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Physiological studies and pathogenicity of *Botryodiplodia theobromae* causing post-harvest stem end rot of mango

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Abstract
Stem end rot (SER) is one of the most frequently occurring post-harvest disease of mango in all parts of the world including India affecting quality of mango fruits. Three fungi namely *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides* and *Alternaria alternata* were isolated from naturally infected mango fruits showing SER symptoms with maximum frequency of *B. theobromae* (68.8%) followed by *C. Gloeosporioides* (20.0%) and *A. alternata* (9.3%). Significant variations in mycelia growth of *B. theobromae* was recorded in six culture media, six carbon sources, four temperatures and five pH levels. Maximum mycelia growth of the fungus was recorded in oat meal agar (66.7mm) followed by potato dextrose agar (55.3 mm) and mango leaf extract agar (45.7mm). Dextrose (79.2 mm) and glucose (78.0 mm) supplemented potato agar media supported the highest mycelia growth of *B. theobromae*. Highest mycelia growth was recorded in potato dextrose agar (PDA) incubated at 30 °C (79.7 mm) temperature followed by 25 °C (65.5 mm) and lowest growth was at 20 °C (32.3 mm). Mycelia growth of the fungus was maximum in PDA adjusted at pH 6 (59.5 mm) followed by pH 6.5 (55.8 mm) and pH 7 (49.5 mm). Pathogenic potential of B. *theobromae* was assessed in five popular mango varieties namely *Fazali*, *Langra*, *Chousa*, *Neelam* and *Dashahari*. Significant variation in lesion area (cm^2), physiological loss in fruit weight (PLW %) and total soluble solids (TSS) content were found in all the varieties. Maximum lesion area (73.7 cm^2) was recorded in *Chousa* followed by *Neelam* (69.9 cm^2). Physiological loss in fruit weight (PLW %) and reduction in TSS content were 9.6to 19.0% and 5.1 to 25.3%, respectively in five varieties of mango with maximum in *Neelam* followed by *Chousa*. On the basis of these characters *Dashahari* was resistant, *Fazali* was moderately resistant, *Langra* was moderately susceptible. Whereas *Chousa* and *Neelam* were susceptible to SER.

Keywords: Mango, stem end rot, *botryodiplodia theobromae*, cultural characteristics, pathogencity

Introduction
Mango *(Mangifera indica* L), belonging to the family Anacardiaceae is an important fruit crop mostly grown in tropical and subtropical areas of the world. Asia is the largest mango producer representing more than75% of global production. In India, the area of mango was 2350 thousand hectares with production of 20772 thousand MT, whereas the area and production of mango in Madhya Pradesh was 60.33 thousand hectares and 842.33 thousand MT, respectively during 2021-22 (agricoop.nic.in). India is a prominent exporter of mangoes and exported 22963.76 MT of fresh mangoes to the world during the year 2022-23 (apeda.gov.in). Mango is the national fruit of India and considered as *king of fruits* due to its delicious taste, flavor, aroma and attractive colour. Ripe mango fruit is considered to be invigorating and freshening. Since it is a perishable fruit with high sugar contents, it has incredible susceptibility towards fungal diseases that harms the fruit during postharvest processes (Sharma, 2014) [19]. Mango is vulnerable to a number of diseases occurring almost at all phases of its growth and development including post-harvest handling stage (Alemu, 2014) [3]. Prabhakar *et al.* (2005) [14] recorded post-harvest losses of fresh mango fruits to be 25-40% in India and microbial decay accounts for 17.0 to 26.9% of the total post-harvest losses in Asian countries. In India, post-harvest stem end rot caused by *Botryodiplodia theobromae* (Syn. *Lasiodiplodia theobromae* Pat.) is the wide spread fungal disease of great economic value. Physiological studies of the pathogen and its pathogenicity in different varieties of mango were undertaken in the present investigation,

Materials and Methods
In the present study, stem end rot affected fruits of mango varieties *Chousa* and *Neelam* were collected and used to isolate associated fungi. Infected fruit tissues were cut into small pieces

