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Effect of isolation media composition on sporulation of *Colletotrichum gloeosporioides* penz and Sacc. causing post-harvest anthracnose disease in mango.

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Abstract
Effect of isolation media composition on sporulation formation of Colletotrichum gloeosporioides Penz and Sacc. On 8th day of inoculation period basically were use different sources of sugar Mannose, Dextrose, Sucrose, Fructose, D-Xylose, Galactose, Mannitol, Lactose, Arabinose and Control Containing only Culture media among Dextrose source of sugar were maximum utilization by fungi and produce maximum sporulation because maximum utilization of carbon and energy source for growth and reproduction then next were Fructose, Mannose And Galactose utilized source of sugar for production of mycelium of fungi and followed by Sucrose, Mannitol And Least In Control And Arabinose Was Poor Sugar Sources For the growth Colletotrichum gloeosporioides Penz and Sacc, All Results Summerized In below Table. The Results Indicates That Dextrose Was Significant Effect on Growth of Colletotrichum gloeosporioides Penz and Sacc.

Keywords: Isolation media, sporulation, nutrients, *Colletotrichum gloeosporioides* Penz and Sacc and effects etc.

Introduction

Fungi require different nutrient source for their growth and reproduction development and critical knowledge of nutritional patterns and factors influencing the growth of fungi is a prerequisite for any study leading to the understanding of host-pathogen relationship. Not much attention has been given on the culture and growth media parameters of the pathogen. Hence, thorough knowledge on the influence of various culture media on growth of the fungus as well as sporulation and colony characteristics of the fungus needed to be developed for suitable management strategies of the disease and may help in taxonomical and physiological study of the fungus. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation.

therefore, present investigation on Mango (*Mangifera indica* L.) belonging to Family *Anacardiaceae* is the most important commercially grown fruit crop of the country. It is called the king of fruits. India has the richest collection of mango cultivars. Cultivation of mango is believed to have originated in South East Asia. Mango is being cultivated in southern Asia for nearly six thousand years. The fruit is very popular with the masses due to its wide range of adaptability, high nutritive value, richness in variety, different cultivar of mango varieties contains 20% of total soluble sugars. The acid content of ripe desert fruit varies from 0.2 to 0.5 % and protein content is about 1 % it's totally defended upon the quality of fruit. But other mango producing countries in the world major production losses due to different pest attacks and diseases. *Colletotrichum gloeosporioides* Penz and Sacc. is one of the most important causal pathogens for disease of mango. About 25 to 30% loses of total mango production has been reported due to anthracnose and stem end rot which can spread with rain drops. Several pre-harvest and post-harvest management approaches has been used to control this anthracnose disease of mango fruits including chemical treatments. Introduction have been reported in Bangladesh. Anthracnose disease of mango is one of the major pre- and post-harvest disease of mango fruit throughout the world and also in Bangladesh which is caused by Colletotrichum gloeosporioides (Ploetz RC,2003) [5].

Anthracnose attacks flowers, young fruits, leaves and twigs, even this disease can also appear in the storage of mature fruits (Chowdhury MNA and MA Rahim,2009) [2]. Disease symptoms appear as slightly, black, sunken irregular shape lesions, which gradually enlarge and developed, leaf spotting, blossom blight, fruit staining and rot. So, present study focused on isolation of *Colletotrichum gloeosporioides* Penz and Sacc. from infected mango fruit in different sugar sources in isolation media and this work are helpful in taxonomical and physiological studies of fungi and also, help in use any single culture medium a combination of two or more media will be more appropriate for routine cultural and morphological characterization of fungi to observe different colony features (G. Sharma and R. R. Pandey, 2010).

