DISTRIBUTION OF MAIZE GREY LEAF SPOT AND MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *Cercospora zeae-maydis* Tehon and Daniels ISOLATES IN WESTERN OROMIA REGION, ETHIOPIA

M.SC. THESIS

BY

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JIMMA, ETHIOPIA

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A Thesis

Submitted to the Department of Horticulture and Plant Sciences, College of Agriculture and Veterinary Medicine, Jimma University, in Partial Fulfillment of the Requirements for the Degree of Master of Sciences in Agriculture (Plant Pathology)

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisors. Hence, I hereby kindly request the Department to allow me to present the findings of my work and submit the thesis.

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DEDICATION

This thesis is dedicated to, my mother Kuleni Ite'a, and my beloved wife Shewaye Gadisa whom my academic success is attributed to.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Plant Pathology at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and is deposited at the University Library to make it available to borrowers under the rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of the academic degree, diploma or certificate.

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ABBREVIATION AND ACRONYMS

AUDPC	Area Under Disease Progress Curve
BNMRC	Bako National Maize Research Center
CIMMYT	International Center for Maize and Wheat Improvement
CLR	Common Leaf Rust
CSA	Central Statistics Agency
CV	Coefficient of Variance
DAI	Days after Inoculation
DI	Disease Incidence
DSI	Disease Severity Index
EIAR	Ethiopian Institute of Agricultural Researches
FAO STAT	Food and Agriculture Organization Statistics
GLS	Grey Leaf Spot
GPS	Geographical Positioning System
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
PLS	Phasphorea Leaf Spot
RGB	Red-Green-Blue
USDA-FAS	United States Department of Agriculture –Foreign Agricultural Service

BIOGRAPHICAL SKETCH

The author Midekssa Dida Ardessa was born on 25 June 1985 in Toke-Kutaye district Western Showa Zone Oromia region from his father Dida Ardessa Abdi and forms his mother Kuleni Ite'a Wako at Lencha Kebele. He attended his Primary educations at Hamus Gebiya elementary school from 1990-1996 and his secondary School at Guder Senior Secondary School from 1997- 2001. After taking Ethiopian School Leaving Certificate Exam (ESLCE), he joined Nedjo ATVET College and obtained Diploma in Plant Science in July 2005.

Thereafter, he employed by the Ministry of Agriculture and worked in Dire Inchini district Agricultural and Rural Development Office as Development Agents from 1 August 2005 to Sep.2009. Then, he joined Ambo University to pursue his BSc and graduated in Plant Science in February 2012.

He joined the Ethiopian Institute of Agricultural Researches (EIAR) based at Bako National Maize Research Center from 1 August 2012 until he joined Jimma University College of Agriculture and Veterinary Medicine in September 2016 to study Masters of Science degree in Plant Pathology. During his stay in EIAR he has got the chance to carry out research activities and published four journals in collaboration with other staff colleagues. He is married and a father of three children.

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ABSTRACT

Maize (Zea mays) is one of the most widely cultivated crops in the world. It occupies an important position in the world economy serving as food, feed, and industrial grain crop. In Ethiopia, an average productivity is about 3.94 t ha^{-1}, which is below the world average of 5.78 tones ha^{-1}. A significant portion of this yield gap is attributable to biotic and abiotic stresses. Diseases play a major role among the biotic constraints. Of these, grey leaf spot (Cercospora zeae-maydis) is one of the major foliar diseases threatening maize production in Ethiopia. Most of the researchers estimated that losses as high as 100% occurred when the pathogen attacked before the flowering stage. Also, in Ethiopia the loss caused due to GLS reached 49.5%. Therefore, the objective of this study was to assess the distribution, of GLS and morphological and cultural characterization, of maize GLS isolates. The field assessment was conducted during 2017 main cropping season by sampling 81 maize fields in 9 districts from 3 zones. Morphological and cultural characterization studies of the 5 isolates were done at JUCAVM Plant Pathology lab and greenhouse, respectively. BH540 moderately susceptible to GLS maize variety was used to confirm the Koch's postulate of the isolates. The survey result showed the highest disease prevalence 62.96 % was recorded in East Wollega zone and the lowest (22.22%) in West-Wollega zone. The highest mean incidence 35.06 % was recorded in East Wollega zone whilst the lowest 9.51% was found in West-Shewa, The maximum disease Severity index of 31.43% in East Wollega zone followed by West-Shewa having (11.98%) and West-Wollega (10.05%) zones. At a district level, the highest prevalence was recorded in Leka-Dulecha followed by Gobu-Seyo with disease prevalence of 88.9% and 66.7%, respectively, whereas the minimum prevalence was at Ayra-Guliso Gimbi and Bako-Tibe, each with 22.2 % prevalence. The highest mean disease incidence 48.15% was recorded at Gobu-Seyo district followed by Leka-Dulecha 47.77% whereas the lowest was recorded in Gimbi 1.1% districts. The highest mean disease severity index was recorded in Leka-Dulecha 46.11% followed by Gobu-Seyo 39.15%, whilst the lowest in Gimbi 1.78%. From 155 samples collected during the assessment, 52 isolates were re-cultured and grouped into 5 isolates. Colony color, shape, elevation, edge, conidial shape and number of septa were used to characterize the isolates. Light grey, grey and dark grey were major colony color revealed by the isolates. Based on the Conidial shape, isolates LD-G and DN-H revealed slightly curved whereas isolates GS-O, IG-3, and LA-Ay were straight shape of the conidia. The highest AUDPC and disease severity index were recorded by LD-G and DN-H isolates, with 1540 and 80 %, respectively. The pathogen was re-isolated and Koch's postulate was proved. From the result, it can be concluded that GLS is prevalent with different intensity and different characteristics across the study areas. Thus, there is a need to develop management strategies like crop rotation with non-host, use of tolerance varieties to reduce the impact of GLS on maize production in the study area.

Key Words: Cercospora zeae mayds, Incidence, Severity Index

1. INTRODUCTION

Maize (*Zea mays*) is one of the most widely cultivated crops in the world. It is one of the three most popular cereal crops next to wheat, and rice in the world (FAOSTAT, 2016). Maize occupies an important position in world economy as a food, feed and an industrial grain crop (Tolesa *et al*., 1993). It serves as a vital source of proteins, calories and some of the important vitamins and minerals to billions of people worldwide, particularly in Africa, South America and Asia, and has been considered a 'poor man's nutricereal' (Prasanna *et al*. 2001).

Maize is grown worldwide approximately on 226.94 million hectares (ha) annually with the production of 1291.94 million metric tons (FAOSTAT, 218). In sub-Saharan Africa, maize is produced on an estimated area of about 26 million ha with an average of 460 million metric tons. In East Africa, maize occupies about 17 million of production area and 30.4 million tons of total production and with 1.8 tons per ha (FAOSTAT, 2016).

In Ethiopia, maize is the second largest food security crop after teff, (*Eragrostis teff* (Zucc.) Trotter. It is primarily produced and consumed by the small-scale farmers that comprise about 80% of Ethiopia's population (Alemu *et al.*, 2008). The mid-altitude sub-humid agro-ecology is considered to be the major maize growing zone in Ethiopia. Owing to its importance, wide adaptation, total production, and productivity, maize is regarded as one of the high priority food security crops in Ethiopia (CSA, 2016). The major production areas are Western, Southern, Southwestern, Eastern and in some Northwestern and Eastern part of the country (Worku *et al.*, 2012). Maize has been selected as one of the National commodity crops to satisfy the food self-sufficiency program of the country to feed the alarmingly increasing population (Tolesa *et al*., 1993; Tegene *et al.*, 2008).

Currently, about 2.13 million ha of land is covered by maize with an average production of 8.39 million tons, and the average national yield of maize is very low under small-scale farmers, which is 3.94 t/ha (CSA 2018) in the country. In Oromia region maize is produced on 1.14 million ha and 4.67 million tons. An average productivity in Oromia region is 4.07 ton per ha (CSA, 2018). However, it is very low as compared to the potential of maize (8 – 11 t ha-1) in the high rainfall and irrigated areas and also low as compared to world average productivity 5.78 ton ha-1 (USDA-FAS 2017).

A significant portion of this yield gap is attributable to biotic and abiotic stresses. The major abiotic factors include nutrient deficiency and drought stress, which could be aggravated by land degradation. Among biotic constraints, foliar diseases play a major role in contributing to the reduction of maize production and productivity across the world (Berger *et al.*, 2014; Masuka *et al.*, 2017).

The major diseases include Turcicum leaf blight (*Exserohilum turcicum* (Pass) Leonard & Suggs), grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels), leaf rust (*Puccinia sorghi* Schr.), maize streak virus disease (Wegary *et al.*, 2001; Tewabech *et al.*, 2012). Recently *Maize Lethal Necrosis* Disease (MLND), which is caused by the double infection of maize Chlorotic mottle virus (MCMV) and the cereal Potyvirus is also among factors threatening maize production (Demissie *et al.*, 2016).

Foliar diseases, particularly GLS caused by *Cercospora zeae-maydis* (Tehon and Daniels, 1925) is one of the necrotrophic and polycyclic foliar diseases of maize that poses a serious problem to maize production in tropical maize production (Renfro and Ullstrup, 1996). This pathogen causes intense water loss from the plant thereby leading to severe blighting of the leaves and reduced photosynthesis. This eventually leads to undersized ears, low grain yield and premature death of maize plants. Severe blighting of the upper eight or nine leaves that contribute 75 to 90% of the photosynthates for grain fill may lead to stalk weakening or even infectious stalk rot diseases leading to premature stalk death and lodging (Lipps *et al.*, 1996: Ward *et al.*. 1999; Poland *et al.*, 2009).

In Ethiopia, a major epidemic occurred in the early 2000s and made considerable maize grain yield losses 36.9 % and 49.5% and there have been extensively disseminated through severe outbreaks every year, particularly in the warm and humid areas of the country (Tilahun *et al.*, 2012; Negash, 2013).

The potential threat of GLS to maize production was started and identified with a survey carried out during the year 1997-1998 to know the distribution and importance of the disease in most maize growing regions of Ethiopia (Wegary *et al.*, 2001). The severity of grey leaf spot was high in the warm humid maize belt areas of the country which adversely affecting farmers who live with limited resources. Currently, GLS, caused by *Cercospora zeae-maydis*, is among the major maize foliar disease in South and Southwest Ethiopia (Nega *et al.*, 2016).

In Ethiopia, Wegary *et al.* (2004) reported that yield loss due to GLS on resistant, moderately resistant and susceptible varieties was between 0-14.9%, 13.7-18.3%, and 20.8-36.9% respectively during 2003/2004 cropping seasons at Bako areas. Similarly, the research carried out at South Ethiopia in the year 2004-2006 showed that the yield loss due to GLS is 29.5% (Tilahun *et al.*, 2012).

The survey study showed that the highest GLS prevalence, incidence and severity were 74%, 71.2% and 45.13% respectively in South and Southwest Ethiopia. Similarly, morphological characterization of GLS isolates were carried out and 10 different isolates were identified (Nega et *al*., 2016). Therefore, this study showed the importance of the pathogen distribution and diversity of the isolates in the country. Even though Western Oromia is a potential to produce maize crop, importance of the pathogen is not assessed and determined to take a measure.

Studies have been conducted on major foliar diseases of maize since late 1990's in the western part of the country (Wegary *et al.*, 2001). However, it was little indicated specifically for GLS and no updated information on the distribution and disease intensity. Additionally, there is a gap of information on the existence of different isolates of the pathogen and, on its distribution map in Western Oromia. This thesis work will come with some information on the variation of maize Gey leaf spot isolates, besides studying the distribution and intensity of the disease in western part of Oromia region. Therefore, the present study was designed with the following general and specific objectives:

General objectives

- To assess the distribution and morphological and cultural characterization of Maize Grey Leaf Spot (*Cercospora zeae-maydis*) in Western Oromia.

Specific objectives

- To assess the distribution and disease intensity of Maize Grey Leaf Spot in western Oromia
- To identify and morphologically and culturally characterize *Cercospora zeae-maydis* isolates associated with Grey Leaf Spot of maize in Western Oromia

2. LITERATURE REVIEWS

2.1. Historical Perspectives and Importance of GLS

Grey Leaf Spot caused by *Cercospora zeae-maydis* Tehon& Daniels, is one of the most important foliar diseases of maize world-wide (Ward *et al.*, 1999). The pathogen was first identified from a sample collected by Tehon & Daniels and confirmed by Chupp in 1953. It was first observed in Illinois, the USA in 1925 (Tehon and Daniels, 1925). The economic importance of the disease in the US was recognized in the first half of the 1970's when it was described as the most destructive disease of maize after increased incidence and epiphytotic in North Carolina (Leonard, 1973). Since then, the disease has steadily spread in the US and other parts of the world. The disease was endemic in proportion and occasional outbreak during the period of the 1970s (Latterell and Rossi, 1983). The disease was recognized as destructive and yield limiting when increased incidence occurred in North Carolina (Leonard, 1973).

The severity and distribution of the pathogen have been increasing and the disease has become the most destructive throughout the maize growing regions of USA (Ward *et al.*, 1999). In Africa, it was first observed at Grey town, South Africa, during the 1988/89 season, and then at Cedara in 1992 (Ward & Nowell, 1997). It has since spread throughout the province of KwaZulu-Natal and has also been identified in neighboring provinces and countries (Ward & Nowell, 1997). Similarly, it was recognized as a yield-limiting disease of maize in Ethiopia, since 1998 particularly in major maize growing parts of the country (Wegary *et al.*, 2001).

