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Studies on cultural variability of *Exserohilum turcicum* (Pass.) Leonard and Suggs. causing turcicum leaf blight of sorghum

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Abstract
Eight solid and liquid media were tested for growth and sporulation of *E. turcicum* **(**Et-8). Among the solid media, potato dextrose agar supported the maximum radial growth (87.67mm) and excellent sporulation. The least growth was recorded on host leaf extract agar and Richard's agar media which are on par with each other by supporting the radial growth of 39.67 mm. whereas no sporulation was observed in Malt extract agar and czapeck's dox agar. With respect to cultural characteristics, the colony colour varied from grey to black. Mycelial growth varied from fluffy to slightly raised colony. Among the liquid media, highest dry mycelial weight with excellent sporulation was recorded in potato dextrose broth (689.67 mg). The least growth with no sporulation was observed in malt extract broth (239.67 mg). Among the eight isolates (Et1-Et8) tested for cultural variability Et4 and Et6 recorded maximum colony diameter of 90.00 mm. The poor growth was observed in Et1 isolate (70.00mm) after 12 days of incubation. Shape of conidia in all the isolates revealed that the conidia were straight, ellipsoidal, oblong or oval shape, round at one end and another end protruding, brownish to dark brown in colour and symmetrical in shape, pale to slight brownish in appearance. Pigmentations varied from greyish, greyish black, whitish Gray, blackish and dark blackish colours. Excellent sporulation was exhibited by the isolates Et7 and Et8. However poor sporulation was noticed in the isolate Et3.

Keywords: Cultural variability, turcicum leaf blight, *Exserohilum turcicum*

Introduction
Sorghum (*Sorghum bicolor* Linn. Moench) popularly known as Jowar, is the major cereal consumed in India and ranks fifth after wheat, rice, maize and pearl millet. The world production of grain sorghum is 70.83 million tons from 44.8 million ha area of land (Faostat, 2014) [6]. India is major producer of sorghum, ranks fifth after, wheat, rice, maize and pear millet cultivated in 6.16 million hectares in both *kharif* (2.26m.ha) and *Rabi* (3.89m.ha) with an annual production of 5.44 million tons of grain with productivity of 8.44 kg per hectare (Indiastat, 2015) [10].

In India the sorghum is cultivated in Maharashtra, Karnataka and Andhra Pradesh as rainfed crop to an extent of 85 per cent (4.93m.ha). In Karnataka sorghum production is about 1.32 million tons in an area of 1.04 million ha with the average productivity of 1180 kg per ha. The sorghum is the main food crop of Hyderabad-Karnataka region and occupies an area of 5.6 lakh hectares with production of 5.5 lakh tons and productivity of 1122kg per ha (Anon., 2014-15) [1].

As the *Rabi* sorghum produces the white pearly grains which is mainly used for food in India for the preparation of roti. It is also an important animal feed (swine, poultry and cattle) used in countries like U.S., Mexico, South America and Australia. Sorghum, as a food, feed and bio fuel crop with excellent drought resistance compared to other cereals, is considered as a "failsafe crop" (Burke *et al*., 2010) [3].

Sorghum grain is a principal source of energy, protein, vitamins and minerals for the poor people living in the semi-arid tropics. It is nutritionally superior to rice because of its high mineral and fiber content. Starch (60-75%) is the main component of sorghum grain, followed by proteins (7-15%), non-starch polysaccharides (2-7%) and fat (1.5-6%). The average energetic value of whole sorghum grain flour is 356 kcal/100gm (Dicko *et al*., 2006) [4]. Sorghum is a good source of vitamins, notably the B vitamins (thiamin, riboflavin, pyridoxine and niacin) and the liposoluable vitamins A, D, E and K. Unique property of sorghum grain makes it well suited to prepare various food items such as porridge, unleavened bread, cookies, cakes, couscous and malted beverages, etc.