Disease cycle of mango anthracnose 1. Dissemination: Conidia (spores) of Colletotrichum gloeosporioides pathogen are dispersed passively by rain splashing or water during irrigation. 2. Inoculation: Pathogenic spores land on the sites of infection such as panicles, leaves, branch terminals. 3. Infection and pathogen development: After germination of the spores, they penetrate through the cuticle and epidermis to ramify through the tissues on immature fruits and young tissues. Infection appears after the spores penetrate the cuticle on mature fruits and remain quiescent until ripening of the climacteric fruits begins (Sepiah M.1986) [15]. 4. Symptom and disease development: rapidly expanding black and sunken lesions develop on affected parts of plants and fruits. 5. Pathogen reproduction: Sticky masses of conidia are produced in acervuline on symptomatic tissue, especially during rainy or humid weather. Many diseases can occur as the fungus continues to multiply during the season. 6. Pathogen survival: The pathogen of Colletotrichum gloeosporioides survives between seasons on affected and defoliated branch terminals and on mature leaves. The symptoms are numerous oval or irregular vinaceous brown or deep brownish spots of various sizes scattered all over the leaf surface under damp conditions. The post-harvest anthracnose of mango incited by latent infection is developed during ripening and transportation to distant markets. The symptom appeared black round or irregular, sometimes sunken spots on the epicarp upon which salmon buff masses of spores developed. As the fruits ripe, these spots may extend over whole surface accompanied by the fruit softening and rotting. Under moist conditions, the blackened areas become covered with minute pinkish reproductive bodies of the fungus, staining, russeting and tear streaking. Conidia of Colletotrichum gloeosporioides produces and develops on mummified inflorescences, panicles, branch terminals, twigs, flower bracts, mummified fruit and leaves are main sources of fungal inoculum. Their production is most in free moisture condition and lower at relative humidity percentage of 95%. Conidia are spread by rains plash and for infection it requires free moisture. As appressoria age, they become melanized. It strengthens the appressorium and helps in penetration of the cuticle by infection pegs that the appressoria produce. Small fruit, disease can produce minute brown spots and abort if infected early in their development. Once an appressorium is developed and fruit exceed 4 - 5 cm in diameter in size, infections stop development. Quiescent infections restart development once concentrations of preformed fungal inhibitors in fruit declining during ripening. On larger fruit, lesions can be developed anywhere, but linear smears that radiate from the stem end to the apex of mango fruit are common.

Mango anthracnose cycle

Conidia/spores are the most important type of inoculum in mango orchards. They are produced on lesions on leaves, twigs, panicles and mummified fruits. The conidia can be rain-splashed to other leaves or flowers to cause secondary infections, thus making the disease polycyclic in these organs Developing fruits can be infected and some aggressive isolates can cause pre-harvest fruit losses.

In the case of postharvest mango anthracnose, developing fruit are infected in the field, but infections remain quiescent until the onset of ripening, which occurs after harvest. Once the climacteric period of the fruit starts, lesions begin to develop. There is usually no fruit-to-fruit infection, hence postharvest anthracnose is considered a monocyclic disease. Mango fruit can also be infected with conidia from isolates of *Colletotrichum* sp. from other host plants like as avocado, papaya and citrus. Wet, humid, warm weather conditions favor mango anthracnose infections in the field.

Losses in quantity and quality (or post-harvest losses) occur after harvest at different points in the handling chain. A loss assessment study conducted under this project in 2015, showed that losses amounted to 31 percent due mainly to decay that manifested at the retail level (Photo 2). Bruising and weight loss which represent loss in marketable weight are the other nature of losses. These losses can be attributed to poor harvesting, rough handling, and poor packaging and transport conditions. The high incidence of decay mainly in the form of anthracnose and stem end rot can be attributed to the high level of pre-harvest infection due to improper disease management during production. Anthracnose is a latent infection and symptoms of the disease become apparent only as the fruit ripens.

Estimated loss caused by Anthracnose disease has been reported 60% or higher in the heavy rainy season (MNA Chowdhury *et al*,2009) [2]. Crop losses generally occur in the form of direct reduction in quantity or quality of the harvested produce. The disease incidence from different countries has been reported to be 32% in South Africa, 64.6% in Costa Rica which can reach almost 100% under wet or Product Loss Caused by Colletotrichum gloeosporioides highly humid condition. 50.28% yield loss caused by anthracnose has been reported in Gondunglegi of Indonesia (Pavitra Kumari Rakesh and Rajender Singh,2017) [17]. and 29.6% post-harvest loss has been reported from Himachal Pradesh of India during 1990-92 [23]. Prakash., *et al*. reported 20 - 30% rotting of mango fruits caused by Colletotrichum gloeosporioides from Hyderabad. Colletotrichum gloeosporioides also causes reduction in flower set, losses in yield and damage foliage, serious problems may appear under crowded and moist conditions in nurseries and orchards. In 2010, incidence and severity of anthracnose were 37 - 57% and 16 - 31% respectively. In 2011, anthracnose disease incidence ranged from 33% to 65% while severity varied between 17% and 35%. Incidence disease and its severity were 77% and 46%, respectively, during surveys at market. Figure 5: Symptoms of Anthracnose Disease on Mango Fruits caused by *Colletotrichum gloeosporioides*. Use of resistant cultivars is an ideal, simplest and cheapest method for the control of plant disease. Heat treatment reduced disease incidence in plantain banana, mango, lychee and longan. Anthracnose is one the most important diseases of mango fruit that affects pre-harvest and post-harvest quality of fruits. It also affects the flowers, leaves and inoculum year-round throughout the canopy. Anthracnose disease is prevalent but well controlled and non-significant in dry regions.

Material and methods

The present experiment conducted *In Vitro* at Department of Botany, ASC college Badarpur district Jalna, Maharashtra, India. During this experiment, sample were collected from infected mango fruit are collected from local market of mango infected by Anthracnose disease caused by *Colletotrichum gloeosporioides* Penz and Sacc. fungi in growing track of Marathwada region.

