"CHARACTERIZATION OF *Colletotrichum gloeosporioides* Penz. and Sacc. CAUSING DIE BACK AND FRUIT ROT OF CHILLI (*Capsicum annum* L.) UNDER SOUTH GUJARAT"

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TO

MY

PARENTS AND TEACHERS

"CHARACTERIZATION OF *Colletotrichum gloeosporioides* Penz. and Sacc. CAUSING DIE BACK AND FRUIT ROT OF Chilli (*Capsicum Annum* L.) UNDER SOUTH GUJARAT"

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A B S T R A C T

Chilli (*Capsicum* anuum L.) is one of the important spice crop, grown extensively in different location of south Gujarat. Occurrence of die back and fruit rot disease in chilli was observed in serious proportion infecting heavy losses in south Gujarat. Considering the seriousness of the disease, the present investigation was carried out to pin point exact cause, morphological, cultural, pathological variability and molecular variation.

The pathogenicity of ten isolates collected from different locations in south Gujarat from different varieties were confirmed by three different artificial inoculation methods on fruit *viz*, injury by pin prick, tooth brush and carborandum powder, where injury by pin prick was found more efficient in proving pathogenicity and fruit inoculation. The cultures were also tallied by microscopic morphological observations. Cultures

of the isolates were also sent to I. T. C. C., New Delhi and it was identified as *Colletotrichum gloeosporioides* Penz. and Sacc., (ITCC No. 8775-12). These 10 isolates were designated, as CCG-1 to CCG-10 (Chilli *Colletotrichum gloeosporioides*).

There was a remarkable difference in pathogenic, cultural, morphological and molecular characteristics within the isolates. The pathogenic variability study was carried out on ten different varieties of chilli. Ten isolates showed differential disease reactions against ten varieties. Isolate CCG-2, CCG-9 and CCG-10 showed resistance reaction against variety Chilli-4884, and Chilli-516, respectively other ten isolates showed moderately resistant to moderately susceptible disease reaction against all other varieties.

Different isolates showed different growth pattern on PDA and PDB. Various isolates produced cottony, fluffy, fluffy cottony, fluffy black or suppressed growth on the PDA with colour range of grey, greyish black to greyish white. The isolate CCG-1 showed maximum growth (85.37 mm) on PDA when incubated at 27 ± 2^{0}C temperature for ten days. The isolate CCG-9 showed maximum sporulation (23.90 million spores/ml), while maximum dry mycelial weight was observed in isolate CCG-1 (188.84mg) on PDB incubated at 27 ± 2^{0}C temperature for 15 days.

The isolates showed variation in morphological characters. Maximum length of the conidia was observed in isolate CCG-8 (29.26 µ) while the maximum width was in isolate CCG-5 (6.07 µ). Conidia of all the isolates showed oblong structure with oil globules. The maximum length of conidiophore

was recorded in isolate CCG-8 (56.60 µ), while maximum width of conidiophore was in isolate CCG-5 (6.64 µ). The maximum length of acervulus was observed in the isolate CCG-7 (74.16 µ), while maximum width was observed in isolate CCG-9 (53.85 µ). The isolate CCG-3 showed maximum length of appresoria with 12.95 µ while maximum width was shown in isolate CCG-4 (12.10 µ).

The molecular characterization study was carried out by using five ISSR (Inter Simple Sequence Repeat) primers. The primer UBC-850 and UBC-854 (100.00%) showed the highest percentage of polymorphism. The dendogram showed isolate CCG-9 and CCG-10 were situated separately with similarity matrix of 0.378 and 0.419 with CCG-1. Other eight isolates were clustered in two group and sub-groups.

The study clearly indicated the variation among ten isolates of *C. gloeosporioides* Penz. and Sacc., collected from south Gujarat region in terms of pathogenic, cultural, morphological and molecular levels. Further extensive study is required to identify the variation among *C. gloeosporioides* Penz. and Sacc., up to races, which helps in the development of race specific resistance varieties.

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C E R T I F I C A T E

This is to certify that the thesis entitled **"CHARACTERIZATION OF *Colletotrichum gloeosporioides* Penz. and Sacc. CAUSING DIE BACK AND FRUIT ROT OF CHILLI (*Capsicum annum* L.) UNDER SOUTH GUJARAT"** submitted by **MR. NAVEEN KUMAR PARASHAR** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **HORTICULTURE PATHOLOGY** of the **NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafide research work carried out by him under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place : Surat
Date : 20/07/2013

(Pushpendra Singh)
Major Advisor

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **HORTICULTURE PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. Pushpendra Singh**, **Associated Professor, Dept. of Plant Pathology, Gujarat Agriculture Biotechnology Institute, Navsari Agricultural University, Surat** and no part of the work has been submitted for any other degree so far.

Place : Navsari

Date : 20 July, 2013 **(Naveen kumar Parashar)**

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Place: Navsari

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(Naveen kumar Parashar)

C O N T E N T S

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

I. INTRODUCTION

Chilli, popularly known as 'Mirchi' (*Capsicum annum* L.) is an important spice cum vegetable crop, often referred as capsicum, hot pepper or sweet pepper. It is commonly known as red pepper in western countries belongs to family Solanaceae, which is native of Tropical America and West Indies. It is an important vegetable crop, because of its adaptability to various climatic conditions. Today chilli crop is grown in all subtropical and tropical regions in many countries of the world.

Chilli is mainly grown in India, Mexico, Japan, Indonesia, Pakistan and China. India ranks second in area and production after China. Andhra Pradesh is the leading chilli producing state contributing to about 58.0 per cent of the country's total production. Karnataka is the second largest chilli producer (13.0%) followed by Orissa (4.8%), West Bengal (4.8%), Maharashtra (3.6%) and Gujarat (3.5%). During 2011, India account 40 per cent of the total chilli area in the world, which is about 7.92 lac hectares with an annual production of 12.23 lac MT of ripe dry chillies (Anonymous, 2012).

In Gujarat, the area under chilli crop is about 38670 hectares with total production of 48051 MT of ripe chillies. The area under cultivation is mostly in Anand, Surat, Himmatnagar, Kheda, Valsad and Navsari districts. In south Gujarat, the crop is cultivated in an area of 2200 hectares mainly for green chilli during post rainy season. (Anonymous, 2012).

It is a popular vegetable valued around the world for the colour, flavor, spices and nutritional value and contributes to many meals. Chilli fruits green or dry fruit powders have become indispensible adjunct to the diatory requirement of the people. It contains large amounts of vitamin-C and small amounts of carotene (pro-vitamin A). In addition, peppers are a good source of B vitamins, particular vitamin B6. They are rich source of potassium, magnesium and iron as well as vitamin-C.

Both, fresh and dried fruit contain phenolic compound 'Capsicin' in the placenta, which is responsible for pungency in chilli. The bright red color at the ripening stage is due to the pigment of 'Capsanthin'. Capsicin, the active principle of chilli is an effective counter irritant and hence chilli extract used in pharmaceutical drugs as a powerful stimulate carminative. It is an important ingredient for flavoring meat, vegetable soups and essence of capsicum are used to increase the pungency, ginger soda, rum etc. (Liverseege, 1932). Internally it is irritant and large doses produce gastroenteritis. Due to its medicinal properties, vitamin content and colouring matter, the demand for chillies has been fast increasing throughout the world.

Chilli is generally grown throughout year. The crop is vulnerable to many pests and pathogens due to its extreme delicacy and succulence. Numerous arrays of fungi, bacteria and viruses frequently invade chilli crop, from prior sowing to harvesting as on post-harvest chilli fruit rots and cause inflicting sizable economic losses.

It is attacked by several fungal diseases *viz*; Die back or ripe fruit rot [*Colletotrichum gloeosporioides* Penz. and Sacc.], Fusarium wilt [*Fusarium oxysporum*, Schlecht.], Alternaria leaf spot and fruit rot [*Alternaria alternata* Fr. Keissler], Damping off [*Pythium aphanidermatum* (Eds.) Fitzp], Collar rot or stem rot [*Sclerotium rolfsi* Sacc.], Powdery mildew [*Leveillula taurica* (Lev.) Arn.], Cercospora leaf spot [*Cercospora capsici* Heald and Wolf], Grey mould [*Botrysis cinerea* Pers], Verticillium wilt [*Verticillium albo-atrum* Reinke and Berhold] and among bacterial diseases *viz;* Bacterial leaf spot [*Xanthomonas compestris* pv. *vesicatoria* (Doidge) Dye], Soft rot [*Erwinia carotovora* sub sp. *carotovora* Jones] and Bacterial wilt [*Rolstonia solanacearum* Smith], while, viral diseases *viz;* Mosaic [Cucumber mosaic virus] and Leaf curl [Tobacoo leaf curl virus] (Sharma and Verma 1999).

Die back and ripe fruit rot caused by *Colletotrichum gloeosporioides* Penz. and Sacc. is one of the important disease of chilli growing area of south Gujarat. Symptoms are brown lesions surrounded by a yellow halo develop on the fruit. The lesions enlarge and result in the formation of irregular sunken patches with a dark brown margin and light grey centre which is covered later by olive brown fungal growth. In the fruit, the infected parts turn black and become depressed or wrinkled. Ultimately the diseased fruits shrivel and dry up. The disease usually develops under high humid conditions when rain occurs after the fruits have started to ripen. Maximum disease development takes place

at 28^0C and 95.7 per cent relative humidity. Yield loss by this disease is upto 50 per cent (Gupta and Thind 2006).

The name *Colletotrichum gloeosporioides* was first proposed in Penzig (1882), based on *Vermicularia gloeosporioides*, the type specimen of which was collected from *Citrus* in Italy. The fungus was identified as *C. gloeosporioides* [*Glomerella cingulata*] based on morphology.

There are many reports of genetic characterization of other species of *Colletotrichum*, but very little informations are available on the relative importance of *C. gloeosporioides* pathotype races - their distribution and diversity. Variability in *C. gloeosporioides* isolates have been characterized by using morphological characters, host reaction, arbitrary marker system such as Inter Simple Sequence Repeat (ISSR) marker have also been successfully used as tools for understanding the phylogenetic relationship of fungi. ISSR is simple technique, requires no sequence information and is carried out using a single primer based on a simple repeat. Only small amounts of DNA templates are required and the results are clearly scorable and reproducible. (Ratanacherdchai *et al.*, 2010).

Now a day's the cultivation of chilli is increased under south Gujarat condition. But, die back and fruit rot caused by *C. gloeosporioides* is on of the important threating disease occurring prevalently and causing economic damage. So, considering the above facts and seriousness of problem, the present investigation is carried out by following objectives under south Gujarat heavy rainfall agro climatic zone.

Objectives:

1) Collection, isolation, purification, Identification and pathogenicity.
2) To study the morphological, cultural and pathogenic variation among isolates of *Colletotrichum gloeosporioides*.
3) To study the molecular characterization of *Colletotrichum gloeosporioides* isolates.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Diseases are one of the major constraints in the crop production, causing enormous losses by reducing the yield quantitatively and qualitatively. Chilli attacked by several fungal diseases *viz*; Die back and fruit rot [*Colletotrichum gloeosporioides* Penz. and Sacc.], Fusarium wilt [*Fusarium oxysporum,* Schlecht.], Alternaria leaf spot [*Alternaria alternate* Fr. Keissler], Damping off [*Pythium aphanidermatum* (Eds.) Fitzp], Collar rot or stem rot [*Sclerotium rolfsi* Sacc.], Powdery mildew [*Leveillula taurica* (Lev.) Arn.], Cercospora leaf spot [*Cercospora capsici* Heald and Wolf], Grey mould [*Botrysis cinerea* Pers], Verticillium wilt [*Verticillium albo-atrum* Reinke and Berhold], and Bacterial diseases *viz*; Bacterial leaf spot [*Xanthomonas compestris* pv. *vesicatoria* (Doidge) Dye], Soft rot [*Erwinia carotovora* sub sp. *carotovora* Jones] and Bacterial wilt [*Rolstonia solanacearum* Smith]; Viral diseases *viz*; Mosaic [Cucumber mosaic virus] and Leaf curl [Tobacoo leaf curl virus] etc., (Sharma and Verma, 1999). Among all these diseases, Die back and fruit rot is one of the important diseases of chilli occurring in chilli growing area of south Gujarat in serious proportion.

Research work carried out by various workers on different aspect of die back and fruit rot disease caused by *C. gloeosporioides is* reviewed and presented as under:

2.1 Occurrence

Colletotrichum capsici, C. graminicola, C. ateramentarium and *C. gloeosporioides* were found to associated with chilli fruit and die back (Verma, 1966). Natarajan and Subramanian (1973) reported fruit rot of chilli caused by *C. capsici, C. piperatum* EII and Eve; *Alternaria solani* (EII and Mart) Jones and Grout, *Alternaria tenuis* and *C. gloeosporioides*.

Roy (1974) reported anthracnose and die back symptoms caused by *C. gloeosporioides* from Jorhat, Assam.

Thind and Jhooty (1985) reported that anthracnose and fruit rot incidence was more of *C. capsici* as compared to *C. gloeosporioides* and *C. piperatum* in Punjab.

Thind and Jhooty (1990) reported that die back and fruit rot of chilli is caused by three species of *Colletotrichum,* namely *viz C. capsici,* C. gloeosporioides *and C. piperatum* in Punjab, while *C. gloeosporioides* Penz. has been found associated with this disease for the first time from this state.

Shukla et al. (1991) reported the occurrence of *C.* gloeosporioides on the leaves of *Rosa indica* Linn. cause of die back disease of rose.

Sinha and Singh (1992) reported *Ricinus communis* L. as a new host of anthracnose caused by *C.* capsici.

Monaco et al. (2004) reported *C.* gloeosporioides was found to cause strawberry anthracnose from North West Argentina.

Pandey (2006) reported *C.* gloeosporioides and *C. dematium* are also known to cause fruit rot of tomato. For the first time, severe fruit rotting symptoms was observed on tomato in Varanasi region.

Talukdar et al. (2012) reported that die back symptoms caused by *C.* gloeosporioides were identified in chilli variety Bhut jolokia from Jorhat, Assam and revealed that highest disease incidence were found from Jorhat district with an average disease incidence of 4.25 per cent.