(1-2 cm). Samples were surface sterilized by dipping in 1% NaOCl solution for 3 min, rinsed 2 times with sterile distilled water and dried on sterilized blotter paper. The samples were placed in petri dishes containing potato dextrose agar medium and incubated at 28 °C for 4 days under alternating 12-h light and dark periods. The hyphal tip transfer method was used for purification of isolated fungal pathogens. The sub culturing was done at an interval of 15 days. The purified fungal pathogens were identified on the basis of cultural and morphological features of the fungus. Frequency of isolated fungal pathogens was calculated using following formula.

$$\text{Frequency}(\%) = \frac{\text{Number of rotten fruits}}{\text{Total number of fruits}} \text{ x } 100$$

Effect of six culture media i.e. Potato dextrose agar (PDA), Mango leaf extract agar (MLEA), Oat meal agar (OMA), Corn meal agar (CMA), Richards agar (RA) and Czapeck's dox agar (CDA), six carbon sources namely glucose, Fructose, Sucrose, Maltose, Lactose and Dextrose, four temperature *viz*. 20, 25, 30 and 35 °C and five pH levels 6.0, 6.5, 7.0, 7.5 and 8.0 were studied on mycelia growth of *Botryodiplodia theobromae*. Six culture media were poured into 9 mm diameter sterilized petri plates and allowed to solidify. Five mm disc of seven days old culture was picked up with a sterile cork borer from the edges of the fungus colony and placed in the centre of each petri plate containing autoclaved media. The Petri plates were wrapped with aluminum foil and incubated at 28+2 °C. In another experiment, about 15 to 20 ml potato agar media containing different carbon sources were poured into 9 mm diameter sterilized petri plates. Five mm disc of culture was placed in the centre of each petri plate. The Petri plates were then wrapped with aluminum foil and incubated at 28±2 °C. The effect of temperature on mycelia growth of *B. theobromae* was studied on PDA medium containing mycelia disc. The Petri plates were incubated at 20, 25, 30 and 35 °C in BOD incubator. The effect of different pH levels i.e. 6.0, 6.5, 7.0, 7.5 and 8.0, on mycelia growth of *B. theobromae* was studied. To get the desired pH levels, the pH of PDA medium was adjusted by adding 0.1 M HCl and 0.1 M NaOH. Litmus stripes were used to confirm the pH of the media prior to sterilizing. The experiments were laid out in 4 replications. The diameter of the fungal colony was recorded in mm from each plate at 24 h interval starting from 48 h of incubation to 146 h.

Mature mango fruit of five varieties namely *Fazali, Chousa, Langra, Neelam* and *Dashahari* were collected from the orchard at Kuthulia farm, Rewa (M.P.). The fruits were thoroughly washed with tap water, sterilized in 0.1% sodium hypochlorite solution for two minutes and rinsed twice in sterilized water. After drying in blotter paper, the pedicel of mango fruit was removed and fruits were inoculated by macerated seven days old culture of *B. theobromae* at the point of style region. After inoculation, the fruits were placed in plastic boxes lined with water soaked blotting paper. Non-inoculated fruits were kept as control. Initial fruit weight of each mango varieties was recorded. Four replications for each variety were maintained. Three days after inoculation, lesion area (cm^2) and fruit weight (g) were recorded up to 6 days of inoculation (DAI). Physiological loss in weight (PLW) of mango fruits was estimated using following formula (Dukare *et al.*, 2019) [5].

$$\text{PLW}(\%) = \frac{\text{Initial fruit weight - Fruit weight on the day of observation}}{\text{Initial fruit weight}} \text{ x } 100$$

A hand refractometer was used for the estimation of total soluble solids (TSS) from healthy and infected mango fruits. The collected data were statistically analyzed for their significance

Results and Discussion

Three fungi were isolated from naturally infected mango fruits showing SER symptoms with maximum frequency of *Botryodiplodia theobromae* (68.8%) followed by *Colletotrichum gloeosporioides* (20.0%) and *Alternaria alternata* (9.3%) in both the varieties of mango (Fig.1). On artificial inoculation with *B. theobromae*, dark brown to black spots appeared on the tip of the mango and subsequently extended on the entire fruit. Maqsood *et al.* (2014) [10] also reported similar symptoms of SER disease caused by *B. theobromae* in the mango. Sangchote (1991) [18], Naznin *et al.* (2007) [12] and Hong *et al.* (2012) [7] also consistently isolated *B. theobromae* from SER infected mango fruits and reported dominant fungal species. Pure culture of *B. theobromae* was initially white to smoke ray with fluffy aerial mycelium which turn grayish black in later stages on PDA medium. Black pigmentation was also observed in old culture.

