Pak. J. Bot., 46(5): 1915-1920, 2014.

PHYSIOLOGICAL ATTRIBUTES OF FUNGI ASSOCIATED WITH STEM END ROT OF MANGO (*MANGIFERA INDICA* L.) CULTIVARS IN POSTHARVEST FRUIT LOSSES

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Abstract

Stem end rot majorly contributes in post-harvest losses of mango during storage conditions. Maximum disease incidence (70%) was recorded in Sindhari cultivar, followed by Chaunsa (64%), Fajri (62.5%) and 50% in both Langra and Anwar ratol. *In vitro* studies were carried out to identify fungal pathogens responsible for rotting and decaying mango fruits during storage along with isolation and testing their pathogencity on healthy fruits. Results revealed that all selected commercial mango varieties infected by stem end rot. *Colletotrichum gloeosporioides, Lasidodiplodia theobromae, Alternaria alternata, Aspergillus niger, Dothiorella domonicana* were identified from Sindhri mango fruits, in which of *C. gloeosporioides* was found the most prevalent. *Phomopsis mangiferae, Botryodiplodia theobromae, Altrnaria* spp., *Aspergillus niger, A. flavis* were found in Chaunsa and *Phomopsis mangiferae* was most prevalent, while *Botryodiplodia theobromae* caused infection to locally consumed Fajri variety. Effect of abiotic factors like pH, temperature, light intensity and carbon sources were tested against these isolates. The most efficient carbon source was glucose, which supported the maximum growth of the *P. mangiferae* and *L. theobromae*, while *C. gloeosporioides* had maximum growth on lactose. All fungi had maximum growth at pH range of 6-6.5 and temperature range of 25-30^{o}C on PDA medium. Alternate cycles of 12hr light and 12 hr darkness resulted maximum mycelial growth as compared to the 24 hour continuous exposure to either dark or light. Susceptibility of fungi with cultivars and intensity of spread under specific abiotic conditions provides basic information in this paper to minimize stem end rot of mango in field and storage conditions.

Key word: Physiological attributes, Mango cultivars, Postharvest diseases, Fungi, Stem end rot.

Introduction

The Mango (*Mangifera indica* L.) is cultivated in tropical and subtropical areas of Pakistan on 170.1 ha (Anon., 2012). Mango possessed 2nd position amongst the fruits produced in Pakistan with its 1729.9,000 tonnes production that ranked 4th in export (Maqbool *et al.*, 2007 and Anonymous, 2012). Among the world mango producers, Pakistan is the 4th largest producer after India, China and Thailand (FAO, 2010). Mango industry in Pakistan is facing several challenges, especially in postharvest loss of mango fruits caused by diseases and insect pest (Ishaq *et al.*, 2004; Iqbal *et al.*, 2004). Susceptibility of mango fruit to post harvest diseases results in physiological changes in the fruit that favors pathogen development and ultimately cause loses (Ecker *et al*., 1996). Proper handling and storage at optimum temperature leads to extension of shelf life. Storage temperatures below 12°C cause chilling injury in some cultivars (Medlicott, 2003). Post-harvest dip with fungicide has been relatively effective in protecting mango against post-harvest pathogen infection (Swart *et al*., 2002).Various varieties such as Sindhri, Chaunsa (Samar Bahisht, Black and White), Saharni, Alphonso, Langra, Anwar ratol, Dosehri, Fajri, Fazli, Neelam etc are grown in Pakistan, of which some of them are exported to USA, EU, Middle East (Kuwait/Saudi-Arabia/UAE) and South-East Asia (Malaysia/Singapore, China-Hong kong). Sindhri, Sammar bahist, Chaunsa have good export value (Amin & Hanif, 2002; Nafees, *et al*., 2013).

