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Morphological and Cultural Variability among *Exserohilum turcicum* Isolates Causing Turcicum Leaf Blight Disease of Maize in Manipur

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ABSTRACT

This present experiment was conducted May–October, 2021 and April–October, 2022) in the Laboratory, Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India to study the different morphological and cultural characterizations of the pathogen, *Exserohilum turcicum* responsible for causing the Northern corn leaf blight (NCLB)/ Turcicum leaf blight (TLB) disease of maize. A total of 10 accessions were used for this experiment which were collected from different districts of Manipur. Based on the colony colour, they were classified as black, greyish-black and light greyish. Pigmentation was the same, i.e., black for all. Based on sporulation they were categorised as excellent, good and moderate. Different types of growth patterns were also observed from different types of accessions, i.e., flattened, slightly raised fluffy and raised cottony. The margins were recorded as regular and irregular. The range of fresh and dry weight, varied from 12.12 g–5.15 g and 1.61 g–0.82 g, respectively. Among six different types of media used to measure radial colony growth, maize leaf extract was found to be the highest and corn meal agar was the least. The growth rate for up to 7 days was observed and it was found that OQ689065 was considered as fastest-growing among all while, OQ689062 was considered as slowest. As per the length of the conidia, the maximum and minimum length was found to be 91.70 µm and 57.35 µm. Similarly, the maximum and minimum width was found to be 18.01 µm and 11.09 µm. The average septation of 10 accessions varied from 6.4 to 4.3. The statistical analyses performed during this study were found significant.

KEYWORDS: Colony, cultural, *exserohilum*, maize, morphological, sporulation

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1. INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop next to wheat and rice (Suganya et al., 2020). The average maize yield in India is 25.09 q ha^{-1}, which is much lower than most of the maize-growing countries of the world (Anonymous, 2016). Many pathogens (bacteria, fungi, viruses, nematodes) were identified affecting maize globally (Anwer et al., 2022). Out of 62 diseases identified, only 16 diseases were reported to infect this crop in India (Shekhar and Bagaria, 2017). Among all foliar fungal diseases reported Turcicum leaf blight (TLB), which is also known as Northern corn leaf blight (NCLB) disease is of serious concern (Kumar et al., 2022). Moreover, it also affects the straw which is of great value and used as a feeding ingredient for cattle (Ahangar et al., 2016). The disease epidemics at an early stage cause premature death of blighted leaves which lose their value as fodder (Hooda et al., 2017). TLB is caused by the pathogen *Exserohilum turcicum* (Pass.) Leonard and Suggs (Synonyms: *Helminthosprium turcicum* (Pass.) Leonard and Suggs.) [Perfect stage: *Setosphaeria turcica* (Luttrell): Leonard and Suggs. The causal agent of turcicum leaf blight on maize is identified by its imperfect stage i.e. *E. turcicum* because *Trichometasphaeria turcica* Lutrell, the fungus' sexual stage, is very rarely found in nature (Luttrell, 1958). The pathogen has a wide host range and high pathogenic variability (Muiru et al., 2010). This disease was first reported from the USA in New Jersey in 1878. This disease is widely prevalent in the Northeastern United States, in sub-Saharan Africa, China, Latin America, and India (Adipala et al., 1993, Dingerdissen et al., 1996). In India, for the first time, this disease was reported by Butler in 1907 (Butler, 1907). In India, this disease was widely distributed in Karnataka, Maharashtra, Andhra Pradesh, Bihar, Uttarakhand, Uttar Pradesh, Odisha, Punjab, West Bengal, Himachal Pradesh, Jammu and Kashmir, Sikkim, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Assam (Kaul, 1997, Mitra, 1981, Chenhulu and Hora, 1962, Payak and Renfro, 1968, Laxminarayana and Shankarlingam, 1983). In the NER of India, maize is grown in rainfed hilly uplands conditions and Jhum fields (Ramkrushna et al., 2022). It is the most potential and predominant rainy season crop, which not only ensures food security but is also used for direct consumption as well as for feed ingredients (Erenstein et al., 2022). In Manipur, maize is cultivated on around 25.53 t ha of land (Anonymous, 2022). *E. turcicum* was capable of infecting maize plants at all stages of crop growth, from seedlings to maturity (Kumar, 2018). Although TLB in maize is widely distributed across the world, however, the severity of the disease is more in the regions where the temperature is low with high humidity and cloudy weather (Jeevan et al., 2023). In Manipur, a maximum DI of 51–71% was reported for this disease (Nongmaithem et al., 2022). The estimated grain yield loss is reported to be up to 70% (Yeshitila, 2003). The yield losses may exceed 50% if the symptoms appear before the flowering stage (Raymundo et al., 1981, Tefferi et al., 1996), it also reduces the rate of photosynthesis by 91% when the severity was more than 50% (Pant et al., 2001). Yield losses approached 50%, when the disease is severe at 2–3 weeks after pollination (Shurtleff, 1980, Dey et al., 2017). Under severe epiphytotic conditions, the losses may vary from 28–91% (Ribeiro et al., 2016). Therefore, the main purpose of this study is to understand the biology, and ecology of this fungal pathogen, which can aid in protecting and implementing effective disease management strategies.

