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EFFECT OF DIFFERENT MEDIA ON THE GROWTH OF *COLLETOTRICHUM GRAMINICOLA* (CES.) WILSON CAUSING ANTHRACNOSE DISEASE OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH]

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SUMMARY

Sorghum anthracnose disease is one of the major fungal disease of sorghum. The variability of different three districts isolates (Nine-9) of *C. graminicola* were studied on different culture media *viz*., potato dextrose agar, Richard's agar, Czapek's agar, malt extract agar, oat meal agar and sorghum seed extract agar. Potato dextrose agar, oat meal agar and malt extract agar supported maximum growth for all isolates. Isolate ST-1 showed maximum growth of 88.75 mm diameter on PDA and least growth on sorghum seed extract agar with least growth of 33.00 mm diameter in ST-2 isolate. Isolate NRM-3 recorded maximum growth mycelial diameter of 89.80 mm on oat meal agar and least on Czapek's agar with 36.00 mm. Isolate BH-2 recorded maximum growth of 89.20 mm mycelial diameter on PDA and least on sorghum seed extract agar with 35.00 mm in BH-3 isolate.

Key words: *Colletotrichum graminicola*, sorghum, anthracnose, isolates, media

Sorghum (*Sorghum bicolor* L. Moench) is a major cereal crop grown under rainfed conditions and marginal lands due to its drought resilience and adaptability. In India, sorghum plays a critical role as a food, fodder, and industrial crop, especially in Gujarat, Karnataka, Maharashtra, and Andhra Pradesh. However, the productivity of sorghum is severely constrained by diseases, among which anthracnose caused by *Colletotrichum graminicola* is one of the most prevalent and economically important.

The pathogen attacks aerial parts including leaves, stems, and panicles, leading to significant reductions in yield and fodder quality. Losses ranging from 30–60% have been reported depending on cultivar susceptibility and environmental conditions. Considering the limitations of sole reliance on fungicides, the identification of resistant genotypes coupled with effective fungicidal strategies offers a sustainable solution. The present investigation was undertaken to screen sorghum genotypes under field conditions for resistance and to evaluate fungicides for their efficacy in managing the disease.

MATERIALS AND METHODS

Collection of the infected leaves

Fresh samples of diseased leave showing typical anthracnose symptoms were collected from each village of Surat, Bharuch and Narmada districts of South Gujarat during survey. Each sample was wrapped in brown paper and kept in polypropylene bag and brought to the laboratory of department of plant pathology, NMCA, NAU, Navsari for further investigation.

Isolation of *Colletotrichum graminicola*

For isolation of the fungal pathogen, the collected disease sample were cut into small bits 3×3 mm from the lesion margins (single lesion), surface sterilized by using 0.1 per cent sodium hypochlorite solution for 1 minute followed by rinsing three times in sterile distilled water. The sections were allowed to dry in a laminar flow before plating on Oat Meal Agar

(OMA) plates under aseptic condition. After incubation for 7 days at temperature of 27±1 °C, fungal growth appearance was purified by single lesions isolation technique. The isolate collected from these single lesions were separated as monoconidial isolates from each districts. The well isolated single spores were marked and then transferred on OMA medium separately under aseptic conditions of Laminar–air-flow cabinet. By following single hyphal tip technique, test pathogen was transferred aseptically on the OMA slants in test tubes. Through sub culturing, the pathogen was purified and pure culture was maintained on agar slants in test tubes and stored in refrigerator for further studies. Per districts three isolates were separated which were named as ST-1, ST-2 and ST-3 for Surat district, NRM-1, NRM-2 and NRM-3 for Narmada district and BH-1, BH-2 and BH-3 for Bharuch district. The slides were prepared from 15 days old culture of the fungal pathogen with lacto-phenol cotton blue solution. The morphological, cultural and formation of conidia were the principle characters to identify the pure cultures and pathogen identification characters were compared with the standard reference description (Holliday, 1980). These cultures were maintained on OMA slants at 4 ºC for further studies.

