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Studies on morphological, cultural, physiological and pathological variability in isolate of *Exserohilum turcicum*, incitant of northern leaf blight of maize

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Abstract
Maize is a major cereal crop and India's third most significant crop after rice and wheat. The crop is afflicted by many fungal diseases, one of which is leaf blight, also known as northern maize leaf blight or turcicum leaf blight, which affects photosynthesis and reduces grain output by 28 to 91%. Disease symptoms first emerge on the leaves at any stage of plant development, but most commonly at or after anthesis. For the variability experiments, nine maize isolates of *E. turcicum* were used. All of the isolates differed in terms of cultural and physical characteristics. All nine isolates were classified into five groups based on colony colour. The pigmentation of the isolates SEt2, GEt3, KEt5, VEt6, SoEt9 and KhEt8 was noticeably different. Nine isolates had three different conidial shapes: curved, spindle and elongated. Conidia has an average length of 73.79 µm and a width of 22.42 µm. The number of septa discovered ranged from 3 to 9. Conidia were found in all of the isolates. Conidia size was greatest in isolate PhEt7 (97.96 µm), with an average of 3-8 septation. Temperature is a key component in regulating the fungus's development and reproduction. The effect of several temperatures on the mycelia growth of *Exserohilum turcicum*, namely 20 °C, 25 °C and 30 °C, was investigated. Temperatures of 25-30 °C promote larger mycelial growth of fungus, but temperatures of 20 °C are less favourable.

Keywords: NLB (northern leaf blight), isolate, temperature, septation and conidia

Introduction
Maize (*Zea mays* L.) is among the most adaptable growing crops, with a wide range of adaptability under various agro-climatic conditions. Maize is regarded as the "Queen of Cereals" worldwide because it has the most significant genetic yield potential of any cereal. It is grown on about 150 million hectares in around 160 countries with diverse soil, environment, habitats, and management methods, accounting for 36% (782 million tonnes) of the global grain supply. The United States of America (USA) is the world's largest producer of maize, accounting for approximately 35% of overall demand, and maize is the engine that drives the US economy. The United States has the most significant productivity (> 9.6 t ha^{-1}) and is twice as productive as the rest of the world (4.92 t ha^{-1}). Maize, botanically known as Zea mays, is a member of the Gramineae grass family. It is a South American native (Galinat, 1988) [4] and has been the primary food for most people in Mexico, Central America, and Latin America since ancient times. Maize is extensively grown in the world and has the highest yield of any cereal crop, with 972.40 million tonnes (Anonymous FAO, 2018-19).

Maize is a potential source of carotenoids like beta-carotene, lutein, zeaxanthin and cryptoxanthin which have highly varied health benefits such as maintaining normal vision and lowering oxidative stress (Chaudhary *et al*. 2014) [2]. Maize demand in India is increasing as a result of evolving food preferences and diverse maize uses in industry. Maize is susceptible to 112 diseases in various parts of the world, which are caused by fungi, bacteria, viruses and nematodes and cause significant damage. Approximately 61 crop diseases have been reported in India. These include seedling blights, stalk rots, foliar diseases, downy mildew and ear rots (Payak *et al*. 1973, Payak and Sharma, 1980) [13, 14]. In contrast to America and Europe, India's maize production is very poor due to a variety of biotic and abiotic stresses. Among the fungal diseases, Northern leaf blight caused by *Exserohilum turcicum* (Pass.). Leonard and Suggs is one of the important foliar disease causing a severe reduction in grain and fodder yield to the tune of 16-98% (Kachapur and Hegde, 1988) [9]. The pathogen has a wide host range and high pathogenic variability (Muiru *et al*. 2010) [10]. The pathogen affects the whole plant, but the most visible symptoms/lesions are located on the foliage.

Lesions defoliate the leaves, causing Yield declines owing to a shortage of carbohydrate to fill the crops. Heavily infected fields present a scorched or burnt appearance resulting in the premature death of leaves (Harlapur *et al*. 2007) [8].

