



**SURVEY OF TURCICUM LEAF BLIGHT (*Exserohilum turcicum*) ON
MAIZE (*Zea mays*) AND EVALUATION OF MAIZE VARIETIES AND
FUNGICIDE SPRAY FREQUENCIES FOR ITS MANAGEMENT**

MSc THESIS

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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MAIZE (*Zea mays*) AND EVALUATION OF MAIZE VARIETIES AND
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SCHOOL OF PLANT AND HORTICULTURAL SCIENCES
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DEDICATION

I dedicate this thesis to my wonderful parents (To my father, Aliyi Mohammed and my mother Kediya Hussein) who have raised me to be the person I am today. And to my brother Mohammed-Hussein Aliyi for his support, warm heartedness and consistent care in the success of my life.

STATEMENT OF AUTHOR

Firstly, I declare and assure that this thesis is my bona fide work and all sources of materials used for this thesis have been duly acknowledged.

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LISTS OF ABBREVIATIONS AND ACRONYMS

APPRC	Ambo Plant Protection Research centre
AUDPC	Area Under Disease Progress Curve
CSA	Central Statistical Authority
DAP	Days after planting
EC	Emulsifiable Concentrate
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
GIS	Geographical Information System
GPS	Global Positioning System
PSI	Percentage Severity Index
RCBD	Randomized Complete Block Design
RLY	Relative Yield Loss
SSA	Sub-Saharan Africa
TKW	Thousand Kernel Weight
TLB	Turcicum leaf blight
WP	Wettable Powder

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Survey of Turcicum Leaf Blight (*Exserohilum turcicum*) on Maize (*Zea Mays*) and Evaluation of Maize Varieties and Fungicide Spray Frequencies for its Management

By Tajudin Aliyi

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ABSTRACT

*Turcicum leaf blight (*Exserohilum turcicum*), TLB, is a major disease affecting maize (*Zea mays*) in western Ethiopia. This study was designed to: assess the incidence and severity of TLB in major maize growing agro-ecologies of Western Oromia, evaluate the reaction of maize varieties under production to TLB, and determine optimum propiconazole (tilt 250 EC) frequency as management option to TLB. Survey was conducted on 172 farmers' fields in 29 districts and five zones of major maize growing agro ecologies in western Oromia region of Ethiopia. Survey results revealed the prevalence of TLB across all the districts even though at different levels. Mean TLB incidence ranged from 16.3% in Abay chomen to 96.67% in Wayu Tuka and mean percent severity index varied between 3.1% in Abay chomen and 57.5% in Lalo Assoabi district. TLB was relatively more severe on maize varieties BH-540, Limu and Kolba on surveyed areas. In addition, the effects of maize varieties, fungicide frequencies and their interactions on TLB development were assessed in a field experiment during the 2016/17 main growing season at Ambo Plant Protection Research Centre (APPRC). The experiment involved three maize varieties and three fungicide (Tilt, propiconazole) frequencies arranged in a factorial setting in randomized complete block design with three replications. TLB was initiated following artificial inoculation. Data were recorded on disease incidence and severity and severity scores were used to calculate area under disease progress curve (AUDPC) and infection rate. Grain yield and yield components were measured after harvest. Finally correlation and economic analyses were carried out. Disease parameters varied markedly across treatments, especially variety-fungicide combinations had a highly significant variation ($p \leq 0.01$) in disease parameters. The highest terminal percent severity index (PSI) (86.7%), AUDPC value (2614.4 %-day) and disease progress rate ($0.09339 \text{ units-day}^{-1}$) were recorded on unsprayed variety AMHQ-760. On the other hand, the same variety had significantly lower disease level and gave the highest grain yield (9.424 t ha^{-1}) when it was treated three times with propiconazole. TLB resulted in grain yield losses of up to 62.4% on AMHQ-760. PSI, AUDPC, incidence and disease progress rate were negatively correlated with yield and yield components. The highest marginal benefit (ETB 63,089 ha^{-1}), and marginal rate of return (ETB 14.53) were obtained from variety AMHQ-760 with thrice application of propiconazole. Based on current results, three propiconazole applications were found effective to manage TLB even on susceptible maize variety. However, additional experiments should be carried out to verify the current results.*

Key words: Area under disease progress curve, severity, Rate of infection, leaf blight, Propiconazole

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most widely grown crops in the world, ranking third next to wheat and rice (Reeves *et al.*, 2016). In sub-Saharan Africa (SSA), maize is the most widely-grown staple food crop occupying more than 33 million hectares each year (FAOSTAT, 2015). It is among the most important and widely grown crops in Ethiopia too ranking first in total production with over 7.23 million tones of produce and second in area coverage next to Teff (*Eragrostis tef* (Zucc.)Trotter) (Mosisa *et al.*, 2012; CSA, 2014). Considering its importance, wide adaptation, total production and productivity, maize is regarded as one of the high priority food security crops in Ethiopia, the second-most populous country in SSA after Nigeria (CSA, 2011). However, maize production has remained low, with the estimated national average yield of 3.4 t ha⁻¹ (CSA, 2014) compared to the world average yield estimated at 5 t ha⁻¹ (FAOSTAT, 2015), due to several major diseases, including foliar diseases.

Turcicum leaf blight (TLB) is a major foliar disease of maize in most production areas worldwide (Jakhar *et al.*, 2017). It is a fungal disease caused by *Exserohilum turcicum* (Pass.) K. J. Leonard and E.G. Suggs. The pathogen was formerly known as *Helminthosporium turcicum* Pass (Khedekar *et al.*, 2010). The disease is more prevalent in humid areas with moderate temperatures (Pataky *et al.*, 2006). It is widely distributed, however, sporadic in nature and its development mostly depends on weather conditions, stage of plant growth and level of resistance in maize cultivars (Perkins *et al.*, 1987). The pathogen has wide host range and a high pathogenic variability (Muiru *et al.*, 2010).

The pathogen attacks all parts of the plant but the most conspicuous symptoms/lesions are found on the foliage. Lesions destroy the leaves, resulting in yield losses due to lack of

carbohydrate to fill the grains. Heavily infected fields present a scorched or burnt appearance resulting in premature death of leaves (Harlapur, 2007). TLB causes extensive leaf damage and defoliation during the grain filling period, and yield losses due to necrosis or chlorosis of leaves premature death of the leaves and loss of nutritive value even as fodder (Patil *et al.*, 2000) has been reported. Yield losses of up to 28 to 91 % due to TLB have been reported in Italy, mostly when heavy infection occurred before tasselling (FAO, 2010).

Ramathani *et al.* (2011) reported the prevalence of TLB in highlands and wetter areas of the Kenya and Uganda. Previous reports have also shown that *E. turcicum* is a serious pathogen in highlands associated with cool, high relative humidity, mid-altitudes and cloudy weather conditions (Palaversic *et al.*, 2012). Extreme impact of TLB on maize in the highland agro-ecologies have also been reported in Uganda, Kenya, Ethiopia and Zambia and South Africa (Ramathani, 2011).

In Western Ethiopia, TLB (*E. turcicum*) is the most important maize disease reported. Farmers in this area (46.7%) indicated TLB as the major leaf disease on maize while gray leaf spot (GLS) caused by *Cercospora zea-maydis*, is ranked as the second most important leaf disease in the area (Wende *et al.*, 2013). Therefore, TLB ranked as the number one problem and is considered as a high research priority of maize in Ethiopia (Wende *et al.*, 2013).

TLB incidence ranges from 95 to 100% in areas with constant moisture and high humidity and the yield loss can reach up to 70% (Tewabeche *et al.*, 2012). It is reported to cause devastating damage on most commercial varieties of maize released in the country (Tewabeche *et al.*, 2012).

Resistant cultivars are primarily used to control TLB. Both major genes and partial resistance

can be combined for disease control but identifying partial resistance has been prioritized due to practical limitations of *Ht* genes. The genetics of resistance is determined in most of maize genotypes quantitatively and has been used for control of this disease (Kumar *et al*, 2011).

TLB varies in incidence and severity from year to year and from one locality to another depending largely on genetic makeup of the plants and prevailing environmental conditions. It is a multiple cycle disease and new repeated inoculations are needed for disease development making it highly dependent upon sporulation from other lesions (Ullstrup, 1966).

Previously the TLB disease was limited to specific areas and varieties, but currently the disease has become very important almost in all maize growing agro-ecologies. Most released varieties under production are affected by the disease. Although, it becomes very important in the nation, there is limited quantified data that reflect the extent of its distribution across highlands and mid-altitude maize growing agro ecologies and the reaction of maize varieties to this disease under this production system need to be studied.

Application of fungicides is the way to manage TLB and specially Propiconazole 25 EC (Tilt) was effective and the best against *E. turcicum* on maize of all tested fungicides (Wani, 2015). Three sprays of propiconazole at 3-week intervals were effective in reducing the rate of turcicum leaf blight development in maize (Bowen and Pederson, 1988). Maize treated with propiconazole at 3-week intervals had less severe epidemics than maize treated with mancozeb at 1-week intervals (Bowen and Pederson, 1988).

Integration of host resistance with three times sprays of foliar fungicide (propiconazole) protected the maize varieties from high TLB epidemics, increased yield, yield components and maximized marginal benefit (Megersa *et al.*, 2017).

Maize production under judicious use of fungicide application program improves the producers' income and help consumers to access year round high yield quality product. Introduced in the 1970s, demethylation inhibitors (DMIs) fungicides have broad-spectrum activity against many fungal pathogens and are currently registered for use on many crops (Munkvold *et al.*, 2001). Although DMIs have traditionally been used to control many foliar diseases of maize (Raid, 1991), resistance in *E. turcicum* has not yet been reported. Systemic fungicides were necessary to prevent high levels of disease when conditions were favorable for disease and were more cost-effective for growers. Propiconazole was reported to have longer effective period and significantly increased yields of maize against TLB compared to mancozeb (Bowen and Pedersen 1988). However, appropriate frequency of application of widely used propiconazole (tilt) fungicide is not yet determined for several maize production systems in Ethiopia. Thus, optimum fungicide (propiconazole) application frequencies need to be studied to enhance maize production and productivity under rain feed condition in Ethiopia. Thus, the present investigation was undertaken with the following objectives:

General objective: To contribute towards improved and sustainable maize production through effective management of TLB.

Specific objectives:

1. To assess the incidence and severity of TLB in major highland and mid-altitudes of maize growing agro-ecologies of Western part of Oromia, Ethiopia
2. To map the geographical distribution of the TLB disease in the study area
3. To assess the reaction of maize varieties under production to TLB in surveyed area
4. To determine optimum propiconazole (tilt 250 EC) spray frequency for integrated management of TLB

2. LITERATURE REVIEW

2.1. Historical Perspective

Turcicum leaf blight (TLB) of maize caused by *Helminthosporium turcicum* (Pass.) was first reported by Passerini (1876) in Parma, Italy. Later on, Pammel *et al.* (1910) and Drechsler (1923) regarded it to be the same as *Trichometasphaeria turcica* Luttrell. Further, Leonard and Suggs (1974) renamed the perfect stage as *Setosphaeria turcica* (Luttrell) Leonard and Suggs and described the conidial stage as *Exserohilum turcicum* (Pass.) Leonard and Suggs in which the conidial hilum is strongly protuberant.

The disease was reported in New Jersey in the USA in 1878 and a serious outbreak occurred in Connecticut in 1889 (Drechsler, 1923). Jordhan *et al.* (1983) conducted survey and collected turcicum leaf blight pathogen isolates from corn fields of Florida, Illinois, Indiana, Iowa, Minnesota, New York and Pennsylvania. An epidemic appeared in North Carolina (USA) destroying up to 75% of leaf areas by the second week of August (Leonard *et al.*, 1985). TLB is one of the major diseases affecting maize and sorghum in warm and humid parts of the world (Ceballos *et al.*, 1991; Adipala *et al.*, 1993).

De Vries and Toenniessen (2001) reported that there is empirical information on severity and distribution of TLB in African countries such as Kenya, Uganda, Ethiopia, Zambia and South Africa. Nwanosike *et al.* (2015) reported that TLB incidence and percent severity reach 57.5 - 96 % and 33.4 – 62.2 %, respectively, and the disease was more severe in humid highlands of Tanzania.

2.2. Economic Importance and Yield Loss Due to TLB

Under severe conditions, the whole leaf can be covered by the disease and dry up. The accompanying TLB effects can reduce photosynthetic area, increase plant lodging, expose to secondary infection, stalk rot, plant death and subsequently can incur high grain yield loss per unit area. Under concomitant infection, TLB causes blighting of especially leaf tissues which may lead to coalescence of large patches of the leaf blade reducing the functional leaf area for photosynthesis and under severe epidemics, TLB causes significant grain yield losses as high as 70%, through reduced kernel weight (Rajeshwar *et al.*, 2014).

TLB causes yield reduction associated with necrosis or chlorosis of leaves in the upper two-thirds of the canopy (Levy and Pataky, 1992). The disease can cause extensive defoliation during grain filling period, resulting in grain yield losses of up to 50% or more (Raymundo and Hooker, 1981). If infection is delayed by 6-8 weeks after silking, yield losses may be minimal. Turcicum leaf blight also predisposes plants to stalk rots caused by other pathogens (Gowda *et al.*, 1992; Cardwell *et al.*, 1997).

The magnitude of yield losses caused by TLB depends on two factors: (1) the stage of maize growth when the infection occurs; and (2) the severity of the disease. If the disease establishes before silking, yield reduction of up to 40% may occur, but if infection delays until 6-8 weeks after silking, yield losses would be minimal (Raymond and Hooker, 1981). A fresh weight yield loss of 0.3 to 0.75% for every 1% of the leaf area affected occurs in sweet corn (Pataky, 1992).

2.3. Symptoms of TLB

TLB disease symptoms primarily appear on the leaves. Plants may be infected at any growth stage, but usually at or after anthesis or male flowering. The disease starts first as a small elliptical spot on the leaves, grayish green in colour with water soaked lesions. The spots turn greenish with age and increase in size, finally attaining a spindle shape. Individual spots are usually 3/4" wide and 2" to 3" long. Spores of the fungus develop abundantly on both sides of the spot. Heavily infected field presents a scorched appearance (Rajesh *et al.*, 2012).

The most observed symptom of *E. turcicum* is long elliptic tan lesions that develop first on the lower leaves and progresses upward. The earliest symptoms of infection are slightly oval, water-soaked, small spots on leaves. These grow into elongated, spindle-shaped necrotic lesions. They may appear first on lower leaves and increase in number as the plant develops and this can lead to complete blighting of the foliage (Richards and Kucharek, 2006).

The disease begins as long, slender, grayish or tan leaf lesions that run parallel to the mid vein. Lesions can eventually expand to a more oblong or “cigar” shape and may also form on husks (Jakhar *et al.*, 2017).

2.4. The Pathogen: Taxonomy, Host Range and Pathogen Variability

E. turcicum is an ascomyceteous pathogen of cereals. It is a heterothallic facultative parasitic fungus (Luttrell, 1958). The fungus belongs to division Eumycota, sub-division Deuteromycotina, order Moniliales and family Dematiaceae. The teleomorph *Setosphaeria turcica* belongs to division Eumycota, sub-division Ascomycotina, order Pleosporales and family Pleosporaceae. *E. turcicum* is a common pathogen of sorghum, *Paspalum*, and *Zea* in

nature. In addition, *Triticum*, *Hordeum*, *Avena*, *Saccharum*, and *Oryza* are susceptible to *E. turcicum* when artificially inoculated (Leonard and Suggs, 1974).

Isolates from different agro-ecological zones showed variation in morphology, pigmentation, growth rate and sporulation rate in different media (Muiru *et al.*, 2008). The different light regimes had significant effect on the growth rate and sporulation of *E. turcicum* isolates. The type of media and incubation temperatures had a significant effect on the growth rate of different isolates. The optimum temperature was 25°C and only one isolate had minimal growth below 10°C and no growth was observed in all the isolates at 40 °C (Muiru *et al.*, 2008).

The germination of conidia of *E. turcicum* (*Setosphaeria turcica*), causing TLB of maize, was tested out at various incubation periods starting from 4 to 36 h at an interval of 4 h. The maximum conidial germination (94.20%) was observed after 36 h of incubation, while the least germination (7.67%) was noticed after 4 h. More than 50% germination was observed after 16 h of incubation. However, there was no significant increase in the germination of conidia from 28 to 36 h of incubation (Harlapur and Kulakarni , 2009).

Misra (1979) reported that *E. turcicum* can infect maize, several millet species such as *Setaria italic*, *Eleusine coracana*, and *Paspalurn scrobiculatum*, Sudan grass, Johnson grass, and teosinte. Shankerlingam and Balasubramanian (1984) reported that a sorghum isolate of the fungus infected maize. Sisterna (1985) tested the pathogenicity of isolates of *E. turcicum* from maize and sorghum on a range of cereals in a greenhouse, and found that only sorghum and maize were infected with similar symptoms.