Stem end rot is caused by several fungi that infect before and after harvest (Coates & Johnson, 1993). Stem end rot is caused by *Botryodiplodia theobromae* that can be controlled by dip in solution of mancozeb and iprodione. In Taiwan's two popular cultivars viz., kiet and irwin, *Phomopsis mangiferae* caused disease (Ko *et al*., 2009). Various authors reported *Dothiorella dominicana*, *Colletotrichum gloeosporioides* and *Aspergillus niger* that caused stem end rot in mango fruit (Gangolly *et al.*, 1957; Kurup *et al.*, 1967; Pathak & Sarivastava, 1968 and Vock, 1978). The optimum temperatures for the growth of *Lasidodiplodia theobromae* were 200C, 25^{0}C and 30^{0}C, while light intensity has no effect on growth (Adeniyi *et al*., 2011). Among several carbon sources, glucose and sucrose were found superior for growth. The potato dextrose agar with pH 6.0 was recorded the most suitable medium for the production of conidia of *L. theobromae* (Shah *et al*., 2008). This study was planned to investigate pathogens which cause stem end rot of mango in Pakistan along with physiological study of associated fungi.

Materials and Methods

Potato Dextrose Agar Medium (PDA) of fallowing composition (15, 20 and 20 g) of agar, starch and glucose respectively was prepared in 1000 ml of distilled water. Glassware used for media pouring and PDA were sterilized at 121°C and 15psi pressure for 15 minutes in the autoclave and poured into Petri plates. For the isolation of the causal organism of stem end rot, healthy, full-bearing mango trees of Sindhri, Chaunsa and Fajri cultivars (25 years old) growing under similar conditions were selected from a commercial orchard in district Multan Punjab province (30.15°N; 71.36°E), for

postharvest studies. These selected fruits were placed in boxes (10 per box) and stored for 40 days of in cold store.

Disease assessment study: Immature healthy green mangoes of Sindhri, Chaunsa, Langra, Anwar Ratol, Saroli and Fajri varieties were collected from different orchards of Punjab and Sindh and stored in cold storage at 20°C for 15 days. Rotted mango fruits of selected cultivars were collected from cold storage and causal pathogens were isolated from rotten portions of fruits along with healthy areas and cut into pieces of 5 mm size. These were sterilized in 0.1% Sodium hypochlorite (NaoCl) solution for about 2 minutes and rinsed twice in autoclaved water. After drying in blotter paper, these were placed on PDA. The fungi associated with the diseased pieces were isolated and identified with the help of available literature (Barnett, 1967). The frequency of pathogens isolated from each variety was collected on overall basis. Pathogenecity of the isolated organisms was confirmed in the laboratory by wound inoculation method (Moalemiyan *et al*., 2007). Apparently healthy and uninjured fruits were washed in tap water, followed by surface sterilized by using NaoCl for 30 seconds and rinsed thrice in sterile distilled water. The spore suspension of each fungus was prepared from 7 days old culture by adding 25 ml distilled sterilized water in each Petri dish. The fruits were inoculated with 105 spore suspension with the help of camel hair brush after making injuries with sterilized needle. The untreated fruits were used as check. All the mango fruits were placed on newspaper under bell jar and in incubator at 25°C for daily observation and recording of data. The pathogens were re-isolated from the diseased portion and compared with the original cultures to prove the Koch's postulates (Koch ,1876).

Following parameters were observed in this study:

1. Effect of different carbon sources on mycelial growth: The five carbon sources i.e. sucrose, glucose, lactose, maltose, fructose was studied to assess the most suitable one for the growth of pathogenic fungi. The trial was replicated four times in 9 cm Petri plates. Observations were recorded in centimetres on daily basis until any of the dish filled with mycelial colony. For each plate two readings at right angle to each other and their means were taken and data was statistically analysed.

2. Effect of different temperature on mycelial growth: The growth of fungi was calculated on PDA, at various temperatures ranging from 0, 5, 10, 15, 20, and 25 to 30°C. Data was recorded after 24, 48, and 72 hours after incubation.

