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Studies on morphological and cultural characteristics of *Colletotrichum gloeosporioides* Penz. causing leaf blight of sapota

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Abstract
During morphological studies it was revealed that, mycelium of *C. gloeosporioides* was septate, branched, hyaline, becoming gray in colour in the later phase. Conidiophores were simple hyaline, short, non-septate and closely packed together in rows Conidia were hyaline, single celled, thin walled, oval to oblong, often contained with 1-2 oil globules at the centre. Whereas in cultural studies it was revealed that, Richard's medium supported the most abundant growth and the best sporulation as compare to rest of the medias.

Keywords: Sapota, Richard's agar, *Colletotrichum gloeosporioides*, sporulation, growth characters

Introduction
Sapota (*Manilkara achras* (Mill.) Forseberg) is a member of the Sapotaceae family. India accounts for 10 percent of the world fruit production with first rank in the production of sapota (APEDA Database, 2020). According to NHB Database, India has 826.92 metric tons production in the year 2020-21. In India, Gujarat ranks first in the production with 273.87 metric tons in 2021-22 (NHB, 2021). In Maharashtra's sapota region covered 70 thousand hectares, producing 322 thousand tonnes with average yield 4.6 tons per hectare. (Surwase *et. al.*, 2015) [10].
In India, around 35 cultivars are widely produced, ranging from which Kalipatti 'Kirthabarthi,' and 'PKM-1' are the most famous varieties. The fruit of Kalipatti' is oval in shape, with a pleasant flesh and a few seeds (one to four). Kirthbharthi contains small to medium fruit with a rough skin whereas, PKM-1 has thin skin and a buttery, highly sweet pulp.
Pune is the second largest district in Maharashtra in area and production of sapota. It is cultivated in Narayangaon, Haveli, Mulashi, Velha, Indapur, Baramati, Shirur and Haveli tehsils of Pune district. Because of the increased area under the sapota cultivation the incidence of various diseases on this crop is increased. As like the disease management in vegetable and field crops farmers are not that much aware about the management of sapota diseases therefore they are not getting optimum yield from this crop.
After 2 to 3 years from plantation, yield of sapota starts but actual economical yield can be obtained from 7 years onwards. Among the various factors for low yield, the maximum reduction in yield occurred by pathogenic factors. The intensity of the leaf blight of sapota in Pune district is found increases from last few years by considering this it, was decided to study the disease in detail by investigating the morphological and cultural characters of the *Colletotrichum gloeosporioides*.

Materials and Methods
Present investigations were carried out in the Department of Plant Pathology and Agricultural microbiology, College of Agriculture, Pune-05 during the year 2022.

Morphological Studies
The morphological characteristics *viz*. sizes, shape, colour of conidia, number of transverse and longitudinal septa were recorded. The size of conidia was measured by using ocular micrometer (calibrated using stage micrometer) under the compound microscope at 400X magnification. Slide culture technique described by Riddle (1950) [8] was employed to obtain details of morphological characters. The morphological traits of isolated fungus with respect to shape, colour and size were recorded.

Cultural studies

The cultural characters of *Colletotrichum gloeosporioides* were studied on six non-synthetic/semi-synthetic and two synthetic solid media. The non-synthetic/semi-synthetic media *viz.* Potato dextrose agar, Malt extract agar, Yeast dextrose agar, Oatmeal Agar and synthetic media *viz.* Richard's agar, Czapek's agar were used.

All media were sterilized at 1.054 Kg/cm^2 pressure at 121^{o}C for fifteen minutes. To carry out the study, 20 ml of each of medium was dispensed in 90 mm petri plates. Such petri plates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1 ^{o}C. Each treatment was replicated thrice. Observations were taken after seven days of incubation. The colony diameter was recorded. The fungal colony colour, margin and sporulations were also recorded. The data on radial fungal growth was assessed statistically. The composition and preparation of the above mentioned synthetic and non-synthetic or semi-synthetic media was taken from 'Ainsworth and Bisby's Dictionary of the Fungi' by Ainsworth (1967) [2] and 'Plant pathological methods: fungi and bacteria' by Tuite (1969) [11].