The existence of special forms of *E. turcicum* in maize in the Bajio region of Mexico causing damage to sorghum was also reported by Ayala *et al.* (1997). On the basis of pathogenicity, three special forms were distinguished. *Setosphaeria turcica* f.sp. *sorghii*, specific for sorghum and Johnson grass; *Setosphaeria turcica* f.sp. *zea*, specific for maize; and *Setosphaeria turcica* f.sp. *complexain* sorghum and maize or sorghum and Johnson grass. No isolate was pathogenic to all three hosts (Ayala *et al.*, 1997).

Gowda *et al.* (2010) studied the cultural and morphological variation of the 13 isolates of *E. turcicum*. The cultural variability was carried out on five solid media namely Czapek's medium, glucose peptone medium, maize leaf extract medium, potato dextrose agar medium and Richard's medium. Variations in mycelia weight, sporulation and morphological characters were recorded. The isolates from Almora, Bajaura and Nagenahalli exhibited faster growth as compared to other isolates while the Coimbatore and Udaipur isolates were grouped in the slow growing category.

Muiru *et al.* (2010) evaluated aggressiveness of 89 *Exserohilum turcicum* isolates comprising 59 Kenyan, 26 German and seven Austrian isolates in green house condition and reported that isolates from the three countries showed a great variation in aggressiveness with incubation periods ranging from 2 to 6 days, lesion size ranging from 1.81 mm² to 57.04 mm², rate of lesion expansion ranging from 0.29 mm² day⁻¹ to 21.67 mm² day⁻¹. Twelve races namely 0, 1, 2, 3, N, 12, 13, 13N, 3N, 123, 23, 23N were identified from the three countries. Race 2 was the most common and had 27% frequency of occurrence followed by race 0 and 1 which had frequency occurrence of 22% and 12%, respectively.

2.5. Biology and Morphology of *Exserohilum turcicum*

The TLB pathogen reproduces both sexually and asexually but the sexual (perfect) stage rarely occurs in nature. In the laboratory, it may occur as black, globose pseudothecia. Conidia from diseased maize leaves are straight or slightly curved nearly cylindrical, tapering only slightly from the middle. The conidia walls are thin and light olivaceous (of a deep shade of green) to light olivaceous brown in mature spores. The hilum protrudes distinctly from the conidia to bluntly rounded basal cells (Leonard and Suggs, 1974).

Conidia of the fungus are olive-Gray and spindle shaped, measuring 5 x 20 µm with one septum to nine septa. Presence of protruding hilum is the identifying feature due to which it has been designated as *Exserohilum turcicum* (Leonard and Suggs, 1974). Conidiophores are simple, cylindrical, olivaceous brown and 47-443 by 46 µm in size. Single conidium is formed terminally on the conidiophore, which then resumes growth to one side of the conidial attachment and eventually produced another conidium at the new tip. Circular conidial scars are evident on the conidiophore after the abscission of the conidia (Leonard and Suggs, 1974). The ascigerous state usually occurs in culture by pairing isolates from compatible mating types. The ascocarps are globose, black uniloculate stromatic bodies 260-400 µm in diameter often with a distinct globose or cylindrical beak. Mating types appear to be controlled at a single chromosome locus (Leonard and Suggs, 1974).

2.6. Epidemiology of TLB

Moderate temperature (18-27°C), relative humidity from 90 to 100%, low luminosity, the presence of large amount of inocula and long dew periods favour TLB epiphytotics (Hennessy *et al.*, 1990; Bentolila *et al.*, 1991). Heavy dew on the growing plant has also been cited as one

of the factors leading to high TLB severity (Levy and Cohen, 1983; Dingerdissen *et al.*, 1996). Further spread of the disease within and between fields occurs by conidia produced abundantly on leaf lesions.

TLB is generally known to be sporadic in occurrence, depending on the environmental conditions and the level of disease resistance in the variety (Degefu *et al.*, 2004). Levy (1989) mentioned that high relative humidity and presence of susceptible hosts are important factors that influence the disease epidemiology. Ceballos *et al.* (1991) reported that turcicum leaf blight severity is high on early maturing maize varieties as compared to the late maturing varieties.

The role of residue-borne pathogens in the epidemiology of maize diseases has been extensively documented, especially in the USA. *E. turcicum* infected maize leaves have been used to induce turcicum leaf blight epidemics (Lipp, 1983). Nevertheless for both maize and sorghum residue-borne pathogens, the final disease severity can be influenced by the amount of infected residue on soil surface, which in turn, can be influenced by the cropping system and tillage practices. Diseases such as anthracnose, turcicum leaf blight, Gray leaf spot and brown leaf spot are more severe under minimum tillage than in conventional tillage where maize debris is buried (De Nazareno *et al.*, 1993). Therefore, it is possible that deep ploughing and crop rotation may control some of the residue-borne pathogens. Under continuous cereal cultivation, deep ploughing may reduce inoculum levels and the incidence of early season disease development but this may not necessarily result into complete disease control (Lipp, 1983).

The dispersal potential of a pathogen is often quantified in field studies, where gradients of inoculum or diseases are measured at various distances from a point source of inoculum. Inoculum gradients have the advantage of measuring directly the number of propagules transported to different distances from the source. Disease gradients have the advantage of accounting for all sub-processes leading to the spread of disease including the release, transport and deposition of inoculums, as well as the infectiveness of that inoculum (Mundt *et al.*, 1998). Primary gradients measure dispersal potential in a monocycle, whereas secondary and subsequent gradients are to be interpreted in the context of the more complex relations between temporal and spatial increase of disease (Minogue and Fry, 1983; Campbell and Madden, 1990).

Both inoculum and disease gradients are often fitted to simple empirical models so as to quantify the steepness of the gradient with a slope (Gregory, 1968) and this has been measured for many plant pathogens/diseases, most of which are fungal (Fitt *et al.*, 1987). Decrease in spore concentration over distance away from the inoculum foci may result in decrease in spore deposition on the ground or crop. Local sources of inoculum, secondary dispersal, population of susceptible hosts, background contamination flattens primary gradient and when disease incidence is expressed as fraction of individuals infected, the multiple infection transformation has to be applied. These are some of the basic principles in dispersal gradient in plant pathosystem (Gregory, 1968).

Plant disease epidemics may also be described by analyzing disease spread over time (Campbell and Madden, 1990). Such analysis often referred to as temporal studies. Several disease progress models have been proposed for characterizing increase in disease over time

for polycyclic diseases with the logistic and Gompertz models being most frequently used (Campbell and Madden, 1990). These models define disease progress in terms of rate of disease increase and estimated disease level at the observed start of the epidemic. A pathogen with ability to complete several generations in the course of the epidemics can best be described by the logistic model (Vander Plank, 1963). A related approach is to calculate the area under disease progress curve (AUDPC), which describes disease progress in terms of disease levels, integrated over the assessment time (Campbell and Madden, 1990).

2.7. Disease Development

Infection by germinating conidia occurs when free water is present on the leaf surface for 6-18 hours and the temperature is between 66 and 80°F (18-27°C). Under favorable conditions, lesions develop and produced a new crop of spores within 7-12 days on susceptible cultivars, causing the disease to spread rapidly. As temperatures rise in the spring and early summer, the fungus produces spores on residue, and then the spores are splashed or wind-blown onto leaves of the new maize crop. Infection occurs during periods of moderate (64° to 81°F), wet and humid weather (Jakhar *et al.*, 2017). The germination of *E. turcicum* conidia is bipolar and occurs within 3-6 hours after inoculation on leaves of maize (Muiru, 2008) and 8-12 hours on sorghum (Shree, 1987). Germination occurs in the presence of free moisture with the optimum temperature of 25°C. The percentage of conidial germination is not affected by the age of the plants and leaves (Levy and Cohen, 1983). After germination, spores produce germ tubes which are 20–150 µm long and, in general, grow at an angle rather than parallel to the veins of the leaf. Germ tubes develop mostly from apical cells of the spores and produce simple or forked terminal appressoria (Shree, 1987).

According to Muiru (2008) infection may take place through the stomata or directly by penetration of the cuticle with appressoria of germinating conidia. The penetration is mostly direct and occurs 12-20 hours after inoculation, and is similar in resistant and susceptible leaves of young and mature maize and sorghum plants (Hilu and Hooker, 1964; Knox-Davies, 1974; Shree, 1987). However, Levy and Cohen (1983) showed in vivo appressorium formation to be associated with collapse of epidermal cells.

Appressoria play an auxiliary role in survival and increase the chances of successful infection (Emmet and Parbery, 1975). However, Levy and Cohen (1983) reported that the appressoria of *E. turcicum* were very sensitive to desiccation. They also reported that appressoria were formed abundantly on leaf surfaces due to the stimulating effect of the leaves, but not on 2% water agar. Infection pegs develop from the appressoria, which grow into or between epidermal cells on either the dorsal or ventral sides of the leaf (Shree, 1987; Muiru 2008). Knox-Davies (1974) reported the formation of more than one infection hyphae from the appressorium of the fungus. Inoculum is the most important factor influencing infection during dew period (Levy and Cohen, 1983). Growth chamber studies show that the minimal dew period, with the optimum temperature of 20 °C, the fungus requires five hours for infection (Levy and Cohen, 1983).

After penetration, the fungus produces a vesicle-like structure of 10-30 µm diameters in or between the epidermal cells. Hyphae grow slowly in the mesophyll cells but rapidly in xylem cells. The entrance of the fungus into the xylem vessels and tracheids is observed 2-3 days after inoculation. Within six days a lesion occupies the area between 2-3 small veins, and later enlarges due to the growth of the hyphae from xylem to the surrounding healthy tissue (Shree,

1987; Muiru, 2008). The hyphae of the fungus establish early in the xylem of a susceptible variety with mycelial growth filling the vessels and tracheids, but in a resistant variety hyphae do not grow laterally or longitudinally and rarely branch (Shree, 1987).

2.8. Factors that Influence TLB Development

Generally, turicum leaf blight severity depends on weather conditions, plant growth stage, pathogenicity and genotype susceptibility (Levy, 1989). Previous studies showed that the turicum leaf blight is most favoured in the areas with high humidity and low temperature (Dunn and Namm, 1970; Raymundo and Hooker, 1981; Esele, 1995). Ngugi *et al.* (2000) reported that TLB is sporadic in occurrence, depending on the environmental conditions and the level of disease resistance in the variety. Severity of the disease occurs when conditions are favorable. High humidity associated with moderate temperature and cloudy weather is conducive conditions for disease development on the host plant (Singh *et al.*, 2004). Heavy dew on the growing plant has also being cited as one of the factors leading to TLB or northern leaf blight (NLB) disease severity (Dingerdissen *et al.*, 1996).

But it is also known that the disease has resurged and is affecting all maize growing regions in the world (Mwangi, 1998). Researchers have been associating the resurgence of TLB disease with many factors, among many factors; the most important ones are type of resistant gene, development of new races, land scarcity, trade liberalization, climate changes, pathogen environmental competence and mutation (Mwangi, 1998).

Many resistant cultivars were developed to confer race specific type of resistance. This type of resistance can easily be succumbed (failed to resist an attack) to new emerging races (Brown, 2002; Campana and Pataky, 2005). Susceptible genotypes act as the source of inoculums build

up, which, in turn, could result in disease resurgence. High amount of inoculum from different sources have the possibility of increasing new race development in the area (Robinson, 1987). There are several reports of new TLB disease races around the world. Emergence of new races of TLB such as 0, 1, 23 and 23N has posed a constant threat to the efforts of controlling the disease in maize breeding programmes worldwide (Ferguson and Carson, 2007). In East Africa, Mwangi (1998) observed races 0, 1, 2, 3, 12 and three unknown races.

Some studies have shown that land scarcity is another source of disease resurgence; land scarcity forces farmers to practice intensive farming, while compromising crop rotation, improved fallow and rotational woodlots (Kimaro *et al.*, 2008). This tendency has resulted in the increased number of fungal spores sufficient to cause the disease outbreak (Esele, 1995)

The effects of trade liberalization on agricultural sector could have contributed significantly to TLB resurgence. Trade liberalization has been associated with double effects on TLB disease occurrence. First, trade liberalization was accompanied with the reduction of agricultural input subsidy that resulted in the increased input prices and lower crop yields (Jean and Christina, 1991). Increase in input prices has forced farmers to resort to cheap and TLB disease susceptible cultivars, which increase inoculum in the field. The second effect is agricultural policy regulations to attract investors in agriculture sector. Governments were obliged to reduce the strict importation and crop inspection rules and regulations. The result was the introduction of inferior and TLB susceptible genotypes in agricultural fields, which lead to the increased TLB fungal inoculum (Love, 1994).

Pathogen environmental competence has been cited by researchers as one of the reasons of disease resurgence in crops (Jackson, 1999). Robinson (1987) further reported on a maize

landrace, which was highly resistant to disease in Malawi, where the same variety was highly susceptible to the same disease in Kenya. This implies that the pathogen has high environment competence and increased pathogenicity in Kenya than in Malawi. The same scenario can be used to explain the susceptibility to TLB of genotypes being currently introduced in different countries without enough testing in the target countries. This scenario results in the build-up of inoculum in maize growing areas.

Mutation can be another source of TLB resurgence in maize germplasm. Advanced breeding in maize have resulted in more uniform genotypes. Genetic uniformity increases the chances of pathogen mutations, new race emergence and increase in pathogenicity (Smale and De Groote, 2003). Pataky *et al.* (1998) reported epidemics of TLB in Florida due to mutation of pathogen on a super sweet hybrid maize cultivar. Mutation can also occur on plants themselves through altering the genetic structure and thus become more susceptible to diseases. Jenks *et al.* (1994) reports on the effect of reduced epicular wax structure in sorghum which exposed the genotype to *E. turcicum* attack.

2.9. Disease Cycle

Exserohilum turcicum, the cause of TLB, remains dormant during the dry period in infected residue of maize from the previous season. In the following growing season, the fungus sporulates on the residue. Conidia are carried by wind over long distances and deposited on leaves of maize and sorghum plants. If environment is favourable, conidial germination occurs 3-6 hours after inoculation (Hilu and Hooker, 1964).

E. turcicum overwinters as mycelium and chlamydospores in infected crop debris. At the onset of the subsequent season, fungi in crop debris begin to sporulate in response to higher

temperatures and humidity. Spores (conidia) are then disseminated by wind and rain splash to freshly planted maize. Conidia can be carried vast distances in the wind. They germinate in temperatures ranging from 17 to 27°C and during periods of extended leaf wetness (6 to 18 hours), infecting host tissue. Secondary cycles of disease occur where conidia produced in disease lesions are disseminated within the crop and to other fields by rain splash and wind (Gregory, 1968).

Once a dead lesion develops on a leaf, the fungus produces spores that can infect more leaf tissue (Gregory, 1968).

According to Fullerton and Fletcher (1974) chlamydospore were observed in conidia and these may be a factor for survival. The pathogen over-winters as mycelia and conidia in infected leaves, husks and other plant parts (Levy, 1984). According to Frederiksen (1980) conidia of *E. turcicum* are known to thicken their walls and become chlamydospore; however, diseased plant residues appear to be a more important source of infection than the conidia. Different races of the TLB possessed varying abilities to over-winter and survive being influenced by the host genotype (Nelson and Schiefele, 1970).

Initial lesions develop on lower leaves. Macroscopic symptoms appear first as minute light green to whitish flecks. Hyphae grow in the mesophyll cells in these flecks and in cells beyond the necrotic tissue of the flecks (Levy and Cohen, 1983). Flecks enlarge either singly or coalesce to form long elliptical, Grayish-green tan lesions ranging from 2.5 to 15cm in length. Once a dead lesion develops on a leaf, the fungus produces spores that can infect more leaf tissue (Gregory, 2004). Over time, the disease progresses to the upper part of the plant, kills

tissue on leaves above the ear. The dull green cast on lesions indicates sporulation of the fungus on lesions surfaces.

2.10. Managements of TLB

Various methods are used to manage turcicum leaf blight. These are cultural, chemical and biological methods. Cultural methods aim at reducing the amount of inoculum in the field. Biological control includes the use of natural enemies and resistant cultivars. The use of fungicides and tolerant genotypes has been reported to manage turcicum leaf blight on maize which holds some promise. Effective management practices that reduce the impact of TLB include selecting resistant cultivars, reducing maize residue, timely planting and applying foliar fungicides (Jakhar *et al.*, 2017). However, while the individual measures can be applied, integration of the components of the different management measures seems to give the highest benefit. Integrated disease Management (IDM) strategies including host resistance, fungicides and cultural practices, can serve a vital role in the control and management of TLB (Pratt *et al.*, 2003).

2.10.1. Cultural practices

Destruction of crop residues, crop rotation, weeding and intercropping practices has shown some effects in managing TLB disease on maize (Sharma and Duveiller, 2004). According to Sharma and Duveiller, (2004) optimal fertilizer rates, moisture management and timely planting decreases TLB and increased yield on maize production. However, cultural management measures are sometimes limited due to land availability and labour shortages.