3. Effect of different pH levels on fungal growth: The colony growth of the isolated fungi was studied on PDA, at different pH ranges i.e. 5, 5.5, 6, 6.5, 7, 7.5 and 8. The data was recorded after 24, 48, and 72 hours after incubation.

4. Effect of Light intensity on mycelial growth: Inoculated petriplates were exposed to various periods of light and dark alternate cycles 12 h for each, continuous light and continuous darkness in incubator and data was recorded after 24,48 and 72 hours after incubation.

Results

Disease incidence: Data revealed that all mango varieties exhibited 100% prevalence of the disease significantly in the cold storage of postharvest laboratory, Institute of Horticulture, University of Agriculture Faisalabad. Maximum incidence (80%) was recorded in black Chaunsa followed by Sindhri (70%), Fajri (65.2%) and Saroli (64%), while least incidence (30%) showed by white Chaunsa (Fig. 1).

The fungal rotting pathogens that infested the mangoes were mainly composed of *Aspergillus niger*, *Alternaria* spp., *C. gloeosporioides*, *B. theobromae*, *Dothiorella* sp., *P. mangiferae*, *Stemphylium* sp. and *Dreschlera* sp. *C. gloeosporioides* caused dark-brown to black decay spots. They coalesced sometimes and penetrated deep into fruit resulting in extensive fruit rotting. Fruits infected by *Alternaria* sp. showed small black spots. The flesh beneath showed no changes either in color or in consistency. Other fungi like *Drechslera* sp. and *Stemphylium* sp. were isolated from a few fruits with spots similar to those caused by *Alternaria* sp. These fungi infest fruits from injured parts. *A. niger* is essentially saprophytic that penetrated into fruits through injuries or the peduncle. It is responsible for light brown round shaped spots showing a depression. The fungus weakens the mango flesh below and at high moisture and sporulation occurred directly on the fruit. *P. mangiferae* caused irregular brown spots that affected deeply the flesh beneath. *B. theobromae* is the causal agent of stem end rot of mango. Once initiated, the rotting process is capable of affecting the entire fruit within 2 to 3 days. *Dothiorella* sp. was also isolated from mangoes showing similar symptoms.

The examination of infected portion of mango fruit revealed the presence of fungi such as *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Phomopsis mangiferae*, *Aspergillus niger*, *Aspergillus flavis* and *Alternaria* spp. Majority of the selected samples represented by *Botryodiplodia theobromae*, which was isolated from all cultivar. Infection frequency of the *Botryodiplodia theobromae* in three cultivars of 300 samples was 67.70%, followed by *Alternaria* spp. (47.20%). *Colletotrichum gloeosporioides* was the most prevalent fungus (79%) on sindhri cultivar, while *Phomopsis mangiferae* (78%) was the most prevalent fungus that was associated with Chuansa cultivar.

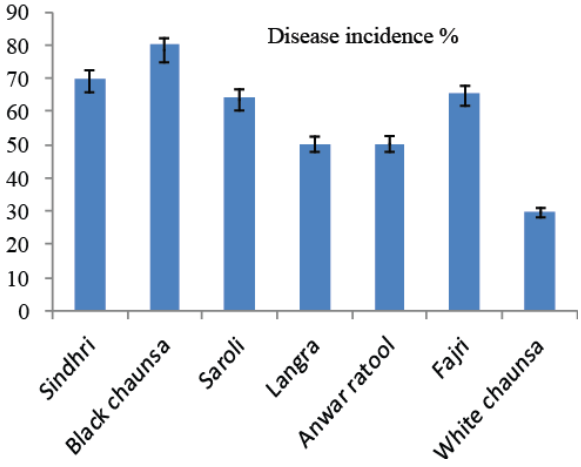

Fig. 1. Incidence of rotting in mango fruits.