Documented yield losses of maize attributed to GLS vary from 11 to 69% (Ward *et al.*, 1999). Most of the researchers estimated that losses as high as 100% occurred when the pathogen attacked before the flowering stage (Stromberg and Donahue, 1986, and Lipps *et al.*, 1996). GLS has been rigorous in recurrence and distribution and has led to economic yield losses: over 60% in western Kenya (Kinyua *et al.*, 2010), 10 to 60% in Tanzania, and 49.5% in Ethiopia (Sibiya *et al*, 2012; Negash, 2013).

Researchers reported that grain yield loss was found high when disease severity occurs during vegetative and tasseling /silking to grain filling stage and low grain yield loss was found after grain filling stage. Yield potential of the cultivars, growth stage of crops and the ability of leaf blighting to predispose the variety to stalk rots contribute to this response (Dhami *et al.*, 2015).

The assessment carried out at a different time in Ethiopia also showed 29.5% yield loss as reported by (Tilahun *et al.*, 2012). Wegary *et al.*, (2004) due to grey leaf spot on resistant, moderately resistant, and susceptible varieties were between 0-14.9%, 13.7-18.3%, and 20.8-36.9%, respectively during 2003/2004 cropping seasons at Bako and its nearby areas. The blighting of leaves and stalk rotting caused the premature death of leaves which reduced the amount of sugar and resulted in significant yield loss. Early blighting of leaves above the ear has led to yield losses of more than 50%. Late planted maize has greater GLS severity and a higher reduction in yield than earlier planted maize (Lipps, 1995; Manandhar, 2007). The study carried out at Bako showed early planting date, mid-May significantly increased the disease incidence, and severity, AUDPC, and grain yield, while delayed planting in early June reduced disease development and grain yield, but economic analysis is very important (Sisay *et al*., 2012).

2.2. Taxonomy of Grey leaf spot

Kingdom: Fungi
- Phylum: Ascomycota
  - Class: Dothideomycetes
    - Order: Capnodiales
      - Family: Mycosphaerellaceae
        - Genus: Cercospora
          - Species: *Cercospora zeae-maydis* (Luttrell, 1951).

2.3. Species of Cercospora

The genus Cercospora is a member of Deuteromycetes and belongs to one of the largest groups of plant pathogenic fungi. Initially, *Cercospora zeae-maydis* was considered to be the sole causal agent of grey leaf spot, but recently it was accepted that three genetically distinct species of Cercospora are associated with this disease. Among the two sibling species of *Cercospora zeae-maydis*; Group I *Cercospora zeae-maydis* and Group II *Cercospora zeina* and *Cercospora sorghi* var. maydis were associated with this pathogen (Wang *et al*., 1998; Crous *et al.*, 2006). The two sibling species (Group I and Group II) are genetically distinct but morphologically similar and uniform internally with a genetic similarity of approximately 93 to 94% (Carson *et al.*, 1997; Wang *et al.*, 1998).

The genus Cercospora sorghai var. maydis is saprophytic and found in maize tissues (Carson and Goodman, 2006). Group I can be distinguished from Group II by its faster growth rate of conidia (8-12mm per week) when compared to that of *C. Zeina* (4-5 mm per week) in artificial media. Also Group I has the ability to produce cercosporin, longer conidiophores and broadly fusiform conidia, whitish to greyish mycelia, irregular edge and visible quantities of reddish toxin (cercosporin) whereas Group II contains mycelia whitish to greyish in color with olive green mycelia, irregular edges on top and no visible reddish toxin (Carson *et al.*, 1997; Wang *et al.*, 1998).

Although two isolates have some differences in morphology and the production of cercosporin. They produce exactly the similar symptoms in maize. Group I is prevalent and predominates over *C. Zeina* throughout the maize growing areas of the eastern and Midwest regions in the USA, Latin America, China, India, Nepal, and Bhutan (Dhami *et al*., 2015). Group II species are confined to Africa and the Eastern US. (Crous *et al.*, 2006) found that Group II (*Cercospora Zeina*) is the causal agent of GLS in Southern Africa.

2.4. Disease Symptoms

Symptom expression depends on the genetic background of the genotype (Kim *et al.*, 1989). Genotypes with resistant gene express the fleck type of lesions due to resistance gene (Latterell and Rossi, 1983). Also, moderately resistant genotypes exhibit Chlorotic lesions

(Roane *et al.*, 1974) and necrotic spots displayed on the susceptible genotypes (Latterell and Rossi, 1983). The symptoms of GLS can be confused at earlier with symptoms of other foliar diseases particularly with southern leaf blight and northern leaf blight (Stromberg, 1986). The disease has two distinct features or symptoms: (1) Lesions may be occurred as grey to tan in color and are distinctly rectangular in shape (5-70 mm long by 2-4 mm wide), and (2) tan spot running parallel to leaf veins (Latterell and Rossi, 1983; Stromberg, 1986).

Generally, the fungus produces spores on the lower side of leaves and the spore-bearing structures may appear as small black specks. Early symptoms of infection include pinpoint lesions surrounded by yellow haloes. The early lesions are transparent when the leaf is held against the light while mature lesions are completely opaque (Latterell and Rossi¸1983). Leaf veins restrict pathogen growth and lesion width, but lesion width may vary with the distance between veins and proximity to other lesions (Dhami *et.al*, 2015). The lesions merge and kill entire leaves during favorable weather condition. The severe blighting of leaves and leaf sheaths are followed by stalk rotting and severe lodging, and premature death of leaves (Stromberg and Donahue, 1986; Stromberg, 2000). If the incidence and severity of disease are high during anthesis, the affected plants are fully dried but the ears have green husks, fresh silks, barren or partially filled ears and shrunken kernels (Manadhar, 2007).

2.5. Disease Cycle

Grey leaf spot caused by *Cercospora zeae-maydis* fungi overwinters as mycelium and stromata in infected maize residues left over the soil surface (Payne and Waldron, 1983). After harvesting maize fungus colonize residues and produces conidia and disease cycle starts in spring (Stromberg, 2000). The conidia disseminated to new corn plants by wind and splashing raindrops (Lipps, 1998). These newborn conidia provide primary inoculums to infect newly planted maize fields (Latterell and Rossi, 1983; Payne and Waldron, 1983). The spores (conidia) infect the lower leaves through the stomata and colonize leaf tissues. Conidia are produced from two to four weeks after initial leaf infection. Sporulation may be delayed in genotypes with moderate levels of resistance (Beckman and Payne, 1983).

During the dry part of summer, the fungus can remain dormant and then become active when conditions are favorable (Stromberg, 2000). The latent period of the pathogen is longer and can take as long as 14-28 days after infection for lesions to sporulate (Stromberg, 1986). In about two weeks, these lesions will generate new spores and produce appressoria over stomata before penetrating the host tissue. Secondary cycles of disease are initiated by conidia produced within the lesions. Prior to grain filling very few infection cycles occur because of the long latent period (Beckman and Payne, 1982).

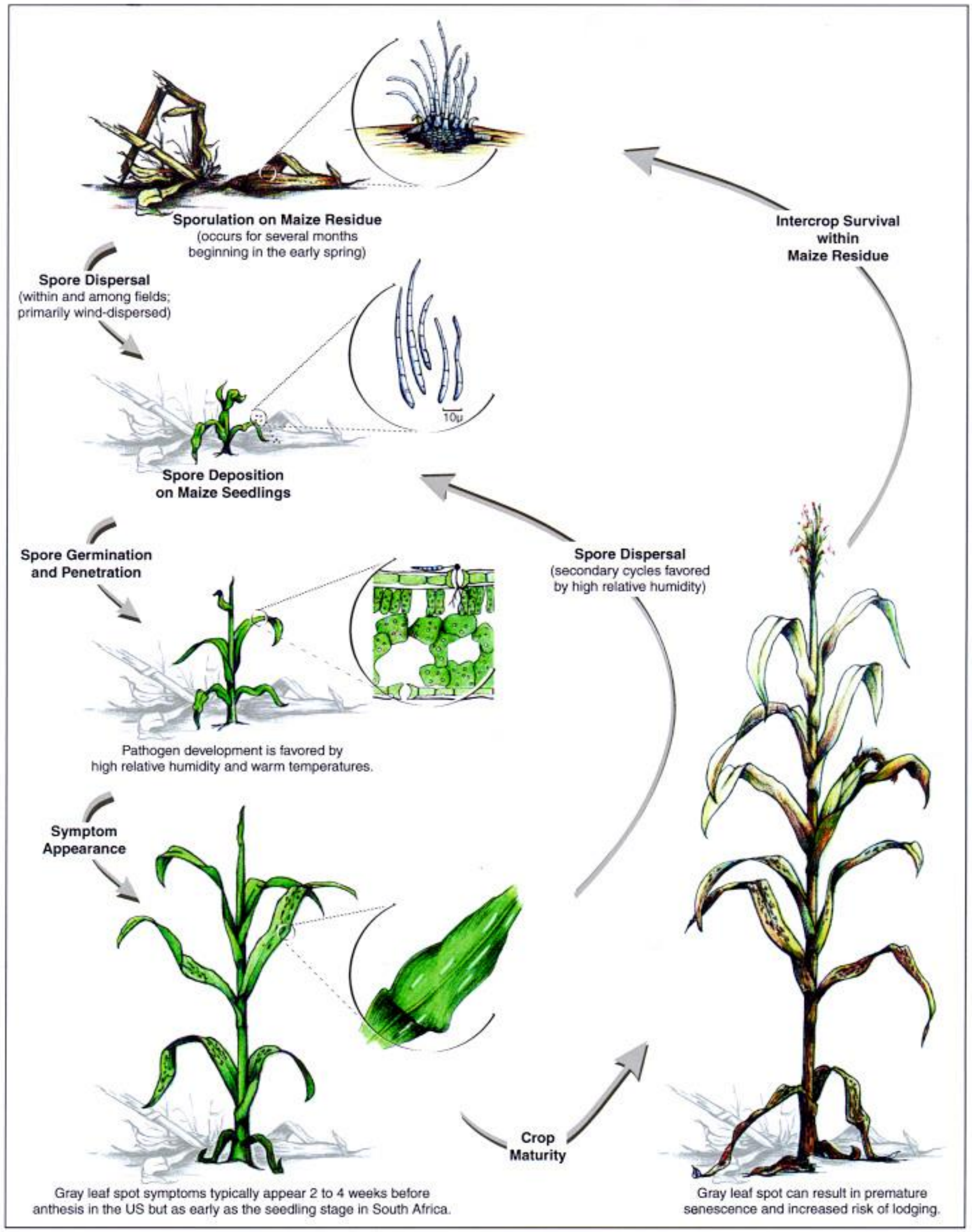

Figure 1. Disease cycle of Grey Leaf Spot in maize.

Source: (Ward *et al*., 1999)

2.6. Epidemiology and Factors Associated With Disease Development

The GLS epidemic primarily depends upon three factors that interact with time and space. The initial amount of inoculums, a rate of reproduction of the pathogen within a season and, a portion of healthy tissues remaining to be infected, high relative humidity, temperature (Stromberg, 2000). Wide adoption of minimum and conservation tillage and maize monoculture are equally important for GLS pathogen development (Ward *et al.*, 1999).

Results of studies conducted to determine the effects of moisture on pre-penetration events supports the idea that prolonged periods of high relative humidity, but not free water, are necessary for gray leaf spot development. Thorson and Martinson (1993) reported that germ tube elongation and appressorium formation were favored by extended periods of 95% relative humidity. Germ tubes were significantly longer at 95% relative humidity than at 90 and 80% relative humidity. Appressoria were formed at 95% relative humidity after 48 and 72 hrs of exposure, but none were observed at relative humidities less than 95%. The number of appressoria formed per germ tube increased as exposure time increased. When compared to 95% relative humidity, fewer, but larger appressoria were formed in the presence of free water.

Sporulation is high at 100% relative humidity and 25 °C-30°C temperature but the number of lesions and lesion expansion were not significantly different with >25 °C temperature (Paul *et al.*,2005). When the mean daily temperature dropped below 20 °C GLS was slow to develop (Ward, 1996; Nowell, 1997). Generally, disease severity increases during mid to late summer due to favorable conditions for lesions expansion (Paul *et al.*, 2005). Ward and Nowell (1998) reported that incidence and severity of disease are usually associated with the amount and distribution pattern of rainfall. Early rains favor the development of primary lesions.

In temperate regions maize monoculture, growing susceptible, local cultivars, plowing by locally fabricated plows and other biophysical factors favor pathogen development (Manadhar, 2007). Residues in neighboring fields may serve as a potential source of inoculums (De Nazareno *et al.*, 1993). Blowing wind in the dry season may facilitate the dissemination of the pathogen up to 80-160 km each year (Ward *et al.*, 1999). The deficiency of mineral nutrients may have a potential role in GLS epidemics. Gray leaf spot severity increases as the levels of nitrogen and potassium increase (Smith, 1989; Ward, 1996).

2.7. Disease Management

2.7.1. Crop rotation and cropping pattern

Maize is the only host crop this fungus is known to attack, therefore, rotation with the non-host crop for two years can reduce the disease inoculums effectively where the management of conservation tillage and field sanitation is equally important (Lipps, 1998; Wolf, 2002). Soybean and potato are the possible crops for rotation. Mixed cropping of soybean with maize, relay and intercropping of finger millet are widely used practices. Mixed or intercropping hinders air circulation inside the crop field which helps to increase relative humidity and favors disease development (Dhami *et al*., 2015).