Even though the crop is robust and versatile, it has faced drawbacks in terms of yield and reduction in acreage due various diseases. The major diseases that affect sorghum include downy mildew, turcicum leaf blight, anthracnose and sorghum smuts (covered kernel smut, loose smut, long smut and head smuts). Turcicum leaf blight (TLB) is one of the most destructive foliar diseases of maize and sorghum. It can cause yield reduction more than 50% in susceptible varieties and is favoured by mild temperatures and humid weather conditions with heavy dews (Bergquist, 1986) [2]. The disease occurs as long elliptic tan lesions that develop on lower leaves and progress upwards. Susceptibility to *Exserohilum turcicum* is reported to decrease with crop maturity (Frederiksen, 1980) [5]. Hence studies on cultural variability of the pathogen is important for documenting virulent isolate of the pathogen and resistant source.

Material and Methods

Growth characters of *E. turcicum* on solid media

To study growth characters on different solid and liquid media, Et-8 isolate from Raichur local was used and eight isolates (Et1-Et8) were used for studying variability in cultural characteristics.

Non-synthetic media

1. Potato dextrose agar (PDA), 2. Host leaf extract agar (HLEA), 3. Potato carrot agar (PCA), 4. Malt extract agar (MEA), 5. Oat meal agar (OMA) 6. Yeast extract potato agar (YEPA)

Synthetic media

1. Czapeck's Dox agar (CDA), 2. Richards's agar (RA)

The composition and preparation of the above mentioned synthetic and non-synthetic/semi- synthetic media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawksworth *et al*. (1983) [7].

Twenty ml of medium listed above was poured in to the Petri dishes for solidification. Five mm discs of *E. turcicum* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 27±2ºC for ten days. Observations were taken on parameters such as colony diameter, colony colour, nature of mycelial growth and sporulation was graded as follows.

Table 1: Observations of colony diameter, colony colour, nature of mycelial growth and sporulation

Grade	Description	Score
Excellent sporulation	>20 spores/ microscopic field (10X)	++++
Good sporulation	15-20 spores/ microscopic field(10X)	+++
Fair sporulation	10-15 spores / microscopic field (10X)	++
Poor sporulation	<10 spores / microscopic field (10X)	+

Growth characters of *E. turcicum* on liquid media

The composition and preparation of different liquid media used were the same as that of solid media except that agar was not added. Fifty ml of different liquid media were added into each of 100 ml conical flasks. These flasks were then sterilized at 1.1 kg per cm^2 pressure for 20 min. The flasks were then inoculated with five mm mycelial discs obtained from periphery of 10 days old culture and and incubated at 27 ± 2 ºC for 10 days. Each treatment was replicated thrice. Cultures were filtered through Whatman No. 1 filter paper, which were dried to a constant weight in an electric oven at 60 ºC and weighed immediately on an electric balance and weight of dry mycelia was recorded. The data was analyzed statistically.

Cultural variability

The TLB infected leaf samples were collected from different locations of Karnataka and pure hyphal tip cultures of 8 isolates were maintained on PDA and later on variability in cultural characteristics were carried out in the laboratory.

Table 2: The details of 8 isolates of *E. turcicum* used for the study

Sl. No.	Place of collection	Designation of isolates
1	Hitinhalli	Et1
2	Jevoor	Et2
3	Malked	Et3
4	Sannur	Et4
5	Polkamdaddi	Et5
6	Gabbur	Et6
7	Chittapur	Et7
8	Raichur local	Et8

To carry out the study 20 ml of medium was poured in to the Petri plates for solidification. Five mm discs of different isolates of *E. turcicum* were placed at the centre of the each plate. These plates were incubated at 27 ± 2 ºC for 10 days. The cultural characteristics such as colony diameter, colony colour and pigmentation were recorded. The colonies were characterized for phenotype growth pattern and different morphotypes, shape (irregular and regular); growth pattern (circular and feathery); texture (velvety and cottony) were observed *in vitro*. Similarly, colour was differentiated into blackish, dark blackish, grey, whitish grey and greyish black. The sporulation was graded as described earlier.

Results and Discussion

Growth characters of *E. turcicum* on different solid media

Among the different solid media (Table 1) tested potato dextrose agar was significantly superior which supported the maximum radial growth (87.67mm) of *E. turcicum*. However there was stastically no significant difference between the radial growths of Oat meal agar (85.00mm) and potato carrot agar (84.67mm).

The least growth was recorded on host leaf extract agar and Richard's agar media which are on par with each other by supporting the radial growth of 39.67 mm. The excellent sporulation was observed in potato dextrose agar. On Oat

meal agar sporulation was good and in host leaf extract and Richard's agar sporulation was poor whereas no sporulation was observed in Malt extract agar and czapeck's dox agar.