2.2 Causal organism

The genus: *Colletotrichum* belongs to the Order: Melanconiales, Class: Deuteromycotina, Phylum: Ascomycota and having Kingdom: Fungi. (Agrios, G.N., 2005).

Verma (1966) reported die back and fruit rot of chilli caused by *C. gloeosporioides* in Jabalpur.

Roy (1974) showed anthracnose and die back symptoms caused by *Glomerella cingulata* (Stonem) Spuid and Vschr (*C. gloeosporioides*). While, Grover and Bansal (1970) showed die back and fruit rot *(C. capsici*) was found in serious proportion in Haryana and other states of India.

Hong and Hang (1998) observed that Different *Colletotrichum* species may also play important roles in different disease of mature stages of chilli fruit. *C. capsici* is widespread in red chilli fruits whereas *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits of chilli.

Rajapakshe (1998) found that two species of *Colletitrichum i.e. C. capsici,* and *C. gloeosporioides* has been identified as causal agent of anthracnose and fruit rot of chilli in Sri lanka.

Sharma and Verma (1999) reported that die back and fruit rot of chilli caused by *C. capsici*, *C. gloeosporioides, C. acutatum* Simmond and Coccodes (Wallr). Shahzad (2002) reported *C. capsici* and *C. gloeosporioides* isolates from betelvine.

Gopalkrishan and Prakasam (2007) isolated the causal agent from leaf spots of various host plants like chilli fruits, green pods of french bean, lablab bean, turmeric leaves and sugarcanes aseptically in sterilized petri plates containing PDA by infected tissue isolation techniques, the pathogen identified as *Colletotrichum* sp. and purified by adopting singal hyphal tip method and maintained in PDA slants for further studies.

Than *et al.* (2008) reported anthracnose and fruit rot of chilli caused by *Colletitrichum* sp. including *C. acutatum, C. capsici, C. gloeosporioides* and *C. coccodes.*

2.3 Pathogenicity:

Various workers have proved the pathogenicity of fruit rot pathogen *Colletotrichum* sp. on chilli and different hosts by following methods.

Natarajan and Subramanian (1973) proved pathogenicity of *C. gloeosporioides* successfully on leaves, on

green and ripe fruits of chillies by spray inoculation with conidia after slight injury by pin prick method.

Roy (1974) proved pathogenicity of *C. gloeosporioides* successfully on wounded ripe fruits of chilli on which infection appeared after four days at 30°C but not on unwounded fruits.

Patel (1978) proved pathogenicity of *C. gloeosporioides* isolated from mango on both detached and attached leaves by using brush and pin pricking injury.

Thakare and Patil (1995) proved pathogenicity of *C. gloeosporioides* on chrysanthemum by spraying spore suspension on injured leaves. Typical watery circular to irregular dark brown spot were appeared after four days of inoculation.

Patel (2000) proved the pathogenicity of *C. gloeosporioides* isolated from turmeric on detached leaves with and without injury by pinprick and tooth brush and reported that tooth brush injury method appeared more superior followed by pin picking and without injury method.

Manaco *et al.* (2004) proved pathogenicity of *C. gloeosporioides* by spray inoculation with conidial suspension (10^6 conidia/ml), which regulated the disease symptoms 7 days after inoculation.

Freeman *et al.* (2006) proved the pathogenicity of *C. gloeosporioides* and *C. acutum* on strawberry seedling by foliar dip and root soak method at 19^0C and 25^0C. They found that mortality of young seedling was observed four days after

inoculation, reaching 50 per cent within ten days using both techniques at 25^0C.

Gopalkrishnan and Prakasam (2007) proved the pathogenicity of *C. capsici, C. falcatum C. lindemuthianum,* isolated from leaves of turmeric and fruits of chilli. In all these interactions, symptoms were observed after 5 days using pinpricking method of inoculation.

Patel *et al.* (2007) proved pathogenicity of *C. gloeosporioides* by making injury on chilli fruit by pin prick.

2.4 Varietal screening:

Patil and Patil (1983) screened eleven turmeric cultivars from Digraj, Sangli district (Maharashtra) by mean of natural incidence of leaf spot disease (*C. capsici*) and found that Kasturi (2.3%) and Gadhavi (2.7%) showed lowest leaf spot infection and higest in Erode (10.66%).

Hartman and Wang (1999) observed that ten red-ripe detached fruits from each of 305 field-planted accessions were inoculated with *C. capsici* and *C. gloeosporioides* using the fruit puncture method. The accessions, C01826 and CO2220, had no visible lesions while eight other lines had less than 0.5 mm diameter lesions. All of these lines were considered highly resistant.

Sharma and Singh (2001) screened 800 rose cultivars, hybrids and selections for resistance to dieback disease and found that 88.0 percent of the cultivars, hybrids and selections were

susceptible, 8.5 percent were moderately susceptible and 3.5 percent were tolerant.

Venkataravanappa (2002) evaluated ten mango cultivars against *C. gloeosporioides* by detached leaf method. Among ten genotypes evaluated Mallika and Alampur beneshan recorded moderately susceptible reaction.

Sarath *et al.* (2004) graded disease severity on 0-5 scale and found that Dolichos bean accessions, *viz.*, IC-383192 (BSBS-151) and IC-369641 (NDS-236) showed consistent resistant reaction to anthracnose disease.

Onyeka *et al.* (2006) screened 60 cultivars of *Dioscorea alata* L. by using a 0-6 grade scale at the seventh days after inoculation and recorded that 12 cultivars including DA 27, DA 26, and DA 32 were resistant to yam anthracnose by *C. gloeosporioides.*

Kaur *et al.* (2011) seventeen chilli genotypes from various sources in India were screened against *C. capsici* with respect to physiological and biochemical traits. A visual observation had identified four chilli genotypes: LLS, Breek-1, Breek-2 and Jaun were resistant to die back and fruit rot.

2.5 Pathogenic variability

Latha *et al.* (2002) characterized eighteen isolates of *Colletotrichum graminicola* infecting sorghum and compared for their morphological, pathogenic and genetic diversity and found that these isolates exhibited significant variation for disease reaction on a set of sorghum differential line.

Mohan *et al.* (2006) studied pathological behavior of 12 representative isolates of *C. capsici* four groups were compared on detached red ripe fruit of chilli cultivars Punjab Lal, Punjab Surkh and CH-1. The incubation period, percent infected fruits and disease severity presented revealed that all the 12 isolates of *C. capsici* were pathogenic and exhibited variable pathogenic response on three chilli cultivars.

Ratanacherdchai *et al.* (2010) studied that thirty-four of *Colletotrichum* sp. including two species, *C. gloeosporioides* and *C. capsici,* from anthracnose on bell pepper, long cayenne pepper and bird's eye chilli were isolated. They found that pathogenic variability of *Colletotrichum* isolates was grouped into three categories *viz;* two isolates from bell pepper were grouped in low virulent, four in moderately virulent and one in highly virulent group. For *Colletotrichum* sp. isolate from long cayenne pepper, 13 isolates were placed in the moderately virulent and four in the highly virulent group. Whereas, four from bird's eye chilli showed low virulence while six isolates were moderately virulent.

Sangdee *et al.* (2011) reported variation amoung 10 isolates of *C. capsici* on chilli fruit. The isolates were evaluated for their pathogenic variation and produced typical anthracnose symptoms small lesions and tissue collapse, acervulus production and sporulation on chilli fruits after inoculation. Various disease scores based on the acervulus development time on inoculated fruits were observed and categorized into three groups *viz;* mildly virulent, moderately virulent and severely virulent strain.

Masoodi *et al.*, (2012) studied pathological variability among the twenty isolates of *C. capsici* by recording the response on a set of 20 chilli genotype and found that ten groups of *C. capsici* on the basis of varied pathogenic response.

2.6 Cultural and morphological variation

2.6.1 Cultural variation:

Ekbote *et al.* (1997) evaluated ten different synthetic and non-synthetic media for sporulation and growth of *Colletotrichum gloeosporioides*. Among the tested media maximum radial growth of *C. gloeosporioides* was recorded on Richards', Browns' and Potato Dextrose Agar, followed by Czapek (Dox) agar.

Jeyalakshmi and Seetharaman (1999) revealed that there is variability among 5 isolates of chilli fruit rot pathogen *C. capsici* on the bases of their cultural characteristics. Which can be divided in five groups as far as mycelial growth and pigmentation is concern.

Kumar *et al.* (2010) studied that 21 isolates of *Colletotrichum* species collected from different infected crop plants were evaluated for their cultural characterization. Such as growth, variation in the colour pigments within the agar and colour of the aerial mycelium and variation in the shape of colony and were characterized into four different groups.

Sangdee *et al.* (2011) studied that cultural morphology, spore shape and size showed an overlap in colony colour and conidial shape and size among the six groups studied. This result

was found a morphometric overlap of conidial size within *Colletotrichum* species.

2.6.2 Morphological variation:

Natarajan and Subramanian (1973) showed that chilli fruit rot incitant *C. gloeosporioides* produce acervuli which were rounded or irregular measuring 121.3 to 223.9 µ with an average hyaline, aseptate and measure 11.3-21.6 µ x 3.6-5.7 µ with an average of 17.4 x 4.3 µ. Setae were absent.

Garcia-Jimenez and Alfaro (1985) observed that *C. gloeosporioides* produced hyaline, single celled and bacilliform conidia with truncated base and averaged 13.1 x 3.9 µm in size. Acervuli were 60.0-180.0 µm long and had few or only rudimentary seate.

Patel (2000) reported that *C. gloeosporioides* causal organism of anthracnose of tumeric produce light gray to dirty white and cottony mycelium. The hyphae were thin, branched, hyaline, one called, thin walled, straight, oblong, slightly depressed in the middle with rounded ends and granular protoplasm contents measuring 13.44-16.66 µm x 3.8-4.5 µm in size and oil droplets in the middle. The acervuli were dark brown, disk shaped produced in groups or singly with straight or slightly curved, measuring 82.8-140.76 µm producing hyaline, short cylindrical conidiophores and dark brown setea measuring 39.33-51.75 µm x 3.11-5.89 µm.

Bag (2004) reported that *C. gloeosporioides* incitant of flower and fruit drop of papaya, produce brown to black septate

mycelium. While acervulus was found dark with black coloured long, slender septate setae. Conidia were short, cylindrical, hyaline and single celled measurning, 13-19 × 4-5 µm.

Wijesekara and Agarwal (2006) found that conidia variable, in shape and size. Single celled, hyaline, straight, cylindrical, ellipsoidal or slightly curved. Conidia apices round or obtuse with narrow truncate base. Conidia measurements of *C. gloeosporioides* in range of 8.54-21.95 x 2.44-7.32 µm. Appressoria brown, clavate or irregular, something becoming complex, 7.32-14.63 x 7.32-10.98 µm. setae were not observed in any of the cultures studied.

Akhtar *et al.* (2007) were found that the morphological characters are *C. capsici* full development of colonies, sporulation was evident. The conidia were gently harvested and measured. Conidia measured 25.27-26.15 x 3.14-3.67 µm. There were only very minor differences in the size of conidia of the isolates grown on different media.

Shenoy *et al.* (2007) were found that the morphological characters are *C. capsici* of fresh specimen are compared with those of the lectotype. Acervuli on chilli fruit, 85-245 µm in diam. Setae 70-135 µm long x 5 µm wide at base, abundant, dark brown, rigid, smooth-walled,1-5 septate, slightly swollen at base, tapered to the paler acute apex. Conidia 17-26 µm long x 3.75 µm wide, one called, smooth walled, hyaline, falcate, sometimes fusiform, tapered toward both ends, acute at the apex.

Kumar *et al.* (2010) studied that a total of 21 cultures of *Colletotrichum* sp. were isolated. Cultures showed dense, cottony puffy and white colour aerial mycelium. On the reverse side of the plate, colonies were white to pale grey mycelium and dark grey at the inoculation point. Isolates in mostly medium size conidia which ranged from 7.5-22.5 x 2.5-7.5 µm most of the isolates in these group had cylindrical conidia tapered at one end (C/T) and a few isolates had cylindrical conidia with rounded end white a few other had egg shaped conidia.

2.7 Molecular variation

Pandey (2006) reported that the pathogen *Colletotrichum capsici* was characterized on molecular basis by specific PCR. The DNA was extracted from pure mycelial mat by CTAB method. This primer was used as forward primer while ITS4 was used as reverse primer. The pathogen was amplified as single band at 450bp DNA and size.

Souza Serra *et al.* (2006) studied molecular methods to be efficient in differentiation of the *C. gloeosporioides* isolates in relation to host specificity. In the analysis of the ITS sequence of the ribosomal DNA, all the isolates amplified with the CgInt and ITS 4 primers, confirming that they pertain to *C. gloeosporioides*. The results from this study suggest that methods based on pathogenicity, isozyme analysis and RAPD are effective in differentiating *C. gloeosporioides* isolates from cashew and mango trees.

Shenoy *et al.* (2007) reported that the phylogenies based on the combined partial β-tubulin gene and ITS

nu-rDNA sequence data suggest a close phylogenetic relationship of the epitype with *C. capsici* strains.

Eight random primers were tested by Gupta *et al.*, (2010) in the genome of *C. gloeosporioides* isolates collected from different locations generated distinct fingerprints. Primers OPA-1, OPA-3 and OPA-18 produced reproducible polymorphic major bands among the selected isolates. Amplicons of 2138, 1202 and 955 bp as generated by OPA-3, 2291 and 1995 bp was obtained with OPA-18 which was shared by all isolates except for isolates 7 and 8 as for the set of amplicons generated by primer OPA-1. Overall fingerprints obtained from *C. gloeosporioides* with the selected primers were unique and species-specific.

Kumar *et al.* (2010) observed that twenty one isolates of *Colletotrichum* sp. were obtained from infected vegetable and spice plants. The experiment to studied ITS- RFLP analysis with three restriction enzymes *viz*, *TaqI*, *HinfI*, *MspI* and *EcoRI* provided greater resolution for distinguishing the *C. gloeosporioides* and unidentified *Colletotrichum*. All *C. gloeosporioides* isolates produced identical RFLP pattern, while other unidentified *Colletotrichum* sp. showed unique ITS-RFLP banding pattering. Sequence analysis of the ITS region revealed that isolates in group I and III showed high similarity with the reference isolates of *C. gloeosporioides*.

