Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2018, 5(12):218-226**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Morphological Characterization of Sorghum *E. turcicum* Isolate from Different Areas of Tharaka Nithi County, Kenya

Fredrick O. Ogolla*[1], Benson O. Onyango[3], Moses M. Muraya[2], Sioma Mulambula[2]

[1]Department of Biological Sciences, Chuka University, P.O. Box 109-60400, Chuka, Kenya
[2]Department of Plant Sciences, Chuka University, P.O. Box 109-60400, Chuka, Kenya
[3]Department of Biological Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O. Box 210 – 40601, Bondo – Kenya

Abstract Low sorghum production in Kenya is attributed to devastation by TLB. Reaction similar to those of TLB have been observed in different farms in Tharaka Nithi County with varied lesion sizes. However, little scientific attention has been expended to characterize the pathogen and provide knowledge on TLB pathogen occurrence in the area. The study was conducted to characterize *E. turcicum* pathogen from different regions of Tharaka Nithi County in Kenya using cultural and morphological characteristics. Pathogen isolation from symptomatic leaves collected between April and July 2018 was done at Chuka University microbiology laboratory. Six out of nine samples collected from different regions of Tharaka Nithi county were typical *E. turcicum*. Data on conidia area was subjected to one-way analysis of variance using SAS software version 9.3 and LSD used to separate significantly different means at 5% probability level. T*here was statistically significant difference on conidia length among E. turcicum isolates (P<0.05).* The conidia length and width for isolates (K005, T002 and G001) were within those already documented range. However, isolates G002 and M004 recorded longer length of up to 200.83 and 184.63um respectively. *Mycelium appeared septate, branched and brownish while the Conidiophores was simple, cylindrical and septate*. Variations were observed on isolates pigmentation, growth pattern and conidia sizes. The results show that morphologically different TLB pathogen are responsible for causing the disease in Tharaka Nithi County. Molecular characterization is recommended to provide information on genetical variability of TLB pathogen in Tharaka Nithi county.

Keywords *E. turcicum*, Morphological, Characterization, Tharaka-Nithi, Kenya

Introduction

Turcicum leaf blight of sorghum is caused by the heterothallic ascomycete *Exserohilum turcicum* the pathogen formerly known as *Helminthosporium turcicum* [1-2]. It also causes northern corn leaf blight (NCLB) on maize and related wild grasses [3] Turcicum Leaf Blight is among the most destructive foliar diseases of maize and sorghum [4]. *Exherohilum turcicum* has been reported to produce non-specific toxic compounds in culture [5] [6] as well as cultivar-specific toxin [7]. The efficacy and production of these toxins greatly depend on temperature and pH [6,8]. Enzymatic and toxic activities of the fungus accounts for the pathogenicity [6]. The susceptible plant varieties such as sorghum experiences physiological challenges which include their capacity to prevent infection if inoculated with the pathogen [4]. Infection of plant's leaves by TLB leads to reduced green leafy area, increased leaf transpiration, limited translocation and uptake of essential plant nutrients to affected leaves and plant cells [4]. Yield reductions due to TLB can be significant depending on disease severity, timing, and plant susceptibility [9]. Although symtoms similar to *E. turcicum* have been observed in a prelimianry investigation in sorghum plantas in Tharaka Nithi County in Kenya, little scientific attaention has been given to isolate and characterize the pathogen.

Exserohilum turcicum belongs to the division Eumycota, subdivision Deuteromycotina, Class Deuteromycetes, order Moniliales and family Demetiaceae. It is a polycyclic, heterothallic and facultative parasite with three distinct mating types: MAT 1, MAT 2 and MAT 1, 2 [10]. The fungus overwinters as mycelia, conidia and chlamydospores in infected leaves, husks and other plant parts left on the soil surface providing innoculum for primary infection [11]. The disease is characterized by long spindle shaped necrotic lesions on leaves measuring up to 12.5 x 2.2 cm [12]. Pseudothecia are black, globose to ellipsoidal, 40–56 × 12–15 µm with clearly visible setae around the ostiole. Asci are cylindrical, clavate, short pedicellate and bitunicate and 1–8 ascospores [10].