Field sanitation and tillage ensures adequate handling of infected crop residue and deep ploughing. Such measures reduced initial inoculum of the pathogen and incidence of early season disease development (De Nazareno *et al.*, 1993)

Adjusting date of planting have also been reported effective in the management of turcicum leaf blight disease of maize (Fininsa and Yuen, 2001; Rai *et al.*, 2002). Crop rotation using soybean, beans and sunflower effectively reduced the NLB disease of maize (Pataky *et al.*, 2006). Assefa (1997) stated in Ethiopia that maize planting after maize would result in high TLB intensity, while planting maize following noug-maize-noug (Niger Seed) system result in lower severities of TLB.

Timely planting can often help cultivars escape the most severe damage from TLB if crop development outpaces normal disease progression. The latest-planted maize in an area may be infected when plants are smaller, resulting in the disease progressing more rapidly relative to the crop. However, in cases of high disease incidence, both early- and late-planted maize may be severely damaged (Jakhar *et al.*, 2017).

Conventional tillage to bury infected residue may also be helpful where erosion is not a problem, while crop rotation is interrupt the disease cycle. Cultural practices do not give complete protection. Significant yield losses still occur when the environmental conditions are favourable for the disease (Sharma and Duveiller, 2004).

2.10.2. Host resistance

Currently the recommended management measurements of the turcicum leaf blight of maize are the use of moderately resistant or tolerant cultivars, which is environmentally friendly as

well as economically feasible way of controlling diseases worldwide (Sharma and Duveiller 2004; Silva *et al.*, 2015).

Previous breeding studies have significantly contributed for TLB disease management (Welz *et al.*, 1999). In maize, breeding for TLB resistance started much earlier than 1961 (Ceballos *et al.*, 1991). But more efforts are still needed as new challenges arise. Following the difficulty in managing TLB due to high input prices and unreliable biological control, breeding for disease resistance is highly demanded (Mwakalobo and Kashuliza, 1999). The use of maize resistant varieties to TLB is cheaper and more reliable approach than other methods towards combating the disease (Welz and Geiger, 2000).

Genetic resistance is the most effective strategy for breeding foliar diseases of maize especially among resource-contributed farmer's (Silva *et al.*, 2015). However, resistance is hampered by high genetic variability of pathogen populations (Costa *et al.*, 2009). Information on management practices for maize disease is very scarce (Silva *et al.*, 2015). Host resistance strategies available for maize disease management include crop and cultivar rotation cultivation that involve varieties pyramided with different resistance genes and/or differ in the types of resistance genes (gene rotation). However, genetic resistance to some foliar diseases has not been stable in certain situations due to the high variability present in the pathogen population (Casela *et al.*, 2001). Therefore, the instability of race specific resistance, often associated with vertical resistance has promoted the search for more stable forms of resistance (Ramathani, 2011). A continuous process of identification of sources of resistance to fungal pathogens is needed not only to be incorporated in breeding programs, but also to be explored in management strategies to increase the stability of this resistance (Ramathani, 2011). On the

other side, molecular marker technology greatly facilitates the study of multiple diseases resistance and has made it possible to dissect the polygenes controlling such traits into individual Mendelian factors (Paterson *et al.*, 2008).

Studies by Carson (1995) indicated that the latent period is related to partial resistance, which suggested that selection for increased latent period length would be more beneficial than selecting for reduced disease severity. Selection for increased latent period length could be done in environments without severe disease epidemics, and also breeding material can be assessed at seedling stage for latent period length in the greenhouse during the off season.

Planting resistant cultivars is the most effective method for controlling TLB. Partial resistance protects against all four of the known races of the fungus and reduces the rate of disease development by reducing the number and size of the lesions and the amount of spores produced in these lesions, as well as increasing the length of the incubation and latent periods (Jakhar *et al.*, 2017). Race-specific resistance protects against specific races of the fungus, reducing disease development by producing chlorotic lesion with limited and delayed sporulation. Resistant cultivars should be planted in all commercial dent maize production fields. Select cultivars with high levels of partial resistance in combination with *Ht1*, *Ht2* or other *Ht* genes (Jakhar *et al.*, 2017).

Host plant resistance and tolerant hybrids of sorghum have been reported to be the most efficient and sustainable approach for management of northern leaf blight (Ramathani *et al.*, 2011). Such maize varieties delayed onset of the disease and reduced severity at crop maturity. However, the pathogen is known to produce new races that can overcome existing resistance. Hence the alternative option to the rapid breakdown of resistance is to cultivate varieties that

can withstand resistance for a long time in an environment favorable to TLB (Pratt *et al.*, 2003).

2.10.3. Biological control method

Various biological control agents have been tested to combat turcicum leaf blight. Biological controls have the advantages that they are environmentally friendly, do not require industry processes and do not develop resistance to pathogens (James, 2003).

The most promising bio-control agent is the use of *Bacillus subtilis* inoculum (Reis *et al.*, 1994). Mahamood *et al.* (1995) reported that *Trichoderma* sp., *Aspergillus* sp. and *Cladosporium* sp. were highly effective in inhibiting mycelial growth and sporulation of *Exserohilum turcicum* causing leaf blight of maize. Harlapur *et al.* (2008) reported that, maximum mean percent inhibition of mycelial growth of *E. turcicum* was recorded in *Trichoderma harzianum* (65.17%), followed by *T. viride* (56.95%). However, bio-control agents have slow and unpredictable actions; they require specialized skills for rearing (mass culturing) and ways of releasing them to the field. Furthermore, biological agents can multiply excessively and could be pathogens of other crops (Jutsum *et al.*, 1988).

2.10.4. Chemical control

TLB can be managed by using a number of fungicides. The use of fungicides has managed the disease to a remarkable point. Foliar fungicides have seen the most drastic increase in use over the past 10 years compared to other pesticides in corn (Wise and Mueller, 2011). In 2007, use of fungicides increased in corn and now a day corn crop is typically produced with higher fungicide inputs than the last five years (Wise and Mueller, 2011).

Raid (1991) studied the efficacy of mancozeb, chlorothalonil and propiconazole for control of rust and NLB in corn and found that all fungicides could reduce disease severity. Rehman *et al.* (1993) observed that propiconazole (Tilt) was effective against *E. turcicum* on maize *in vitro* conditions. Three sprays of propiconazole at three week interval were effective in reducing the rate of turcicum leaf blight development in maize (Bowen and Pederson, 1988).

Kachapur and Hegde (1988) tested seven fungicides and observed that mancozeb and captafol were the most effective fungicides for controlling turcicum leaf blight of maize. Sharma and Mishra (1988) found that *E. turcicum* infection of maize was effectively managed by six sprays of mancozeb (0.2% a.i.) at 10 days interval, starting from three days after inoculation at 30 days after sowing, although four sprays were most economical. The infection rate and severity rating were reduced and grain yield was increased with treatment. The average grain yield loss due to this disease was 24.49%.

Rehman *et al.* (1993) evaluated five fungicides for the management of *E. turcicum* on artificially infected susceptible maize cultivars. All of them reduced disease intensity and increased grain yield with mancozeb being distinctly the most effective, followed by carbendazim (Bavistin), zineb (Tierene, Aspor), thiophanate methyl (Topsin) and lastly copper oxychloride (Vitigran, Miltox, Cobox Blue). Finally he observed that propiconazole was effective against *E. turcicum* on maize under *in vitro* conditions.

Harlapur *etal.* (2011) conducted an experiment to assess the field efficacy of new generation fungicides for effective management of TLB caused by *E. turcicum* (Pass.) Leonard & Suggs and common rust caused by *Puccinia sorghi* Schwein. Seven fungicides were evaluated in field during *kharif* seasons of 2009 and 2010. The test fungicides were sprayed twice at 35 and

50 days after sowing. Results revealed that the disease severity was significantly reduced by fungicidal sprays. The triazole group of fungicide, Tebuconazole @ 0.1% treated plot resulted significantly lowest disease severity (18.97% TLB and 18.23% common rust) and recorded higher grain yield (71.66 q/ha). It is thus concluded that the fungicide Tebuconazole 250 EC @ 0.1% can be sprayed twice for economical and effective management of turcicum leaf blight and common rust.

The *in vitro* efficacy of various non-systemic and systemic fungicides, bio-agents and plant extracts was carried out and among non-systemic fungicides, mancozeb was found best in inhibiting the mycelial growth of *E. turcicum* (95.23% mean inhibition), propiconazole among systemic fungicides was found best (96.51% mean inhibition), *Trichoderma harzianum* among bio-agents was found best (76.38% inhibition) and among plant extracts neem oil inhibited maximum mycelial growth of *E. turcicum* (62.33%). The integrated disease management capsule was developed which showed that seed treatment with mancozeb followed by two foliar sprays at 40 and 50 DAS with propiconazole could effectively manage the disease with lowest disease intensity of 3.57 per cent giving highest grain and stover yield of 56.95 q/ha and 15.57 t/ha, respectively, as compared to control plot having highest disease intensity of 20.45 per cent with lowest grain and stover yield of 45.20 q/ha and 11.45t/ha, respectively (Wani ,2015).

Megersa *et al.* (2017) reported that using six maize varieties (BH-540, BH-543, BH-546, BH-660, BH-661 and AMHQ-760) integrated with foliar sprays of the systemic fungicide propiconazole at the rate of 350 ml ha⁻¹ and contact fungicide mancozeb at 2.6 kgha⁻¹ and integration of host resistance with foliar sprays protected the maize varieties from high TLB

epidemics, increased yield, yield components and maximized marginal benefit. The highest (11383 kg ha⁻¹) grain yield was obtained from propiconazole treated hybrid maize variety BH-546. And the highest marginal benefit (ETB 48,801.28 ha⁻¹) and marginal rate of return (ETB 6.33) were obtained from propiconazole treated variety BH-543 and BH-546, respectively.

Propiconazole (tilt) absorbed by assimilating parts of plant, the majority within one hour. It acts on the fungal pathogen inside the plant at the stage of first haustoria formation. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membrane (Anonymous, 2016).

3. MATERIALS AND METHODS

3.1. Survey of Turcicum leaf blight in Western Oromia

3.1.1. Study areas and sampling system

Field surveys were carried out during the 2016/17 growing season in major maize growing agro-ecologies of Western part of Oromia region of Ethiopia (Fig. 1). West Shewa, South West Shewa, Horro Guduru Wollega, East and West Wollega zones were assessed to determine the incidence and severity of TLB of maize. Zones and districts were selected based on the differences in production (farming) systems, weather condition (Relative humidity, maximum and minimum temperature), altitudes and major vegetation cover (availability of maize crop) (Ramathani, 2011). However, some farming systems tend to overlap between districts.

From each representative zone, 2-12 districts were surveyed based on production of maize crop and 8-73 fields each averaging 1ha at the interval of 5-10 km along road side were assessed per zone. Such fields were randomly selected on both sides of the road. A total of 172 farmers' fields, 29 districts and 5 zones were surveyed when the crop was at flowering to grain filling stage. Each sampling point was marked with the global positioning system (GPS) using GPS receiver for altitude and co-ordinates. In each field, 9 m² areas were marked out in three randomly selected points in a diagonal form using meter tap. Ten stands of maize plant in the middle of each marked area were randomly selected and assessed for incidence and severity (Nwanosike *et al.*, 2015). Each marked area was regarded as a replicate for disease assessment.

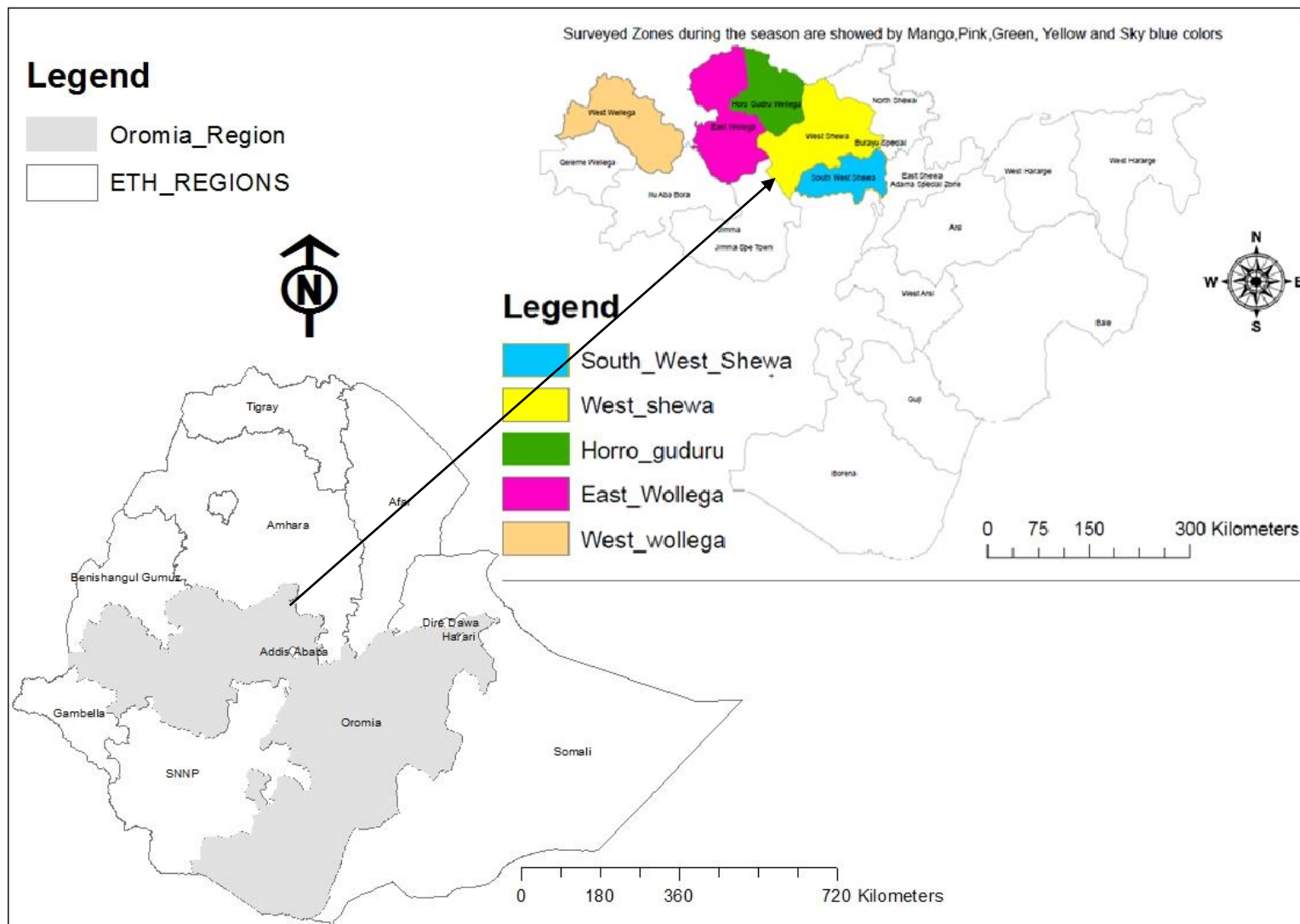


Figure 1: Map of Ethiopia showing the survey area

3.1.2. Assessment of turcicum leaf blight

Diseases incidence

Disease incidence was assessed as proportion of plants showing symptoms in the field. The number of plants within 10 randomly selected stand showing TLB symptoms were counted and expressed as a percentage of the total number of stands per plot using the following formula (Nwanosike *et al.*, 2015).

$$DI(\%) = \frac{\text{Number of Diseased plants}}{\text{Total number of plants observed}} * 100$$

Where, DI is disease incidence

Disease severity

Disease severity on whole plant basis was rated using a visual scale of 0-5; where, 0 = all leaves free from infection, 1= a few restricted lesions on the lower leaves ($\leq 5\%$), 2= several small and large lesions on many leaves (5.1-10 %), 3= numerous small and large lesions on many leaves (10.1-25 %), 4 = many enlarged and coalesced lesions on many leaves above the cob (25.1-50 %), 5 = several coalesced lesions, leaf showing wilting, tearing and blotching typical blight symptoms ($> 50\%$) (Muiru, 2007; Durrishahwar *et al.*, 2008). Severity scores were converted to percent disease index, PDI, (Wheeler, 1969;Kumar, *et al.*, 2011).

$$PSI(\%) = \frac{\text{Sum of numerical rating}}{\text{Total number of plant observed} \times \text{maximum rating}} * 100$$

Where: PSI is percent severity index

Varieties with ratings; 0-1.4 ($<30\%$) were considered resistant, 1.5-2.4(30-50%) =moderately resistant, 2.5-5($>50\%$) = susceptible (Muriithi, 2001).

3.1.3. Data analysis

Disease data (incidence, severity and PSI) were entered into Excel to calculate means and percentages. Correlation analysis was carried out between disease data and weather parameters using the Proc. Corr. procedures of the SAS software (SAS, 2002). Disease distribution maps were generated using the Arc GIS 10.3 software with spatial analyst by interpolating the surface from GPS points and the associated field severity data using the inverse distance weighted (IDW) interpolation method.

