen, 1980). In literature (Sutton 1980), it is mentioned that *C. dematium* species are saprophytic rather than pathogenic. Inability of *C. falcatum* species to infect any of the dicot plants tested showed their host specificity towards sugarcane. The present study on morphological and pathogenic characters revealed, that *Colletotrichum* species survive during unfavourable conditions as sclerotia and chlamydospores. Casela and Frederiksen (1993) reported that sclerotia of *Colletotrichum graminicola* might act as primary source of inoculum in the field. Due to the hyaline nature of conidia, it is susceptible to sunlight, high temperatures and low relative humidity. Therefore, these cause infection during current season. Smith and Black (1990) also reported that spraying of spore suspension on to strawberry plants caused severe disease.

# SUMMARY

*Colletotrichum* Corda is an important fungal genus comprising of many pathogenic species causing diseases in cereals, legumes, fruits, vegetables, forest trees, humans and animals. Its taxonomy is confusing and the characters used in its identification are shape and size of conidia, appressoria, setae, sclerotia, and chlamydospores. Knowledge of host plant and perfect state also contribute immensely to its taxonomy. Presently genetic characters of fungi are widely used in it's taxonomy especially when the morphotaxonomic characters are insufficient for segregation of individuals.

Objectives of the present study were to investigate morphological and cultural characters of commonly available *Colletotrichum* isolates of different species collected from several places of India and testing their pathogenicity on common crop species. To further this cause, the use of random amplified polymorphic DNA (RAPD) markers for discrimination of *Colletotrichum* species was also undertaken. Light microscopic studies coupled with Camera Lucida drawings were made and photomicrographs of important morphotaxonomic characters were taken.

1. Five species of *Colletotrichum* comprising of fifteen isolates viz., *C. capsici* (ITCC No. 4764, 4871, 5008, 5107, 5227), *C. dematium* (ITCC No. 4970, 5306), *C. falcatum* (ITCC No. 4800, 4803, 4893), *C. gloeosporioides* (ITCC No. 4573, 5132, 5213, 5255) and *C. lindemuthianum* (ITCC No. 4765) were obtained from the Indian Type Culture Collection. Another ten isolates (ITCC No. 6082-05, 6083-05, 6084-05, 6085-05, 6086-05, 6087-05, 6088-05, 6089-05, 6090-05,

6091-05) were collected from different places of India. As a result of this investigation, it was observed that ten isolates collected from field comprised of four species of *Colletotrichum* viz., *C. dematium* (6088-05), *C. falcatum* (6085-05, 6086-05, 6087-05), *C. gloeosporioides* (6082-05, 6083-05, 6084-05) and *C. lindemuthianum* (6089-05, 6090-05, 6091-05).

2. Colour and appearance of *Colletotrichum* isolates were found to be variable within a species and it was not possible to correlate colour and the appearance of the colonies with any of the characters studied.

3. The host range of *C. capsici* isolate ITCC No. 5008 was identified as broad and it also infected chilli and soybean in addition to *Melia* leaves.

4. The measurements of conidia and appressoria for *C. capsici* were found to be larger than that observed by earlier workers. The description of the fungus *C. capsici* was amended in respect to conidia and appressoria.

5. Isolates of *C. dematium* grouped together with *C. capsici* isolates in RAPD profiles indicating relatedness of both species as observed by Noble and Richardson and von Arx.

6. Conidial dimensions of *C. dematium* observed in the present study were found to be larger than described by earlier workers. Hence, the description of the *C. dematium* was amended for its conidial size.

7. Present studies also revealed that conidial and appressorial dimensions of *C. falcatum* were more than those observed by previous workers. Therefore, the limits of the conidia and appressoria of *C.*

*falcatum* have been expanded and the description of the fungus was amended.

8. RAPD profile of *C. gloeosporioides* isolates showed that these are highly variable in natures. The hyper variability may be due to the fact that these have been isolated from different host plant species. In the present study, wider conidial dimensions were also observed. Therefore, description of *C. gloeosporioides* was amended for its conidial size.

9. In the case of *C. lindemuthianum,* broader range of conidia and appressoria were observed. Hence, the description of *C. lindemuthianum* was amended.

10. Sclerotia of some species act as propagules, which survive adverse environmental conditions. They readily produced colonies when plated in fresh media plates. Some isolates produced sclerotia abundantly and very few conidia.

11. Survival on wide host range was observed as another method of pathogenic fitness.

12. Clustering of *Colletotrichum* isolates with respect to RAPD profiles could not be correlated with the geographical origin of the isolate.

13. In the primer studies, use of primers OPN3 and OPN11 was found to be a suitable tool for differentiation of *Colletotrichum* species.

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