Conidia of the fungus are olive grey and spindle shaped, curved, elongated and measuring 5 x 20 µm with one to nine septa [13]. Conidiophores are simple, cylindrical and olivaceous brown [14]. Germination of *E. turcicum* conidia is bipolar and occurs 3-6 hours after inoculation. Germ tubes are 20-150 µ long and in general, grow at an angle rather than parallel to the veins of the leaf [15]. It has a single conidium formed terminally on the conidiophore, which resumes growth to produce new conidium at the new tip [12]. Conidia has a hilum that protrudes distinctly from the conidia to bluntly rounded basal cells useful for identification [1]. Circular conidial scars are evident on the conidiophore after the abscission of the conidia [1, 14].

E. turcicum isolates show variation in; colony character, colony diameter, mycelial dry weight, spore germination, mean length and conidia width [10]. However, morphological characterisation of *E. turcicum* affecting sorghum in Tharaka Nithi County have not been done to deterimine existance of variation. Investigation on the variability of fungal pathogens is necessary to delineate phylogenetic relationships amongst the local species, and to provide useful taxonomic information for disease management.

Materials and Methods

Tharaka Nithi County borders the County of Embu to the South and South West, Meru to the North and North East, Kirinyiga and Nyeri to the West and Kitui to the East and South East. The county lies between latitude 000 07’ and 000 26’ South and between longitudes 37^0 19’ and 37^0 46’ East. The highest altitude of the county is 5,200m while the lowest is 600 m Eastwards in Tharaka. The average annual rainfall of 717 mm. The highlands (upper zone) comprise of Maara and Chuka which receive adequate rainfall for agriculture. The semi-arid (lower zone) covers Tharaka receiving less rainfall. The high-altitude areas have reliable rainfall. The lower regions receive low, unreliable and poorly distributed rainfall. Temperatures in the highland areas range between 14^oC to 30^oC while those of the lowland area range between 22^oC to 36^oC. Tharaka constituency experiences temperatures of up to 40^oC at certain periods. The county has a bi-modal rainfall pattern with the long rains falling during the months of April to June and the short rains in October to December [16].

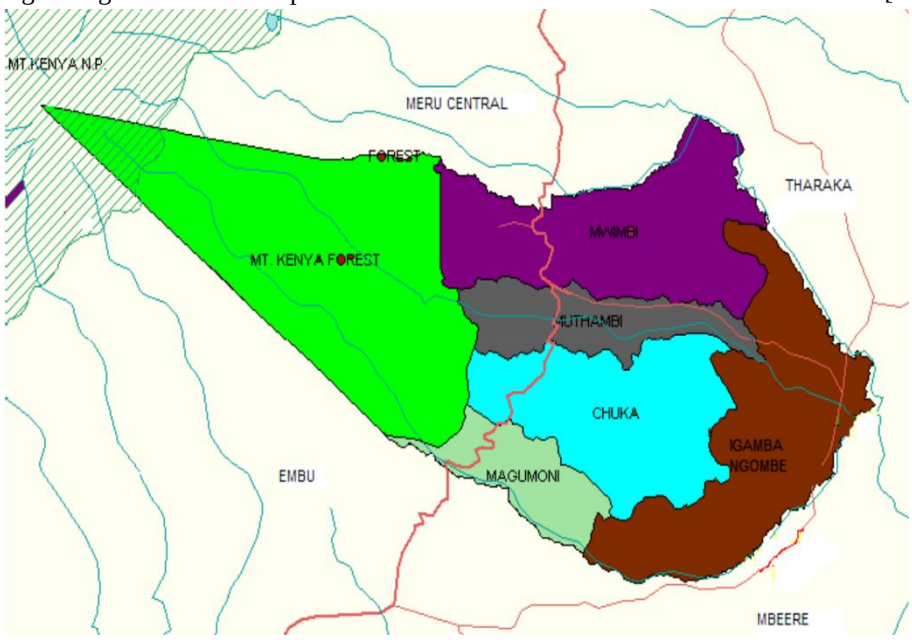


Figure 1: Map of Tharaka Nithi County [17]