2. MATERIALS AND METHODS

The research activities were carried out during *kharif* seasons (May–October, 2021 and April–October, 2022) in the Laboratory, Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur. The details of the materials used and the methodology adopted during investigations are presented below.

2.1. Sample collection and isolation of pathogens

Plants showing heavy infestation with the TLB disease were collected from five different districts of Manipur i.e., (Imphal East, Imphal West, Senapati, Bishnupur and Kangpokpi). The details of the areas from which diseased samples were collected for variability studies are given in (Table 1). The collected samples were labelled then wrapped in blotter papers and brought to the laboratory for further studies (Navarro et al., 2021).

2.2. Isolation and identification of E. turcicum

The isolation of fungus was done by using the method described by Manamgoda (Manamgoda et al., 2012). First,

Table 1: Details of the places for the collection of turcicum leaf blight samples

Sl. No.	Districts	Location	Accession No.
1.	Imphal East	Andro	OQ689025
2.	Imphal East	Huikap	OQ696339
3.	Imphal West	Iroisemba	OQ689029
4.	Imphal West	Shantipur	OQ689065
5.	Kangpokpi	Daili	OQ689028
6.	Kangpokpi	Gorkha Harup	OQ689027
7.	Bishnupur	Leimaram	OQ689064
8.	Bishnupur	Kumbi	OQ689061
9.	Senapati	Vakho	OQ689062
10.	Senapati	Christian Colony	OQ689066

these collected leaves were washed with tap water followed by sterilized distilled water. The infected leaf tissues along with the adjacent healthy portions were cut into small pieces 0.5×0.5 cm^2 in diameter. For 1 min the leaf portions were surface sterilized by dipping them with 1% sodium hypochlorite (NaOCl). Then the desired portions were handled with the help of sterilized forceps and transferred into distilled water for 1–2 m. Those portions were collected in sterilized filter paper in order to absorb the moisture after that they were finally placed on a suitable nutrient potato dextrose agar (PDA) medium and the plates were completely sealed by using Para film®. Then the plates were kept in the incubation chamber at 26±2°C. After 7–10 days when the mycelia growth is full, a small portion of fresh mycelia was transferred aseptically to PDA of fresh culture medium to obtain pure cultures of *E. turcicum* (Anwer et al., 2022). Pure cultures of fungus were maintained on PDA slants at 4°C for further studies.

2.3. Cultural characters

Observations for different cultural studies like colony colour, pigmentation, sporulation, growth pattern, colony diameter, growth rate, fresh weight and dry weight were observed 12 days after incubation.

2.3.1. Colony colour and pigmentation

Colony colour and pigmentation of all the accessions were determined with the help of Munsell's colour chat (Anonymous, 1951). The colony colour was observed from the upper side of the Petri plate whereas for pigmentation under the surface of the Petri plate was observed.

