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Research Article

Variation in Temperature and Nutrient Source Influence the Growth of Exserohilum Turcicum Mycelia Isolated from Sorghum

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Abstract *Turcicum* leaf blight (TLB) caused by the fungus *Exserohilum turcicum* is a serious threat to production of maize and sorghum, since it damages photosynthetic leaves. Growth and development of *E. turcicum* pathogen is influenced by factors such as light, temperature, dew period, plant age and inoculums concentration. Tharaka Nithi county in Eastern Kenya where sorghum is actively grown has heterogenous climatic and edaphic conditions. It is currently unclear if variations in temperature and media type may influence growth, development and virulence of *Exserohilum turcicum*. Thus, this study was carried out to investigate the effect of media type and different temperature variations on the growth and development of mycelia of *E. turcicum* isolates from Tharaka Nithi county in Kenya. Results showed that the effect of temperature was significantly differences for development of *E. turcicum* ($Pr < 0.05$) mycelia. Media type had significant effect on growth of *E. turcicum* isolates ($p<0.05$). Corn meal agar with mean of 4.233 cm was the best growth media followed by Malt extract agar at 3.3200 cm, while the most preferential ($p<0.05$) temperature for mycelia growth was 30 ^{o}C. The study recommends in cooperation of wider environmental factors in future studies involving TLB pathogen from Tharaka Nithi county.

Keywords Temperature, Nutrients, *E. turcicum*, Sorghum, Tharaka-Nithi, Kenya

1. Introduction

Turcicum leaf blight (TLB) caused by the fungus *Exserohilum turcicum* is a serious threat to production of maize (*Zea mays*) and sorghum (*Sorghum bicolor*), since it damages photosynthetic leaves. Growth of TLB pathogen is influenced by both biotic and abiotic factors; light, temperature, dew period, plant age, inoculums concentration and mutations [1, 2, 3]. Optimal temperature of between 6-18 hours are conducive for the establishment of TLB infection [4]. Conidia germination on leaves is high when the temperature ranges from 18 to 27 °C. Optimal temperature for *E. turcicum* growth in laboratory vary [5, 6, 4]. Isolates of *E. turcicum* from Zimbabwe and those from Uganda varied at temperatures of 20-15 °C and 25- 30 °C respectively in Potato Dextrose Agar (PDA) [6]. Pandey and Shukla [5] reported optimum temperature ranges for growth of sorghum *E. turcicum* isolates to be at 20 – 30 °C, with no growth at 40 °C. Gregory [7] reported that temperature range of 18-27 ^{o}C favours sporulation of TLB fungus. In Kenya, temperature of between 11 to 27 °C is ideal for infection and dispersal of *E. turcicum* in maize [8]. Setyawan *et al* [4] reported temperature range of 20-26 ^{o}C to be optimum for conidial formation. Tharaka Nithi County has heterogeneous environmental conditions that may bring about fungal pathogen variability. The lowest temperature reported is 14 ^{o}C with the highest temperature of 40 ^{o}C [9]. Nonetheless, the information on the impact of temperature variation on the growth of *E. turcicum* isolates from Sorghum plants Tharaka Nithi County is scarce.

Media type is essential for studying fungal morphological and cultural characteristics such as pigmentation, growth pattern, conidia sizes and shapes [10]. Both clinical and environmental fungal isolates varies depending on media type [11]. Colony mycelia growth and sporulation of soil fungi were optimal on Sabouraud's dextrose medium followed by Potato dextrose medium and Richard medium [11]. Sporulation of plant fungal isolates varried on the following media; Rose Bengal medium, Kaefer's Medium, Enriched Soil Medium, Malt extract Medium and Czapek Dox medium [12]. Isolates of *E. turcicum* from different agro-ecological zones also varies in morphology, pigmentation, growth rate and sporulation in different media [13]. Rani [2] observed this variation in isolates of *E. turcicum* from maize. The study reported that the pathogen growth characteristics differed on Potato Carrot Agar (PCA), Malt Agar Medium (MAE), Corn Meal Agar (CMA), Czapeck's Malt Agar (CzMA) and in PDA [2]. Report by Rani *et al.,* [2] showed that growth of three out of 12 isolates were better in PDA while the rest of isolates were irregular on PDA. Knowledge of influence of temperature and media type on development of *E. turcicum* isolates of sorghum in Tharaka Nithi County is limited. Studies on temperature assay is important in disease management since different disease pathogens thrives well at different optimal temperatures. Tharaka Nithi county have heterogenous temperatures and edaphic factors, information on temperature assay will be useful in guiding fungicide application time based on prevailing temperatures.

