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EFFECT OF DIFFERENT ABIOTIC FACTORS ON THE GROWTH AND SPORULATION OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING ANTHRACNOSE OF MANGO

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ABSTRACT

The present study was carried out to investigate the influence of physiological factors on vegetative growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose rot of mango. Among ten culture media, the highest colony growth of the fungus was observed on corn meal agar, malt extract agar, Sabouraud's agar and oat meal agar followed by potato carrot agar. Significantly higher number of conidia was observed on Waksman's agar and corn meal agar, followed by oat meal agar. Corn meal broth medium produced maximum dry mycelial weight of the test pathogen followed by Waksman's and Czapek's broth. The incubation of the test fungus under '12 h light 12 h dark' produced significantly more colony growth than other treatments. The minimum, optimum and maximum temperatures for *C. gloeosporioides* were 20 °C, 30 °C and 40 °C, respectively. It grows best at pH 5 followed by pH 6.

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INTRODUCTION

Mango, being a perishable fruit, is subjected to various diseases caused by different pathogens that results in the loss of a huge quantity of produce during postharvest processes like grading, packing, marketing, storage and shipping to distant markets especially through sea shipment (Jha et al., 2010). Although, Pakistan is the 4th largest producer and exporter of mango, but still facing several challenges specifically in term of postharvest diseases which limit its share in international markets (Maqsood et al., 2014). A wide diversity of pathogens attack various parts of nursery and adult mango trees. Anthracnose, blossom blight, powdery mildew, malformation, cankers, twig dieback and bacterial black spot are some of the main problems faced by mango producer's worldwide (Prakash and Srivastava, 1987; Wolstenholme and Whiley, 1995). Mango is also subjected to the attack of numerous postharvest diseases; however, stem end rot, anthracnose rot, Phomopsis rot, Alternaria rot and Aspergillus rots are more important in Pakistan. Postharvest disease development is a major constraint to the quality and shelf life of mango fruit thereby limiting its domestic and export marketing (Bally et al., 2009), as well as resulting in heavy economic losses (Barkai-Golan, 2001; Narayanasam, 2006). Like other fresh commodities, mango has also been found prone to postharvest fruit decay due to rapid disease development

during storage and ripening (Prusky et al., 2009).

Anthracnose is regarded one of the major postharvest diseases of mango (Bally et al., 2009). Stem end rot (SER) and black spots (i.e., Alternaria rot) have also been reported to cause significant postharvest decay in mango (Prusky et al., 2009). Recently, mango sea-freight trial shipments from Pakistan to Germany also indicated SER and anthracnose rot as the major concern for high postharvest losses (Malik et al., 2010). Anthracnose rot of mango fruits, which is due to pre-harvest infection develops as fruit ripen, first appearing as superficial black spots and streaks that then become sunken (Fitzell and Peak, 1984). It also attacks flowers, young fruits, leaves and twigs. On fruits, symptoms appear as black, slightly sunken lesions of irregular shape, which gradually enlarge and cause fruit rot. In Bangladesh, about 25 to 30% losses (Reza and Kader, 1995) of total production due to anthracnose and stem end rot has been recorded. Its intensity was enhanced in humid production areas (Arauz, 2000; Lim and Khoo, 1985; Ploetz, 2003; Ploetz and Freeman, 2009; Ploetz and Prakash, 1997).

Two closely related species of fungi i.e., *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*) (Ploetz and Prakash, 1997) and *C. acutatum* (teleomorph: *Glomerella acutata*) are responsible for anthracnose rot of mangoes (Ploetz and Prakash, 1997; Tarnowski and Ploetz, 2008). The fungal growth is greatly influenced by nutritional factors, such as a nitrogen source, carbon source, pH of the substrate and temperature. All these factors play important role in fungal growth, sporulation and other activities of fungi. The production rate of spores, which is of elementary importance for the fungal dissemination and subsequent infection, also depends on nutritional and environmental factors. These factors determined whether a species will sporulates under a given set of conditions or not and what would be its rate of multiplication (Eicher and Ludwig, 2002). The determination of these requirements of plant pathogens is necessary to develop an appropriate control strategy (Jaruhar and Prasad, 2011). Therefore, present studies on the influence of physiological factors on vegetative growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose rot of mango was carried out.

