PREVALENCE, INTENSITY, AND MORPHOLOGICAL VARIABILITY OF WHEAT BLOTCH (ZYMOSEROPTORIA TRITICI) IN OROMIA, ETHIOPIA

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Studies of the wheat Zymoseptoria tritici blotch (ZTB) status in different locations, on agronomic practice, and pathogen variability has not yet been studied in Ethiopia. As a result, the goal of this study was to determine ZTB’s distribution and intensity, as well as the morphological variability of isolates. In Oromia’s central-southeastern region, zones and districts were purposefully chosen, whereas kebeles were determined via a systematic sampling procedure. In a generalized linear model (GLM), the mean comparison of fixed effects was examined using least significant difference (LSD) tests. Colony texture, shapes, and colors were used to identify isolate variability. Pearson correlation was used to examine the relationship between disease intensity and the independent variable, and multiple regression analysis was used to estimate the magnitudes of the association. A total of 108 fields were examined, with the percent occurrence of zones (88.9 to 100%) and districts (77.8 to 100%) recorded. ZTB intensity was not significantly different across districts (p < 0.05) while severity was significantly different across zones (p < 0.01). Weed infestation (r = 0.78 and r = 0.20) and growth phases (r = 0.72 and r = 0.36) had a positive correlation, although plowing frequency (r = -0.77 and r = -0.43) had a negative correlation with incidence and severity. There are 43 isolates classified into four colors, three textures, and three growth forms. The ZTB epidemics in current research areas are need more consideration and they should be prioritized for integrated management. Our data suggest that weed control, soil tillage, and crop rotation are all effective ways to mitigate the effects of wheat ZTB.

Keywords
Prevalence
Intensity
Variability
ZTB
Wheat

INTRODUCTION

Wheat Zymoseptoria tritici blotch (ZTB) is a devastating disease that causes problems in many parts of the world (McDonald et al., 2015; Mehra et al., 2018; McDonald and Mundt, 2016; Dalvand et al., 2018). It is a hemibiotrophic fungal pathogen (Zhong et al., 2017) that causes significant yield loss in wheat by disrupting the photosynthetic component of the plant (Griffiths and Ao, 1980; Eyal, 1981). ZTB epidemics in wheat fields are mostly determined by host vulnerability and climatic factors (Eyal, 1987). Inoculum density, strain pathogenicity, and cultural practices are all factors that influence it (Kema and van Silfhout, 1997; Harrat and Bouznad, 2018). The principal inoculums are obtained through diseased plant residue, seeds, and alternate hosts (Ponomarenko et al., 2018).
ZTB infestations have been related to wheat yield losses of 30 to 54% (Eyal, 1987) and even greater than 60% (Shipton et al., 1971). ZTB causes 25 to 82% wheat yield loss in Ethiopia, with incidence and severity increasing in the key production areas (Abebe et al., 2017; Abeyo et al., 2011; Hailu and Woldeab, 2015; Takele et al., 2015; Said and Hussien, 2013). The losses in yield related to severe ZTB occurrences have been found to vary from 31 to 53% (Babadoost and Hebert, 1984) to 56% (Eyal, 1981). ZTB can be found all around the world (Ponomarenko et al., 2011).

For the first time, ZTB was discovered in 1956 in Ethiopia (Stewart and Yiroou, 1967). Nowadays, ZTB is distributed in Oromoia, Amhara, SNNPR regions of Ethiopia (Tadesse et al., 2018; Said and Hussien, 2013; Azanaw et al., 2017). Its severity is highest in Ethiopia’s central highlands (Ayele et al., 2008; Ababa Tarafa, 2020) and in environments with high humidity, altitude, and warmer temperatures (Azanaw et al., 2017; Eyal, 1987; Ponomarenko et al., 2011; Ghini et al., 2008). The pathogen’s diverse population is to account for the high intensity. *Z. tritici* exhibits distinct growth forms, hues, and textures, according to investigations of colony morphology on various media (Harrat and Bouznad, 2018; Ayad et al., 2014; Bentata et al., 2011). This suggests that the pathogen is very variable among the population due to genetics (Kema and van Silfhout, 1997; Mekonnen et al., 2020).

Aside from the assessment, one of the few types of ZTB research done in Ethiopia was the evaluation of fungicides and wheat cultivars under natural infection. However, there have been no morphological or pathogenic variability studies of *Z. tritici* isolates yet. Because the disease is dynamic, ongoing disease assessment and studies of disease variability are utilized to alert farmers and governments early, devise management practices, and conduct additional research. The goal of this research was to evaluate ZTB distribution and intensity in a previously unstudied location, as well as to identify the variety of collected isolates based on colony colors, growth patterns, and textures.

**MATERIALS AND METHODS**

**Description of the survey areas**

During the 2019 cropping season, ZTB field surveys were done in central-southeastern Oromia, Ethiopia. Arsi, West Arsi, Bale, and West Shoa zones were all surveyed (Figure 1).
Sampling method and strategy
From flowering until maturity, wheat ZTB survey was carried out. The four zones and three districts were chosen from the region using a purposive sampling method. At 5-10 km intervals along the main, available, and accessible roadsides, three kebeles within each district and three farms within each kebele were assessed (Table 1). Farmers’ training centers and research stations were also surveyed at the same time. Infected wheat leaf tissues were collected, as well as 91 green leaves with pycnidia and a few dried samples from 108 farmers’ fields in paper bags for pathogen isolation (Figure 2A and B).

