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Nutritional studies of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the causal agent of leaf and fruit spot of pomegranate

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Abstract
Leaf and fruit spot caused by *C. gloeosporioides* is second important disease of pomegranate. Carbon and Nitrogen are required for structural framework and vital metabolic processes of any cell. The study was taken up to know the best carbon and nitrogen source of the pathogen. Among 16 isolates, 4 isolates preferred sucrose. Maltose was most preferred carbon source and Ammonium nitrate was most preferred nitrogen source.

Keywords: *Colletotrichum,* pomegranate, carbon, nitrogen, growth

Introduction
Pomegranate is generally grown in tropical and sub-tropical regions of the world. The Annual production of Pomegranate in our country is 13.45 lakh Tones. In India anthracnose, caused by *C. gloeosporioides* is the second most important disease of pomegranate next to bacterial blight commonly referred as oily spot caused by *Xanthomonas auxonopodis* pv. *punicae.* The severity of anthracnose has also been reported by several workers (Nargund *et al*. 2012; Raghuvanshi *et al*. 2005; Mandhare *et al*. 1986) [7, 8, 5]. The average intensity of anthracnose and fruit rot of pomegranate under Rahuri (Maharashtra) conditions during 2006 was to the tune of 20.78%. The fungi have specific requirement for its nutrition. Carbon and nitrogen are the most important and essential element, besides others, for their sources infection, growth and reproduction. So to know the best source of carbon and nitrogen for the rapid growth of *C. gloeosporioides*, the study was taken up.

Material and method
This experiment was conducted to find out the source of carbon and nitrogen which can be most efficiently utilized by the fungus for its growth and sporulation. The samples were collected from 10 districts of western Maharashtra. The details regarding location of sample collection is presented in Table given below.

Table 1

Isolate No's	Village	Taluka	District
Pcg 1	Loni	Rahata	Ahmadnagar
Pcg 2	Vilad	Rahuri	Ahmadnagar
Pcg 3	Kolhar	Rahuri	Ahmadnagar
Pcg 4	Bhabareshwar	Rahata	Ahmadnagar
Pcg 5	Kopargaon	Kopargaon	Ahmadnagar
Pcg 6	Pravranagar	Rahata	Ahmadnagar
Pcg 7	Pathri	Sinner	Nashik
Pcg 8	Satana	Satana	Nashik
Pcg 9	Sri krushna nursery	Khatau	Satara
Pcg 10	Shrushti nursery	Khatau	Satara
Pcg 11	Chikhli	Mohol	Solapur
Pcg 12	Kasbe	Miraj	Sangli
Pcg 13	Belhe	Karveer	Kolhapur
Pcg 14	Shinban	Sakri	Dhule
Pcg 15	Chalisgaon	Chalisgaon	Jalgaon
Pcg 16	Shirur	Shirur	Pune

The samples were isolated on Potato Dextrose Broth. Monosporic culture of *C. gloeosporioides* were used from infected Pomegranate fruit and Six different carbon sources viz., fructose, glucose, lactose, maltose, mannitol and sucrose were incorporating into Richard's liquid basal medium. Potassium nitrate was added as a source of nitrogen in all the treatments. Carbon sources were added to the basal medium @ 21.053 grams carbon per liter of medium. Ammonium nitrate, Aspartic acid, L-Asparagine, L-Proline, Potassium nitrate and Ammonium nitrate were used as different nitrogen sources incorporated into Richard's liquid medium @ 1.3855 grams of nitrogen per liter of the medium. In control, no nitrogen source was added. Sucrose was used as source of carbon in all the treatments.

Twenty-five milliliter of Richards agar medium was poured into 100ml flasks, plugged with non-absorbent cotton and autoclaved at 121°C (15psi pressure) for 20 minutes. All the flasks were aseptically inoculated with 5mm fungal discs from an actively growing zone of seven day old culture. Inoculated flasks were incubated at room temperature (27±1 °C) for ten days. The fungal mycelial mat was filtered medium through Whatman No. 42 filter paper and the dry mycelial weight was recorded after drying it in hot air oven maintained at 60 °C for 24 hours. The data thus recorded was statistically analyzed. (Sangeetha C.G., 2009) [9].

Results

Carbon is the most important component in the structural framework of any fungal cell. The different carbon sources (three monosaccharides and three disaccharides) were incorporated in the Richard's agar broth *viz*; Glucose, Fructose, Mannitol, Lactose, Maltose and Sucrose. The data is presented in the Table 1 and Figure 1.