2.7.2. Residues and weed management

The infected residue of a previous crop left over the soil surface is the principal source of inoculums. There was a strong positive correlation between the amount of infected maize residue and disease inoculums (Asea *et al*., 2002). They reported that disease intensity was higher in a high residue treated plot than a non-treated plot. The collection of stovers which are stacked in the field and near the homestead is a common practice. This practice may help to keep the field clean and reduce disease inoculums. But, the practices of using maize stalks for mulching, animal bedding, and undecomposed compost harbor and disseminate the inoculum. This is because the absolute rate of disease development increases as the amount of infested crop residue increases (Ward *et al*., 1977; Denazaro *et al.*, 1993; Ward *et al*., 1999). Weed management practices increase airflow within the crop canopy, reduce relative humidity and help limit the time period favorable for pathogen infection (Wolf, 2002).

2.7.3. Adjustment in time of planting

Most of the researchers reported that late planted maize is more affected than early planted maize because disease development is slow due to unfavorable environmental condition early in the season (Payne and Waldron, 1983). They also suggested planting early maturing cultivars earlier in the season to minimize the yield loss. The late planted maize tended to develop more severe GLS because the plants experienced initial infection at earlier stages and there was a greater opportunity for multiple cycles of infection before the plants reached their physiological maturity (Stromberg and Donahue, 1986; Bhatia *et al.*, 2002). Early maturing cultivars escape from disease because plants face the first cycle of infection at physiological maturity stage.

2.7.4. Selection and development of tolerant cultivars

Genetic resistance is the most cost-effective strategy for managing gray leaf spot, and numerous studies have been conducted to improve hybrid resistance. The development of locally adapted tolerant cultivars enhances the durability of resistance (Ayers *et al*., 1984; Nowell, 1997). The breeder should practice selecting the tolerant genotypes from adapted germplasm based on yield potential and standability under disease pressure. Plants with mild symptoms of the disease and good yield at maturity will have the highest tolerance (Kim, 2000).

The incorporation of new genotypes, either local or exotic, in the evaluation of a breeding program increases the availability of genes for resistance that were not previously available. Improved open-pollinated varieties should be crossed with GLS resistant materials either locally developed or introduced (Elwinger, *et al*., 1990). As the inheritance of GLS resistance is mainly quantitative in nature, the frequency of resistant alleles in a population can be increased by population improvement techniques. Recurrent selection can be an effective method to incorporate and accumulate the resistant genes in elite breeding materials if several genes with additive gene action are involved (Goodman, 1999).

2.7.5. Use of fungicides

Fungicides have been used to delay the development and severity of GLS in the United States (Stromberg, 1986). However, only surface protectant, non-systemic fungicides have until recently been registered for use on commercial maize in the United States and must be applied as a series of preventive treatments at 7- to 10-day intervals for effective disease control (Thorson, 1989). The cost of fungicide and its application is therefore high and, for this reason, chemical control of GLS on commercial maize crops is not an acceptable method of control (Lipps & Pratt, 1989). However, in years of highly favorable for disease spread, chemical control may be a way of preventing excessive yield losses. Hybrid maize seed is a commodity of high economic value and seed producers often apply fungicides to reduce yield losses due to various foliar fungi (Rivera-Conales, 1993). Rivera-Conales (1993) reported that application of propiconazole, a systemic fungicide has proven efficacy in the control of foliar diseases of maize and was subsequently registered for use in maize. Propiconazole, however, may only be applied until the tassel stage of development. It could be more economically attractive than protectant fungicides, as fewer spray treatments are necessary.

In South Africa, systemic fungicides are registered for the control of GLS on maize and, having a "curative" action, can be applied after the onset of the disease, to provide cost-effective control. The object of the fungicide programme is to delay the rate of disease development until the grain is physiologically mature. The effectiveness of the programme depends on the correct timing and application of sprays and when correctly carried out, the programme is cost-effective (Ward, *et al.*, 1997). Chemical control of GLS in South Africa has become widely accepted by farmers in areas in which GLS is a problem, and until resistant hybrids are 25 developed for commercial use, fungicides are likely to be widely used (Ward &Nowell, 1997).

3. MATERIALS AND METHODS

3.1. Survey of Grey Leaf Spot of Maize

3.1.1. Description of the Survey Area

The survey covered the most important maize growing zones of West-Shewa, East Wollega and West-Wollega Zone of Oromia Regional administration which covered 9 districts namely Dano Ilu-Gelan, Bako-Tibe, Gobu-Seyo, Sibu-Sire, Leka-Dulecha, Gimbi, Lalo-Asabi, Ayra-Guliso and a total of 27 PAs each district having 3 PAs (Table 1). Similarly, agro-ecology of the study area was also summarized below in Table 2.

Table 1. Description of the study site

Zone	District	PAs / Locality
West-Shewa	Dano	Haro Jibat, Dirre Hareyyu, Danno-Shenan
	Ilu-Gelan	Abako-Anno, Refiso-Kamino, Guba Washemo
	Bako-Tibe	Oda-Korma, Tullu-Sengota, Gajo Kuyi
East-Wollega	Gobu-Seyo	Sombo-Kejo, Agolaften, Ongobo-Bekenisa
	Sibu-Sire	Cheri-Jarso, Felamo-Yubdo, Lalisa
	Leka-Dulecha	Badh’oo, Gudina, Halle-Kewisa
West-Wollega	Gimbi	Gerjo-Siban, Harrojji-Sardo, Lelisa-Yesus
	Lalo-Asabi	Harojji-Harowwa, Abba-Odo, Mogga
	Ayra-Guliso	Ketta, Wayyu-Koli, Seda-Birbir

Table 2. Geographical and climatic description of the study area

Zone	District	Location (Geographical co-ordinates)	Altitude (m.a.s.l)	Annual Rainfall (mm)	Mean T (^{O}C)	
					Max	Min
West-Shewa	Dano	8° 43' 13.46"N to 8° 52'50.57"N 37° 17' 3.76"E to 37° 21' 5.46"E	1600-2600	1200-2300	28	22
	Ilu-Gelan	8°99’90”N and 37°32’98”E	1500-2200	1400-2300	27	13
	Bako-Tibe	9° 1' 49.91"N and37° 12' 21.19"E	1600-2800	1000-1200	27.2	13.2
East-Wollega	Gobu-Seyo	9°3'52.65"N to 9°9'24.74"N 36°2'33.23"E to 36°59'45.34"E	1650-2300	1200-2400	27	18
	Sibu-Sire	9°2'25.06"N to 9°5'27.079"N 36° 47' 57.73"E to36°55'3.33"E	1700-2300	1350-2300	28	16
	Leka-Dulecha	8°51'17.56"N to 8°59'9.06"N 36°28'30.81"E to36°29'37.11"E	1850-2800	1500-2600	26	12
West-Wollega	Gimbi	9°11'22kk.61"N to 9°14'0.37"N 35°42'21.88"E to 35°46'45.66"E	1300-2600	1200-1800	27	18
	Lalo-Asabi	9°10'9.67"N to 9°15'41.84"N 35°30'30.21"E to 35°42'4.11"E	1450-2950	1750-2200	30	25
	Ayra-Guliso	9°6'49.60"N to 9°10'9.67N 35°24'13.80"E to 35°33'22.33E	1500-1750	1000-2000	28	13

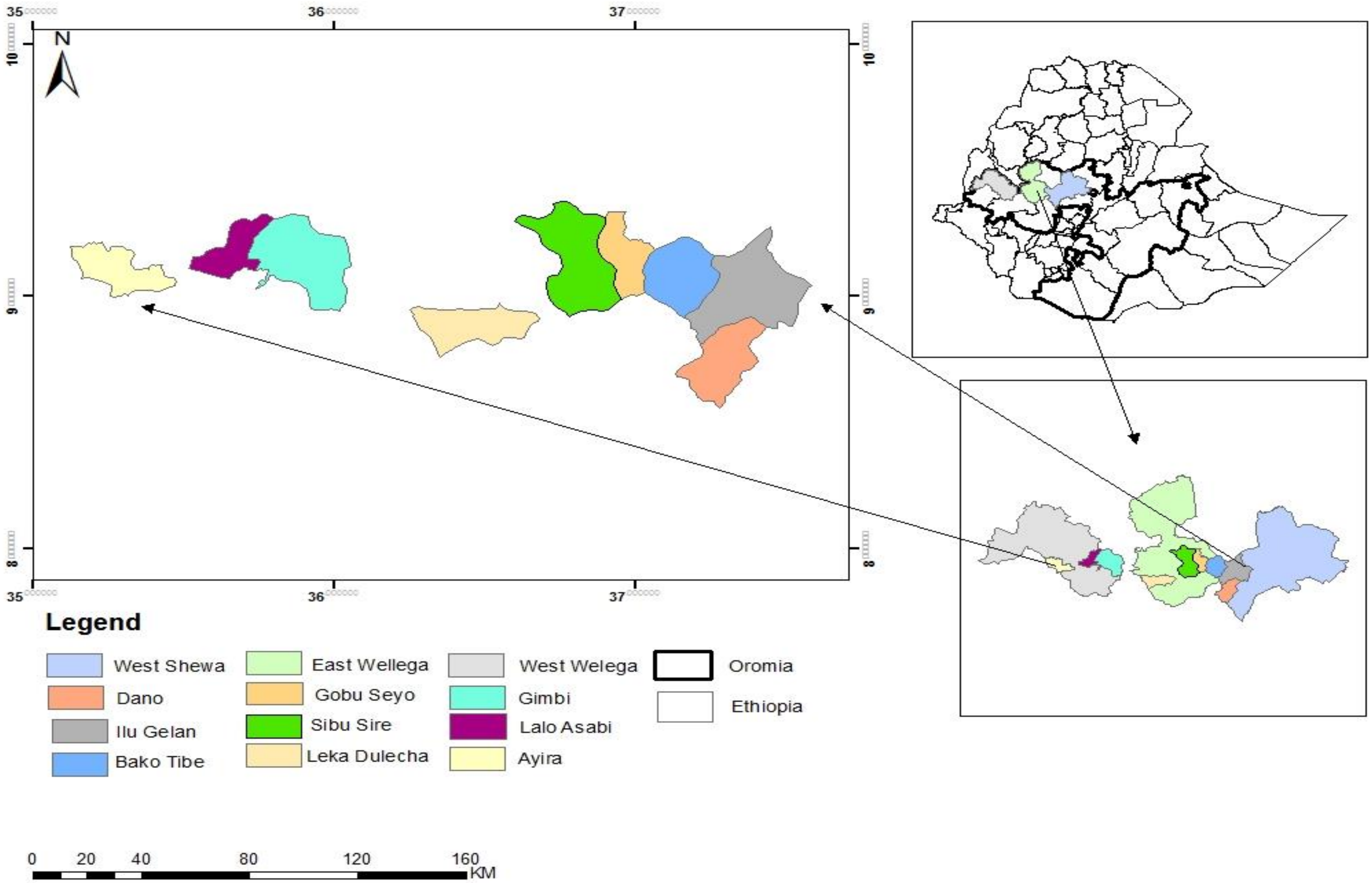

Figure 2. Map of survey areas of Grey Leaf Spot of maize in Western Oromia, Ethiopia in 2017.

3.1.2. Sampling and Sampling Techniques

Purposive multistage sampling method was used to reach the Peasant Associations (PAs). Potential districts were selected with in collaboration with Zonal experts, whereas particular PAs were selected with experts from each district based on the potential to produce maize crop and accessibility. Maize fields were selected through random sampling technique with frequent stopping at 3-5 km intervals depending on the variability of fields in terms of altitude and cropping systems (Kinyua *et al*., 2010). The survey route followed major roads and accessible roadside to towns and localities in each zone and district across different altitude. Five representative maize leaf samples with symptoms recognized as those of GLS (principally, pale brown or grey to tan, long narrow streaks that become dark, grayish-brown rectangular lesions as the disease develops) per field were collected along the survey routes. A total of 81 fields were assessed from 27 PAs 3 farms from each PAs and 155 leaf samples were collected from 31 fields those revealed GLS disease symptom.

Samples were air dried and kept in a pressing board after covered with newspaper in the form of the herbarium. Information like Zone, district, PAs, sample code, farm number, name of the collector, date of collection, were written on the label paper and attached to the margin of folded newspaper used to cover the leaves sample (Lyimo *et al*., 2013). Samples were transported to Jimma University Plant Pathology Laboratory and kept in a refrigerator at 4°C for further isolation and identification.

3.1.3. Disease Assessment

For the assessment of disease incidence and severity, the fields were selected randomly, and the incidence and severity of GLS on maize plants were assessed. A total of 30 plants per field were assessed by visual scale moving in "W" pattern in the field (Cardwell *et al.*, 1997). Severity of GLS on maize leaf was assessed by using a 1-5 visual disease rating scales in all fields, where: 1 = no symptoms (Very slight to slight infection, one or two to few Scattered lesions on lower leaves); 2 = moderate lesion development below the leaf subtending the ear; 3 = heavy lesion development on and below the leaf subtending the ear with a few leaves, 4=severe lesion development on all but the uppermost leaves, which may have a few lesions; and 5 = all leaves dead (Maroof *et al*., 1993).