With respect to cultural characteristics, the colony color varied from grey to black. Mycelial growth varied from fluffy growth to slightly raised colony. The fungus showed greyish colour with irregular margin and slightly raised mycelium on Yeast extract potato agar, Malt extract agar, potato dextrose agar and also on Richard's agar. Mycelial growth on potato carrot agar and Oat meal media was whitish gray colour with regular margin. Mycelial growth on Czapeck's Dox agar and host leaf extract media showed blackish colour with regular and irregular margin respectively having slightly raised and flattened mycelium respectively. Effect of different solid media on cultural characters of *E. turcicum* is represented in the Table 2. Similar variability results was obtained by Harlapur *et al.* (2007) [8] who studied on 16 isolates of *E. turcicum* for colony diameter, mycelial dry weight *etc.* The results are in accordance with previous workers (Khedekar, 2009, Hulagappa 2012 and Nataraj 2014) [11, 9, 12].

Growth characters of *E. turcicum* on different liquid media

Among the different liquid media (Table 3) tested significantly higher dry mycelial weight of the test pathogen was recorded in potato dextrose broth (689.67 mg) followed by Oat meal broth (639.67 mg). The least growth was observed in malt extract broth (239.67 mg).

With regard to sporulation, excellent sporulation was observed on potato dextrose broth. On Oat meal broth sporulation was good and in Richard's broth and Yeast extract potato broth sporulation was fair whereas in potato carrot broth, host leaf extract broth and Czapeck's Dox broth poor sporulation was observed. No sporulation was observed in malt extract broth. Similar results were obtained by Nataraj (2014) who reported that potato dextrose broth supported an excellent dry mycelia weight to all isolates. This may be due to changes in nutrient content in different media.

Cultural variability of *E. turcicum*

The diversity in cultural characters was studied by growing different isolates of *E. turcicum* on potato dextrose agar at 27 ± 1 ^{0}C. All the isolates showed considerable variability in their growth and cultural characters. The observations on radial growth of the different isolates of *E. turcicum* and cultural characters such as colony character, Pigmentation, type of margin, margin colour, spore colour and sporulation are presented in the Table 4. Further these isolates of *E. turcicum* were categorized into different groups based on cultural characteristics as presented in the Table 5.

Colony growth

Regarding colony character two isolates Et4 and Et6 showed excellent growth and recorded maximum colony diameter of 90.00 mm followed by Et7 and Et8 which produced good growth and recorded colony diameter of 85.00mm.Whereas Et2, Et3 and Et5 isolates showed moderate growth and there colony diameter are in the range of 87-88.00mm. The poor growth was observed in Et1 isolate and recorded colony diameter of 70.00mm after 12 days of incubation (Table 5a).

These results are in similar with findings of Harlapur *et al.* (2007) [8] who observed variation in morphological and cultural characters of 16 isolates of *E. turcicum.* He also studied colony character, colony diameter, mycelial dry weight, spore germination and sporulation. Similar variation in the isolates of the pathogen in different location by the Khedekar (2009) [11] and Hulagappa (2012) [9].

Shape of conidia

It has been observed that the shape of conidia in all the isolates revealed that the conidia were straight, ellipsoidal, oblong or oval shape, round at one end and another end protruding, brownish to dark brown in colour and symmetrical in shape, pale to slight brownish in appearance. These results are in similar with findings of Harlapur *et al.* (2007) who observed variation in morphological and cultural characters of 16 isolates of *E. turcicum.* He also studied colony character, colony diameter, mycelial dry weight, spore germination and sporulation. Similar variation in the isolates of the pathogen in different location by the Khedekar (2009) [11] and Hulagappa (2012) [9].

Pigmentation (Colour)

Isolates of *E. turcicum* exhibited various pigmentations such as greyish, greyish black, whitish gray, blackish and dark blackish colors. The greyish pigmentation of mycelium was observed in Et5, Et7 and Et8. The greyish black pigmentation was noticed in Et3 and Et6 isolates. Whitish gray pigmentation was observed in Et2. The blackish pigmentation of the mycelium was observed in Et4 isolate and dark blackish pigmentation was found in Et1 isolate (Table 5b). These results are in similar with findings of Harlapur *et al.* (2007) who observed variation in morphological and cultural characters of 16 isolates of *E. turcicum.* He also studied colony character, colony diameter, mycelial dry weight, spore germination and sporulation. Similar variation in the isolates of the pathogen in different location by the Khedekar (2009) [11] and Hulagappa (2012) [9].