Preparation of different media and inoculation

Oat meal agar was used as basal culture medium for isolation, purification and maintenance of the pure culture of *C. graminicola*. The growth characters of *C. graminicola* were studied on six solid media which included both synthetic and non-synthetic media. Synthetic media *viz*., Richard's agar and Czapek's agar media and non-synthetic media *viz*., potato dextrose agar, sorghum seed extract agar, oat meal agar and malt extract agar were used. All the media were sterilized at 1.1 kg/m² pressure for 15 minutes. 20 ml of each of the medium was poured 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 °C.

RESULTS AND DISCUSSION

The result presented in Table 1, 2 and 3 and Photo 1, 2 and 3 revealed that all the nine isolates of *C. graminicola* showed variation in the mycelial growth and colony diameter on six different synthetic and non-synthetic media *viz*., potato dextrose agar, Richard's agar, Czapek's agar, malt extract agar, oat meal agar and sorghum seed extract agar. Colony diameter was recorded, when the maximum growth was attained after seven days of incubation period in all the tested media.

TABLE 1
Effect of various culture media on growth of different isolates of *C. graminicola* from Surat district

S. No.	Culture media	Mean colony diameter (mm)		
		Surat Isolates		
		ST-1	ST-2	ST-3
1.	Potato dextrose agar	88.75	88.60	84.35
2.	Oat meal agar	86.25	81.23	76.15
3.	Malt extract agar	84.07	87.60	71.48
4.	Richards agar	61.00	46.30	59.33
5.	Czapek's agar	53.00	50.00	72.98
6.	Sorghum seed extract agar	74.50	33.00	61.57
	SE(m) ±	1.15	0.87	1.07
	CD at 5%	3.60	2.73	3.33
	CV %	2.68	2.36	2.61

The mean colony diameter of three isolates from Surat district in various media ranged from 33.00 mm to 88.75 mm. The isolate ST-1 showed significantly highest mean colony diameter (88.75 mm) on potato dextrose agar which was at par with oat meal agar (86.25 mm). The next best in the order of merit was malt extract agar (84.07 mm). The least effective media was Czapek's agar (53.00 mm). However, in isolate ST-2, maximum colony diameter was observed in potato dextrose agar (88.60 mm) which was at par with malt extract agar (87.60 mm). The next best in the order of merit was oat meal agar

TABLE 2
Effect of various culture media on growth of different isolates of *C. graminicola* from Narmada district

S. No.	Culture media	Mean colony diameter (mm)		
		Narmada Isolates		
		NRM-1	NRM-2	NRM-3
1.	Potato dextrose agar	89.10	88.25	89.60
2.	Oat meal agar	88.50	87.25	89.80
3.	Malt extract agar	89.70	86.25	89.40
4.	Richards agar	60.50	58.00	48.00
5.	Czapek's agar	40.00	60.50	36.00
6.	Sorghum seed extract agar	50.00	70.00	49.00
	SE(m) ±	1.21	1.16	1.01
	CD at 5%	3.78	3.62	3.13
	CV %	3.02	2.68	2.60

TABLE 3
Effect of various culture media on growth of different isolates of *C. graminicola* from Bharuch district

