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# Assessment of Maize (*Zea mays* L.) *Exserohium Turcicum* (Pass.) Leonard and Sugg. Isolates on Different Culture Media in Tanzania

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**Abstract** Morphological characteristics of twenty five isolates of *E. turcicum* collected from Kilimanjaro, Arusha, Morogoro, Iringa, Njombe and Mbeya Regions in Tanzania were studied in four solid media namely; V8 vegetable juice agar, malt extract agar, maize leaf extract agar and potato dextrose agar. The experiment was conducted twice and replicated three times (3 replicate × 25 isolates × 4 media) for each medium, making a total of 300 plates. The inoculated cultures were arranged in a Complete Randomized Design (CRD) and incubated at 25±1°C. The statistically significant differences ($P \leq 0.05$) in colony growth, conidia germination, dry mycelial weight and rate of sporulation on the four solid media indicated the possibility of different strains of *E. turcicum* in the studied areas. However, colony growth was aggressive in V8 juice agar (5.7 cm) but conidia germination and rate of sporulation were high in malt extract agar. No isolate of *E. turcicum* germinated or sporulated on PDA. Isolates such as $KHK_{10}$, $KHK_{18}$, $KHN_{17}$, $KHN_3$, $KMM_{18}$ (Kilimanjaro Region), $MMU_{13}$ and $MRI_4$ (Mbeya Region), $INM_8$ (Iringa Region) and $MMM_{18}$ from Morogoro Region significantly yielded more colony growth, conidial germination, sporulation and dry mycelia compared to the other isolates. Molecular studies are needed to confirm the genetic variations amongst the isolates for sustainable maize breeding in Morogoro, Tanzania.

***Keywords:*** *Northern leaf blight, solid medium, Isolates, maize, Tanzania*

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## 1. Introduction

*Exserohilum turcicum* Pass. (Syn. *Helminthosporium turcicum* (Pass) Leonard and Suggs, *Bipolaris turcica* (Pass) Shoemaker, i (Pass.) Subram and Jain.) teliomorph (sexual stage) *Setosphaeria turcica* (Luttrell) Leonard and Suggs. (Syn. *Trichometasphaeria turcica* Luttrell.), is the fungal pathogen that causes northern leaf blight of maize globally [1,2,3,4]. *Exserohilum turcicum* is an ascomycete pathogen of cereals and is a heterothallic facultative parasite [5,6].

The anamorph phase of the fungus belongs to the division Eumycota, sub-division Deuteromycotina, order Moniliales and family Dematiaceae [7,8] while the teliomorph *Setosphaeria turcica* belongs to the division Eumycota, sub division Ascomycotina, order, Pleosporales and family Pleosporaceae. The sexual stage of the fungus, *Setosphaeria turcica* Luttrell rarely occur in nature [5,9] but when it occurs, it produces black globose pseudothecia mostly under laboratory conditions.

The conidiophores of *E. turcicum* are single or in groups of 2-6, straight or flexuous, brown up to 300 µ long. Conidia are straight or slightly curved, pale to mid strew coloured, olive grey and spindle shaped, 3-8 pseudo-septate, 50-144 µ long and a hilum protrudes distinctly from the conidia to bluntly rounded basal cells [8,10]. Ellis [11] reported that the conidia of *E. turcicum* is ellipsoidal to obclavate, smooth, 4-9 pseudo-septate, 40-144 µ long, 18-23 (commonly 22-24 µ) thick in the broadest portion.

Variation in size of conidia due to environmental factors and the existence of several strains of the pathogen has been reported [12,13,14,15,16]. Beside primary host such as maize, pearl millet and sorghum, *E. turcicum* also infects wild hosts [8,17,18,19,20,21], thereby encourage diversity of the pathogen.

High genetic variation in terms of virulence, genetic structure, races, cultural characteristics and pathogenicity exist [22,23,24], single strain from the same single conidial culture differed in colour, type of mycelium, rate of growth and sporulation in culture. Strains from different geographic areas showed different parasitic fitness and virulence [25,26], possibly due to heterokaryons and their perpetuation through conidia [27].

The genetic variations among pathotypes of *E. turcicum* were reported on the basis of ecologically important traits and selectively neutral genetic markers [28,29]. The use of race differentials for *E. turcicum* is dependent on ecologically selective traits based on differences in pathogenicity among pathotypes [29], hence frequent emergence of new races of the pathogen has posed a big challenge in the management of NLB [30,31].

