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Variations in Morphology and Growth Pattern of *Bipolaris cynodontis* Isolates

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A B S T R A C T

Phenotypic variations among fungal species provide important insights into the existence of distinct races or strains in fungi. *Bipolaris cynodontis* isolates from Bermuda grass and sugarcane were evaluated for their growth rate, colony morphology and conidial characteristics on different culture media. Isolates were divided into four different groups based on colour and growth pattern. Growth rate among different isolates varied in the same medium indicating aggressiveness of the isolate. At the same time growth pattern and colony morphology differentiated greatly among the isolates. Growth and colony morphology was also influenced by the nutrients in the media. Significant differences in the conidial shape, size and number of septa provided additional parameters to differentiate isolates. These morphological differences may be due to parasexuality among naturally occurring isolates of *B. cynodontis.* The results indicate importance of growth pattern to assess variability in *B. cynodontis,* providing opportunity to understand fungal biodiversity with implications in agriculture.

Introduction

Variability is the major attribute of biodiversity. Extent of variations among species of fungi provide an essential insight into differentiation. Several phytopathogenic fungi exhibit inter- and intra-species variations.

The variations could be generated due to mutations, recombinations, heterokaryosis, parasexuality and gene flow at the molecular level in fungi (Tinline, 1962; Burdon and Silk, 1997; Deacon, 2005). Assessment of variability is essential to understand pathogenic variations and formulate disease management strategies. Various techniques have been used for analysing variations in phytopathogenic fungi (Gautam *et al.,* 2022; Janowski and Leski, 2023). However, the use of morphological characters based on growth parameters can be employed for assessing variations among the species (Aryani *et al.,* 2015; Sharmin *et al.,* 2022). Morphological characteristics exhibit true potential of the organisms even though the characters may get influenced by nutritional and environmental factors.

Bipolaris cynodontis is a phytopathogen known to cause leaf spot disease in maize, sorghum, rice and other grasses of the family Poaceae. The pathogen is also

reported to cause diseases on plants of other families including Asteraceae, Mytraceae and Oleaceae (Farias *et al.,* 2011; Manamgoda *et al.,* 2014; Bhunjan *et al.,* 2020). Variability studies have been reported using pathological, morphological and molecular techniques in *B. sorokiniana* (Jaiswal *et al.,* 2007; Pandey *et al.,* 2008; Aggarwal *et al.,* 2009; Poloni *et al.,* 2009). Morphology-based differentiation can serve as a criterion for studying variations in fungi (Narayanasamy, 2011).

Morphological variations are key parameters in detection and differentiation of etiologic agent. Pathogenic variability is crucial in the development of pathotypes/races. Such studies have not been carried out in *B. cynodontis* in spite of its pathogenicity on various crops. The present study was conducted to establish morphological variations among the isolates of *B. cynodontis* based on the growth pattern in different media. The growth rate and colony morphology variations were reported at intra-specific level. Influence of media on conidial characteristics was also considered.

Materials and Methods

Isolation of fungi

Necrotic tissues from the leaves of Bermuda grass (*Cynodon dactylon*) and sugarcane (*Saccharum officinarum*) were collected from different regions of Davangere District, Karnataka, India. Diseased plant materials were placed on moistened blotter paper in a Petri dish and incubated for three days at room temperature. The fungi expressed on the leaf samples were observed through stereo-binocular microscope (Magnus MS 24).

Fungi with the characteristics of the genus *Bipolaris* were isolated and pure cultures were established on potato dextrose agar (PDA) medium. Monoconidial cultures of the isolates were established by isolating a single conidium from water agar medium and then establishing pure culture on PDA. Single conidium with germ tube developed from the polar ends were selected (Goh, 1999).

The fungi were sporulated on water agar medium provided with sterilized sorghum seeds and leaf fragments. Conidia were stained with lactophenol cotton blue and observed under light microscope (Olympus S803281). A total of 100 conidia were observed for their size, shape, colour and septations. Length and breadth of conidia were measured by micrometry. The isolates were identified based on the parameters considered for the detection and differentiation of the *Bipolaris* species (Sivanesan, 1987; Manamgoda *et al.,* 2014). The isolates showing characteristics of *B. cynodontis* were selected for further study. The isolates were allotted with Davangere University Microbiology (DUMB) culture collection numbers.