Physiological studies of *B. theobromae*

The mycelia growth of *B. theobromae* was recorded in six culture media after 24, 48, 72, 96, 120 and 146 hrs. of incubation and data are presented in Table 1. Significant variations in colony diameter (mm) of fungus were recorded in different media. Average colony diameter ranging from 0.0 to 11.3 mm was recorded after 24 hrs. of incubation. Whereas it was 14.3 to 22.3 mm, 17.0 mm to 30.7 mm, 24.0 to 40.7 mm, 28.0 mm to 51.3 mm and 31.3 to 66.7 mm after 48, 72, 96, 120 and 146 hrs. of incubation, respectively. The maximum growth of *B. theobromae* (66.7 mm) was recorded in Oat Meal Agar followed by Potato Dextrose Agar (55.3mm), and Mango Leaf Extract Agar (45.7 mm). Least mycelia growth was recorded in Richard's medium (31.3 mm) followed by Corn Meal Agar (35.0 mm) and Czapeck's Dox Agar (35.7 mm) which were statistically at par. Mazumdar and Mandal (2018) [9] also recorded fastest mycelia growth of *B. theobromae* on Oat Meal Agar. Maximum growth of *B. theobromae* in Potato Dextrose Agar was also reported by Qureshi and Meah (1991) [15], Alam *et at* (2001) [1], Saha *et al.* (2008) [17], Chukunda and Onyeizu (2019) [4] *and* Ekanayake *et al.* (2019) [6] in mango isolate

The data presented in Table 2 revealed that fungi differ in their ability to utilize carbon sources for growth. Dextrose supplemented media exhibited maximum mycelia growth of *B. theobromae* (79.2 mm) and was statistically at par in glucose supplemented media (78.0 mm). Mycelia growth of the fungus was at par in Sucrose (57.0 mm), Maltose (56.5 mm) and Fructose (52.0 mm) supplemented medium after Dextrose and glucose. These results are in agreement with those of Meah *et al.* (1991) [11] and Maqsood *et al.* (2014) [10], who reported that *B. theobromae* had the best growth on glucose, fructose and sucrose. In the present study, lactose supplemented media showed lowest growth (44.5 mm) of *B.*

theobromae and is supported with the findings of Maqsood *et al.* (2014) [10].

Mycelia growth of *B. theobromae* was recorded at four temperature i.e.20, 25, 30 and 35 °C after 24, 48, 72, 96, 120 and 146 hrs. of incubation on potato dextrose agar medium and data are presented in Table 3. Significant differences among the temperature levels that affected the growth of *B. theobromae* were recorded. Average mycelia growth ranging from 8.0 to 10.3 mm, 10.3 to 33.5 mm, 14.7 to 58.5 mm, 19.0 to 68.5 mm and 27.0 to 70.5 mm was recorded after 24, 48,72, 96, 120 and 146 hrs. of incubation, respectively. Maximum growth of fungus was recorded on potato dextrose agar incubation at 30 °C (79.7 mm) after 146 hrs. of incubation followed by 25 °C (65.5 mm) and 35 °C (48.3 mm). Minimum growth was recorded when pathogen was incubated at 20 °C (32.3 mm). Khanzada *et al.* (2006) [8] recorded highest fungal growth of *B. theobromae* at temperature range from 30 to 40 °C. Maqsood *et al.* (2014) [10] also recorded maximum mycelia growth on media incubated at 28 °C followed by 25 °C and 30 °C. Rajmane and Korekar (2016) [16] reported severe SER and fruit rotting in mango at 30 °C temperature. Ullah *et al.* (2017) [22] observed maximum growth at 30 °C. These results support the present findings.