*Exserohilum turcicum* is mostly controlled by resistant varieties derived from qualitative and quantitative genes acting together or separately [32,33,34]. Qualitative resistance is typically race-specific and controlled by single genes (monogenic) whereas quantitative resistance is race non-specific and controlled by many genes [35,36]. However, [26] reported that host plant resistance is based on the effectiveness of resistance against all the virulence of the pathogen present in a region, therefore understanding the variability of *E. turcicum* will enhance breeding for resistance against the pathogen.

One of the major factors that reduce maize yield globally is diseases, particularly northern leaf blight due to reduced photosynthetic surface of the leaves. In Tanzania, northern leaf blight is prevalent, widespread and caused grain loss of up to 62.8 % on susceptible maize in the Southern Highlands [37]. However, information on the morphological and cultural characteristics is lacking. Such information is necessary to understand pathogenic variation, management and research on *E. turcicum*. Hence the study aimed at using different culture media to study morphological behaviour of the pathogen mostly, colony growth, rate of sporulation, dry mycelia weight and conidia germination.

# 2. Material and Methods

This study was carried out at the Plant Pathology-Mycology Laboratory, African Seed Health Centre, College of Crop Science and Horticulture, Sokoine University of Agriculture, Morogoro, Tanzania.

## 2.1. Sample Collection

Maize leaves bearing typical symptoms of the northern leaf blight disease were collected at silking/grain filling stage from farmers' field in 2012 and 2013 growing seasons in Arusha, Kilimanjaro, Morogoro, Iringa, Njombe, Mbeya and Coastal Regions [38,39] and used for the study. Such samples were sterilized using 70 % alcohol to suppress saprophytic invasion, rinsed in distilled water, dried on the laboratory bench at room temperature and stored at 4°C until required. Of the 480 samples, twenty five isolates from the different regions were selected and coded based on location and morphological differences (Table 1).

## 2.2. Isolation of the Pathogen

Tissue segment of about 2 $mm^2$, consisted of infected and healthy parts were excised from advancing lesion margins on symptomatic maize leaves, surface sterilized with 3 % sodium hypochloride solution for two minutes, rinsed three times in sterile distilled water and dried between sterilized blotter filter papers. Such segments were aseptically plated in 9 cm diameter petri dishes containing three layers of moist blotter papers (Ramathani *et al*., 2011) and incubated for 48 hours at 25±1°C in 12 hours alternating light and darkness to promote sporulation [40]. Pure isolates of *E. turcicum* were obtained using complete vegetable V8 agar medium and purified by standard hyphal tip isolation technique [8,41]. The cultures free from saltation or sectoring were preserved in V-8 agar slants at 4°C.

**Table 1. Strains of *Exserohilum turcicum* and their locations in Tanzania**

| S/No | Isolate code | Region | District | Village |
|---|---|---|---|---|
| 1 | KHK10 | Kilimanjaro | Hai | Kimashuku |
| 2 | KHK18 | ,, | ,, | ,, |
| 3 | KHN1 | ,, | ,, | Nshara |
| 4 | KHN17 | ,, | ,, | ,, |
| 5 | KHN3 | ,, | ,, | ,, |
| 6 | KMM18 | ,, | Moshi | Marangu |
| 7 | KMU10 | ,, | ,, | Uchira |
| 8 | AAE10 | Arusha | Arusha Municipal | Ekenywa |
| 9 | AAE16 | ,, | ,, | ,, |
| 10 | AAM2 | ,, | ,, | Mateves |
| 11 | AAM8 | ,, | ,, | ,, |
| 12 | AMN16 | ,, | Monduli | Ngarah |
| 13 | MMU13 | Mbeya | Mbeya Rural | Uyole |
| 14 | MMN16 | ,, | ,, | Nsongwi juu |
| 15 | MMI11 | ,, | ,, | Itanji |
| 16 | MRI3 | ,, | Rungwe | Ilinga |
| 17 | MRI4 | ,, | ,, | ,, |
| 18 | MRKi9 | ,, | ,, | Kikota |
| 19 | MRK8 | ,, | ,, | Katumba |
| 20 | MRK2 | Morogoro | Morogoro Rural | Kiroka |
| 21 | MRK7 | ,, | ,, | Kiziwa |
| 22 | MRK11 | ,, | ,, | Kasanga |
| 23 | MMM18 | ,, | Morogoro Municipal | Mkwujuni |
| 24 | MMM20 | ,, | ,, | Mbuyuni |
| 25 | INM8 | Iringa | Njombe | Mjimweme |