The present investigation revealed that the isolates had varied ability to utilize different carbon sources. The 16 isolates were grown in different carbon sources. The maximum mean dry weight of isolates PCg 1 (0.660 g), PCg 2 (1.649 g), PCg 5 (0.759 g), PCg 13 (0.675g) and PCg 14 (0.448 g) was observed in maltose incorporated broth whereas the mean dry weight of isolates-PCg3 (0.425 g), PCg 5 (0.456 g) and PCg 8 (0.759 g) was maximum in lactose incorporated broth. Similarly, the mean dry weight of isolate Pcg 4 (0.314 g), PCg 6 (0.475 g), PCg 7 (1.513 g) and PCg 10 (0.799 g) more in sucrose and that of isolate PCg 9 (2.103 g) and PCg 12 (0.717 g) in glucose. The maximum mean dry weight of isolates PCg 11 (0.600 g) and PCg 16 (0.993 g) in mannitol as carbon source. Fructose was less preferred by all isolates.

Table 1: Dry weight (in gram) of all isolates in different carbon sources

Isolates	Lactose	Maltose	Sucrose	Glucose	Mannitol	Fructose
PCg 1	0.403	0.660	0.202	0.202	0.160	0.027
PCg 2	0.345	1.649	0.422	0.037	0.238	0.046
PCg 3	0.425	0.281	0.067	0.204	0.204	0.014
PCg 4	0.124	0.160	0.314	0.252	0.193	0.274
PCg 5	0.456	0.759	0.078	0.327	0.357	0.045
PCg 6	0.127	0.252	0.475	0.091	0.112	0.215
PCg 7	0.386	0.262	1.513	0.322	0.248	0.239
PCg 8	0.456	0.196	0.056	0.018	0.229	0.048
PCg 9	0.412	1.534	0.109	2.103	0.243	0.236
PCg 10	0.266	1.503	0.799	0.606	0.254	0.222
PCg 11	0.256	0.290	0.173	0.224	0.600	0.143
PCg 12	0.163	0.461	0.259	0.717	0.247	0.403
PCg 13	0.191	0.675	0.382	0.546	0.278	0.035
PCg 14	0.43	0.448	0.199	0.123	0.201	0.078
PCg 15	0.314	0.193	0.259	0.184	0.226	0.184
PCg 16	0.126	0.106	0.204	0.297	0.993	0.076
SE	0.03	0.06	0.05	0.04	0.03	0.02
CD @ 5%	0.09	0.18	0.14	0.13	0.10	0.06

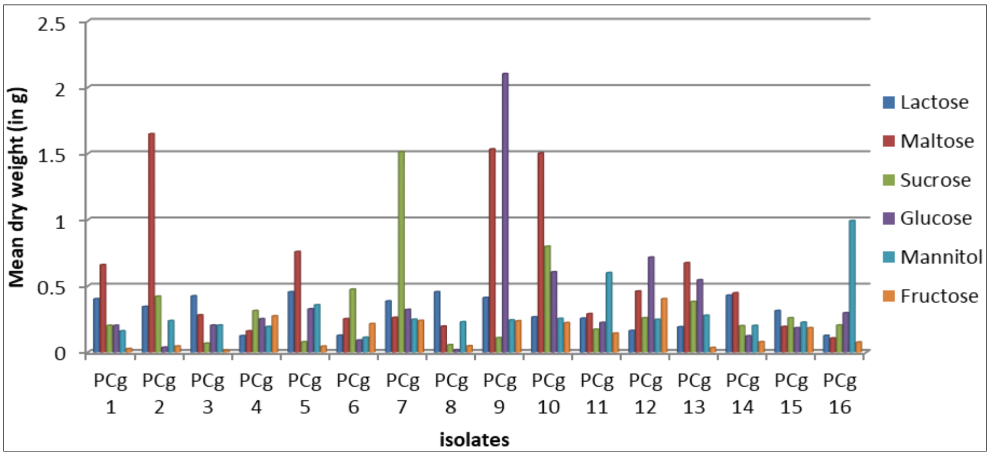

Fig 1: Effect of different carbon sources on growth of Colletotrichum gloeosporioides

Nitrogen is required for many vital processes of fungal cells. The isolates also varied in case of utilizing nitrogen sources. The data is presented in table 2 and figure 2.

The study revealed that Potassium nitrate was most preferred Nitrogen source. The isolate PCg 2 (0.465 g), PCg 5(0.232 g) PCg 6 (0.475 g) and PCg 10 (1.791 g) had maximum mean dry weight in Potassium nitrate whereas PCg 1 (0.349 g) had in L-asparagine. The isolates PCg 3 (0.367 g), PCg 9 (0.567 g), PCg 11 (0.282 g) and PCg 15 (0.461 g) had the maximum mean dry weight in proline and PCg 4 (0.643 g), PCg 7 (0.650 g), PCg 13 (0.698 g), PCg 14 (0.224 g) and PCg 16 (0.396 g) in sodium nitrate. However, isolates PCg 8 (0.553 g) and PCg 12 (0.460 g) had maximum mean dry weight in ammonium nitrate.