Pandey *et al.* (2010) reported that genomic DNA from 12 isolates of *C. gloeosporioides* belonging to different agroclimatic regions was amplified by PCR with *C. gloeosporioides* species-specific primer. All the isolates

amplified have a uniform DNA fragment of size 450 bp. PCRRFLP using the restriction endonuclease *Alu*I, *Hae*III, *Msp*I, *Rsa*I and *Taq*I reliably reproduced unique restriction patterns specific for *C. gloeosporioides*.

Ratanacherdchai *et al.* (2010) studied ISSR for comparison of cross-inoculation potential of *Colletotrichum capsici* causing chilli anthracnose and observed that inter simple sequence repeat (ISSR) analysis indicated that there are two distinct groups of *C. gloeosporioides* and *C. capsici*. Furthermore, genetic diversity was correlated with geographic distribution, while there was no clear relationship between genetic diversity and pathogenic variability.

MATERIAL AND METHODS

III. MATERIALS AND METHODS

The details of the materials used and the methods adopted in the present investigation are described here as under.

GLASSWARES

Different types of glasswares *viz.*, Petri plates, test tubes, conical flasks, measuring cylinders, glass rods, slides, cover slips etc., used throughout the study were cleaned by overnight soaking in six per cent chromic acid solution followed by washing with tepole and finally rinse with distilled water. These glasswares were air dried before use at room temperature.

CHEMICALS

All chemicals used were of either "Fisher's" or proanalytic quality of E-Merck. The chemicals used for molecular study were from Bangalore Genei Pvt. Ltd. Bangalore, India.

STERILIZATION

Glasswares

The glasswares were sterilized in electrical hot air oven at 180°C for an hour.

Media

The liquid and solid media used throughout the study were sterilized by autoclaving at 1.045 kg / cm^2 for 15 minutes.

Plasticwares

The plasticware used for molecular study were sterilized by autoclaving at 1.045 kg/ cm^2 for 15 minutes.

Experimental location

The laboratory experiments were conducted in Department of Plant Pathology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. The molecular study was conducted at Department of Molecular Biology and Plant Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari.

The pot experiment was laid out in green house near Forestry College, Navsari Agricultural University, Navsari.

3.1 Pathological investigations

3.1.1 Collection of samples

The diseased samples of chilli showing die back and ripe fruit rot symptoms of different varieties were collected from different location of south Gujarat region. The infected chilli fruits were brought into the laboratory. The chilli fruits were split open and prepared the slide for the microscopic examination. The symptoms and signs on infected chilli fruits, found in nature were critically observed and recorded.

3.1.2 Isolation of pathogen

External as well as microscopic examination of the infected chilli fruits were carried out and symptoms association with the fungus were studied. The samples were subjected to

Table 1: Different isolates of *Colletotrichum gloeosporioides* Penz. and Sacc. causing die back and fruit rot in chilli collected from different places and geographical locations of south Gujarat

Sr. No.	Isolate	Variety	Place	Geographical coordinates
1.	CCG – 1	GVC- 111	Regional Horticulture Research Station, NAU, Navsari.	20°55′ 38″ N 72°53′ 54″ E
2.	CCG – 2	G- 4	Bardoli	21° 7′ 12″ N, 73° 7′ 12″ E
3.	CCG – 3	Sitara	Chikhli	20° 45′ 0″ N, 73° 4′ 12″ E
4.	CCG – 4	Sulekha	Bardoli	21° 7′ N, 73° 7′ E
5.	CCG – 5	Suryamukhi	Gandevi	20° 49′ 12″ N, 72° 58′ 48″ E
6.	CCG – 6	Local	Patari	23.18^0 N 71.08^0 E
7.	CCG – 7	Local	Mandvi	21° 15′ 11″ N, 73° 18′ 1″ E
8.	CCG – 8	Local	Maroli	28° 0′ 30″ N, 76° 0′ 51″ E
9.	CCG – 9	Chilli 4884	Valsad	20° 36′ 36.18″ N, 72° 55′ 33.14″ E
10.	CCG - 10	GVC- 121	Regional Horticulture Research Station, NAU, Navsari.	20°55′ N 72°53′ E

Note: CCG = Chilli *Colletotrichum gloeosporioide*

tissue isolation from infected chilli fruits. The infected tissue due to die back and fruit rot were cut into small pieces, surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 second followed by three subsequent washing of sterile distilled water, then aseptically transferred under laminar air flow system (cabinet manufactured by Klenzoid contamination control Ltd.) to sterile petriplates containing 20 ml of Potato Dextrose Agar (PDA) medium (Peeled potato 200 g + dextrose 20 g + agar agar 20 g + distilled water 1000 ml). The petriplates were incubated at 27 + 2°C temperature. The growth of the fungus developed after an incubation of 48 hrs, were sub-cultured on

PDA. The cultures were further purified by single spore isolation technique and pure isolates were maintained on PDA slants for further studies.

3.2 Identification of the pathogen

Identification of the pathogen causing die back and fruit rot of chilli was carried out by studying the cultural and morphological characters. The cultural characters were recorded right from the initiation of mycelial growth till the period of 15 days. The morphological characters of spore *viz.*, length and diameter were measured under low power magnification (100 X) from 10 days old culture, using stage and occular micrometer. The characteristics of mycelium and conidiophore were also observed and recorded. These were compared with those described in literature. The photomicrographs of the mycelium and spores were also taken. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 for further identification and confirmation.

3.3 Pathogenicity assay

In order to test the pathogencity of ten isolates collected from different locations of south Gujarat, the pin pricking, injury tooth brushing and carborandum method of inoculation technique and best superior method was used in pathogenic variability. The chilli plant of highly susceptible variety GVC-111 was grown in the pot. The spore suspension of $1x10^6$ spores/ml was used for the inoculation of the chilli fruits. Two drops of spore suspension were put in the hole by a needle.

The symptoms were observed as that of isolated fruits. The infected fruits were subjected to the re-isolation of the pathogen. The re-isolated cultures showed same cultural and morphological characters thus proved the pathogenicity test.

3.4 The pathogenic, cultural and morphological variation.

3.4.1 Pathogenic variability

To study was pathogenic variability, ten different varieties of chilli were used. Spore suspensions ($1x10^6$ spores/ml) from 9 days old culture were prepared by through homogenization of mycelia mass in sterile distilled water followed by filtration through muslin cloth to remove mycelium. This was then inoculated on the fruits. The fruits were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution and washed thoroughly with sterile distilled water to remove traces of the mercuric chloride ($HgCl_2$). Inoculation was carried out by putting two drops of spore suspension on fruits by three methods;

(i) Pin pricking

Sterile pins were dipped in spore suspension and pricked immediately on chilli fruit at 6 to 8 place.

(ii) Tooth brush injury method

Sterile tooth brush was dipped in spore suspension and inoculated with gentle rubbing on chilli fruit at 6 to 8 place.

(iii) Injury by carborandum powder

Fruit were injured with carborandum powder (600 mesh) and spore suspension was placed on the injured area.

The per cent disease intensity (PDI) for severity was calculated using the formula;

$$PDI = \frac{\text{Sum of all numerical rating} \times 100}{\text{Total no. of observation} \times \text{Max. rating observed}}$$

The fruits are scored on a 0-10 rating described by scale (Paul *et al.*, 2008):

Severity scale	**Fruit surface affected (%)**	**Category**
0	0	Highly resistant
1	1-10	Resistant
2	11-20	
3	21-30	Moderately Resistant
4	31-40	
5	41-50	Moderately Susceptible
6	51-60	Susceptible
7	61-70	
8	71-80	Highly Susceptible
9	81-90	
10	91-100	

3.4.2 Cultural variation

The isolates were separately cultured on potato dextrose agar (PDA) media, at 27±2°C for seven days. After seven days of incubation diameter of the mycelial growth and the growth pattern of pathogen were recorded.

The isolates were also cultured in liquid media in 100ml flask containing 20ml of potato dextrose broth (PDB). These flasks were incubated at 27±2°C for fifteen days. In case of liquid media, the mycelial mat was removed by filtering through Whatman No. 1 filter paper after fifteen days of incubation and dried in hot air oven till consistant weight was obtained. The numbers of spores were counted with the help of haemocytometer. Three replications maintained for each isolates, the results were tabulated and data were analyzed statistically using complete randomized design.

3.4.3 Morphological variation

The *Colletotrichum gloeosporioides* isolates were cultured on potato dextrose agar in Petri plates at 27±2 ^{0}C for seven days. After incubation, average measurements were taken by the micrometry method.

The morphological characters like size (length and width) and shape of the conidia and conidiophore, size of acervuli, size of the setae and shape (length and width) of appressoria were recorded. The observations were recorded in three replications within each isolate. The study was carried out using ocular and stage micrometer after mounting them on the

slides containing sterile distilled water at magnification of 450X. Data were analyzed statistically using complete randomized design.

3.5 Molecular variation

3.5.1 Study of molecular variation by using ISSR primers

3.5.1.1 Genomic DNA isolation and purification

Ten purified isolates of *C. gloeosporioides* were grown on potato dextrose broth at 27 ± 2°C for 10 days. Young growing mycelia were filtered through autoclaved muslin cloth, and about 1 g mycelia were ground in a pre-chilled mortar with liquid nitrogen. The powder was transferred to an Eppendorf tube and resuspended in 500 µl of extraction buffer (0.5% SDS, 200 mM Tris–HCl pH 8.5, 250 mM NaCl, and 25 mM EDTA) (Raeder and Broda 1985). The tubes were incubated at 65°C for 30 min with occasional gentle swirling. Two third volumes of chloroform: isoamylalcohol (24 : 1, v / v) was added to this sample and mixed for 15–25 min and then centrifuged at 10000 rpm for 10 min. For purification, 10 µl RNAse (10 mg/ml) was added to the supernatant and incubated for 30 min at 37°C. The incubated sample was again extracted with chloroform : isoamyl alcohol solution and centrifuged at 10000 rpm for 10 min. Chilled isopropanol (0.57 volume) was added to the upper phase, mixed by inverting and centrifuged at 10000 rpm for 10 min at 4°C. The DNA pellet was rinsed twice with 70% ethanol for 10–15 min. and then dried at room temperature. The dried pellet was dissolved in 250 µl TE buffer (pH 8.0). The purified DNA was checked by running 2 µl of the sample on a 0.8% agarose gel. The

quality and quantity of DNA were measured at 260/280 nm and 260nm respectively using nanospectophotometer.

3.5.1.2 Molecular analysis

Twenty ISSR primers were screened for the amplification of genomic DNA with one isolate of *C. gloeosporioides* five of them gave reproducible results. The annealing temperature of primers varied according to GC content (Table 2).

For ISSR, PCR amplifications were carried out in a reaction volume of 25 µl containing 25 ng of fungal DNA. The PCR reaction mixture consisted of 10X PCR buffer (0.01% gelatin 20 mM Tris–HCl, pH 8.4; 50 mM KCl, 1.5 mM $MgCl_2$), 10 mM dNTPs, 1 U Taq DNA polymerase, and 0.2 µm primer. All the PCR reaction components were obtained from Bangalore Genei Pvt. Ltd. Bangalore, India. The amplifications were performed using a Thermal cycler (T-Gradient Biometra; GmBh, Goettingen, Germany). For ISSR markers, PCR temperature profiles were as follows: initial DNA denaturation at 94^{o}C for 5 min, followed by 35 PCR cycles at 94^{o}C for 1 min, 1 min at the primer specific annealing temperature 72^{o}C for 2 min and a final cycle at 72^{o}C for 7 min. All the amplified PCR products were resolved by electrophoresis in 1.4% Agarose gel containing 5µg ethidium bromide/100ml for 2 h in 0.5X TBE buffer at 60 V. the standard DNA marker (Fermentus) was also run along with the sample. The separate bands were visualized under UV light and photographed by Biorad gel documentation system (Biorad GelDoc XR+).

Table 2: Details of primers used for ISSR analysis of *Colletotrichum gloeosporioides* Penz. and Sacc.

Sr. No.	Code	Sequence (5'-3')
1	UBC-801	5'-ATA TAT ATA TAT ATA TT-3'
2	UBC-802	5'-ATA TAT ATA TAT ATA TG-3'
3	UBC-807	5'-AGA GAG AGA GAG AGA GT-3'
4	UBC-810	5'-GAG AGA GAG AGA GAG AT-3'
5	UBC-816	5'-CAC ACA CAC ACA CAC AT-3'
6	UBC-817	5'-CAC ACA CAC ACA CAC AA-3'
7	*UBC-819	5'-GTG TGT GTG TGT GTG TA-3'
8	UBC-821	5'- GTG TGT GTG TGT GTG TT-3'
9	*UBC-825	5'-ACA CAC ACA CAC ACA CT-3'
10	UBC-827	5'-ACA CAC ACA CAC ACA CG-3'
11	UBC-837	5'-TAT ATA TAT ATA TAT ART-3'
12	UBC-843	5'-CTC TCT CTC TCT CTC TRA-3'
13	*UBC-850	5'-GTG TGT GTG TGT GTG TYC-3'
14	*UBC-854	5'-TCT CTC TCT CTC TCT CRG-3'
15	UBC-857	5'-ACA CAC ACA CAC ACA CYG-3'
16	UBC-873	5'-GAC AGA CAG ACA GAC A-3'
17	*UBC-875	5'-CTA GCT AGC TAG CTA G-3'
18	UBC-877	5'-TGC ATG CAT GCA TGC A-3'
19	ITS-4	5'-TCC TCC GCT TAT TGA TAT GC-3'
20	ITS-5	5'-GGA AGT AAA AGT CGT AAC AAG G-3'

*Primers selected for further study

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

Chilli is one of the most extensively grown spice crops in south Gujarat. It is severely affected by die back and fruit rot disease infecting fruits resulting in fruit loss and posing a serious threat to its cultivation in south Gujarat. Choudhary (1957), Gupta and Thind (2006) and Masoodi *et al,* (2012) earlier reported highest incidence, intensity of die back and fruit rot of chilli and fruit yield losses, (61.36%, 28.75%, 76.29%), respectively. Considering the seriousness of the problem and lack of systematic studies on this disease under south Gujarat condition, present investigation were carried out with a view to study variability in die back and fruit rot fungus. Variability in the pathogen is main cause of deterioration of cultivated chilli varieties, considered disease resistant at the time of their release. Thus, appearance of new race of *Colletotrichum gloeosporioides* Penz. and Sacc., is an important factor in the die back and fruit rot epidemic and the failure of popular varieties.