2. Materials and Methods

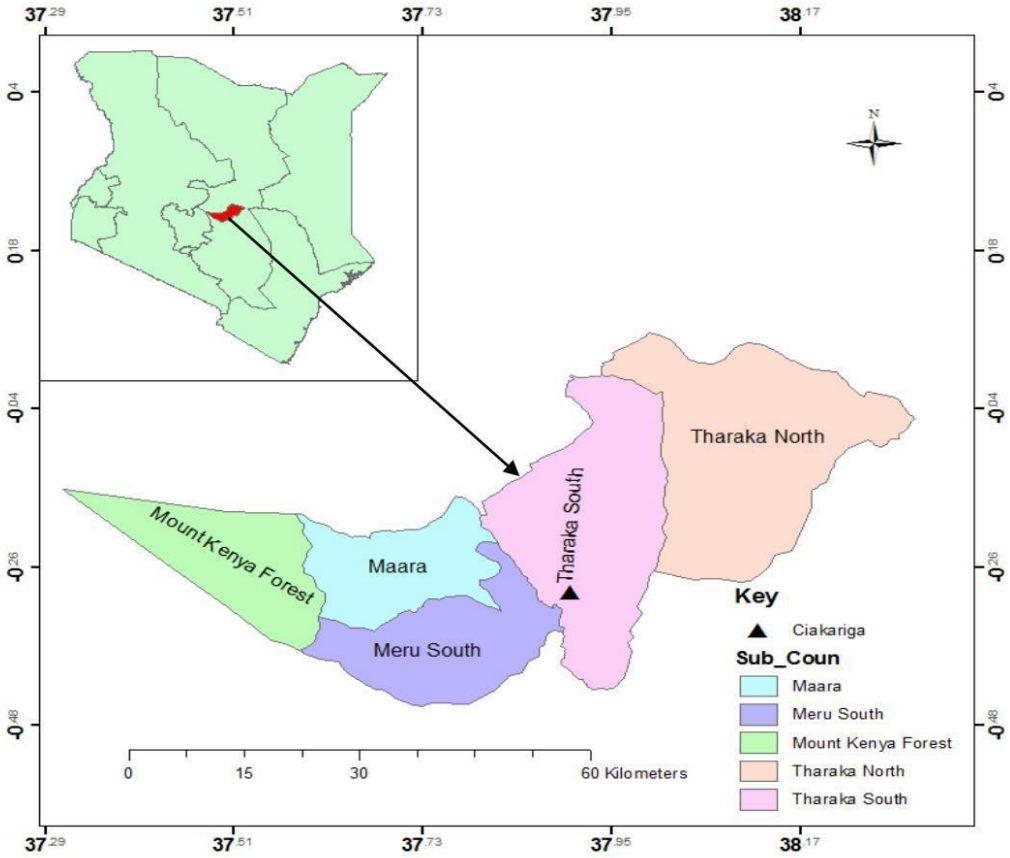

Figure 1: Map of Tharaka Nithi County

Tharaka Nithi 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 5,200 m 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 while the lower region receive low, unreliable and poorly distributed rainfall. Temperatures in the highland areas range between 14 ^{O}C to 30 ^{O}C while those of the lowland area range between 22 ^{O}C to 36 ^{O}C (CGoK, 2018). Ferrasols soils which are highly weathered and leached is predominant in

Tharaka Nithi County [14]. The soil is infertile and deficient in nitrogen (N) phosphorus (P) and zinc (Zn). Major crops in the area are; *Phaseolus vulgaris*, *Zea mays*, *Vigna unguiculate*, *Manihot esculenta*, *Cajanus cajan*, *Sorghum spp*., *Eleusine coracana* among others [9].