The results of experiment of pathogenecity on Sindhri cultivar are presented in Fig. 2a. All the treatments produced the disease symptoms variably except in treatment 5, in which no inoculation was occurred. *Colletotrichum gloeosporioides* was inoculated alone that caused 79.22% infection in sindhri variety revealing its importance in sindhri variety during storage in Pakistan, followed by *Botryodiplodia theobromae* (44.45%). Mixed culture showed 77.2% infection. No symptoms were appeared on control.

The results of pathogenecity test on Chaunsa cultivar are presented in Fig. 2b. All the treatments produced disease symptoms variably except in treatment 5, in which no inoculation was occurred. *Phomopsis mangiferae* caused 92.90% infection in Chaunsa cultivar which shows that it is the most important pathogen for Chaunsa cultivar during storage in Pakistan, followed by *Botryodiplodia theobromae* (44.45%). Mixed culture showed 81.25% infection, while no symptoms were appeared in control.

Physiological studies of isolated fungi

Effect of different carbon sources: The effect of five carbon sources such as Glucose, Lactose, Maltose, Fructose, Sucrose was recorded on the growth of C. *gloeosporides, P. mangiferae* and *B.theobromae* after 24, 48 and 72 hours on PDA medium. Lactose supplemented media exhibited maximum growth (9.00 cm) for *C. gloeosporioides*, followed by Glucose (7.54 cm) and relatively less growth (7.22 cm) was noticed on Sucrose. Minimum growth was obtained on Fructose supplemented media (6.10 cm), however it was statically same as in the case of Maltose (6.80 cm). *Phomopsis mangiferae* exhibited maximum growth (8.70 cm) on Glucose supplemented media, followed by Sucrose (8.24 cm) and Fructose (7.28 cm), however it was statistically same as in the case of Lactose (7.08 cm). Minimum growth was obtained on Maltose supplemented media (2.62 cm). In the case of *Botryodiplodia theobromae*, maximum growth (8.44 cm) was recorded on Glucose supplemented media, followed by Fructose (8.33 cm) and Sucrose (7.76 cm), although it was statically same as in the case of Maltose (7.10 cm). Lactose supplemented media showed minimum growth (5.66 cm) as indicated in Fig. 3.

Effect of different pH: The effect of six different pH viz., 5, 5.5, 6, 6.5, 7 and 7.5 was recorded on the growth of C. *gloeosporides, P. mangiferae* and *B.theobromae* after 24, 48 and 72 hours on PDA medium and shown in Fig. 4. Maximum growth (8.83 cm) of *Colletotrichum gloeosporioides* was recorded on media with adjusted pH of 6.5, followed by 6.60 cm at pH 6 and 5.3cm at 7, while minimum growth (4.8 cm) was obtained at 5.5 pH. *Phomopsis mangiferae* expressed maximum growth (8.1 cm) on the media adjusted with 6.5 pH, followed 7.83 cm at pH 6 and 5.16 cm at 5.5, while minimum growth (4.56 cm) was obtained 5. The maximum growth of *Botryodiplodia theobromae* (8.86 cm) was recorded on the media having pH of 6, followed by 6.43 cm at pH 6.5 and5.10cmat 5.5 pH.

Effect of different temperature levels on mycelial growth: The effect of six different temperature such as 0^oC, 10^oC, 15^oC, 25^oC, 28^oC and 30^oC was recorded on growth of C. *gloeosporides* after 24, 48 and 72 hours on PDA medium (Fig. 5). The ANOVA indicated highly significant differences among the temperature levels that affected the growth of *C. gloeosporioides B. theobromae*. Maximum growth (8.83 cm) of *Colletotrichum gloeosporioides* was obtained on the media incubated at 28^oC, followed by 30^oC (8.2 cm) and minimum growth (5.40 cm) was obtained when *B. theobromae* was incubated at 25^oC. The pathogens failed to grow when it was incubated at 0^oC. *B. theobromae* was heavily infested (8.5 cm) when media was incubated at 28^oC, followed by 25^oC and 30^oC (8.2 cm), while minimum growth (2.26 cm) was obtained at 15^oC. The fungi failed to at 0^oC. Maximum growth (8.33 cm) of *P. mangiferae* was obtained on media when it was incubated at 25^oC followed by 28^oC (7.8 cm) and minimum growth (2.0 cm) was obtained when *P. mangiferae* was incubated at 15^oC. The fungus failed to grow when incubated at 0^oC.