Material and Methods

Collection of diseased samples

The maize plants severely infected by *E. turcicum* showing typical symptoms of Northern leaf blight necrotic lesions were collected from nine tehshil of Aurangabad district *viz*., Aurangabad, Sillod, Gangapur, Paithan, Kannad, Vaijapur, Phulambri, Khultabad and Soegaon during the survey programme. The fungus *E. turcicum* was isolated by standard tissue isolation procedure and also by hyphal tip isolation procedure and later nucleus culture was maintained on potato dextrose agar slants and kept it in a refrigerator at 4 ºC for all laboratory as well as other studies.

Isolation of the pathogen

The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized in 0.01 per cent with mercuric chloride solution for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of mercuric chloride. Then such bits were aseptically transferred to sterilized Petri plates (1 to 2 infected bits per Petri dish) containing potato dextrose agar (PDA) in aseptic condition under laminar hood. The Petri plates were incubated at room temperature (27 ± 1 ºC) for 15 days for fungal growth. The pure colonies developed from the bits were transferred to PDA slants and incubated at room temperature for 15 days. Abundant sporulation was observed after 15 days of incubation. The pathogen was purified following hyphal tip isolation technique as described below.

Hyphal tip isolation and maintenance of culture

This method was followed for maintaining of pure culture, hyphal tip isolation was done on water agar plates. Spore suspension of the pathogen was prepared in sterilized distilled water containing eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and observed for spores under the microscope.

Single spore was marked with a marker on back side of the Petri plate and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae growing from each cell of the single spore was traced and marked with marker. The growing tip of the hyphae was cut carefully by cork borer and transferred to PDA plates and incubated at 27 ± 1 ºC for 10 days. Saltation or sectoring was also observed in the culture plates. Further the fungus was sub-cultured on PDA slants and kept in laboratory at 27 ± 1 ºC for 15 days. Such mother culture slants were preserved at 4 ºC in refrigerator. Further, these cultures were sub-cultured once in a month and used for further studies.

Identification of the fungus

In order to confirm the identity of the fungus the conidia and conidiophores were observed under the high power (40X) microscope from the infected leaves of maize plant. Observations such as length, breadth and number of septa were recorded using image capturing microscope or ocular micrometer and were compared with original description of the fungus.

Variability of *E. turcicum*

Northern leaf blight infected leaf samples were collected from nine tehshil of Aurangabad district at the time of survey during *kharif* 2021 and pure culture of 09 isolates were maintained on PDA and later cultural, morphological, physiological and pathological studies were carried out in the laboratory.

Cultural Variability

Among the different media tested PDA was found to be effective for growth and development of *E. turcicum*. Hence this media was selected to study the variability of cultural and morphological characteristics of pathogen. The potato dextrose agar medium was prepared and 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 ± 1 ºC for 10 days. The variation in cultural characteristics of *E. turcicum* was investigated by selecting isolates of fungus. The cultural characteristics such as colony diameter, colony colour and pigmentation were recorded.

Morphological Variability

Pure culture of 09 isolates of *E. turcicum* was selected for morphological variations such as shape, size of conidia and number of septa. The size of conidia was also measured using stage and ocular micrometer under compound microscope. The colonies were characterized for phenotype growth pattern and different morphotypes, shape (irregular and regular); growing pattern (circular and feathery); texture (velvety and cottony) were observed under *in vitro*. Similarly, colour was differentiated into black and grayish black. The sporulation was graded as ++++ (>20 spores/microscopic field), +++ (15-20 spores/microscopic filed), ++ (10-15 spores/microscopic field) and + (<10 spores/microscopic field).

Physiological Variability

The cultural characters of *Exserohilum turcicum* were studied on the Potato Agar (PDA) media at different temperature viz. 20º, 25º and 30 ºC.

Pathological Variability

Studies on pathogenic variability were carried out in pot at the College of Agriculture, Badnapur. Pathogenicity of the nine isolates was determined by spray inoculation of spore suspension of *E. turcicum*.

Preparation of Inoculum

The inoculum of each isolate was prepared by flooding 12-day old culture plates of *E. turcicum* grown on potato dextrose agar (PDA) medium with sterile distilled water and scrapping the spore mass with the scalpel. The spore suspension was filtered through a muslin cloth. The conidial concentration was adjusted to 4 × 105 spores/ml using a haemocytometer.