Source: https://image.slidesharecdn.com/breedingfordiseaseresistanceinmaize

Figure 3. A visual scale to estimate the severity of Grey Leaf Spot of maize

3.1.4. Data Collection

3.1.4.1. Disease Incidence

During the field survey, incidences, visual scale of severity, information of the field such as GPS readings (Altitude, Latitude, and Longitude) were recorded. Additionally, farming practices, maize varieties, previous cropping history, and management practices like weed management were recorded through visual observation and by interviewing the farmers.

Disease incidence was computed as a percentage of infected maize plants out of total maize plants in the sample plot shown as follow:

$$\text{Disease Incidence}(\%) = \frac{\text{Number of diseased plants}}{\text{Total Number of Plants inspected}} \text{X}100$$

3.1.4.2. Disease Severity

During field survey, data on disease severity were recorded by evaluating the field using 1-5 disease recording scale in all assessed fields of the districts.

The numerical rating was converted to percentage severity index (PSI) using the following equation suggested by Wheeler (1969).

$$PSI = \frac{\text{Sum of all Numerical rating}}{\text{Total Number of rated piants X Maximum disease score on scale}} X100$$

3.1.4.3. Disease Prevalence

The prevalence of the disease was measured by using the number of fields affected divided by a total number of fields assessed and expressed in percentage:

$$\text{Disease Prevalence (\%)} = \frac{\text{Number of infected fields}}{\text{Total number of fields assessed}} x100$$

3.1.5. Data analysis

The survey data on incidence and severity index of GLS were analyzed using three-stage nested design by nesting districts in Zone, peasant Association (PAs) within districts and farms within PAs with the following model to see their interaction.

$$y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)}$$

Where: y_{ijk} is the GLS disease intensity where peasant association *k* is nested within district *J* nested within zone *i*, μ is the overall mean, τ_i is the effect of the i^{th} zone, $\beta_{j\,(i)}$ is the effect of the j^{th} district within the i^{th} zone, and $\gamma_{k(ij)}$ is the effect of the k^{th} peasant association within the j^{th} district and i^{th} zone, and $\varepsilon_{l(ijk)}$ is the error term.

Analysis of variance (ANOVA) was performed using SAS V 9.2 statistical (SAS 2008). Means were separated using LSD t-test at significance levels of 0.05. The associations of GLS disease incidence and severity with altitude, maize variety, and previous crop were computed using Pearson correlation analysis. In addition, the current extent and the distribution of GLS isolates across Western Oromia region were mapped by using Arc GIS software 10.1 from the collected GPS coordinates.

3.2. Morphological and Cultural Characterization

3.2.1. Isolation, Identification, and Characterization

3.2.1.1. Isolation and Culture of *Cercosporra zeae-maydis* Isolates

A total of 155 leaf samples obtained from the study area were air dried and thereafter kept in a refrigerator at 4 °C. Three lesions were cut out from each leaf sample, surface sterilized using 5% sodium hypochlorite solution for 30 Sec and rinsed three times in sterile distilled water, placed on moist filter paper in a Petri dish and incubated for 5 days to stimulate sporulation (Asea *et al*., 2005) (Appendix Figure 3). Conidia were dislodged from the lesions by adding sterilized distilled water and hand shook. Samples were placed on 3% Water Agar plates and incubated for 48 hours. Mono-conidial cultures of the isolates were then established by sub-culturing germinated distinct colony characteristics of *C. zeae-maydis to* fresh potato dextrose agar plates amended with 250 mg/500 ml chloramphenicol when the medium cool down to 50 °C. Then the culture-dishes were sealed using parafilm and labeled and incubated at 25 °C for 7 days under alternative 12hrs dark and white light to allow the pathogen to grow and sporulate (Beckman and Payne, 1983). The isolates were sub-cultured from single conodium on sterilized PDA medium using hyphal tip transfer from distinct colonies with three replications using Completely Randomized Design (CRD). These hyphal tip purified isolates were used for examination of morphological and microscopic characterization.

3.2.1.2. Characterization of *Cercospora zeae-maydis* Isolates

Characterization of *Cercospora zeae-maydis* isolates was done by grouping the culture isolates, after carefully observing the colony features (growth, a color of the top and reverse side) of the culture plates and comparing with the color illustrated on RGB chart (Anonymous, 2013) (Figure 6). The colony shape and elevation were also characterized, and isolate grouping was done and colonial growth diameter was tested on both PDA and Malt

Extract Agar (MEA). Similarly, specimen from cultures plate was taken and diluted with sterile water and drops of the suspension and placed on slides and put under a microscope for further identification of the conidia. Finally, identification of macro and micro-conidial features of *Cercospora zeae-maydis* such as conidial shape, and septa per conidia were morphologically examined at 400x magnification using a compound microscope. The purified isolates were kept at 5 °C and sub-cultured at some intervals onto fresh PDA medium to maintain fungal viability (Kinyua *et al.*, 2010).

3.2.2. Data collection and Analysis

Data on colony/mycelial growth such as color (pigmentation) shape (form), elevation and margin were recorded, and the colony diameters of every culture of the isolates were recorded. Similarly, conidial shapes/forms, and a number of septa were recorded following (Crous *et al.*, 2006) manual on species of *Cercospora zeae-maydis* was used. Analysis of variance (ANOVA) was performed using SAS V 9.2 statistical SAS (2008). Means were separated using LSD t-test at significance levels of 0.05.

3.3. Pathogenicity Test

3.3.1. Soil Media Preparation and Maize Variety

A greenhouse experiment was conducted at JUCAVM to evaluate the pathogenicity of *C. zeae-maydis* isolates collected from the major maize growing areas of Western Oromia region. A mixture of sterilized top soil, manure, and sand at a ratio of 2:1:1 v/v was used to fill the pots and then placed in a greenhouse. BH540 maize variety that is moderately susceptible to the pathogen was used in the study (Wegary *et al*.,2008; Bekeko *et al*., 2018). Prior to sowing, seeds were washed under a running tap water, then disinfected by 75% ethanol for 30 seconds and finally rinsed twice in distilled sterilized water. Two seeds per pot were sown at 5 cm depth in 20 cm diameter with 0.01256 m^3 volume capacity plastic pots with soil which was thinned to one plant per pot later. Complete Randomized Design (RCBD) with five replications having *Cercospora zeae-maydis* isolates as treatments were used and DAP fertilizer was applied at the rate of 1.5 g per pot during planting and UREA at the rate of

2.5g per pot during four leaves growth stage (Lyimo *et al.*, 2013). Agronomic practices like watering the pots and weed management were carried out on time.

3.3.2. Preparation of Inoculums

Five identified and characterized *Cercospora zeae-maydis* isolates were re-cultured on Potato dextrose agar and the plates were incubated for 21 days at 25 ºC for sporulation. Thereafter, 10 ml of sterilized distilled water was poured onto each plate and the conidia were dislodged from the surface of the agar with the help of a sterile spatula and then filtered through 2 layers of sterile cheesecloth. Quantification of conidia was made prior to the inoculation by using hemocytometer (Mod-Futchs Rosenthol Cristalite double cell 85748 Weber) model and was adjusted to the final concentration of 5×10^4 conidia per ml and was kept in tightly covered 50 ml Falcon tube at 4 ºC awaiting for inoculation (Bair and Ayers,1986).

3.3.3. Inoculation and Inspection of symptoms development

Each of maize plant was inoculated at the V4 growth stage (three weeks after emergence) of the plant at the level of 1 ml of conidia per plant (Ritchie *et al.*, 1989). Hand sprayers were used to apply the conidial suspension while puncturing the whorl was done using a hypodermal syringe. The control maize plant for this experiment was wounding plants and sprayed with sterilized distilled water without adding the inoculums (Asea *et al.*, 2005).
Thereafter, the inoculated plants were placed in the plastic chamber (250 cm length × 150 cm width × 120 cm height) constructed using polythene sheets and plastic tubes for 72 hours. Each Isolate was isolated by plastic not to mix-up one isolate with other (Beckman and Payne, 1983; Bair and Ayers, 1986). wet sacks made of jute spread on the floor was done to keep relative humidity ≥ 95 % and temperature 28 ºC under the chamber for the successful infection of the pathogen by watering daily, in the morning and in the late afternoon.

Leaf spot due to the infection of inoculated *Cercospora zeae-maydis* isolates were carefully inspected at 21, 28, 35, 42, and 49 days after inoculation. The severity caused by each isolate was determined and finally, re-isolation was done to fulfill the Koch's postulate.

3.3.4. Data collection and Analysis

Under greenhouse, disease symptoms were observed 21 days after inoculation, and disease severity was recorded every 7 days starting from 21 days after inoculation (DAI), subsequently for five times on 21, 28, 35, 42, and 49 DAI.

Pathogenecity of *Cercospora zeae-maydis* isolate was quantified as disease severity index and area under the disease progress curve (AUDPC) (Campbell and Madden, 1991) were computed as follows:

$$AUDPC = \sum_{i=1}^{n-1}\left[\frac{(x_{i+1} + x_i)}{2}\right] * [t_{i+1} - t_i]$$

Where xi is the cumulative disease severity expressed as a proportion at the i^{th} observation, ti is the time (days after inoculation) at the i^{th} observation and n is a total number of observations. AUDPC values were then used in the analysis of variance (ANOVA) to compare amounts of disease among isolates. Logistic equation, ln [(Y/1-Y)], (Van der Plank, 1963; Madden *et al.*, 2007) was used for estimation of infection rate from each treatment.

The severity and AUDPC data were used to determine the pathogenesity of *Cercospora zeae-maydis* isolates (Shaner and Finney, 1977). The general linear model (GLM) procedure of RCBD design was used to perform AUDPC, infected leave severity data analysis using SAS 9.2 version statistical software (SAS 2008) using the following model. Means were separated using the LSD test at significance levels of 0.05.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where: Y_{ij} is the response (AUDPC) for treatment i observed in block j, μ is the overall mean, α_i is the effect of the j^{th} block, β_j is the effect of the i^{th} treatment, ε_{ij} is the error term for the i^{th} treatment in the j^{th} block.

4. RESULTS AND DISCUSSION

4.1. Disease Intensity, Distribution of Maize GLS and effect of different variables

4.1.1. Disease Intensity and prevalence of GLS at Zone level

The study revealed that Grey leaf spot disease was found in all assessed zones of Western-Oromia. Statistically, there was highly significant ($p<0.001$) difference among districts in terms of maize grey leaf spot incidence and severity. Grey Leaf spot of maize was observed with an incidence ranging from 0–76.67% in West-Shewa zone, 0–80% in East Wollega zone, and 0–70% in West-Wollega zone. The highest mean incidence of Grey leaf spot disease was recorded in East Wollega zone (35.06 %) followed by West-Shewa zone (12.09%). The minimum mean incidence was observed in West Wollega zone with 9.51%. Disease Severity index of Grey leaf spot was observed to be in the range (0-72%) in west Showa zone, whilst (0-79.67%) in East Wollega and (0-74.84%) in West Wollega zone. The highest mean severity index (31.43%) was recorded in East Wollega Zone followed by West Showa zone having 11.98% severity, whereas the minimum severity index was recorded in West Wollega zone with 10.05% (Table 3).

Grey Leaf Spot disease prevalence across Zones showed significant differences with the highest value in East Wollega Zone with 62.96 % followed by West-Shewa Zone with 29.63% disease prevalence while the lowest was recorded in West Wollega Zone with 22.22% of GLS disease prevalence (Table 3).

Table 3. Mean disease Incidence and Severity of GLS of maize across study zones in 2017

Zone	Prevalence (%)	Mean Incidence	Mean Severity
West Showa	29.63	12.09^{b}	11.98^{b}
East Wollega	62.96	35.06^{a}	31.43^{a}
West Wollega	22.22	9.51^{b}	10.05^{b}
LSD		3.41	1.67
CV (%)		75.4	64.39

LSD= Least significance difference Figures followed by the same letter are not significantly different at 0.05.

4.1.2. Disease Prevalence and Intensity at district level

The highest GLS disease prevalence of 88.9% was found in Leka-Dulecha district followed by Gobu-Seyo district with 66.7% prevalence. Also, the medium prevalence were recorded in Dano, Sibu-Sire, and Lalo-Asabi with 44.44%, 33.33%, and 33.33%, respectively. On the other hand, less GLS prevalence was recorded in Ilu-Gelan, Gimbi, Ayra-Guliso, and Bako-Tibe districts respectively having 22.2%, 22.2%, 22.2%, and 11%. (Table 4). The variation in the disease prevalence among the districts is due to the variation in agro-ecology. For instance, Leka-Dulecha had the highest altitude range which ranges from (2031–2422 m.a.s.l) which associated with high rainfall and also farming practices enhanced the disease. Other things kept constant, the use of specific maize variety had its own effect as disease prevalence is minimum with shone and Limu maize varieties as compared to others.

The prevalence of high incidence of grey leaf spot in cool humid high-altitude areas had also been reported (Lipps 1995; Asea *et al*. 2005). Incidence ranges of GLS in maize fields were varied from 0–50%, 0–60% and 0–76.7% in Dano, Ilu-Gelan, and Bako-Tibe districts of West-Shewa zone, respectively. Similarly, Gobu-Seyo, Sibu-Sire and Leka-Dulecha districts of East-Wollega zone had shown incidences that range from 0–80%, 0–46.7% and 0–73.3%, respectively. Also, districts of West-Wollega zone namely Gimbi, Lalo-Asabi, and Ayra-Guliso had an incidence ranging from 0–6.7%, 0–40% and 0–70%, respectively (Table 4).