Table 1. Description of surveyed areas in 2019 in Oromia, Ethiopia.

| Zones     | Districts | No. of farmers | No. of farmers | Longitude | Latitude | Altitude range |
|-----------|-----------|----------------|----------------|-----------|-----------|----------------|
|           |           | field assessed/kebele | field assessed/district |           |           |                |
| West Shoa | Welmera   | 3              | 9              | 03°28'60" | 09°52'6" | 2252-2577     |
|           | Tokekutaye| 3              | 9              | 03°43'45" | 08°51'31"| 2245-2792     |
|           | Ambo      | 3              | 9              | 03°50'49" | 08°53'26"| 2463-2988     |
|           | Adaba     | 3              | 9              | 03°26'59" | 07°01'33"| 2357-2498     |
| West Arsi | Dodola    | 3              | 9              | 03°03'36" | 07°59'33"| 2410-2573     |
|           | Assassa   | 3              | 9              | 03°09'29" | 07°02'28"| 2386-2573     |
|           | Sire      | 3              | 9              | 03°30'69" | 08°15'53"| 2018-2366     |
| Arsi      | Hetosa    | 3              | 9              | 03°14'37" | 08°10'45"| 2123-2244     |
|           | Lemunabilbilo | 3    | 9              | 03°16'22" | 07°18'46"| 2602-2938     |
|           | Sinana    | 3              | 9              | 04°17'48" | 07°64'30"| 2481-2625     |
| Bale      | Goba      | 3              | 9              | 03°58'23" | 07°11'40"| 2392-2472     |
|           | Agarfa    | 3              | 9              | 03°56'53" | 07°16'36"| 2344-2462     |
| Total     |           | 36             | 108            |           |           |                |

Figure 2. Symptoms of Zymoseptoria tritici blotch on the leaves of the wheat.

Diseases Assessment
Depending on the size of the field, 1 m² quadrant was thrown at three to five spots at random, with 15 meter intervals along the section. Each 1 m² quadrant had 14 plants randomly selected and analyzed for ZTB incidence and severity (Eyal, 1987). ZTB prevalence was estimated by dividing the number of infected fields by the total number of fields examined, and incidence was obtained by dividing the number of infected plants by the total number of plants assessed from three quadrants (Cooke, 2006). Severity was measured on a two-digit scale (Saari and Prescott, 1975). The first digit (0-9) represents the ZTB upward migration on the plant, and the second digit (0-9) determines the severity of the total foliar infection on the whole plant (Eyal, 1987).

Its severity index was determined by the formula:

\[
\% \text{Severity Index} = \frac{D_1}{Y_1} \times \frac{D_2}{Y_2} \times 100
\]

Where, D1 representing STB upward movement,
whereas D2 is the severity. Y1 represents the maximum ZTB upward movement and Y2 represents the maximum severity (Sharma and Duveiller, 2007). Data of agronomic practice (Table 2), altitude (Table 1), and crop growth stage were gathered to do an association with ZTB intensity. The longitude and latitude coordinates of each field were taken using a global positioning system (GPS) (Table 1).

### Table 2. Descriptions of agronomic practice and crop growth stage with their qualitative measurement and quantitative levels.

| Plowing frequency | Weed infestation | Crop growth stage |
|-------------------|------------------|------------------|
| Qualitative       | Quantitative     | Qualitative      | Quantitative |
| measurement       | levels           | measurement      | levels       |
| One time          | 1                | Low              | 1            | Flowering    |
| Two times         | 2                | Medium           | 2            | Milking      |
| Three times       | 3                | High             | 3            | Dough        |
| Four times        | 4                | Very high        | 4            | Maturity     |

### Isolation process

Isolation was carried out in the Holeta National Biotechnology Research Center’s Microbiology Laboratory at Holeta, Ethiopia. With a little modification from the original protocol, the isolation was completed (Eyal, 1987). The filter paper was placed on the Petri plate and wetted with distilled water in the first stage. The wheat leaves were then placed on the wetted filter paper in a 7 cm segment. For enhancing pycnidiospore oozing from an opening of the pycnidium (ostiole), the petridish was incubated at 24 °C for 2 to 8 hours depending on the stages of leaves. The produced oozes were transferred to potato dextrose agar (PDA) supplemented with chloramphenicol succinate 250 mg for 1 liter distilled water using a dissecting microscope or stereoscope (Eyal, 1987). Pycnidia that did not generate ooze, on the other hand, were extracted from the leaf epidermis and placed onto PDA plates using a sterile needle. The colony was picked via sterile loops and smeared onto PDA plates after seven days. The streaked plates were incubated for seven days in a 24 °C incubation chamber to promote fungal growth. The single pinkish-orange, dark hard color colony that matched (HARRAT and BOUZNAD, 2018) were streaked on PDA plates and then chosen and distributed on new PDA plates without antibiotics.

### Colony morphology

On PDA, cultural appearances (colony color, shapes, and texture) were identified based on macroscopic inspection. The colony morphology was described using both a laboratory manual and a graphical atlas for fungal identification (Watanabe, 2010).