Effect of light intensity on fungal growth: The effect of light intensity on mycelial growth of *C. gloeosporioides P. mangiferae* and *B. theobromae* was studied (Fig. 6). All these fungi grew best at the exposure of 12 hr light and 12 hr darkness (9 cm), followed by 24 hr light exposure (7.5 cm), whereas, their growth was found at minimum at the exposure of 24 dark (4.6 cm).

Discussion

Mango stem end rot is sever postharvest problem in mango growing areas of Southern Punjab. The fungal flora responsible for the infestation is diverse and seems to be strongly influenced by the climatic conditions. The harvesting of early varieties coincided with the end of dry season. The fungal species that infect mangoes during dry season are *Alternaria* sp. *Botryodiplodia theobromae, Dothirella* sp., *Aspergillus niger, Aspergillus flavis* and *Colletitrichum gloeosporioides.* The fruit is harvested during the humid season in which late varieties were found heavily infested by *Colletotrichum gloeosporioides, Phomopsis mangiferae* and *Botryodiplodia theobromae*. *C. gloeosporioides* was the most prevalent fungus on cv. Sindhri, while *Phomopsis mangiferae* was found associated with Chounsa. Similar results were reported by Raza *et al*., (2013) and (Estrada 1994) suggesting that anthracnose becomes more competitive than stem end rot under humid conditions. According to Dodd *et al.,* (1997), *C. gloeosporioides* is dispersed by rain drops, whereas spores of *Alternaria* sp. are spread by wind (. Fruit infections caused by *C. gloeosporioides* during rainfall season that occur from fruit set until harvesting, with dead leaves entangled in the tree canopy, defoliated branch terminals, mummified inflorescences and flower bracts constituting the main source of inoculum (Dodd *et al*., 1997). Conidia spread throughout the orchard by means of heavy dew, irrigation and light rain, with rainy weather being conducive to conidium production, dispersal and infection (Prusky, 1996). Interactions between *C. gloeosporioides* and the climate may lead to a variation of disease incidence and severity throughout the growing season as a result some export consignments being virtually disease-free and others, completely unmarketable depending on degree of infection (Kotze, 1978; Cappellini *et al*., 1988). Incidence of anthracnose can reach up to 100% in fruits produced under wet or very humid conditions (Arauz, 2000). The association between dry season and the occurrence of *Alternaria* sp. confirmed earlier results according to which mango black spot caused by *A. alternate* is prevalent in dry countries (Dodd *et al*., 1997).

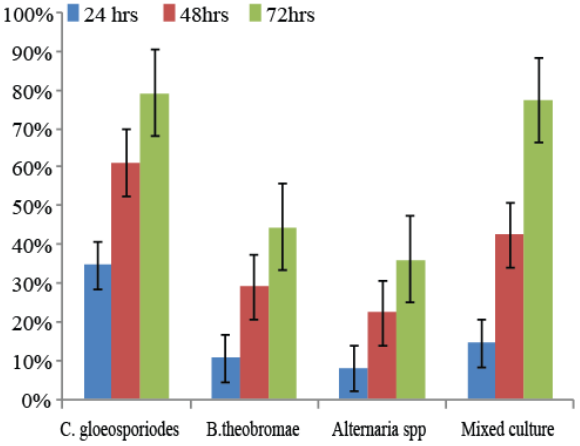

Fig. 2a. Pathogenicity test on Sindhri variety.

