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Management of Turcicum Leaf Blight (*Exserohilum turcicum*) of Maize (*Zea mays*) through Evaluation of Maize Varieties and Fungicide Spray Frequencies at Ambo, Ethiopia

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Abstract

Turcicum leaf blight (Exserohilum turcicum), TLB, is a major disease affecting maize (Zea mays) in western Ethiopia. The aims of this study wereto: evaluate maize varieties and propiconazole spray frequencies on epidemics of TLB, determine optimum propiconazole (tilt 250 EC) spray frequency for integrated management of TLB, and assess the cost/benefit of using propiconazole for each spray frequency against TLB. The effects of maize varieties, fungicide frequencies and their interactions on TLB development were assessed in a field experiment during the 2017 main growing season at Ambo Plant Protection Research Centre (APPRC). The experiment involved three maize varieties(AMHQ-760,Wonchi and Jibat) and three fungicide (Tilt, propiconazole) frequencies arranged in a factorial setting in randomized complete block design with three replications. One unsprayed check for each hybrid varieties was included. TLB was initiated following artificial inoculation. Data were recorded on disease incidence and severity. Six time severity scores were used to calculate area under disease progress curve (AUDPC) and infection rate. Grain yield and yield components weremeasured after harvest. Finally correlation and economic analyses were carried out. Disease parameters varied markedly acrosstreatments, especially variety-fungicide combinations had a highly significant variation ($p<0.01$) in disease parameters. The highest terminal percent severity index (PSI) (86.7%), AUDPC value (2614.4 %-day) and disease progress rate (0.09339 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 thrice 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, thrice propiconazoles pray were found effective to manage TLB even on susceptible maize variety. However, additional experiments should be carried out to verify the current results.

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Keywords

Area under disease progress curve, Fungicide, Severity, Rate of infection, Leaf blight, Propiconazole

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 (*Eragrostistef* (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, 2012), 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 *Heliminthosporium 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.

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).

TLB (*E.turcicum*) is the most important maize disease reported in Western Ethiopia. Farmers in this area (46.7%) indicated TLB as the major leaf disease on maize while gray leaf spot (GLS) caused by *Cercosporazeae-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% (Tewabech *et al.*, 2012). It is reported to cause devastating damage on most commercial varieties of maize released in the country (Tewabech *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).

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 sever 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 and economic analysis need to be studied to enhance maize production and productivity under rain feed conditionin Ethiopia. Thus, the present investigation was undertaken with the following objectives:

To evaluate maize varieties and propiconazole spray frequencies on epidemics of turcicum leaf blight

To determine optimum propiconazole (tilt 250 EC) spray frequency for integrated management of TLB.

To assess the cost/benefit of using propiconazole for each spray frequency against TLB.

Materials and Methods

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)

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 hybrid released varieties used were AMH-851(Jibat), AMH-850(Wenchi), and AMHQ-760(Webi).

These hybrid 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.

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 $9m^2$(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.

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).

Data collection

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):

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

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):

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

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.

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:

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

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}).

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):

$$RYL(\%) = \frac{(Y_1 - Y_2)}{Y_1} * 100$$

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)

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:

$$MRR = \frac{DNI}{DIC}$$

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.

Results and Discussion

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

Disease incidence

Maize varieties tested in the current experiment exhibited significant difference ($p \leq 0.01$) in terms of TLB incidence (Table 2). 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 3). 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 based on previous recommendation. 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 spray of propiconazole (Table 3). 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 spray frequencies did not show any significant effect on TLB incidence during the first assessment regardless of the maize variety. However, at 90DAP, fungicide spray frequencies exhibited significant ($p \leq 0.05$) differences (Table 2). At the last TLB incidence assessment the highest mean per cent incidence of the disease (100%) observed on unsprayed, one time and two times spray plots of susceptible and moderately susceptible varieties of AMHQ-760 and Wenchi respectively, but still thrice spray 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 thrice spray of this fungicide significantly reduced TLB incidence on moderately resistant variety, Jibat (Table 2 and 3).

Terminal percent severity index

Analysis of variance (ANOVA) showed that there was significant difference ($p \leq 0.01$) across varieties and fungicide frequencies in PSI at the last date of disease assessment (Table 2). The combination of varieties with frequencies also showed significant difference ($p \leq 0.05$) with respect to terminal PSI. The highest (86.9%) TLB

PSI was scored on untreated plot of AMHQ-760followed by 58.8% on unsprayed plot of Wenchi variety and 52.5% when AMHQ-760 was treated once with propiconazole (Table 4). In contrast, the lowest terminal PSI (15%) was recorded on maize variety Jibat treated thrice 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, (Table 4).