### Data analysis

The data was analyzed using SAS version 9.3 statistical software (Stokes et al., 2012). The survey data were converted using ARCSINE after Kolmogorov-Smirnov analysis showed the substantial differences (p < 0.05) and exhibited a non-normal distribution (Kema and van Silfhout, 1997). Fixed factors were structured in three phases of nested design (Tsedaley et al., 2016), with the exception of farmers' fields, which were regarded as a random effect (Table 3). Kebeles were nested under districts in the three levels of nested design, while districts were nested under zones. Pearson correlation was used to examine the relationship between ZTB intensity and agronomic practice, altitude, and crop growth phases, and multiple regressions were used to predict the magnitudes of ZTB intensity.

### RESULTS

#### Distribution of *Zymoseptoria tritici* blotch across a location

Wheat ZTB was found in all of the investigated areas, with prevalence rates of 100%, 88.8%, and 96.3% in Bale, Arsi, and both West Arsi and West Shoare, respectively. The over all of the surveyed zones had the highest ZTB prevalence (95.4%). It was found to be 100% prevalent in eight districts (Tokekutaye, Ambo, Welmera, Adaba, Dodola, Hetosa, Goba, Agarfa, and Sinana) but Lemunabilbilo district having the lowest prevalence (77.8%) (Figure 3).
Table 3. Nested ANOVA for the disease intensity of wheat Zymoseptoria.

| Source of variation          | Degree of freedom | Mean square     |
|------------------------------|-------------------|-----------------|
|                              |                   | Disease Incidence | Disease Severity |
| Model                        | 35                | 478.04ns        | 401.2ns          |
| Zone                         | 3                 | 637.6ns         | 1969.9**         |
| District(Zone)               | 8                 | 611.7ns         | 245.9ns          |
| Kebele(Zone*District)        | 24                | 413.6ns         | 256.8ns          |
| Error                        | 72                | 426.5           | 313.1            |
| Corrected Total              | 107               |                 |                 |

The intensity of Zymoseptoria tritici blotch across a location

The incidence of ZTB was not substantially different at the zone and district levels (p < 0.05). This indicated that it has infected wheat crops in all of the surveyed areas in a similar manner. West Shoa, West Arsi, Arsi, and Bale zones had ZTB incidences of 95.7%, 94.7.9%, 87.7%, and 99%, respectively. The maximum incidence (100%) was recorded in three districts (Tokekutaye, Dodola and Sinana), while the lowest incidence (75%) was recorded in Lemunabilbilo (Figure 3).

Between the four zones, ZTB severity index revealed highly significant (p < 0.01) differences. The severity indexes of the Arsi and Bale zones were notably different, but the severity indexes of the other zones were similar (Table 4). The severity index of the districts, on the other hand, did not differ substantially (p < 0.05). At district level, Tokekutaye received the highest severity rating of 42%, while Lemunabilbilo received the lowest severity index of 12% (Figure 3).

Table 4. The effect of four zones on disease severity.

| Zones   | Disease Severity Index (%) |
|---------|---------------------------|
| West Shoa | 31.69<sup>ab</sup>       |
| West Arsi | 23.5<sup>ab</sup>         |
| Arsi     | 15.64<sup>b</sup>        |
| Bale     | 34.57<sup>a</sup>        |
| CV       | 40.7                      |

Association of Zymoseptoria tritici blotch with agronomic practices, altitude, and wheat growth stages

ZTB severity score showed a positive correlation (r = 0.78) and a highly significant difference (p < 0.001) with weed infection levels. Plowing frequency was found to have a negative relationship with ZTB severity index (r = -0.77) and incidence (r = -0.43). ZTB severity and wheat crop stages showed strong positive relationships (r = 0.72). According to our current findings, the increase in altitude in meters has no significant relationship with disease severity (p < 0.05) (Table 5).
Multiple regression
The degree of disease intensity was predicted, and there was a highly significant negative relationship between disease incidence and plowing frequency (p < 0.01). There was no correlation between disease incidence and other parameters. The disease severity predicted increased considerably (p < 0.05) as weed infestation grew, decreased significantly (p < 0.001) as plowing frequency increased, and increased significantly (p < 0.001) as crop growth stages increased, but no significant (p < 0.05) as altitude increased (Table 6).

Table 5. Pearson’s correlation coefficients of Zymoseptoria tritici blotch intensity over agronomic practice, altitude, and crop growth stages.

| Variables | ALT | WIL | PF | GS | DSI | DI |
|-----------|-----|-----|----|----|-----|----|
| ALT       | 1   | 0.01ns | 0.012ns | -0.002ns | -0.008ns | -0.14ns |
| WIL       | 1   | -0.66*** | 0.69*** | 0.78*** | 0.2* |
| PF        | 1   | -0.68*** | -0.77*** | -0.43*** | |
| GS        | 1   | 0.72*** | 0.36*** | |
| DSI       | 1   | 0.36*** | |
| DI        | 1   | | |

DI - Disease incidence, DSI - Disease severity index, WIL - Weed infestation level, PF - Plowing frequency, ALT - Altitude, and GS - Growth stage. * Significant level at p < 0.05, ** Significant level at 0.01, and ***Significant level at 0.001.