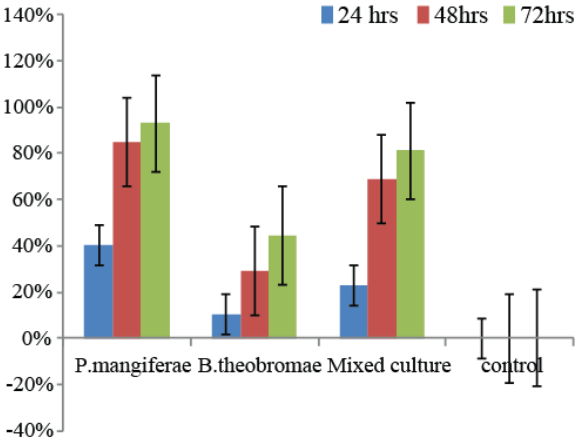

Fig. 2b. Pathogenecity on Chaunsa variety.

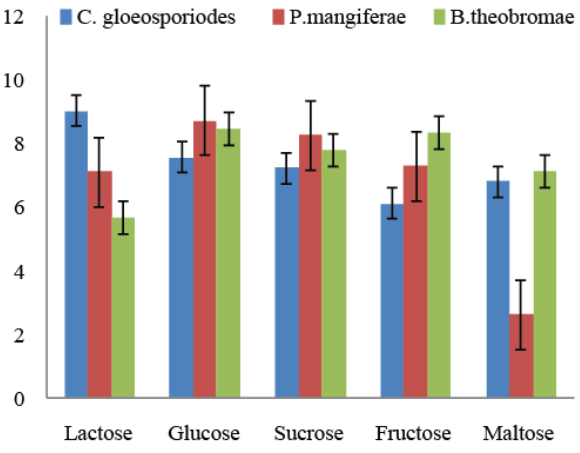

Fig. 3. Effect of different carbon sources on the fungal growth.

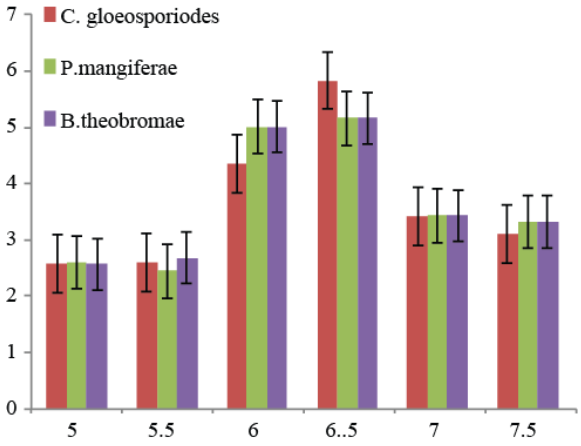

Fig. 4. Treatments Means of different pH on the fungal growth.

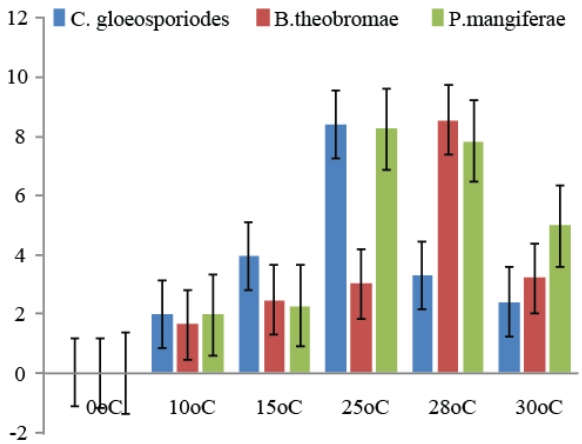

Fig. 5. Effect of different temperature levels on mycelial growth (cm) of fungi.