Inoculation

Thirty days old different plants of maize were inoculated with spore suspension (4 × 105 spores per ml) in the hand sprayer in the evening hours. The inoculated plant was be bagged with a polythene bag to create high humidity at 24 hrs interval for three days.

Observation

The pathological variability of each isolate was categorised based on five virulent scales, weak (DSI=1-10%), mild (11-20%), moderate (21-30%), virulent (31-50%) and highly virulent (> 50%) (Shah *et al*., 2006) [15].

Disease severity was estimated on inoculated leaves 14 days after inoculation by using the 1-5 disease rating scale.

Result and Discussion

Cultural characteristics

The cultural characteristics of isolates are presented in Table 3Plate 1A and 1B The following observations were made on Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony. (M.R. Vinay and A.R. Sataraddi, 2019) [16] and (Geeta D. S., 2019) [5].

Incubation period (days) for maximum growth

All the nine isolates produced good growth on PDA, but the period taken by different isolates to completely cover the 9 cm petridish were different based on aggressiveness of the isolate. Among isolates KEt5 and PhEt7 shown lowest Incubation period of 7 days and AEt1 shown highest Incubation period. In an average known to take 14 days of Incubation period to completely cover the 9 cm petridish.

Colony colour

The colony colour of fungus was recorded based on dominant spectral colour from Munsell's soil colour chart (1954), 14 days after incubation on PDA medium and the results are presented in Table 4.6. The colony colour varied from grey to black colour. Based on the colony colour all the nine isolates were grouped in 8 categories *i.e*., dark Grey, light greenish, white to grey, yellow, blackish, creamy white, white to black and black. The KEt5 (Kannad), KhEt8 (Khultabad) and SoEt9 (Soegaon) showed Black colony colour where isolate AEt1 shown Dark greyish to black colony colour.

The isolates SEt2 from Sillod showed yellow colony colour and GEt3 from Gangapur showed light greenish colony colour which was distinctly different from all other isolates. The isolates PEt4 (Paithan) showed white to grey colony colour while creamy white colony colour was observed in the isolates VEt6 from Vaijapur. PhEt7 from Phulambari showed white to black colony colour.

Pigmentation

Based on the pigmentation *E. turcicum* isolates were grouped into 6 groups *i.e*., Black, yellowish, light green, Brown, creamy, blackish, and Black red. The isolates SEt2, GEt3, KEt5, VEt6, SoEt9 and KhEt8 was in a distinctly different pigmentation *i.e.* yellowish, light green, Brown, Creamy, yellowish and Black red. With regard to the isolate AEt1, PEt4 and PhEt7 showed Black to Blackish pigmentation.

Sporulation

All the nine *E. turcicum* isolates were classified into three groups based on the sporulation as mentioned in Table 4.6. Three isolates KEt5, VEt6 and PhEt7 produced good sporulation while the isolates SEt2, KhEt8 and SoEt9 exhibited moderate sporulation and isolate AEt1, GEt3 and PEt4 produced poor sporulation.

Surface texture

On Potato Dextrose Media (PDA) majority of the isolates showed irregular shape. The isolates SEt2, VEt6, KhEt8 and SoEt9 produced rough texture and GEt3, PEt4 and PhEt7 produced fluffy texture whereas smooth surface texture produced by AEt1 and KEt6.

Colony texture

On PDA majority of the isolates produced distinct wavy and moderately wavy zonation are SoEt9, SEt2, VEt6 and KhEt8 and with regards to AEt1 which produced ad pressed colonies. GEt3, PEt4 and PhEt7 produced cottony colony growth and AKEt5 produced smooth colony growth.

Edge of colony

The isolates when grown on PDA shown sparsely branched to highly branched edges of the colony. The isolate having edge of colony sparsely branched are AEt1, PEt4 and PhEt7 and moderately branched edge of colony in isolate GEt3, VEt6 and KhEt8. Isolate SEt2 having branched edges of colony and isolate SoEt9 having heavily branched edges of colony. And one isolate that No branching edges of colony *i.e*. KEt5. The nine isolates did differ in different prospect such as Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony. Such variations have been reported by Gowda *et al*., (2010) [7].