Table 6. Multiple regression analysis of Zymoseptoria tritici blotch intensity over agronomic practice, altitude, and crop growth stages.

| Predictor | Incidence | Severity |
|-----------|-----------|----------|
| Constant  | 167       | 42       |
| GS        | 4.44ns    | 3.19*    |
| PF        | -11.8**   | -10.4*** |
| WIL       | -4.54ns   | 9.73***  |
| ALT       | -0.0141ns | -0.00075ns |

Disease Incidence = 167 + 4.44 GS - 11.8 PF - 4.54 WIL - 0.0141 ALT;
Determination coefficient R² = 0.22; WIL-Weed infestation level, PF-Plowing frequency, ALT - Altitude, and GS - Growth stage; Disease severity index = 42.0 + 3.19 GS - 10.4 PF + 9.73 WIL - 0.00076 ALT; Determination coefficient R²= 0.74; WIL-Weed infestation level, PF-Plowing frequency, ALT - Altitude, and GS - Growth stage; Ns indicates nonsignificant.

Microscopic and Morphological variability

Zymoseptoria tritici isolates produced very thin pycnidiospores with more than three septation and few curves in form. The shapes, size and septa of pycnidiospores of ZTB isolates are the same. The Zymoseptoria tritici isolates were produced macropycnidiospores of very thin, and more than three septation and erect in shape. Also, the isolates were produced micropycnidiospores in those are without septa (Figure 4).

Six pinkish colony isolates had a creamy texture and three different growth forms: dense, medium, and sparse. The whitish color isolates had a creamy texture, and the ooze floods the sowing lines. On PDA, dark-colored isolates grow compactly, densely, and sparsely. Brown color isolates have an intermediate, solid, and creamy texture, with sparse and thick growth patterns (Table 7 and Figure 5).

Only two (4.5%) of the total isolates showed whitish colony color. A colony of black color was composed of 28 isolates (63%) of the total isolates, and this colony became the most dominant. Out of the total isolates analyzed, 8 (18.2%) have a brown color and 6 (14%) have a pinkish color.
Figure 4. Pycnidiospores of wheat Ethiopian *Zymoseptoria tritici*.

Figure 5. The colors of wheat Ethiopian *Zymoseptoria tritici* isolates on PDA; Pinkish, brown, whitish and black colors.

Table 7. Morphological variability of Ethiopian *Zymoseptoria tritici* isolates.

| Zones      | No. of isolates | Colony color          | Colony growth          | Texture                             |
|------------|-----------------|-----------------------|------------------------|-------------------------------------|
| West Shoa  | 23              | Black, pinkish, and brown colors | Dense and intermediate sparse | compact, cream, and intermediate    |
| West Arsi  | 6               | Brown, black colors   | Dense intermediate and sparse | intermediate and compact            |
| Arsi       | 6               | Whitish, pinkish, and Black color | Dense intermediate and sparse | Cream and compact                  |
| Bale       | 9               | Pinkish, brown, and Black color | Dense intermediate and sparse | Cream, intermediate, and compact    |

The colors of nine isolates generated from Bale samples varied. Four isolates were pinkish in color, three were brown, and two were black in color. The isolates were taken from the Arsi zone, and one was whitish, three were black, and two were pinkish. One brown and five black colors were found in West Arsi isolates. Eighteen
isolates from West Shoa produced black colonies, while one and four isolates produced pinkish and brown colonies, respectively (Table 7 and Figure 5). A total of 44 *Z. tritici* isolates were obtained from 91 samples collected across the Oromia region (Table 8 and Figure 6).

![Map showing the geographic locations of the Zymoseptoria tritici isolates in 2019 in Oromia Ethiopia.](image)