3.2. Field experiments

3.2.1. Description of the Experimental Area

The field experiment was conducted at Ambo Plant Protection Research Centre (APPRC). APPRC is found between 8°57' 58" N latitude and 37° 51' 33" E longitudes and at an altitude of 2175 m.a.s.l. The annual average temperature and rain fall was 27.54°C and 1077.68 mm, respectively (Ambo Plant Protection Research Centre, Metrology Station Data Base)

3.2.2. Description of experimental materials and treatments

Twelve treatment combinations which consisted of three varieties, three fungicide spray frequencies and one untreated check for each variety were used for field experiment (Table 1). The three highland maize released varieties used were AMH-851(Jibat), AMH-850 (Wenchi), and AMHQ-760(Webi). These released varieties were reported to have moderately resistant, moderately susceptible and susceptible reactions to TLB, respectively based on previous recommendation (Source APPRC, Highland Maize Research Program). Wenchi and Jibat grow at an altitude of 1800-2600 m.a.s.l. and both had potential yields of 8-12 t/ha, whereas

AMHQ-760 is a hybrid quality protein maize (QPM) which grows at an altitude of 1650-2400 m.a.s.l. and has a potential yield of 9-12 t/ha under good management condition on research fields (Source APPRC, Highland Maize Research Program).

The fungicide used in the experiment was Propiconazole (Tilt® 250 EC at 500 ml ha⁻¹). 500 ml dissolved in 200L of water per hectares. It was applied at three different spray frequencies which were one time, two times, and three times applications at 10 days interval. Fungicide application was started from the time lesions were visible on the three to five basal leaves of the susceptible variety (AMHQ-760, Webi). It was applied using Manual Knapsack Sprayer of 15 liters capacity. Control plots were sprayed with water only in the same manner with that of fungicide sprayed plots to minimize difference due to moisture. The area of sprayed plot was bordered by plastic sheet at the time of fungicide spraying to minimize the risk of fungicide drift to adjacent plots.

Table 1: Treatment numbers and their descriptions

Treatment numbers	Treatment descriptions
1	Maize Variety AMHQ-760(Webi) + one time application
2	Maize Variety AMHQ-760(Webi) + two times application
3	Maize Variety AMHQ-760(Webi) + three times application
4	Maize Variety AMH-851(Jibat) + one time application
5	Maize Variety AMH-851(Jibat) + two times application
6	Maize Variety AMH-851(Jibat) + three times application
7	Maize Variety AMH-850(Wenchi) + one time application
8	Maize Variety AMH-850(Wenchi) + two times application
9	Maize Variety AMH-850(Wenchi) + three times application
10	Maize Variety AMHQ-760(Webi) + untreated check
11	Maize Variety AMH-850(Jibat) + untreated check
12	Maize Variety AMH-850(Wenchi) + untreated check

3.2.3. Experimental design and management of the experiment

Treatments had a factorial arrangement in randomized complete block design (RCBD) with three replications. Each plot consisted of four rows of 3m long with 75 cm spacing between rows. The distance between adjacent hills was 25cm. Plot size was 9m² (3mx3m). The spacing between plots and blocks were 1m and 1.5m, respectively. Susceptible maize variety AMHQ-760 was planted perpendicular to the experimental blocks one week before the experimental plots to serve as spreader rows. All plots were planted by hand. At planting, two seeds were placed per hill and thinned to one at establishment. A 200kg ha⁻¹ nitrogen fertilizer was applied in two splits: half at planting and half at 40 days after emergence. Diammonium phosphate (DAP) fertilizer was applied at the rate of 150 kg ha⁻¹ at planting. Weeds were controlled three times by hoeing and hand weeding.

3.2.4. Inoculum preparation, inoculation and disease establishment

Maize leaves infected with TLB were identified by their classical symptom and collected in the previous season. The presence of spores and mycelium of TLB was observed under compound microscope from collected and stored maize leaves. The infected leaves were dried under shade, grounded in to powder and placed in the paper bag and preserved at 4 °C in refrigerator until inoculation date. The whole infected leaf powder (WILP) inocula were used for inoculation. According to Dutta *et al.* (2010) infected leaf powder (ILP) inocula are effective in inducing infections under field conditions. Maize plants in the border rows were inoculated first with TLB ILP inoculum. Pinches (Tea spoon quantity) of pulverized leaves were added or dusted in to the whorls of maize plants in the border rows and to plants of the experimental plots (Megersa *et al.*, 2017). Inoculations were performed twice at seven days interval starting from 3-5 leaves stage of the plant and were done in the evening or late afternoon to allow successful infection when dew and ambient temperature is optimal (Carson, 1995).

3.2.5. Data collection

3.2.5.1. Disease data

Disease incidence: Initial scoring for disease incidence was conducted when lesions were visible on the three to five basal leaves of maize plants. The number of infected plants in each plot was recorded and their values converted into percentage of the total number of plants inspected according to the formula suggested by Nwanosike *et al.* (2015) as follows.

$$DI(\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants per plot}} * 100$$

Where:- DI is disease incidence

Disease severity: Severity was recorded on eight randomly tagged plants per plot. Disease severity was recorded six times at seven days interval starting at about 2-3% infection on the lower leaves of the susceptible variety, AMHQ-760. Plants were assessed for disease severity using a 1 to 5 scale (Payak and Sharma, 1983).Where:

1 = very slightly infected, one or two restricted lesion on lower leaves or trace.

2 = slight to moderate infection on lower leaves, a few scatter lesions on lower leaves.

3 = abundant lesions on lower leaves, a few on middle leaves.

4 = abundant lesions on lower and middle leaves extending to upper leaves.

5 = abundant lesions on all leaves, plant may be prematurely killed by blight.

The severity scales were converted in to percentage severity index (PSI) for analysis using the following formula (Wheeler, 1969):

$$\text{PSI}(\%) = \frac{\text{Sum of numerical rating}}{\text{Total No. of plant observed} \times \text{maximum rating}} * 100$$

Area under disease progress curve (AUDPC): The disease percent severity index scores were used to calculate AUDPC for each treatment. The area under disease progress curve was used to quantify the beginning of epidemic and the time until disease reached maximum stage. AUDPC was calculated with the formula suggested by Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i=1}^{n-1} 0.5[(x_{i+1} + x_i)(t_{i+1} - t_i)]$$

Where, x_i = is the cumulative percent severity index expressed as a proportion at the i^{th} observation, t_i = is the time (days after sowing) at the i^{th} observation, and n = is total number of observations. Since the percent severity index was expressed in percent and time (t) in days, AUDPC values were expressed in %-days (Wilcoxson *et al.*, 1975).

Apparent Infection Rate: The six disease severity observations recorded at 7 days interval were regressed over time and the apparent infection rates as the coefficient of the regression line, $\ln [X/(100 - X)]$, where X is average coefficient infection plotted against time in days (Vander Plank, 1963) were calculated for each treatment.

3.2.5.2. Yield and Yield Components

Days to maturity: recorded as a number of days after planting to when 90% of the plants in a plot form black layer at the point of attachment of the kernel with the cob (i.e. 90% physiological maturity).

Stand count at harvest: Stand count was determined by counting the plants from all rows in the plot just at harvesting. Total stand count per net plot area was converted into total stand count per hectare.

Number of ears per plant: Number of ears per plant of eight tagged plants was counted from each plot.

Number of rows per ear: The average numbers of rows per ear from the eight pre-tagged maize plants of each plot were counted.

Ear length (cm): The average length of the ear from its base to the tip from the eight randomly tagged plants in each experimental unit was measured.

Moisture contents: Actual moisture contents of the seeds from each plot was measured immediately at harvesting date

Thousand kernel weights (gm): Kernels were drawn randomly from each plot, counted manually and weighed in grams using sensitive balance just on the day of harvest.

Yield per plot and per hectare: Total grain yield harvested from the two middle rows was determined and adjusted to 12.5% moisture content as follows:

$$\text{Adjusted yield per plot} = \frac{(\text{FW}(100 - \text{AMC}) * 0.8)}{\text{RDW}}$$

Where:

FW= Field Weight

AMC= Actual moisture content

RDW= Recommended dry weight (100-12.5) =87.5

0.8= Shelling %(Given)

Then the yield per plot was converted into yield per hectare (t ha⁻¹).

3.2.6. Relative yield loss

Losses in grain yield were calculated as the difference between mean yield of protected plots(plots that received thrice application of propiconazole fungicide) and unprotected (unsprayed plot) plots of respective variety. Losses were calculated separately for each of the treatments using the formula developed by Harlapur (2005):

$$\text{RYL}(\%) = \frac{(Y_1 - Y_2)}{Y_1} * 100 \quad \text{Where:-}$$

RYL= relative yield loss, Y_1 = Mean yield of protected plots (plot with maximum protection, plots that received thrice application of propiconazole fungicide)

Y_2 = Mean yield of unprotected plots (i.e. unsprayed plots)

3.2.7. Data analysis

Analysis of variance: Data on TLB initial and final incidences, terminal percent severity index, AUDPC values, apparent infection rate, yield and yield components were subjected to analysis of variance (ANOVA) using SAS software version 9.0a (SAS, 2002). Mean separation was made based on the LSD at the 5% probability level and interaction effects were separated by SAS extension software *Pdmix800.sas* ($p \leq 0.05$).

Correlation analysis: Correlation analysis was carried out using SAS PROC CORR (SAS, 2002) to determine the relationship among disease parameters (disease incidence, percent severity index, Progress rate and AUDPC) with yield and yield components.

Cost-benefit analysis: Economic decision was based on price of produce at field level, cost of fungicide (i.e. when applied one time, two times and three times), and labor at local market. Accordingly, price of grain (ETB kg⁻¹) obtained from local market and total sale from one hectare was computed and expressed as Ethiopian birr per kilogram (ETB kg⁻¹). Price of seed of each variety was collected from local market and farmers union in the localities. Price of propiconazole fungicide per liter was assessed, labor to spray this fungicide, the total price incurred to spray one hectare of maize was calculated as well (Appendix Table 4). Partial budget or marginal rate of return (MRR) analysis was applied based on the input and outputs of the products for production profitability decisions (CIMMYT, 1988).

The cost/benefit analysis for integrated TLB management options was performed using partial budget analysis or marginal rate of returns. Partial budget analysis is a method of organizing data and information about the cost and benefit of various agricultural alternatives (CIMMYT, 1988). It is a useful technique to determine the costs and benefits of use of new technologies compared to the traditional methods. Partial budgeting is employed to assess profitability of any new technologies (practices) to be imposed to the agricultural business. Marginal analysis is concerned with the process of making choice between alternative factor-product combinations considering small changes. Marginal rate of return is a criterion which measures the effect of additional capital invested on net returns using new managements compared with the previous one (CIMMYT, 1988). It provides the value of benefit obtained per the amount of additional cost incurred percentage. The formula is as follows:

$$\text{MRR} = \frac{\text{DNI}}{\text{DIC}} \quad \text{Where:-}$$

MRR is marginal rate of returns, DNI = difference in net income compared with control and DIC= difference in input cost compared with control.

The following points were considered during cost/benefit analysis using partial budget.

- Since the experiment were planted on a research field; yield produced were adjusted to 10% lower than values from research field, assuming the farmers farming condition.
- Costs for all agronomic practices were uniform for all varieties and treatments within site;
- Costs of labor and spray equipment was taken based on the price in the locality; and Costs return and benefit were calculated per hectare basis.

4. RESULTS AND DISCUSSION

4.1. Field Surveys

4.1.1. Status of turicum leaf blight of maize (TLB) in surveyed areas

Turicum leaf blight of maize was prevalent in all surveyed agro-ecologies in 2017 cropping season at varying levels (Table 2). The mean TLB incidence in the surveyed districts ranged between 16.3% (Abay chomen district of Horro Guduru Wollega Zone) and 96.7 % (Wayu Tuka district of East Wollega Zone). Amongst all the surveyed districts, the highest disease incidence (96.7%) was recorded in Wayu Tuka followed by Lalo Assabi, Sibu Sire, Diga and Bako tibe with disease incidence of 94%, 92.5%, 91.8% and 90%, respectively. On the other hand, Abay chomen, Horro, Bacho and Jima rare districts had relatively lower TLB incidence of 16.25%, 20%, 25% and 26.7%, respectively.

Percent severity index (PSI) ranged from 3.13% in Abay chomen district to 57.50% in Lalo Assabi district. The highest mean PSI (57.5%) was recorded in Lalo Assabi district followed by Sibu sire, Bako Tibe, and Toke Kutaye, which had 50.7%, 46% and 45% PSI, respectively. In contrast the mean minimum PSI was recorded in the Abay Chomen district (3.13%) followed by Bacho (4%), Dandi (6.33%) and Jima rare (6.67%).

The highest maize TLB incidence and PSI were recorded in areas with a mean maximum temperature of 31.12-34.93°C and humid areas (Table 2). The finding of the present study is in agreement with Nwanosike *et al.* (2015) who stated that turicum leaf blight of maize is widely distributed, and the level of intensity varied across locations in Tanzania and the highest TLB incidence and percent severity index recorded at maximum temperature up to (31-35°C) and high relative humidity (75-100%). Earlier researchers (Harlapur, 2005;

Khedekar *et al.*, 2010 ; Rani, 2015) suggested that prevailing environmental conditions (relative humidity, maximum and minimum temperature) during cropping season could be reasons for higher disease incidence. Ullstrup (1966) also reported that TLB incidence vary in prevalence and severity from year to year and from one locality to another, depending largely on environmental conditions (i.e. Humid weather along with heavy dew favored the spread and development of the disease in an epidemic form). TLB has particularly been noticed to cause significant maize yield reduction in many production regions including in sub-Saharan Africa (SSA), especially in the humid mid-attitude and highland regions (Latterell and Rossi, 1983; De Vries and Toenniessen, 2001).

Beshir *et al.* (2015) reported that *E. turcuicum* was the causative agent of leaf blight observed in central Sudan. Furthermore, the TLB occurred in all the study locations with incidence and severity ranging from 45 to 100, and 65 to 100, respectively.

In Africa, where maize and sorghum are the staple foods, TLB is reported to be wide spread in the warm and humid growing regions of Ethiopia, Tanzania and Uganda (Adipala *et al.*, 1993; Tewabech *et al.*, 2001).

Table 2: Mean incidences and percent severity index of Turcicum leaf blight on maize across survey districts and zones during the 2017 growing season

Zones	Districts	Field No.	Altitude range (m.a.s.l)	Min.T (°C)	Max T (°C)	RH%	Disease incidence (%)			PSI (%)		
							Mean	Range	St dev.	Mean	Range	St dev.
West	Bako Tibe	10	1623-1708	22.14	33.90	68.10	91.00	50-100	16.63	46.00	10-75	23.66
Shewa	Ilu Galan	8	1707-1859	24.53	31.05	61.38	63.75	30-100	27.22	19.00	10-52	14.96
	Dano	7	1600-1739	24.00	35.59	70.00	75.71	50-100	18.13	31.71	8-60	19.70
	Cheliya	2	2140-2561	23.40	35.60	65.00	80.00	60-100	28.28	32.50	20-45	17.68
	Liban Jawi	4	2269-2455	23.45	35.68	65.00	62.50	30-90	27.54	23.75	10-40	16.01
	Toke Kutaye	11	1947-2416	21.15	33.29	68.82	79.09	30-100	27.00	45.00	10-70	21.56
	Ambo zuria	10	2053-2624	20.45	27.14	67.40	73.00	30-100	26.69	34.10	4-60	21.52
	Dire Inchini	5	2413-2491	16.20	34.72	79.00	72.00	30-100	30.33	32.40	10-55	21.82
	Shenen	1	2495	16.20	36.00	79.00	80.00	80	—	40.00	40	—
	Jibat	5	2100-2565	16.20	36.00	81.00	90.00	60-100	17.32	35.00	10-55	20.31
	Nono	7	1525-2062	19.03	36.00	81.00	68.57	50-100	21.93	22.57	8-60	19.36
	Dandi	3	2257-2592	15.40	36.00	81.00	43.33	30-50	11.55	6.33	4-10	3.22
South West	Waliso	3	2174-2342	15.40	36.00	81.00	73.33	50-90	20.82	18.33	10-25	7.64
Shewa	Bacho	2	2159-2268	15.40	36.00	81.00	25.00	20-30	7.07	4.00	4	0
	Dawo	3	2175-2203	15.40	36.00	81.00	43.33	40-50	5.77	8.66	8-10	1.16
East	Diga	11	1193-2237	27.89	31.63	57.00	91.82	80-100	7.51	39.27	25-60	14.62
Wollega	Kiramu	4	1987-2084	22.20	26.25	50.50	70.00	0-100	46.90	42.50	0-65	29.58

Table 2: Continued.....