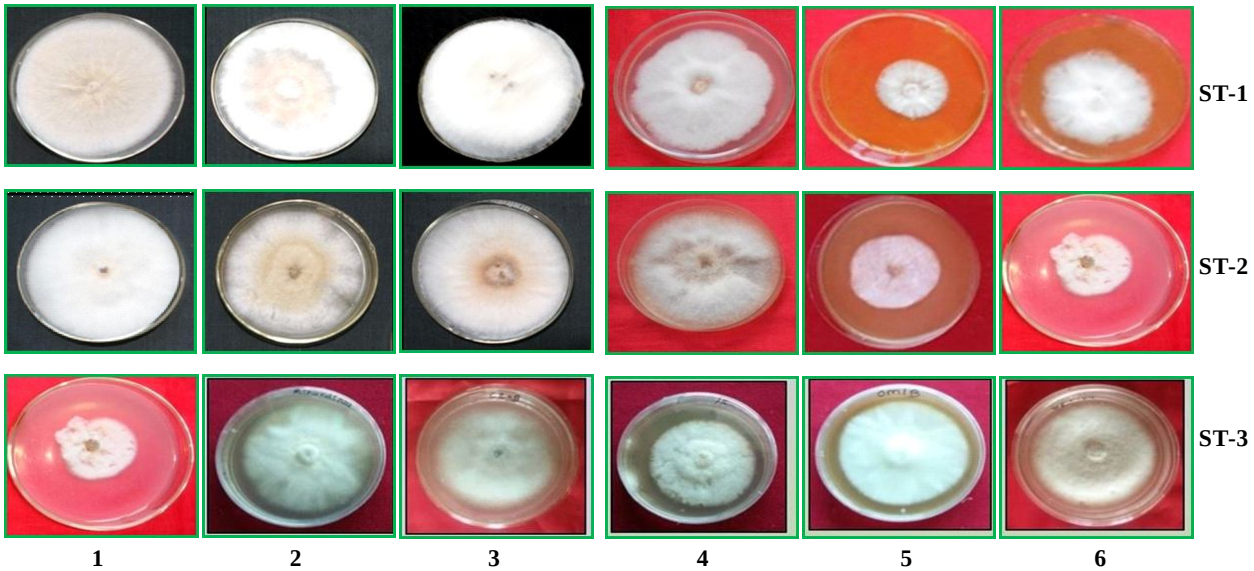
S. No.	Culture media	Mean colony diameter (mm)		
		Bharuch Isolates		
		BH-1	BH-2	BH-3
1.	Potato dextrose agar	88.70	89.20	87.40
2.	Oat meal agar	88.50	89.10	85.80
3.	Malt extract agar	88.60	82.10	86.20
4.	Richards agar	68.90	57.00	44.00
5.	Czapek's agar	71.30	39.00	40.00
6.	Sorghum seed extract agar	40.00	44.00	35.00
	SE (m)+	1.15	1.12	0.87
	CD at 5%	3.58	3.49	2.72
	CV %	2.68	2.91	2.39

(81.23 mm). The minimum colony diameter was observed in sorghum seed extract agar which was 33.00 mm. In isolate ST-3, the maximum colony diameter of the fungus was observed on potato dextrose agar (84.35 mm) which was significantly superior to all other media. Least colony diameter was observed in Richard's agar (59.33 mm).

The mean colony diameter of three isolates from the Narmada district varied between 36.00 mm and 89.80 mm, with isolate NRM-1 showing the highest diameter of 89.70 mm on malt extract agar, similar to results on potato dextrose agar and oat meal agar. Isolate NRM-2 had maximum growth on potato dextrose agar (88.25 mm), closely followed by oat meal agar and malt extract agar. The minimum growth for NRM-2 was on Richard's agar (58.00 mm). For isolate NRM-3, the largest diameter was recorded on oat meal agar (89.80 mm), comparable to potato dextrose agar and malt extract agar, while the smallest was again in Czapek's agar (36.00 mm).

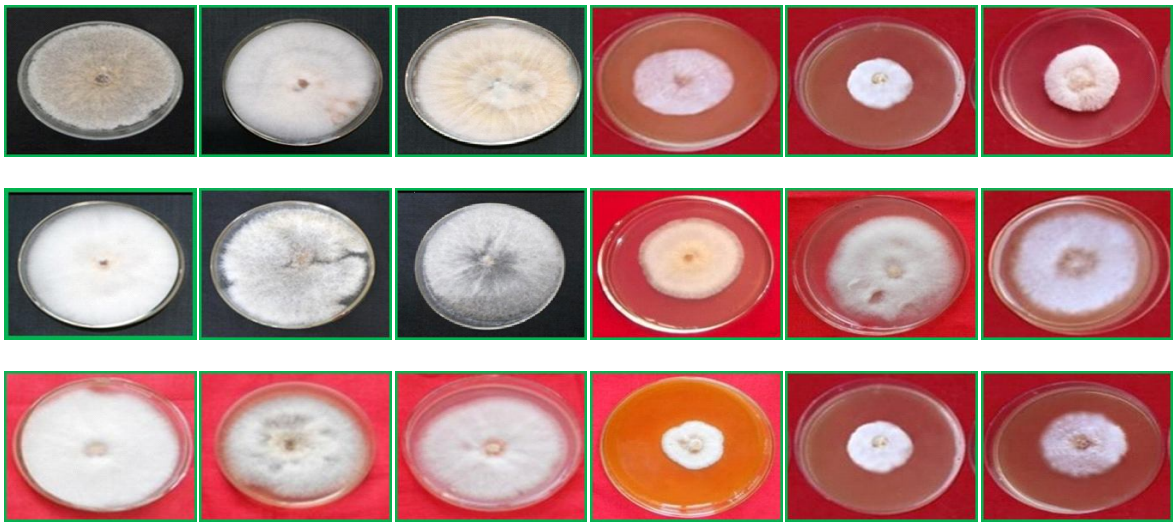
The mean colony diameter of three isolates from Bharuch district varied between 35.00 mm and 89.20 mm across different media. Isolate BH-1 had the largest diameter (88.70 mm) on potato dextrose agar, similar to malt extract agar (88.60 mm) and oat meal agar (88.50 mm). For isolate BH-2, the maximum was also in potato dextrose agar (89.20 mm), comparable to oat meal agar (89.10 mm). The smallest diameter recorded was 39.00 mm in Czapek's agar. Isolate BH-3 showed the maximum diameter on potato dextrose agar (87.40 mm), closely followed by malt extract agar (86.20 mm) and oat meal agar (85.80 mm), while sorghum seed extract agar had the minimum at 35.00 mm.