MATERIALS AND METHODS

Collection of diseased fruits: Ripe mango fruits showing typical symptoms of anthracnose rot of mango were collected from local markets.

Isolation, identification and purification of anthracnose causing pathogen: The fruits showing, symptoms of anthracnose rot were used for isolation of causal pathogen. For this purpose, the affected portions of the fruit skin having disease lesions including some symptomless part were detached with the help of a sterilized scalper's knife (Waller et al., 1998). These skin portions divided into small pieces of about 0.5 cm. Under aseptic conditions they were surface sterilized with the help of 5% Sodium hypochlorite solution for 2-3 minutes, dried on sterilized blotting paper and placed on the surface of PDA plates. These plates were incubated at ambient on laboratory benches. The appearing fungal colonies were purified on fresh PDA plates and identified on the basis of the morphological characters after reference to Johnson et al. (2012) and Barnett and Hunter (1972).

Physiological Studies: In the first phase of the physiological studies, different culture media were evaluated for the colony growth and sporulation of the *C. gloeosporioides.* The resulting best culture medium was used for studying the effect of different incubation temperature, light regimes and pH on the growth and sporulation of test pathogen. Similarly, experiments will be carried out to evaluate the effect of different culture media, temperature, light regimes and pH on the dry mycelial weight of *C. gloeosporioides* when grown in liquid culture. There were replications of each treatment arrange in randomized complete design (RCD).

Effect of different solid culture media: Ten different culture media i.e., Waksman's agar [Peptone 5 g, Glucose 10 g, Potassium dihydrogen phosphate 1.0 g, agar 20 g, distilled sterilized water 1000 ml], Potato dextrose agar [potato 200 g, dextrose 20 g, agar 20 g, distilled sterilized water 1000 ml], Oat meal agar [rolled oat 75 g, agar 20 g, distilled sterilized water 1000 ml], Sabouraud's agar [peptone 10 g, dextrose 40 g, agar 15 g, Glucose or maltose 40 g, distilled sterilized water 1000 ml], Corn meal agar [corn meal 50 g, agar 15 g, dextrose 10 g, distilled sterilized water 1000 ml], Gulcose peptone agar[dextrose 20 g, peptone 5 g, yeast extract 2 g, Potassium phosphate 1 g, Magnesium sulfate 0.5 g, agar 15 g, distilled sterilized water 1000 ml], Potato carrot agar [potato 20 g, carrot 20 g, agar 20 g, distilled sterilized water 1000 ml], Czapek's dox agar [sucrose 30 g, Sodium nitrate 3.0 g, agar 15 g, Ferric 0.5 g, Dextrose 12 g, Dipotassium hydrogen phosphate 0.5 g, distilled sterilized water 1000 ml], Malt extract agar [malt extract 20 g, dextrose 20 g, peptone 6.0, agar 15 g, Magnesium

sulfate 2.5 g, Maltose 5.0 g, Monopotassium phosphate 0.25 g, distilled sterilized water 1000 ml] and Rhichard's agar [Sucrose 50 g, agar 15 g, , Potassium nitrate 10 g, Monopotassium dihydrogen phosphate 5.0 g, Magnesium sulfate 2.5 g, Ferric chloride 2.5 g, distilled sterilized water 1000 ml] were prepared according to their recipes. After preparation, each medium was sterilized in the autoclave. Sterilized medium was poured in 9 cm Petri dishes, before pouring antibiotics (Streptomycin sulfate @ 1 ml L^{-1} medium and Penicillin @ 1000,000 units L^{-1}) were added to avoid bacterial contamination. After solidifying of medium, a 5 mm diameter agar disk of *Colletotrichum gloeosporioides* was cut from 8-10 days old culture plate by using sterile cork borer and placed in the center of the culture plate and incubated at ambient temperature on laboratory benches. The radial colony growth of test fungus in each treatment was recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the center of the plate. The data on colony growth was recorded after each 24 hours until the plates were filled in any treatment; while, data on sporulation was recorded after 15 days of incubation. For this purpose, conidia from Petri dishes were harvested by adding sterilized water. The numbers of conidia in each treatment were determined with the help of hemocytometer (Waller et al., 1998). The plates were arranged in CRD with five replications.