	Gida Ayana	15	1267-1911	23.04	28.49	52.67	80.00	40-100	22.04	38.93	10-75	26.08
	Guto Gida	3	1342-1394	28.40	33.77	57.00	53.33	30-100	40.41	30.00	10-55	22.91
	Wayu Tuka	3	1888-2067	21.87	34.93	67.00	96.67	90-100	5.77	18.33	10-25	7.64
	Sibu sire	8	1722-1834	22.40	36.30	78.38	92.50	80-100	8.86	50.75	10-70	17.56
	Gobu Sayo	3	1746-1857	19.43	31.60	59.67	66.67	20-100	41.63	42.33	5-70	33.56
Horro	Horro	3	2390-2400	29.80	31.70	61.33	20.00	0-30	17.32	6.67	0-10	5.77
Guduru	Abay choman	8	2308-2418	28.74	31.70	67.25	16.25	0-40	15.05	3.13	0-5	2.59
Wollega	Guduru	4	2251-2285	25.25	25.43	58.75	35.00	0-80	33.17	10.00	0-25	10.80
	Choman Guduru	2	2229-2302	23.00	28.45	75.00	35.00	30-40	7.07	7.50	5-10	3.54
	Jima rare	6	2267-2622	23.00	31.03	84.00	26.67	20-40	8.17	6.67	5-10	2.58
West	Gimbi	11	1746-1857	27.04	32.78	64.73	67.27	40-100	21.95	32.27	10-60	16.64
Wollega	Lalo Assabi	10	1773-1900	25.67	31.12	75.10	94.00	60-100	13.49	57.50	25-75	15.68

PSI=percent disease index, RH=relative humidity, Min.T=minimum temperature, Max.T=maximum temperature, St dev. = Standard deviation

4.1.2. Geographical distribution of TLB of maize in surveyed areas during the season

Disease incidence, PSI and GPS records were used to construct disease map in surveyed zones and districts. The disease map illustrates severity and incidence levels over the agro-ecologies and was used to study epidemic patterns at the time of the study. Results showed spatial pattern of epidemics.

TLB incidence was categorized on the map as described by Harlapur (2005), where, 0-5 % = slight/trace infection, 5.1-15 % = light infection, 15.1-30 % = moderate infection, 30.1-75 = severe infection, >75 = very severe infection.

In terms of incidence, virtually all districts had very high level of disease. The TLB incidence in vast majority of districts studied recorded disease incidence of 75% and above (Fig. 2). In Ambo zuria, Ilu Galan, Dire Inchini, Nono, Dandi, Dawo, Waliso, Guduru, KIRAMU, Gobu Sayo, Guto Gida, and Gimbi districts severe infection (30.1-75%) of TLB were recorded which was indicated by orange colour on the map. Toke kutaye, Sasiga, Diga, Wayu Tuka, Sibu Sire, Bako Tibe, Jibat, Lalo Assabi, Dano, and Chelia recorded above 75% which were very severe infection indicated by red colour on the map (Fig. 2). On the other hand, moderate infections (15.1-30%) of TLB were recorded in a few districts such as Becho, Abay chomen, Horro and Jimma Rare. This was due to moderately resistant to resistant hybrid varieties or cultivar grown in the districts.

The disease map also illustrates spatial patterns of epidemics with some districts showing high variation in PSI. PSI levels ranged from as low as 3.13% in Abay Chomen to as high as 57.7% in Lalo Assabi district. It was described on the map according to Nwanosike *et al.* (2015), where, 0-29=low severity, 30-49=moderate severity and 50-100=high severity (Fig. 3).

In general low value of disease incidence and severity suggests that a number of varieties grown by farmers appear to be resistant or tolerance to TLB. Second possible explanation for the observed data could be crop rotation (especially preceding crop).

The distribution of TLB and pathotypes of *E. turcicum* have been identified in Kenya (Ngugi *et al.*, 2000) and Uganda (Sserumaga *et al.*, 2013). According to Wende *et al.* (2013) turcicum leaf blight is ranked as the number one problem and is considered a high research priority of maize in Ethiopia.

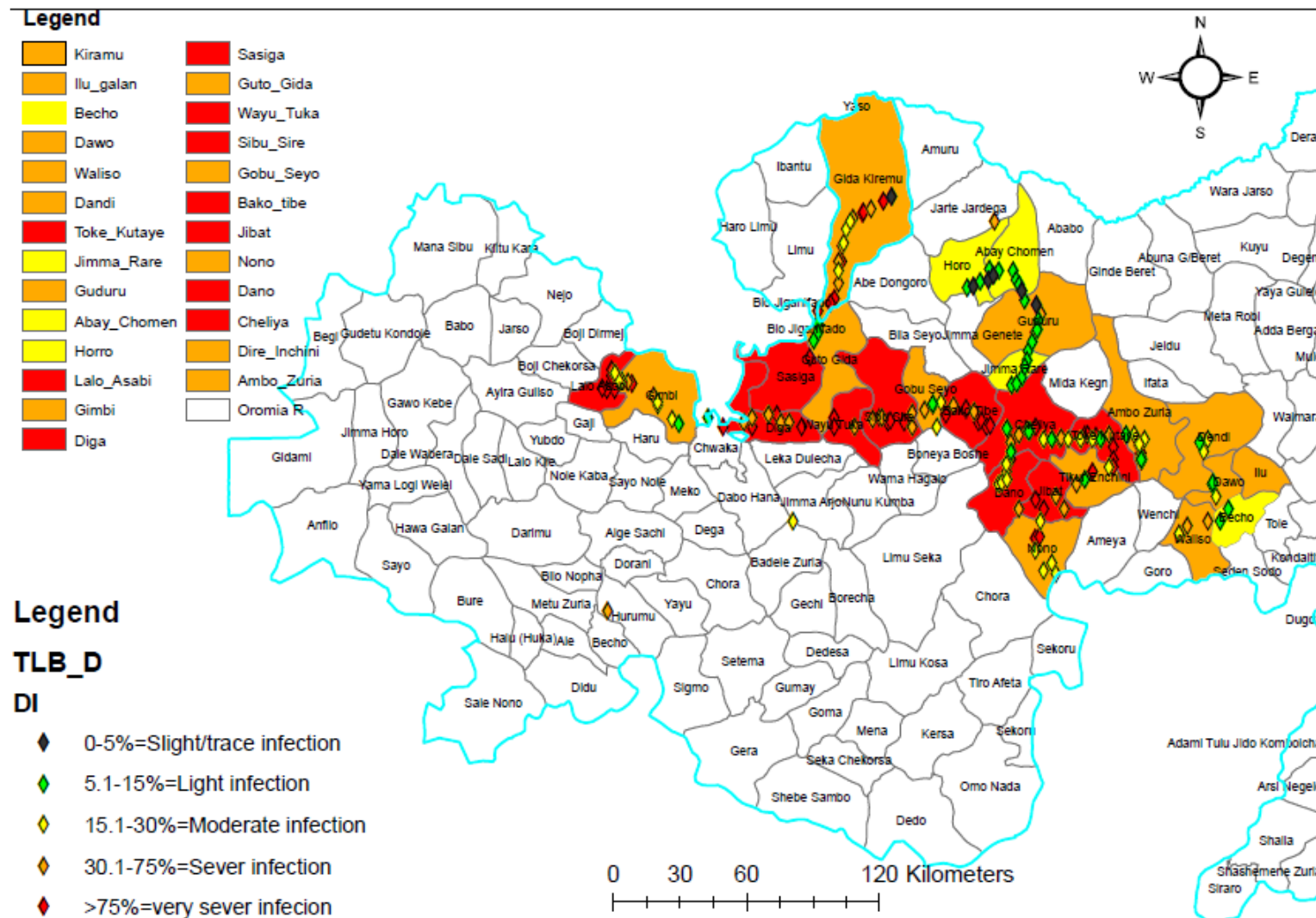


Figure 2: Disease map based on incidence of Turcicum leaf blight of maize in 29 districts of (5 different Agro-ecologies of zones) of Western Oromia region of Ethiopia during 2017 main growing season.

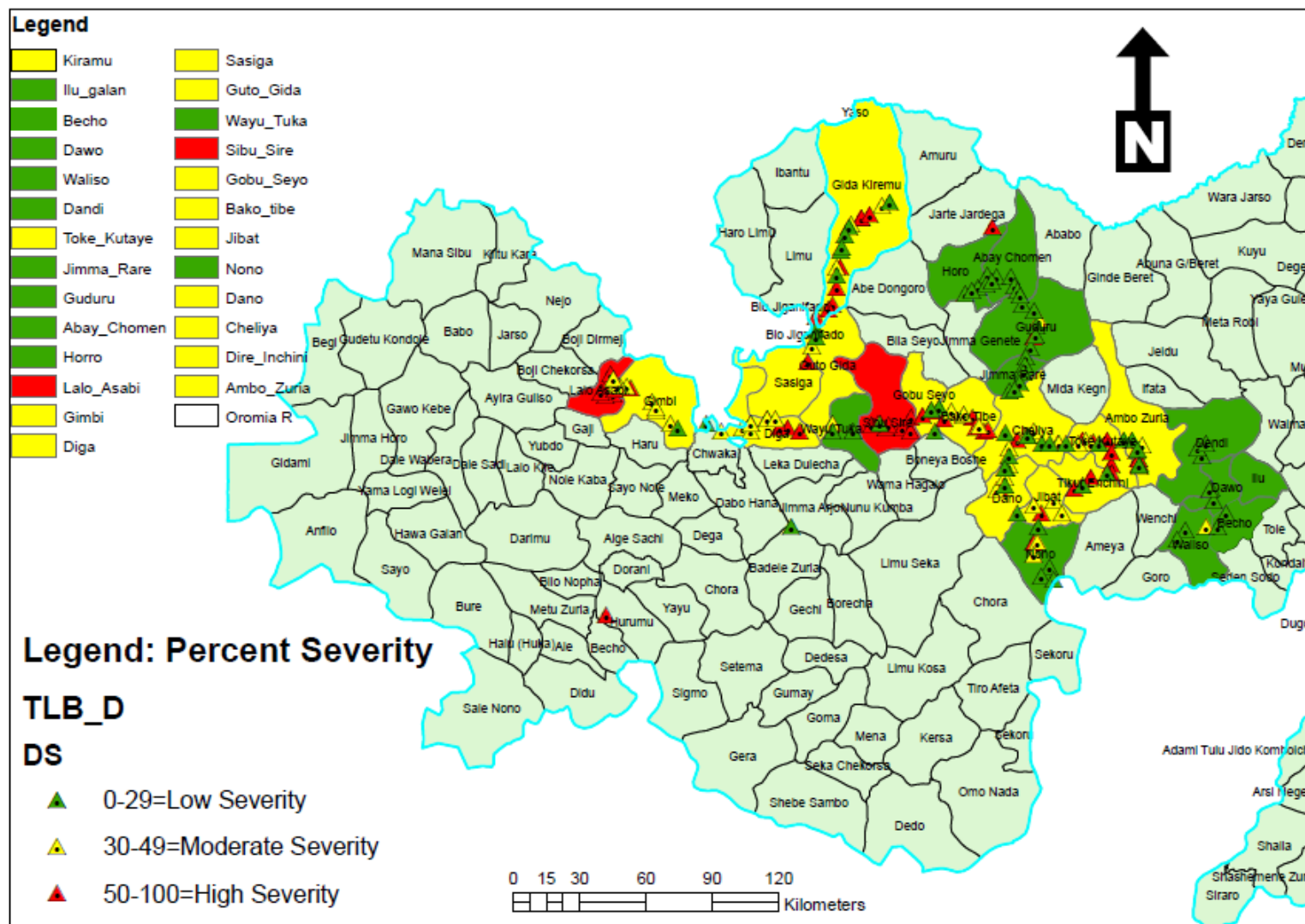


Figure 3: Disease map based on Percent disease index of Turcicum leaf blight of maize in 29 districts (5 different Agro-ecologies of zones) of Western Oromia region of Ethiopia during 2017 main growing season.

4.1.3. Status of TLB in relation to maize varieties under production

Survey result on incidence and percent severity index of TLB revealed that maize varieties under production varied in their reaction to TLB. The highest mean disease incidence (90%) was scored on variety designated as Oromia, whereas the lowest (30%) was recorded on variety denominated as Shashamane (Fig. 4). Varieties such as Limu, Wenchi and BH-540 had 84%, 80% and 79.1% disease incidences, respectively. TLB incidences of 37.5% and 47.5% were recorded on unspecified hybrid and Jibat varieties, respectively.

TLB index expressed as PSI ranged from 10% on variety Shashamane to 42% on BH-540. Maize varieties Limu and Kolba also had higher PSI of around 40%. The study revealed that TLB was more common and severe on varieties such as BH-540, Limu, Kolba and Local. In contrast varieties such as Shashamane, Global and Jibat performed relatively better than others with respect to the TLB reaction in the surveyed areas (Fig. 4). This indicated that the upsurge in the TLB on maize was highly influenced by the nature of susceptibility/resistance of the varieties and conducive environmental conditions for the development of the disease in the location. Earlier survey reports (Harlapur *et al.*, 2000; Ramathani, 2011) indicated that cultivar susceptibility and weather parameters play an important role in influencing TLB severity. Turcicum leaf blight is reported to cause devastating damage on most commercial varieties of maize released in Ethiopia (Tewabeche *et al.*, 2012).

Krausz *et al.* (1993) reported an epidemic of turcicum leaf blight from Texas and reported loss in grain yield of susceptible hybrids ranging from 40 to 50%, whereas hybrids with either Ht1 resistance gene or significant polygenic resistance had minimum disease incidence. Babu *et al.*

(2004) reported TLB incidence on maize at Almora and it attained epidemic proportion resulting in 83% yield reduction.

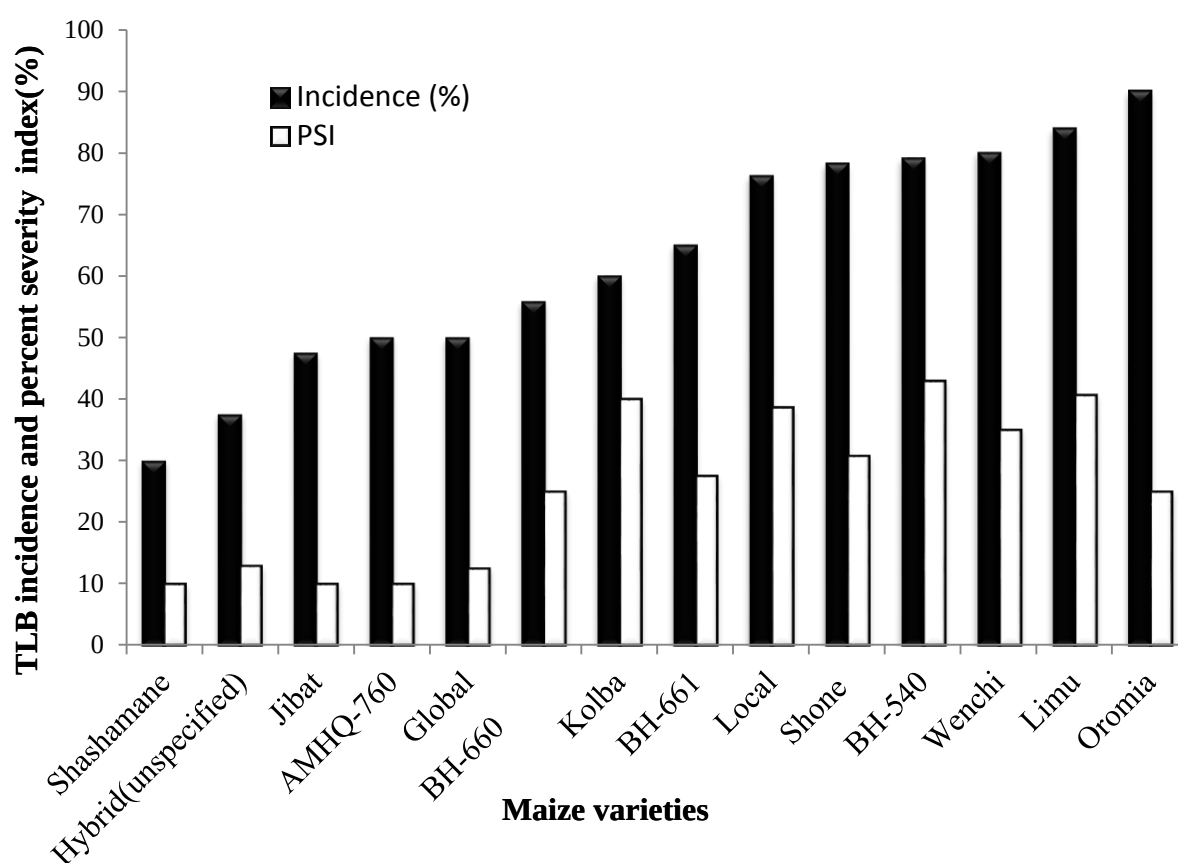


Figure 4: Incidences and percent severity index of *Turcicum* leaf blight on maize varieties under production in surveyed districts

4.1.4. Intensity of TLB at different growth stages of Maize

TLB incidence and PSI showed marked variations across the different maize growth stages. This revealed that the development of TLB also relies on the maize growth stage. The overall mean incidence and PSI were high (up to 74.1% and 34.7%, respectively) during the grain

filling stage of maize compared to tasseling and silking stage of the crop (Fig. 5). Kalappanavar (2017) conducted survey in India and reported that the maximum disease severity was recorded at grain filling stage (22.06 and 15.50 %) and minimum at vegetative stages (14.31 and 9.49 %).