**Table 8.** Collection area and varieties source of *Zymoseptoria tritici* isolates in 2019 in Oromia, Ethiopia.

| Sr. No | Isolate code | Zone     | District | Kebele                      | Varieties source |
|--------|--------------|----------|----------|-----------------------------|------------------|
| 1      | EtAm-1       | West Shoa| Welmera  | Holeta agricultural research center in the station | Alidoro          |
| 2      | EtAm-2       | West Shoa| Tokekutaye | Handersa                  | Danda’a          |
| 3      | EtAm-3       | West Shoa| Tokekutaye | Maruf                   | Danda’a          |
| 4      | EtAm-4       | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 5      | EtAm-5       | West Shoa| Ambo     | Yaechbo                  | Hidase           |
| 6      | EtAm-6       | West Shoa| Tokekutaye | Malkedera              | Danda’a          |
| 7      | EtAm-9       | West Shoa| Ambo     | Kuregatira               |                  |
| 8      | EtAm-10      | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 9      | EtAm-11      | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 10     | EtAm-12      | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 11     | EtAm-13      | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 12     | EtAm-14      | West Shoa| Ambo     | Bojibilo                  | Danda’a          |
| 13     | EtAm-16      | West Shoa| Ambo     | Kibakube                 | Kingbird         |
| 14     | EtAm-19      | West Shoa| Ambo     | Yaechbo                  | Danda’a          |
| 15     | EtAm-20      | West Shoa| Tokekutaye | Malkedera          |                  |
| 16     | EtAm-21      | West Shoa| Tokekutaye | Maruf                   | Hidase           |
| Location Code | Location Type | Village Name | Host Plant | Pathogen Name |
|---------------|----------------|--------------|------------|---------------|
| EtAm-22       | West Shoa      | Tokelutaye   | Maruf      | Digalu        |
| EtAm-23       | West Shoa      | Tokelutaye   | Maruf      | Huluka        |
| EtAm-26       | West Shoa      | Tokelutaye   | Gorobiyo   | Gololcha      |
| EtAm-27       | West Shoa      | Tokelutaye   | Adersabila | Hidase        |
| EtAm-28       | West Shoa      | Tokelutaye   | Adersabila | Danda’a       |
| EtAm-29       | West Shoa      | Tokelutaye   | Adersabila | Hidase        |
| EtAm-30       | West Shoa      | Tokelutaye   | Adersabila | Hidase        |
| EtB-1         | Bale           | Goba         | Sinja      | Hidase        |
| EtB-2         | Bale           | Sinana       | Shalo      | Ogolcho       |
| EtB-3         | Bale           | Agarfa       |            |               |
| EtB-4         | Bale           | Goba         | Sinja      | Candidate     |
| EtB-5         | Bale           | Agarfa       | Ilani      | Ogolcho       |
| EtB-6         | Bale           | Sinana       | Amalama    | Ogolcho       |
| EtB-7         | Bale           | Sinana       | Robearea   | Ogolcho       |
| EtB-8         | Bale           | Gasera       | Wute       |               |
| EtB-10        | Bale           | Goba         | Misira     | Ogolcho       |
| EtA-3         | Arsi           | Hetosa       | Hatehandode| Ogolcho       |
| EtA-4         | Arsi           | Hetosa       | Hatehandode| Kubsa         |
| EtA-7         | Arsi           | Hetosa       | Seruanketo | Ogolcho       |
| EtA-8         | Arsi           | Lemunabilbilo|            |              |
| EtA-11        | Arsi           | Hetosa       | Hatehandode| Kubsa         |
| EtA-19        | Arsi           | Tiyo         | Doshia     | Danda’a       |
| EtSh-1        | West Arsi      | Assassa      | Debara     | Ogolcho       |
| EtSh-2        | West Arsi      | Dodola       | Bekola     | Paven-76     |
| EtSh-4        | West Arsi      | Kechamachare |           | Ogolcho       |
| EtSh-5        | West Arsi      | Assassa      | Edobelio   | Kubsa         |
| EtSh-6        | West Arsi      | Assassa      | Tuse       | Kubsa         |
| EtSh-7        | West Arsi      | Assassa      |            |              |

**DISCUSSION**

The significant prevalence of ZTB in the examined locations can be attributed to favorable environmental conditions for ZTB development (regular rains and mild temperatures) (Gilchrist and Dubin, 2002; Teklay et al., 2015).

In the altitude range of 2072 to 3043 m.a.s.l, (Tadesse et al., 2018) reported a 38 to100% ZTB incidence. Furthermore, the current findings demonstrate that ZTB is found in 100% of the assessed locations, indicating that it is a severe danger to wheat production in the country. The ZTB disease is very important in the entire world. Argentina, Ethiopia, Iran, the United States, the Netherlands, Russia, New Zealand, and Australia are among the largest wheat-producing countries on the planet. In Iran, Tunisia, and Morocco, it is a major issue with durum wheat (Ponomarenko et al., 2011; Eyal, 1987).

High inoculum levels associated with farming methods, particularly in the examined areas, are thought to be the cause of the high incidence. Farmers, in general, do not use appropriate crop rotation systems with non-pathogen host plants and cultivate wheat from year to year, particularly in the Arsi and Bale zones. Because it overwinters in the soil and decaying plant residues as pycnidia, has a higher chance of inoculum survival (Ponomarenko et al., 2011).

The high ZTB incidence found in this study is due to high inoculum build up, susceptible cultivars planted by farmers, and favorable environmental conditions across all agro-ecologies in the examined areas of the country. Crop rotation with non-host crops was not practiced by the majority of farmers in the examined area, regardless of zone, and poor weed management and low plowing
frequency were also prevalent. In comparison to Ethiopia’s central highlands, monocropping is typical in the Arsi and Bale zones. Greater weed population can exacerbate the severity of ZTB. This could be due to wheat competing with weeds for nutrients, water, space, and sunlight, resulting in increased wheat succulence and less ability to resist the pathogen physically (Agrios, 2012). The plant’s canopy draws the wheat leaves closer together, making it simpler for rain splashes to disperse spores and altering the pathogen’s life cycle (Ponomarenko et al., 2011; Eyal, 1987). In dense plant population, the microclimate, such as high moisture, was always present, providing a favorable setting for the disease. It is possible that the higher plant density leads to a more suitable microclimate within the leaf canopy, which promotes ZTB development (Ansar et al., 2010).

Many researches on the impact of environmental conditions on Z. tritici have found that temperature fluctuations play the most crucial function. The Z. tritici body temperature is the wheat leaf temperature that develops into plant leaves, influencing their life cycle significantly (Pietravalle et al., 2003; Gladders et al., 2001; Lovell et al., 2004). Aside from temperature, moist leaf surface plays a significant role in early infections, necessitating a total of 10 mm of rain during three consecutive rainy days with at least 1 mm of rain (Pietravalle et al., 2003).