3. Experimental Design

A factorial experimental design laid out in Complete Randomized Design (CRD) with temperatures (20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C) being main plot factor and media type (six media types: SDA, PDA, CzMA, CMA, PCA, MEA) was used as the subplot factor. The treatments were replicated three times.

Preparation of Different Media

Potato Carrot Agar (PCA) was prepared from Carrot infusion (50 g) peeled, Potato (200 g) Agar agar (20 g) and distilled water (1000 ml). The medium was prepared by boiling 200 g of clean sliced potato in 500 ml of distilled water, filtered and 20 g agar added to the filtrate. The final volume of the medium was made up to one litre by adding distilled water. The pH of the medium was adjusted to 6.8 before sterilization using 1 Molar HCL and 1 Molar NaOH. The medium was sterilized in an autoclave at 15 psi at 121 ºC for 20 minutes. Medium was left to cool before addition of 25mg/l antibiotic made up of Asdoxin and Ampicillin of equal ratio [15].

Corn meal agar was prepared from the corn meal infusion (30g), oxoid agar gar (20g) and 1000 ml distilled water. The medium was prepared by boiling 30 g of corn meal in 500 ml of distilled water, filtered and 20 g agar was added to the cornmeal filtrate before heating to dissolve the agar. The final volume of the medium was made up to one litre by adding distilled water. The pH of the medium was adjusted to 6.8 before sterilization using 1 Molar HCl and1 Molar NaOH added in drops until the right pH was attained. The medium was sterilized in an autoclave at 15 psi at 121 ºC for 20 minutes. The medium was left to cool before addition of 25mg/l antibiotic made up of Asdoxin and Ampicillin of equal ratio.

Malt Extract Agar was prepared using; Malt extract (30 g), Mycological peptone (5.0 g), Agar (15.0 g). The medium was prepared by soaking 30 g of malt extract, mycological peptone (5 g) and 15g of agar in 700 ml of distilled water, then filtered through muslin cloth. The final volume of the medium was made upto one litre by adding distilled water. The pH of the medium was adjusted to 5.6 before sterilization using 1 Molar HCl and 1 Molar NaOH. The medium was sterilized in an autoclave at 15 psi at 121 ºC for 20 minutes. The medium was left to cool before addition of 25mg/l antibiotic made up of Asdoxin and Ampicillin of equal ratio.

Czapeck's Malt Agar was prepared from; Malt extract (40.00 g), Sucrose (30.0g) Sodium nitrate (2.00 g), Potassium chloride (0.50 g), Magnesium sulphate (0.50 g), Ferrous sulphate (0.01 g), Dipotassium phosphate (1.00 g) and agar (20.00g). The ingredients totalling to 94.01 g were suspended in 700 ml distilled water, heated to boiling to dissolve the medium completely. The final volume of the medium was made up to one litre by adding distilled water. The pH of the medium was adjusted to 6.8 before sterilization using 1 Molar HCl and 1 Molar NaOH. The medium was sterilized in an autoclave at 15 psi at 121 ºC for 20 minutes. The medium was left to cool before addition of 25mg/l antibiotic made up of Asdoxin and Ampicillin of equal ratio.