Effect of different liquid culture media: Ten different liquid media, i.e., Waksman's broth, Potato dextrose broth, Oat meal broth, Sabouraud's broth, Corn meal broth, Gulcose peptone broth, Potato carrot broth, Czapek's dox broth, Malt extract broth and Rhichard's broth were prepared and evaluated for their effects on biomass production of anthracnose rot pathogen. For this purpose each broth medium was prepared as per their recipes given in table 1. Each medium was then poured into 100 ml conical flask and sterilized in the autoclave. After sterilization, when the medium becomes cool, a 5mm diameter agar disk of *Colletotrichum gloeosporioides* was cut from 8-10 days old culture plate by using sterilized cork borer and placed in the conical flask containing broth medium and incubated at ambient temperature on laboratory benches.

After 15 days on incubation, the fungal mycelial mat growing on the surface of the broth medium was harvested. For this purpose, the contents of the conical flask were filtered through a Buchner funnel by pre-weighed 9-cm Whatman No. 1 filter paper. Dry weights of the test fungus were obtained by placing the mycelial pellet in a forced-air drying oven at 70 °C for 24 h. The dry weight of the fungus was calculated by using the following formula:

$$\text{Dry weight} = (\text{weight of filter paper} + \text{mycelial weight}) - (\text{weight of filter paper})$$

Effect of different light regimes: Different light regimes such as 24 h darkness, 24 h light, 12 h light/12 h dark, 16 h light/8 h dark, 8 h light/16 h dark were evaluated for their effects on colony growth of test pathogen. For this purpose, the best favourable culture medium was selected on the basis of the result of an earlier experiment. The selected agar culture medium was prepared, sterilized and poured as described above. Similarly, a 5 mm disk of test pathogen was placed in the center of each culture plate and data on colony growth was recorded.

Effect of different temperatures: The test fungus was inoculated on most suitable agar medium as well as broth medium, incubated at different temperature regimes such as 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. Data on colony growth and biomass production was recorded as described above.

Effect of different pH levels: The effects on different pH levels (5 to 9) on the colony growth and biomass production of causal pathogen were determined. The pH of most favourable culture medium growing medium was adjusted as pH5, pH6, pH7, pH8 and pH9. Data on colony growth and biomass production was recorded as described above.

RESULTS AND DISCUSSION

Symptoms of anthracnose rot: On fully ripe mango fruits the pathogen infection appears as small black spots on the fruit surface. With the advancement of disease, they become enlarged and became slightly sunken lesions of irregular shape.

Isolation of *Colletotrichum gloeosporioides*: Isolation from affected fruits showing typical symptoms of anthracnose rot yielded frequent recovery of *Colletotrichum gloeosporioides.* On PDA, it produced cottony or wooly growth of pale oliveaceous gray to olivaceous gray colour. Conidiophores irregularly branched, conidia hyaline, smooth sub-cylindrical with rounded ends measuring 12-17 x 4.5-6 µm.

Effect of different culture media on colony growth: Among ten culture media significantly maximum colony growth of test fungus was observed on Corn meal agar, Malt extract agar, Sabouraud's agar and Oat meal agar

followed by Potato carrot agar. On the other hand, Czapek's produced least colony growth of 47 mm followed by Glucose peptone agar (52.75 mm). While other media such as Potato dextrose agar, Waksman's and Richard's agar produced moderate growth ranging from 73.75-68.5 mm (Figure 1).