The results presented in Table 4 showed that pH levels significantly affected the mycelia growth of the fungus. Mycelia growth of *B. theobromae* was 5.3 to 11.0 mm, 6.8 to 15.0 mm, 15.9 to 19.0 mm, 23.5 to 31.5 mm, 32.5 to 42.2 mm and 35.8 to 59.5 mm after 24, 48, 72, 96, 146 and 146 hrs. after incubation in different pH levels. Maximum growth of the fungus was recorded on potato dextrose agar adjusted with pH of 6.0 (59.5 mm) followed by 55.8 mm at pH 6.5 and 49.5 mm at pH level 7.0. Minimum growth of the fungus was recorded at pH level 8.0 (35.8 mm) closely followed by pH levels 7.5 (36.5 mm).Hydrogen ion concentration (pH) of the medium is one of the important factor for growth and sporulation of a particular fungus. Maqsood *et al.* (2014) [10] also recorded most suitable pH 5.5 to 6.5 for maximum growth of *B. theobromae*. Ullah *et al.* (2017) [22] observed maximum growth of *B. theobromae* at pH 6.0. On contrary, best growth of *B. theobromae* isolated from die back infected mango (Patil *et al.*, 2006) [13] was at pH level 7.

Table 1: Growth of *Botryodiplodia theobromae* isolated from infected mango fruits on different culture media

Culture media	Colony diameter (mm)					
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	146 hrs.
Potato dextrose agar	7.0	20.5	30.7	38.0	49.0	55.3
Mango leaf extract agar (10%)	5.7	20.5	27.3	31.7	41.3	45.7
Oat meal agar	11.3	22.3	24.3	40.7	51.3	66.7
Corn meal agar	0.0	17.3	22.0	24.0	32.3	35.0
Richards agar	3.3	14.3	17.0	26.3	30.0	31.3
Czapeck's dox agar	4.0	15.3	18.7	24.7	28.0	35.7
SEm±	0.657	1.078	1.233	1.413	1.595	1.719
CD (5%)	1.952	3.203	3.663	4.199	4.739	5.108

Table 2: Growth of *Botryodiplodia theobromae* isolated from infected mango fruits on different carbon sources

Carbon sources	Colony diameter (mm)					
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	146 hrs.
Glucose	15.0	20.3	32.3	40.0	67.2	78.0
Maltose	10.0	17.5	28.5	37.5	45.2	56.5
Fructose	0.0	17.5	29.5	38.5	44.4	52.0
Lactose	0.0	15.0	25.0	25.0	34.6	44.5
Dextrose	0.0	20.0	30.0	42.0	68.3	79.2
Sucrose	0.0	18.0	29.7	38.0	46.2	57.0
S.E.m±	0.595	1.094	1.393	1.605	1.820	1.952
CD (5%)	1.768	3.252	4.141	4.770	5.410	5.799

Table 3: Growth of *Botryodiplodia theobromae* isolated from infected Mango fruits at different temperature

Temperature (°C)	Colony diameter (mm)					
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	146 hrs.
20	8.0	10.3	14.7	19.0	27.0	32.3
25	10.0	26.7	35.7	45.3	55.5	65.5
30	10.3	33.5	58.5	68.5	70.5	79.7
35	8.6	19.6	23.3	40.6	44.3	48.3
S.E.m±	0.809	1.243	1.538	1.732	1.815	1.857
CD (5%)	2.428	3.728	4.611	5.193	5.443	5.568

Table 4: Effect of different pH levels on mycelia growth of *Botryodiplodia theobromae*

pH level	Colony diameter (mm)					
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	146 hrs.
6.0	9.6	11.6	19.0	30.6	42.2	59.5
6.5	11.0	15.0	17.5	27.5	40.5	55.8
7.0	10.5	14.5	18.0	23.5	38.0	49.5
7.5	6.0	9.8	19.0	31.5	34.5	36.5
8.0	5.3	6.8	15.9	30.5	32.5	35.8
SEm±	0.564	0.724	0.938	1.052	1.243	1.729
CD (5%)	1.700	2.183	2.829	3.171	3.747	5.212