Experimental Design

Cleaned glassware was sterilized at the temperature of 160^{o}C in hot air oven (Model Memmert UNB400) for 2 hours. Potato Dextrose Agar (PDA) medium was prepared using the following ingredients for culturing the fungi in the laboratory; Pealed and washed Potato: 200 g, Dextrose: 20 g, Agar agar: 20 g and 1000 ml distilled water. Peeled potato pieces were boiled in 500 ml distilled water in a 1000 ml beaker to make the pieces soft. The extract was filtered through a double layered muslin cloth. To another 500 ml of distilled water in another 1000ml beaker, 20g of agar agar was added and melted till it got dissolved. Both the solutions were mixed in another 1000 ml beaker into which 20 g of dextrose was added. The final volume of the medium was made up to 1000 ml by addition of sterile distilled water. The pH of the medium was adjusted to 6.8 with 1 N NaOH or 1 N HC1 as the case may be with the pH meter. The medium was transferred into the media preparation bottles and sterilized at 121^{o}C and pressure of 15 psi for 15 minutes in an autoclave (Model X280A). The media was then transferred to the water bath for media tempering at 50^{o}C for 30 minutes. Antibiotic (25mg/l made up of Asdoxin and Ampicillin of equal ratio) was incorporated in the media to inhibit bacteria contaminants. Samples collected from different villages were grouped into two categories (Regions); Upper zones (Maara and Chuka area including– Kanwa, Mikuu, Miranji, Gatuntu and Gituntu villages) with adequate rain; Lower zone (Tharaka area including– Kanyiritha, Tunyai, Kithinge, Kamwati, Nkairini villages) representing semi-arid area. From these groupings nine samples were randomly selected, four from upper zone and five from lower zone respectively for pathogen isolation and characterization. These samples were K004 (Kamwati), MJ001 (Miranji) M004 (Mikuu), G002 (Kanwa), G003 (Gatuntu) T002 (Tunyai), G001 (Kithinge), NK001 (Nkairini), KH001 (Kanyiritha).

Section of the leaf of about 5 mm^2 (infected and healthy) parts of the leaves was cut and surface sterilized with 0.5% sodium hypochloride chloride solution for 1 minutes then washed in a series of sterile distilled water to remove the disinfectant. The pieces were transferred to sterilized blotting paper in a Petri dish to remove the excess water. Finally, one piece was aseptically plated on potato dextrose agar (PDA) for growth. Inoculated plates were incubated in the incubator (Memmert TYP INB200) at room temperature and monitored for fourteen days. After the fungus has grown, cultural characteristics such as growth pattern, colour of conidia and pigmentation were observed. Other than cultural characteristics, conidia properties such as length, width, shape, type of germination, type of mycelium and size, presence of hilum as well as number of segments for each isolate were accessed using adhesive tape method under LCD Inversed Biological microscope (Model A33.1005) after staining with methylene cotton blue.

Results

The isolates varied in colony morphology traits characteristics such as fungal colour, fungal pigmentation, margin, mode of growth (profuse or sparse). Isolate G001 had regular margin, was grey in colour with brownish pigmentation and had profuse growth. Isolate K005 was light and grey with regular margin and had black pigmentation. Isolate M004 was light grey with circular margin and had golden brown pigmentation and showed profuse growth. Isolate G002 was dark grey in colour with irregular margin with greenish pigmentation and showed profuse growth. Isolate T002 was greyish in colour, had brownish to blackish pigmentation with irregular margin while the growth was sparse (Table 1; Plate 1). Three isolates MJ001, NK001 and KH001 did not show cultural and morphological characteristics of *E. turcicum*. *The morphological characteristics of the E. turcicum studied on potato dextrose agar medium in the laboratory are tabulated below*

Table 1: Cultural characteristic observation of *E. turcicum* Isolates from Different Location in Tharaka Nithi County