The severity of ZTB reduced as the frequency of plowing increased, and similar trend was seen with Z. tritici (Bailey et al., 2001; Fernandez et al., 2016; Bankina et al., 2014). The effects of soil tillage on ZTB have been researched in a variety of locations. The severity of ZTB was higher in plowed plots under conventional tillage (Gilbert and Woods, 2001; Bürger et al., 2012; Fernandez et al., 2016) than in alternative tillage systems, notwithstanding the contradicting results.

Increased soil tillage is utilized for a variety of reasons during crop cultivation, including exposing inoculum to sunlight and removing inoculum sources from the soil. As a result, reducing the amount of inoculums in the soil may hinder the ZTB life cycle (Fernandez et al., 2016; Mergoum et al., 2007). As the frequency of plowing increased, the incidence of ZTB reduced once more. Rotation to non-hosts and agricultural debris sanitation achieved by deep plowing can reduce the quantity of inoculums available to start a new ZTB life cycle. Due to the long-distance spread of ascospores, this may be less effective in the field, but it may be beneficial if used within a region (Ponomarenko et al., 2011).

Some research have found a low incidence of ZTB under zero tillage or conservation tillage, but this outcome varied (Gilbert and Woods, 2001). The incidence of tan spot and powdery mildew is reduced as the plowing frequency is raised in farmer’s fields, but the incidence of ZTB is increased (Krupinsky et al., 2007). Conservation tillage is encouraging the over-summering of Z. tritici, according to (Mergoum et al., 2007).

Throughout the survey effort, the majority of the district’s wheat growth stages were at the dough stage. Although the crop had reached full maturity in certain districts, particularly in the midlands. The severity of ZTB was influenced by the variation in growth stages. Because of senescence, the positive association shows that as the crop stage progressed, the severity of the ZTB increased as well. The reason for this is because as the crop matures, it loses its physical and chemical defenses, allowing the disease to easily penetrate and develop on the crop (Agrios, 2012).

The different reports showed that the increment of altitude in meter negatively correlated with wheat stem rust (Hirpa, 2018) but from our study, the ZTB intensity is not correlated with altitude in the surveyed areas.

We measured disease severity and concluded that when plowing frequency increased, disease incidence decreased by 11.84%. As weed infection levels increased, disease severity increased by 9.73%. Conversely, as plowing frequency increased, disease severity decreased by 10.42%. Other effects included an increase in disease severity of 3.19% as crop growth stages progressed from flowering to maturity.

Zymoseptoria tritici pycnidiospore differed from Parastignospora nodorum pycnidiospore, which were thick, had less than three septations, and had an erect morphology. The germinated spores of the Septoria tritici isolates had the different number of septations, shape, and thickness from Parastignospora nodorum isolates (Eyal, 1987).

On a solid PDA media, the colony morphology of 44 isolates revealed a wide range of textures, growth patterns, and colors (Figure 5). The whitish color isolates were discovered in the current experiments and had never been reported before (HARRAT and BOUZNAD, 2018). EtAm-14 and EtA-4 had the pinkish color similar to Bale Zone and EtA-3, EtA-8, and EtSh-1 also had the black
color similar to the West Shoa zone. This indicates that location may not affect the outcome of colonies of various colors resulting from isolates plated on PDA media, meaning that isolates collected from different locations and plated on PDA media could have the same or various colors, or isolates from the same location had different colors and from the same causative agent (Saidi et al., 2012). When *Z. tritici* isolates were plated on PDA growth media, they showed morphological differences.

**CONCLUSION**
The *Z. tritici* disease was prevalent in the most of the wheat production areas and its intensity also very high in most of the areas where wheat production is known such as Bale, Arsi and west arsi Ethiopia. Furthermore *Z. tritici* has a wide range of colony shape, which is new to our country. The morphologic heterogeneity of wheat *Z. tritici* isolates in Ethiopia was validated by the current finding. Because wheat *Z. tritici* is extremely common and severe in all of Ethiopia’s central-southeast regions, and wheat is the country’s most important crop, focusing on building an effective ZTB management strategy is crucial.

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**DATA AVAILABILITY**
The data that support the findings of this study are available on request from the corresponding author.