Pathogenicity of *B. theobromae* causing post-harvest stem end rot of mango

Data on disease development in terms of lesion area (cm^2) were recorded at 3, 4, 5 and 6 days after inoculation (DAI) in five varieties of mango. The results are presented in Table 5. Lesions were initiated 2 DAI only in mango varieties *Chausa* and *Neelam*. Complete disease development was observed 3 DAI in all the varieties. Significant variation in lesion area was recorded after inoculation in all the varieties. Lesion area ranging from 1.3 to 13.8 cm^2 with a

mean of 6.5 cm^2, 3.5 to 42.4 cm^2 with a mean of 19.0 cm^2, 4.9 to 58.7 cm^2 with a mean of 29.7 cm^2 and 11.6 to 73.7 cm^2 with a mean of 39.1 cm^2 were recorded 3, 4, 5 and 6 DAI in all varieties of mango. After 6 DAI maximum lesion area was recorded in mango variety *Chausa* (73.7 cm^2) followed by *Neelam* (69.9 cm^2). Whereas minimum lesion area was noted in *Dashahari* (11.6 cm^2) followed by *Fazali* (13.1 cm^2) and *Langra* (27.3 cm^2). On the basis of disease development (SER), mango variety *Dashahari* and *Fazali* were found resistant to SER. Whereas *Neelam* and *Chausa* were susceptible to SER. Mango variety *Langra* was moderately susceptible to SER. Data of average fruit weight (g) and physiological loss in weight (%) due to infection of *B. theobromae* in five varieties of mango are presented in Table 6. Initial fruit weight of *Fazali*, *Langra*, *Chausa*, *Neelam* and *Dashahari* varieties was 220.0g, 182.2g, 194.5g, 102.0g and 153.4g, respectively before inoculation. Gradual decrease in fruit weight from 220.0g to 198.9g in Fazali, 182.2g to 162.2g in Langra, 194.5g to 159.5g in Chausa, 102.0g to 82.6g in Neelam and 153.4g to 141.9g in Dashahari was recorded. Average physiological loss in weight (PLW) was 1.7%, 5.1%, 8.6% and 9.6% in *Fazali* at 3, 4, 5 and 6 days after inoculation (DAI), respectively. In *Langra* variety average PLW was 2.9%, 5.5%, 8.1% and 11.0% at 3, 4, 5 and DAI, respectively. In *Chausa*, average PLW was 2.7%, 8.2%, 14.3% and 18.0% at 3, 4, 5 and 6 DAI, respectively. Average PLW was 5.1%, 10.7%, 16.4% and 19.0% at 3, 4, 5 and 6 DAI, respectively in *Neelam*. Whereas minimum loss was recorded in *Dashahari* and it was 1.4%, 3.0%, 6.1% and 7.5% at 3, 4, 5 and 6 DAI, respectively. Maximum PLW was recorded in mango variety *Neelam* (19.0%) followed by 18.0% in *Chausa* and 11.0% in *Langra*. Whereas minimum loss in PLW was recorded in *Dashahari* (7.5%) followed by *Fazali* (9.6%). Tandel (2017) [20] reported significant negative correlation of SER with fruit weight (-0.30g), and shelf life of mango variety *Dashahari* (-0.70g), *Neelam* (-0.80g) and *Amprapali* (-0.83g). Ullah *et al.* (2017) [22] recorded mean lesion length and disease area in white *Chausa* and *Sindhuri* variety of mango caused by SER pathogen and reported that white Chausa is prone to SER. Dukare *et al.* (2019)[10] reported that progress of lesion development was faster in mangoes stored under ambient condition than the controlled condition. They recorded 29.97% PLW in *Dashahari* and 1.1% PLW for *Safeda* stored in controlled condition. Alam *et al.* (2020) [2] verified that *Lasiodiplodia theobromae* was most aggressive and virulent pathogen with the largest lesion on inoculation fruits in pathogenicity assay. All these studies are in agreement with the present findings.

Biochemical quality attribute total soluble solids (TSS) was recorded in healthy and stem end rot infected fruits (6 DAI) of five mango varieties i.e. *Fazali*, *Langra*, *Chausa*, *Neelam* and *Dashahari* which ranged 17.6 to 21.9% in healthy fruits and 15.9 to 19.1% in inoculated fruits (Table 7). TSS was more in *Chausa* (21.9%), *Neelam* (21.3%) and *Dashahari* (20.6%). Maximum reduction in TSS value was recorded in mango fruit of *Neelam* (25.3%) followed by *Chausa* (23.7%), *Langra* (11.4%), D*ashahari* (7.3%) and *Fazali* (5.1%). Tongdee *et al.* (1980) [21] also reported that varietal susceptibility was closely related to the amount of sucrose and reducing sugars in fruits.