Moreover, the highest mean incidence of GLS in West-Shewa zone was recorded at Ilu-Gelan with 15.2%, followed by Dano (10.74%), whereas the smallest mean incidence was recorded in Bako-Tibe district with 10.37%. In East-Wollega zone, Gobu-Seyo district had the highest mean incidence of 48.15%, followed by Leka-Dulecha district with 47.77%, whereas the minimum mean incidence was recorded in Sibu-Sire district with 9.26%. Similarly, in West-Wollega, the highest mean incidence of 17.41% was recorded in Ayra-Guliso followed by Lalo-Asabi (10%), but the minimum mean incidence of 1.11 % was found in Gimbi district (Table 4). The difference in disease intensity and prevalence among zones and districts could be due to the differences in environmental factors (altitude, temperature, humidity), and farming practices at the study area.

Those districts with comparatively high disease incidence and severity index were practicing monoculture under high relative humidity. For instance, in Leka Dulecha and Gobu-Seyo the farmers were mainly practicing using BH660 maize variety which is relatively with high disease intensity. On the other hand, in Ilu-Gelan and Bako-Tibe farmers were mainly practicing using Shone and Limu maize varieties. De Nazareno *et al*., (1993) also reported that when the weather conditions were favorable for the development of the disease, there was a significant positive relationship between the relative humidity and disease severity.

Disease Severity index of Grey leaf spot was observed with a range of 0 – 72% in West-Shewa zone, 0–79.67% in East-Wollega and 0–74.84% in West-Wollega zone. The highest mean severity index was recorded in East-Wollega zone with 31.43% followed by West-Shewa zone having 11.98%, whereas the minimum severity index was recorded in West-Wollega zone with 10.05% (Table 4). The range of disease Severity of Grey Leaf Spot of Maize was varied from 0–65%, 0–57.5%, 0–72.8% in Dano, Ilu-Gelan Bako-Tibe districts of West-Shewa zone, respectively, whereas in Gobu-Seyo, Sibu-Sire, and Leka-Dulecha of East-Wollega zone it varied from 0–79.7%, 0-48.3%, and 0–74.8%, respectively. Similarly, in West-Wollega zone, Gimbi, Lalo-Asabi, and Ayra-Guliso districts disease severity ranges from 0–8%, 0–48% and 0–74.9%, respectively (Table 4).

The highest mean disease severity of Grey Leaf Spot in West-Shewa zone was recorded at Dano with 16.33% value followed by Ilu-Gelan with 12.00% whereas the smallest mean incidence was recorded in Bako-Tibe district with 7.59%. In East-Wollega zone Leka-Dulecha district the mean GLS disease severity was the highest with 46.11% followed by Gobu-Seyo with mean severity value of 39.15%, whereas the minimum disease severity was recorded in Sibu-Sire district with 9.37%.

Similarly, the highest mean severity index in West-Wollega zone was recorded in Ayra-Guliso with 16.68% followed by Lalo-Asabi with 11.75% whereas severity the minimum value in Gimbi district with 1.78 % (Table 4). Also percent of disease severity index of the districts were showed on (Figure 5) with their respective level. Accordingly, Leka-Dulecha and Gobu-Seyo districts were grouped under high severity index, whereas Dano, Ilu-Gelan, Lalo-Asabi and Ayra-Guliso were under moderate severity index. On the other hand, Bako-Tibe, Sibu-Sire and Gimbi districts were under lower disease severity index (Figure. 5).

Those districts with maximum disease incidence and severity index had relatively high relative humidity, less crop rotation, and use of susceptible maize varieties as compared to those districts recorded with the minimum. Results from various surveys conducted in most maize growing regions of Ethiopia indicated that the disease has a wide distribution and significant impact on maize yield reduction on both local and improved susceptible varieties (Tadesse, 2008). Survey of maize diseases in Western and Northwestern Ethiopia by Tefferi (1999) showed that disease incidence and severity were relatively high. Similarly, in the U.S.A. (Iowa), the epidemic of grey leaf spot is severe under monoculture maize with no rotation practices and minimum tillage practices (Perkins *et al*., 1995).

All of the surveyed districts had shown severity ranges from mild to moderately severe grey leaf spot infection indicating the potential of the disease in hindering maize productivity. Agro-ecology of the assessed areas was conducive to the current disease prevalence and intensity. Comparatively warm temperature and high rainfall could give rise to high relative humidity which results in a conducive environment for the pathogen epidemic and enhance disease intensity. Reportedly, warm temperature, well-distributed rainfall, and high relative humidity are weather conditions favoring this disease development (Beckman and Payne 1983).

Table 4. Disease Prevalence, incidence and Severity index of Grey Leaf spot across the study area in 2017

Districts	Altitude Range	Prevalence (%)	D I (%)		SI (%)	
			Range	Mean	Range	Mean
Dano	1630 – 2131	44.44	0-50	10.74[cd]	0-65.0	16.33[cd]
Ilu-Gelan	1685 – 1923	22.22	0-60	15.19[bc]	0-57.5	12.00[cde]
Bako-Tibe	1657 – 1900	11.11	0-76.7	10.37[cd]	0-72.8	7.59[e]
Gobu-Seyo	1646 – 1959	66.67	0-80	48.15[a]	0-79.7	39.15[b]
Sibu-Sire	1668 – 1983	33.33	0-46.7	9.26[d]	0-48.3	9.37[e]
Leka-Dulecha	2031 – 2422	88.89	0-73.3	47.77[a]	0-74.8	46.11[a]
Gimbi	1846 – 1920	22.22	0-6.7	1.11[e]	0-8.00	1.78[e]
Lalo-Asabi	1550 – 1852	33.33	0-40	10.00[cd]	0-48.0	11.70[de]
Ayra-Guliso	1543 – 1631	22.22	0-70	17.41[b]	0-74.9	16.68[c]
LSD (0.05)				5.91		4.76
CV				74.43		64.39

LSD=Least significant difference DI=disease incidence SI=severity index

CV=coefficient of variance

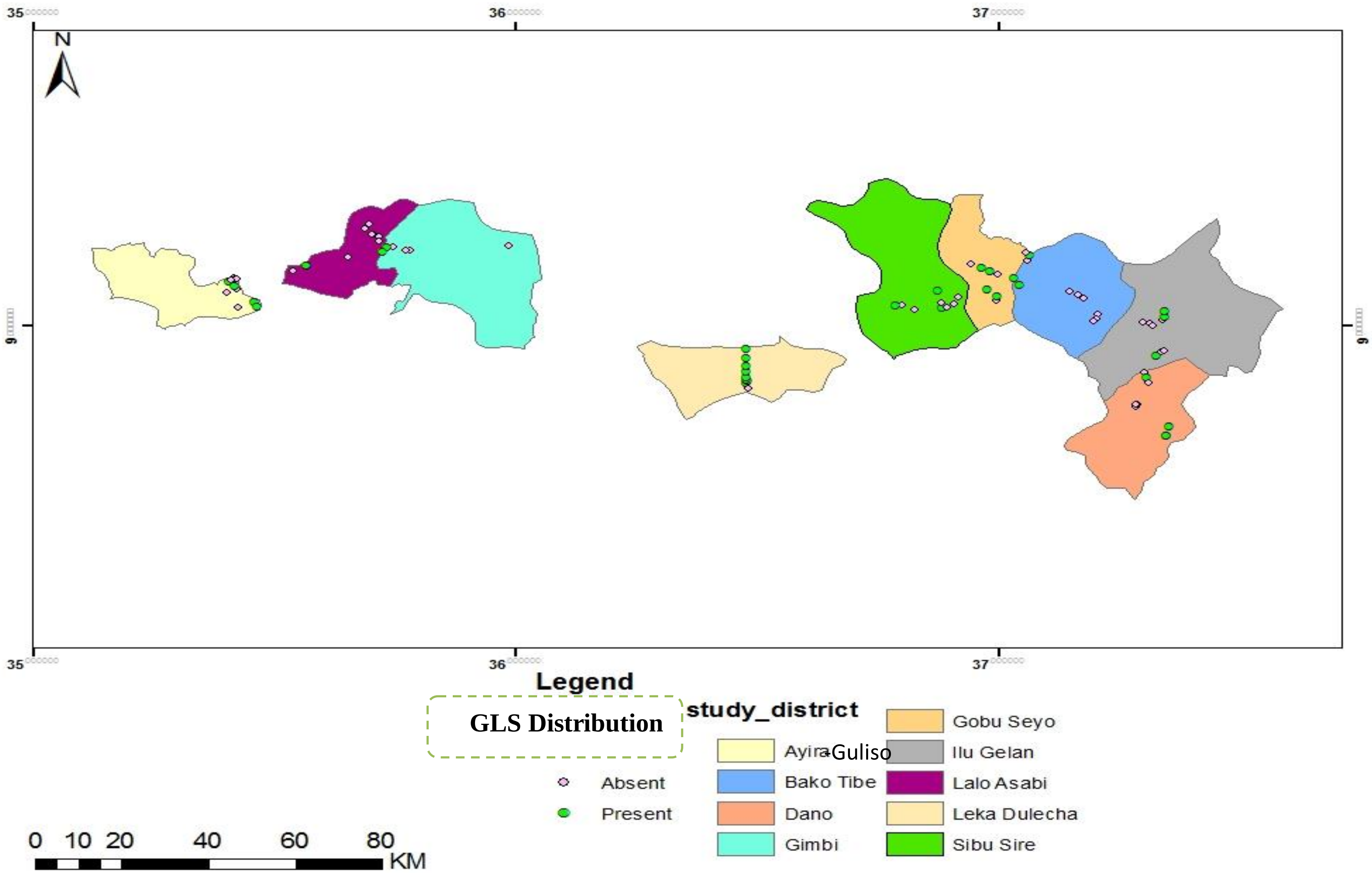

Figure 4 Map of disease distribution of study area of Grey Leaf Spot of maize in 2017

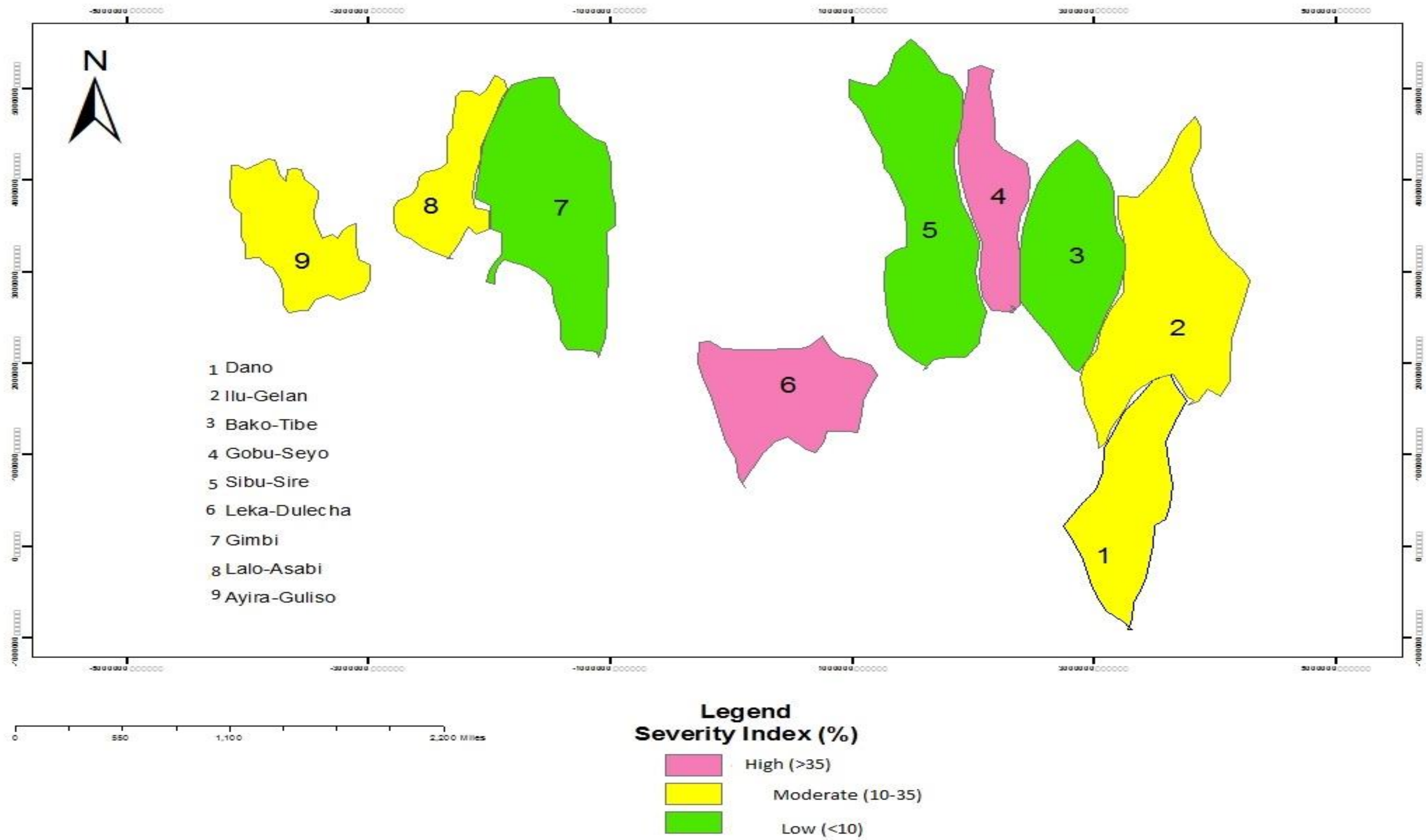

Figure 5 Map of Disease Severity of Grey Leaf Spot of Maize across surveyed districts

4.1.3. Effects of different practices on disease incidence and severity of GLS

4.1.3.1. Effect of Farming Practices

The result from the study revealed 54.32% of the farmers were practiced monoculture, whereas 38.27% were practiced crop rotation. Similarly, 4.94% of the farmers practice intercrop with rotation whereas, 2.47% were practice fallow. The highest disease incidence and severity index was recorded on monoculture farming practices with 35.00% and 47.50%, respectively, followed by intercropping having 20.80% and 21.26% respectively. The minimum disease incidence and severity index was recorded in fallow practice with (0.00%) for both (Table 6). The increase in disease intensity on monoculture practice was due to the high inoculums developed in the field year after year whereas the decrease of the disease intensity in the rotation and fallow practices were due to maize is the only host crop this fungus is known to attack. Grey leaf spot of maize is known to be pathogenic only to maize; rotation of the non-host crop for two years can reduce the disease inoculums effectively (Latterell and Rossi, 1983). Cropping methods such as mono or inter-cropping and use of cultivar mixture are also recognized to supply to disease pressure in positive or negative ways (Agrios, 2005). Similarly, mixed or intercropping also important in disease intensity because it hinders air circulation inside the crop field which helps to increase relative humidity and favors disease development (Wolf, 2002; Dhami *et al*., 2015).