4.1 Pathological investigations

4.1.1 Symptoms

Under the natural conditions, the symptoms observed in the infected chilli fields during the survey are as under.

Die back:

Symptoms on the branches first appeared as yellowing of the upper leaves on showed blighting of leaves in advance stage. (Plate-I A). Sunken lesions developed from the apex which

becomes dark black coloured in advance stage of disease resulting in quick dried up portion when the typical die back set in. A few branches of the same plant might show these symptoms simultaneously. Acervuli appeared as minute grey to almost black dots on the discoloured twigs. These symptoms are more or less similar with the symptoms described by Roy (1974) and Gupta and Thind (2006).

Fruit rot:

On ripe fruit, the lesions appeared as slightly sunken, circular to irregular discoloured or yellowish straw coloured spots on fruits later on turning light brown with acervuli either scattered (Plates I-B). These are similar to those described by Natarajan and Subramanian (1973). In severe case fruits get detached from the petioles and fall on the ground.

4.1.2 Collection of die back and fruit rot samples and isolation of pathogen

The sample of infected chilli leaves and fruits showing die back and fruit rot symptoms collected from the various locations and from different varieties from south Gujarat region and disease sample were subjected to tissue isolations, yielded pure culture of *Colletotrichum* sp., which was further purified by single spore isolation technique and maintained on potato

A. Initial symptons of die back

B. Fruit rot infected on chilli fruit.

Plate I: Symptons of die back and fruit rot infected on chilli fruit caused by *C. gloeosporioides* Penz. and Sacc.

dextrose agar (PDA) slants for further investigation.

The samples showing typical die back and fruit rot symptoms on all infected varieties which were collected from different locations and varieties yielded pure culture indicating its constant association with the disease.

4.1.3 Cultural characters

Colonies grew fairly fast on PDA covering whole Petri plate within 9-10 days at temperature 27 ± 2°C. Initially the fungus produced white cottony mycelium, which turned light grey and finally ashy grey. Acervulus produced 12 days after inoculation (Plate II).

Plate II: Photograph showing of growth of *C. gloeosporioides* on PDA at 27±2⁰ C temperature after ten days of incubation.

4.1.4 Morphological characters

In all the cases, mycelium was light grey to ashy grey and cottony. The hyphae were thin, branched, hyaline, septate containing oil droplets. Conidia were hyaline, one celled, oblong in shaped. The acervuli consisted of a mass of short, closely packed, short conidiophores having conidia and dark brown to almost black setae (Plate VI and VII).

4.1.5 Taxonomy and identification

The isolates of *Colletotrichum* sp. obtained by tissue isolation from the leaves and fruits of infected chilli were purified by single spore isolation technique. The morphological and cultural characters of the pure culture grown on PDA were studied for identification and taxonomy.

The studies on morphological and cultural characters of isolated *Colletotrichum* sp. showed its close identity with *Colletotrichum gloeosporioides* Penz. and Sacc. As described by earlier workers (Verma, 1966; Natarajan and Subramanian, 1973; Roy *et al.*, 1974; Sharma and Verma, 1999; and Pandey, 2006).

Further, pure culture was sent for identification to Indian Type Culture Collection (I. T. C. C), Division of Mycology and Plant Pathology, I. A. R. I., New Delhi-110 012 and was identified as *Colletotrichum gloeosporioides* Penz. and Sacc. (ITCC No. 8775-12). The fungus also produced die back and fruit rot symptoms under pathogenicity tests. Thus *Colletotrichum* sp. under study was identified and confirmed as *Colletotrichum gloeosporioides* Penz. and Sacc.

4.1.6 Pathogenicity test

For proving the pathogenic ability of the fungus *Colletotrichum gloeosporioides* isolated from the die back and fruit rot infected fruits, inoculation were carried out on healthy fruits with and without injury by pin pricking, tooth brush and carborandum powder method. The results are presented in Table-3

It is clearly indicated from the data presented in table-3 that the fungus *Colletotrichum gloeosporioides* was capable of infecting fruit of chilli. The pathogen invaded the host tissues through the intact as well as injured surface of fruits showing clearly their pathogenic ability. All the three different method of inoculation *viz.*, pin pricking, tooth brush and carborandum powder injury method confirmed its pathogenic nature producing fruit rot symptoms on the inoculated green fruits. All the method appeared successfully in reproducing the symptoms on artificially inoculated healthy green fruits. Among the three method; pin prick method is most superior followed by tooth brush and carborandum powder injury method. The disease producing efficiency by these three method reported here is in line with the finding of Natarajan and Subramanian (1973), Patel (1978), Patel (2000) and Patel (2004) who reported for proving the pathogenicity of chilli, mango, and turmeric, respectively.

Table 3: Pathogenicity test of inoculation of *Colletotrichum gloeosporioides* by different inoculation method on fruit of chilli for fruit rot symptoms after ten days inoculation.

Sr. no.	Inoculation method	Number of green fruit		Per cent Infection
		Inoculated	Infected	
1.	Injury by pin prick	10	10	100
2.	Injury by tooth brush	10	06	60
3.	Injury by carborandum powder	10	06	60
4.	Control (Spray sterilized distilled water on uninjured surface)	10	00	00

4.2 Pathogenic, cultural and morphological variation:

4.2.1 Pathogenic variation

Pathogenic variability of ten isolates of *C. gloeosporioides* were carried out on a chilli fruit of ten differential varieties by using pin pricking method of inoculation as deseriabed earlier. The data of the disease score indicated the differential reactions on host genotype. Isolates of *C. gloeosporioides* indicating, differential pathogenic behavior within isolates (Table 4).

The data of the disease score indicated that all the ten isolates showed more or less pathogenic behaviour against ten different varieties. In the present study, all the isolates were

showed moderately to highly susceptible reaction against variety SG-5, GVC-121 and GVC-111. Isolate CCG-2 (20 PDI), CCG-9 (20 PDI) and CCG-10 (20 PDI), showed resistance reaction against variety Chilli-516 and Chilli-4884, respectively (Table 4). All the ten isolates were shown moderately resistant to moderately susceptible reaction against all other different chili varieties showed in table.

Plate III: Pathogenic varibility of 10 isolates of *C. gloeosporioides* Penz. and Sacc. against 10 host different varieties of chilli.

Table 4: Pathogenic variability among different isolates of *Colletotrichum gloeosporioides* Penz. and Sacc. on ten different varieties of chilli (in severity per cent).

Isolates / Varieties	CCG-1		CCG-2		CCG-3		CCG-4		CCG-5		CCG-6		CCG-7		CCG-8		CCG-9		CCG-10	
	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE	PDI	RE
Chilli-516	30	MR	20	R	45	MS	60	S	40	MR	45	MS	46	MS	25	MR	30	MR	54	S
SG-5	67	S	65	S	50	MS	50	MS	75	HS	45	MS	55	S	75	HS	75	HS	60	S
GVC-121	70	S	80	HS	90	HS	54	S	66	S	55	S	70	S	75	HS	67	S	66	S
GAVCH-1	80	HS	90	HS	50	MS	90	HS	90	HS	76	HS	76	HS	35	MR	73	HS	46	MS
Suryamukhi	40	MR	34	MR	88	HS	45	MS	75	HS	35	MR	55	S	30	MR	30	MR	40	MR
Jwala	50	MS	60	S	40	MR	50	MS	50	MS	40	MR	70	S	80	HS	50	MS	50	MS
Chilli-4884	80	HS	60	S	35	MR	67	S	60	S	30	MR	30	MR	27	MR	20	R	20	R
S-49	66	S	74	HS	62	S	40	MR	70	S	30	MR	50	MS	60	S	60	S	40	MR
GVC-111	42	MS	80	HS	68	S	65	S	62	S	46	MS	70	S	65	S	68	S	70	S
G-4	50	MS	30	MR	40	MR	47	MS	35	MR	35	MR	30	MR	34	MR	30	MR	46	MS

R= Resistant, **MR**= Moderately Resistant, **MS**= Moderately Susceptible, **S**= Susceptible, **PDI**= Percent Disease Inensity) **RE**= Reaction.

The study conducted by Manandhar, *et al.* (1995) suggested that the isolates of anthracnose and die back pathogen with differential pathogenicity were in existence in the country. It appears necessary to exercise much great care and discretion in selection of *C. gloeosporioides* isolates for screening chilli varieties. It appears desirable to make regular collection of prevailing isolates of *Colletotrichum gloeosporioides* pathogen in nature to keep the track for determining the existence and changes in population of parasitic races in the country or region.

Pathogenicity tests divided the pathogenic potential of *C. gloeosporioides* into low, medium and high-virulence groups. It clearly revealed that ten isolates of *C. gloeosporioides* inoculated on twenty four standard differentials were the most virulent isolates. Pathogenicity behaviour is supported by the earlier studies (Thakare and Patil, 1995 and Than *et al.*, 2008).

Masoodi *et al.* (2012) evaluated twenty isolates of die back and fruit rot pathogen and observed that twenty genotype of chilli exhibited susceptible reaction, on the other differential line *viz.*, SH-SC-17, SH-SC-17, KL-1, SH-SC-111, SH-SC-23, SH-SC-7, SH-SC-4, SH-SC-13, SH-SC-444, SH-P-104 and SH-P-201 whereas other genotype of chilli produced resistance reaction on above genotype of chilli. Smith and Black (1990) evaluated the pathogenic variability in different isolates of *C. gloeosporioides Penz. and Sacc.* showing different pathogenic behaviour of the isolates on different host. Manadhar *et al.* (1995) evaluated response of chilli varieties to different isolates of *C. gloeosporioides Penz. and Sacc.* in which they observed that

different isolates gave various degree of resistance or susceptible reaction on different varieties.

Rao *et al.* (1998) investigated pathogenic diversity among isolates of *C. graminicola* and observed that the isolates Cg 042(5.0), Cg 044(5.1), Cg 015(6.0) Cg 035(5.4) and Cg 048(5.9) were more virulent than other isolates. All other isolates were significantly varied in pathogenicity.

Jayalakashmi and seetharaman (1999) have also evaluated 40 chilli varieties against *Colletotrichum* sp. and found reistance only in eight cultivar, whereas remaining cultivars were either moderately susceptible or highly susceptible.

4.2.2 Cultural characteristics

The morphological and cultural variation serves as an aid in differentiation of isolates. All the isolates of *C. gloeosporioides* possessed characteristic feature when culture on PDA. The differences in colony characteristics were observed *viz.*, colony colour, growth, sporulation, acervuli frequency and colony diameter.

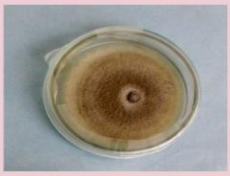
Various isolates produced cottony, fluffy, fluffy cottony, fluffy black and suppressed growth on the PDA with colour range of grey, greyish black to greyish white (Plate IV) on ventral surface whereas the reverse of the colonies were mainly creamish white with some black spots. The colony diameter of different isolates ranged between 65.33 mm to 85.37 mm after ten days of incubation at 27±2^{o}C temperature (Table 5). The isolate CCG-1 showed maximum growth (85.37 mm) which was at par

with CCG-10 and CCG-5 with 82.68 mm and 82.09 mm radial growth respectively. The isolate CCG-7 showed minimum radial growth (65.33 mm).

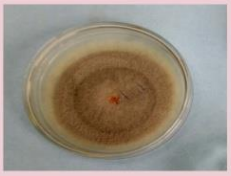
The isolate CGG-9 was showed maximum sporulation (23.90 million spores/ml) which is at par isolates CCG-5 values 23.07 million spores/ml. Minimum sporulation was observed in isolate CCG-7 (8.73 million spores/ml).

Dry mycelial weight was observed in the range of 140.48 mg to 188.84 mg when isolates were cultured on 20 ml PDB for 15 days at 27±2^{o}C temperature. Maximum dry mycelial weight was observed in isolate CCG-1 (188.84 mg) which is at par isolates CCG-10 (182.67 mg). The colony of isolates CCG-1, CCG-2, CCG-3, CCG-4, CCG-5, CCG-8, CCG-9 and CCG-10 showed more or less orange coloured pigmentation while CCG-6 and CCG-7 colonies had no pigmentation (Table 5).

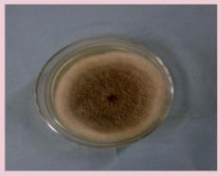
Findings of the cultural variation are correspondence in case of the mycelial growth, colour, sporulation with previous workers. Fokunang *et al.* (2000) observed that maximum sporulation observed in isolate-5 (20.36 million/mg) while minimum in isolate-15 (1.10 million/mg). The colony colour on Potato Dextrose Agar media was white to grayish and fluffy to white while Smith and Black (1990) observed the colony colour of *C. fragariae* isolates was olive to dark gray.