Inoculation and Incubation

Six different *E. turcicum* fungal isolates were obtained from TLB symptomatic infected sorghum leaves by cutting 3 mm thin sections which were surface cleaned using tween 20 for 30 seconds. Sections were then rinsed in sterile water before surface sterilization in 70% alcohol for 30 second. Surface sterilized sections were rinsed in three changes of distilled water and placed on the surface of solidified PDA plates. Fungal isolates (*E. turcicum)* were then allowed to grow at room temperature for two weeks. Upon growth pure isolates were obtained by fungal hyphal transfer method into fresh PDA media for growth. Using cork-borer, 2.5 mm diameter pure isolates of *E. turcicum* was obtained and used to inoculate six different media to study their effect on mycelial growth of different *E. turcicum* isolates under five different temperatures. Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Corn Meal Agar (CMA), Malt Extract Agar (MEA), Czapeck's Malt Agar (CzMA) and Sabouroud agar (SDA) media were used. The inoculated media were incubated in an incubator model Memmert TYP INB200 at temperatures 20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C and 40 ^{o}C for two weeks at Chuka

University Microbiology laboratory. The criterion for temperature selection was based on temperature ranges in Tharaka Nithi County. The mycelia growth of the fungus isolate on different solid media under different temperatures were measured (in cm) after two weeks of incubation. Five isolates were further evaluated using corn meal agar which was the best media. The five fungi isolates were selected based on their variation in conidial sizes and difference in colony pigmentation.

4. Results

Mycelia Growth of E. turcicum at Different Temperatures and Different Media Types

The effect of different temperatures and different media types were statistically significant ($p<0.05$). The means of temperatures ranged from 3.3573 cm at 30 ^{o}C, to 0.0101 cm at 40 ^{o}C being the lowest mean **(Table 1)**. Corn Meal agar (CMA) with mean of 3.1311cm mycelia growth was the best media for growing of *E. turcicum* isolates. Potato dextrose agar with mean of 1.7744 mm mycelia growth was the least in promoting the growth of TLB pathogen (Table 2). Under 5 different temperatures and six media type, isolate M004 gave the highest mycelia growth of 2.8300 while isolate T002 gave the lowest growth of 1.9578 which was below the overall mean of 2.3933 (Table 3). The best isolates, best media and the best temperature were selected for further evaluation.

Table 1: Effect of varying Temperature on Growth of *E. turcicum* Isolates

Temperature	Growth (cm)
30 ^{o}C	3.3537 [a]
25 ^{o}C	3.1093 [b]
35 ^{o}C	2.7213 [c]
20 ^{o}C	2.4574 [d]
40 ^{o}C	0.0101 [e]
Mean	2.3304
LSD	0.1721
CV (%)	27.6258

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

Table 2: Effect of Different Media Type on Growth of Different *E. turcicum* Isolates

Media Type	Means Difference (cm)
CMA	3.1278 [a]
MEA	2.8078 [b]
PCA	2.4067 [c]
CzMA	1.9633 [d]
SDA	1.9022 [d e]
PDA	1.7744 [e]
Mean	2.3304
LSD	0.1886
CV (%)	27.6258

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

Table 3: Variations in mycelia Growth of Different *E. turcicum* Isolates on Different Media Types

Level of Isolate	Measurement Mean
M004	2.8300 [a]
G002	2.5104 [b]
G003	2.3278 [b c]
K005	2.3111 [c]
G001	2.0119 [d]
T002	1.9578 [d]
Mean	2.3933
LSD	0.1887
CV (%)	26.1446

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

Effect of Temperatures on mycelia Growth *E. turcicum* Isolate M004

The effect of five incubation temperatures on the growth of *E. turcicum* was investigated and there was a significant difference ($p<0.05$) in the mycelia growth of *E. turcicum* mycelia at different temperatures. Means of all the temperatures ranged from 5.0000 cm at 30 ^{o}C being the highest mean to 0.03333 cm at 40 ^{o}C which was the lowest mean (Table 4).

Table 4: Effect of Different Temperature on Growth of *E. turcicum* Isolate M004 from Tharaka Nithi County

Temperature	Growth (cm)
30 ^{o}C	5.0000 [a]
25 ^{o}C	3.9000 [b]
20 ^{o}C	2.6000 [c]
35 ^{o}C	2.5000 [d]
40 ^{o}C	0.03333 [e]
Mean	2.806667
LSD	0.5076
CV (%)	9.604545

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

Effect of Media Types on Growth of *turcicum* Isolate M004

Growth of *E. turcicum* on different media at the temperature of 30 ^{o}C was significantly different ($p<0.05$). Corn meal agar with mean of 4.133 cm was the most preferred while SDA (2.4933 cm) was the least preferred (Table 5).