Results and Discussion

Morphological characters of the test fungus

Morphological study of the isolated fungus was done by using slide culture technique (Riddell, 1950) [8] during this study various morphological traits such as shape, colour and size of mycelium, conidiophores, conidia and acervuli were recorded.

Mycelium

In an early phase the culture of test fungus developed copious amounts of branching, hyaline mycelial growth, which subsequently took on a faintly black hue. The 1.8 to 2.6 m wide, immature hyphae were first thin before becoming broad.

Conidiophores

Conidiophores were arranged in rows between the acervuli's setae and were short, straight, hyaline, non-septate, and tightly spaced.

Conidia

Hyaline conidia were one celled, thin walled, oval to oblong, somewhat constricted in the middle, and rounded at the ends. They ranged in size from 12.5 to 18.5 3.5 to 5.2 m and frequently included 1-2 oil globules in the centre.

Acervuli

In the centre of the places on the leaves that were impacted, there were a lot of little black dots, which were really the asexual fruiting bodies of the fungus. Acervuli had a cushion form with bristly, dark brownish or blackish, pointy setae that tapered toward the tip and mixed with conidiophores. The test pathogen's growth into its ideal condition was not seen during the course of the investigation.

These findings are in conformity with that of Pawar (1998) [7] who observed that, individual hyphae were thin, septate, conidiophores were non-septate, single celled, hyaline individually but brick red in bulk, oval to cylindrical, and rounded at both ends. Venkatravanappa & Nargund (2007) [12] who reported that, the conidia of *C. gloeosporioides* infecting mango were oblong or cylindrical, one celled, hyaline straight with one to two oil globules. In addition, these findings are exactly matching with the earlier report of Patil *et al.* (2009) [6] who also studied morphology of *C. gloeosporioides* isolated from sapota leaves and stated that, mycelium of *C. gloeosporioides* was branched, septate, hyaline and grey in colour in the later phase. Similarly, Adhikary *et al*. (2017) [1], Kimaru *et al.* and (2018) [5] Dharbale *et al.* (2019) [3] who studied the morphology of *C. gloeosporioides* isolated from mango fruit.

Cultural characters of the test fungus

The study on cultural characters of the test fungus was carried out on six different media which were used in both solid and liquid state. Observations regarding mean colony diameter (mm) and colony growth was recorded on solid medium and observations regarding dry weight of mycelium (mg) was recorded on liquid medium whereas on both the media (solid and liquid) observation regarding sporulation was recorded.

Effect of different solid media on growth of and sporulation *C. gloeosporioides*

From the data (Table.1) regarding the effect of different solid media on colony diameter, colony growth and sporulation it was observed that, Richard's medium recorded maximum mycelial growth to the tune of 83.00 mm and was significantly superior to rest of the media. Potato dextrose agar ranked next superior medium in which 80.25 mm mycelium growth was recorded. This was followed by Oat meal agar (76.50 mm) and Czapek's dox agar (75.50 mm) which were statistically on par with each other and significant over Yeast Dextrose Agar medium in which 73.00 mm and colony diameter was recorded. Least mycelial growth (65.25 mm) was recorded in case of Malt Extract Agar Medium.

Richard's agar medium, Oat meal agar, and Potato dextrose agar supported excellent sporulation, whereas Czapek's dox supported moderate sporulation. While sporulation was poor with Malt Extract Agar Medium. No sporulation was seen in yeast extract glucose agar medium.

As per as the observations regarding colony growth concern, it was observed that the growth of *C. gloeosporioides* exhibited considerable variation on six different synthetic and semi synthetic media.

Table 1: Effect of different solid media on growth and sporulation of *Colletotrichum gloeosporioides* Penz

Tr. No.	Name of medium	Colony diameter on 7th day (mm)*				Mean of four replicates	Sporulation
		RI	RII	RIII	RIV		
T_1	Richard's medium	83.00	82.00	84.00	83.00	83.00	++++
T_2	Potato dextrose agar medium	81.00	79.00	80.00	81.00	80.25	+++
T_3	Czapek's dox medium	76.00	75.00	76.00	75.00	75.50	++
T_4	Malt Extract Agar Medium	65.00	65.00	66.00	65.00	65.25	+
T_5	Yeast Dextrose Agar medium	73.00	72.00	74.00	73.00	73.00	-
T_6	Oat meal agar medium	76.00	77.00	76.00	77.00	76.50	++++
	S.E (m). ±					0.36	
	C.D. at 1%					1.48	

Sporulation: - No, + Poor, ++ Moderate, +++ Good, ++++ Excellent

The colony growth on Richard's medium was white, slightly granular to filamentous with wavy surface due to formation of sectors. The colony was thick raised at the central half position and was tapering and becoming flat at the margins. Colony reverse with light brick red coloured development exhibiting typical star like growth due to sector formation, the development of sporulation in the form of masses of acervuli only observe at the centre.