Colony growth rate and morphological variations in *Bipolaris cynodontis* isolates

Growth rate and colony morphology of *Bipolaris cynodontis* isolates were assessed on the four different media. The isolates were point inoculated on different media namely potato dextrose agar (PDA), Czapek-Dox agar (CZA), Sabouraud's dextrose agar (SDA) and Malt extract agar (MEA). Plates were incubated for eight days at 25± 2ºC in a BOD incubator (Model CI-3S, Remi Instruments Ltd).

For measuring growth rate of the isolates, the colony diameter on the reverse side of the plate was recorded at every 24 h. for the initial five days of incubation. Three replicates were maintained for the isolates on each medium and mean values of the colony measurements were considered. Morphological characteristics of the colony was recorded on the eighth day by examining colour and growth pattern. Considering the colony characteristics of colour and growth pattern in *B. sorokiniana,* five morphological groups were made by Jaiswal *et al.,* (2007). They were Group-I: colony black with suppressed growth; Group-II: colony brown/dull black with suppressed growth; Group-III: colony grey with white spots and cottony growth; Group-IV: colony dull white/greenish black with fluffy growth and Group-V: colony white with fluffy growth. The same criteria were applied for grouping of *B. cynodontis* isolates cultured on different media.

Conidial morphology and size variations in *Bipolaris cynodontis* isolates

For observing conidial morphological characters, conidia were harvested into lactophenol and observed in a compound microscope (Olympus GB 803281). Conidial morphological characters like shape, number of septa, presence of hilum and colour were assessed. Size of the conidia were considered by measuring length and breadth of 100 conidia by micrometry and the results were expressed as mean± standard error values.

Results and Discussion

Isolation

Based on morphological characters of conidia and conidiophore, the fungi isolated were identified as *Bipolaris cynodontis.* The conidia of the fungi were slightly curved, cylindrical in shape with the broadest part at the middle and tapering at the ends.

Conidiophores were flexuous, simple, smooth and cylindrical shaped. Colour of the conidia were pale to mid golden brown with 4-9 distoseptate and size ranged from 47-81 x 15-18 µm. Out of the six isolates, DUMB 101 was obtained from Bermuda grass and all other five isolates numbered DUMB 104, DUMB 105, DUMB 107, DUMB 111, DUMB 113 were isolated from sugarcane.

Colony growth rate and morphological variations in *Bipolaris cynodontis* isolates

Growth rate

Visible growth appeared from all the isolates of *B. cynodontis* on different media by 24 h. of incubation. On PDA isolates showed similar growth during initial period of incubation. Differences in growth was observed from the 72 h. of incubation (Fig. 1A). At this time of incubation, two of the isolates DUMB 101 and DUMB 104 showed increased growth compared to the other isolates and the size of the colony were found to be 52 mm and 57 mm respectively. The isolate with least colony diameter was in the isolate DUMB 107 (45 mm). On the 120 h. of incubation variations in growth were observed among the isolates. The highest growth was showed by the two isolates namely DUMB 111 and DUMB 113 with a colony diameter of 88 mm and the least was by the isolate DUMB 105 (55 mm) (Fig. 2).

Analysis of growth rate on the fifth day of incubation showed variations among the isolates. The peak growth rate was showed by DUMB 111 (18 mm/day) and the lowermost growth rate was showed by DUMB 105 (11 mm/day).

