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In vitro studies on various factors affecting growth of *Exserohilum turcicum*, causing Northern leaf blight disease of maize

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Abstract
Exserohilum turcicum, causing Northern leaf blight of maize is one of the most devastating pathogen of maize. A number of factors like light, source of nutrition, temperature, moisture etc greatly influence the growth and metabolism of the fungus. The responsive behaviour of pathogenic fungi to various physical factors can be great use to study the pathogen and to counteract its growth by managing the physical conditions accordingly. The effect of different media, temperature, light and pH were examined to find the conditions conducive to the growth of *Exserohilum turcicum.* The study reveals that 25-30^0 C temperature is most favourable for mycelial growth of the fungus and maximum radial growth was found in Oat meal agar media. However, no fixed pattern of fungal growth under different light wavelengths and different media interactions was observed. The growth of fungus was maximum at pH 7 and minimum mycelial growth was observed at pH 5.

Keywords: *Exserohilum turcicum*, pH, temperature, light, media

Introduction
Maize constitutes a major part of diet and nutrition in many countries. Maize is not only utilised as food for humans but also as fodder for animals. It is also processed as variety of industrial products such as starch, sweetners, beverages, oil, industrial alcohol, glue and ethanol fuel [1]. Northern leaf blight of maize caused by a fungal pathogen *Exserohilum turcicum*, is one of the most devastating diseases as it results in reduction of grain yield by 28 to 91 per cent [2, 3]. The genus *Exserohilum turcicum* for *Helminthosporium* species was established by Leonard and Suggs (1974) [4] in which the conidial hilum was strongly protuberant. The perfect state *Setosphaeria turcica* (Luttrell) Leonard and Suggs is rarely found in nature. The causal agent of Northern leaf blight of maize is normally the imperfect stage *Exserohilum turcicum.*
Information about various physical factors affecting the growth of the fungus and can be of great use for *in vitro* studies of fungus and can also be utilised in management aspects. Thus the experiment was performed to study the effect of various factors such as media, light, temperature and pH on growth of the pathogen. Fungal growth and sporulation is affected by a number of physical factors like light, temperature, the source of food, pH and humidity. All these factors not only affect the growth of the pathogen but may also play major role in virulence. The study of all these factors can greatly help to understand the physiology of the fungus.

Materials and Methods
Isolation of pathogen
The leaf portion with lesion was cut with help of sterilised blade into pieces of 2-3mm size having half healthy and half diseased tissues. The small pieces were sterilised with Sodium hypochlorite solution 2% for 30 seconds and thoroughly washed in sterilised water three times. Then the pieces were placed between two layers of sterilized blotter paper to remove excess of water. These pieces were then transferred to slants and Petri plates containing PDA medium inside laminar flow chamber under aseptic conditions. The inoculated plates were incubated at 28±2 ºC for 7 days and then subcultured in fresh PDA.

Purification of fungus by single spore isolation method
Spore suspension was prepared by adding 5ml sterilised distilled water to plate containing seven days old culture and was filtered with muslin cloth to get spores in a beaker. The suspension obtained was then diluted to reduce the spore count to get 10-15 spores per microscopic field from the suspension.

One ml of suspension was taken and then was uniformly spread on 2 per cent solidified water agar plates and incubated at 28±2 ^{0}C for 12 hours. The plates were then examined under stereoscopic microscope and single spores were marked by using marker. The marked spores were then picked up by cork borer and aseptically transferred to PDA medium in sterilized petriplates, for further growth and incubated at 28±2 ^{0}C. The pure culture obtained, was used for further studies.

Effect of different media and different temperatures on radial growth

The growth of *Exserohilum turcicum* was studied in six different synthetic media *viz*; Potato Dextrose Agar, Czapex Dox Agar, 2% Malt Extract Agar, Oat Meal Agar, Corn Meal Agar and Richard's Agar at six different temperatures *viz;* 10 ^{0}C, 15 ^{0}C, 20 ^{0}C, 25 ^{0}C, 30 ^{0}C and 35 ^{0}C.The media prepared were subjected to moist heat sterilisation in an autoclave at a temperature of 121.6 ^{o}C for 15 minutes. The medium was sufficiently cooled and 20ml of each was poured in 90mm sterilised petri plates and allowed to solidify. After solidification, 5mm discs of test fungus from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petriplate. Each set of experiment was replicated thrice and plates were incubated at different temperatures *viz*; 10, 15, 20, 25, 30, 35 ^{0}C. The measurements of the colony diameter were taken at 7 days after incubation.

Effect of different media and light quality on growth of *Exserohilum turcicum*

Petri plates were poured with six different types of media and allowed to solidify. After solidification, 5mm discs of *Exserohilum turicum* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petriplate. These Petri plates were then incubated for seven days at 28 ^{0}C ± 2 ^{0}C enclosed in the chambers providing different coloured lights. Three replications were maintained for each treatment. These chambers were prepared by covering them with coloured cellophane sheets of varying spectral characteristics. All types transmitted different spectra of visible light depending on cellophane colour: 400–500nm (blue); 480–540nm (green); 500–700nm (yellow); 600–700nm (red). Observations of colony diameter in different treatment were taken from the third day of inoculation and final reading was taken on the seventh day of inoculation.

Effect of different pH on radial growth of *Exserohilum turcicum*

Five pH levels *viz.*, 5, 6, 7, 8, 9 were adjusted in Potato Dextrose Agar medium with the help of pH meter by adding 0.1 N Sodium hydroxide or 0.1 N Hydrochloric acid. The media having different pH values were sterilised and poured in petri plates. After solidification, 5mm discs of *Exserohilum turicum* from actively growing culture were cut using a cork borer and a single disc was placed at the centre of petriplate. Three replication of each treatment were maintained and incubated at 28 ± 2 ^{0}C. Colony diameter was measured from third till seventh day of incubation.