Significant variations ($p \leq 0.05$) also existed in terminal PSI across fungicide spray frequencies regardless of maize varieties, although reductions in TLB PSI as a result of fungicide applications had marked differences across varieties (Table 2 and 4). For instance, thrice 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, Wenchi, thrice sprays 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, once 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 thrice sprays of propiconazole caused significant reduction in TLB severity over the two times and one time sprays on the susceptible and moderately susceptible varieties but not on the moderately resistant variety.

Area under Disease Progress Curve (AUDPC)

Area under disease progress curve (AUDPC) showed significant difference $(p \leq 0.01)$ among maize varieties and spray frequencies (Table 2). The two-way interaction of treatment combinations effects of propiconazole spray schedules indicated that there were significant differences ($p \leq 0.05$) between treatment combinations with respect to AUDPC values (Table 4). The mean 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 thrice applications (Table 4). The AUDPC value for the variety Jibat was lower by 873.56and 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(resistant) were lower by 368.44 and 317.53%-days than the values for BH-543 and AMHQ-760, respectively.

Progress Rate of TLB

The apparent infection rate of TLB in this investigation ranged from 0.01614 to 0.09339 units-day^{-1}(Table 4). 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 spray frequencies and their interactions differed significantly ($p<0.05$) in terms of TLB progress rate (Table 4). Accordingly, the fastest disease progress rate (0.093391units-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 sprays 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 sprays 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 sprays. This pointed that thrice spray of propiconazole reduced the TLB progress.

Effect of Maize Hybrid Varieties, Fungicide spray Frequencies and Their Interactions on Yield and Yield Components against TLB

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 spray frequencies did not yield significant difference($p \leq 0.05$) in terms of number of ears per plant (Table 5 and 6). 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 (Table 6).

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 hybrid varieties and fungicide frequencies. Thrice propiconazole spray yields the longest (19.68cm) whereas the shortest (12.22cm) recorded on unsprayed plots (Table 5). The hybrid AMHQ-760 maize variety had the longest mean ear length (20.25cm) when treated with thrice sprays of propiconazole. 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 sprays (Table 6).

Number of rows per ear also showed significant difference ($p \leq 0.05$) across propiconazole spray frequencies and maize varieties combinations. Thrice sprays recorded the highest (15.33) number of rows per ear while unsprayed plots recorded the lowest (12.00) number of rows per ear (Table 5). The highest (16.00) mean number of ears per row was obtained from the susceptible AMHQ-760 variety that received three times spray of propiconazole. The lowest (12.00) was recorded on unsprayed plots of all tested maize hybrid varieties (Table 6).

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 spray frequencies, significantly increased these yield parameters. These findings support the report of Megersa *et al.*, (2017) which suggested that thrice spray 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.

Grain yield

The yield obtained from the three tested varieties showed significant difference ($p \leq 0.01$) among fungicide frequencies and fungicide-variety interactions (Table 5 and 7). Each level of propiconazole frequencies significantly ($p \leq 0.05$) differ in grain yield tone per hectares. Three times spray increased grain yield up to 8.76 t ha^{-1} whereas the minimum yield harvested from unsprayed plots (Table 5). Integrated effects also showed significant ($p \leq 0.05$) difference on grain yield. The highest (9.424 t ha^{-1}) mean grain yield was obtained from AMHQ-760 sprayed with three times propiconazole followed by 8.430 t ha^{-1}, 8.413 t ha^{-1} from Jibat and Wenchi hybrid varieties sprayed with thrice 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 7). 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 fungicide.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.

Thousand Kernel Weight (TKW)

Mean thousand kernel weight showed significant difference ($p<0.05$) among maize varieties, fungicide frequencies and treatment combinations (Table 5 and 7).

The maximum (525.4g) mean TKW was recorded from plots of AMHQ-760 that received three times application of propiconazole whereas the minimum (330.0g) was obtained from unsprayed plots of the same variety (Table 7). The hybrid maize varieties significantly ($p \leq 0.05$) differed from each other. Each variety when sprayed thrice with propiconazole fungicide produced significantly higher ($p \leq 0.05$) TKW as compared to the unsprayed plots. On the other hand, twice propiconazole sprays significantly increased TKW only on moderately resistant (Jibat) maize variety over the untreated control and one time spray.