Fungal isolate	Culture Growth characteristics	Place of Collection
G001	Fungal colour: Greyish Margin: Regular margin, Pigmentation: Brownish Mode of Growth: Profuse,	Kithinge
K005	Fungal colour: Grey (light and dark), Margin: Circular Pigmentation: Black Mode of Growth: Profuse,	Kamwati

G003	Fungal colour: Greyish with whitish shades Margin: Irregular Mode of Growth: Profuse Pigmentation: Greenish	Gatuntu
M004	Fungal colour: Light Grey Margin: Circular Mode of Growth: Profuse, Pigmentation: Golden brown	Mikuu
G002	Fungal colour: Dark Grey Margin: Irregular Mode of Growth: Profuse, Pigmentation: Greenish	Kanwa
T002	Fungal colour: Greyish Margin: Irregular Mode of Growth: Sparse Pigmentation: Brownish- black	Tunyai
MJ001	Fungal colour: Greyish Margin: Irregular Mode of Growth: Sparse Pigmentation: Brownish- black	Miranji
NK001	Fungal colour: White Margin: Circular Mode of Growth: profuse Pigmentation: colourless	Nkairini
KH001	Fungal colour: Brownish Margin: Regular with concentric rings Mode of Growth: Profuse Pigmentation: Brownish- black	Kanyiritha

Plate 1: Cultural Characteristics of E. turcicum Isolates from Different Location in Tharaka Nithi County

Lengths, Width and Area of Conidia of E. turcicum Isolates

The length of *E. turcicum* isolates were statistically significance (p<0.05; Table S7a*)*. The length of the conidia observed ranged from 71.32 *um* in isolate K005 to 200.83 um in isolate G002 (Table 2). The width of *E. turcicum* isolates were not statistically significance (p>0.05*)*. The smallest width of the conidia observed was 15.43um in isolate K005 while the largest width was 30.42 in isolate G002 (Table 2). *The results morphological characteristics revealed that* there was statistically significant difference among conidia sizes of *E. turcicum* isolates from different area of Tharaka Nithi County using morphological criteria with (P < 0.05). The largest isolate was G002 with Means of conidia area of 6130 um while the smallest isolate was G001 with conidia area of 2055.78 um (Table 2).

Table 2: Variation in Conidia Length, Width and Area (in µm) of *E. turcicum* Isolates from Different location in Tharaka Nithi County

Isolate ID	Conidia Length (µm)	Conidia Width (µm)	Area of Conidia (µm)
G002	200.83 a	30.4167 a	6130.19 a
M004	184.63 a	27.0233 ab	4975.16 ab
K005	71.32 b	15.4300 b	3505.75 ab
T002	100.13 b	22.1500 ab	2196.01 b
G003	95.56 b	30.1100 a	2877.11 b
G001	100.01 b	27.8033 ab	2055.78 b
Mean	129.1463	24.91125	3023.6
LSD	64.223	13.911	3716.611
CV (%)	23.67241	26.58261	38.727

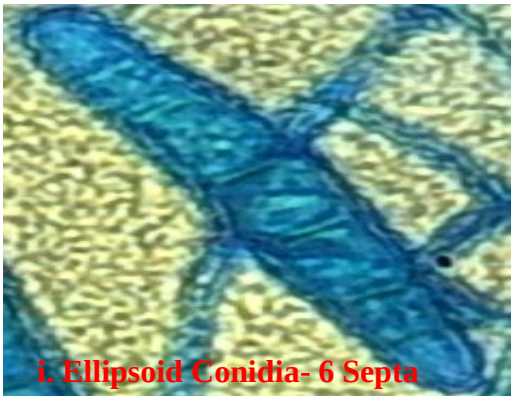
[a]Means followed by the same letters are not significantly different at 5% probability level.