Results and Discussion

The study concludes that 25-30 ^{0}C temperature supports greater mycelial growth of the fungus whereas 10 ^{0}C is not favourable for the growth of fungus (as in Table1). These results were in accordance with the results obtained by Misra and Singh (1963) [5] who observed that the optimum temperatures for spore germination, growth of the fungus in culture, and for infection and development of disease were 20-30 °C, 25-30 °C, and 30 °C respectively.

Table 1: Effect of different media and temperature on radial growth of *Exserohilum turcicum*

Sl. No.	Media	Temperature					
		10 ^{o} C	15 ^{o} C	20 ^{o}C	25 ^{o}C	30 ^{o}C	35 ^{o}C
		Colony diameter (mm)					
1	Potato Dextrose Agar	27.83	44.00	31.67	70.00	87.33	44.50
2	Richard's Synthetic Agar	20.50	53.83	42.67	57.50	77.17	73.33
3	Oat Meal Agar	46.17	63.67	54.83	85.33	86.67	64.67
4	Corn Meal Agar	23.00	53.17	31.83	75.00	60.00	54.00
5	2% Malt Extract Agar	27.17	40.33	28.17	35.00	41.00	38.00
6	Czapex Dox Agar	29.17	54.50	42.50	81.00	85.00	63.60
		Temperature (a)		Media (b)		Interaction (a×b)	
	SEM±	0.59		0.59		1.46	
	CD at 5%	1.68		1.68		4.13	
	CV	4.93					

Pandey and Shukla (1982) [6] reported that optimum temperature for colony growth of sorghum isolate of *E. turcicum* was 20- 30 ^{0}C, and no growth was observed at 40 ^{o}C. Nisikado (1927) [7] observed that rice decoction agar was more favourable for mycelial growth compared to Hopkin's nutrient solution. Champi (1939) [8] reported good growth of the fungus on various standard media. Best growth of the fungus was observed in Richard's and Czapek's media [9]. Isolates from different agro-ecological zones showed variation in growth rate, morphology, pigmentation, and sporulation rate in different media [10].

Light is an important environmental factor for most living organisms, including fungi, which use light as a signal in many metabolic pathways. It controls many metabolic activities, asexual conidiation, pigmentation, secondary metabolism and sexual development [11]. Special light regime is experienced by the fungi pathogenic on plants as the pathogen has to adapt to the optimum light conditions and environment required by the host for photosynthesis [12]. There have been reports of more than 100 fungal species, representing all phyla, to be reactive towards light [13].

The study concluded that there was no fixed pattern of increased colony diameter of the fungus under different light wavelengths and different media interactions (as in Table 2). There have been reports of limited effects of light quality on virulence or fungal pathogenicity [14].

Table 2: Effect of different media and light wavelength on radial growth of *Exserohilum turcicum*

Sl. No.	Media	Light Quality			
		Red	Yellow	Green	Blue
		Colony diameter (mm)			
1	Potato Dextrose Agar	44.01	51.83	44.5	45.83
2	Richard's Synthetic Agar	55.53	68.22	48.20	84.80
3	Oat Meal Agar	88.67	88.93	90.00	89.08
4	Corn Meal Agar	76.24	82.17	81.33	88.00
5	2% Malt Extract Agar	55.67	54.65	51.93	51.50
6	Czapex Dox Agar	88.67	89.67	88.67	88.03
		Light Quality (a)	Media (b)	Interaction (a×b)	
	SEM±	0.52	0.43	1.06	
	CD at 5%	1.51	1.23	3.01	
	CV	2.59			

Fungi generally utilise substrates in the form of solution only if condition of solution is conducive for fungal growth and metabolism. Hydrogen ion concentration (pH) either in food or habitat is known to affect the growth and development of micro-organisms. The results reveal that the colony diameter was maximum at pH 7 (87.17mm) and minimum mycelial growth was observed at pH 5 (63.67mm) (as in Table 3). The growth gradually decreases with decrease in pH from 9 to 5, which is in conformity with the results obtained by Kutawa *et al.,* (2017) [15] who observed that pH 7 was found to be the best for growing *E. turcicum* with mycelial growth rate of 4.72 mm/day, 6.36 mm/day and 6.96 mm/day at the 3rd, 5th and 7th day after incubation, respectively.

Table 3: Effect of pH on radial growth of *Exserohilum turcicum*

S. No.	pH	Colony diameter (mm)
1	5	63.67
2	6	72.33
3	7	87.17
4	8	80.00
5	9	82.67
	SEM±	2.16
	CD at 5%	6.81
	CV	4.85

Conclusion

Like other organism fungi too has its likes and dislikes regarding the source of nutrition and the environmental conditions which affect the growth of the fungus. All these physical factors not only affect the growth of the fungus but also affect sporulation, pigmentation and metabolism of fungi. In this study, the maximum radial growth of the fungus was observed in Oat meal agar at 25 ^{0}C and in Potato dextrose agar at 30 ^{0}C. No fixed pattern was observed in relation to light quality and pH 7 was most favourable for growth. Different fungi also prefer different media for growth. Light tends to play an important role in one or the other aspect of fungal metabolism. Other factor such as pH has been reported to play an important role not only in growth and metabolism of the fungus but also virulence of the pathogen. Temperature has its own role to play in germination of spore, growth and to infection in host. All these factors have an eminent role to play in study and management of pathogenic factors.

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