Table 5. Mean of disease incidence and severity index across farming practices

Variable	Class	% Farms	Incidence (%)	Severity Index (%)
	Monoculture	54.32	35.00^{a}	47.50^{a}
Farming Practice	Intercrop	38.27	20.80^{ab}	21.26^{b}
	Rotation	4.94	11.33^{b}	12.36^{b}
	fallow	2.47	0.00^{b}	0.00^{b}
	LSD		22.05	21.76

LSD=Least significant difference Figures followed by the same letter are not significantly different

4.1.3.2. Effect of Maize Varieties

During the assessment, there were about nine hybrids and one local maize variety grown over the surveyed districts. Accordingly, Limu variety had the highest coverage with 35.80% followed by BH661 variety having 30.86% of the total assessed farms. Shone and BH660 with 14.81% and 9.88% had the medium coverage, whereas BH140, BH543, BH545, BH546 and Local maize varieties with 1.23% each, had the minimum coverage under the surveyed districts (Table 4). The highest disease incidence and severity index was recorded on BH545 (70% and 79.67%), BH546 (80% and 68%), BH540 (63.33% and 61.07%), BH140 (76.67% and 73.67%) and local maize varieties with (70 and 74.87%), respectively. On the other, the minimum disease incidence and severity index were recorded on Limu with (2.29% and 2.77%), respectively (Table 7). This result implies that there is a great variation among maize varieties in reaction to the pathogen which could be due to genetic variation within varieties. Increased incidence of GLS has been associated with the continuous cultivation of maize, and use of susceptible maize cultivars (Gevers *et al*., 1994; De Nazareno *et al*., 1993; Wegary *et al*., 2008). The responses of some commercial varieties were showed different resistance and susceptibility level among different maize hybrids (Wegary *et al*., 2004; Ward *et al*., 1996). Similarly, according to field survey report, Tefferi (1999) of all released hybrid maize, only BH-660, and PHB-30H83 were found relatively tolerant to grey leaf spot.

Table 6. Mean of DI and DSI of GLS across assessed maize varieties.

Maize Variety	Farm (%)[a]	DI (%)	DSI (%)
Limu	35.80	2.29 [d]	2.77[d]
BH660	30.86	26.67[cb]	27.52[bc]
Shone	14.81	10.30[cd]	10.12[cd]
BH661	9.88	13.75[cbd]	19.13[cd]
BH540	2.47	63.33[a]	61.07[a]
BH543	1.23	33.33[b]	39.33[b]
BH545	1.23	70.00[a]	79.67[a]
BH546	1.23	80.00[a]	68.00[a]
BH140	1.23	76.67[a]	73.67[a]
Local	1.23	70.00[a]	74.87[a]
LSD (0.05)		19.9	20

DI=disease incidence DSI=severity index BH=Bako Hybrid [a] =of total farms assessed
Figures followed by the same letter are not significantly different at 0.05.

4.1.3.3. Effect of Previous crop history

In surveyed districts majority of the farmers (51.85%) grow maize after maize and 14.81% grow maize after pepper and 12.35% grow maize after teff whereas 4.94% and 3.70% of the farmers grow sorghum and Common bean, respectively. Similarly, Noug and fallow, each had (2.47%) whereas potato and wheat, (4.96% & 2.63%) in the previous cropping history. The highest disease incidence and severity index was recorded on rotation maize after maize cropping history with (40% and 45.4%) whilst the minimum incidence and severity index were recorded on maize grown after potato, Noug, and fallow each account 0% (Table 8).

The highest result in disease intensity on rotating maize after maize could be due to increase in the amount of inoculum in the field year after year, whereas growing maize after non-host crops to GLS, like Potato, Noug and fallow were showed the lowest disease intensity. On some of the non-host crops like pepper and C. bean GLS incidence and severity were shown high in relation to fallow and Noug. This implies that the pathogen may over winter in maize residue if not properly removed and decomposed. Therefore, it is better to completely remove maize residue and/or deep ploughed to improve the decomposition residue effectively.

Increased incidence of GLS in Africa has been associated with cultural practices such as reduced tillage, continuous cultivation of maize, and use of susceptible maize cultivars (Gevers *et al*., 1994). Smith, (1989); Ward, (1996) also reported the deficiency of mineral nutrients required by the pathogen may have a potential role in GLS epidemics. Similarly, maize is the only host crop this fungus is known to attack, therefore, rotation with non-host crop Soybean and potato can reduce the disease inoculums effectively (Lipps, 1998; Wolf, 2002; Dhami *et al.*, 2015).

Table 7. Mean of disease incidence and severity index of GLS across assessed previous crop varieties at study area in 2017

Variables	Class	% of Farms	Incidence (%)	Severity index (%)
	Maize	51.85	40.00[a]	45.00[a]
	Teff	12.35	16.67[ab]	20.76[ab]
	Pepper	14.81	19.74[ab]	17.19[b]
Previous Crop [a]	Sorghum	4.94	24.44[ab]	24.93[ab]
	C. bean	3.70	18.00[ab]	22.87[ab]
	Potato	4.92	0.00[b]	0.00[b]
	Fallow	2.40	0.00[b]	0.00[b]
	Noug	2.40	0.00[b]	0.00[b]
	Wheat	2.63	17.62[ab]	18.53[ab]
	LSD		27.37	26.96

LSD = Least significance difference [a] = crop sown before the surveyed maize Figures followed by the same letter are not significantly different at 0.05.

4.1.3.4. Effect of Altitude

The result from the altitude of the survey area revealed that comparatively the highest disease incidence and disease severity index was recorded at high altitude ranges from 2301-2422 m.a.s.l with 40.00% and 46.30%, respectively. The lowest disease incidence and severity index was recorded at the mid-altitude ranges from 1543-2300 m.a.s.l having 12.8% for both respectively (Table 9). This result implies that the pathogen is more severe on the higher altitude might be due to the higher relative humidity associated with a higher altitude than with the mid-latitudes of the study area. The prevalence of high incidence of grey leaf spot in cool humid high-altitude areas has also been reported by Lipps (1995) and Asea *et al.* (2005) whereas the results by Lyimo *et al*. (2013) have shown increasing severity of grey leaf spot in low-altitude warmer areas. Therefore, altitude association with other environmental factors like relative humidity and temperature has its own effect in increasing or decreasing disease intensity.

Table 8. Mean disease incidence and severity index of GLS across the agro-ecology in 2017

Variable	Class	Incidence (%)	Severity Index (%)
Altitude	1543-2300	12.85[b]	12.87[b]
	2301-2422	40.00[a]	46.30[a]
	LSD (0.05)	10.36	10.21

LSD= Least significance difference

4.1.3.5. The Relationship between Altitude, Variety and GLS Intensity

Pearson correlation analysis indicated that there is highly significant ($P < 0.01$) and strong direct relationship between disease incidence and severity index ($r = 0.98$), Altitude ($r = 0.38$) and maize varieties ($r = 0.58$). Similarly, disease severity index with altitude ($r=0.46$) and maize varieties ($r = 0.58$) had a significant positive and strong correlation (Table.10).

This result indicates that some variables like altitude have a strong correlation with disease incidence and severity index this is due to factors associated with altitude like high rainfall could give rise to high relative humidity which results in high disease intensity. Similarly, warm temperature, well-distributed rainfall, and high relative humidity are weather conditions favoring this disease development (Wheeler, 1969).

Table 9. Pearson correlation association between variety, altitude, GLS incidence, and severity

	Severity.	Altitude.	Maize variety
Incidence	0.98**	0.38**	0.58**
Severity		0.46**	0.58**
Altitude			0.06[ns]
Maize Variety			

ns= non-significant, ** highly significant p <0.01

4.2. Morphological and cultural characteristics of *Cercospora zeae-maydis*

Of 155 Grey Leaf Spot samples collected from 31 maize fields across Western Oromia, a total of fifty two purified isolates were recovered. Based on the similarity of colony appearance on PDA and conidial characters, all the isolates were grouped into 5 *C. zeae-maydis* isolates.

4.2.1. Colonial Characteristics

The isolates were then assigned tentative identities by examining their cultural and morphological features such as growth habits, color, elevation, and edge of the fungal colonies were studied. Accordingly, 5 isolates were showed different colony appearance on PDA both at upper and the reverse sides.

Therefore, the upper colors of the two isolates (LD-G, LAAY) were recorded as Grey color which consists of 38.71% of the total isolates, and the rest three isolates (GS-O, IG-3 DN-H) were brown, Cornsilk and white gross color which comprises 19.35%, 25.81% and 16.13% of the total isolates, respectively. Similarly, the reverse sides of the two isolates (LD-G, LAAY) colony colors were recorded as Cornsilk which comprises 41.94% of the total isolates, and the rest three isolates (GS-O, IG-3 DN-H) were recorded as Dark Grey, Cornsilk3 and White smoke which comprised 25.81%, 16.13%, 16.83 %, respectively (Table 11).

Overall, the study of cultural characteristics showed the existence of variation among the five isolates of *Cercospora zeae-maydis* in colony growth, and colony color. The reason for the variation in morphology could be related to genetic and /or environmental factors. Similarly Colonial gross color appeared cottony and grey in color, with a greyish white cast on colony surface was the result reported by (Kinyua *et al*., 2010). This result also supported by Latterell and Rossi, (1983) who reported that the cultural performances of *Cercospora zeae-maydis* grow on agar media with the dense, sluggish growing colony type characteristics of the genus. Growth character ranges from black, densely sporulating cushion like colonies to white, cottony mycelial growth. Intermediate types include gray, moderately sporulating colonies often with pink, red, or purple pigment, depending on the substrate, due to the formation of cercosporin crystals. Similarly, Lyimo *et al. (*2013) reported that most isolates he identified were grey to light grey colony color.

Table 10. Colony color of *C. zeae-maydis* isolates collected from western Oromia in 2017

Isolate code	Colonial color on PDA		
	Front view	Reverse view	proportion of isolates (%)
LD-G, LA-AY	Grey	Dark grey	41.94
GS-O	Light brown	Brown	25.81
IG-3	Cornsilk	Cornsilk3	16.13
DN-H	white	Light grey	16.83

LD-G=Leka-Dulecha_ Gudina GS-O= Gobu-Seyo _Ongobo
IG-3=Ilu-Gelan DN-H= Dano_Haro LA-AY=LaLo-Asabi__Yesus

Colony Shape, Edge, and elevation were also used as a means of cultural characteristics of the Isolates. Accordingly, based on colony shape LD-G, LAAY, IG-3 isolates were categorized as round which comprises 61.29% and DN-H, GS-O isolates were having 38.71% of the total isolates had an irregular shape. Similarly, Colony Edge and Elevation were used as means of characterizing the isolates as flat with LD-G, LAAY, IG-3, GSO isolates comprising 80.65% flat and one isolate was Unbonate with 19.5% of the total isolates based on colony elevation and four isolates (83.87%) entire and one isolate (16.13%) undulate based on colony edge (Table 13). Even though, the isolates are with some differences in colonial morphology, they are known to produce the same symptoms on maize. The colonies were compact (hard), dome-like, well raised from the medium surface and dark grey to black in color. Some sectoring was exhibited in culture, whereby whitish-grey mycelial patches developed from the typical grey black colonies (Kinyua *et al*., 2010).