CCG.-1

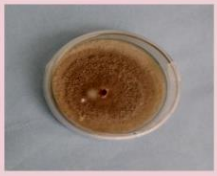


CCG.-2



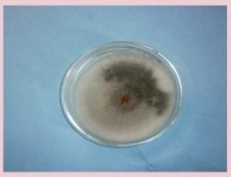
CCG.-3

CCG.-4

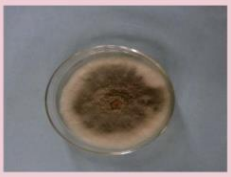


CCG.-5

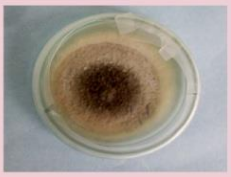
CCG.-6



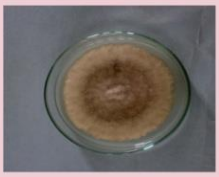
CCG.-7



CCG.-8



CCG.-9



CCG.-10

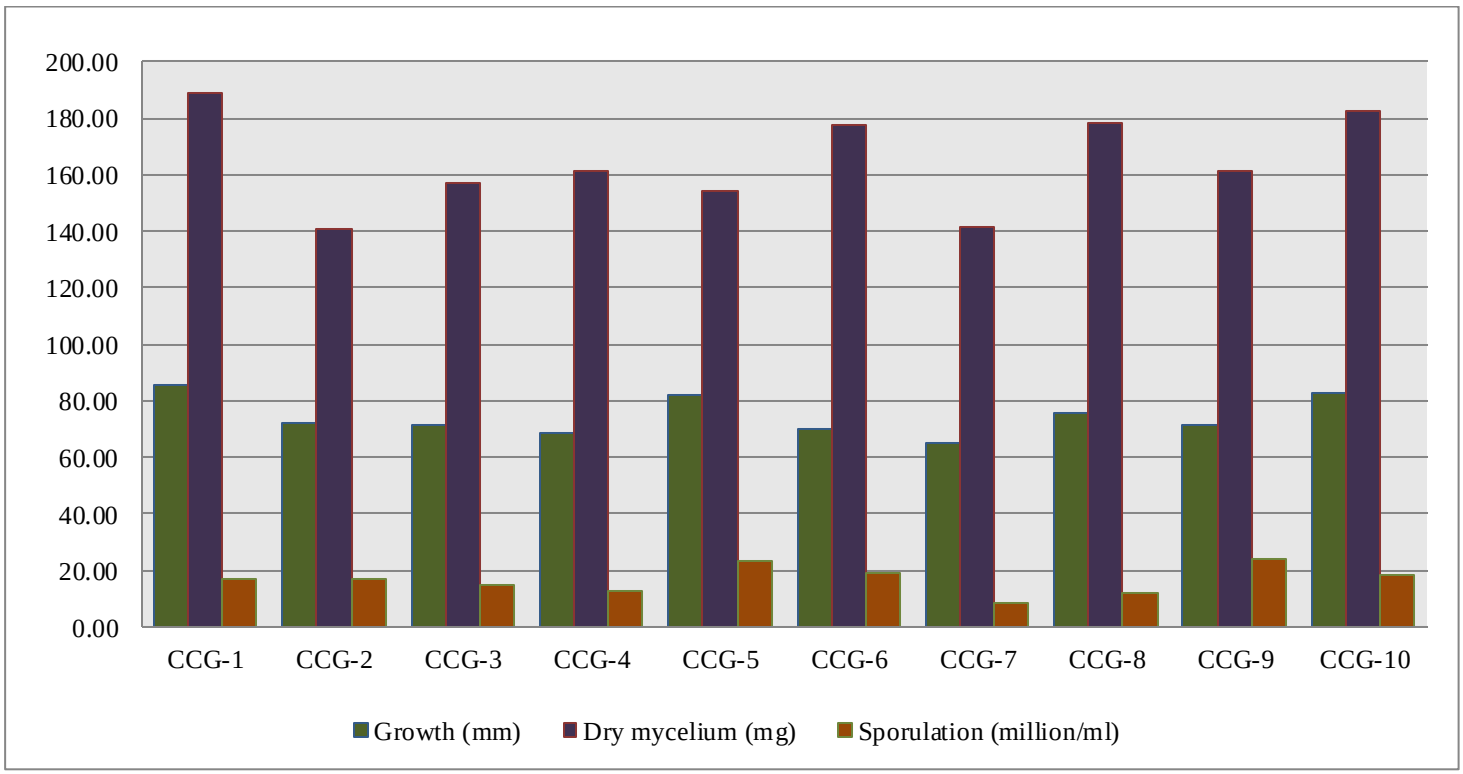
Plate IV: Growth of different isolates of *C. gloeosporioides* Penz. and Sacc. on PDA at 27±2⁰ C temperature after ten days of incubation.

Table 5: Growth, sporulation and growth character of different isolates of *C. gloeosporioides* on PDA at 27±2°C temperature for ten days of incubation and on PDB for fifteen days of incubation

Sr. No.	Isolates	*Growth (mm)	*Dry mycelium weight (mg)	**Sporulation (million/ml)	**Sporulation catagory	*Pigmentation	*Colony colour	*Colony growth
1	CCG – 1	85.37	188.84	17.07	++++	Dark orange	Greyish white	Fluffy cottony
2	CCG – 2	72.03	140.48	17.16	+++	Light orange	Greyish black	black
3	CCG – 3	71.55	156.92	15.07	+++	Light orange	Grey	Fluffy
4	CCG – 4	68.78	160.94	12.73	++	Light orange	Grey	Fluffy
5	CCG – 5	82.09	154.22	23.07	++++	Dark orange	Greyish white	Fluffy cottony
6	CCG – 6	70.18	177.84	18.81	+	Absent	Whitish grey	Suppressed
7	CCG – 7	65.33	141.33	8.73	+	Absent	Whitish grey	Suppressed
8	CCG – 8	75.32	178.00	12.07	++++	Light orange	Greyish white	Fluffy
9	CCG – 9	71.46	160.94	23.90	+++	Light orange	Greyish white	Fluffy
10	CCG - 10	82.68	182.67	18.07	++	Light orange	Greyish white	Fluffy cottony
	S. Em. ±	1.143	2.887	0.449				
	C.D. at 5%	3.37	8.52	1.32				

* On PDA (Average of three replications)
** On PDB (Average of three replications)
Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abudent

Fig 1: Growth, sporulation and dry mycelial weight of different isolates of *Colletotrichum gloeosporioides* Penz. and Sacc., on PDA and PDB at 27±2°C temperature.



Adaskaveg *et al.* (1997) observed arial mycelium in *C. acutatum* isolates with grayish to pink with bright orange spore masses produced outward from the centre of the culture, whereas the reverse was orangish or occasionally reddish to gray.

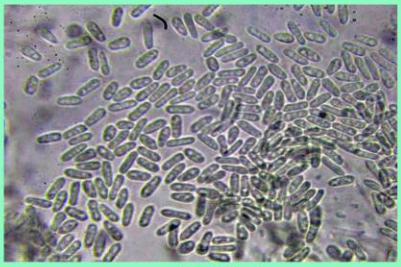
4.2.3 Morphological characteristic

4.2.3.1 Conidia

The variation in conidial measurement like length and breadth between the isolates has been reported by several workers. In the present study also conidial length among the isolates varied from 14.67 µ to 29.26 µ while width of conidia varied from 2.82 µ to 6.07 µ. The length of the conidia was

bigger in the isolate CCG-8 (29.26 µ) than other isolates which is at par with isolate CCG-5 (28.60 µ). Smaller conidial length was observed in isolate CCG-3 (14.67 µ). The larger conidial width was observed in isolate CCG-5 (6.07 µ) which is at par with isolates CCG-8 (5.79 µ) and CCG-4 (5.72 µ) (Table 6).

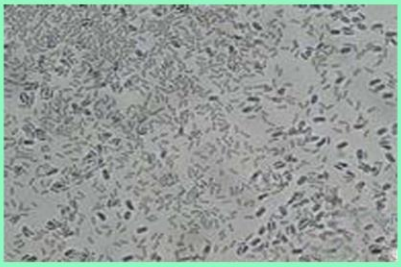
The structure of conidia was oblong with rounded ends shaped among the ten isolates. There was difference in the presence of oil globules in conidia. The isolates CCG-1, CCG-5 and CCG-10 were showed larger and less oil globules while other were showed very small and more number of oil globules (Plate V).



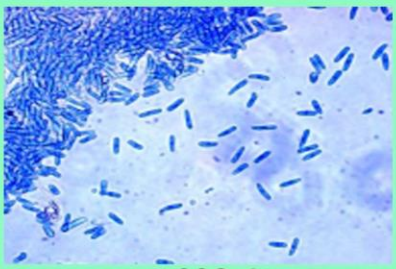
CCG.-1

CCG.-6

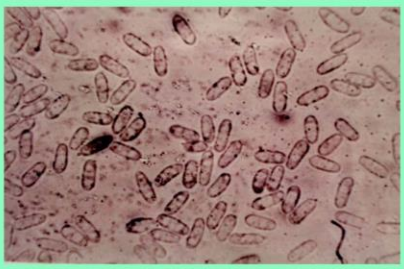
CCG.-2



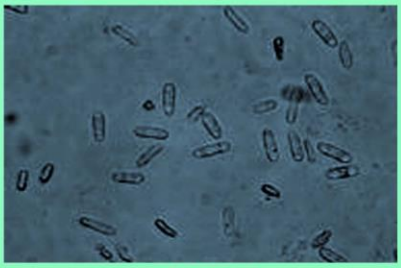
CCG.-7



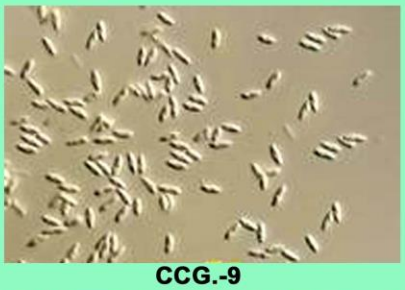
CCG.-3



CCG.-8

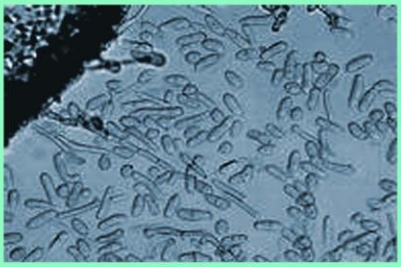


CCG.-4



CCG.-9

CCG.-6



CCG.-10

Plate V: Photomicrograph showing conidia of different isolates of *C. gloeosporioides* Penz. and Sacc. on PDA at 27±2^0 C temperature after ten days of incubation (450X)

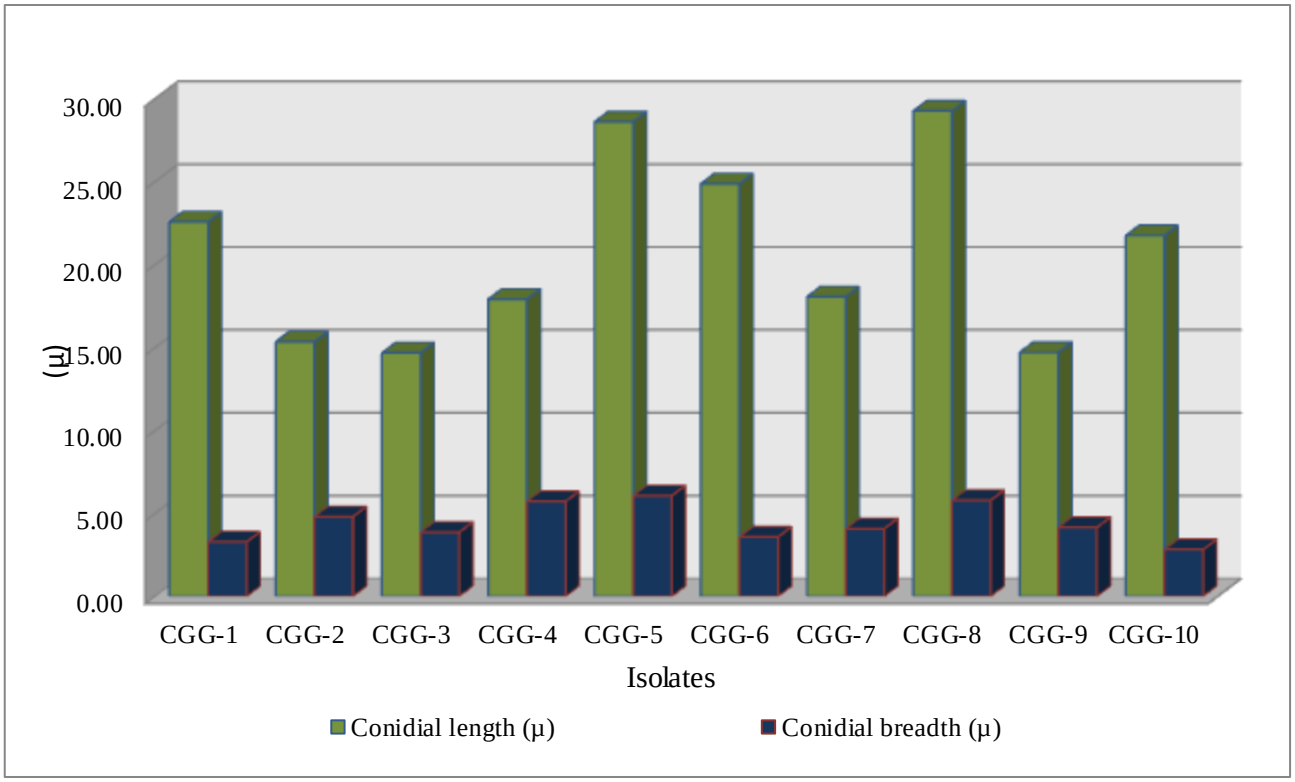
Table 6: Variation in length and breadth of conidia (µ) among isolates of *C. gloeosporioides* Penz and Sacc. on PDA at 27±2°C temperature after ten days of incubation

Sr. no.	Isolates	**Conidial length (µ)	*Mean	**Conidial breadth (µ)	*Mean
1	CCG-1	21.77-23.44	22.57	3.11-3.47	3.28
2	CCG-2	14.61-16.11	15.34	4.62-4.99	4.80
3	CCG-3	13.99-15.42	14.67	3.63-4.08	3.87
4	CCG-4	17.01-18.86	17.91	5.68-5.76	5.72
5	CCG-5	27.65-29.68	28.60	5.65-6.34	6.07
6	CCG-6	23.98-25.99	24.86	3.33-3.88	3.58
7	CCG-7	17.34-18.88	18.06	3.92-4.28	4.08
8	CCG-8	28.16-30.40	29.26	5.71-5.84	5.79
9	CCG-9	14.01-15.44	14.70	3.98-4.34	4.15
10	CCG-10	20.87-22.58	21.75	2.62-2.99	2.82
	S. Em. ±		0.51		0.12
	CD at 0.05%		1.51		0.36
	CV %		4.26		4.77

*The values are average of three replications

**The values are the range of minimum and maximum value

Fig 2: Variation in length and breadth of conidia in µ among isolates of *C. gloeosporioides* Penz. and Sacc., on PDA at 27±2°C temperature after ten days of incubation.