On CZA, the isolates began to show differences in the growth from the 48 h. of incubation (Fig. 1B). The colony diameter of the isolates differed from each other. During the incubation time, the highest colony diameter was showed by the isolate DUMB 113 whereas the least colony diameter was showed by the isolate DUMB 107. It was observed that at the 48 h. of the incubation, isolates DUMB 107 and DUMB 111 exhibited the smallest colony diameter of 11 mm. However, with the continued incubation, the growth of the two isolates diverged. DUMB 107 maintained limited growth, consistently showed the least colony diameter while DUMB 111 showed increased growth by 72 h. of incubation. At the 120 h. of incubation, five of the isolates showed highest colony diameter whereas the least colony diameter was exhibited by DUMB 107 (Fig. 2). Differences in growth rate was also found among the isolates. The maximum growth rate was showed by DUMB 111 and DUMB 113 (17 mm/day) and the minimum growth rate of 5 mm/day was showed by DUMB 107.

Differences in growth of the isolates were also marked on SDA (Fig. 1C). Although only minor growth differences were noted at the 48 h. on incubation, the continued incubation showed distinct growth differences among the isolates. The highest growth at the 72 h. of incubation was showed by the isolate DUMB 104 with a colony diameter of 39 mm. At the 120 h. of incubation, isolate DUMB 105 showed the highest growth with a colony diameter of 67 mm (Fig. 2). Throughout the incubation period, the lowest growth was exhibited by the isolate DUMB 107. On the other hand, differences in growth rate were also obvious among the isolates. The highest growth rate of 14 mm/day was exhibited by the isolate DUMB 113 whereas the lowest growth rate was exhibited by the isolates DUMB 101 and DUMB 107 (12 mm/day).

Growth of the isolates on MEA differed markedly when compared with the other three media (Fig. 1D). Clear differences in the growth were observed from the 48 h. of incubation. Beginning from the 72 h. of incubation, isolates DUMB 101 and DUMB 104 showed the highest growth on the medium. In contrast, the lowest growth at the 72 h. was showed by the isolate DUMB 111 and DUMB 113 with a colony diameter of 10 mm. At the 96 h. of incubation, isolates DUMB 107 and DUMB 113 showed the lowest colony diameter of 16 mm. At 120 h. the lowest colony diameter of 23 mm was showed by the isolate DUMB 107 (Fig. 2). The highest growth rate of 8 mm/day was showed by DUMB 101 and the lowest growth rate of 5 mm/day were showed by isolates DUMB 105 and DUMB 107. Among the media tested, the minimum colony growth was observed on MEA medium by all the isolates of *B. cynodontis.*

Colony Morphology

Isolates of *B. cynodontis* exhibited different colony morphological characters on the four media tested (Fig. 3). Based on the colour and growth pattern developed on PDA, the isolates were divided into different groups. On PDA, six isolates of *B. cynodontis* belonged to three groups (Table 1). Group-I had three isolates DUMB 101, DUMB 105 and DUMB 111. Group-II had two isolates, DUMB 104 and DUMB 113. Group-IV had one isolate-DUMB 107. Groups with grey cottony spots and white fluffy growth were not found on PDA. One of the isolates DUMB 105 was distinct from other isolates with the characters of constricted colony growth. On CZA six isolates of *B. cynodontis* belonged to only two groups (Group-I and Group-II). In Group-I the isolate DUMB 107 was found and all other five isolates belonged to Group-II. Two of the isolates from Group-II had developed unique characters where one of the isolates DUMB 107 formed constricted colony growth on the medium and the other isolate DUMB 113 developed a crater at the centre of the colony (Table 1).

In case of SDA, three morphological groups were observed. Group-I comprised of isolate DUMB 101 and Group-II had isolates DUMB 111 and DUMB 113. Group-III involved three isolates DUMB 104, DUMB 105 and DUMB 107. None of the isolates belonged to the Group-IV and Group-V on SDA medium (Table 1).

Poor growth of *B. cynodontis* isolates was observed on MEA. Group-I, II and III was represented by two isolates each. Group-I had isolates DUMB 101 and DUMB 104, Group-II had isolates DUMB 111 and DUMB 113 whereas Group-III had isolates DUMB 105 and DUMB 107. Two of the isolates DUMB 105 and DUMB 107 showed constricted colony development but share similar colony morphological characteristics of Group-III. No isolates showed characteristics of Group-IV and Group-V on MEA medium (Table 1).