## 2.3. Media Preparation

Four solid media namely; Complete Vegetable V-8 agar [42], Potato Dextrose agar (PDA) [40], Malt Extract agar (MEA) [42] and 10 % Maize Leaf Extract agar (MLEA) [37,40] were used to study morphological cultural variation among 25 isolates of *E. turcicum* obtained from Tanzania.

## 2.4. Inoculation of the Media

The centre of each medium was inoculated with *E. turcicum*, using 5 mm disc from 15-day-old cultures of each isolate with a sterilized corks borer. The experiment was conducted twice and replicated three times (3 replicate × 25 isolates × 4 media) for each medium, making a total of 300 plates. The inoculated plates were arranged in a Complete Randomized Design (CRD) and incubated at 25±1ºC for 12 hours of alternate light and darkness. Data on colony diameter, conidia germination and rate of sporulation were recorded after 15 days of incubation while dry mycelia weight was determined after drying and harvesting the cultures.

## 2.5. Data Collection on Morphological Characteristics of *Exserohilum turcicum*

Data on radial colony growth of *E. turcicum* were obtained by multiplying vertical and horizontal growth of hyphea, measured with centimetre calibrated plastic ruler. The values were rated as excellent (≥7.6 cm²), good (6.6-7.5 cm²), moderate (5.0-6.5 cm²) and poor (< 5.0 cm²) growth as described by [26] with modification. Conidial suspensions were prepared from 15-day-old cultures on infected sorghum seeds [43,44] and adjusted to $10^5$ conidia/ml based on counts made with a hemacytometer. Thereafter, 5 ml of the conidial suspension were pipetted into a 9 cm petri dish containing V-8 agar, malt extract agar, leaf agar and potato dextrose agar [25]. Inoculated plates were incubated for 24 hours under light and darkness at 25°C and used for percentage germination based on 100 conidia per petridish. Temporary slides of each treatment were viewed under binocular Leica microscope at 40 x and the sporulation were rated excellent for ≥ 20 conidia per microscopic field (++++), good (15-20 conidia per microscopic field +++), fair (10-15 conidia ++), poor ≤ 10 conidia +) and – for no sporulation, as described by [26]. Sporulated cultures were arranged at room temperature to dry; using hairbrush dry mycelia were carefully removed and weighed to obtain the dry mycelia weight. Each of the experiments were repeated twice and statistically analysed using ANOVA while means were separated with Turkey test ($P \leq 0.05$) level of confidence.

# 3. Results

## 3.1. Morphological Variation of Different Isolates of *Exserohilum turcicum*

Morphological and cultural characteristics of 25 mono-conidial hyphal tip strains of *E. turcicum* showed significant ($P \leq 0.05$) variations in colony diameter, dry mycelia weight, conidia germination and sporulation on V-8 agar, malt extract agar (MEA), maize leaf extract agar (LEA) and potato dextrose agar (PDA) media at 24±1°C (Table 2). The study probably suggested diversity and variation in the virulence of the isolates. Colony growth (5.7 cm) and dry mycelia weight (3.2 g) of the isolates were high in V-8 agar, while conidial germination (30.9 %) and sporulation (++++) were high in Malt extract agar. Although colony characteristics varied with isolates, colonies were initially fluffy white with a greenish tint, which later became dark greyish black on sporulation with regular and irregular margins, similar to the description by [26].