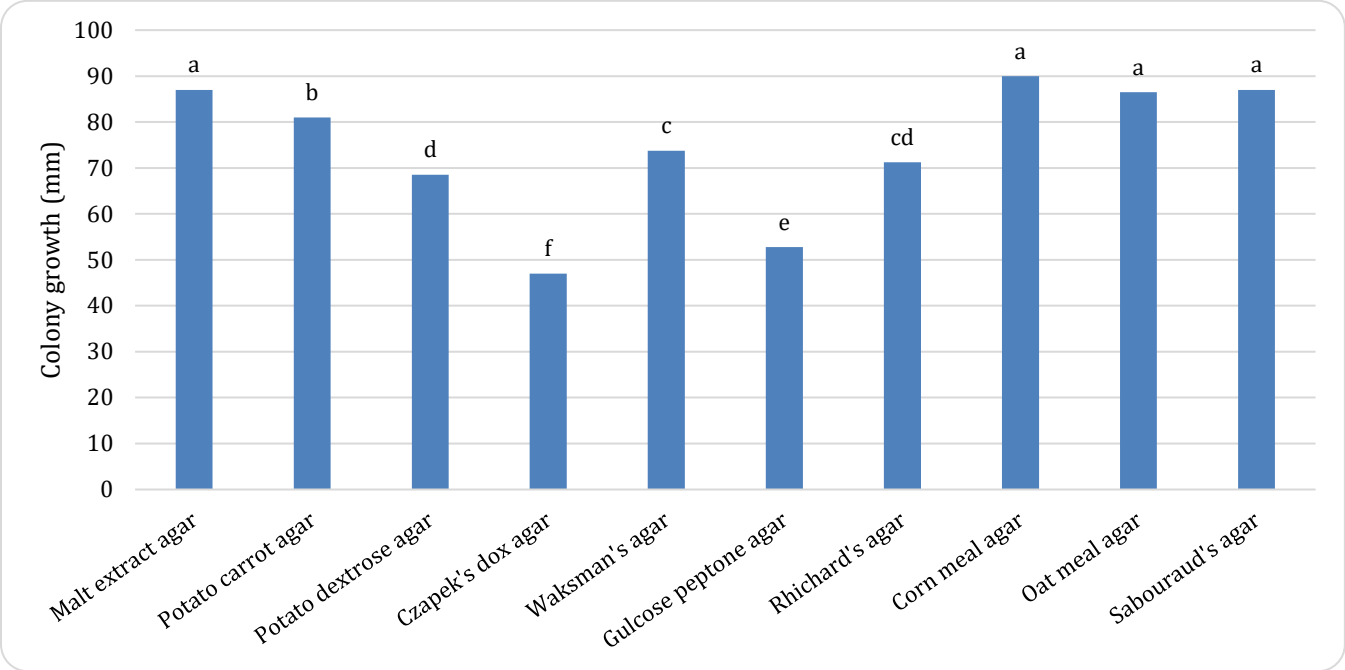

Figure 1. Effect of different culture media on the colony growth of *C. gloeosporioides.*

Effect of different culture media on sporulation: The responses of different media to the sporulation of test fungus were differed from colony growth. Significantly higher numbers of conidia were observed on Waksman's agar and Corn meal agar, followed by Oat meal agar. While the lowest number of conidia were recorded on Sabouraud's agar followed by Glucose peptone agar and Potato carrot agar. While Potato dextrose agar, Malt extract agar, Czapek's and Richard's agar produced moderate number of conidia of *Colletotrichum gloeosporioides* (Figure 2).