From the current study data higher yield loss due to TLB could be expected in many of the surveyed location because the disease occurred before silking stage of maize. This is in agreement with the study of Raymond and Hooker (1981) who stated that if the TLB disease establishes before silking, yield reduction of up to 40% may occur but if infection delays until 6-8 weeks after silking, yield losses would be minimal. The present study confirmed wider distribution of TLB in surveyed areas as associated with plant stages. This is in line with Perkins *et al.* (1987) who stated that TLB disease is widely distributed, however, sporadic in nature and mostly depends on weather conditions, stage of plant growth and level of resistance in maize cultivars.

In Uganda, overlapping of growing seasons and presence of off-season maize resulted in infection before tasseling stage, consequently resulting to higher yield loss (Adipala *et al.*, 1993b). Yield loss is caused predominantly through loss of photosynthetic leaf area due to blighting (De Vries and Toenniessen, 2001). Loss of photosynthetic tissue can result in decreased yield, and silage quality can be affected (Jakhar *et al.*, 2017).

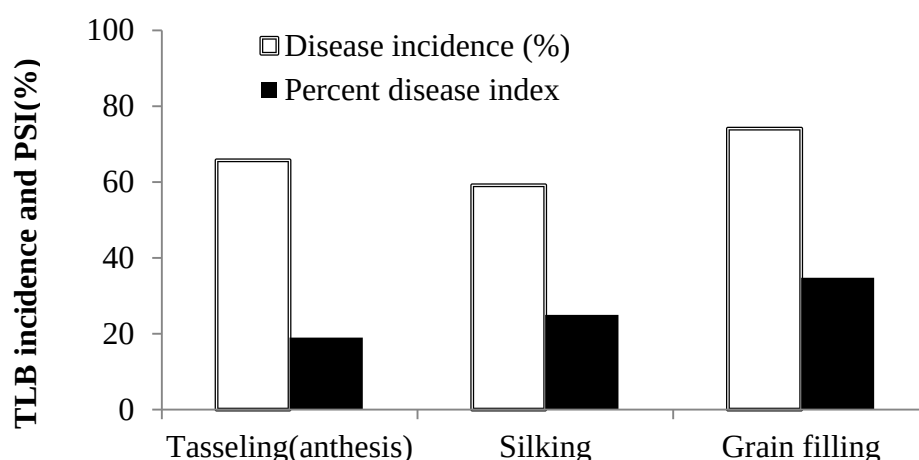


Figure 5: Mean incidence and percent severity index of *Turcicum* leaf blight on maize at different growth stages of maize growing in surveyed areas

4.1.5. Effects of cropping system and preceding crop on maize TLB incidence and PSI

Types of cropping system, previous crop, and residue left in the field played important role for the development of maize TLB in the surveyed areas. PSI ranged from 12.3% to 60% during the current survey (Table 3). The maximum PSI was recorded in fields where maize is intercropped with sorghum while maize-kale intercropping was associated with the lowest PSI. On the other hand, most of the intercrops failed to reduce TLB level as compared to mono cropping. The maximum TLB severity in sorghum-maize intercropping might be due to sorghum's ability to serve as collateral host for *E. turcicum*, the cause of TLB. However, no definite pattern was established in the current experiment as far as the effect of intercropping on TLB is concerned. Assefa (1997) also reported that planting maize and sorghum together showed higher TLB intensity and lower common rust (CR). Ramathani (2011) also suggested that cropping systems significantly influenced the patterns of spread of TLB disease.

With respect to effect of preceding crop, the current work failed to establish a pattern with respect to effect of rotation on TLB development. The only exceptions were maize following fenugreek, which had 30% incidence and 5% PSI, a much lower score as compared to other systems. TLB is residue born disease, as a result alternatively sowing non cereal crops in the same field can reduce the availability of host residue from the field, thereby affecting the chance of the pathogen to over season. This obviously reduces the inoculum level (source) and the incidence of early season development of the disease. Studies on TLB on maize indicate that maize residues are an important factor for the survival of the pathogen and initiation of the epidemic (Fullerton and Fletcher, 1974). Assefa (1997) stated that maize planting after maize would result in high TLB intensity, while planting maize following noug-maize-noug (Niger Seed) system result in lower severities of TLB. High percent severity index on maize was due to intensive cultivation of maize crop season after season, every year, narrow genetic makeup of the commercial hybrids and non-adoption of disease management practices by the farmers (Kalappanavar, 2017). Increased severity of *Turcicum* leaf blight in Africa associated with continuous cultivation of maize and susceptible maize cultivar has also been reported by Danson *et al.* (2008). However, lack of marked reductions in TLB level across most of the rotation schemes, included in the current survey, might be because of short durations for rotating maize with other crops. The fact that most of the rotating crops were cereals and might have also contributed to the lack of any significant effect by the rotation scheme on TLB development.

Table 3: Mean incidences and percent severity index of Turcicum leaf blight on maize under different production systems in major maize growing areas

Particulars	No. of fields	DI (%)	PSI (%)
Cropping system			
Mono cropping	50	69.936	31.854
Inter cropping			
Maize (<i>Zea mays</i>) with Ethiopian Kale (<i>Brassica carinata</i>)	25	43.33	17.33
Maize with ground nut (<i>Arachis hypogaea</i>)	19	100	55
Maize with haricot bean (<i>Phaseolus vulgaris</i>)	24	96.67	31.67
Maize with Faba bean (<i>Vicia fabae</i>)	15	53.33	12.33
Maize with sorghum (<i>Sorghum bicolor</i>)	25	90.00	60.00
Maize with potato (<i>Solanum tuberosum</i>)	14	100.00	55.00
Previous crop (preceding crop)			
Barley (<i>Hordeum vulgare</i>)	6	80.00	35.40
Dagusa, Finger millet (<i>Eleusine coracana</i>)	20	100.00	40.00
Ethiopian kale, Gomen, (<i>Brassica carinata</i>)	10	60.00	20.00
Faba bean (<i>Vicia faba</i>)	8	61.00	21.20
Fallowing	9	62.5	15.75
Fenugreek (<i>Trigonella foenum-graecum</i>)	20	30.00	5.00
Groundnut (<i>Arachis hypogaea</i>)	9	100.00	55.00
Haricot bean (<i>Phaseolus vulgaris</i>)	12	70.00	27.00
Maize (<i>Zea mays</i>)	17	72.61	34.28
Noug (<i>Guizotia abyssinica</i>)	5	55.63	24.19

Table 3:Continued

Pea (<i>Pisum sativum</i>)	4	80.00	25.00
Pepper (<i>Capsicum frutescens</i>)	5	82.00	47.00
Potato (<i>Solanum tuberosum</i>)	6	50.00	21.00
Rice (<i>Oryza sativa</i>)	8	100.00	25.00
Sesame (<i>Sesamum orientale</i>)	10	100.00	70.00
Sorghum (<i>Sorghum bicolor</i> L.)	15	82.50	38.75
Teff (<i>Eragrostis tef</i>)	4	71.33	31.40
Wheat (<i>Triticum aestivum</i>)	4	60.00	23.00

DI=disease incidence, PSI= percent severity index

4.1.6. TLB incidence and percent disease index on maize in relation to fertilizer application

Fertilized and non fertilized maize fields assessed during the survey varied in the level of TLB incidence and PSI. The TLB disease incidence and PSI were relatively low in fertilized fields compared to non-fertilized fields (Fig. 6). Mean minimum TLB incidence (67.7%) and PSI (29.9%) were recorded from fertilized fields. On the other hand, maximum disease intensity, 86.7% TLB incidence and 44.8% PSI were recorded in non-fertilized maize fields. It is known that plants suffering from nutrient deficiencies are weaker, slow in growing, and faster in aging. Such plants are susceptible to pathogens (Agrios, 2005). Assefa (1998) studied the effects of farm-yard manure (FYM), and nitrogen and phosphorus rates (N/P) on the intensity and frequency of turicum leaf blight and reported that the incidence was low (26.4%) at 20/46 kg/ha N/P₂O₅ plus 24 t/ha FYM as compared to the other combinations (with a maximum incidence of 33.6%) and the two checks (40/46 and 75/75 kg/ha N/P₂O₅). Plant

population and fertilize application influence the incidence and severity of turcicum leaf blight of maize. TLB of maize percent disease index was significantly reduced in treatments that received optimum fertilizer dosage compared to others (Kumar *et al.*, 2017).

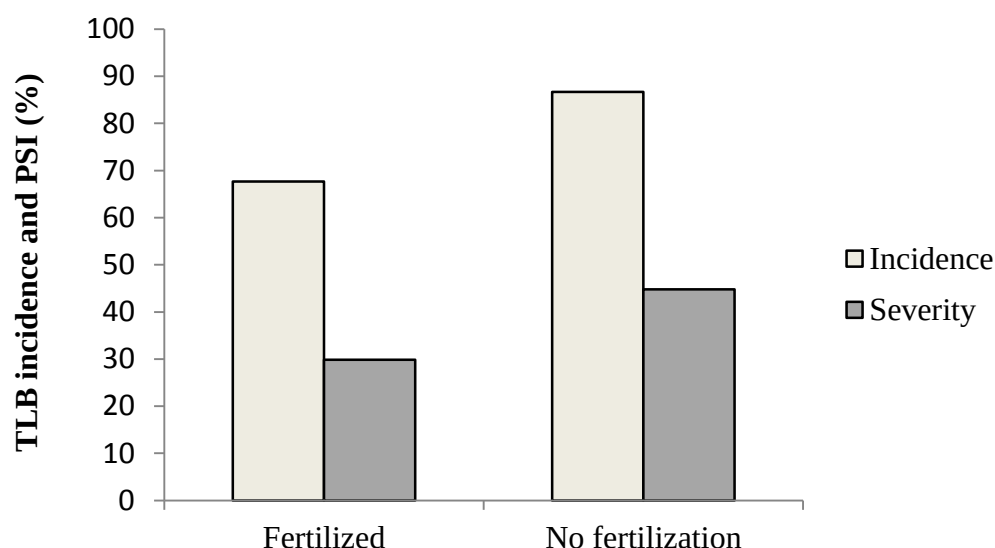


Figure 6: Mean disease incidence and percent severity index of TLB on maize fields with and without fertilizer applications

4.1.7. Correlation between Turcicum leaf blight of maize and weather parameters

TLB incidence and PSI positively correlated with relative humidity although not significantly differ (Table 4). On the other hand TLB incidence negatively correlated with maximum and minimum temperatures. Similarly TLB PSI was negatively correlated with maximum and minimum temperatures, while altitude had significant and negative relationship with both TLB incidence and PSI. In general, the disease levels were significantly influenced by altitude but not weather conditions according to results of the current survey. Reddy *et al.* (2013) observed negative correlation between disease incidence and maximum temperature and positive

correlation with high relative humidity. They reported that the mean minimum temperature had no effect on the disease development.

The results are contrary to those reported by Nwanosike (2015) in which TLB was highly influenced by relative humidity. Rani (2015) also reported positive and significant relationship between relative humidity and disease intensity and significantly negative correlations between minimum and maximum temperature on one hand, and disease intensity on the other. Rai *et al.* (2002) have also revealed definite relations between environmental parameters and disease development with the maximum initial disease incidence (100%) and significant disease severity (4.00) recorded during February.

The deviations of current survey results from those of previous studies might be because weather data were recorded only using portable devices during disease assessment dates in the current survey. It should be noted that disease development in general is influenced by long term and short term weather conditions that span longer than the few particular dates. However, absence of weather stations in the survey areas, make it impossible to have weather records throughout the growing period and beyond.

Table 4: Correlation coefficient between TLB disease of maize and weather parameters during assessment in 2017 growing season in surveyed area

Variables	Incidence	PSI	Altitude	Max.temp.	Min.temp.	RH
Incidence						
PSI	0.809*					
Altitude	-0.319*	-0.233*				
Max.temp.	-0.069Ns	-0.091Ns	-0.055Ns			
Min.temp.	-0.109Ns	-0.064Ns	-0.390*	0.025Ns		
RH	0.084Ns	0.089Ns	0.284*	0.068Ns	-0.496*	

*significant at $p \leq 0.05$, Maximum and minimum temperature ($^{\circ}\text{C}$), Relative humidity (%), PDI= percent disease index, Incidence (%)

4.2. Effect of Maize Varieties, Fungicide Frequencies and Their Interactions on TLB Development

4.2.1. Disease incidence

Maize varieties tested in the current experiment exhibited highly significant difference ($p \leq 0.01$) in terms of TLB incidence (Appendix Table 1). During the first assessment (55 DAP), TLB incidence was the highest (74.3%) on the susceptible variety, AMHQ-760, and the lowest (19.7%) on the moderately resistant variety (Jibat) (Table 5). Variety Wenchi had intermediate TLB incidence (67.3%). This result was in line with the expectation of Wenchi is known as a moderately susceptible variety to TLB. During the last assessment (90 DAP), TLB incidence reached 100% on unsprayed plots of varieties AMHQ-760 and Wenchi, while it was 56.7% on Jibat, which received thrice application of propiconazole (Table 5). Bowen and Pederson (1988) reported that three times sprays of propiconazole at regular interval were effective in reducing the rate of TLB development in maize.

Propiconazole application frequencies did not show any significant effect on TLB incidence during the first assessment regardless of the maize variety. However, at 90 DAP, fungicide application frequencies exhibited significant differences ($p \leq 0.05$) among and within each variety. At the last TLB incidence assessment the highest mean per cent incidence of the disease (100%) observed on unsprayed, one time and two times plots of susceptible and moderately susceptible varieties of AMHQ-760 and Wenchi respectively, but still thrice application reduced the TLB incidence even on susceptible released variety, AMHQ-760 and the lowest (56.67%) was on Jibat variety which received thrice application of propiconazole fungicide plot. Thus three times application of this fungicide significantly reduced TLB incidence on moderately resistant variety, Jibat.

Table 5: Effects of maize varieties, fungicide application frequencies and their combinations on TLB incidence (%) at Ambo during the 2016/17 cropping season

Varieties	Fungicide frequencies	DI at 55 DAP	DI at 90DAP
AMHQ-760	One time	74.333 ^a	100.000 ^a
AMHQ-760	Two times	70.000 ^{ab}	100.000 ^a
AMHQ-760	Three times	72.667 ^a	94.333 ^{ab}
Jibat	One time	25.667 ^d	60.000 ^{de}
Jibat	Two times	19.667 ^d	71.66 ^{cd}
Jibat	Three times	22.667 ^d	56.667 ^e
Wenchi	One time	59.000 ^c	100.000 ^a
Wenchi	Two times	67.333 ^{abc}	100.000 ^a
Wenchi	Three times	61.333 ^{bc}	90.667 ^{ab}
AMHQ-760	Unsprayed	68.333 ^{abc}	100.000 ^a
Jibat	Unsprayed	20.000 ^d	83.333 ^{bc}
Wenchi	Unsprayed	58.667 ^c	100.000 ^a
LSD (5%)		9.7848	12.48
CV (%)		11.19	8.37

Mean values with the same letter within the column are not significantly different at described probability level; LSD=least significant difference; CV=coefficient of variation; DAP=dates after planting; DI= disease incidence

4.2.2. Terminal percent severity index

Analysis of variance (ANOVA) showed that there was highly significant difference ($p \leq 0.01$) across varieties, fungicide frequencies and their interactions in PSI at the last date of disease assessment (Appendix Table 1). The highest (86.9%) TLB PSI was scored on untreated plot of AMHQ-760 followed by 58.8% on unsprayed plot of Wenchi variety and 52.5% when AMHQ-760 was treated once with propiconazole (Table 6). In contrast, the lowest terminal PSI (15%) was recorded on maize variety Jibat treated three times with propiconazole. This variety had PSI 18.49%, 30.5% when it was treated with propiconazole twice and once, respectively, PSI reached 41.7% on unsprayed plots of same variety (Jibat).

Significant variations ($p \leq 0.05$) also existed in terminal PSI across fungicide application frequencies regardless of maize varieties, although reductions in TLB PSI as a result of fungicide applications had marked differences across varieties (Table 6). For instance, three time of application of propiconazole fungicide on susceptible variety AMHQ-760 reduced PSI by 59.79, 25.42 and 11.67% over unsprayed, one time and two times application, respectively. On moderately susceptible variety, Wench, thrice application of propiconazole reduced PSI by 38.75, 22.5, and 11.25% over unsprayed, one time, and two times of application, respectively. Whereas on moderately resistant variety, Jibat, the same application frequency reduced PSI by 26.67, 15.54, and 3.39% over untreated control, one time application and twice application, respectively. The present findings demonstrated that the reduction in PSI increased with the susceptibility of the variety. It should also be noted that three applications of propiconazole caused significant reduction in TLB severity over the two times and one time application on the susceptible and moderately susceptible varieties but not on the moderately resistant variety.