Table 11. Colonial shape, edge, and elevation of isolates of *Cercospora. zeae-maydis* in 2017

Isolates	Based on Colony	Morphology	proportion of isolates (%)
LD-G, LAAY, IG-3	Shape	Round	61.29
DN-H, GS-O		Irregular	38.71
LD-G, LAAY, IG-3, GSO	Edge	Entire	83.87
DN-H		Undulate	16.13
LD-G, LA-AY, IG-3, GS-O	Elevation	Flat	80.65
DN-H		Umbonate	19.5

The highest colony growth was recorded on LD-G isolate on PDA and MEA having 55.67 mm and 54mm, respectively, followed by DN-H with 54.67mm and 52.33mm on PDA and MEA respectively, whereas the minimum colony diameter growth was recorded on IG-3 and GS-O isolates with 51.67mm and 51.33mm on PDA and 51mm and 49.67mm on MEA respectively (Table 13). There were statistical differences ($p<0.01$) among the isolates on both media. Overall, the result revealed that there was significant variation between growth on the two media investigated with the highest vegetative growth on PDA and the lowest growth occurred on MEA and also we observed an effect of medium on colony growth. This result is supported by Latterell and Rossi (1974) and Nega *et al*. (2016) who reported that various media supported different types or degrees of development of erect, or submerged stromata and of sub-spherical bodies containing either macro or micro spermatia of *Cercospora zeae-maydis*.

Table 12. Effect of PDA and MEA on mycelial growth diameter (mm) of *C. zeae-maydis* isolates

Isolate	PDA	MEA
DN-H	54.67[ab]	52.33[ab]
IG-3	51.67[ab]	51.00[ab]
GS –O	51.33[bc]	49.67[b]
LD- G	55.67[a]	54.00[a]
LA- AY	50.00[c]	50.67[b]
CV (%)	4.09	3.17
LSD (0.05)	4.12	3.27

Mean Values in the same letter within a column are not significantly different at 5% Probability level. LSD= Least significant difference.

4.2.2. Conidial Characteristics

Conidial shape and number of septa of the conidia were used as a means of characterizing GLS isolates. Accordingly, three isolates showed straight conidial shape whereas two isolates showed a slightly curved conidial shape. The maximum mean number of septa was 6 and the minimum was 4.7 (Table 14). The mean variation in the shape of conidia and number of septum reveal the presence of variation in the conidial morphological characteristic among 5 isolates of *Cercospora zeae-maydis*. Although the isolates are with some differences in conidial morphology, they are known to produce the same symptoms on maize. Similar to the current study, the result reported by Kinyua *et al*. (2010); Nega *et al*. (2016) indicated, significant variation in the mean number of septa of conidia which ranges (3-10).

The conidial shapes of IG-3, GS-O, LA-Ay isolates were showed straight, whereas LD-G, DN-H isolates were identified to be a slightly curved in shape. Straight and slightly curved, hyaline, subcylindrical in shape, with gradual tapering shape, were observed on IG-3, GS-O, LA-Ay and LD-G, DN-H, respectively (Table 14 and Figure 6). The variation in colony color also backed by variation of the conidia shapes. The widest part of the conidia was around the one-third position along the spore length from the base. This variation might be due to the difference in the agro-ecology from where the pathogen came and/or inherent variation among the isolates. This study is supported by Ward and Nowell, (1998), who reported that the ecological conditions have a consequence on the development of grey leaf spot disease on maize. In addition, there was a comparable difference in conidial shape and number of septa of the *C. zeae-maydis* isolates. Similarly, Donahue *et al*. (1991), also reported that conidial shape and septa can vary among different isolates of maize GLS.

Table 13. Mean number of septum and conidial shape of 5 isolates of *C. zeae-maydis* collected in 2017

Isolates	Conidial shape and No. of Septum		
	shape	Septum range	Mean septum
DN-H	Slightly curved	3-9	4.7
IG-3	Straight	3-7	5.2
GS –O	Straight	3-8	5.8
LD- G	Slightly curved	3-9	6.0
LA- Ay	Straight	3-7	5.2

Figure1 LD-G Isolate

Figure2. DN-H Isolate

Figure3. IG-3 Isolate

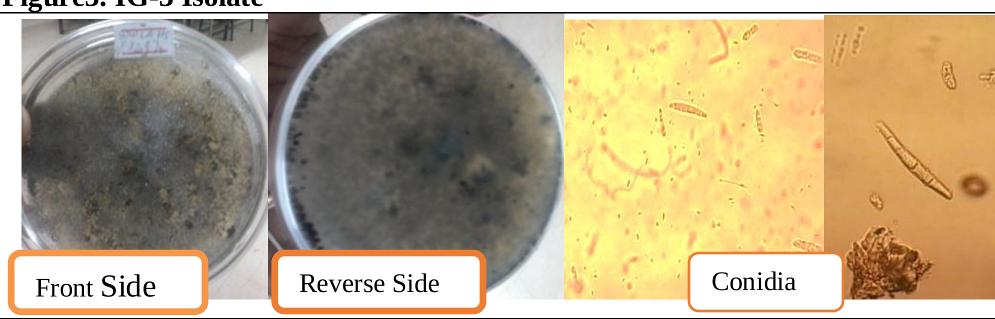

Figure 4. LA-AYIsolate

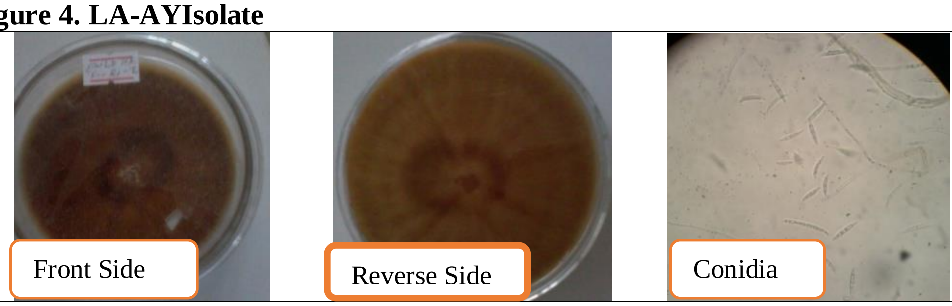

Figure 5. Isolte GS-O

Figure 6. Pictorial illustration of 5 Isolates of *C. zeae-maydis* collected from western Oromia in 2017.

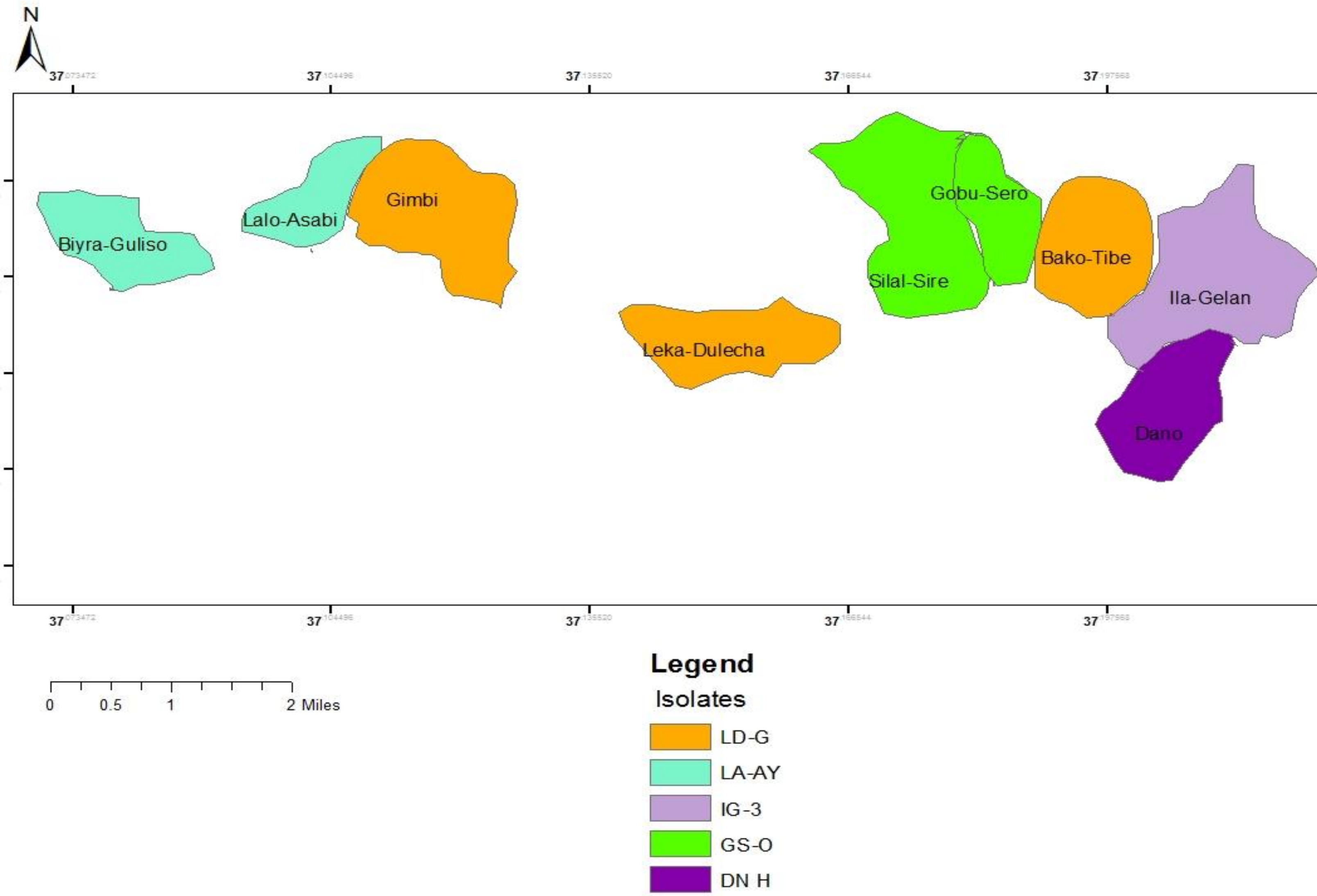

Figure 7. Distribution of maize GLS isolates across surveyed districts in 2017

4.3. Pathogenecity Test

Pathogenecity of all the isolates of *Cercospora zeae-maydis* identified in this study was assessed. The result indicated that all the tested isolates were caused GLS symptoms on the leaves of moderately susceptible maize variety BH540. However, no GLS symptoms were observed on maize leaves inoculated with sterile distilled water (control). The highest and the minimum AUDPC value and disease progress rate were constructed from the severity value of the isolates on BH540. The same symptom was observed on the infected plants with those found on naturally infected plants in the field (Appendix Figure 4). Re-isolation from the inoculated maize leaves were agreed with descriptions of the inoculated isolate, which confirms their Pathogenecity in greenhouse conditions.

4.3.1. Area Under Disease Progress Curve (AUDPC)

Disease severity scores were used to calculate AUDPC and disease progress rate per each isolate. All of the isolates were able to cause GLS symptom at 21 days after inoculation (DAI) and disease severity recording was started from that day up to 49 DAI. The first characteristic symptoms of GLS were observed within a period that corresponded to the reported latent period or incubation period of this disease under field conditions (14-28 days), according to Latterell & Rossi (1983), thus indicating that the infection process in the greenhouse environment was similar to that expected in the field.

Isolates LD-G and DN-H showed the highest AUDPC value with 1540 for each of the isolates, but there was no significant variation between severity indexes of both isolate, whereas GS-O, LA-Ay, and IG-3 isolates revealed relatively the minimum value having 1134 each. Similarly, the highest AUDPC 1650.17 and the lowest AUDPC 1369.16 was reported by (Bekeko *et al.*, 2018).

This variation could be due to the difference in the isolates intrinsic nature and environmental conditions like relative humidity as well as the minimum and maximum temperatures in the study area. The pathogenesity parameters showed significant variations between isolates collected at different altitudes indicating that the environment could have a major influence on the expression of these parameters. For example, lesions of isolates from samples collected in cooler high-altitude areas were generally longer than lesions of isolates collected in warmer low-attitude areas (Lyimo *et al*., 2013).

This study is supported by Ward and Nowell, (1998) who reported that the ecological conditions have a consequence on the development of grey leaf spot disease on maize. Differences in Pathogenecity among isolates of *C. zeae-maydis* were reported earlier. Bair & Ayers (1986), for instance, inoculated 15 isolates on susceptible maize hybrid, under the greenhouse and found significant variations in disease severity among isolates. Okorai *et al*. (2004) studied 27 African isolates of *C. zeae-maydis* and also reported significant differences in aggressiveness between isolates. Finally, Carson *et al*. (2002) also reported on the variation in aggressiveness among isolates.

Table 14. Means of area under disease progress curve for *Cercospora zeae-maydis* under greenhouse condition in 2018

Isolates	AUDPC
DN-H	1540[a]
IG-3	1120[c]
GS-O	1330[b]
LD-G	1540[a]
LA-AY	1134[c]
Water /Control	0[d]
LSD (0.05)	67.44
CV (%)	4.6

Mean Values in the same letter within a column are not significantly different at 5% Probability level. LSD= Least significant difference.

Disease severity index was recorded under greenhouse condition and there was a significant difference among the isolates. Accordingly, the mean disease severity index of LD-G and DN-H isolates revealed the maximum disease severity index with (80%) whereas, IG-3, GS-O, and LA-AY isolates had modest disease severity index having (60%) respectively (Figure 8).