## 3.2. Colony Diameter

Eleven isolates of *E. turcicum*, namely $KHK_{10}$, $KHK_{18}$, $KHN_{17}$, $KHN_3$, $KMM_{18}$, $MMM_{18}$, $INM_8$, $MMU_{13}$ $MRI_4$, $KMM_{18}$ and $KMU_{10}$ indicated profuse and excellent (> 7.6 cm²) growth on V-8 juice agar, malt extract agar and leaf extract agar media (Table 3). The isolates were consistently aggressive in growth and may probably suggest same strain *E. turcicum*, although they are from different regions (Kilimanjaro, Iringa, Mbeya and Morogoro). Such profuse isolates covered 9 $cm^2$ petridish within and between 10-12 days after inoculation. Isolates $MMN_{16,}$ $MRK_7$ $KHN_{3,}$ $MRK_2$ indicated good growth (6.6-7.5 cm) on the four media while twelve strains, $KHN_{1,}$ $MRK_{11,}$ $AAE_{16}$, $KHN_1$, $MMU_{16}$, $MRK_{11,}$ $MRKi_{9,}$ $AAM_8$, $KHN_1$, $KMU_{10}$, $MMN_{16}$ and $MRKi_9$ were moderate (5.1-6.5 cm²) in growth. Generally, isolates reacted differently in different medium, but $AAM_8$ and $MRK_7$ recorded minimum growth on V-8 (1.1 cm²), MEA (0.0 cm²) and LEA (0.01 cm²). Although there were significant differences ($P \leq 0.05$) in mycelial growth on potato dextrose agar (PDA), restricted colony growth were recorded with average growth of 2.7 cm. Cultural growth of the isolates conformed to report by [8,20].

## 3.3. Dry Mycelial Weight

The dry mycelia weight significantly ($P \leq 0.05$) varied among the isolates on the four media (Table 4). V-8 juice agar ranged from 0.3 g – 9.5 g, with a mean of 3.2 g. Isolates $KMM_{18}$ and $KMM_{18}$ recorded 9.5 g and 8.2 g dry weight respectively while isolate $MRK_2$ recorded the minimum weight of 0.3 g. On malt extract agar, dry weight, varied from 0.3 g to 2.2 g with an average of 1.02 g. Isolates $MRK_{11}$ and $KMU_{10}$ significantly yielded 2.2 g mycelial weight followed by $MRKi_9$ and $KMM_{18}$ (2.1 g) while minimum dry weights of 0.3 g were observed for strains $AAE_{16}$, $AAM_2$ and $MRK_2$. Leaf extract agar (0.2 g to 2.5 g) and PDA (0.0-2.8 g) also varied significantly ($P \leq 0.05$) with mean of 0.9 g and 1.3 g, respectively (Table 4). However, isolate$MRK_2$ recorded maximum dry weight (2.5 g) while $MMI_{11}$ recorded minimum weight (0.2 g) on LEA. On the other hand, isolate $MMM_{18}$ (2.8 g) gave maximum dry weight followed by $KHK_{18}$ and $MRK_{11}$ (2.7 g) compared while Isolate $AAE_{10}$, $MRK_2$ and $MRK_8$ recorded minimum dry weight (0.2 g).

**Table 2. Morphological and cultural variations among different strains of *Exserohilum turcicum* on four different media**

| | | Morphological characteristics | | | |
|---|---|---|---|---|---|
| **S/No** | **Media** | **Colony growth (cm)** | **% germination** | **Dry mycelia weight (g)** | **Rate of sporulation (%)** |
| 1 | V-8 juice | 5.7a | 28.1b | 3.2a | +++ |
| 2 | Malt extract agar | 5.1b | 30.9a | 1.0c | ++++ |
| 3 | Leaf extract agar | 4.5c | 3.7c | 0.9d | + |
| 4 | Potato dextrose agar | 2.7d | 0.0d | 1.3b | - |
| Mean | | 4.5±0.01 | 15.7±0.1 | 1.6±0.01 | - |
| CV | | 2.6 | 5.7 | 7.5 | - |
| Fpr. | Media | <.001 | <.001 | <.001 | - |
| | Strains | <.001 | <.001 | <.001 | - |
| | Media. Strains | <.001 | <.001 | <.001 | - |

Each medium had three replicates of 25 isolate, Means of two repeated experiments, totaling 300 analyzed isolates, Means followed by the same letter in the same column are not significantly different according to Turkey test 95 % confidence level of statistics.