The present results are similar as reported by Rewale *et al.* (2018) that among the different media tested the maximum mycelial growth (89 mm) of *C. graminicola* of sorghum was recorded in potato dextrose agar media. Begum *et al.* (2015) observed highest mycelial growth (90 mm) of *C. capsici* in potato dextrose agar and oat meal agar media. Perane *et al.* (2015) studied effect of seven different media on growth on *C. gossypii* of cotton. Among them



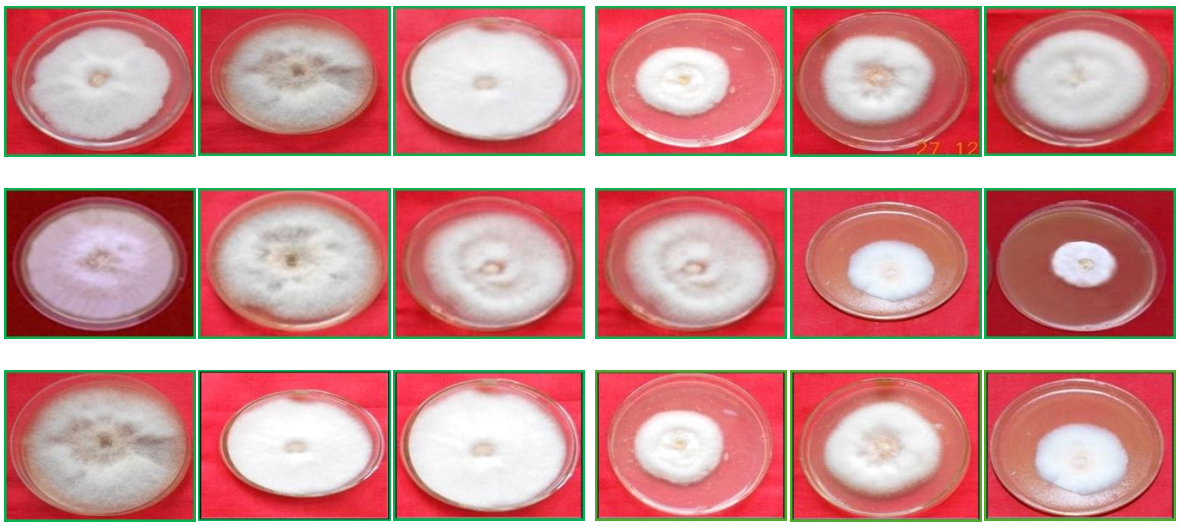
1. Potato Dextrose Agar media
2. Oat Meal Agar media
3. Malt Extract Agar media
4. Richard's Agar media
5. Czapek's Agar media
6. Sorghum Seed Extract Agar media

Photo 1. Effect of different culture media on growth of *C. graminicola* isolates from Surat district.