Table 2: Dry weight (in gram) of all isolates in different nitrogen sources

Isolates	Ammonium Nitrate	L Asparagine	Proline	Potassium Nitrate	sodium nitrate
PCg 1	0.324	0.349	0.111	0.202	0.210
PCg 2	0.247	O.104	0.226	0.465	0.189
PCg 3	0.042	0.112	0.367	0.139	0.237
PCg 4	0.204	0.061	0.457	0.312	0.643
PCg 5	0.068	0.052	0.231	0.232	0.145
PCg 6	0.231	0.202	0.345	0.475	0.108
PCg 7	0.035	0.023	0.634	0.023	0.650
PCg 8	0.553	0.499	0.456	0.055	0.301
PCg 9	0.094	0.234	0.567	0.109	0.334
PCg 10	0.235	0.120	0.624	1.791	0.226
PCg 11	0.118	0.226	0.282	0.173	0.120
PCg 12	0.460	0.153	0.186	0.259	0.441
PCg 13	0.061	0.048	0.012	0.382	0.698
PCg 14	0.007	0.056	0.195	0.199	0.224
PCg 15	0.450	0.048	0.461	0.259	0.326
PCg 16	0.120	0.156	0.197	0.204	0.396
SE	0.02	0.02	0.03	0.05	0.04
CD @ 5%	0.07	0.05	0.10	0.15	0.11

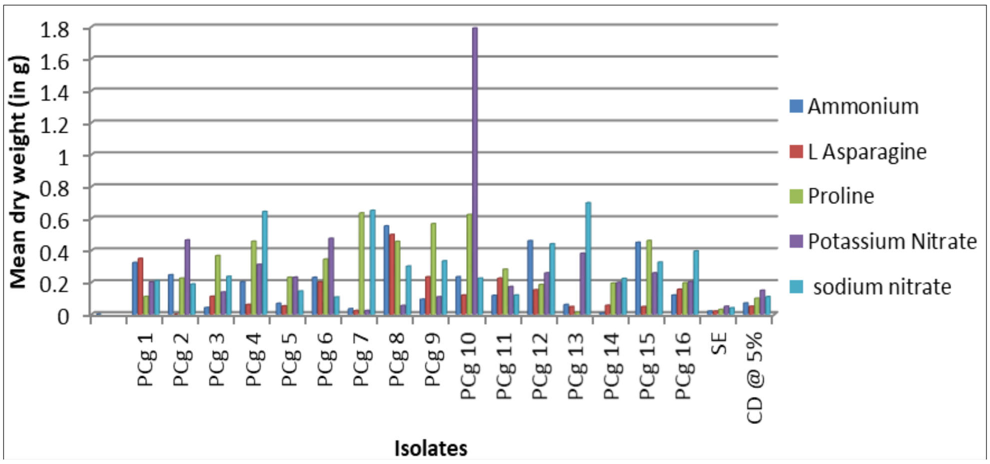

Fig 2: Effect of different nitrogen sources on growth of Colletotrichum gloeosporioides

Discussion

Sucrose was found to be the best source of carbon for growth of *C. gloeosporioides* by many workers like Durairaj, Naik and Saxena but these workers have not used mannitol as a source of carbon in their studies. The results are in collaboration with the findings of Hegde *et al*. (1990) [2] stated dextrose and sucrose were the best C sources for *C. gloeosporioides* [*Glomerella cingulata*] isolated from *Areca catechu.*

In the nitrogen requirement of isolates, Potassium nitrate was most preferred Nitrogen source. Next preferred as Nitrogen source was Ammonium nitrate. The results are in agreement to Wasantha Kumara and Rawal (*2008*) [12] who found aspartic acid supported the maximum growth of isolates followed by potassium nitrate and proline. Sangeetha and Rawal (2009) [9] stated that Potassium nitrate and sodium nitrate also showed good growth. Udhayakumar and Usha Rani (2010) [11] studied the different carbon and nitrogen sources and found out that ammonium nitrate (86.6mm and 680.8mg) as the best nitrogen source. Kuberan *et al*. (2012) [4] found Sodium nitrate was to be the best one followed by potassium nitrate for growth. The amino acid sources aspartic acid provided maximum growth followed by proline. Jahanara Begam and Sharma (2015) [3] found Ammonium nitrate has yielded highest mycelia followed by malt extract, $NaNO_3$, KNO_3, Ammonium sulphate and peptone.

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