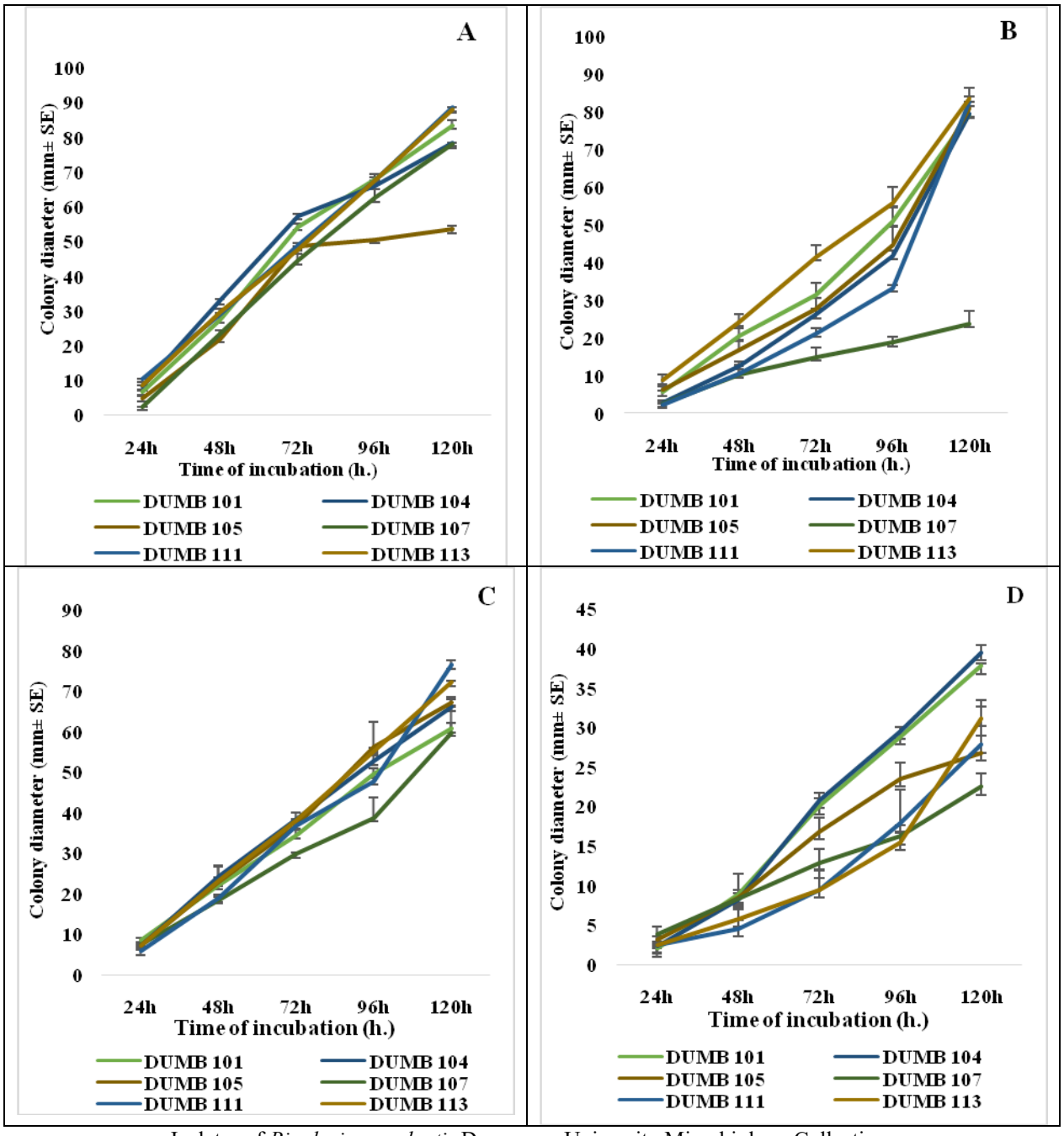
Conidial morphology and size variations in *Bipolaris cynodontis* isolates