1. Potato Dextrose Agar media
2. Oat Meal Agar media
3. Malt Extract Agar media
4. Richard's Agar media
5. Czapek's Agar media
6. Sorghum Seed Extract Agar media

Photo 2. Effect of different culture media on growth of *C .graminicola* isolates from Narmada district.



1. Potato Dextrose Agar media
2. Oat Meal Agar media
3. Malt Extract Agar media
4. Richard's Agar media
5. Czapek's Agar media
6. Sorghum Seed Extract Agar media

Photo 3. Effect of different culture media on growth of *C. graminicola* isolates from Bharuch district.

abundant sporulation and maximum colony diameter (82 mm) was noticed on potato dextrose agar medium. Rajamanickam and Sethuraman (2015) reported significantly higher radial growth (81.5 mm) of *C. capsici* in potato dextrose agar media followed by oat meal agar (81.4 mm). The growth of different isolates of *Colletotrichum* sp. depended upon the different media which was attributed by the difference in media nutrient composition- carbohydrates, lipid, proteins and other mineral elements which were needed for the growth of fungi. They provide energy for biosynthesis and cell metabolism (Udhaykumar and Rani, 2010). Not all the media support luxuriant growth of fungi. 65

But non-synthetic media in general support the growth of fungi as compared to synthetic media (Lokare and Fatima, 2021). So, the present study showed that all the isolates of *C. graminicola* grew well in potato dextrose agar, malt extract agar and oat meal agar.

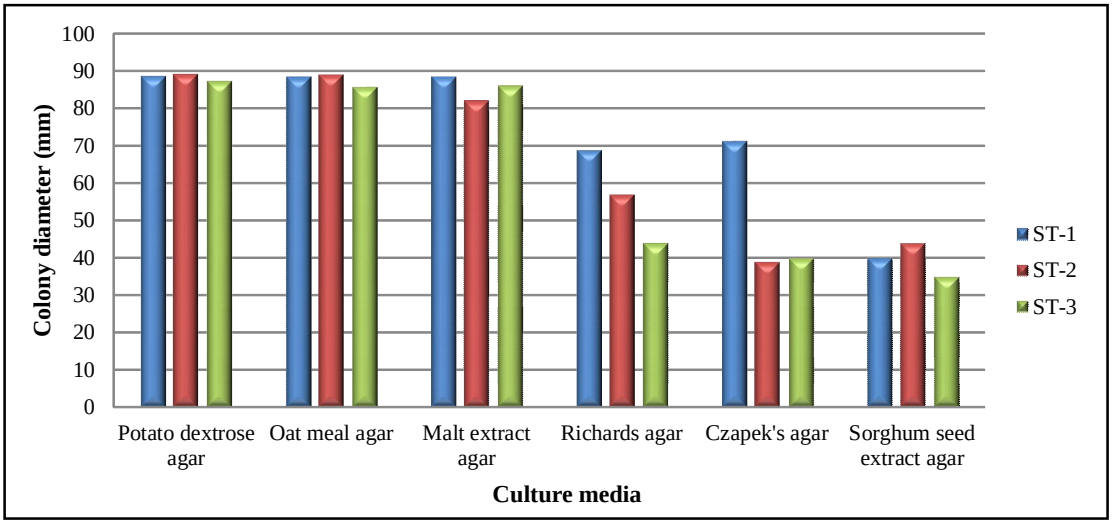


Fig. 1. Effect of various culture media on growth of *C. graminicola* isolates from Surat district.