Sporulation

Excellent sporulation of *E. turcicum* was exhibited by the isolates Et7 and Et8, while good sporulation was recorded in Et5 isolate. It was fair sporulation in Et1, Et2, Et4 and Et6 isolates. However poor sporulation was noticed in the isolate Et3 (Table 5f).

Based on the cultural characteristics of 8 isolates, they were further categorized into seven different groups. These results are in similar with findings of Harlapur *et al.* (2007) [8] who observed variation in morphological and cultural characters of 16 isolates of *E. turcicum.* He also studied colony character, colony diameter, mycelial dry weight, spore germination and sporulation. Similar variation in the isolates of the pathogen in different location by the Khedekar (2009) [11] and Hulagappa (2012) [9].

Out of eight isolates, maximum sporulation was observed in the isolates of Et7 of Kalaburagi district and Et8 of Raichur district which helps to perpetuate and survival of *E. turcicum* which belonged to Kalaburagi and Raichur districts. So these isolates may cause highest severity of leaf blight in future under favourable environmental conditions.

Table 1: Growth and sporulation of *E. turcicum* (Et8) on different solid media

Sl. No.	Media	Radial growth (mm) *	Sporulation
1	Potato carrot agar	84.67	++
2	Czapeck's Dox agar	78.67	-
3	Yeast extract potato agar	73.67	++

4	Malt extract agar	44.67	-
5	Potato dextrose agar	87.67	++++
6	Host leaf extract agar	39.67	+
7	Oat meal agar	85.00	+++
8	Richard's agar	39.67	+
S.Em±		0.37	
C.D. at 1%		1.54	

*Mean of three replications ++++ - Excellent, > 20 conidia per microscopic field, +++ - Good, 15-20 conidia per microscopic field, ++ - Fair, 10-15 conidia per microscopic field, + - Poor, < 10 conidia per microscopic field

Table 2: Cultural characters of *E. turcicum* on different solid media

Sl. No.	Media	Colony character	Margin	Margin colour
1	Potato carrot agar	Excellent growth, whitish gray, slightly raised, cottony growth colony	Regular	Gray
2	Czapeck's Dox agar	Excellent growth, blackish, slightly raised growth colony	Regular	Black
3	Yeast extract potato agar	Good growth, greyish, slightly raised colony	Irregular	Light gray
4	Malt extract agar	Good growth, greyish, slightly raised cottony growth colony	Irregular	Light gray
5	Potato dextrose agar	Excellent growth, greyish, slightly raised growth colony	Irregular	Light gray
6	Host leaf extract agar	Poor growth, blackish, flattened colony	Irregular	Black
7	Oat meal agar	Excellent growth, whitish gray, fluffy with raised cottony growth colony	Regular	Gray
8	Richard's agar	Good growth, greyish, slightly raised cottony growth colony	Irregular	Light gray

Table 3: Growth and sporulation of *E. turcicum* (Et8) on different liquid media

Sl. No.	Media	Dry mycelial weight (mg) *	Sporulation
1	Potato carrot broth	469.67	+
2	Host leaf extract broth	329.67	+
3	Oat meal broth	639.67	+++
4	Richard's broth	259.67	++
5	Czapeck's Dox broth	619.67	+
6	Yeast extract potato broth	319.67	++
7	Malt extract broth	239.67	-
8	Potato dextrose broth	689.67	++++
S. Em±		0.33	
C.D. at 1%		1.38	

*Mean of three replications ++++ - Excellent, > 20 conidia per microscopic field, +++ - Good, 15-20 conidia per microscopic field, ++ - Fair, 10-15 conidia per microscopic field, + - Poor, < 10 conidia per microscopic field

Table 4: Cultural variations in different isolates of *Exserohilum turcicum* on PDA