**ETHICAL STATEMENT**
This study did not engage in any human or animal testing.

**REFERENCES**
Ababa Tarafa, G. 2020. Distribution, Intensity and Variability of Septoria Blotch (*Septoria tritici*) in Central-Southeastern Oromia, Ethiopia, and Seedling Resistance of Wheat Cultivars, Jimma University.
Abebe, T., S. Alamerew and L. Tulu. 2017. Genetic variability, heritability and genetic advance for yield and its related traits in rainfed lowland rice (*Oryza sativa* L.) genotypes at Fogera and Pawe, Ethiopia. Advances in Crop Science and Technology, 5: 1-8.
Abeyo, B., E. Firdisa, T. Kebede and G. Solomon. 2011. Screening wheat germplasm for Septoria resistance in Ethiopia. International Symposium on Mycosphaerella and Stagonospora Diseases of Cereals, 8; Mexico City (Mexico); 10-14 Sep 2011. Book of Abstracts. ^TInternational Symposium on Mycosphaerella and Stagonospora Diseases of Cereals, 8; Mexico City (Mexico); 10-14 Sep 2011. Book of Abstracts^ ADuveiller, E. Singh, PK^AMexico, DF (Mexico)^ BCIMMYT^ C2011.
Agrios, G. 2012. Plant pathology. Elsevier.
Ansar, M., N. M. Cheema and M. H. Leitch. 2010. Effect of agronomic practices on the development of septoria leaf blotch and its subsequent effect on growth and yield components of wheat. Pakistan Journal of Botany, 43: 2125-38.
Ayad, D., R. Sayoud, K. Benbelkacem and Z. Bouznad. 2014. La tache septorienne du blé: Première signalisation de la présence en Algérie des deux Mating types du téloomorphe Mycosphaerella graminicola (Fuckel) Schröter,(anamorphe: Septoria tritici Rob. ex Desm.) et diversité phénotypique de l’agent pathogène. Nature & Technology: 34.
Ayele, B., B. Eshehu, B. Betelem, H. Bekele, D. Melaku, T. Asnakech, A. Melkamu, A. Amare, M. Kiros and A. Fekede. 2008. Review of two decades of research on diseases of small cereal crops. Increasing crop production through improved plant protection, 1: 375-416.
Azanaw, A., Y. Ebabuye, A. Ademe, S. Gizachew and Z. Tahir. 2017. Survey of Septoria Leaf Blotch (Septari atritici Roberge in Desmaz) on Wheat in North Gondar, Ethiopia. Abyssinia Journal of Science and Technology, 2: 11-18.
Bailey, K., B. Gossen, G. Lafond, P. Watson and D. Derksen. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analyses. Canadian Journal of Plant Science, 81: 789-803.
Bankina, B., Z. Gaile, O. Balodis, G. Bimšteine, M. Katamadze, D. Kreita, L. Paura and I. Priekule. 2014. Harmful winter wheat diseases and possibilities for their integrated control in Latvia. Acta Agriculturae Scandinavica, Section B—Soil &
Plant Science, 64: 615-22.
Bentata, F., M. Labhilili, A. Merrahi, F. Gaboun, J. Ibibijben, A. El Aissami, S. Amiri, M. Boulif and M. Jliben. 2011. Determination of the genetic diversity of a population of Septoria tritici on broad wheat via cultural and pathogenic characterization. Revue Marocaine de Protection des Plantes, 2: 1-10.

Bürger, J., A. Günther, F. de Mol and B. Gerowitt. 2012. Analysing the influence of crop management on pesticide use intensity while controlling for external sources of variability with Linear Mixed Effects Models. Agricultural Systems, 111: 13-22.

Cooke, B. 2006. Disease assessment and yield loss. In, The epidemiology of plant diseases. Springer.

Dalvand, M., D. Zafari, M. Soleimani Pari, R. Roohparvar and S. Tabib Ghafari. 2018. Studying Genetic Diversity in Zymoseptoria tritici, Causal Agent of Septoria Tritici Blotch, by Using ISSR and SSR Markers. Journal of Agricultural Science and Technology, 20: 1307-16.

Eyal, Z. 1981. Integrated control of Septoria diseases of wheat. Plant Disease, 65: 763-68.

Eyal, Z. 1987. The Septoria diseases of wheat: concepts and methods of disease management. Cimmyt.

Fernandez, M. R., C. F. Stevenson, K. Hodge, F. Dokken-Bouchard, P. G. Pearse, F. Waelchli, A. Brown and C. Peluola. 2016. Assessing effects of climatic change, region and agronomic practices on leaf spotting of bread and durum wheat in the western Canadian Prairies, from 2001 to 2012. Agronomy Journal, 108: 1180-95.

Ghini, R., E. Hamada and W. Bettiol. 2008. Climate change and plant diseases. Scientia Agricola, 65: 98-107.

Gilbert, J. and S. Woods. 2001. Leaf spot diseases of spring wheat in southern Manitoba farm fields under conventional and conservation tillage. Canadian Journal of Plant Science, 81: 551-59.

Gilchrist, L. and H. Dubin. 2002. Fusarium head blight. Bread wheat: Improvement and production (9251048096).

Gladders, P., N. Paveley, I. Barrie, N. Hardwick, M. Hims, S. Langton and M. Taylor. 2001. Agronomic and meteorological factors affecting the severity of leaf blotch caused by Mycosphaerella graminicola in commercial wheat crops in England. Annals of Applied Biology, 138: 301-11.

Griffiths, E. and H. Ao. 1980. Variation in Septoria nodorum. Annals of Applied Biology, 94: 294-96.

Hailu, E. and G. Woldeab. 2015. Survey of Rust and Septoria Leaf Blotch Diseases of Wheat in Central Ethiopia and Virulence Diversity of Stem Rust Puccinia graminis f. sp. triticici. Adv Crop Sci Tech 3: 166. doi: 10.4172/2329-8863.10001 66 Page 2 of 5 Volume 3• Issue 2• 1000166 Adv Crop Sci Tech ISSN: 2329-8863 ACST, an open access journal identify. Puccinia graminis.

HARRAT, W. and Z. BOUZNAD. 2018. Prevalence, cultural and pathogenic characterization of Zymoseptoria tritici, agent of wheat septoria leaf blotch, in Algeria. African Journal of Agricultural Research, 13: 2146-53.