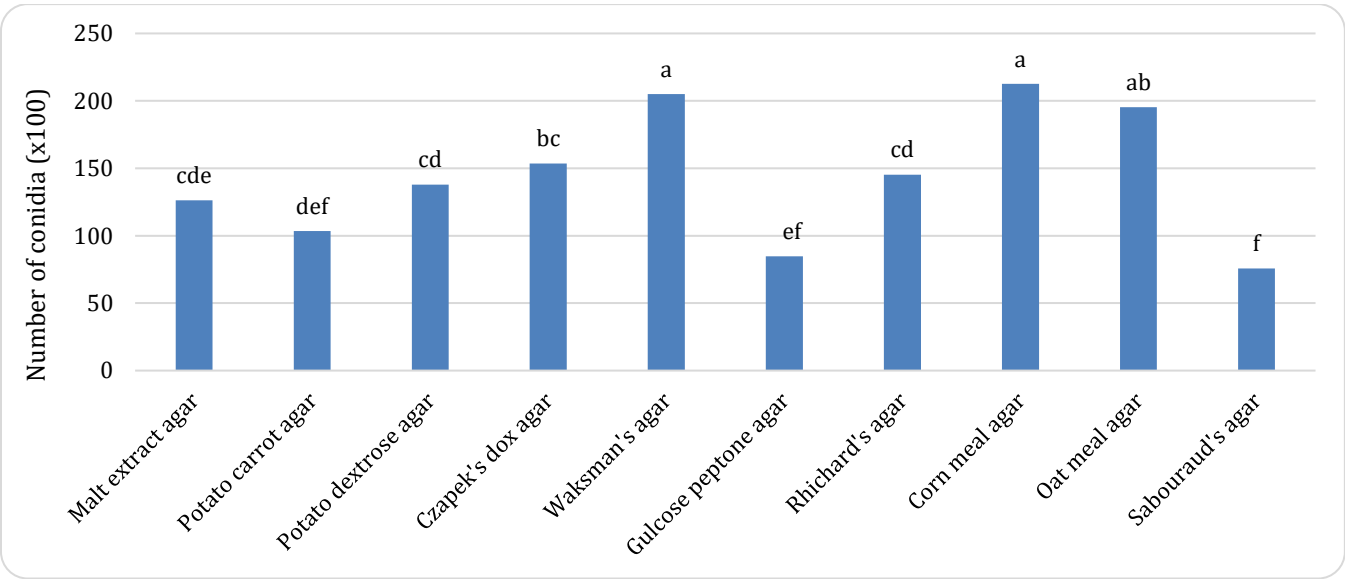

Figure 2. Effect of different culture media on the sporulation of *C. gloeosporioides.*

Effect of different culture media on biomass: Biomass production of *Colletotrichum gloeosporioides* greatly varied with different growing broth media. Corn meal broth medium produced maximum dry mycelial weight of test pathogen (44.33 g) followed by Waksman's broth (43 g) and Czapek's broth (42 g). While Oat meal broth and Malt extract broth media appeared as the least suitable culture medium for the biomass production of test pathogen. They produced only 16.67 and 17.67 g dry mycelial weight, respectively. After these two media, the least biomass production was occurred in Potato carrot broth (24.67g) followed by Potato dextrose broth (34 g) (Figure 3).

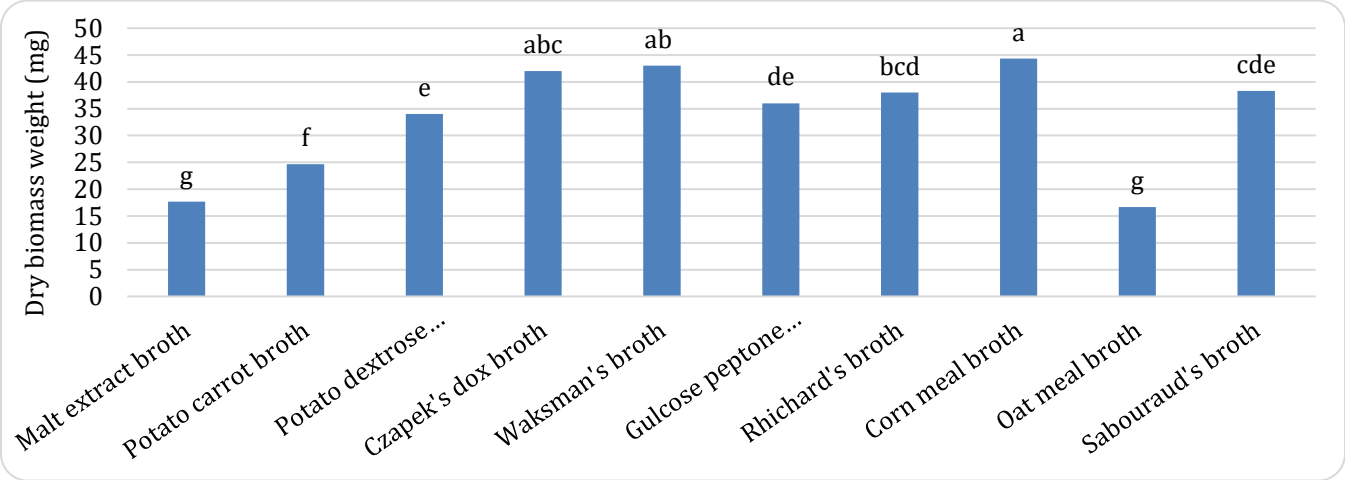

Figure 3. Effect of different broth culture media on the biomass production of *C. gloeosporioides.*

Effect of different light regimes on colony growth: The growth of *Colletotrichum gloeosporioides* varied with different light regimes. The incubation of test fungus under '12 h light 12 h dark' produced significantly higher colony growth than other treatments. While placing of culture under continuous light (24 h light) yielded least colony growth of the test fungus. Other light regimes produced intermediate colony growth (Figure 4).