General Microscopic Observation of *E. turcicum* Isolates

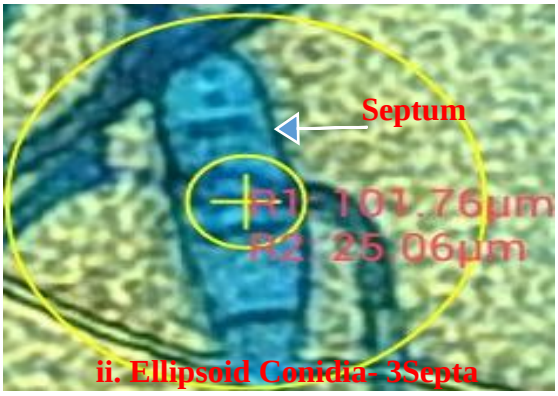
Generally, microscopic study revealed mycelium which appeared septate, branched and greyish while the conidiophores was simple, cylindrical and septate (plate1-VI). Conidiophores suspended conidia which appeared singly at the tips. The conidia observed in this study were straight to slightly curved and spindle shaped (plate1- VII), the septation in the conidia ranged from 3-7 septate and conidia had protruding hilum at one end (Table 3; Plate I-II). Bipolarly conidial germination was observed in isolate K005 (Plate 1- IV) while the rest of the conidia from other isolates showed unipolar germination (plate1- III).

Table 3: Microcopy Characteristics of *E. turcicum* Isolate from Different Location in Tharaka Nithi County

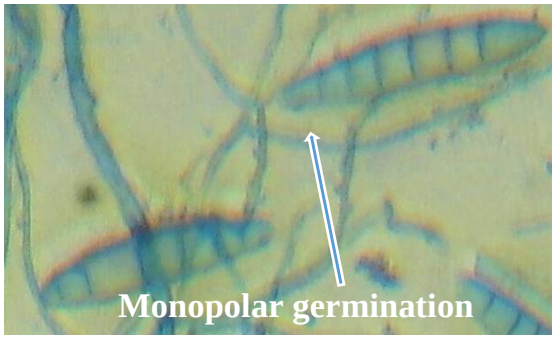
Isolate	Conidia length/Width	Other Microscopic Features
G001	Length; 100.04 um Width: 27.80 um	Conidia: Straight to slightly curved, protruding hilum, 3-7septa Mycelium: Branched, Septate, grey in colour Conidiophore: Septate, greyish Conidia Germination: Unipolar
G002	Length: 200.50 um Width: 30.28 um	Conidia: Straight or slightly curved, protruding hilum, 7 septa Mycelium: Branched, Septate, brownish to grey Conidiophore: Septate, Dark grey Conidia Germination: Unipolar
K005	Length: 71.32 um Width: 15.43 um	Conidia: Straight or slightly curved, protruding hilum, 3-5septa Mycelium: Branched, Septate, brownish to grey Conidiophore: Septate, greyish Conidia Germination: Bipolarly
T002	Length: 101.13 um Width: 22.15 um	Conidia: Straight or slightly curved, protruding hilum, 3-7septa Mycelium: Branched, Septate, brownish to grey Conidiophore: Septate, greyish Conidia Germination; Unipolar
M004	Length: 184.63 um Width: 27.47 um	Conidia: Straight or slightly curved, protruding hilum7-8septa Mycelium: Branched, Septate, brownish to grey Conidiophore: Septate, Dark grey Conidia Germination: Unipolar
G003	Length: 95.56 um Width: 30.11 um	Conidia: Straight or slightly curved, protruding hilum, 7 septa Mycelium: Branched, Septate, brownish Conidiophore: Septate, Dark grey Conidia Germination: not observed