Differences in conidial morphology were found among the isolates on each medium (Table 2). It was found that on PDA, the shape of conidia was cylindrical for the isolates DUMB 101 and DUMB 107 and rest of the isolates showed elliptical conidia. Three of the isolates DUMB 101, DUMB 104 and DUMB 107 were pale brown in colour and the other three isolates were brown in colour (Table 2). Differences in conidial measurements were also observed. Five different measurements of conidial length were found and the isolate DUMB 111 showed the largest conidial length of 81 µm and the smallest length of 69 µm was showed by the isolate DUMB 104. Two of the isolates DUMB 105 and DUMB 111 showed maximum of 10 number of septa and a minimum of 4 number of septa. Hilum with 6 µm diameter was found in DUMB 101, DUMB 104 and DUMB 111 isolates and hilum in other isolates was not conspicuous.

On CZA, shape of the conidia was different among the isolates. Subcylindrical shaped conidia was showed by the isolates DUMB 107 and DUMB 113 whereas the other four isolates showed elliptical shaped conidia. Dark brown colour conidia were present in the isolates DUMB 107 and DUMB 113 and in other four isolates conidia were pale brown. Three different conidial length was observed among the isolates. The largest conidial length of 66 µm was in DUMB 101 and DUMB 104 whereas the smallest conidial length of 46 µm was in the isolate DUMB 105. Maximum of 9 septa were present in the isolate DUMB 111 and minimum of 4 septa were in the three isolates DUMB 107, DUMB 111 and DUMB 113. The hilum was present in the isolates DUMB 101, DUMB 104 and DUMB 111 with the diameter of 6 µm and in other isolates hilum was not clearly visible.

Conidial morphology on SDA was not much diverse in shape or colour. Isolate DUMB 101 had elliptical shaped conidia whereas other five isolates were with cylindrical shaped. Brown colour conidia were showed by the isolates DUMB 104 and DUMB 111 whereas the other isolates were with dark brown coloured conidia. No similarity in conidial length among the isolates was found. The largest conidial length of 88 µm was showed by the isolate DUMB 111 and the smallest length of 53 µm was showed by the isolate DUMB 105. Highest numbers of septa of 10 were found in the isolate DUMB 111. Conspicuous hilum was seen on the conidia of all the isolates except on DUMB 107 and the diameter was 6 µm. Differences in conidial morphology were not observed on MEA. Isolates were ellipsoidal in shape with dark brown in colour. Isolates were also distinct from each other in conidial length. The largest conidial length of 72 µm was in the isolate DUMB 111 whereas the smallest length of 50 µm was showed by the isolate DUMB 101. Number of septa in the conidia ranged from 4 to 9 among the isolates. Conspicuous hilum of 6 µm diameter was found in the isolate DUMB 101.

Figure.1 Growth rate of *Bipolaris cynodontis* isolates on different media.