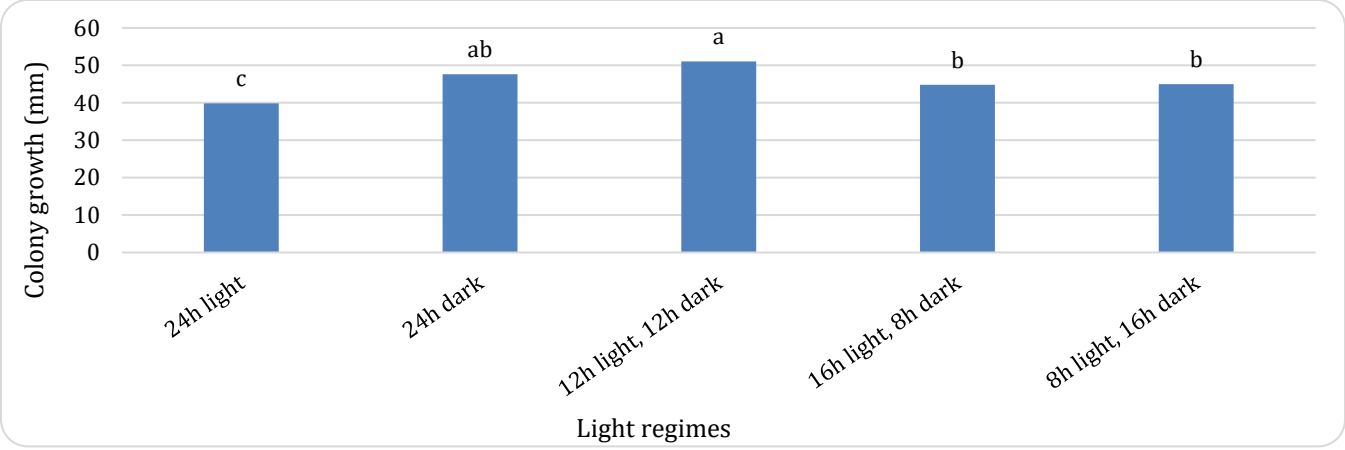

Figure 4. Effect of different light regimes on the colony growth of *C. gloeosporioides.*

Effect of different temperatures on colony growth: The incubation temperatures remarkably influenced on the colony growth of test pathogen. The maximum colony growth of *C. gloeosporioides* (90 mm) was recorded at 30 °C. It was followed by 74.67 mm and 54 mm observed at 35 °C and 20 °C, respectively. The extreme incubation temperatures such as 15 °C, 40 °C and 45 °C produced no or very rare growth. The minimum, optimum and maximum temperatures for *C. gloeosporioides* are 20 °C, 30 °C and 40 °C, respectively (Figure 5).

Effect of different temperatures on fungal biomass: In broth culture, *Colletotrichum gloeosporioides* produced significantly higher biomass when incubated at 30 °C and 25 °C, followed by 20 °C and 25 °C. The extreme low or high temperatures yielded significantly minimum biomass of test pathogen (Figure 6).

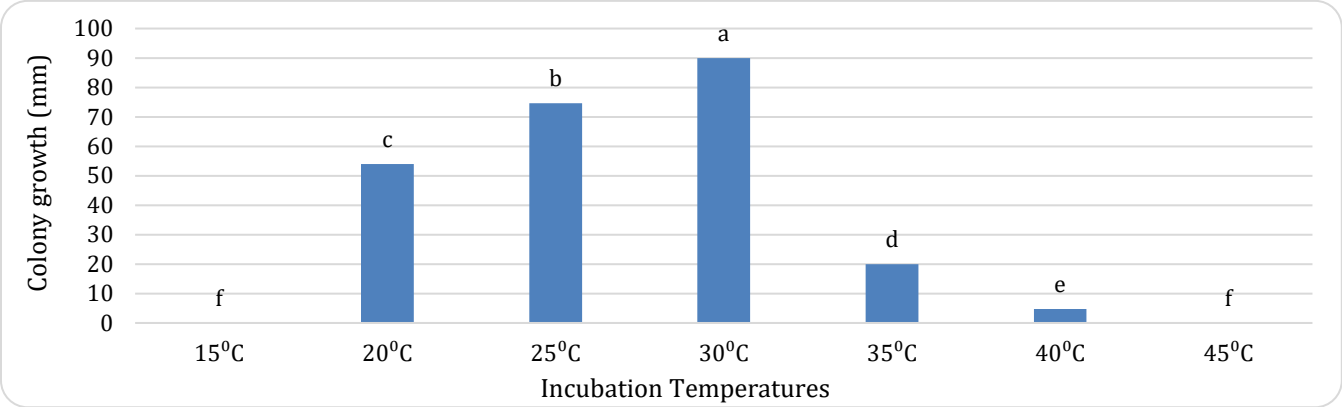

Figure 5. Effect of different temperatures on the colony growth of *C. gloeosporioides.*