2.3.2. Sporulation

For sporulation of different accessions, discs of 5 mm size were cut from 10 day old culture. Three such discs were placed in a 15 ml test tube containing sterilized distilled water and were vortexed to dislodge the spores from the mycelial mat. Then the spore load prepared was measured by using a haemocytometer. The rating for sporulation was given as per the standards described by Reddy in (Table 2) (Reddy et al., 2014).

2.3.3. Fresh and dry weight

Fresh weight and dry weight was taken on PDB (Potato Dextrose Broth) nutrient medium with the help of digital weighing balance. The mycelial dry weight was obtained by subtracting the weight of the filter paper.

$$W=W_2-W_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, in equation (1), W=Dry weight of the mycelial mat, W_2=Weight of the filter paper, and W_1=Total weight of the fungal mycelial mat and filter paper.

2.3.4. Growth rate

The growth rate of different accessions was recorded on 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h after incubation, cultured on PDA (Potato Dextrose Agar) growth medium.

Table 2: Score given for sporulation (Reddy et al., 2014)

Score	Grade	Description
++++	Excellent	>20×10^4 conidia/microscopic field (40x)
+++	Good	15–20×10^4 conidia/microscopic field (40x)
++	Moderate	10–15×10^4 conidia/microscopic field (40x)
+	Poor	<10×10^4 conidia/microscopic field (40x)

2.3.5. Effect of different growth media on radial colony diameter of E. turcicum

The colony diameter was observed in different growth mediums like PDA (Potato Dextrose Agar), CMA (Corn Meal Agar), PCA (Potato Carrot Agar), PSA (Potato Sucrose Agar), SDA (Sabouraud Dextrose Agar), and MLE (Maize leaf extract). The composition of the growth mediums are as follows: PDA (Potato, 200 g, Dextrose, 20 g, Agar-Agar, 20 g, distilled water, 1000 ml), CMA (Corn meal, 20 g, Peptone, 20 g, Agar-agar, 20 g, glucose, 20 g, distilled water, 1000 ml). PCA (Carrot infusion form, 50 g, Potato, 200 g, Agar-Agar, 20 g, Distilled water, 1000 ml), PSA (Potato, 200 g, Sucrose, 20 g, Agar-Agar, 20 g, Distilled water, 1000 ml), MLE (Agar-Agar, 20 g, Dextrose, 20 g, Maize leaves, 200 g, Distilled water, 1000 ml), SDA (Peptone, 10 g, Dextrose, 40 g, Agar-Agar, 15 g, Distilled water, 1000 ml), incubated at 26±2°C for 12 days.

2.4. Morphological characters

Growing cultures of 7–10 days old were used for different morphological studies like conidia length, width, and septation. Temporary slides were prepared in water mount by using cotton blue. Data on length, width and septation of conidia were recorded by ocular micrometer by using a precalibrated compound microscope.

2.5. Statistical analysis

All the experiments were conducted with three replications and the data was statistically analysed wherever necessary. Completely Randomized Design (CRD) was used for the analysis of different cultural and morphological variations, to determine significant differences among accessions except for the radial colony diameter measurement using different media, where Factorial Randomised Block Design (FRBD) was used for evaluating significance.

3. RESULTS AND DISCUSSION

3.1. Cultural characters

The cultural variability studies were necessary to move further to the next step towards developing any new approaches for its management. After 12 days of incubation

on a PDA nutrient medium at 26±2°C different cultural studies were done. The detailed cultural study was summarised in (Table 3).

3.1.1. Growth pattern type and margin

Accessions grown on PDA nutrient media were observed 12 days after incubation to study the type of growth pattern and margins. Based on the type of growth pattern all ten fungal specimens were classified into 3 types flattened, slightly raised fluffy and raised cottony. OQ689025, OQ689027, OQ689064, OQ689061, and OQ689066 show a flattened type of growth pattern, whereas OQ696339, OQ689029, OQ689028 and OQ689062 show a slightly raised fluffy type and the raised cottony type of growth pattern was observed by OQ689065. Based on the margin they were classified as regular and irregular. OQ689025, OQ689065, OQ689027, OQ689064, OQ689061, and OQ689066 show a regular type of margin whereas, OQ696339, OQ689029, OQ689028 and OQ689062 show an irregular type of margin (Plate 1).