Table 1: Cultural characters of nine isolates *Exserohilum turcicum* on potato dextrose agar (PDA)

Location	Isolate	Incubation period (days) for max. growth	Mean Colony diameter (mm)	Colony colour	Pigmentation	Surface texture	Colony texture	Edge of colony
Aurangabad	AEt1	14	22	Dark grey	Black	Smooth	Pressed	Sparsely branched
Sillod	SEt2	12	25	Yellow	Yellowish	Rough	Moderately wavy	branched
Gangapur	GEt3	9	33	Light green	Light green	Fluffy	Cottony	Moderately branched
Paithan	PEt4	14	23	White to grey	Black	Fluffy	Cottony	Sparsely branched
Kannad	KEt5	7	90	Blackish	Brown	Smooth	Smooth	No branching
Vaijapur	VEt6	10	39	Creamy white	Creamy	Rough	Moderately wavy	Moderately branched
Phulambri	PhEt7	7	87	White to black	Blackish	Fluffy	Cottony	Sparsely branched
Khultabad	KhEt8	11	43	Black	Black to red	Rough	Moderately wavy	Moderately branched
Soegaon	SoEt9	10	63	Black	Yellowish	Rough	Wavy	Heavily branched

Morphological variability

Nine isolates shown three types of conidial shapes *viz*., curved, spindle and elongated. The size of the conidia averaged 73.79 µm in length and 22.42 µm in width. The number of septa was found to range from 3 to 9. Conidia were observed in all the isolates. Morphological studies data of various location given in Table 2 and Plate-2.

Among the isolates, conidia size was maximum in isolate PhEt7 (97.96× 26.52µm) with an average of 3-8 septation and minimum in isolate AEt14 (49.62×15.55 µm) with 3-5 septation.

Table 2: Morphological variability in different isolates of *Exserohilum turcicum*

Sr. No.	Isolate code	Spore colour	No. of septa	Size of conidia µm (10X)	
				Length	Breadth
1.	AEt1	Dark brownish	3-5	49.62	15.55
2.	SEt2	Brownish	3-6	57.56	23.62
3.	GEt3	Brownish	3-6	58.63	25.51
4.	PEt4	Brownish	3-5	52.49	18.65
5.	KEt5	Dark brownish	3-9	97.50	25.32
6.	VEt6	Brownish	3-5	77.48	20.51
7.	PhEt7	Brownish	3-8	97.96	26.52
8.	KhEt8	Dark brownish	3-7	86.52	22.45
9.	SoEt9	Dark brownish	3-7	86.43	23.68

Physiological variability

Effect of temperatures on growth of *E. turcicum* isolate

In order to culture pathogenic fungi in the laboratory it is necessary to furnish essential elements and compounds in the medium which are required for growth and other life processes and temperature is also one of the most important factor for regulating growth and reproduction of the fungus thus effect of different temperatures *i.e.* 20 °C, 25 °C and 30 °C on the mycelia growth of nine isolate of *Exserohilum turcicum* was studied. Nine isolate were taken and each one of them was kept at three different temperatures and difference in colony diameter of the pathogen among them was observed.

Isolate AEt1 showed maximum colony diameter of 86.77 mm and Minimum mycelial growth in isolate KEt5 were 40.71 mm was observed at 30 °C.

Isolate GEt3 showed maximum colony diameter of 85.22 mm and Minimum mycelial growth in isolate KEt5 were 34.45 mm was observed at 25 °C.

Isolate GEt3 showed maximum colony diameter of 54.39 mm and Minimum mycelial growth in isolate KEt5 were 27.10 mm was observed at 20 °C.

The cultural characteristics of isolates were also change according to temperature changes. *I.e.* Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony.

The study concludes that 25-30 °C temperature supports greater mycelial growth of the fungus whereas 20 °C is less favourable for the growth of fungus.