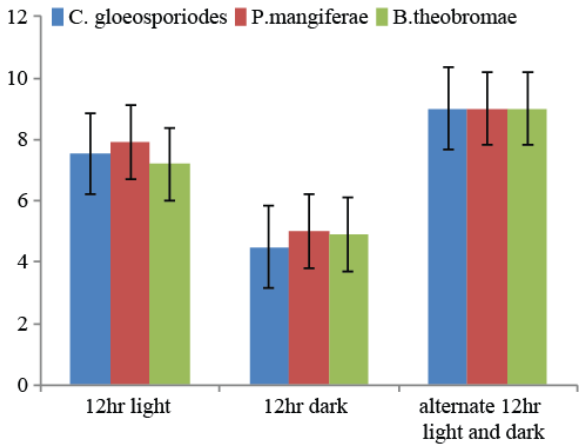

Fig. 6. Effect of light intensity on mycelial growth of fungi.

Fungi differ in their ability to utilize carbon compounds in growth, but it is frequently expected that species of same genus are similar in their ability to utilize carbon in different carbon sources. After detecting the role of different carbon sources it is found that *B. theobromae* had the best growth on glucose and fructose. These carbon sources are also found in mango fruit. Sporulation occurred on all carbon sources but it was best on sucrose and fructose followed by glucose. These results are in agreement with those of Ekundayo (1973) who reported that *B. theobromae* pycnidia had maximum size on sucrose supplemented with Na_2NO_3, + $MgSo_4.7H_20$+K_3HP0_4. The

maximum (9.00 cm) growth of *C. gloeosporioides* was recorded on lactose supplemented media, followed by glucose (7.54 cm). The minimum growth was obtained on fructose supplemented media (6.10 cm). Sporulation occurred on all sources but it was the best on lactose, followed by glucose. *C. gloeosporioides* had the best growth on lactose which is contrary to the findings of Kumara *et al.,* (2008), who found the variations in nutritional and physiological characteristics in different isolates of *C. gloeosporioides*that caused anthracnose disease of papaya in India. Pathogen under study varied in its ability to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and speculations of most of the isolates. These results have also contradiction with the results of Madan (1993), who studied 41 carbon compounds. The pathogen showed excellent growth on starch, maltose, melibiose, dextrose, sucrose, raffinose, and dulcitol; good on tartaric acid, mannose, galactose, fructose, mannitol, and castor oil; fair on inulin, isopropyl alcohol, coconut oil, and pectin; poor on sorbose, *n*-butyl alcohol, arabionose, maleic acid, ethyl alcohol, succinic acid, citric acid, ribose and malic acid, and no growth on the rest of the carbon compounds. In general, these compounds which supported the best mycelial growth, yielded excellent or good sporulation of *C. gloeosporioides* and vice versa. These results was also not matched with the (Sangeeta and Rawal, 2008) her study indicated that out of six different carbon sources, mannitol was found to be the best source of carbon for the growth followed by fructose and sucrose. Heavy sporulation was observed where maltose was used as carbon source followed by moderate sporulation in fructose and lactose. Maximum (8.70 cm) growth of *Phomopsis mangiferae* was recorded on media supplemented with Glucose followed by Sucrose (8.24 cm) while minimum growth was obtained on media supplemented with Maltose (2.62 cm). Sporulation occurred on all sources but it was best on Glucose and Sucrose followed by Fructose. Sporulation on Maltose was poor. *P. mangiferae* had the best growth on glucose. The finding in these studies matched with earlier study hexoses and disaccharides, supported good growth for virtually all cultivated fungi (Cochrane, 1958). Our results also confirmed the finding of (Garraway & Evans, 1994) while they found that the utilization of glucose may be due to ease with which this sugar was metabolized to produce cellular energy.