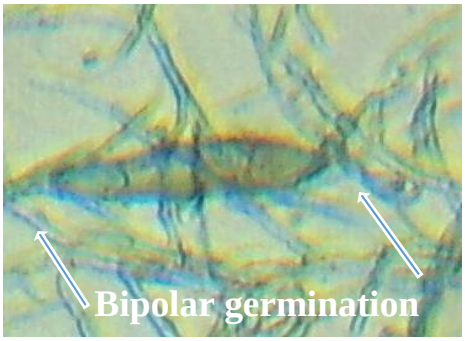
I. Conidia from isolate G002



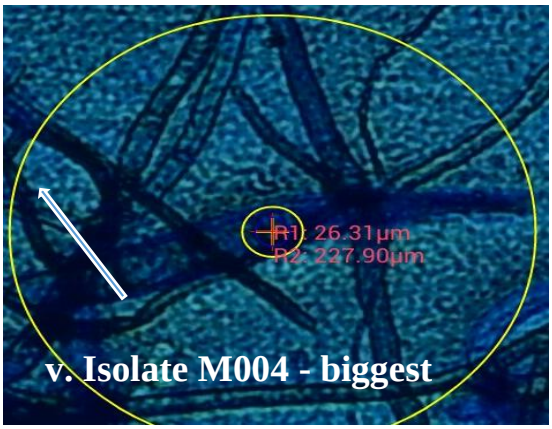
II. Conidia from isolate G001



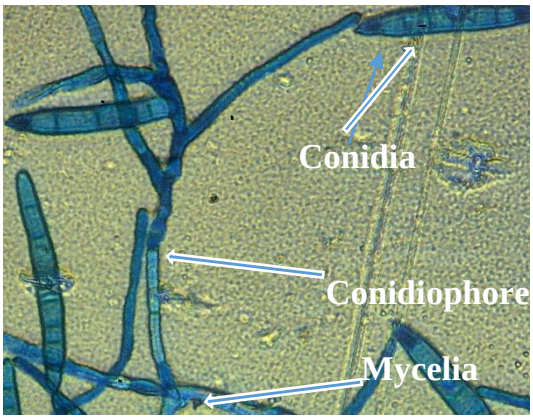
III. Monopolar Conidia germination in isolate T002



IV. Bipolar Conidia germination isolate K005



V. The biggest conidia observed



VI. Conidiophore and mycelia isolate G002

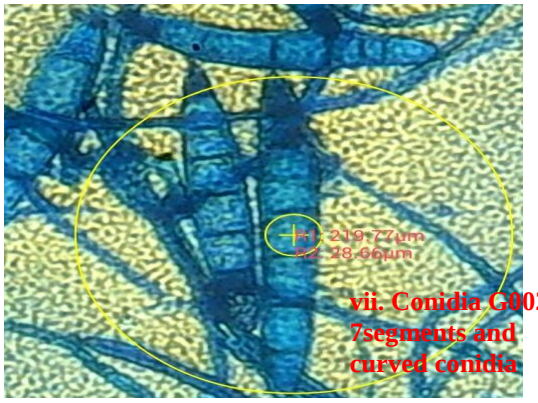


Plate 2: E. turcicum Characteristic Conidium Exhibiting Varied Characteristics (I-IV)

Discussion

Results on the morphology of *E. turcicum* showed significant distinctions between the six isolates cultured. The difference was observed in colony margin, pigmentation and growth pattern (Table 1). The results are in line with those of Rajashwal, [18] and Abdulaziz, *et al*., [19] that there exists variation in *E. turcicum* colony colour, pigmentation and growth pattern. A study by Rajula *et al*., [20] on sorghum *E. turcicum* in Western region of Kenya also reported similar cultural and morphological variation. The conidial shapes observed in this study were curved, spindle or elongated with characteristic protruding hilum on one end which agreed with findings by Sivanesan [21]**;** Abebe and Singburaudom, [22]; Harlapur *et al.* [23**]**; Rajeshwar *et al***.,** [4] and Rani, [14]**.** Bipolar and monopolar conidial germination was observed in this study on isolates K005 and T002 respectively (Plate I-III, IV and VI). Similar observation on conidia germination had earlier been made by Rajeshwar *et al***.,** [4]. Isolate G002 had conidial length of 200.50 um and width of 30.50um while isolate M004 had length of 184.64um and width of 27.47um (Table 3). These conidial lengths were longer than those described on *E. turcicum* by Ellis [24], Shurtleff, [25], Rani, [14], and Wani [12**]**. Variation on morphology of other plant fungal pathogens have also been reported. Bunker *et al.*, [26**]** observed variability in five isolates of *Bipolaris maydis* from three different sites. The variations were on conidia mean, length and pathogen morphological and cultural variations. Variation in pathogen morphology are brought about by changes in environment conditions which may lead to mutations [22] Thus, frequent monitoring is necessary for timely detection and management of fungal pathogen of economically important crops. Frequent monitoring will help to contain emergence of new variants (mutants) that are likely to evade the existing control methods.