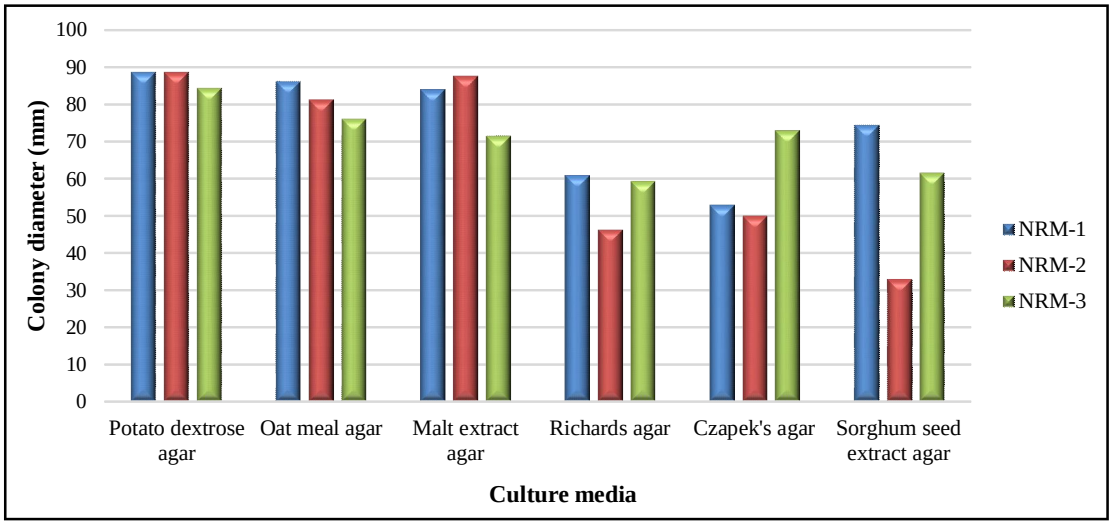


Fig. 2. Effect of various culture media on growth of *C. graminicola* isolates from Narmada district.

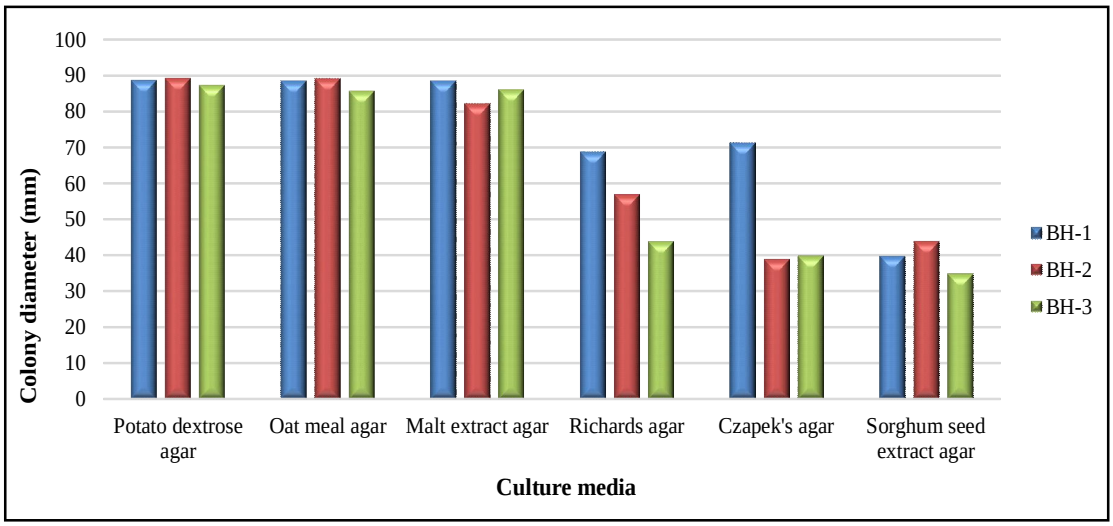


Fig. 3. Effect of various culture media on growth of *C. graminicola* isolates from Bharuch district.

SUMMARY

Six different media viz., non-synthetic (potato dextrose agar, malt extract agar, oat meal agar and sorghum seed extract agar) and synthetic media (Richard's agar and Czapek's agar) were used. Among the isolate collected from Surat district, isolate ST-1 registered maximum growth of 88.75 mm in PDA and the isolate ST-2 registered minimum growth of 33.00 mm in sorghum seed extract agar. Similarly, in Narmada district, isolate NRM-3 recorded maximum growth of 89.80 mm in OMA and least by NRM-3 isolate in Czapek's agar with 36.00 mm. in Bharuch district, isolate BH-2 was found maximum growth with 89.20 mm in PDA and minimum by BH-3 isolate in sorghum seed extract agar with 35.00 mm.

CONCLUSIONS

Sorghum anthracnose samples were collected from three districts *viz*., Surat, Narmada and Bharuch of South Gujarat and nine isolates were identified based on variation in growth and pigmentation in culture media. Among the nine isolates NRM-3 was identified as a virulent isolate.

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