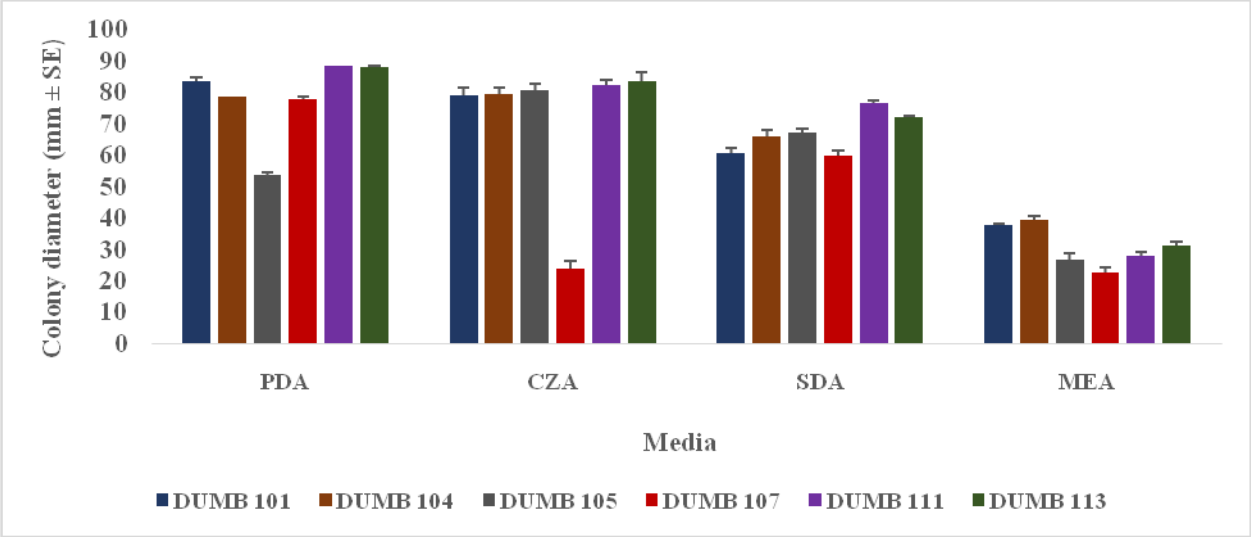
Isolates of *Bipolaris cynodontis* Davangere University Microbiology Collection.
DUMB 101; DUMB 104; DUMB 105; DUMB 107; DUMB 111; DUMB 113.
Media- A: Potato Dextrose Agar; B: Czapek-Dox Agar; C: Sabouraud's Dextrose Agar; D: Malt Extract Agar.

Table.1 Colony growth pattern of *Bipolaris cynodontis* isolate groups on different media

Group	Colony colour	Colony growth pattern	Media			
			PDA	CZA	SDA	MEA
I	Black	Suppressed	DUMB 101 DUMB 105 DUMB 111	DUMB 107	DUMB 101	DUMB 101 DUMB 104
II	Dull black	Suppressed	DUMB 104 DUMB 113	DUMB 101 DUMB 104 DUMB 105 DUMB 111 DUMB 113	DUMB 111 DUMB 113	DUMB 111 DUMB 113
III	Grey	Cottony	-	-	DUMB 104 DUMB 105 DUMB 107	DUMB 105 DUMB 107
IV	Dull White	Fluffy	DUMB 107	-	-	-
V	White	Fluffy	-	-	-	-

DUMB represents Davangere University Microbiology (DUMB) culture collection numbers
Media- PDA: Potato dextrose agar; CZA; Czapek-Dox agar; SDA: Sabouraud's dextrose agar; MEA: Malt extract agar

Figure.2 Colony diameter of *Bipolaris cynodontis* isolates on different media at 120 h. of incubation.



Isolates of *Bipolaris cynodontis* Davangere University Microbiology Collection.
DUMB 101; DUMB 104; DUMB 105; DUMB 107; DUMB 111; DUMB 113.
Media-PDA: Potato Dextrose Agar; CZA: Czapek-Dox Agar; SDA: Sabouraud's Dextrose Agar; MEA: Malt Extract Agar.

Table.2 Size and conidial morphology among *Bipolaris cynodontis* isolates on different media

***Bipolaris cynodontis* isolates**	**Media**															
	PDA				CZA				SDA				MEA			
	Mean conidial size± SE (µm)	No. of septa	Shape[#]	Colour[*]	Mean conidial size± SE (µm)	No. of septa	Shape[#]	Colour[*]	Mean conidial size± SE (µm)	No. of septa	Shape[#]	Colour[*]	Mean conidial size± SE (µm)	No. of septa	Shape[#]	Colour[*]
DUMB 101	71± 0.10 x 15± 0.02	4-9	C	Pb	66± 0.05 x 17± 0.11	6-8	E	Pb	79± 0.05 x 18± 0.01	4-8	E	Db	50± 0.50 x 15± 0.06	6-8	E	Db
DUMB 104	69± 0.14 x 16± 0.05	4-9	E	Pb	66± 0.07 x 16± 0.09	6-8	E	Pb	73± 0.09 x 17± 0.07	4-9	C	B	59± 0.07 x 16± 0.09	6-9	E	Db
DUMB 105	78± 0.07 x 17± 0.02	4-10	E	B	46± 0.70 x 17± 0.06	5-7	E	Pb	53± 0.90 x 17± 0.05	4-9	C	Db	65± 0.50 x 17± 0.70	4-9	E	Db
DUMB 107	76± 0.03 x 17± 0.03	5-8	C	Pb	51± 0.19 x 16± 0.03	4-8	Sc	Db	46± 0.05 x 14± 0.5	4-8	C	Db	68± 0.05 x 17± 0.09	4-8	E	Db
DUMB 111	81± 0.02 x 18± 0.05	4-10	E	B	52± 0.05 x 19± 0.09	4-9	E	Pb	88± 0.50 x 16± 0.2	6-10	C	B	72± 0.05 x 16± 0.9	4-7	E	Db
DUMB 113	75± 0.01 x 17± 0.06	4-9	E	B	47± 0.02 x 18± 0.08	4-8	Sc	Db	84± 0.05 x 15± 0.01	4-6	C	Db	61± 0.05 x 15± 0.5	4-9	E	Db