It may be concluded from the present study that the pathogen responsible for stem end rot (SER) caused significant spoilage in fruits, reduces shelf life and quality of fruits. Maximum frequency of *Botryodiplodia theobromae* (68.8%) was found from the naturally infected mango fruits showing post-harvest stem end rot symptoms followed by *Colletotrichum gloeosporioides* (20.0%) and *Alternaria alternata* (9.3%). Maximum mycelia growth of *B. theobromae* was recorded in Oat meal agar followed by Potato dextrose agar and Mango leaf extract agar at 30 °C temperature and 6 to 6.5 pH level. Dextrose (79.2 mm) and glucose (78.0 mm) supplemented media supported the highest mycelia growth of *B. theobromae*. Lowest stem end rot severity was recorded in mango variety *Dashahari* followed by *Fazali* and *Langra*, whereas *Chousa* and *Neelam* were highly susceptible. Reduction in TSS value was recorded ranging from 5.1 to 25.3% in different varieties of mango infected with stem end rot disease.

Table 5: Progression in mean lesion area (cm^2) on different varieties of mango

S. No.	Variety	Lesion area (cm^2)			
		3 DAI	4 DAI	5 DAI	6 DAI
1	Fazali	1.3	5.7	8.5	13.1
2	Langra	2.8	10.7	21.2	27.3
3	Chausa	13.8	42.4	55.4	73.7
4	Neelam	12.9	32.6	58.7	69.9
5	Dashahari	1.8	3.5	4.9	11.6
6	Mean	6.5	19.0	29.7	39.1
7	SEm±	1.151	3.251	3.690	7.725
8	CD (5%)	3.501	9.888	11.225	23.499

Table 6: Physiological loss in fruit weight of mango due to infection of *Botryodiplodia theobromae*

S. No.	Variety	Initial Fruit Weight (g)	Final fruit weight (g)			
			3 DAI	4 DAI	5 DAI	6 DAI
1	Fazali	220.0	216.3 (1.7%)	208.7 (5. 1%)	201.1 (8.6%)	198.9 (9.6%)
2	Langra	182.2	176.9 (2.9%)	172.2 (5.0%)	167.5 (8.1%)	162.2 (11.0%)
3	Chausa	194.5	189.2 (2.7%)	178.5 (8.2%)	166.6 (14.3%)	159.5 (18.0%)
4	Neelam	102.0	96.8 (5.1%)	91.1 (10.7%)	85.3 (16.4%)	82.6 (19.0%)
5	Dashahari	153.4	151.2 (1.4%)	148.8 (3.0%)	144.1 (6.1%)	141.9 (7.5%)
	Mean	170.4	166.1 (2.5%)	159.9 (6.2%)	152.9 (10.3%)	149.0 (12.6%)

Figures in parentheses are percent loss in fruit weight, DAI = Days After Inoculation

Table 7: Influence of stem end rot infection on TSS (total soluble solids) of mango fruits

S. No.	Variety	TSS		% Reduction
		Healthy	Inoculated	
1	Fazali	17.6	16.7	5.1
2	Langra	19.3	17.1	11.4
3	Chausa	21.9	16.7	23.7

4	Neelam	21.3	15.9	25.3
5	Dashahari	20.6	19.1	7.3
6	SEm±	0.473	0.476	-
7	CD (5%)	1.439	1.449	-

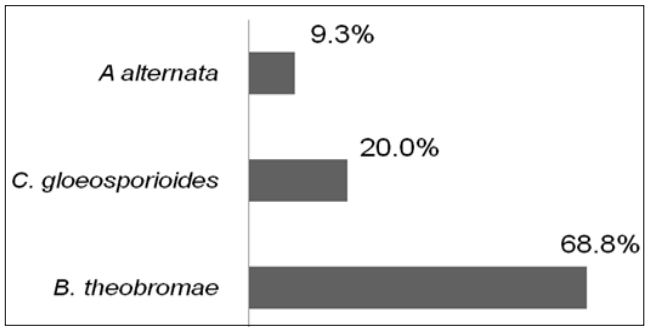

Fig 1: Association of fungi with SER infected mango fruits

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