**Table 3. Mycelial growth of different strains of *Exserohilum turcicum* on four different olid medium**

| | | Growth media and colony diameter (cm²) | | | |
|---|---|---|---|---|---|
| **S/No.** | **Isolates** | **V-8** | **MEA** | **LEA** | **PDA** |
| 1 | $AAE_{10}$ | 3.1j | 2.0n | 4.1h | 0.7l |
| 2 | $AAE_{16}$ | 7.3d | 6.5f | 3.1i | 2.2j |
| 3 | $AAM_{2}$ | 2.3k | 2.0n | 2.5j | 3.2fg |
| 4 | $AAM_{8}$ | 1.1l | 2.1n | 6.2cd | 1.4k |
| 5 | $AMN_{16}$ | 4.4h | 3.8l | 5.0g | 2.3ij |
| 6 | $KHK_{10}$ | 8.9ab | 8.5b | 7.1b | 3.5def |
| 7 | $KHK_{18}$ | 8.6b | 7.5d | 5.0g | 3.8cde |
| 8 | $KHN_{1}$ | 5.2g | 5.1i | 5.8ef | 3.0gh |
| 9 | $KHN_{17}$ | 8.2c | 4.8j | 7.2b | 3.4efg |
| 10 | $KHN_{3}$ | 9.0a | 7.0e | 4.2h | 2.7hi |
| 11 | $KMM_{18}$ | 9.0a | 9.0a | 8.1a | 3.1fgh |
| 12 | $KMU_{10}$ | 7.3d | 8.0c | 6.1de | 3.2fg |
| 13 | $MMI_{11}$ | 1.4l | 1.0p | 1.6k | 1.4k |
| 14 | $MMM_{18}$ | 8.9ab | 8.0c | 4.0h | 4.7a |
| 15 | $MMM_{20}$ | 2.2k | 3.5m | 4.0h | 2.1j |
| 16 | $MMN_{16}$ | 6.6ef | 5.1ij | 5.7f | 4.2bc |
| 17 | $INM_{8}$ | 8.9ab | 7.7d | 3.2i | 4.0cd |
| 18 | $MMU_{13}$ | 9.0a | 8.5b | 4.8g | 0.0m |
| 19 | $MRI_{3}$ | 3.6i | 4.1k | 1.8k | 3.0gh |
| 20 | $MRI_{4}$ | 8.7ab | 7.6d | 5.0g | 4.5ab |
| 21 | $MRK_{2}$ | 0.7m | 1.5o | 6.6c | 0.8l |
| 22 | $MRK_{7}$ | 6.9e | 0.0q | 0.0l | 3.0gh |
| 23 | $MRK_{8}$ | 1.4l | 2.2n | 4.4h | 0.9l |
| 24 | $MRK_{I1}$ | 6.2f | 5.6h | 2.9i | 4.8a |
| 25 | $MRKi_{9}$ | 4.3h | 6.0g | 5.6f | 3.3fg |
| | Mean | 5.7±0.1 | 5.1±0.1 | 4.5±0.7 | 2.7±0.1 |
| | CV | 2.1 | 1.6 | 2.6 | 5.3 |
| | Fpr. | <.001 | <.001 | <.001 | <.001 |

Means followed by the same letter in the same column are not significantly different according to Turkey's 95 % level of confidence. Mean of three replications repeated twice. V-8 = Complete vegetable V8 agar, MEA = Malt extract agar, LEA = Leaf extract agar, PDA = Potato dextrose agar media.