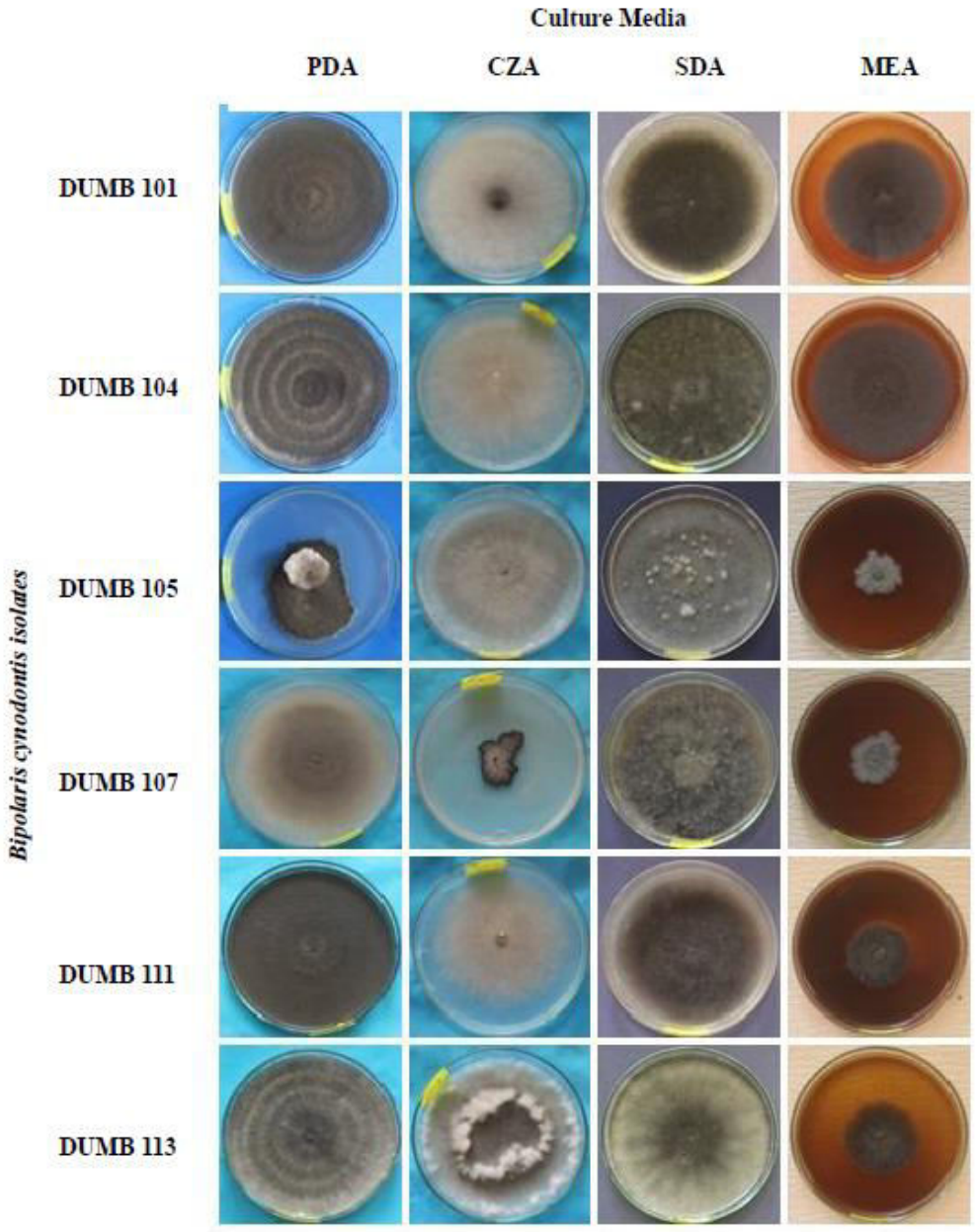
DUMB represents Davangere University Microbiology (DUMB) culture collection numbers