Table 5: Effect of Media Type on Growth of *E. turcicum* M004 Isolates from Tharaka Nithi Count at 30 ^{o}C

Media Type	Means Difference (cm)
CMA	5.7000 [a]
MEA	5.2667 [a]
PCA	4.1667 [b]
CzMA	3.3000 [c]
PDA	3.3667 [c]
SDA	2.9333 [c]
Mean	4.122222
LSD CV (%)	0.6624 8.832173

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

Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Corn Meal Agar (CMA), Malt Extract Agar (MEA), Czapeck's Malt Agar (CzMA) and Sabouroud agar (SDA)

Effect of Corn meal agar on Growth of *E. turcicum* isolates

Corn meal agar selected as the best media after evaluation of effect of all the media was selected for the growth of all of the *E. turcicum* isolates from different regions of Tharaka Nithi county. Effect of CMA was significantly different ($p<0.05$). Isolate M004 had the biggest mycelia growth with mean of mean of 3.2267 cm while isolate T002 had the smallest mycelia growth of mean 2.4733 cm (Table 6). Only isolate M004 had growth of mycelia above the overall mean.

Table 6: Effect of the best media (Corn meal agar) type on growth of different *E. turcicum* isolates from Tharaka Nithi county

Level of Isolate	Measurement Mean
M004	3.2267 [a]
G002	2.6267[b]
K005	2.5933 [b]
G001	2.5733 [b]
T002	2.4733 [b]
Mean	2.6987
LSD	0.239
CV (%)	12.0618

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

5. Discussion

The study revealed that difference in temperature and media types affects growth of different *E. turcicum* isolates from Tharaka Nithi County. Temperature of 30 ^{O}C was most preferred while Corn Meal Agar was the most suitable nutrient media for optimal mycelia growth (Table 1 and 5). The current findings were within the range of reports by Bergquist and Masias [16] that temperature of 28 ^{O}C was ideal for spore germination. A study by Misra and Singh [17] reported that the temperatures of 30 ^{O}C and between 20-30 ^{O}C respectively are suitable for spore development, germination in culture. Rajashwar [15] reported temperature of 30 ^{O}C as being suitable for growth of *E. turcicum*. Poor mycelia growth at extreme temperatures has been attributed to injurious and lethal effect on spores and radial growth of the pathogen [18]. Thus, plant pathogens require optimal temperature ranges to grow, multiply and carry out their physiological activities normally.

Effect of nutrient (media type) on growth and development of mycelia of different *E. turcicum* from Tharaka Nithi in Kenya was significantly different ($p<0.05$). Corn Meal agar (CMA) with mean of 3.1311cm mycelia growth was the best media for growing of *E. turcicum* isolates (Table 5). The effect of different media type on growth of *E. turcicum* corresponds to the finding by Rani [2]. Pathogen growth characteristics differed on Potato Carrot Agar (PCA), Malt Agar Medium (MAE), Corn Meal Agar (CMA), Czapeck's Malt Agar (CzMA) and in PDA [2]. Growth of three out of 12 isolates were better in PDA while the rest of isolates were irregular on PDA. De Rossi *et al.* [19] working with potato dextrose agar (PDA), Reis' medium, Lactose casein hydrolysate agar (LCHA) and semi-selective DRR observed that the semi-selective DRR was most selective. Better growth of the isolates in these media may be due to absence of chemicals inhibitors that retard fungal growth.

6. Conclusion and Recommendations

Corn meal agar was found to be the best media for culturing *E. turcicum*. However, the best temperature was found to be 30 ^{O}C. The study recommends in cooperation of wider environmental factors in future studies involving TLB pathogen from Tharaka Nithi county.

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