Hirpa, G. 2018. Virulence Spectrum of Stem Rust (Puccinia graminis f. sp. tritici) and Reactions of Wheat Varieties to Dominant Races in Tigray Region, Northern Ethiopia.

Holloway, G. 2014. Septoria tritici blotch of wheat. DEPI information note series may.

Kema, G. H. and C. H. van Silfhout. 1997. Genetic variation for virulence and resistance in the wheat-Mycosphaerella graminicola pathosystem III. Comparative seedling and adult plant experiments. Phytopathology, 87: 266-72.

Krupinsky, J., A. Halvorson, D. Tanaka and S. Merrill. 2007. Nitrogen and tillage effects on wheat leaf spot diseases in the northern Great Plains. Agronomy Journal, 99: 562-69.

Lovell, D., T. Hunter, S. Powers, S. Parker and F. Van den Bosch. 2004. Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. Plant Pathology, 53: 170-81.

McDonald, B. A. and C. C. Mundt. 2016. How knowledge of pathogen population biology informs management of Septoria tritici blotch. Phytopathology, 106: 948-55.

McDonald, M. C., B. A. McDonald and P. S. Solomon. 2015. Recent advances in the Zymoseptoria tritici–wheat interaction: insights from pathogenomics. Frontiers in plant science, 6: 102.

Mehra, L., U. Adhikari, C. Cowger and P. S. Ojiambo. 2018. Septoria nodorum blotch of wheat (2167-9843). Peerj Preprints.

Mekonnen, T., T. Haileselfassie, S. B. Goodwin and K. Tesfaye. 2020. Genetic diversity and population structure of Zymoseptoria tritici in Ethiopia as
revealed by microsatellite markers. Fungal Genetics and Biology: 103413.
Mergoum, M., P. Singh, S. Ali, E. Elias, J. A. Anderson, K. Glover and T. Adhikari. 2007. Reaction of elite wheat genotypes from the northern Great Plains of North America to Septoria diseases. Plant Disease, 91: 1310-15.
Pietravalle, S., M. Shaw, S. Parker and F. Van Den Bosch. 2003. Modeling of relationships between weather and Septoria tritici epidemics on winter wheat: a critical approach. Phytopathology, 93: 1329-39.
Ponomarenko, A., S. B. Goodwin and G. H. Kema. 2011. Septoria tritici blotch (STB) of wheat. Septoria tritici blotch (STB) of wheat.
Saari, E. and J. Prescott. 1975. Scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter.
Said, A. and T. Hussien. 2013. TEMPORAL DEVELOPMENT OF SEPTORIA BLotch (Septoria tritici) AND ITS EFFECT ON GRAIN YIELD AND YIELD COMPONENT S OF BREAD WHEAT IN HADDIYA-KAMBATA AREA, SOUTHERN ETHIOPIA, Haramaya University.
Saidi, A., M. Eslahi and N. Safaie. 2012. Efficiency of Septoria tritici sporulation on different culture media. Trakia J. Sci, 10: 15-18.
Sharma, R. and E. Duveiller. 2007. Advancement toward new spot blotch resistant wheats in South Asia. Crop Science, 47: 961-68.
Shipton, W., W. Boyd, A. Rosielle and B. Shearer. 1971. The common Septoria diseases of wheat. The Botanical Review, 37: 231-62.
Steinberg, G. 2015. Cell biology of Zymoseptoria tritici: Pathogen cell organization and wheat infection. Fungal Genetics and Biology, 79: 17-23.
Stewart, R. B. and D. Yiroou. 1967. Index of plant diseases in Ethiopia. Bull. Exp. Stn Coll. Agric. Halle Selassie Univ., 30.
Stokes, M. E., C. S. Davis and G. G. Koch. 2012. Categorical data analysis using SAS. SAS institute.
Tadesse, Y., A. Chala and B. Kassa. 2018. Survey of Septoria Tritici Blotch (Septoria Tritici) of Bread Wheat (Triticum aestivum L.) in the Central Highlands of Ethiopia. American Journal of Bioscience and Bioengineering, 6: 36-41.
Takele, A., A. Lencho, W. Getaneh, E. Hailu and B. Kassa. 2015. Status of wheat Septoria leaf blotch (Septoria tritici Roberge in Desmaz) in south west and Western Shewa zones of Oromiya regional state, Ethiopia. Research in Plant Sciences, 3: 43-48.
Teklay, A., M. Muez and L. Muruts. 2015. Field response of wheat genotypes to septoria tritici blotch in Tigray, Ethiopia. Journal of Natural Sciences Research, 5: 146-52.
Tsadale, B., G. Adugna and F. Lemessa. 2016. Distribution and importance of sorghum anthracnose (Colletotrichum sublineolum) in southwestern and western Ethiopia. Plant Pathology Journal, 15: 75-85.
Watanabe, T. 2010. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press.
Zhong, Z., T. C. Marcel, F. E. Hartmann, X. Ma, C. Plissonneau, M. Zala, A. Ducasse, J. Confais, J. Compain and N. Lapalu. 2017. A small secreted protein in Zymoseptoria tritici is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. New Phytologist, 214: 619-31.

CONFLICT OF INTEREST
The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS
Girma Ababa conducted the practical experiments, collected the data, analyzed the data, and wrote the paper, while Girma Adugna and Bekele Hundie worked as advisors and wrote the paper.

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