Table 3: Cultural characteristics of different accessions of *Exserohilum turcicum*

Sl. No.	Accession No.	Colony colour	Pigmentation	Sporulation	Growth pattern	
					Type	Margin
1.	OQ689025	Black (2.5 Y/1)	Black	++++	Flattened	Regular
2.	OQ696339	Greyish black (10Y/R 5/1)	Black	++	Slightly Raised Fluffy	Irregular
3.	OQ689029	Light greyish (2.5Y 5/1)	Black	++	Slightly Raised Fluffy	Irregular
4.	OQ689065	Greyish black (10Y/R 5/1)	Black	++++	Raised Cottony	Regular
5.	OQ689028	Greyish black (10Y/R 5/1)	Black	+++	Slightly Raised Fluffy	Irregular
6.	OQ689027	Black (2.5 Y/1)	Black	++++	Flattened	Regular
7.	OQ689064	Black (2.5 Y/1)	Black	++++	Flattened	Regular
8.	OQ689061	Black (2.5 Y/1)	Black	++++	Flattened	Regular
9.	OQ689062	Light greyish (2.5Y 5/1)	Black	++	Slightly Raised Fluffy	Irregular
10.	OQ689066	Light greyish (2.5Y 5/1)	Black	+++	Flattened	Regular

3.1.2. Colony colour and pigmentation

The colony colour of the fungus was based on the dominant spectral colour from Munsell's soil colour chart (Anonymous, 1951). Based on the colony colour, the fungal specimens were classified into three different categories, i.e., black, greyish black, and light greyish. The black colour of the colony was observed by OQ689025, OQ689027, OQ689064 and OQ689061, while the greyish-black colour of the colony was observed by OQ696339, OQ689065, and OQ689028, and the light greyish colour of the colony was observed by OQ689029, OQ689062 and OQ689066. The colony colour of all the isolates can be viewed on (Plate 1). Based on pigmentation they were categorised into a single colour i.e. black. Detail about colony colour and pigmentation was mentioned in (Table 3).

3.1.3. Sporulation

Based on sporulation, all 10 *E. turcicum* samples were divided into three categories (Table 3). Excellent sporulation i.e., >20×10^4 conidia/microscopic field (40x) was recorded by OQ689025, OQ689065, OQ689027, OQ689064, and OQ68906, while good sporulation i.e., 15–20×10^4 conidia/

Plate 1: Cultural characteristics of different accessions of *Exserohilum turcicum*

microscopic field (40x) was recorded by OQ689028 and OQ689066. As compared to others, moderate sporulation i.e., 10–15×10^4 conidia/microscopic field (40x) was recorded by OQ696339, OQ689029, and OQ689062.

3.1.4. Fresh weight and dry weight

The fresh weight of the *E. turcicum* samples was taken 12 days after inoculation on the PDB. The fresh weight varies from 12.12 g (OQ689066) being the highest to 5.15 g (OQ689065) being the least similarly the dry weight varies from 1.61 g (OQ689066) being the highest to 0.82 g (OQ689025) being the least. The detailed fresh and dry weight of all accessions was mentioned in Table 4.

Table 4: Fresh weight and dry weight of different accessions of *E. turcicum*

Accession No.	Fresh weight (g)	Dry weight (g)
OQ689025	10.84	0.82
OQ696339	7.27	1.22
OQ689029	10.08	1.42
OQ689065	5.15	1.34
OQ689028	9.15	0.88
OQ689027	9.83	1.41
OQ689064	10.29	1.53
OQ689061	11.36	1.58
OQ689062	8.22	1.43
OQ689066	12.12	1.61
SEm±	1.26	0.10
CD (p≤0.05)	3.71	0.30

3.1.5. Growth rate

The growth rate was observed at every 24 h time intervals after incubation on a PDA at 26±2°C for 7 days. Based on the growth rate, OQ689065 is considered as fastest-growing among all while, OQ689062 is considered as slowest. The comparative growth rate between different accessions was mentioned in (Table 5).