The colony growth on PDA was thick, granular, myceloid with raised central portion. The colony colour was grayish black but no salmon-colored granular development conidial mass was observed. Colony reverse exhibited few concentric rings of black minute acervuli which mainly concentrated a few mm distance from the centre. The overall colony colour was light brick red and pale colored towards the periphery.

The colony growth on Czapek's dox medium was in the form of typical concentric rings, myceloid, thick raised with cottony growth between two successive depressions, colony tending flat, creamy white at periphery. Colony at reverse was also with typical concentric ring. Formation of acervuli was observed prominently forming black dot like structure in the colony. The colony growth on Malt yeast extract agar medium was typically filamentous, somewhat red with oval shape. Colony was completely white without pink- or salmon-coloured conidial masses and black acervuli.

The colony growth on Yeast extract glucose agar medium was uniformly flat slight submerged with uniform growth. The spars hairy filamentous growth was typically observed without salmon masses of conidia. Colony at reverse was milky white without sectoring, without salmon spore masses.

The colony growth on Oat meal agar was thin less filamentous producing abundant salmon coloured spore masses, scattered with less number of black coloured acervuli. The colony at reverse was uniformly creamy red.

Effect of different broth media on growth and sporulation of *C. gloeosporioides* Penz.

Perusal data in Table.2 revealed that, Richard's medium supported maximum vegetative growth (398.50 mg) of the test fungus and was significantly superior to rest of the media. This was followed by Potato dextrose medium (369.75 mg), Czapek's dox medium (334 mg), Oat meal medium (211.75 mg). Remaining two media i.e., Malt yeast extract with 120.20 mg and Yeast extract glucose broth medium with 118.00 mg were found least supportive for the vegetative growth of test fungus. All these media were significantly different from each other except Malt yeast extract medium (120.20 mg) and Yeast extract glucose medium (118.00 mg) which were on par to each other.

In case of sporulation maximum sporulation was recorded in Oat meal medium, Potato dextrose and Richard's media whereas Czapek's dox medium supported moderate sporulation. Malt yeast extracts medium recorded poor sporulation of *Colletotrichum gloeosporioides*. No sporulation was seen in yeast extract glucose medium.

These findings are in agreement with the earlier work did by Ekbote *et al.* (1997) [4] who also studied the cultural aspects of *C. gloeosporioides*, causing mango anthracnose and he observed that, Richard's agar medium, Brow's medium, and PDA showed the greatest radial growth, followed by Czapek's agar. Savale (2006) [9] who studied cultural characters of C. gloeosporioides on eight different solid media and he observed that, Richard's agar medium, PDA medium, Czapek's dox medium and Coon's medium were found most suitable for colony growth of *C. gloeosporioides*.

Table 2: Effect of different broth media on growth and sporulation of *Colletotrichum gloeosporioides* Penz.

Tr. No	Name of the medium	Dry weight of mycelium (mg)*				Mean of four replicates	Sporulation
		RI	RII	RIII	RIV		
T_1	Richard's broth medium	399	404	398	393	398.50	+++
T_2	Potato dextrose broth medium	365	369	374	371	369.75	+++
T_3	Czapek's dox broth medium	333	325	339	335	334.00	++
T_4	Malt yeast extract	120	117	123	121	120.20	+
T_5	Yeast extract glucosebroth medium	121	117	119	115	118.00	-
T_6	Oat meal broth medium	211	214	213	209	211.70	++++
	S.E(m). ±					1.73	
	C.D. at 1%					6.91	

Effect of different solid media on growth and sporulation of *Colletotrichum gloeosporioides* Penz.

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