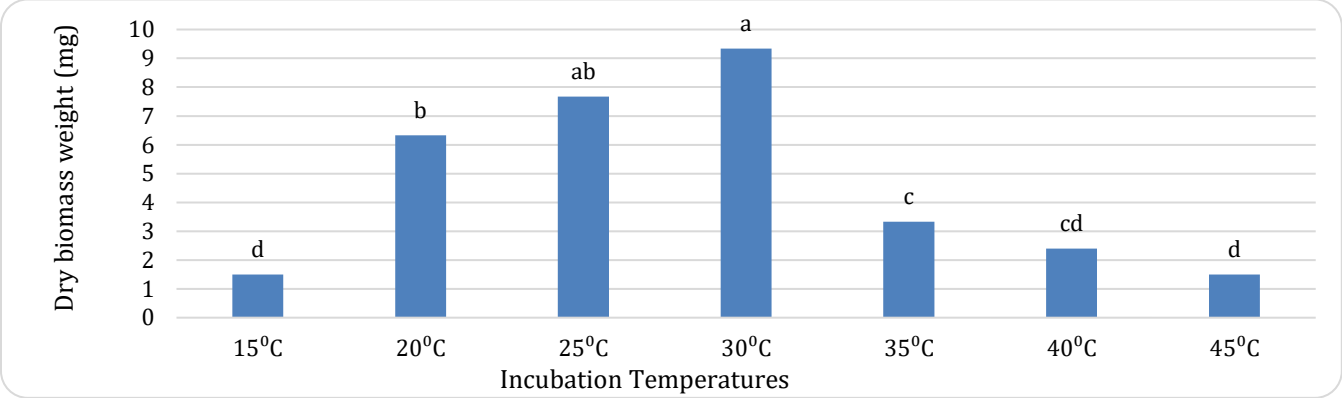

Figure 6. Effect of different temperatures on the biomass production of *C. gloeosporioides.*

Effect of different pH levels on colony growth: The colony growth of *C. gloeosporioides* varied with the changing pH of culture medium when other conditions remained constant. It produced significantly highest colony growth (82.25 mm) at pH5, which gradually decreased with increasing pH of growing medium. The 2nd highest colony growth (75.25 mm) of test fungus was observed at pH6 followed by pH7 (60.75). While pH9 produced significantly minimum colony growth (42.25) followed by pH8 (52.25 mm) (Figure 7).

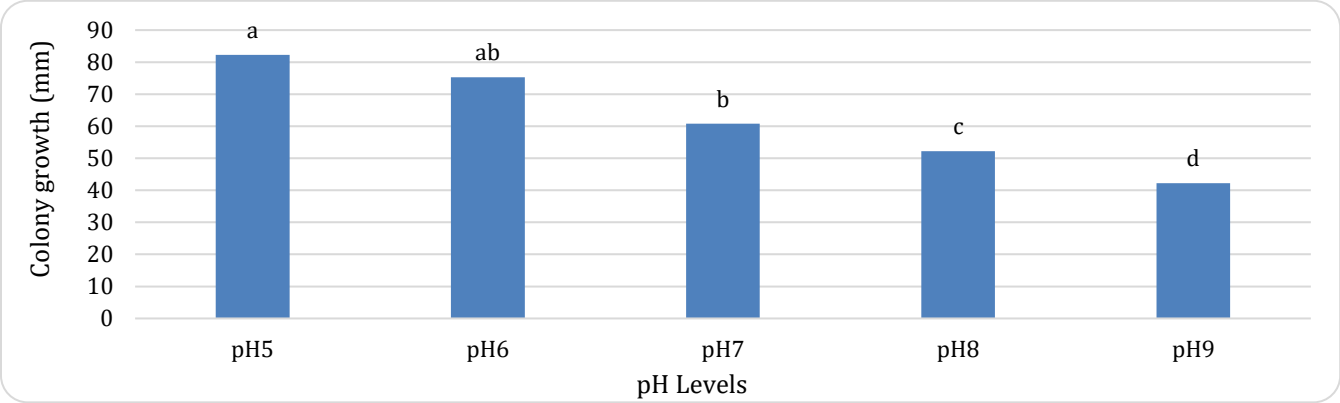

Figure 7. Effect of different pH levels on the colony growth of *C. gloeosporioides.*