Media. PDA: Potato dextrose agar; CZA; Czapek-Dox agar; SDA: Sabouraud's dextrose agar; MEA: Malt extract agar

[#]Shape of conidia. C: Cylindrical; Sc: Subcylindrical; E: Elliptical

[*]Colour of conidia. B: Brown; Pb: Pale brown; Db: Dark brown

Figure.3 Colony morphology of *Bipolaris cynodontis* isolates on different media**.**



Isolates of *Bipolaris cynodontis* Davangere University Microbiology Culture Collection. DUMB 101; DUMB 104; DUMB 105; DUMB 107; DUMB 111; DUMB 113

Media- PDA: Potato Dextrose Agar; CZA: Czapek-Dox Agar; SDA: Sabouraud's Dextrose Agar; MEA: Malt Extract Agar.

Bipolaris cynodontis is an important asexually reproducing fungal pathogen of plants. Differences in growth rate and colony morphology and conidial morphology among *B. cynodontis* isolates was established. Natural populations of *B. cynodontis* from two different plant hosts showed considerable variations under *in vitro* growth conditions. Variability studies conducted by Chand *et al.,* (2003) in *B. sorokiniana* occurring on wheat showed five morphological groups whereas in the present study *B. cynodontis* isolates belonged to four morphological groups. Colony morphology was greatly influenced by nutrient media

used for the cultivation of the fungi. Clonal isolates of *B. sorokiniana* were known to have distinct variations in growth pattern and growth rate (Jaiswal *et al.,* 2007; Pandey *et al.,* 2008).

The present study clearly demonstrated distinct variability of *B. cynodontis* on different media. Maximum growth of all the isolates was found on PDA and least growth was observed on MEA. The isolate DUMB 107 showed least growth on CZA, probably indicating that the isolate to be less aggressive. Faster colonization and rapid host cell death are important in determining variability among *Bipolaris* spp. (Tinline 1962; Aggarwal *et al.,* 2009).

Host differential reactions are the major criteria for pathogenic variability assessment. Irrespective of the host source isolates of *B. cynodontis* showed variations. The present study clearly demonstrated differences in the conidial shape, size, colour and presence of hilum. Such studies are important in establishing intra-specific variations in *Bipolaris* (Jaiswal *et al.,* 2007; Pandey *et al.,* 2008; Poloni *et al.,* 2009). This genus comprised sexually reproducing stages providing opportunity for genetic recombinations (Aggarwal *et al.,* 2009; Farias *et al.,* 2011). It will be highly useful criteria to detect sexual stages in *B. cynodontis*. At the same time assessment of other criteria for genetic variations would provide comprehensive information on variability.

Variations in morphology and growth rate were significant among the isolates of *Bipolaris cynodontis* probably directed by parasexuality. Such differences provide evidence for variations at the intra-specific level. Morphological variations could be used as a tool for differentiating fungi. Polyphasic taxonomy for identification of meaningful species deepens our understanding of fungal biodiversity, with implications in agriculture.

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Author Contributions

Beena Gore: Investigation, formal analysis, writing—original draft. S. Shishupala: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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