4.2.3.2 Conidiophore

The variation in conidiophores also recorded. The length of conidiophores varied from 30.05 µ to 56.60 µ while the width of conidiophores ranged from 3.40 µ to 6.64 µ. Maximum length of conidiophore was recorded in isolate CCG-8 (56.60 µ) which is at par with isolate CCG-4 (54.28 µ). Minimum length of conidiophore observed in the isolate CCG-3 (30.05 µ). Maximum width of conidiophore observed in the isolate CCG-5 (6.64 µ) which is at par with isolates CCG-8 (6.25 µ) and CCG-4 (6.42 µ). The minimum width of conidiophore was observed in isolates CCG-10 (3.46 µ), (Table 7).

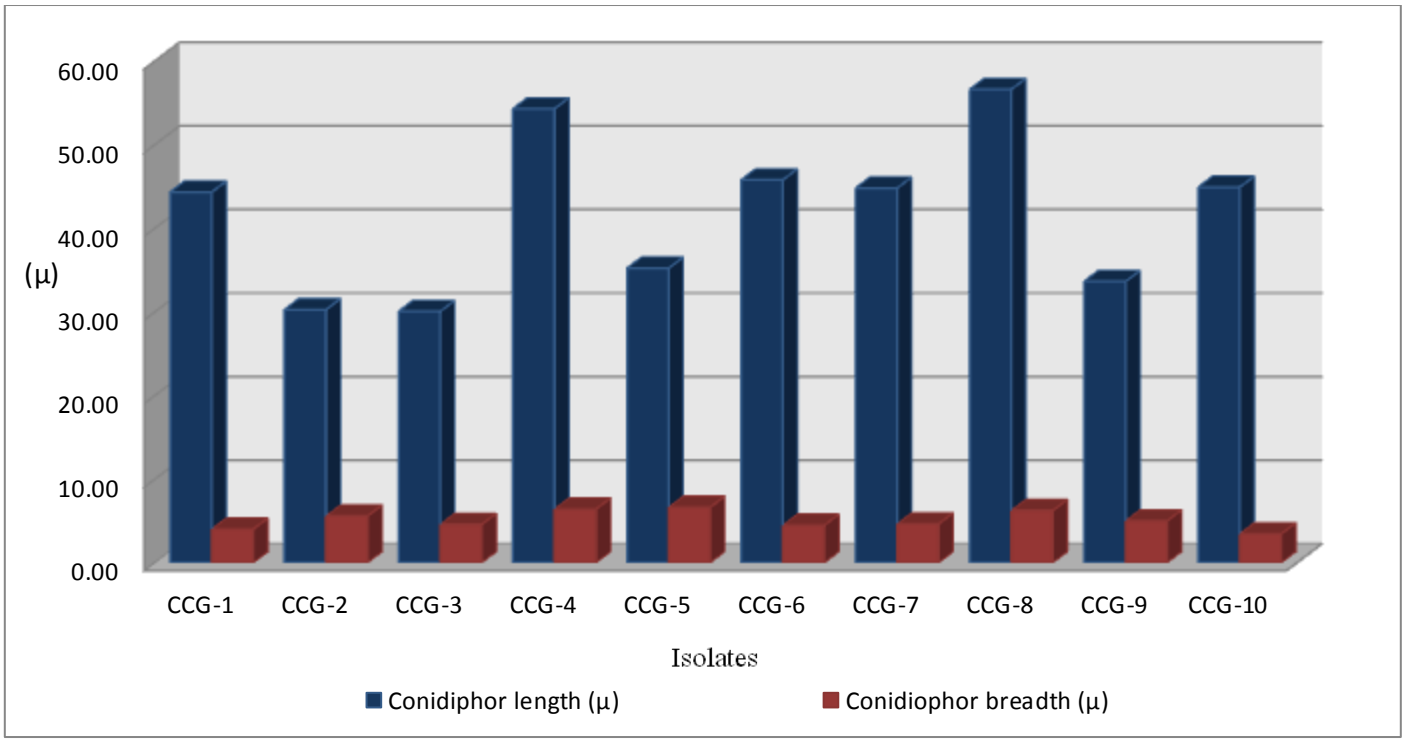
Table 7: Variation in length and breadth of conidiophores (µ) among isolates of *C. gloeosporioides* Penz and Sacc. on PDA at 27±2°C temperature after ten days of incubation

Sr. no.	Isolates	**Conidiophore length (µ)	*Mean	**Conidiophore breadth (µ)	*Mean
1	CCG-1	42.33-46.30	44.33	3.72-4.37	4.03
2	CCG-2	28.49-32.02	30.26	5.38-5.84	5.59
3	CCG-3	28.38-31.70	30.05	4.35-4.85	4.60
4	CCG-4	51.58-56.98	54.28	6.22-6.68	6.42
5	CCG-5	32.89-37.54	35.23	6.41-6.88	6.64
6	CCG-6	43.18-48.38	45.79	4.06-4.99	4.50
7	CCG-7	42.05-47.52	44.80	4.42-4.89	4.65
8	CCG-8	53.85-59.33	56.60	5.94-6.46	6.25
9	CCG-9	31.93-35.31	33.63	4.90-5.16	5.01
10	CCG-10	43.13-46.79	44.91	3.24-3.72	3.46
	S. Em. ± CD at 0.05% CV %	1.30 3.83 5.35			0.16 0.47 5.37

*The values are average of three replications

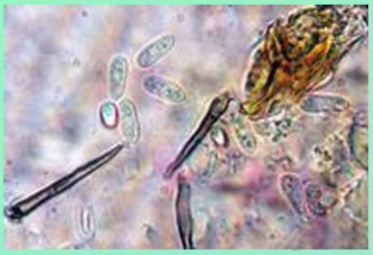
**The values are the range of minimum and maximum value

Fig 3: Variation in length and breadth of conidiophore in µ among isolates of *C. gloeosporioides* Penz. and Sacc., on PDA at 27±2°C temperature after ten days of incubation.



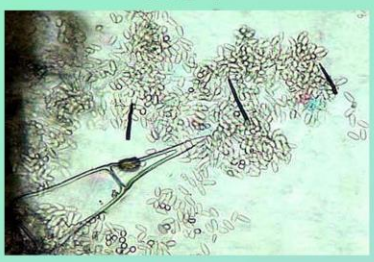
4.2.3.3 Acervulus

The length of the acervuli ranged from 63.77 µ to 74.16 µ as well as width was 40.89 µ to 53.85 µ. The maximum length of acervulus was observed in the isolate CCG-7 (74.16 µ) which is at par with isolate CCG-2 (67.08 µ), CCG-8 (69.80 µ), CCG-4 (70.59 µ), CCG-6 (73.24 µ) and CCG-9 (73.85 µ). Minimum length was observed in the isolate CCG-1 (63.77 µ). Maximum width of acervulus was observed in isolate CCG-9 (53.85 µ) which is at par with CCG-2 (48.61 µ), CCG-7 (53.45 µ), CCG-8 (48.41 µ) and CCG-10 (48.55 µ) while minimum width was observed in isolate CCG-4 (40.89 µ), (Table 8).



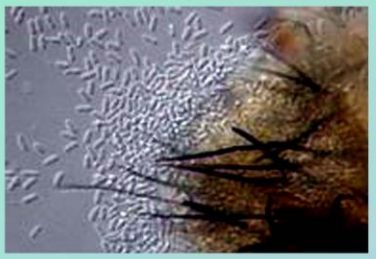
CCG-1

CCG-6



CCG-2

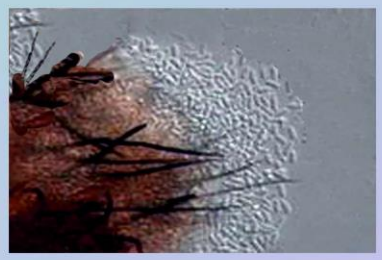
CCG-7



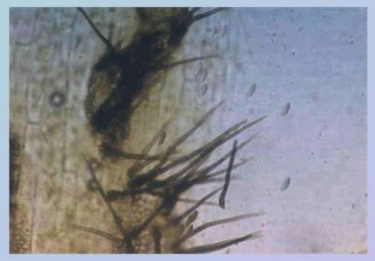
CCG-3

CCG-8

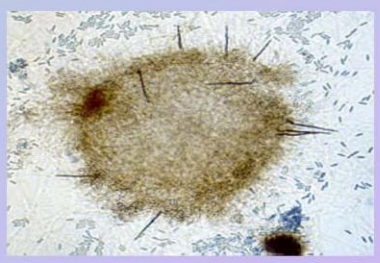
CCG-4



CCG-9



CCG-5



CCG-10

Plate VI Photomicrograph showing acervuli fromed in different isolates of *C. gloeosporioides* Penz. and Sacc. on PDA at 27±2^{0}C temperature after ten days of incubation (450X)

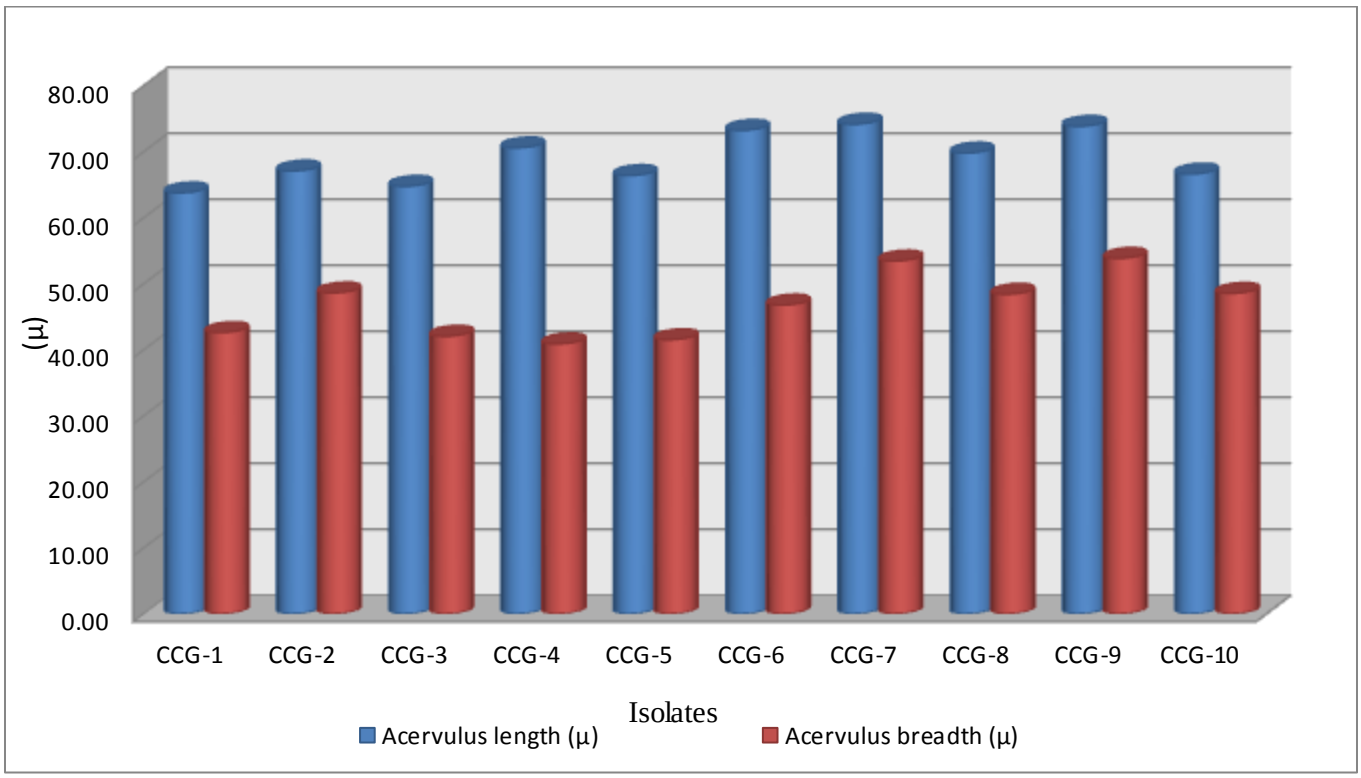
Table 8: Variation in length and breadth of acevulus (µ) among isolates of *C. gloeosporioides* Penz and Sacc. on PDA at 27±2°C temperature after ten days of incubation

Sr. no.	Isolates	**Acervulus length (µ)	*Mean	**Acervulus breadth (µ)	*Mean
1	CCG-1	60.93-66.61	63.77	38.76-46.47	42.61
2	CCG-2	63.08-70.0	67.08	44.15-53.62	48.61
3	CCG-3	58.64-71.24	64.76	39.00-44.98	42.06
4	CCG-4	64.94-77.06	70.59	37.83-43.95	40.89
5	CCG-5	63.58-69.38	66.40	39.57-43.13	41.53
6	CCG-6	68.57-78.20	73.24	43.49-50.42	46.78
7	CCG-7	70.59-78.34	74.16	50.08-56.59	53.45
8	CCG-8	65.02-74.17	69.80	47.50-49.51	48.41
9	CCG-9	70.26-77.45	73.85	50.43-57.28	53.85
10	CCG-10	64.53-68.84	66.62	45.70-51.52	48.55
	S. Em. ± CD at 0.05% CV %		2.47 7.30 6.21		1.85 5.47 6.88

*The values are average of three replications

**The values are the range of minimum and maximum value

Fig 4: Variation in length and breadth of acervulus in µ among isolates of *C. gloeosporioides* Penz. and Sacc., on PDA at 27±2°C temperature after ten days of incubation.