Sl. No	Location	Isolate No.	Colony character	Pigment-ation	Margin	Margin colour	Spore colour	Sporula-tion
1.	Hitinhalli	Et1	Poor growth, blackish, slightly raised growth colony	Dark Blackish	Irregular	Black	Brownish	+
2.	Jevoor	Et2	Moderate growth, whitish gray, slightly raised cottony growth colony	Whitish gray	Regular	Gray	Dark brownish	+
3.	Malked	Et3	Moderate growth, grayish black, slightly raised growth colony	Grayish black	Regular	Gray	Brownish	++
4.	Sannur	Et4	Excellent growth, blackish, slightly raised growth colony	Blackish	Regular	Black	Dark brownish	+
5.	Polkamdaddi	Et5	Moderate growth, grayish black, slightly raised cottony growth colony	Grayish	Regular	Black	Brownish	+++
6.	Gabbur	Et6	Excellent growth, grayish black, slightly raised growth colony	Grayish black	Regular	Black	Brownish	+
7.	Chittapur	Et7	Good growth, grayish, slightly raised cottony growth colony	Grayish	Irregular	Black	Brownish	++++
8.	Raichur local	Et8	Good growth, grayish, slightly raised growth colony	Grayish	Regular	Gray	Dark brownish	++++

* Mean of three replications

++++ - Excellent, > 20 conidia per microscopic field, +++ - Good, 15-20 conidia per microscopic field, Et - *Exserohilum turcicum* ++ - Fair, 10-15 conidia per microscopic field, + - Poor, < 10 conidia per microscopic field

Table 5: Categorization of *E. turcicum* isolates based on cultural characteristics

5a: Based on growth of mycelia

Growth of mycelia	Isolate number	Number of isolates
Excellent growth (90.00mm)	Et4, Et6	2
Good growth (85.00mm)	Et7, Et8	2
Moderate growth (87-88.00mm)	Et2, Et3, Et5	3
Poor growth (70.00mm)	Et1	1
Total		8

5b: Based on pigmentation (colour)

Colony color	Isolate number	Number of isolates
Grayish	Et5, Et7, Et8	3
Grayish black	Et3, Et6	2
Blackish	Et4	1
Whitish gray	Et2	1
Dark blackish	Et1	1
	Total	8

5c: Based on type of topography

Topography	Isolate number	Number of isolates
Slightly raised growth	Et1, Et3, Et4, Et6, Et8	5
Slightly raised with cottony growth	Et2, Et5, Et7	3
	Total	8

5d: Based on type of margin

Type of margin	Isolate number	Number of isolates
Regular	Et1, Et7	2
Irregular	Et2, Et3, Et4, Et5, Et6, Et8	6
	Total	8

5e: Based on colour of margin

Colour of margin	Isolate number	Number of isolates
Gray	Et2, Et3, Et8	3
Black	Et1, Et4, Et5, Et6, Et7	5
	Total	8

5f: Based on Sporulation

Sporulation	Isolate number	Number of isolates
Excellent	Et7, Et8	2
Good	Et5	1
Fair	Et3	1
Poor	Et1, Et2, Et4, Et6	4
	Total	8

5g: Based on spore colour

Colors	Isolate number	Number of isolates
Brownish	Et1, Et3, Et5, Et6, Et7	5
Dark brownish	Et2, Et4, Et8	3
	Total	8

Conclusions

Potato dextrose agar was significantly superior which supported the maximum radial growth (87.67mm) of *E. turcicum*, over all other media tested and it was followed by oat meal agar (85 mm), potato carrot agar (84.67 mm). The excellent sporulation was observed in potato dextrose agar and good sporulation was observed in Oat meal agar The fungus showed grayish colour with irregular margin and slightly raised mycelium on Yeast extract potato agar, malt extract agar, potato dextrose agar and also on Richard's agar. Maximum dry mycelial weight of the test pathogen was recorded in potato dextrose broth (689.67 mg) followed by Oat meal broth (639.67 mg) and Czapeck's Dox broth (619.67 mg). Excellent sporulation was observed on Potato dextrose broth good sporulation was observed in Oat meal broth. Among the eight isolates, tested for mycelial growth on PDA, Sannur (Et4) and Gabbur (Et6) showed an excellent mean radial growth of 90.00 mm and least mean radial growth was obtained in Et1(Hitinhalli) isolate with mycelial growth of 70.00 mm.

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