The study was also conducted to find the role of temperature on growth and sporulation of *C. gloeosporioides, P. mangiferae* and *B. theobromae.* Maximum (8.83 cm) growth of *C. gloeosporioides* was recorded on the media that was incubated at 28^{o}C, followed by 30^{o}C (8.2 cm), minimum growth (5.40 cm) of *B. theobromae* was obtained at 25^{o}C. The fungi failed to grow when incubated at 0^{o}C. These results are further strengthened by the Kumara *et al.,* (2008). They found the temperature ranges from 28^{o}C to 30^{o}C were the most favorable for the spore production of different isolates of *C. gloeosporioides* when grown on solid medium. There was no spore production observed when these isolates were grown at 15^{o}C. Extent of sporulation was high in most isolates at 28^{o}C in liquid culture. These results also confirmed the findings of Sangeeta and Rawal, (2009) that the temperature ranging from 25 to 30^{o}C was favorable for the growth of *C. gloeosporioides.* Maximum (8.5 cm) growth of *Botryodiplodia theobromae* is obtained on media incubated at 28^{o}C, followed by 25^{o}C. Our results are in the line of Alam *et al*., (2001) that the *B. theobromae* was the causal organism of crown rot of banana. The fungi grew and sporulated at 10-40^{o}C, the optimum being 25-30^{o}C and the highest mycelial growth (78-90 mm) and sporulation (27-38 condia/0.01 ml) were observed on PDA medium. The maximum (8.33 cm) growth of *Phomopsis mangiferae* was obtained on the media incubated at 25^{o}C, followed by 28^{o}C (5.96 cm). The fungi failed to grow when incubated at 0^{o}C. These results confirmed the work of Zivkovic (2007). He studied 12 isolates of *Phomopsis* sp. obtained from the branches and the trunk of plums (*Prunus domestica* L.) with decay symptoms in Valjevo, Ljig, Koceljeva and Ub vicinity during 2004-2006.

Morphological, pathogenic and growing characteristics were studied. Pathogen caused tissue necrosis of branches around the inoculate portion/sight, and wrinkling and watering of plum fruits. All media were suitable for pathogen development, except prune agar. The optimal temperature for growth and germination of pycnidiospores was 25˚C. One of the important factors responsible for the growth and sporulation of particular fungus is the hydrogen ion concentration (pH) of the medium. The pH of the medium effect the rate and amount of growth, sporulation and many other life process of the fungi. Different fungi have different pH for their growth. However mostly fungi grew at pH between 4 to 8 although there are some exceptions which have either a narrow or a wide range of tolerance (Bilgrami & Verma, 1981). In the present study, the most suitable pH was 5.5-6.5 for *P. mangiferae, C. gloeosporioides* and *B. theobromae*. At high (Acidic) or Low (alkaline) pH, the mycelial growth decreased drastically. These results matched with the finding of Zivkovic (2007), who studied 12 isolates of *Phomopsis* sp. plums had best growth at medium pH 5.5. These results have contradiction with the finding of Kumara *et al.,* (2008) who reported that the variations in nutritional and physiological characteristics found in different isolates of *C. gloeosporioides* causing anthracnose disease of papaya, in India. Pathogen under study varied in its ability to utilize different carbon and nitrogen sources and pH. *C. gloeosporioides* isolates grew well at pH 5 while sporulation was better at pH 6.Light has profound effect on the mycelial growth of all isolated fungi and present study revealed that maximum mycelial growth was observed when it was exposed to alternate cycles of light and darkness. This was followed by continuous light and continuous darkness.

Orchard sanitation and particularly cleaning and pruning are very useful to decrease the infection rate of fungi causing post-harvest rotting of mango as shown by the multivariate analysis. The most heavily infested mango samples were those from orchards with no care. They showed not only a higher proportion of mango rotting but also a wider range of fungal pathogens, in addition to a high infection rate by fruit flies (Dodd *et al*., 1997). Control measures specific to the pathogens and taking into account their epidemiology should be used to keep the mango business running. Therefore, complementary studies on the efficacy of fungicides as

well as on a treatment schedule considering the interactions between climate and epidemiology of the fungal flora should be carried out.

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(Received for publication 6 July 2013)