**Table 4. Dry mycelial weight (g) of different strains of *Exserohilum turcicum* on four solid medium**

| | | Culture media | | | |
|---|---|---|---|---|---|
| **S/No.** | **Isolate** | **V-8** | **MEA** | **LEA** | **PDA** |
| 1 | $AAE_{10}$ | 0.7lm | 0.3i | 0.3ijk | 0.2ijk |
| 2 | $AAE_{16}$ | 2.1jk | 0.3i | 0.2ijk | 0.4hij |
| 3 | $AAM_{2}$ | 1.2l | 0.3i | 0.9def | 2.0de |
| 4 | AAM8 | 1.2l | 1.5c | 2.0bc | 0.6gh |
| 5 | $AMN_{16}$ | 2.4jk | 0.9de | 0.9efg | 1.0f |
| 6 | $KHK_{10}$ | 5.0de | 1.7b | 2.1bc | 1.1f |
| 7 | $KHK_{18}$ | 6.7c | 0.8ef | 0.4hij | 2.7ab |
| 8 | $KHN_{1}$ | 4.7ef | 0.5gh | 0.4hij | 1.2f |
| 9 | $KHN_{17}$ | 5.5d | 1.1d | 1.2de | 1.3f |
| 10 | $KHN^{3}$ | 2.7j | 0.4hi | 0.2ijk | 1.1f |
| 11 | $KMM_{18}$ | 8.2b | 2.1a | 1.8c | 1.2f |
| 12 | $KMU_{10}$ | 4.9ef | 2.2a | 2.3ab | 1.1f |
| 13 | $MMI^{11}$ | 2.1k | 1.1d | 0.2jk | 0.5hi |
| 14 | $MMM_{18}$ | 9.5a | 0.7fg | 0.5ghi | 2.8a |
| 15 | $MMM_{20}$ | 2.2jk | 0.8ef | 0.9efg | 0.7g |
| 16 | $MMN_{16}$ | 3.3i | 0.4hi | 2.1bc | 1.9e |
| 17 | $INM_{8}$ | 4.4fg | 1.1d | 0.4hijk | 2.2cd |
| 18 | $MMU_{13}$ | 4.0gh | 2.1a | 0.7fgh | 0.0k |
| 19 | $MRI_{3}$ | 0.7lm | 0.8ef | 0.3ijk | 1.0f |
| 20 | $MRI_{4}$ | 1.3l | 0.7efg | 1.3d | 2.5bc |
| 21 | $MRK_{2}$ | 0.3m | 0.3i | 2.5a | 0.2ijk |
| 22 | $MRK_{7}$ | 1.1l | 0.0j | 0.0k | 2.1de |
| 23 | $MRK_{8}$ | 1.9k | 1.8b | 0.5hij | 0.2jk |
| 24 | $MRK_{I1}$ | 0.8lm | 2.2a | 0.4hij | 2.7ab |
| 25 | $MRKi_{9}$ | 3.6hi | 2.1a | 1.1def | 1.1f |
| | Mean | 3.2±0.1 | 1.02±0.03 | 0.9±0.1 | 1.3±0.1 |
| | CV | 5.7 | 6.6 | 12.5 | 6.7 |
| | Fpr. | <.001 | <.001 | <.001 | <.001 |

Means followed by the same letter in the same column are not significantly different according to Turkey's 95 % level of confidence. Mean of three replications repeated twice. V8 = Complete vegetable V-8 agar, MEA = Malt extract agar, LEA = Leaf extract agar, PDA = Potato dextrose agar media.

Results revealed that although, dry weight on V-8 and MEA media were proportional to mycelia growth, it was not consistent. For instance, mean colony growth of 2.7 cm² on PDA yielded high mycelia weight (1.3 g) compared to 4.5 (0.9 g) in LEA. Generally, isolates from Kilimanjaro, Mbeya and MMM18 (Morogoro) yielded higher dry mycelia weight than others.

**Table 5. Conidial germination (%) of different isolates of *Exserohilum turcicum* on four medium**

| | | Conidia germination (%) | | | |
|---|---|---|---|---|---|
| S/No. | Isolate | V8 | MEA | LEA | PDA |
| 1 | $AAE_{10}$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 2 | $AAE_{16}$ | 11.0h | 12.0j | 0.0e | 0.0 |
| 3 | $AAM_2$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 4 | AAM8 | 0.0i | 0.0k | 0.0e | 0.0 |
| 5 | $AMN_{16}$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 6 | $KHK_{10}$ | 81.2b | 75.5c | 0.0e | 0.0 |
| 7 | $KHK_{18}$ | 36.8e | 44.5e | 21.0b | 0.0 |
| 8 | $KHN_1$ | 17.5g | 39.0fg | 24.0a | 0.0 |
| 9 | $KHN_{17}$ | 22.5f | 30.3h | 0.0e | 0.0 |
| 10 | $KHN_3$ | 81.0b | 41.0ef | 13.0c | 0.0 |
| 11 | $KMM_{18}$ | 87.5a | 80.0b | 0.0e | 0.0 |
| 12 | $KMU_{10}$ | 47.5d | 58.5d | 0.0e | 0.0 |
| 13 | $MMI_{11}$ | 47.5d | 30.5h | 0.0e | 0.0 |
| 14 | $MMM_{18}$ | 60.0c | 30.5h | 22.0b | 0.0 |
| 15 | $MMM_{20}$ | 37.3e | 40.0f | 0.0e | 0.0 |
| 16 | $MMN_{16}$ | 21.5f | 20.0i | 0.0e | 0.0 |
| 17 | $INM_8$ | 48.0d | 35.3g | 11.5d | 0.0 |
| 18 | $MMU_{13}$ | 85.3a | 88.0a | 0.0e | 0.0 |
| 19 | $MRI_3$ | 0.0i | 28.5h | 0.0e | 0.0 |
| 20 | $MRI_4$ | 0.0i | 35.0g | 0.0e | 0.0 |
| 21 | $MRK_2$ | 0.0i | 20.5i | 0.0e | 0.0 |
| 22 | $MRK_7$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 23 | $MRK_8$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 24 | $MRK_{11}$ | 0.0i | 0.0k | 0.0e | 0.0 |
| 25 | $MRKi_9$ | 17.5g | 62.5d | 0.0e | 0.0 |
| | Mean | 28.1±0.7 | 30.9±0.8 | 3.7±0.2 | - |
| | CV | 4.0 | 4.4 | 9.9 | - |
| | Fpr. | <.001 | <.001 | <.001 | |