Conclusion

The results obtained from the current study proved that the *E. turcicum* pathogen is the causative agent of turcicum leaf blight of sorghum in Tharaka Nithi County. The *E. turcicum* isolates studied were culturally and morphologically different. The variations were observed in cultural charateristics such as colour, pigmentation, growth and pattern. Morphologically, conidia lengths were significantly different while their width were not significantly different. The variations observed were attributed to the prevailing varied weather conditions in Tharaka Nithi County. The current study recommendation is that molecular characterization should be done for race determination of *E. turcicum* isolates from Tharaka Nithi County since they registered variation both on cultural and morphological aspects.

Acknowledgement

The research was partially funded by Chuka University internal research grant 2017/2018 cycle. I acknowledge the assistance of Mr. Sam Chabari who played key role in sample collection, Ms. Truphena Koech for her significant contribution in laboratory work. Lastly, I acknowledge all the staff at the department of Biological Sciences, Chuka University for their consistent support.

References

[1]. Leonard, K. J. and Suggs, E. G. “Mycological Society of America Setosphaeria prolata, the Ascigerous State of Exserohilum prolatum Setosphaeria prolata. The Ascigerous State of Exserohilum Prolatum,” Mycologia, vol. 66, no. 2, p. 281–297, 1974.

[2]. Khedekar, S., Harlapur, S., Shripad, K. and Benagi, V. “Evaluation of fungicides, botanicals and bioagents against turci Petitprez cum leaf blight of maize caused by Exserohilum turcicum (Pass.) Leonard and Suggs.,” International J. Pl. Protect, vol. 5, no. 1, pp. 58-62, 2010.

[3]. Martin, T. “Setosphaeria turcica , fungal mating and plant defence,” Doctoral Thesis. Swedish University of Agricultural Sciences, 2011.

[4]. Rajeshwar, R., Narayan, P. and Narayan, Narayan, R. “Turcicum leaf blight of maize incited by Exserohilum turcicum: A review,” Int. J. Appl. Biol. and Pharmaceutical Technology, vol. 5, no. 1, pp. 54-59, 2014.