Pandey and Shukla (1982) reported that optimum temperature for colony growth of maize isolate of *E. turcicum* was 20- 30 °C and no growth was observed at 40 °C.

Pathological variability

Reaction of maize variety against *E. turcicum* isolates

It is evident that the four variety of maize included in the present study were able to differentiate the virulence of the pathogen associated with geographical origin of the isolates. The results showed that irrespective of the isolates in susceptible local variety exhibited significantly more mean lesion size of, 1.31 cm^2-3.84 cm^2 respectively. Similarly, smaller mean lesion size 0.04-0.97 cm^2 was recorded in resistant variety Pioneer 3501, Pinnacol and Advanta irrespective of the isolates. Across the maize variety *E. turcicum* isolates from Aurangabad (AEt1), Sillod (SEt2), Gangapur (GEt3), Paithan (Pet4) and Soegaon (SoEt9) produced higher mean lesion size of 3.84, 1.50, 1.31, 1.51 and 1.40 cm^2, respectively whereas small lesion size was noticed in Kannad (KEt5), Vaijapur (VEt6), Phulambari (PhEt7) and Khultabad (KhEt8) isolates are 0.52, 0.97, 0.26 and 0.76 cm^2 (Table-3) On susceptible local variety, some of the isolates such as Aurangabad, Sillod, Gangapur, Paithan and Soegaon produced maximum lesion size and could be considered as most virulent isolates. The results indicated that, there were three virulence patterns exhibited on four maize variety after inoculating with 9 isolates of *E. turcicum*. The isolates from Aurangabad, Sillod, Paithan and Soegaon were highly pathogenic on local variety.

The variety showing disease score between 0.04-0.14 were considered as resistant (R), 0.24-0.33 as moderately resistant (MR), 0.44-0.97 moderately susceptible (MS), 1.31-1.40 susceptible (S) and 1.50-3.84 as highly susceptible (HS).

Based on the disease reaction of these maize variety, the isolates could be grouped into three virulent types viz., the isolates has reaction 3.84 as most virulent, reaction between 1.31-1.40 as moderately virulent and reaction upto 0.97 as less virulent type.

Therefore, the reason for lack of resistance in some of the commercial cultivars of maize may be attributed to the prevalence of virulent isolates of the pathogen *E. turcicum* similar observations were made by Gowda *et al*., (1989) [6].

The results of the present findings were supported by the Daniel and Narong (2006) [3] Present findings are also in conformity with earlier findings of (Muiru *et al*., 2008) [11].

Table 3: Reaction of maize variety against *E. turcicum* isolates

Sr. No.	Isolate code	Varieties				Average
		Pinnacol	Pioneer	Advanta	Local	Mean
1.	AEt1	0.97	0.76	0.73	3.84	1.57
2.	SEt2	0.51	0.42	0.39	1.50	0.70
3.	GEt3	0.51	0.40	0.37	1.31	0.64
4.	PEt4	0.52	0.44	0.40	1.51	0.71
5.	KEt5	0.14	0.12	0.09	0.52	0.21
6.	VEt6	0.33	0.30	0.28	0.97	0.47
7.	PhEt7	0.07	0.05	0.04	0.26	0.10
8.	KhEt8	0.24	0.21	0.17	0.76	0.34
9.	SoEt9	0.44	0.41	0.38	1.40	0.65
	Av. Mean	0.41	0.34	0.31	1.34	0.60
	SE±	0.006	0.005	0.007	0.125	-
	CD@ 1%	0.019	0.016	0.021	0.374	-

The variety showing disease score between 0.04-0.14 were considered as resistant (R), 0.24-0.33 as moderately resistant (MR), 0.44-0.97 moderately susceptible (MS), 1.31-1.40 susceptible (S) and 1.50-3.84 as highly susceptible (HS)