Means followed by the same letter in the same column are not significantly different according to Turkey's 95 % level of confidence. Mean of three replications repeated twice. V-8 = Complete vegetable V-8 agar, MEA = Malt extract agar, LEA = Leaf extract agar, PDA = Potato dextrose agar media.

## 3.4. Conidial Germination

Results showed maximum *E. turcicum* conidial germination of 87.5 % and 85.3 % in isolates $KMM_{18}$ and $MMU_{13}$, respectively, followed by $KHK_{10}$ (81.2 %) and $KHN_3$ (81.0 %). Except for Isolates $AAE_{16}$ (11.0 %) and $MMM_{20}$ (37.3 %), other isolates from Arusha and Morogoro did not germinate on V-8 agar medium (Table 5). On malt extract agar, isolates $MMU_{13}$ (88 %), $KMM_{18}$ (80 %) and $KHK_{10}$ (75.5 %) significantly ($P \leq 0.05$) recorded the maximum conidial germination. Such isolates like $MRK_2$, $MRI_3$ and $MRI_4$ that did not germinate on V-8 agar medium but gave 20.5 %, 28.5 % and 35 % germination on malt agar, respectively (Table 5) and increased germination of $AAE_{16}$ (12 %) and $MRKi_9$ (62.5 %) on MEA. Conidial germination on LEA though very low, were significantly different. Isolates $KHN_1$ (24 %), $MMM_{18}$ and $KHK_{18}$ (21 %) recorded maximum germination while the minimum germination was recorded in INM8. Other *E. turcicum* isolates did not germinate on LEA. On PDA, all isolates of *E. turcicum* did not germinate (Table 5).

## 3.5. *Exserohilum turcicum* Sporulation on four Solid Media

Excellent sporulation was exhibited by isolates $KHK_{10}$, $KHN_3$, $KMM_{18}$ and $MMU_{13}$ and $MMU_{13}$ while good sporulation was observed for isolates $KMU_{10}$, $MMI_{11}$, $INM_8$ $KHK_{18}$, and $MRKi_9$ V-8 and MEA agar media. Results also indicated fair sporulation for isolates $KHK_{18}$, $KHN_1$, $KHN_3$, $MMI_{11}$, $MMM_{18}$, $MMM_{20}$, $MMN_{16}$, $INM_8$, $MRI_3$ and $MRI_4$ and poor in $AAE_{16}$, $KHN_1$, $KHN_{17}$, $MMM_{20}$, $MMN_{16}$, $MRKi_9$ $AAE_{16}$, $KHN_{17}$ and $MRK_2$ on both media (Table 6). Variation was also observed on isolates $MRI_3$, $MRI_4$ and $MRK_2$, that did not sporulate on V8 but showed fair sporulation on MEA. On LEA, sporulation was fair for isolates $KHK_{18}$, $KHN_1$ and $MMM_{18}$ and poor for strain $INM_8$. Sporulation was not observed on PDA (Table 6).