In Isolation of *Colletotrichum gloeosporioides* modified method are used by Kinkel and Andrews, 1988, C.V. Chudhary, 2006, G. Sharma and R. R. Pandey,2010. Pathogen was isolated from infected mango fruit parts on Potato Dextrose Agar (PDA) medium. Diseased parts were cut into small pieces with the help of sterilized blade. Pieces were washed with sterilized distilled water and disinfected with 70% ethanol for 1 min, then transferred with 1 per cent $HgCl_2$ solution for 10 seconds. Thus, obtained disinfected tissues were immediately washed thrice with sterilized distilled water and aseptically transferred on PDA plates. Inoculated Petri plates were incubated at room temperature (27±2 °C). *Colletotrichum gloeosporioides* Penz and Sacc. were identified according to Sutton’s key (Sutton, 1992) [7]. The obtained culture was purified by using hyphal tip culture method, and maintained on same medium for the further investigations.

Inoculation of Pathogen on Various Sugar sources were incorporated molecular weight in Richard.s broth. The quantity of nitrogen required in each case were determined on the basis of their so as to provide equivalent amount of Sugar as that of potassium nitrate present in the basal medium. The Sugar sources were Mannose, Dextrose, Sucrose, Fructose, D-xylose, Galactose, Mannitol, Lactose, Arabinose and control (no sugar) C.D.AT 0.05&0.01 All the above Sugar sources were mixed thoroughly and the pH of medium was adjusted to seven by using 0.1 N sodium hydroxideor 0.1 N hydrochloric acid. The growth of fungus was studied as described under studies of carbon sources. 30 ml of each of the medium was taken in 100 ml flasks, sterilized and then inoculated with 5 mm discs taken from 9 days old culture of *Colletotrichum gloeosporioides* Penz and Sacc. incubated at 27±1°C for 8 days. Three replications were maintained for each treatment. According to H. S. Nagaraj Rao *et al*., 1964 to Dry weights of the mycelium were estimated after filtering, washing and drying of the harvested mats. (K. T. Arunakumara *et al*., 2015).

Results and discussion

Isolated fungi *Colletotrichum gloeosporioides* Penz and Sacc dense, white aerial mycelium with pink, white, grayish colony colour which carry oil globule pale grey in centre these shows on Potato Dextrose Agar (PDA) medium.

Fig 1: Anthracnose symptoms caused by *Colletotrichum gloeosporioides* Penz. on Mango Fruit.

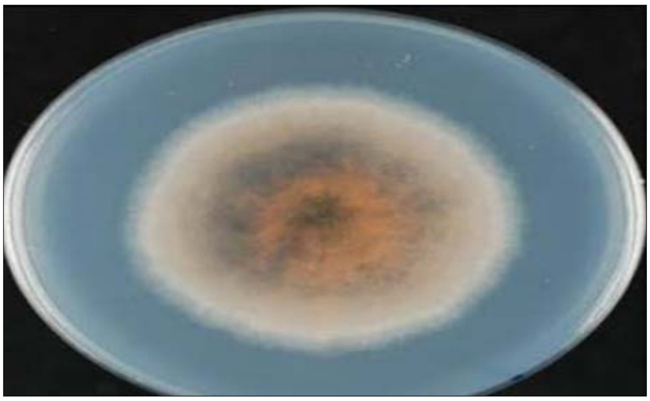

Fig 2: *Colletotrichum gloeosporioides* Penz and Sacc

Effect of isolation media composition on sporulation of *Colletotrichum gloeosporioides* Penz and Sacc. On 8th day of inoculation period.

Effect of isolation media composition on sporulation formation of *Colletotrichum gloeosporioides* Penz and Sacc. On 8th day of inoculation period basically were use different sources of sugar Mannose, Dextrose, Sucrose, Fructose, D-Xylose, Galactose, Mannitol, Lactose, Arabinose and Control Containing only Culture media among Dextrose source of sugar were maximum utilization by fungi and produce maximum sporulation because maximum utilization of carbon and energy source for growth and reproduction then next were Fructose, Mannose And Galactose utilized source of sugar for production of mycelium of fungi and followed by Sucrose, Mannitol And Least In Control And Arabinose Was Poor Sugar Sources For the growth *Colletotrichum gloeosporioides* Penz and Sacc, All Results Summerized In below Table. The Results Indicates That Dextrose Was Significant Effect on Growth of *Colletotrichum gloeosporioides* Penz and Sacc.