4.2.3.4 Appresoria

The length and width of appresoria was set out between 11.06 µ to 12.95 µ and 10.08 µ to 12.10 µ respectively. Maximum length was observed in the isolate CCG-3 (12.95 µ) which was at par with isolate CCG-1 (12.34 µ), CCG-4 (12.60 µ), and CCG-10 (12.21 µ) while minimum length was observed in isolate CCG-6 (11.06 µ). The maximum width of appresoria was recorded in isolate CCG-4 (12.10 µ) which was at par with isolate CCG-5 (11.16 µ), CCG-7 (11.70 µ) and CCG-10 (11.38 µ) respectively. Minimum width was observed in isolate CCG-2 (10.08 µ.) (Table-9).

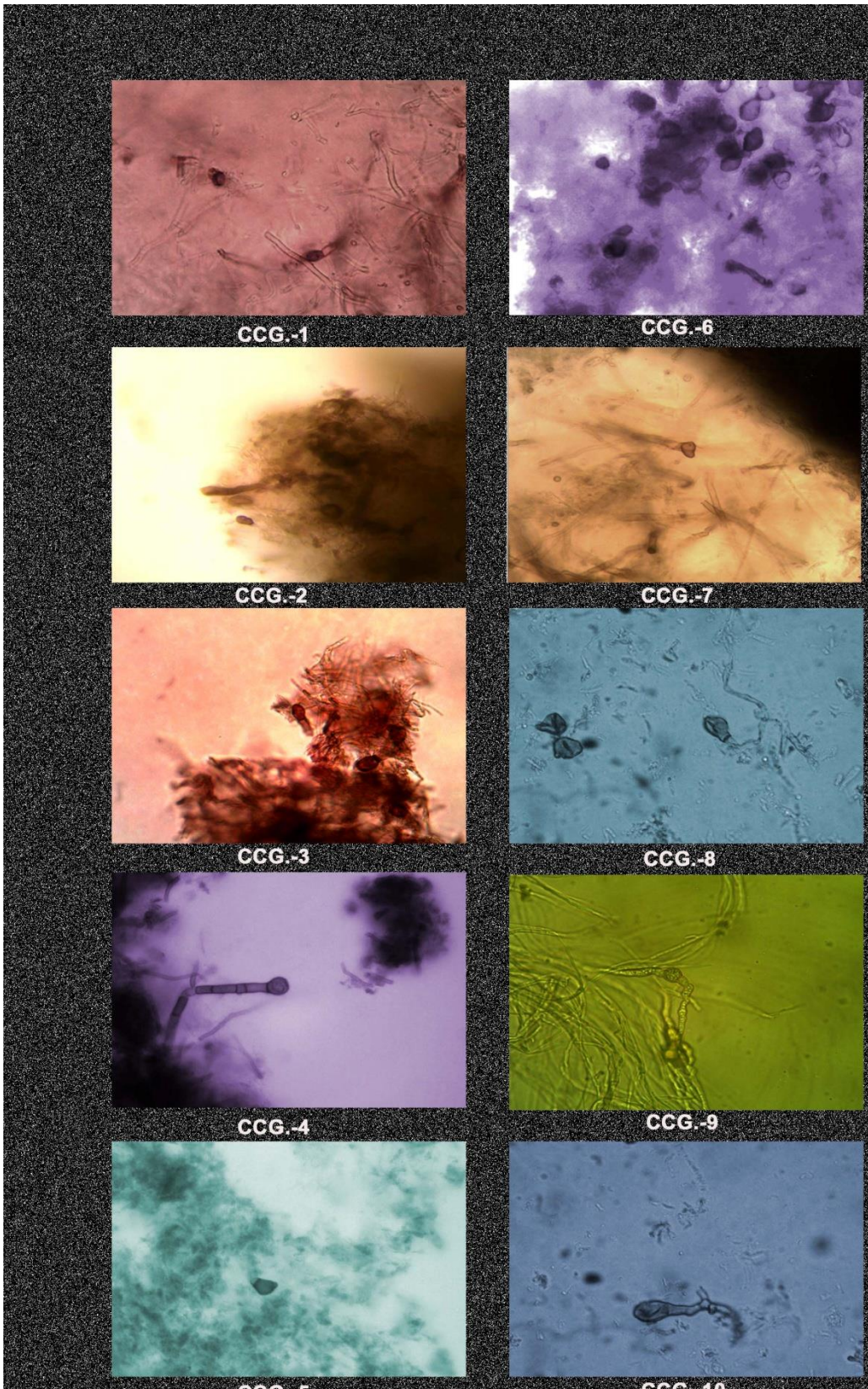


Plate VII: Photomicrograph showing appresoria formed in different isolates of *C. gloeosporioides* Penz. and Sacc. on PDA at 27±2° C temperature after ten days of incubation (450X)

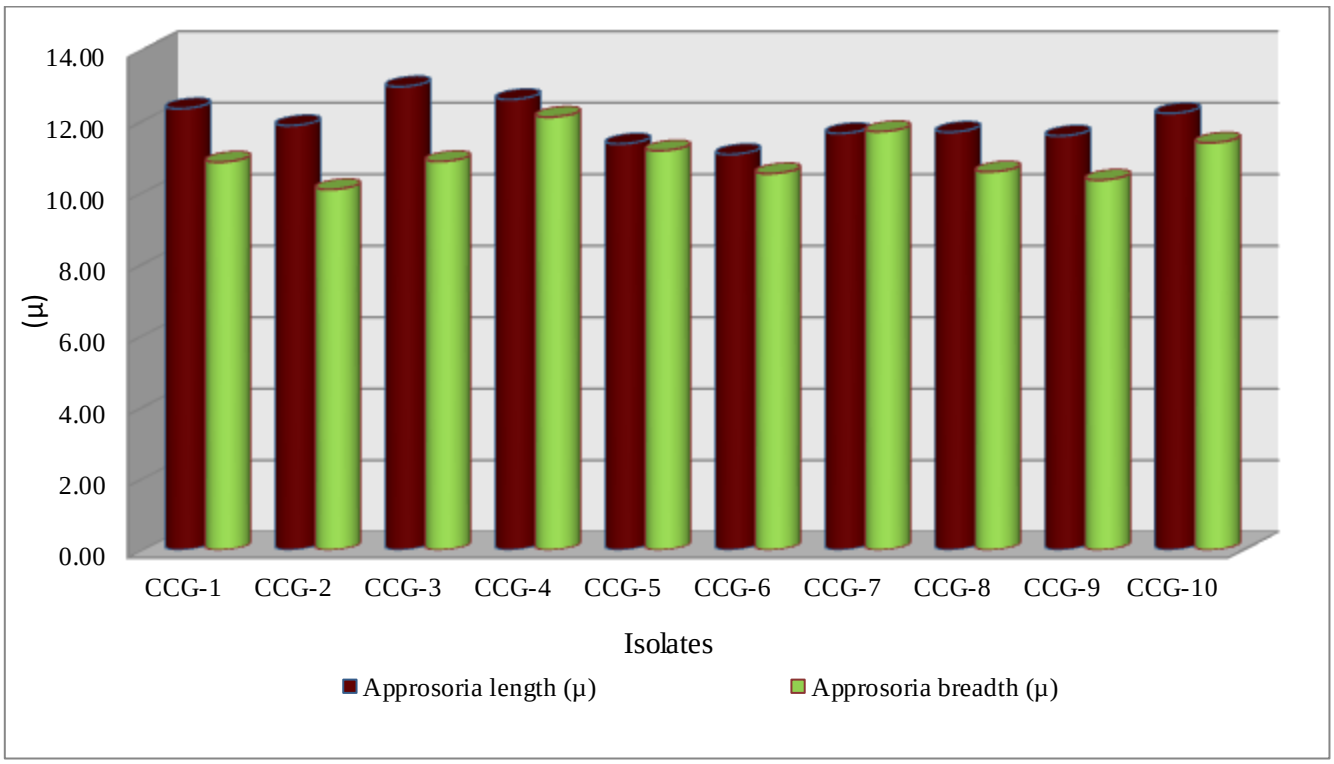
Table 9: Variation in length and breadth of appresoria (µ) among isolates of *C. gloeosporioides* Penz and Sacc. on PDA at 27±2°C temperature after ten days of incubation

Sr. no.	Isolates	**Appresoria length (µ)	*Mean	**Appresoria width (µ)	*Mean
1	CCG-1	11.84-12.82	12.34	10.21-11.30	10.85
2	CCG-2	11.26-12.50	11.87	9.47-10.40	10.08
3	CCG-3	12.26-13.60	12.95	10.20-11.52	10.86
4	CCG-4	12.01-13.18	12.60	11.57-12.63	12.10
5	CCG-5	10.83-11.90	11.35	10.41-11.62	11.16
6	CCG-6	10.60-11.44	11.06	9.96-11.12	10.52
7	CCG-7	11.24-12.05	11.65	11.26-12.13	11.70
8	CCG-8	11.15-12.16	11.68	9.82-12.21	10.57
9	CCG-9	11.09-12.06	11.58	9.54-11.09	10.34
10	CCG-10	11.56-12.78	12.21	11.09-11.63	11.38
	S. Em. ± CD at 0.05% CV %		0.31 0.92 4.53		0.34 1.00 5.35

*The values are average of three replications

**The values are the range of minimum and maximum value

Fig 5: Variation in length and breadth of approsoria in µ among isolates of *C. gloeosporioides* Penz. and Sacc., on PDA at 27±2°C temperature after ten days of incubation.



All the isolates produced hyline, oblong conidia bearing small oil globules. The different isolates showed smaller to higher degree of variation within different parameters like size of conidia, conidiophore, acervuli, appresoria and length of setae. Such morphological variation in *C. gloeosporioides* has also been observed by many workers (Fokunang *et al.*, 2000 and Rao, *et al.* 1998).

Kumar *et al.*, (2010) observed that measurement in conidia, conidiophores, acervulus varied from each other among ten isolates of *C. gloeosporioides.* The size of conidia, length and width of conidiophores and size of acervulus was maximum in group-1.

4.3 Molecular variation

4.3.1 Identification and evaluation of ISSR markers for diversity estimates in ten *C. gloeosporioides* isolates

In recent years, molecular markers have received arable attention and have been used for genetic diversity phylogenetic and evolutionary studies and mapping and tagging of agronomically important traits in different plant species. It can also be used in the study of molecular diversity in microorganism such as fungus, bacteria, viruses, etc. In this study, the present investigation was carried out with the ten different isolates of fungus *C. gloeosporioides* to study the molecular variation in these isolates.

A total of 20 Primers consisted of di-nucleotide repeat motifs were used for initial screening with two isolates. Out of these, fifteen primers gave no amplification at all, while only five primers were found to give clear banding patterns and were subsequently used to analyze the entire set of ten isolates. The oligonucleotide sequences of these primers are given in Table 10. These five ISSR primers amplified a total of 64 scorable bands of which 59 bands were polymorphic. These primers showed variation in the percentage of polymorphism. The percent of polymorphism ranged from 81.25 to 100.00%. The primer UBC-850 and UBC-854 showed the highest value of percentage of polymorphism i.e. (100.00%).

Table 10: Details of amplification obtained with different ISSR primers

Sr. No.	Name of primer	Sequence	Annealing temperature (^oC)	No. of total bands	No. of polymorphic bands	No. of mono morphic bands	Polymorphism percent (P %)	Total No. of bands amplified in 10 isolates
1.	UBC-819	5'-$(GT)_8$ TA-3'	50.3	12	11	1	91.67	67
2.	UBC-825	5'-$(AC)_8$ T-3'	51.4	13	12	1	92.30	70
3.	UBC-850	5'-$(GT)_8$ YC-3'	52.7	11	11	0	100.00	52
4.	UBC-854	5'-$(TC)_8$ RG-3'	54.3	12	12	0	100.00	49
5.	UBC-875	5'-$(CTAG)_4$-3'	43.9	16	13	3	81.25	97
Total				64	59	5	92.19	335

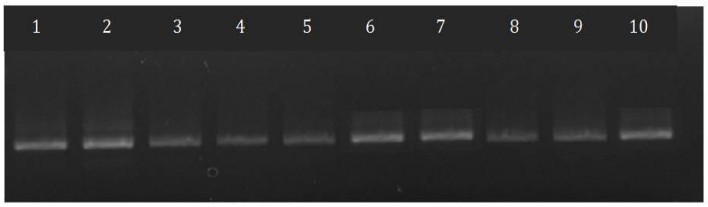


Plate 8 A. Genomic DNA of ten *C. gloeosporioides* isolates

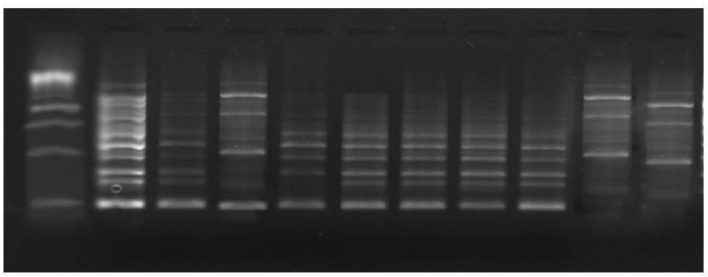


Plate 8 B. ISSR amplification pattern of ten *C. gloeosporioides* isolates using primer UBC-819.

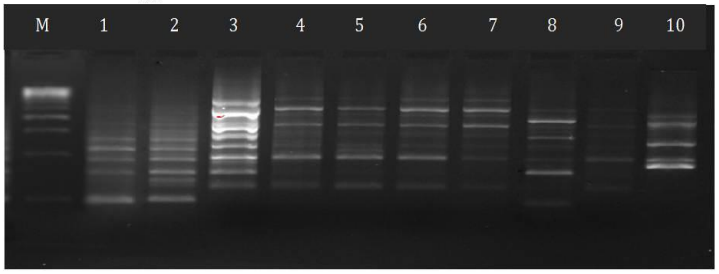


Plate 8 C. ISSR amplification pattern of ten *C. gloeosporioides* isolates using primer UBC-825.