Plate 1A): Growth of nine isolate on cultural media

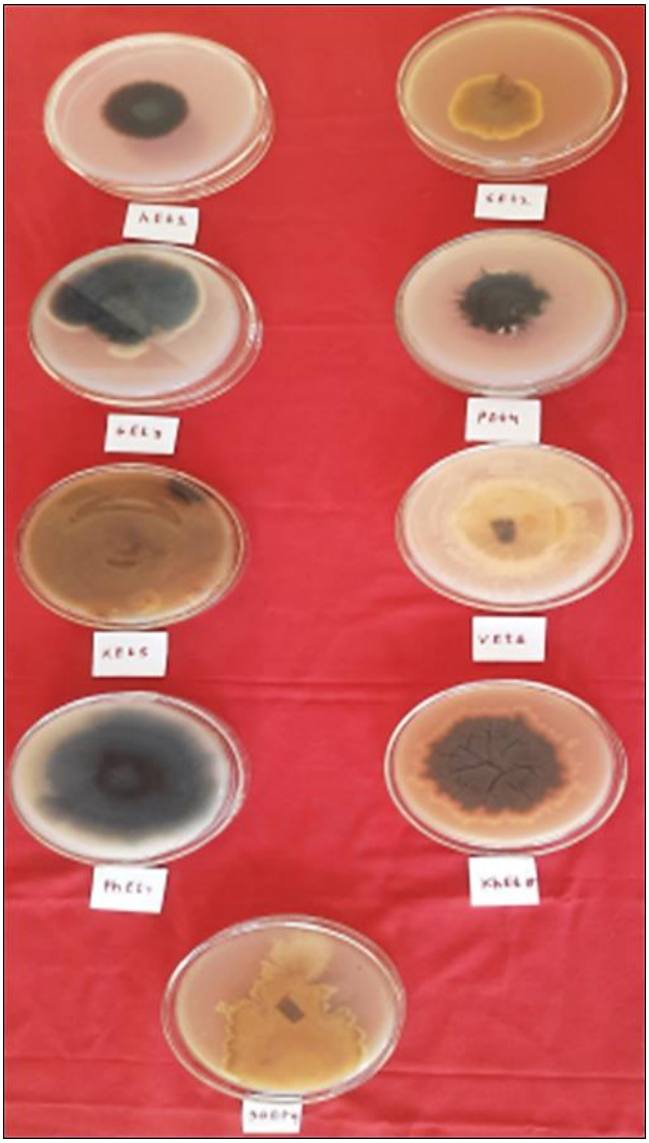

Plate 1B): Pigmentation of Nine isolate on culture media

AEt1	Dark grey
SEt2	Yellow
GEt3	Light green
PEt4	White to grey
KEt5	Blackish
VEt6	Creamy white
PhEt7	White to black
KhEt8	Black
SoEt9	Black

AEt1	Black
SEt2	Yellowish
GEt3	Light green
PEt4	Black
KEt5	Brown
VEt6	Creamy
PhEt7	Blackish
KhEt8	Black to red
SoEt9	Yellowish