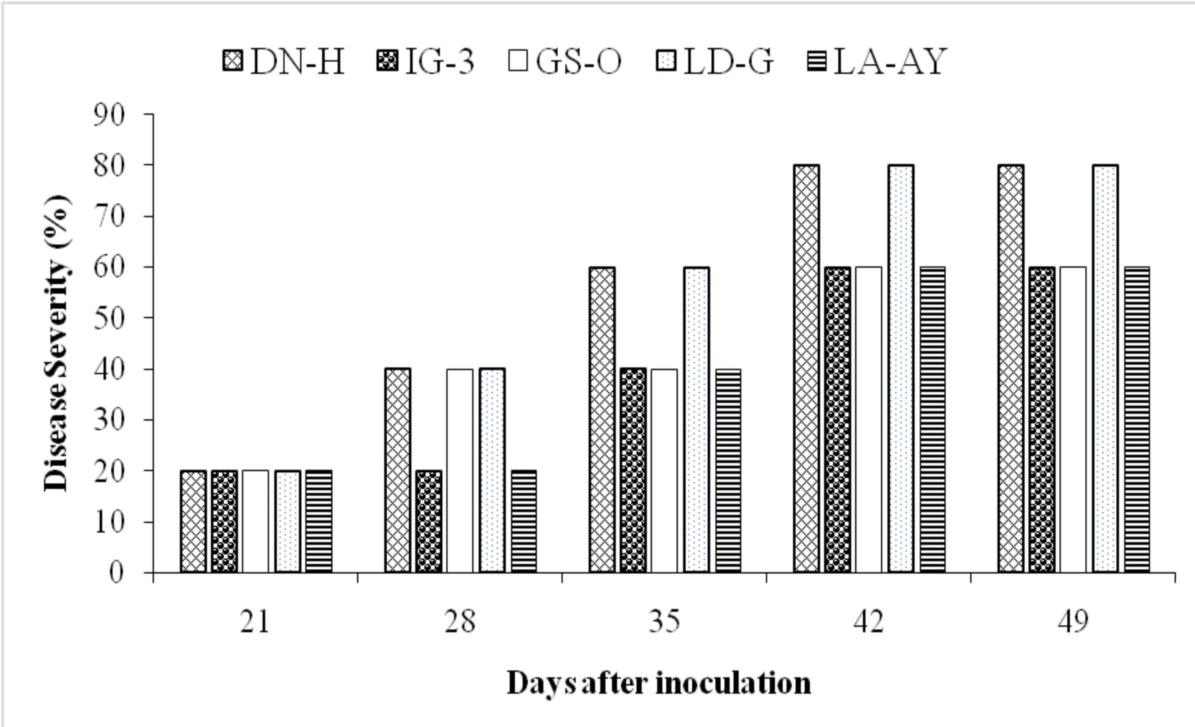

Figure 8. Mean severities of 5 GLS isolates on BH540 variety

4.3.2. Disease progress Rate

Disease progress rates calculated from the data taken 21DAI, 28DAI, 35DAI, 42DAI, 49DAI, of the pathogen isolates showed significant ($p<0.01$) difference on BH540 maize variety. Accordingly, the disease progress rate of DN-H and LD-G isolates revealed the highest disease progress rate with (0.13) each whereas, IG-3, GS-O, and LA-AY isolates showed relatively minimum disease progress rate of (0.09, 0.09 and 0.09 units per day) (Table 15).

This result could imply that there is a variation among the isolates of GLS of maize which could be due to the difference in agro-ecology where the pathogen collected and intrinsic nature of the pathogen. Similar, result reported by De Nazareno *et al.* (1993) revealed the rate of gray leaf spot progress (*r*) ranged from 0.13 to 0.17 logits per day with relatively aggressive isolates and 0.02 to 0.06 logits per day with relatively less aggressive.

Table 15. Disease progress rate of GLS isolates on BH540 maize varieties.

Isolate	Rate	R^2
DN-H	0.13041	99.86%
IG-3	0.09080	87.53%
GS-O	0.09080	87.96%
LD-G	0.13041	99.86%
LA-Ay	0.09080	87.53%

5 UMMARY AND CONCLUSION

Maize (*Zea mays L.*) is one of the most widely cultivated crops in the world. It is the most popular cereal crops ranked third next to wheat, and rice. In Ethiopia, maize is the second largest food security crop after teff. National average productivity of maize in Ethiopia is 3.94 tons per hectare, whereas the world average productivity is 5.78 ton per hectare. A significant portion of this yield gap is attributable to biotic and abiotic stresses. Diseases play a major role among biotic constraints. Of diseases, GLS (*Cercospora zeae-maydis*) is the most important infectious diseases of maize that can cause severe yield loss in the country. There is an information gap on the status and distribution of the pathogen at the study area. Therefore, the survey was conducted in 3 zones of Western Oromia region namely West-Shewa, East-Wollega, and West-Wollega Zone and a total of 9 districts having 27 PAs with a general objective to assess the distribution of maize GLS and morphological and cultural characterization of Maize GLS isolates.

Purposive multistage sampling method was used to reach the PAs and a total of 155 leaf samples were collected from 31 fields out of 81 maize fields assessed. All the leave samples were air dried and kept in a refrigerator and cultured on PDA. A total of 52 isolates were recovered from the leave samples and grouped in to 5 isolates based on colony/mycelia color using RGB color chart. Morphological and cultural characterizations of mycelia and conidia characters were studied. Confirmations of Koch's postulate of the 5 isolates were done on moderately susceptible BH540 maize variety and AUDPC and disease progress rate were also recorded.

Survey result showed that the highest Grey Leaf Spot disease prevalence, incidence, and severity index was recorded in East Wollega Zone, having (62.96%, 35.06%, and 31.43%), respectively. Similarly, at the district level, the highest disease prevalence, incidence, and severity index were recorded in Leka-Dulecha with (88.9%, 47.77%, and 46.11%), respectively. But in contrast, the lowest disease incidence and severity index were recorded in Gimbi having (1.11%, and 1.78%), respectively. The difference in disease intensity and prevalence among zones and districts could be due to the differences in environmental factors (altitude, temperature, and humidity) and farming practices in the study area and maize varieties used. When weather conditions are favorable for the development of the disease, there is a significant positive relationship between the environmental factors, farming practices, and disease intensity.

From 155 samples collected during the assessment, fifty two isolates have been re-cultured and grouped into five isolates and the identity was confirmed as DN-H, IG-3 GS-O, and LD-G, LA-AY. Based on the colony color the upper colors of the two isolates (LD-G, LA-AY) were recorded as Grey, whereas isolates GS-O, IG-3 DN-H were showed brown, Cornsilk and white, respectively. There was also a variation among the isolates based on colony shape as round and irregular for (LD-G, LAAY, IG-3) and (DN-H, GS-O) respectively. Additionally, straight conidial shape for (IG-3, GS-O, LA-Ay) isolates and slightly curved for LD-G, DN-H isolates. Additionally number of conidial septa was also used to characterize the isolates. Greenhouse study revealed that the highest AUDPC and disease severity index were recorded by LD-G and DN-H isolates each with 1540 and 80%, respectively. Those isolates with high AUDPC value and progress rate were also showed comparatively the highest disease incidence and severity index in field during the survey.

This study provides information on distribution of GLS, in Western Oromia and morphological and cultural characterization of GLS isolates. Grey leaf spot does occur in all assessed zones and districts, but the extent of disease prevalence, incidence and severity showed variation across the assessed area. This indicates that there will be a high epidemic when the conditions favor the disease. The result from morphological characterization showed that there are different colonial and conidial growth features among GLS isolates which is supported by the variation in severity among the identified isolates. From the survey result, the farmers at relatively high disease prevalence and intensity area shall better if use those maize varieties with less severity and practicing crop rotation to minimize disease intensity. Besides, there is a need to develop effective, affordable and sustainable management strategies to reduce the effect of GLS on maize production in the study area. Further study would be required in order to see the trend of disease distribution and importance of maize grey leaf spot disease over seasons, as this study only considered one season data. Also, molecular diagnostics to confirm their specific morphological differences and to design appropriate management options.

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7 APPENDICES

Appendix Table 1. Survey questionnaire

Distribution of Maize Grey Leaf Spot (*Cercospora Zeae-Maydis* Tehon and Daniels) and Morphological Characterization and Pathogenecity of GLS Isolates in Western Oromia Region, Ethiopia

Field Coode No.________

1. Date of Assessment (dd/mm/yy)______________
   - Variety__________________
   - Seed source___________________________
   - Crop growth stage ________________
   - Preceding crop and field history/disease history_________________
   - Field area ______________________________
2. Farmer's Name _______________________________
   - Zone ____________________________________
   - District/woreda _________________________
   - PA/ Kebele______________________________
   - Location:_______________________________
3. GPS Co-ordinates:

   Altitude______________Latitude__________________Longitude_______________
4. Topography: Plain (flat) ☐ Gentle ☐ sloppy ☐

   Dell/semi-dell ☐ Mountain ☐
5. How was the rainfall amount and distribution?
   - Normal: time ☐ early ☐ late ☐
   - Distribution: fair ☐ good ☐ bad ☐
   - Amount: fair ☐ good ☐ bad ☐
   - Have you prepared your land on time? Yes ☐ No ☐
6. Sowing time: Early ☐ Normal ☐ late ☐

- Method of planting: row ☐ broadcast ☐
- Plant density: high ☐ Medium ☐ low ☐

7. Weed management: good ☐ bad ☐ fair ☐
8. Crop stand: good ☐ fair ☐
9. Do fertilizer applied?

   If yes, what was the rate applied?

   - Dap________________________kg/ha
   - Urea__________________________kg/ha
   - FYM__________________________kg/ha
   - Compost_______________________kg/ha, mixture________kg/ha
10. Is there disease and inspect pest problems? Yes, ______No________

    Time of disease appearance;
    - Early ☐ mid ☐ late ☐
11. At which crop stage disease appeared?

    Seedling ☐ Vegetative ☐ Dough stage ☐

12. Disease Management Practices If any

    ____

    ____

    ____

    ____

.

Appendix Table 2. Nested ANOVA of Surveyed zone

Source	DF	Mean Square Incidence	Mean Square Severity
Model	80	4633.00**	1118.44**
Zone	2	21930.91**	4136.64**
District(zone)	6	9166.77**	2304.82**
PA(zone*District)	18	3249.02**	773.60**
Farm(zone*District*PA)	54	3949.91**	989.78**
Error	324	216.74	48.92
CV (%)		71.98	24.62378

DF= Degree of Freedom
CV= Coefficient of Variance

..

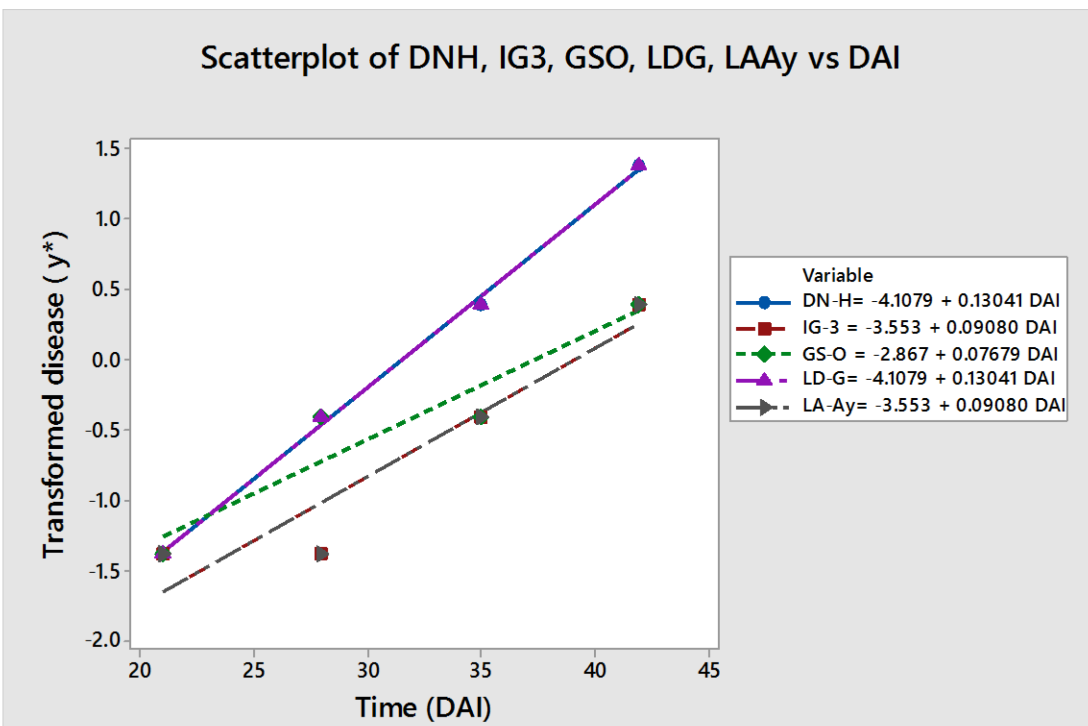
Appendix Table 3. ANOVA for different variables

Source	DF	Mean Square Incidence	Mean Square Severity
Model	23	1573.30**	1680.76
Altitude	1	5383.21**	7258.41**
Maize variety	9	2424.16**	2367.63**
Error	57	274.07	265.97
CV (%)		98.11	91.49

Appendix Table 4. ANOVA of AUDPC mean square for *Cercospora zeae-maydis* isolates

Source	DF	AUDPC
Model	9	918368.88**
Replication	4	2613.33Ns
Treatment	5	1650973**
Error	20	2613.33

Ns at p<0.05 ** Significant at p<0.001
DF= Degree of Freedom



Appendix Figure 1. Mean disease progress rate of the 5 *Cercospora zeae-maydis* isolates

Appendix Figure 2. Data collection and sample taking during the survey.

Appendix Figure 3. Photo during the course of culture plating and characterization.

Appendix Figure 4. Photo during Pathogenecity study