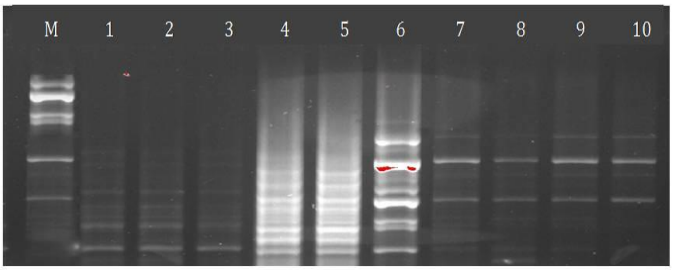


Plate 8 D. ISSR amplification pattern of ten *C. gloeosporioides* isolates using primer UBC-850.

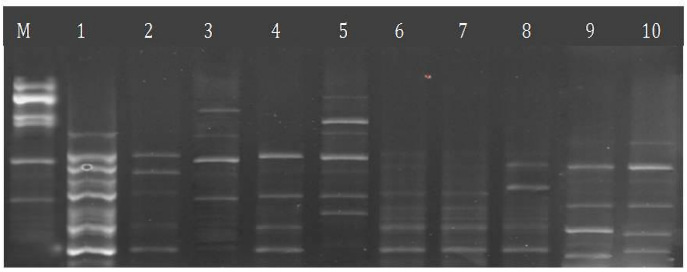


Plate 8 E. ISSR amplification pattern of ten *C. gloeosporioides* isolates using primer UBC-854.

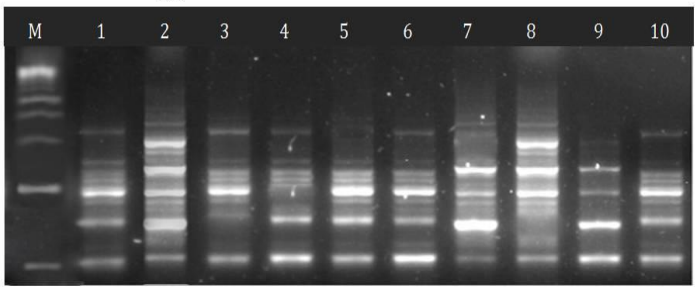


Plate 8 F. ISSR amplification pattern of ten *C. gloeosporioides* isolates using primer UBC-875.

Amplification pattern obtained using primers UBC-819, UBC-825, UBC-850, UBC-854 and UBC-875 are depicted as representative pictures in Plate VIII B to VIII F. This primer UBC-819 has amplified a total number of 12 bands (Plate VIII B). Out of these 12 bands 11 were polymorphic while one was monomorphic amplification pattern. In same Plate, amplification pattern of the primer UBC-825 showed a total of 13 scorable bands, out of which 12 were polymorphic (Plate VIII C). A total of 11 bands were amplified on primer UBC-850 shows in (Plate VIII D), out of which 11 were polymorphic while no one was

monomorphic. The banding pattern of primer UBC- 854 showed total of 12 scorable bands out of which 12 were polymorphic while zero was monomorphic (Plate VIII E). The primer UBC-875 was showed more amplification than other primers with 16 scorable bands of which 13 were polymorphic and three were monomorphic as showed in (Plate VIII F).

The ISSR amplification data were used to obtain a similarity matrix (Table 11). A dendrogram was constructed (Figure VI) using UPGMA method for ten isolates under present study. The dendogram clearly indicated the one big cluster of eight isolates while two isolate was separately situated which similarity matrix 0.378 and 0.419 (CCG-9) and (CCG-10) with CCG-1. In these eight isolates CCG-3 was placed separately while others were sub divided in two sub clusters A and B. The cluster A was contained three isolates (CCG-1, CCG-2 and CCG-8) while cluster B contain four isolates (CCG-4, CCG-5, CCG-6 and CCG-7). These two sub clusters showed similarity coefficient of 0.48. The sub-cluster B consisted of isolates CCG-6 and CCG-7 which were most similar which were diversed from isolateCCG-4 and CCG-5.

ISSR markers are useful for studying genetic diversity in *Colletotrichum* species. These are rapid, reproducible and produce a large number of polymorphic bands and aid the understanding of pathogen population dynamics, which can facilitate the development of effective control strategies. The genetic diversity of *C. gloeosporioides* based on ISSR markers showed a correlation between genetic and geographical

distribution which was supported by Ratanacherdchai *et al.* (2010) who reported that *C. capsici* isolates of chilli formed a sub cluster distinct from isolates from Thailand.

The intra and inter specific polymorphism among fungal pathogen that cause wilt and root rot on chick pea were investigated by using 30 RAPD and 20 ISSR primers. According to ISSR analysis, primers $(GA)_8T$, $(AG)_8G$ and $(AC)_8T$ produced amplification profiles that differentiated each fungal species of *Fusarium* (Bayraktar and Sara Dolar, 2007).

Similarly Kumar *et al.*, (2011) utilized nine ISSR markers out of which two ISSR markers, ISSR 02 (ACTG 4) and ISSR 10 (CAC 5) differentiated all the 25 *C. falcatum* isolates with alleles ranging from 250 to 3500 bp. The average number of bands per primer was eight with 100 per cent polymorphism. ISSR 10 performed better in all the marker efficiency parameters.

Table 11: Jaccard's similarity coefficient between ten isolates of *Colletotrichum gloeosporioide* Penz. and Sacc. based on ISSR data.

	CCG-1	CCG-2	CCG-3	CCG-4	CCG-5	CCG-6	CCG-7	CCG-8	CCG-9	CCG-10
CCG-1	1.000									
CCG-2	0.600	1.000								
CCG-3	0.455	0.447	1.000							
CCG-4	0.426	0.479	0.370	1.000						
CCG-5	0.431	0.453	0.468	0.636	1.000					
CCG-6	0.510	0.560	0.489	0.659	0.646	1.000				
CCG-7	0.426	0.543	0.400	0.571	0.565	0.738	1.000			
CCG-8	0.426	0.578	0.313	0.404	0.412	0.553	0.571	1.000		
CCG-9	0.378	0.467	0.208	0.356	0.367	0.417	0.488	0.488	1.000	
CCG-10	0.419	0.383	0.326	0.395	0.347	0.489	0.429	0.429	0.571	1.000

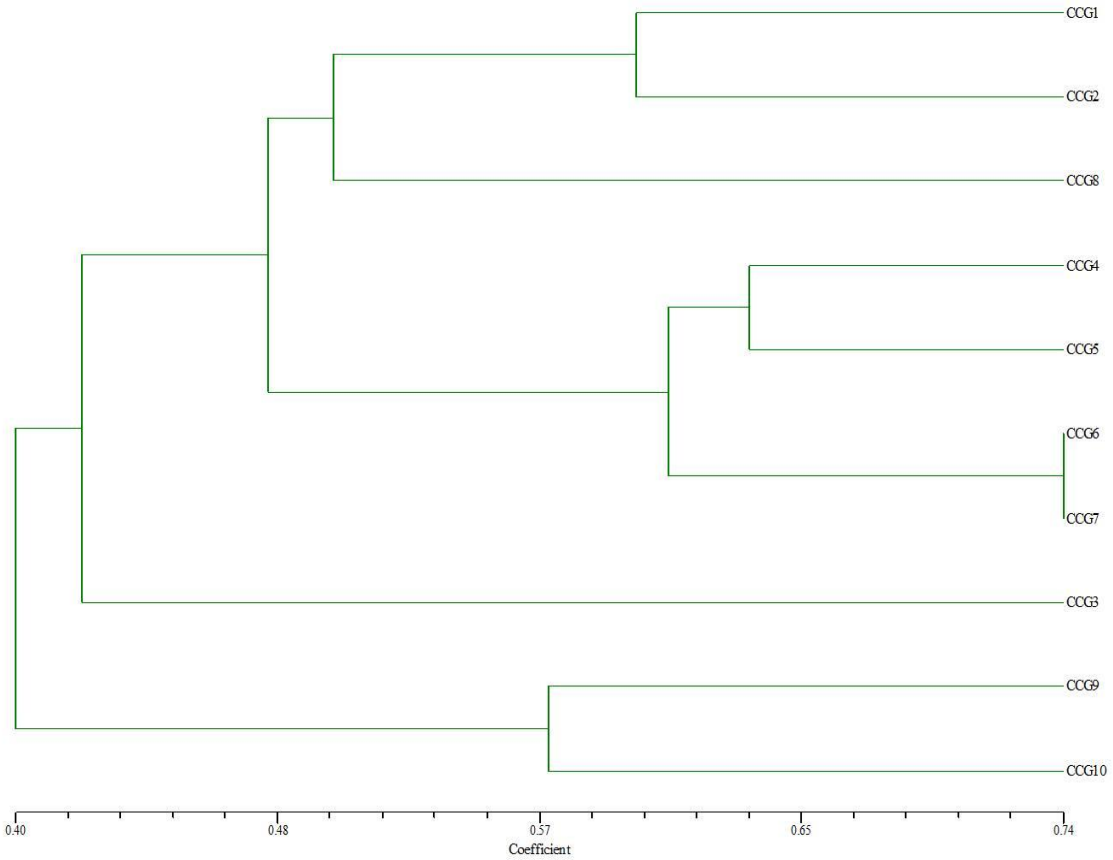


Fig : Dendrogram of average analysis of combined inter Simple Sequence Repeat (ISSR) primers from 10 isolates of *C. gloeosporioides* Penz. and Sacc.

SUMMARY AND CONCLUSION

V. SUMMARY AND CONCLUSION

Chilli is the most important crop grown extensively under South Gujarat conditions. The die back and fruit rot has become the most important disease of chilli during last three years with a severe threat to successful and profitable cultivation of the crop in South Gujarat area. The present investigation was to pin point cause, pathogenic, cultural, morphological and molecular variability among the different isolates of *Colletotrichum gloeosporioides* Penz. and Sacc., which were collected from different locations and from different varieties of chilli south Gujarat.

The microscopic examination and tissue isolation from fruit of the infected chilli plants of different varieties yielded pure culture of *Colletotrichum* sp. The typical symptoms of the disease were observed in the field were on the branches which first appear as yellowing of the upper leaves. Sunken lesion developed from apex which becomes dark black coloured in advance stage of disease resulting in quick dried up portion when the typical die back set in acervuli appeared as minute grey colour to almost black dots on the fruits. On fruits, the lesion appear as slightly sunken, circular to irregular discoloured or yellowish straw coloured, later on turning light brown with acervuli either scattered. In sever cases fruits get detached from the petioles and fall on the ground.

On the basis of morphological and cultural characters *Colletotrichum* sp. was identified as *Colletotrichum*

gloeosporioides Penz. and Sacc. This was also confirmed through identification by I. T. C. C., I. A. R. I., New Delhi (ITCC No. 8775-12). The pathogenicity was tested by injury by pin prick, tooth brush and carborandum powder on fruits. All these methods successfully in reproducing the symptoms on artificially inoculated healthy green fruits. Among the three methods, pin prick method is most superior followed by tooth brush and carborandum powder injury method. Pathogenicity was pin prick method of inoculation produced typical fruit rot symptoms similar to those observed under natural conditions and described in the literature, conferring pathogenic nature of the fungus.

Pathogenic variability of ten isolates on a chilli of differential varieties were carried out by pin prick method of inoculation. The isolates of *C. gloeosporioides* indicating differential pathogenic behaviour. Isolate CCG-2, CCG-9 and CCG-10 showed resistance reaction against variety Chilli-516 and Chilli-4884 respectively.

The isolates of *C. gloeosporioides* possessed characteristic feature when culture on PDA with respect to colony colour, growth, sporulation acervuli frequency, etc. Isolates produced cottony, fluffy, fluffy cottony or suppressed growth with colour grey, greyish black to greyish white the colony diameter ranged between 65.33 mm to 85.37 mm after ten days of incubation at 27 ± 2^{0}C temperature. The isolate CCG-1 was showed maximum radial growth 85.37 mm while isolate CCG-7 was showed minimum radial growth 65.33mm. The maximum sporulation was observed in isolate CCG-9 (23.90 million

spores/ml) while maximum dry mycelial weight was observed in isolate CCG-1 (188.84) when cultured on liquid media. The maximum isolates were showed orangish pigmentation.

Morpholological studies also revealed the variation in size of conidia, conidiophore, acervuli and size of acervulus and appresoria when cultured on PDA at 27±2^{o}C temperature. The maximum length of conidia was observed in isolate CCG-8 (29.26 µ) while maximum breadth was in isolate CCG-5 (6.07 µ). The conidia of *C. gloeosporioides* were falcate in nature with more or less number of oil globules. The isolate CCG-1, CCG-5 and CCG-10 were showed larger and less oil globules while other had small and more oil globules. The maximum length of conidiophore was observed in isolate CCG-8 (56.60 µ) while maximum breadth of conidiophore observed in the isolate CCG-5 (6.64 µ).

The acervulus body also showed variation in different isolates. The maximum length of acervulus was observed in the isolate CCG-7 (74.16 µ) while maximum breadth of acervulus was observed in isolate CCG-9 (53.85 µ).

On the solid culture media also formed appresoria which were varied in their length and width. The length of appresoria was larger in isolate CCG-3 (12.95 µ) while it was smaller in isolate CCG-6 (11.06 µ). The maximum width of appresoria was observed in CCG-4 (12.10 µ) while minimum in isolate CCG-2 (10.08 µ).

The molecular characterization study was carried out by using ISSR primers. The five primers (UBC-819, UBC-825, UBC-850, UBC-854 and UBC-875) were used for analyze the ten

isolates which gave total of 64 scorable bands of which 59 bands were polymorphic. The dendogram was showed isolate CCG-9 and CCG-10 was situated separately with similarity matrix of 0.378 and 0.419 with CCG-1 while other eight were clustered in two groups and sub-groups. The ISSR markers were reveal sufficient genetic diversity. There is need to verify the results with more number of primers as well as isolates.

Present study clearly indicated the variation among ten isolates of *C. gloeosporioides* Penz. and Sacc., collected from South Gujarat region in terms of pathogenic, cultural, morphological and molecular level.

Further extensive study is required to identify the variation among *C. gloeosporioides* Penz. and Sacc., up to races. Therefore, race specific screening programmes are to be started to identify specific resistance or multiple race resistant chilli which can withstand die back and fruit rot disease for long time.

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