Effect of different pH levels on fungal biomass: Similar trend was also observed in term of biomass production of test fungus. The significantly maximum dry mycelial weight of *Colletotrichum gloeosporioides* was recorded at pH5 (40 g) followed by pH6 (35 g) and pH7 (34g). While the lowest dry mycelial weight was observed at pH9 (27.67 g) and pH8 (28 g) (Figure 8).

Plants and pathogens co-evolved in nature. Plant growth conditions may be altered to create the worst conditions for the pathogens development but without sacrificing the yield. Documentation of pH, temperature and other physiological requirements of infecting organisms are essential to develop an appropriate control strategy. On the other hand, developments of reliable and suitable methods of pathogen multiplication are prerequisite for advanced research.

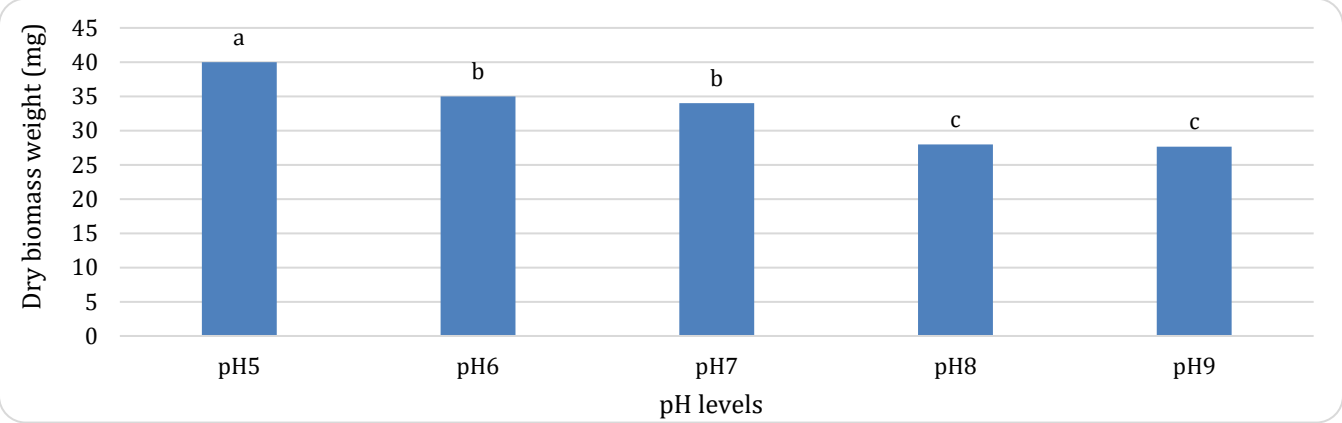

Figure 8. Effect of different pH levels on the biomass production of *C. gloeosporioides.*

Isolation from affected fruits showing typical symptoms of anthracnose rot yielded frequent recovery of *Colletotrichum gloeosporioides* have the same morphological characters as described by Johnson et al. (2012). Our results in confirmation to those reported by Kumara and Rawal (2010) found that most of the isolates of *Colletotrichum gloeosporioides* grow well at 28 °C to 30 °C on Richard's agar medium. Sripromsuk et al. (1999) found that *Colletotrichum gloeosporioides* produced highest mycelial growth when grown on Potato dextrose agar pH 5, 7, 8 at 22-23 °C. They also observed that maximum sporluation of *Colletotrichum gloeosporioides* was occurred on Potato dextrose agar pH 7 at 27-29 °C. However, Kumara and Rawal (2010) observed that pH 5 was best for colony growth and pH 6 for sporulation. Further studies should be carried out to alter the growing conditions prefer by the *Colletotrichum gloeosporioides.*

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