Generally, for isolation of fungi use Potato dextrose agar culture because it is easy for preparation and formulation and good formulation help in growth of fungi, according to Maheshwari *et al*., 1999; Saha *et al*., 2008 PDA media suitable for isolation fungi but most fungi grow on PDA and produce maximum sporulation or mycelium. According to G. Sharma and R. R. Pandey in 2010 rich nutrient can affect on sporulation of fungi but in present study showed that 1% of sugar Dextrose in isolation media given maximum sporulation and dry weight of mycelium. Also, author find out effect of sugar sources On Growth of *Alternaria Solani* the Utilization of Sugars as Carbon Sources Has Been Investigated in Several Ectomycorrhizal Fungi (Martin *et al*., 1998; Deveau *et al*., 2008). Effect of nitrogen and carbon sources on the mycelia growth depends on species, culture media, and growth conditions supported by work of (Lin and Yang ,2006) [13]. who also reported similar findings. All the six species studied showed better mycelial growth (measured as dry mass) when nitrogen was supplied in the ammonical form instead as nitrate. Ammonium is generally recognized as 395 the most readily utilizable N source for the most of ECM fungi (Rangel-Castro *et al*. 2002) [14].

Table 1: Effect of isolation media composition on sporulation of *Colletotrichum gloeosporioides* Penz and Sacc. On 8th day of inoculation period

Sr. no.	Sugar /concentration 1%	Dry weight in mg
1	Mannose	147
2	Dextrose(glucose)	188
3	Sucrose	96
4	Fructose	113
5	D-xylose	59
6	Galactose	114
7	Mannitol	28
8	Lactose	65
9	Arabinose	11
10	control (no sugar) C.D.AT 0.05&0.01	87

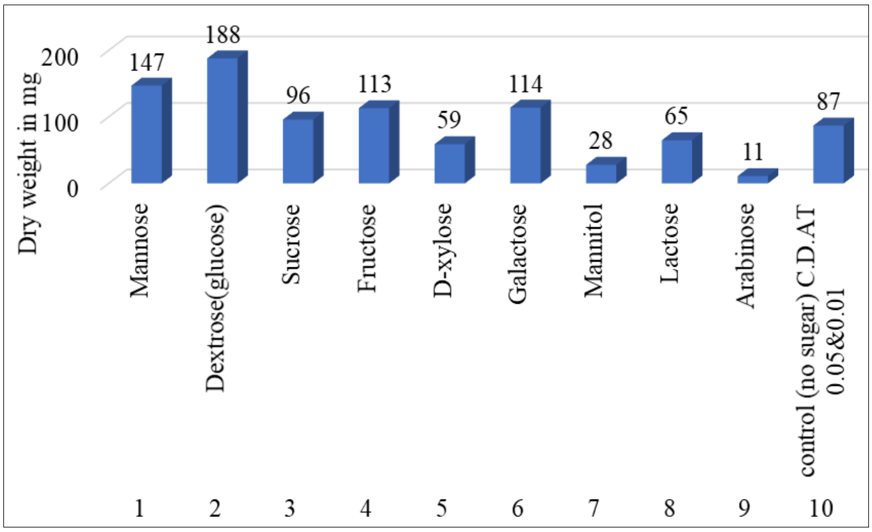

Fig 3: Effect of Media composition on soprulation of *Colletotrichum gloeosporioides* Penz and sacc at 8th day

Conclusion

Results showed that composition of media with 1% dextrose for isolation of fungi, *Colletotrichum gloeosporioides* Penz and Sacc. influenced growth, heavy sporulation and dry weight of mycelium on 8th day inoculation at 27±2 °C temperature and also found that visible colony character so, it is concluded that the media composition with appropriate concentration of sugar source will be suitable for daily practices in culture preparation for isolation of causal organism of disease by plant pathologist.

Prevention of disease

Anthracnose and stem end rot are the two most important post-harvest diseases of mango fruits. These two diseases cannot be detected at the green stage and symptoms of infection appear only as the fruits ripen. An integrated pre- and post-harvest disease management protocol will ensure adequate control of these diseases.

Put the mangoes to be treated in plastic crates with holes on all sides and at the bottom. Do NOT overfill the crate. The crate protects the fruits from heat injury resulting from contact with the hot sides and bottom of the tank.

Dip the crates in hot water for 5 or 10 minutes.

- Stir the water occasionally (if there is no pump to circulate the water) to have uniform temperature within the tank.
- The treatment can be shortened to 5 minutes if the mangoes are produced during the dry hot period when conditions are not favorable for infection in the field.

Remove the crates from the tank

- Cool treated mangoes for 10 minutes in clean tap water if fruits are to be brought to distant markets. Cooling is needed since HWT hastens ripening of the fruit.
- If fast ripening is desired, then fast cooling is not necessary.
- Allow fruits to cool and dry before packing. Faster drying can be achieved by placing crates of mangoes in front of blowers.

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