[5]. Petitprez, M., Gelie, B., Albertini, L. and Barrault, G. "Biological activities of phytotoxins of Exserohilum turcicu.m, a Cytological study," Revue de Cytologie et de Biologie Vegetales Ie Botaniste, vol. 7, pp. 261-270, 1984.
[6]. Bashan, B., Levy, R., Cojocaru, M. and Levy, Y. "Purification and structural determination of a phytotoxic substance from Exserohilum turcicum," Physiological and Molecular Plant Pathology, vol. 47, no. 4, pp. 225-235, 1995.
[7]. Bashan, B. and Levy, Y. "Differential response of sweet corn cultivars to phytotoxic water soluble compounds from culture filtrates of Exserohilum turcicum," Plant Disease, vol. 76, pp. 451- 454, 1992.
[8]. Yoka, P. and Albertini, L. "Enzymatic and toxic activities of Helminthosporium turcicum Pass., parasite of maize," Bulletin de la Societe d'Histoire Naturelle de Toulouse, vol. 111, pp. 255-272, 1975.
[9]. Weems, J. D. "Evaluation of race population distribution, fungicide sensitivity, and fungicide control of Exserohilum turcicum, the causal agent of northern leaf blight of corn," Doctoral dissertation, University of Illinois at Urbana-Champaign, 2016.
[10]. Bunkoed, W., Kasam, S. and Chaijuckam, P. "Sexual reproduction of Setosphaeria turcica in natural corn fields in Thailand. Kasetsart," J. (Natl Sci.), vol. 48, p. 175–182, 2014.
[11]. Jakhar, D. S., Rajesh, S., Saket, K. Pargat, S. and Vivek, O. "Turcicum Leaf Blight: A Ubiquitous Foliar Disease of Maize (Zea mays L.)," International Journal of Current Microbiology and Applied Sciences, vol. 6, no. 3, pp. 825-831, 2017.
[12]. Wani, T. A. "Status and Management of Turcicum Leaf Blight of Maize [Exserohilum turcicum (Pass.) Leonard and Suggs.] in Kashmir Valley (PhD Thesis)," Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, 2015.
[13]. Rajeshwar, T., Reddy, P., Narayan, R. and Reddy, S. "Management of Turcicum leaf blight of maize caused by Exserohilum turcicum in maize," International Journal of Scientific and Research Publications, vol. 3, pp. 1-3, 2013.
[14]. Rani, S. "Epidemiology and management of Turcicum leaf blight (Exserohilum turcicum (Pass.) Leonard and Suggs) of maize in Jammu Sub-tropics (PhD Thesis)," Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, 2015.
[15]. Muiru, W., Mutitu, E. W. and Kimenju, J. W. "Distribution of turcicum leaf blight of maize in Kenya and cultural variability of its causal agent, Exserohilum turcicum," Journal of Tropical Microbiology and Biotechnology, vol. 4, no. 1, pp. 32-39, 2008.
[16]. Tharaka Nithi, GoK. "County Government of Tharaka Nithi Integrated Development Plan," 2013. [Online]. Available: https://cog.go.ke/images/stories/CIDPs/TharakaNithi.pdf. [Accessed 15 08 2018].
[17]. Ombaka, O., Gichumbi J. and Kinyua, C. "Status of Water Quality of Naka River in Meru South, Kenya," International Journal of Modern Chemistry, vol. 3, no. 1, pp. 23-38, 2012.
[18]. Rajeshwar, T. R. "Studies on Variability and Management of Turcicum Leaf Blight (Exserohilum Turcicum (Pass.) Leonard and Suggs) on Maize (Thesis)," Acharya N.G. Ranga Agricultural University, 2012.
[19]. Abdulaziz, B. K., Kamaruzaman, S., Khairulmazmi, A., Zulkifli, A. S., Norzihan, A. and Mohd, S. F. "Characterisation and pathological variability of Exserohilum turcicum responsible for causing northern corn leaf blight (NCLB) disease in Malaysia," Malaysian Journal of Microbiology, vol. 13, no. 1, pp. 41-49, 2017.
[20]. Rajula, J. O., Ochuodho, J. and Kiprop, E. "Morphological and Cultural and Variation of Exserohilum turcicum isolates in Sorghum (Sorghum bicolour L.)," African Journal of Education, Science and Technology, vol. 4, no. 2, p. 207, 2017.
[21]. Sivanesan, A. "Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs," Mycological Papers, vol. 15, pp. 1-261, 1987.
[22]. Abebe, D. and Singburaudom, N. "Morphological, Cultural and Pathogenicity Variation of Exserohilum turcicum (Pass) Leonard and Suggs Isolates in Maize (Zea Mays L.)," Kasetsart J. (Nat. Sci.), vol. 40, pp. 341 - 352, 2006.

[23]. Harlapur, S., Kulkarni, M., Yeshoda, H. and Srikant, K. "Variability in Exserohilum turcicum (Pass.) Leonard and Suggs., Causal Agent of Turcicum Leaf Blight of Maize," Karnataka Journal of Agricultural Sciences, vol. 20, no. 3, pp. 665-666, 2007.
[24]. Ellis, M. "Dematiaceous hyphomycetes. Kew, Surrey," CAB, p. 608p, 1971.
[25]. Shurtleff, M. "Compendium of corn diseases," American Phytopathological Society:St. Paul, p. 105p, 1992.
[26]. Bunker, R., Rathore, R. and Kumawat, D. "Pathogenic and morphological variability of Bipolaris maydis incitant of maydis leaf blight in maize. Journal of Mycology and Plant pathology," Indian Journal of Agricultural Sciences, vol. 41, no. 3, pp. 418-421, 2011.