4.2.3. Area Under Disease Progress Curve (AUDPC)

Area under disease progress curve (AUDPC) showed highly significant difference ($p \leq 0.01$) among maize varieties and application frequencies (Appendix Table 1). The two-way interaction of treatment combinations effects of propiconazole application schedules indicated that there were significant differences ($p \leq 0.05$) between treatment combinations with respect to AUDPC values (Appendix Table 1). The highest AUDPC value (2618.4%-days) was recorded on unsprayed plots of the susceptible variety, AMHQ-760, while the lowest AUDPC value (641.7%-days) was registered on the moderately resistant variety, Jibat, with three propiconazole applications (Table 6). The AUDPC value for the variety Jibat was lower by

873.56 and 461.76 %-days than the values for AMHQ-760 and Wenchi, respectively. Previous works by Daniel *et al.* (2008) indicated varieties considered as susceptible such as Abobako, BH-540 and Local-M had AUDPC values more than resistant variety Kuleni and BH-660. Megersa *et al.* (2017) reported that the AUDPC values for the variety BH-660 were lower by 368.44 and 317.53%-days than the values for BH-543 and AMHQ-760, respectively.

4.2.4. Progress Rate of TLB

The apparent infection rate of TLB in this investigation ranged from 0.01614 to 0.09339 units-day⁻¹ (Table 6). Levy (1989) reported apparent infection rate of TLB varying between 0.05 and 0.20 units-day⁻¹, while Harlapur (2008) reported that apparent infection rate of TLB ranged from 0.009-0.482 units-day⁻¹. Maize varieties, propiconazole application frequencies and their interactions differed significantly ($p \leq 0.05$) in terms of TLB progress rate (Table 6). Accordingly, the fastest disease progress rate (0.09339 units-day⁻¹) was recorded on the unsprayed plot of AMHQ-760 and the slowest (0.016144 units-day⁻¹) was on the same variety treated with thrice application of propiconazole fungicide. The disease progress rate of unsprayed plot of susceptible variety AMHQ-760 was 5.785 time faster than that of three time application of propiconazole fungicide whereas TLB progress rate on the unsprayed plot of the moderately resistant variety, Jibat, was 1.737 time faster than that of same variety with three times applications. This pointed that thrice application of propiconazole reduced the TLB progress.

Table 6: Integrated effect of maize variety by propiconazole application frequencies on TLB terminal percent severity index, AUDPC value and progress rate at Ambo during the 2016/17 cropping season

Varieties	Frequencies	PSI	AUDPC (%-days)	Progress rate (Unit-day ⁻¹)	SE	P
AMHQ-760	Unsprayed	86.875 ^a	2618.4 ^a	0.093391 ^a	0.003896	<.0001
	One time	52.500 ^{bc}	1560.4 ^c	0.038281 ^{ef}	0.002841	<.0001
	Two times	38.750 ^{de}	1394.2 ^{cd}	0.024395 ^{fg}	0.006828	<.0001
	Three times	27.083 ^{fg}	1276.0 ^{de}	0.016144 ^g	0.004812	<.0001
Wenchi	Unsprayed	58.750 ^b	1997.9 ^b	0.073966 ^{bc}	0.004525	<.0001
	One time	42.500 ^{cd}	1225.0 ^{de}	0.058540 ^{cd}	0.009296	<.0001
	Two times	31.250 ^{ef}	1071.9 ^{ef}	0.031771 ^{efg}	0.005659	<.0001
	Three times	20.000 ^{gh}	907.1 ^{fg}	0.027425 ^{efg}	0.004424	<.0001
Jibat	Unsprayed	41.667 ^d	1181.3 ^{ef}	0.076451 ^{ab}	0.007802	<.0001
	One time	30.542 ^{ef}	780.6 ^{gh}	0.078942 ^{ab}	0.003219	<.0001
	Two times	18.396 ^{gh}	751.3 ^{gh}	0.037717 ^{ef}	0.003940	<.0001
	Three times	15.000 ^h	641.7 ^h	0.044007 ^{de}	0.008168	<.0001
LSD (5%)		10.187	262.88	0.017		
CV (%)		15.58	12.09	20.09		

Mean values with the same letter within the column are not significantly different at described probability level; LSD=least significant difference; CV=coefficient of variation; PSI= percent severity index; AUDPC=Area under disease progress curve

4.2.5. Yield and yield components

4.2.5.1. Number of ears per plant (NEPP)

Mean number of ears per plant showed significant difference ($p \leq 0.05$) among varieties but varietal interaction with fungicide application frequencies did not yield significant difference in terms of number of ears per plant (Table 7). Significantly ($p \leq 0.05$) the highest mean number of ears per plant (1.42) was recorded on Jibat hybrid variety while the lowest NEPP (1.00) was harvested from AMHQ-760 and Wenchi varieties.

4.2.5.2. Ear length (EL) and number of row per ear (NRPE)

Ear length and number of rows per ear were significantly different ($p \leq 0.05$) among treatment combinations. The hybrid AMHQ-760 maize variety had the longest mean ear length (20.25cm) when treated with three applications of propiconazole fungicide. On the other hand, the shortest mean ear length (9.67cm) was recorded on the same variety without fungicide application. Generally three times spray of propiconazole significantly ($p \leq 0.05$) increased ear length of tested commercial hybrid maize varieties compared to unsprayed, two and one times applications (Table 7).

Number of rows per ear also showed significant difference ($p \leq 0.05$) across propiconazole fungicide frequencies and maize varieties combinations. The highest (16.00) mean number of ears per row was obtained from the susceptible AMHQ-760 variety that received three time application of propiconazole. The lowest (12.00) was recorded on unsprayed plots of all tested maize hybrid varieties.

Thus, it can be emphasized from the results that severe infection of TLB on maize reduced the number of ears per plant, ear size (length) and number of rows per ear, while checking TLB development with propiconazole, especially with three application frequencies, significantly increased these yield parameters. These findings support the report of Megersa *et al.* (2017) which suggested that thrice application of propiconazole reduced the severity of TLB on hybrid maize varieties and increased the ear length and number of rows per ear on each tested six maize varieties. Pataky *et al.* (1988) also suggested that ear size and weight were reduced by severe TLB attack. Harlapur (2005) reported that highly susceptible lines (> 4.0 score)

failed to produce normal foliage as well as ears as disease covered the entire plant before silking and tasseling stage.

Table 7: Effects of varieties, propiconazole application frequencies and their interaction on number of ear per plant, Ear length and number of rows per ear at Ambo during the 2016/17 main cropping season

Varieties	Frequencies	NEPP	Ear length (cm)	NRPE
AMHQ-760	One time	1.000 ^d	11.50 ^{gh}	12.00 ^f
AMHQ-760	Two times	1.125 ^{bcd}	15.50 ^{cde}	14.00 ^{cd}
AMHQ-760	Three times	1.083 ^{dc}	20.25 ^a	16.00 ^a
Jibat	One time	1.333 ^{ab}	16.00 ^{dc}	12.00 ^f
Jibat	Two times	1.125 ^{bcd}	16.33 ^{dc}	13.33 ^{de}
Jibat	Three times	1.417 ^a	19.17 ^{ab}	15.33 ^{ab}
Wenchi	One time	1.000 ^d	14.83 ^{def}	12.00 ^f
Wenchi	Two times	1.083 ^{dc}	17.50 ^{bc}	12.67 ^{ef}
Wenchi	Three times	1.125 ^{bcd}	19.63 ^a	14.67 ^{bc}
AMHQ-760	Unsprayed	1.042 ^{dc}	9.67 ^h	12.00 ^f
Wenchi	Unsprayed	1.00 ^d	13.33 ^{fg}	12.00 ^f
LSD (5%)		0.2357	2.101	1.179
CV (%)		12.29	7.95	5.29

Mean values with the same letter within the column are not significantly different at described probability level; LSD=least significant difference; CV=coefficient of variation; NEPP=number of rows per ear; NRPE=number of row per ear;

4.2.5.3. Grain yield

The yield obtained from the three tested varieties showed highly significant difference ($p \leq 0.01$) among fungicide frequencies, varieties and interactions (Appendix Table 2). The highest (9.424 t ha⁻¹) grain yield was obtained from AMHQ-760 sprayed with three times propiconazole fungicide followed by 8.430 t ha⁻¹, 8.413 t ha⁻¹ from Jibat and Wenchi hybrid varieties sprayed with three times propiconazole fungicide, respectively. The lowest (3.542 t ha⁻¹) grain yield was harvested from unsprayed plots of the AMHQ-760 variety followed by

3.667 t ha⁻¹, 5.412 t ha⁻¹, from Wenchi and Jibat varieties of unsprayed plots, respectively (Table 8). The variation in mean grain yield between the tested hybrid maize varieties was attributed to their genetic potential for yield, disease resistance and the role of propiconazole. As observed from the current results, thrice propiconazole sprays significantly ($p \leq 0.05$) increased maize hybrid yield (by 266%) as compared to unsprayed plot within the same variety. It has been reported that, under stressful conditions, plants often mobilize nutrients and redirect their metabolism to support active defense mechanisms to the detriment of growth and final yield (Smith and Moser, 1985). However, the application of propiconazole might have prevented the triggering of active defense mechanisms by killing the inoculum prior to infection and/or attempted tissue colonization. This would allow the allocation of more metabolic resources to the sink organ and lead to a better yield. Triazole fungicides (i.e. propiconazole) are also known to positively affect photosynthesis by enhancing the chlorophyll content (Petit *et al.*, 2012). Ali *et al.* (2015) reported that thrice spray of propiconazole resulted significantly higher yield (by 443%) as compared to control in maize crop.

4.2.5.4. Thousand Kernel Weight (TKW)

Mean thousand kernel weight showed significant difference ($p \leq 0.05$) among maize varieties and treatment combinations. The maximum (525.4g) TKW was recorded from plots of AMHQ-760 that received three times application of propiconazole fungicide whereas the minimum (330.0g) was obtained from unsprayed plots of the same variety (Table 8). The hybrid maize varieties significantly ($p \leq 0.05$) differed from each other. Each variety when sprayed three times with propiconazole fungicide produced significantly higher ($p \leq 0.05$) TKW as compared to the unsprayed plots. On the other hand, two times propiconazole

application significantly increased TKW only on moderately resistant (Jibat) maize variety over the untreated control and one time application.

4.2.6. Relative Yield Loss (RYL)

Relative yield losses of maize varieties were calculated from their respective treatments that offered maximum protection and maximum yield (thrice propiconazole application). The highest (62.42%) relative yield loss was recorded from unsprayed plots of the susceptible AMHQ-760 maize variety (Table 8). In general, the respective relative yield losses of tested maize varieties ranged from 35.8% on the moderately resistant variety (Jibat) to 62.4% on the susceptible variety AMHQ-760 on untreated plots. These results are in line with the data on TLB incidence and severity, where disease reduction increased with varietal susceptibility to TLB. Krausz *et al.* (1993) reported grain yield loss of 40-50% on susceptible maize hybrids. In another study, Babu *et al.* (2004) reported 83% yield reduction by turicum leaf blight incidence on maize. Turicum leaf blight incidence ranges from 95 to 100% in areas with constant moisture and high humidity and the yield loss can reach up to 70% (Tewabeche *et al.*, 2012). On susceptible cultivars in Uganda, losses as high as 60% have been recorded on maize (Esele, 1995). Crop loss model is an important tool in the prediction and forecasting of losses due to TLB disease. It is a prerequisite for determining decision in threshold and deployment of cost effective management practices (Rani, 2015).

TLB is one of the most destructive foliar diseases of maize and sorghum. It is the most important fungal disease hampering maize production in East Africa (Adipala *et al.*, 1993a) and the world in general (Chandrashekara *et al.*, 2014). It can cause yield loss of greater than 50% on susceptible varieties (Carson, 1995).

Table 8: Integrated effect of maize variety and propiconazole application schedules on mean thousand kernel weight, grain yield and relative yield loss at Ambo 2016/17 cropping season

Varieties	Frequencies	Yield(t ha ⁻¹)	TKW(g)	RYL (%)
AMHQ-760	Unsprayed	3.5419 ^g	330.00 ^d	62.415
	One time	4.2464 ^f	332.33 ^d	54.939
	Two times	7.3144 ^c	354.53 ^d	22.383
	Three times	9.4238 ^a	525.40 ^a	0.00
Wenchi	Unsprayed	3.6686 ^{fg}	340.00 ^d	56.392
	One time	4.9397 ^e	367.20 ^d	41.282
	Two times	6.3551 ^d	390.73 ^{cd}	24.457
	Three times	8.4126 ^b	443.87 ^{bc}	0.00
Jibat	Unsprayed	5.4116 ^e	335.00 ^d	35.80
	One time	5.4159 ^e	393.13 ^{cd}	35.752
	Two times	6.4248 ^d	444.93 ^{bc}	23.784
	Three times	8.4297 ^b	473.60 ^{ab}	0.00
LSD (5%)		0.6753	71.792	
CV (%)		6.50	10.75	

Mean values with the same letter within the column are not significantly different at described probability level; LSD=least significant difference; CV=coefficient of variation; TKW=thousand kernel weight, RYL=relative yield loss

4.2.7. Association of disease parameters with yield and yield components

Disease incidence, percent severity index, area under disease progress curve and disease progress rate were negatively correlated with yield and yield components of maize varieties except relative yield loss. This finding is in agreement with Megersa *et al.* (2017). In spite of that the significant association depended on the maize varieties and their respective disease parameters (Table 9).

Most of disease parameters on susceptible hybrid maize variety of AMHQ-760 were strongly (negatively or positively) associated with grain yield, relative yield loss, TKW, ear size

(length) and number of rows per ear. Disease incidence, percent severity index, disease progress rate and AUDPC value had strongly and significantly associated with grain yield and relative yield loss for this variety. Percent severity index assessed at the last date had the strongest negative association with maize grain yield($r=-0.94$), ear size($r=-0.86$), number of rows per ear($r=-0.82$) and positively associated with relative yield loss($r=0.92$). More or less similar results were obtained on the other two varieties (Table 9).

Table 9: Association of disease parameters with yield and yield components of maize at Ambo in 2016/17 main cropping season

Disease parameters	Yield(tha^{-1})	RYL (%)	TKW(g)	Ear size	NRPE
AMHQ-760					
Incidence (%)	-0.74**	0.68*	-0.85**	-0.76**	-0.82**
PSI (%)	-0.94**	0.92**	-0.56Ns	-0.86**	-0.82**
AUDPC(%-days)	-0.89**	0.89**	-0.35Ns	-0.74**	-0.65*
Rate(Unit day $^{-1}$)	-0.86**	0.83**	-0.61*	-0.77**	-0.70**
Wenchi					
Incidence (%)	-0.71**	0.74**	-0.65*	-0.72**	-0.69*
PSI (%)	-0.83**	0.82**	-0.79**	-0.82**	-0.73**
AUDPC(%-days)	0.87**	0.87**	-0.82**	-0.79**	-0.56Ns
Rate(Unit day $^{-1}$)	-0.72**	0.67*	-0.66*	-0.77**	-0.55Ns
Jibat					
Incidence (%)	-0.42Ns	0.44Ns	-0.24Ns	-0.57*	-0.23Ns
PSI (%)	-0.78**	0.80**	-0.69*	-0.69*	-0.63*
AUDPC(%-days)	-0.63*	0.66*	-0.59*	-0.63*	-0.42Ns
Rate(Unit day $^{-1}$)	-0.78**	0.79**	-0.63*	-0.68*	-0.69*

RYL=relative yield loss, TKW= thousand kernel weight, NRPE=number of rows per ear, AUDPC=area under disease progress curve, PSI= percent severity index, Ns=non-significant, *=significant ($p<0.05$), **=significant ($p<0.01$)

4.2.8. Cost/Benefit analysis

Treatments tested in the current experiment, as part of integrated management of TLB of maize, resulted in lower disease level, higher maize grain yield, gross revenue, marginal benefit and marginal rate of return (MRR) as compared to the untreated controls (Table 10). The only exception was Jibat maize hybrid coupled with one time propiconazole fungicide frequency that resulted in lower marginal benefit and marginal rate of return than the respective control plots.

Three times spray of propiconazole yields the highest (ETB 63,089.1 ha⁻¹) marginal benefits with AMHQ-760 followed by (ETB 56,155.16 ha⁻¹, and ETB 56,055.89 ha⁻¹) from hybrid varieties Wenchi and Jibat, respectively. The lowest marginal benefit (ETB 24,704.75 ha⁻¹) was obtained from unsprayed hybrid variety of AMHQ-760.

Similarly the highest (1458.8% and 1452.8%) marginal rate of return were obtained from maize hybrid variety of AMHQ-760 that received twice and thrice spray of this fungicide, respectively, followed by Wenchi hybrid sprayed thrice with propiconazole. This means that for every payment of 1.00 ETB for propiconazole cost and 2-3 times spray, there was a gain of ETB 14.59 and 14.53 on the AMHQ-760 maize variety and ETB 10.10 for Wenchi variety.

Accordingly, the highest maize grain yield, highest marginal benefit, and marginal rate of return were obtained from maize hybrid AMHQ-760 sprayed thrice in one cropping season. Of all other varieties this hybrid maize variety is quality protein maize (QPM), contains high content of protein, but it is susceptible to TLB. Hence, from the economic point of view, production of the three tested maize hybrid varieties under propiconazole-sprayed practices is highly profitable when the fungicide was sprayed 2-3 times in one cropping season.