3.1.6. Effect of different growth media on radial colony diameter of E. turcicum

Different growth mediums were used to determine the best media for the growth of *E. turcicum* samples. Out of the six growth mediums used, the best result for the radial colony growth of *E. turcicum* was observed on MLE and PDA, followed by PSA, PCA, and CMA, respectively (Table 6 and Plate 2). In the MLE medium, OQ689061 has shown the highest radial colony growth (74.5 mm) and OQ689028 has shown the least radial colony growth (61.6 mm). In the PDA medium, OQ689065 has shown the highest growth (79.6 mm) and OQ689028 has shown the least growth

Table 5: The growth rate of different accessions of *E. turcicum* recorded at 24 h interval time period

Accession No.	24 h (cm)	48 h (cm)	72 h (cm)	96 h (cm)	120 h (cm)	144 h (cm)	168 h (cm)
OQ689025	2.11	3.06	3.98	4.51	5.26	5.95	6.65
OQ696339	1.16	1.93	2.61	3.23	3.81	4.55	5.28
OQ689029	1.21	1.91	2.46	3.13	3.86	4.55	5.30
OQ689065	2.00	3.03	4.02	5.25	6.23	6.75	7.68
OQ689028	1.31	2.01	2.56	3.36	4.05	4.75	5.60
OQ689027	1.81	2.63	3.56	4.33	5.16	5.88	6.60
OQ689064	1.96	3.18	4.31	5.03	5.76	6.50	7.26
OQ689061	1.41	2.55	3.53	4.26	5.06	5.76	6.50
OQ689062	1.31	1.09	2.28	2.91	3.80	4.51	5.26
OQ689066	1.16	1.08	2.43	3.25	4.21	5.06	5.81
SEm±	0.06	0.12	0.21	0.24	0.28	0.25	0.28
CD (p=0.05)	0.17	0.35	0.62	0.71	0.81	0.75	0.81

(55.3 mm). In PSA medium highest growth was recorded as 81.3 mm for OQ689066 and 30.3 mm was observed as the lowest for OQ689029. In the PCA medium, OQ689061 has shown the highest growth (72.3 mm) and OQ689029 has shown the least colony growth (43.6 mm), whereas, in the CMA medium, (78.6 mm) for OQ689029 and (21.6 mm) for OQ689025 were recorded as the highest and lowest colony growth, respectively. The difference in the radial colony growth was recorded for different types of media used because of the availability of nutrients in different media.

3.2. Morphological characters

3.2.1. Conidia length

The data on the variability in the morphological characters between ten *E. turcicum* samples were presented in (Table 7 and Plate 3) and the differences in conidial length and width between them were found to be significant. OQ696339 recorded the maximum conidial length of 91.70 µm and the minimum conidial length was recorded as 57.35 µm by OQ689029.

3.2.2. Conidia width

The maximum conidial width was observed as 18.01 µm of OQ696339 and the minimum as 11.09 µm of OQ689064 (Plate 4).

3.2.3. Septation

The average septation between ten *E. turcicum* samples varies from 4.3 to 6.5. OQ689064 was recorded as the highest septation of 6.5 among all while, OQ689027 was recorded as the least septation of 4.3 (Plate 5).

Table 6: Effect of different media on the growth of different accessions of *E. turcicum*

Accession No.	PDA (cm)	CMA (cm)	PCA (cm)	PSA (cm)	SDA (cm)	MLE (cm)	Mean
OQ689025	7.03	2.16	5.46	5.23	5.93	7.02	5.55
OQ696339	5.53	4.05	5.86	5.04	3.76	7.36	5.40
OQ689029	5.96	7.86	4.36	7.86	2.06	6.86	5.92
OQ689065	7.96	3.53	5.86	6.03	5.53	6.76	5.95
OQ689028	6.86	6.56	6.00	6.03	3.06	6.16	5.82
OQ689027	7.04	3.13	6.09	3.03	3.43	7.26	5.19
OQ689064	7.63	7.04	6.86	5.05	8.06	6.56	7.00
OQ689061	7.03	7.04	7.23	4.05	6.46	7.43	6.67
OQ689062	6.46	7.83	5.13	7.93	6.26	6.9	6.75
OQ689066	6.83	7.08	5.93	8.13	6.03	7.02	6.98
MEAN	6.09	5.82	5.96	5.99	5.11	6.97	
	Accessions (A)		Media (B)		Inter (A×B)		
SEm±	0.076		0.059		0.186		
CD (p=0.05)	0.212		0.165		0.52		