**Table 6. Sporulation of *Exserohilum turcicum* strains in four different medium**

| | | Rate of sporulation | | | |
|---|---|---|---|---|---|
| S/No | Isolate | V-8 | MEA | LEA | PDA |
| 1 | $AAE_{10}$ | - | - | - | - |
| 2 | $AAE_{16}$ | + | + | - | - |
| 3 | $AAM_2$ | - | - | - | - |
| 4 | $AAM_8$ | - | - | - | - |
| 5 | $AMN_{16}$ | - | - | - | - |
| 6 | $KHK_{10}$ | ++++ | ++++ | - | - |
| 7 | $KHK_{18}$ | ++ | +++ | ++ | - |
| 8 | $KHN_1$ | + | ++ | ++ | - |
| 9 | $KHN_{17}$ | + | + | - | - |
| 10 | $KHN_3$ | ++++ | ++ | + | - |
| 11 | $KMM_{18}$ | ++++ | ++++ | - | - |
| 12 | $KMU_{10}$ | +++ | +++ | - | - |
| 13 | $MMI_{11}$ | +++ | ++ | - | - |
| 14 | $MMM_{18}$ | +++ | ++ | ++ | - |
| 15 | $MMM_{20}$ | + | ++ | - | - |
| 16 | $MMN_{16}$ | + | ++ | - | - |
| 17 | $INM_8$ | +++ | ++ | + | - |
| 18 | $MMU_{13}$ | ++++ | ++++ | - | - |
| 19 | $MRI_3$ | - | ++ | - | - |
| 20 | $MRI_4$ | - | ++ | - | - |
| 21 | $MRK_2$ | - | + | - | - |
| 22 | $MRK_7$ | - | - | - | - |
| 23 | $MRK_8$ | - | - | - | - |
| 24 | $MRK_{11}$ | - | - | - | - |
| 25 | $MRKi_9$ | + | +++ | - | - |

Mean of three replications repeated twice. ++++ = Excellent > 20 conidia per microscopic field view, +++ = Good 15-20 conidia per microscopic field view, ++ = Fair 10-15 conidia per microscopic field view, + = Poor < 10 conidia per microscopic field view and – zero sporulation. V-8 = Complete vegetable V-8 agar, MEA = Malt extract agar, LEA = Leaf extract agar, PDA = Potato dextrose agar media.

## 4. Discussion

Isolates from different agro-ecological zones showed variation in colony diameter, dry mycelial weight, conidial germination and sporulation in different media and probably represent different strains, patho-types and or races of the pathogen in Tanzania. Colony growth was aggressive in V8 juice agar (5.7 cm) but spore germination and rate of sporulation were high in malt extract agar. On the basis of cultural characteristics strains such as $KHK_{10}$, $KHK_{18}$, $KHN_{17}$, $KHN_3$, $KMM_{18}$ (Kilimanjaro Region), $MMU_{13}$ and $MRI_4$ (Mbeya Region), $INM_8$ (Iringa Region) and $MMM_{18}$ from Morogoro Region significantly yielded more colony growth, conidial germination, sporulation and dry mycelia on the artificial media compared to the other strains. Gowda [45] reported a similar observation on 13 isolates of the pathogen on five media.

On the other hand, the Arusha and some other Morogoro *E. turcicum* strains mostly $AAM_8$ and $MRK_2$ showed restricted growth without sporulation and germination on the media and were rated as least virulent. These variations in the cultural behavior of the isolates may be attributed to long term influence of weather conditions of particular location and ability of the pathogen to adapt to the varieties developed in a specific situation [26]. These results are in conformity with earlier reports [8,26,46,47,48].

Muiru [48] observed similar response among *E. turcicum* strains in Kenya and reported that strains from different areas vary in cultural characteristics and parasitic fitness with those from the same locality showing less variation. Therefore, morphological variations observed among the isolates in this present study suggested wide, high genetic variation and virulent isolates in the different location (Mbeya, Arusha and Morogoro), and may be responsible for lack of resistance in some of the maize varieties and commercial cultivars, particularly in Arusha and Mbeya Regions [30,45,50,51,52]. No strain of *E. turcicum* germinated or sporulated on PDA. Similarly, poor development of *E.turcicum* on PDA and LEA was also reported by [40].

The finding of the present study is useful in resistance breeding programme, management and further empirical study on the pathogen maize in Tanzania. This is the first report on morphological and cultural characteristics of maize *E. turcicum* in Tanzania; therefore there is a need for further investigation on variability using molecular approach. There is also a need to determine relationship between *E. turcicum* isolates and PDA medium, preventing germination and sporulation.

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