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

Table.2 Effects of hybrid varieties and propiconazole spray frequencies on TLB incidences, Terminal PSI, AUDPC values and infection rate at Ambo during the 2017 cropping season

Factors	DI(%) at 55 DAP	DI(%)at 90 DAP	Terminal PSI	AUDPC (%-days)	Progress rate (Unit-day^{-1})
Varieties					
AMHQ-760	71.333^{a}	98.583^{a}	51.302^{a}	1712.27^{a}	0.0431b
Wenchi	61.583^{b}	97.667^{b}	38.125^{b}	1300.47^{b}	0.0479b
Jibat	22.00c	67.917^{c}	26.401^{c}	838.71^{c}	0.0593a
LSD (1%)	**4.9236**	**6.2655**	**5.3891**	**145.18**	**0.0077**
CV (%)	**10.72**	**7.99**	**15.69**	**12.71**	**17.26**
Fungicide Frequencies					
Unsprayed	49.00	94.444^{a}	62.431^{a}	1932.53^{a}	0.0813^{a}
One time	53.00	86.667^{bc}	41.847^{b}	1188.69^{b}	0.0586b
Two times	52.33	90.556^{ab}	29.465^{c}	1072.43^{bc}	0.0313^{c}
Three times	52.22	80.556^{c}	20.694^{d}	941.60^{c}	0.0292^{c}
LSD (5%)	**NS**	**7.2347**	**6.2228**	**167.64**	**0.0089**
CV (%)	**10.72**	**7.99**	**15.69**	**12.71**	**17.26**

Mean values with the same letter within the column are not significantly different at described probability level; NS=none significant; LSD=least significant difference; CV=coefficient of variation; DAP=dates after planting; DI= disease incidence; AUDPC=Area under disease progress curve

Table.3 Effects of maize varieties, Propiconazole spray frequencies and their combinations on TLB incidence (%) at Ambo during the 2017 cropping season

Varieties	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

Table.4 Integrated effect of maize variety by propiconazole spray frequencies on TLB terminal percent severity index, AUDPC value and progress rate at Ambo during the 2017 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**	**LSD (1%) 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

Table.5 Effects of hybrid varieties and propiconazole spray frequencies on Number of ear per plant, Ear size, Number of rows per ear, Grain yield and thousand kernel weights at Ambo during the 2017 cropping season

Factors	NEPP	Ear length (cm)	NRPE	Yield(t ha^{-1})	TKW(g)
Varieties					
AMHQ-760	1.0625^{b}	14.2292^{b}	13.50a	6.57	385.567b
Wenchi	1.0521^{b}	16.3250^{a}	12.8333b	6.19	385.450b
Jibat	1.2813^{a}	16.2917^{a}	13.1667ab	6.42	411.667a
LSD (5%)	**0.1299**	**0.9202**	**0.593**	**NS**	**21.433**
CV (%)	**12.91**	**6.62**	**5.06**	**8.99**	**6.11**
Frequencies					
Unsprayed	1.097	12.2222^{d}	12.0000^{c}	4.3407^{d}	335.00^{a}
One time	1.111	14.1111^{c}	12.0000^{c}	5.7784^{c}	364.22b
Two times	1.111	16.4444^{b}	13.3333^{b}	6.6981^{b}	396.73^{c}
Three times	1.208	19.6833^{a}	15.3333^{a}	8.7554^{a}	480.96^{d}
LSD (5%)	**NS**	**1.0625**	**0.6847**	**0.5903**	**24.749**
CV (%)	**12.91**	**6.62**	**5.06**	**8.99**	**6.11**

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 ear per plant; NRPE=number of rows per ear; TKW=Thousand kernel weight

Table.6 Effects of varieties, propiconazole spray frequencies and their interaction on number of ear per plant, Ear length and number of rows per ear at Ambo during the 2017 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 ear per plant; NRPE=number of row per ear

Table.7 Integrated effect of maize variety and propiconazole spray schedules on mean thousand kernel weight, grain yield and relative yield loss at Ambo 2017 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**	