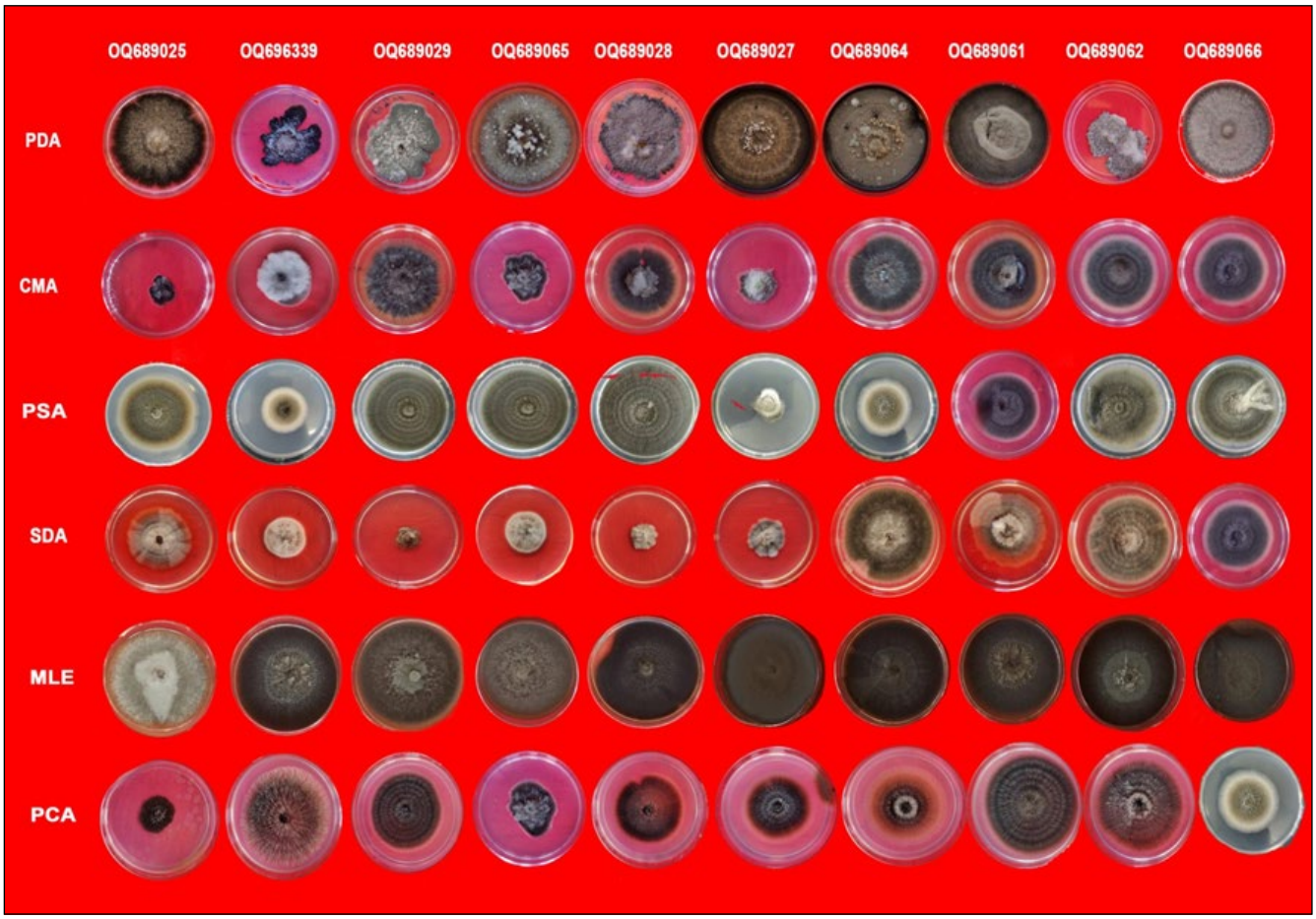

Plate 2: Effect of different media on the growth of different accessions of *E. turcicum*