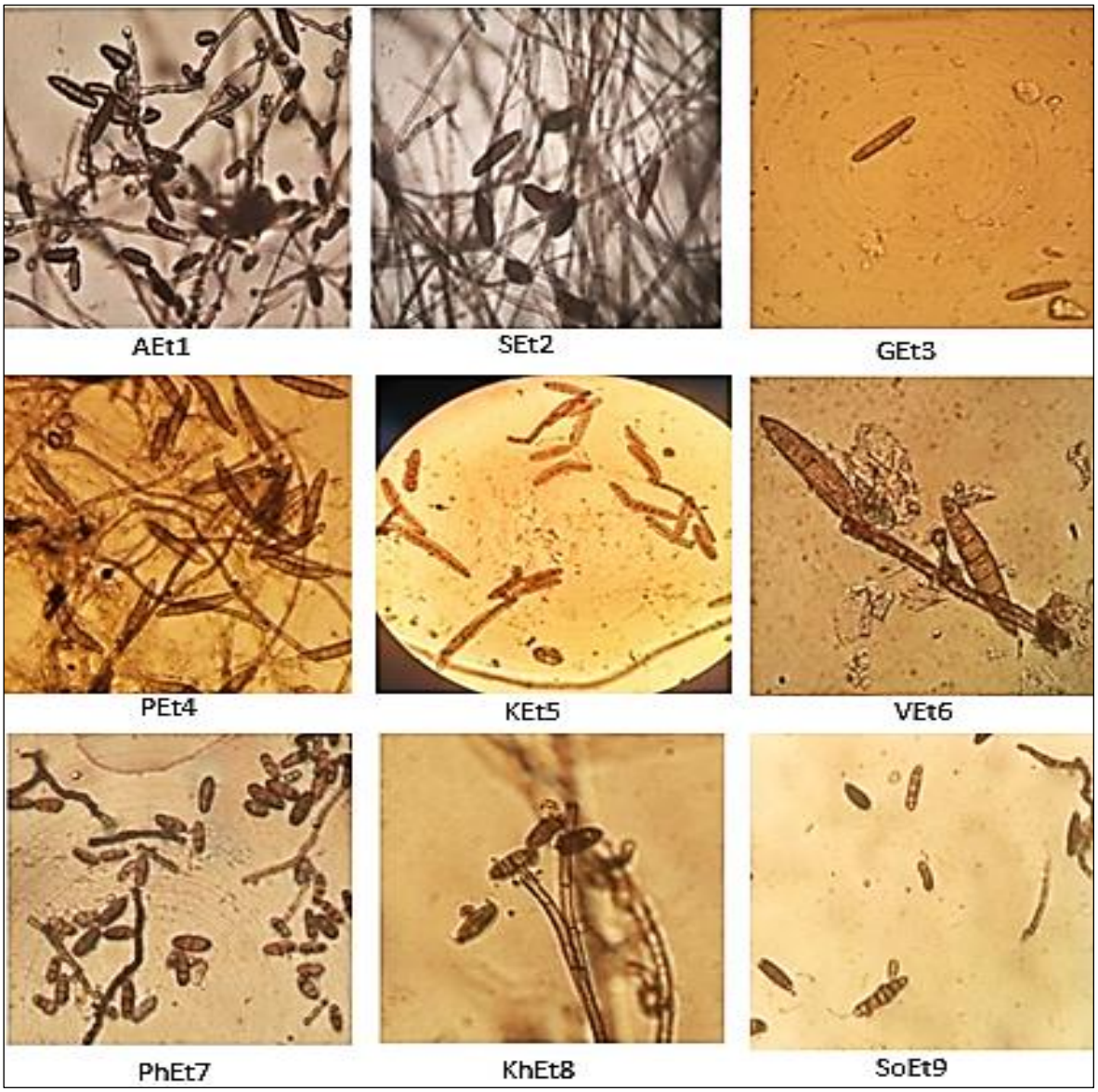

Plate 2: Conidial variability of *E. turcicum* isolate (40x) causing NLB of maize

Conclusion

Maize is one of the world's most valuable cereal crops, contributing to food security in the majority of developing countries. Maize is widely grown throughout the world and produces the most of any cereal crop, with 972.40 MT (Anonymous FAO, 2018-19). Many biotic and abiotic stresses reduce maize production and result in significant economic losses. Northern leaf blight of maize, caused by the Deuteromycetes fungus *Exserohilum turcicum* (teleomorph: *Setosphaeria turcica*), is one of the most serious fungal diseases affecting maize. The disease is found in almost all maize-growing areas and causes significant yield loss. Given the disease's significance, the current research was conducted to classify the causal organism associated with northern leaf blight of maize. The research on "Study on Northern Leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs" was conducted at the College of Agriculture, Badnapur and the NARP, Aurangabad in 2020-21. Systematic research on morpho-cultural, physiological, and pathological variability was carried out. The *in vitro* efficacy of various fungicides, botanicals and biogents against the test pathogen was evaluated at various concentrations and the findings obtained are summarised.

In terms of morpho-cultural, physiological, and pathological diversity, the different test isolates of *E. turcicum* varied greatly. The significant interaction of four different maize varieties (pinacol, pioneer, advanta and local) and nine isolates suggests some kind of specialisation in the fungus population because there are differences in both the resistance level of maize varieties and the aggressiveness of the pathogen isolates. The presence and spread of diverse isolates of *E. turcicum* with wide pathogenic diversity in the field gives critical information for developing an appropriate disease control approach.

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