Table 10: Cost/benefit assessment of propiconazole fungicide application frequencies against TLB on three hybrid maize varieties at Ambo in 2017 main cropping season

Fungicides	Varieties	Yield (t ha ⁻¹)	Adjusted yield (%)	MSP (ETB kg ⁻¹)	SR (ETB ha ⁻¹)	TIC (ETB ha ⁻¹)	MC (ETB ha ⁻¹)	NP (Birr ha ⁻¹)	MB (Birr ha ⁻¹)	MRR (%)
Unsprayed	AMHQ-760	3.541	3.188	7.75	24704.75	4055	0	20649.75	24704.75	0
	Wenchi	4.069	3.302	7.75	25588.49	4055	0	21533.49	25588.49	0
	Jibat	5.411	4.869	7.75	37741.73	4055	0	33686.73	37741.73	0
One time	AMHQ-760	6.013	3.822	7.75	29618.64	4889	834	24729.64	28784.64	489.2
	Wenchi	5.906	4.446	7.75	34454.41	4889	834	29565.41	33620.41	963.1
	Jibat	5.416	4.874	7.75	37775.9	4889	834	32886.9	36941.9	-95.9
Two time	AMHQ-760	7.314	6.583	7.75	51017.94	5743	1688	45274.94	49329.94	1458.8
	Wenchi	6.355	5.719	7.75	44326.82	5743	1688	38583.82	42638.82	1010.1
	Jibat	6.415	5.782	7.75	44812.98	5743	1688	39069.98	43124.98	318.9
Three times	AMHQ-760	9.424	8.481	7.75	65731.01	6697	2642	59034.01	63089.01	1452.8
	Wenchi	8.413	7.571	7.75	58677.89	6697	2642	51980.89	56035.89	1152.4
	Jibat	8.429	7.587	7.75	58797.16	6697	2642	52100.16	56155.16	696.9

MSP=maize selling price, SR = Sale revenue, TIC = Total input cost, MC = Marginal cost, NP = Net profit. MB = Marginal benefit, MRR = marginal rate of return

5. SUMMARY, CONCLUSION AND RECOMMENDATION

Maize (*Zea mays* L.) is one of the most widely grown and regarded as one of the high priority food security crops in Ethiopia, in terms of importance, wide adaptation, total production and productivity. However, its production and productivity has remained low due to several constraints including foliar diseases. Turcicum leaf blight (TLB) caused by *Exserohilum turcicum* is the most important infectious diseases among foliar diseases of maize in the country. Despite its importance, there is limited quantified data that reflect the extent of its distribution across maize growing agro ecologies and the reaction of maize varieties to the disease. As a consequence, the present study was conducted to assess the incidence and severity of TLB in major maize growing agro-ecologies of Western Oromia, evaluate the reaction of maize varieties under production to TLB and determine optimum propiconazole (tilt 250 EC) spray frequency as management option to TLB. The main aim of the study was to contribute towards improved and sustainable maize production through effective management of TLB.

Disease surveys were conducted in 172 farmers' field, 29 districts and five zones in western Oromia region. Across the survey districts, TLB incidence ranged from 16.25% in Abay chomen to 96.67% in Wayu Tuka districts. PSI also ranged from 3.13% to 57.50% in Abay chomen and Lalo Assabi districts, respectively. TLB of maize was believed to be more devastating only in mid-altitude, sub-humid agro-ecologies in Ethiopia. However, the present study demonstrated that the disease was prevalent and intense across survey areas. Most of maize varieties under production were found to be affected by TLB, while fertilizer application reduced the intensity of disease. However, the current work did not demonstrate a clear pattern in terms of effect of intercropping and crop rotation on TLB development except

for kale-maize intercropping and fenugreek-maize rotations that resulted in marked reduction in the disease level as compared to other systems.

In the field experiment, 12 treatments and treatment combinations involving three maize varieties, three fungicide spray frequencies and untreated checks were evaluated for their effect on TLB development and, yield and yield components of maize. Artificial inoculation was used to supplement the natural inoculum. Disease symptoms, water soaked necrotic spots, first appeared on the leaf of susceptible varieties eight days after inoculation and later on moderately resistant varieties. Integrated effect of varieties with application of proiconazole frequencies resulted in significant difference ($p < 0.05$) in reducing disease intensity. The highest (86.88%) TLB PSI assessed at the last date of disease assessment was recorded on unsprayed maize variety AMHQ-760. Likewise the highest (2614.4 %-day) AUDPC values and fastest (0.09339 unit-day⁻¹) TLB progress rate were recorded on the same maize variety without fungicide application. On the other hand, three times propiconazole sprayed on Jibat variety had the lowest (15 % and 641 %-day) PSI and AUDPC, respectively, and the lowest (0.01614 unit-day⁻¹) TLB progress rate was recorded on AMHQ-760 with thrice propiconazole spray per season. In the current experiment, TLB resulted in grain yield losses of up to 62.42, 56.39 and 35.80% on AMHQ-760, Wenchi and Jibat, respectively on unsprayed plots. PSI, AUDPC, incidence and progress rate were negatively correlated with yield and yield components. Partial budget analysis of total variable cost and marginal benefits revealed that the highest (ETB 63,089.1 ha⁻¹) marginal benefit obtained when three times spray of propiconazole coupled with maize variety AMHQ-760, followed by the variety Wenchi (ETB 56,155.16 ha⁻¹) and the variety Jibat (ETB 56,055.89 ha⁻¹) whereas the lowest (ETB 24,704.75 ha⁻¹) marginal benefit was obtained from the unsprayed maize variety AMHQ-760. Similarly

the highest (1458.8% and 1452.8%) marginal rate of return were obtained from maize variety AMHQ-760 that received twice and thrice application of this fungicide, respectively followed by Wenchi (1152.4%) sprayed thrice with propiconazole. Hence, from the economic point of view, production of the three tested maize hybrid varieties under propiconazole-sprayed practices is highly profitable when the propiconazole sprayed 2-3 times for contribution of integrated TLB disease management.

In conclusion survey results revealed the prevalence of TLB across all the districts although at different levels. Mean TLB incidence ranged from 16.3% in Abay chomen to 96.67% in Wayu Tuka and mean percent disease index varied between 3.1% in Abay chomen and 57.5% in Lalo Assoabi district. TLB was relatively more severe on maize varieties BH-540, Limu and Kolba on surveyed areas. In addition, the effects of maize varieties, fungicide application frequencies and their interactions on TLB development were assessed in a field experiment during the 2016/17 main growing season at APPRC. Disease parameters varied markedly across treatments, especially variety-fungicide combinations had a highly significant variation ($p \leq 0.01$) in disease parameters. The highest terminal percent severity index (PSI) (86.7%), AUDPC value (2614.4 %-day) and disease progress rate ($0.09339 \text{ units-day}^{-1}$) were recorded on unsprayed variety AMHQ-760. On the other hand, the same variety had significantly lower disease level and gave the highest grain yield (9.424 t ha^{-1}) when it was treated three times with propiconazole application. TLB resulted in grain yield losses of up to 62.4% on AMHQ-760. PSI, AUDPC, incidence and disease progress rate were negatively correlated with yield and yield components. The highest marginal benefit (ETB 63,089 ha^{-1}), and marginal rate of return (ETB 14.53) were obtained from variety AMHQ-760 with thrice application of propiconazole.

The current experiment revealed the importance of TLB across the study areas and the role fungicide-variety combination plays in managing the disease. Nonetheless, the following recommendations were made to further enhance the knowledge on the pathosystem and come up with effective, affordable and sustainable TLB management strategies:

- Successive survey for TLB in all the maize producing areas across the country should be carried out to have a complete picture on the importance of the disease across geographic regions and agro ecologies, to identify sources of resistant genotypes and the pathotypes/available in Ethiopia, and associate weather variables with the development of TLB
- Management practices tested in the current experiment need to be verified across locations and seasons.
- Host resistance integrated with other cultural practices applicable in the area should be given due attention to provide other alternatives for effective, efficient and sustainable TLB management options. However, selection of effective and safe fungicides that can be integrated with other options should also be carried out in the future.

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7. BIOGRAPHICAL SKETCH

The author was born from his father Aliyi Mohammed Ismael and his mother Kediya Hussien Abdulder at Goro, Bale Zone, Oromia Regional State on 13 January 1989. He attended his elementary and junior education at Goro primary school from 1999-2006 and attended his secondary education at Goro Secondary High School from 2007-2008. He joined Goro Preparatory School from 2009-2010 to attend the preparatory education and studied Natural Science. Upon passing the Ethiopian Higher Education Entrance Examination, he received Ethiopian Higher Entrance Examination Quality Certificate (EHEEQC) in 2010 and then he joined the Ambo University in 2011 and graduated with B.Sc. Degree in HORTICULTURE on July 03, 2013.

Thereafter, he was employed by Ethiopian Institute of Agricultural Research (EIAR) at Ambo Plant Protection Research Centre (APPRC) and was assigned as Junior Researcher in Mycology research section on 15 May 2014. After two years service he joined the School of Graduate Studies at the Hawassa University in September 2016 to pursue a study leading to M.Sc. Degree in Crop Protection.

8. APPENDICES

Questionnaire for Farmers and agricultural workers on Conducting Survey of Turcicum Leaf Blight (*Exserohilum turcicum*) of maize (*Zea mays* L)

Name of Respondent: _____ Village/Kebele: _____

Zone _____ District: _____ Education: _____

Age----- Sex----- Male ☐ Female ☐

Profession _____

1. Cropping system

1.1. Intercropping ☐

1.2. mono cropping ☐

2. Plant stage at assessment: _____

3. Methods of controlling turcicum leaf blight(TLB) in your locality

4. Cultural practices

5.1.Planting date: _____

5.2.Fertilization

5.2.1. Kind of fertilizer

5.2.2. Amount of fertilizer

5.2.3. Time of application

6. Pesticide used: _____

7. Is there any influence of weather factors (temperature, Relative humidity and rainfall) on the population of diseases in maize field? [Yes = 1, No = 2] ☐

8. Cultivation of Maize Variety in your locality: _____

9. Previous/Preceding crop: _____

Appendix Table 1: Mean squares and significant levels of disease incidence, terminal percent severity and AUDPC at Ambo in 2016/17 main cropping season

Source	DI initial				DI final			Terminal PSI			AUDPC(%-day)		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Treatment	11	1298.3	38.88	<.0001	693.29	12.76	<.0001	1045.7	28.89	<.0001	827107.7	34.32	<.0001
Block	2	107.7	3.51	0.0629	58.11	1.17	0.343	21.282	0.59	0.5639	81384.7	3.38	0.0526
Frequencies(A)	3	28.9	0.94	0.4501	315.74	5.81	0.0079	2947.5	81	<.0001	1775044.3	73.65	<.0001
Varieties (B)	2	8191.4	267.4	<.0001	3652.7	67.24	<.0001	1862.3	51.5	<.0001	2291817.3	95.09	<.0001
A*B	6	32.14	1.05	0.4421	107.32	1.98	0.1203	164.0	4.5	0.0132	113477.2	4.7	0.0158
Error	22	33.39			54.323			36.193			24101.99		
Total	35												
R²			95.83			94.17			94.46			95.30	
Mean			51.64			88.05			38.61			1283.813	

Appendix Table 2: Mean squares and significant levels of TLB disease progress rate and number of ear per plant at Ambo in 2016/17 main cropping season

Source	DF	Disease progress rate			Number of ear/plant		
		MS	F	P	MS	F	P
Treatment	11	0.000984	13.12	<.0001	0.06804	3.51	0.0046
Block	2	0.000085	1.13	0.3526	0.14106	7.28	0.0037
Frequencies (A)	3	0.005503	73.37	<.0001	0.02373	1.11	0.3826
Varieties (B)	2	0.000832	11.1	0.0018	0.20095	9.42	0.0035
A*B	6	0.000489	6.5	0.0030	0.02156	1.01	0.4627
Error	22	0.000075			0.01937		
Total	35						
R²			96.18			67.48	
Mean			0.05008			1.13194	

Appendix Table 3: Mean squares and significant levels of ear length, number of rows per ear, thousand kernel weights (gm) and Grain yield (t ha⁻¹) at Ambo in 2016/17 main cropping season

Source	DF	Ear length			Number of rows/ear			TKW			Yield (t ha ⁻¹)		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Treatment	11	26.943	17.49	<.0001	5.564	11.48	<.0001	13411.201	7.46	<.0001	7913.962	23.68	<.0001
Block	2	0.520	0.34	0.7171	0.000	0.00	1.00	19274.634	10.72	0.0006	298.539	0.89	0.4237
Frequencies (A)	3	93.036	60.37	<.0001	22.333	46.06	<.0001	35808.745	19.92	<.0001	30790.55	92.12	<.0001
Varieties (B)	2	17.295	11.22	0.0004	1.333	2.75	0.0878	2737.0744	1.52	0.0308	457.555	1.37	0.2876
A*B	6	5.919	3.84	0.0059	0.444	0.92	0.4682	3815.992	2.12	0.0029	1499.611	4.49	0.0125
Error	22	1.541			0.484			1797.542			334.254		
Total	35												
R²			91.18			87.14			81.51			93.33	
Mean			15.615			13.167			394.228			639.315	

Appendix Table 4: Total marginal cost of propiconazole fungicide at different frequencies for protected plots at Ambo in 2017
main cropping season

Lists of items	One time			Two times			Three times		
	Unit price	Amount ha ⁻¹	Sub total	Unit price	Amount ha ⁻¹	Sub total	Unit price	Amount ha ⁻¹	Sub total
Fungicide cost	800	0.5	400	800	0.5	800	800	0.5	1200
Sprayer rent	50	1	50	50	2	200	50	3	450
Labor cost for sprayers	38	4	152	38	4	304	38	4	456
Labor cost for water hauling	38	4	152	38	4	304	38	4	456
Labor cost for equipment cleaning	20	4	80	20	4	80	20	4	80
Total marginal cost			834			1688			2642

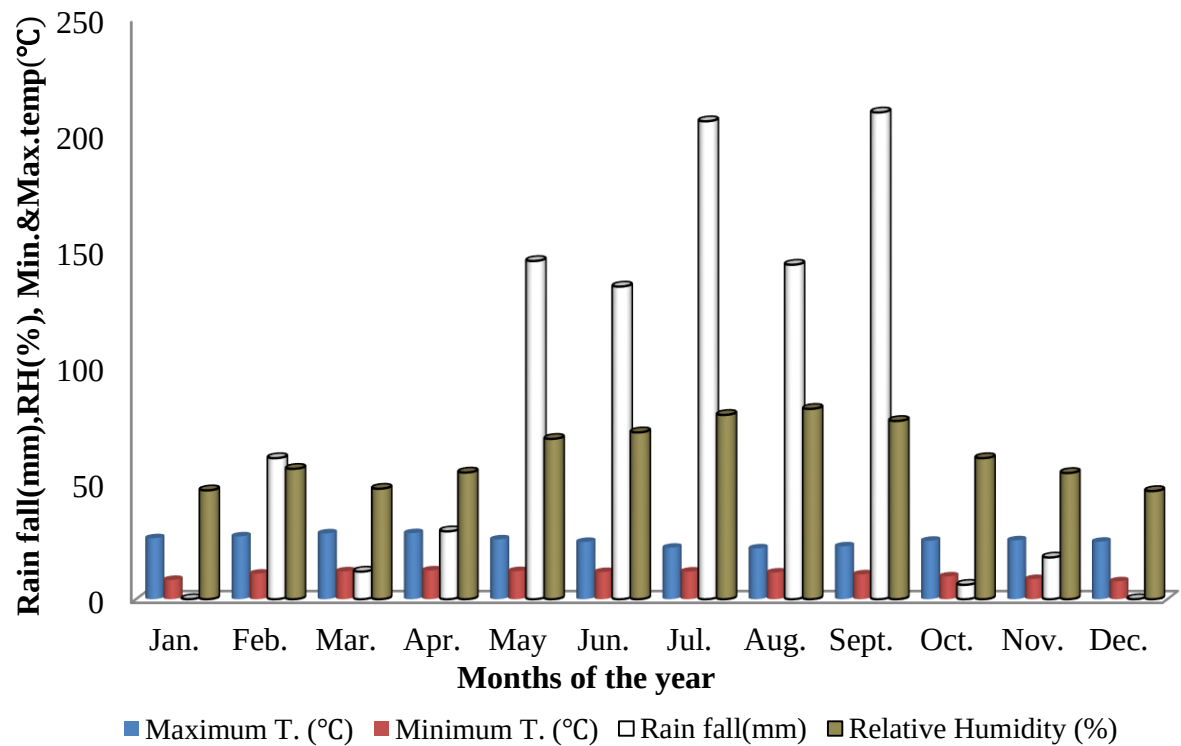


Figure 7: Monthly total rainfall (mm), mean relative humidity (%), mean minimum and maximum temperature (°C) at APPRC in 2017

Source (Ambo Plant Protection Research Center, Meteorology Station Database, 2017)