Table 7: Morphological characteristics of different accessions of *Exserohilum turcicum*

Sl. No.	Accession No.	LC	Width of Conidia (µm)	Septation (Average)	Colour of conidia
1.	OQ689025	80.53	11.19	6.4	Brownish
2.	OQ696339	91.70	18.01	5.5	Brownish
3.	OQ689029	57.35	15.25	6.1	Brownish
4.	OQ689065	71.25	15.24	4.7	Brownish
5.	OQ689028	74.60	14.74	6.3	Brownish
6.	OQ689027	89.67	14.64	4.3	Brownish
7.	OQ689064	61.09	11.09	6.5	Brownish
8.	OQ689061	85.12	13.21	5.2	Brownish
9.	OQ689062	91.64	16.16	6.4	Brownish
10.	OQ689066	82.27	12.55	6.2	Brownish
	SEm±	0.587	0.235		
	CD (p=0.05)	1.745	0.697		

LC: Length of Conidia (µm)

Plate 3: Conidial length of ten different isolates of *E. turcicum*

Plate 4: Conidial width of ten different isolates of *E. turcicum*

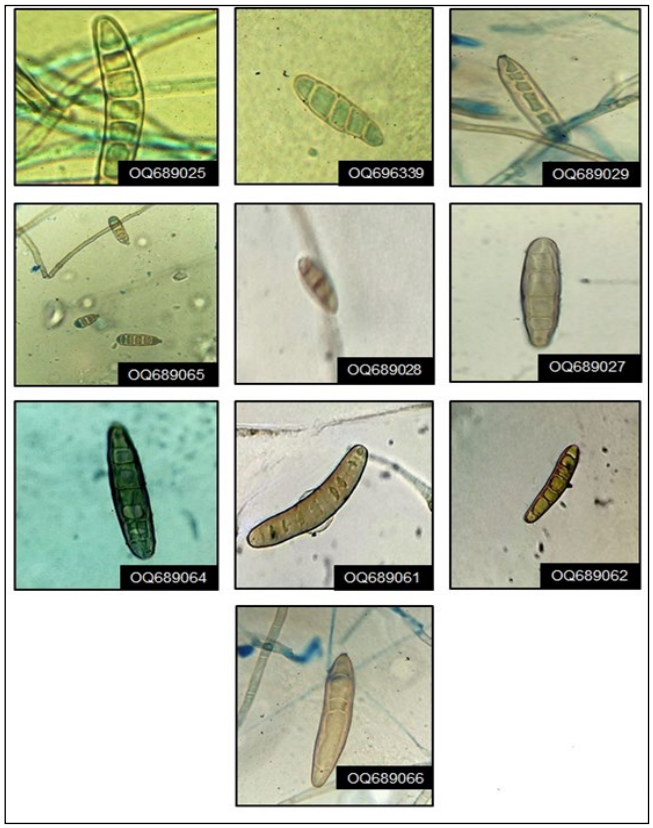

Plate 5: Conidial septation of ten different isolates of *E. turcicum*

Based on the colour of the conidia, all the accessions were found to be brownish.

4. CONCLUSION

The present study was important for the identification and differentiation of *E. turcicum* from other fungal species. *E. turcicum* produced greyish-green to brownish-black colonies having curved or slightly bent spores. It grew well on potato dextrose agar as well as in maize leaf extract and required a slightly acidic pH for optimum growth. It also preferred cooler temperatures (15–20°C). This knowledge was important for developing effective disease management strategies and reducing the economic losses caused by northern corn leaf blight.

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