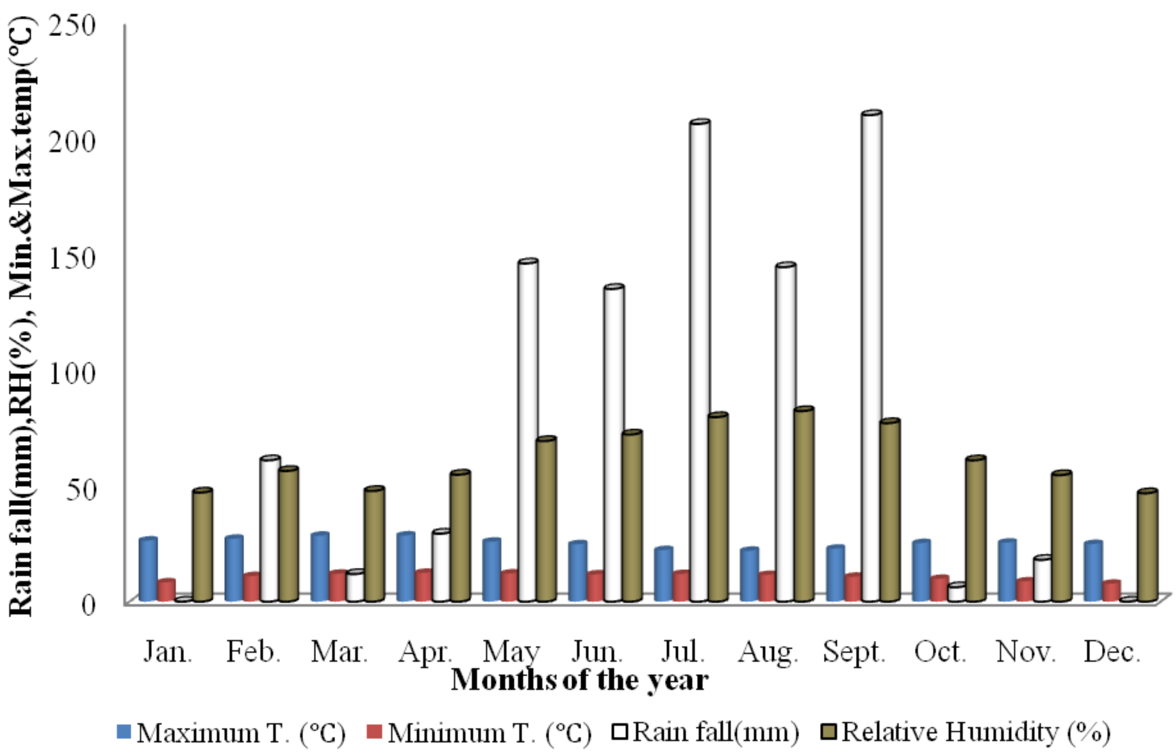
Mean values with the same letter within the column are not significantly different at described probability level; LSD=least significant difference; CV=coefficient of variation; TKW=thousand kernel weight, RYL=relative yield loss

Table.8 Association of disease parameters with yield and yield components of maize at Ambo in 2017 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 ($p<0.01$)

Fig.1 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)

Table.9 Cost/benefit assessment of propiconazole fungicide spray 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

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 7). 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 turcicum leaf blight incidence on maize. Turcicum 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% (Tewabech *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.,* 1993) and the world in general (Chandrashekara *et al.,* 2014). It can cause yield loss of greater than 50% on susceptible varieties (Carson, 1995).

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 8).

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 maize varieties (Table 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 9). The only exception was Jibat maize hybrid coupled with once propiconazole fungicide frequency that resulted in lower marginal benefit and marginal rate of return than the respective control plots.

Thrice 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(Table 9).

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 sprays 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. However, 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 9).

The effects of maize varieties, fungicide spray frequencies and their interactions on TLB development were assessed in a field experiment during the 2017 main growing season at APPRC. Disease parameters varied markedly across treatments, especially variety-fungicide combinations had a highly significant variation ($p<0.01$) in disease parameters. 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 hybrid variety without fungicide spray. On the other hand, thrice sprayed of propiconazoleon 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 sprays 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 thrice sprays of propiconazole coupled with maize varietyAMHQ-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 applications 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.

The current experiment revealed the role of fungicide-variety combination plays in managing the disease. However, the following recommendations were made to come up with effective, affordable and sustainable TLB management strategies:

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.

Conflicts of Interests

The authors have not declared any conflict of interests.

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