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

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ABSTRACT

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.

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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.*, 2011; Holloway, 2014; Steinberg, 2015).

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).

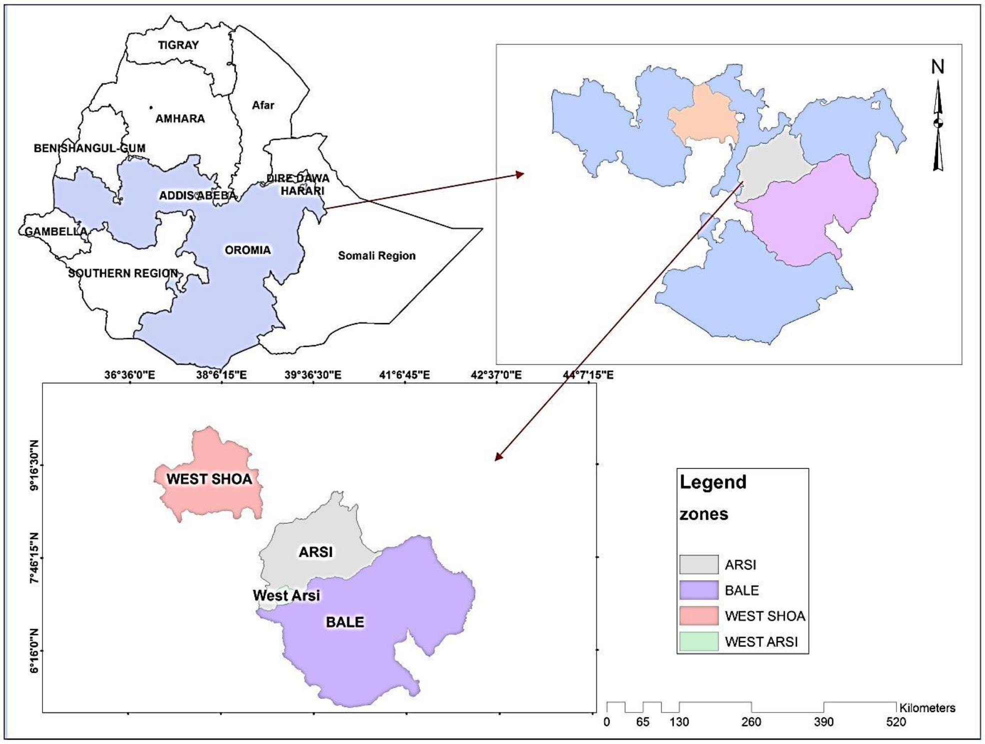

Figure 1. Map showing the geographic locations of the survey zones in 2019 in Oromia, Ethiopia.

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 field assessed/kebele	No. of farmers field assessed /district	Longitude	Latitude	Altitude range
West Shoa	Welmera	3	9	038^{0}28'60''	09^{0}52'6''	2252-2577
	Tokekutaye	3	9	037^{0}43'45''	08^{0}51'31''	2245-2792
	Ambo	3	9	037^{0}50'49''	08^{0}53'26''	2463-2988
West Arsi	Adaba	3	9	039^{0}26'59''	07^{0}01'33''	2357-2498
	Dodola	3	9	039^{0}03'36''	07^{0}59'33'	2410-2573
	Assassa	3	9	039^{0}09'29''	07^{0}02'28''	2386-2573
Arsi	Sire	3	9	039^{0}30'69''	08^{0}15'53''	2018-2366
	Hetosa	3	9	039^{0}14'37''	08^{0}10'45''	2123-2244
	Lemunabilbilo	3	9	039^{0}16'22''	07^{0}18'46''	2602-2938
Bale	Sinana	3	9	040^0'17'48'	07^{0}64’30”	2481-2625
	Goba	3	9	039^{0}58'23''	07^{0}11’40”	2392-2472
	Agarfa	3	9	039^{0}56'53''	07^{0}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 m^2 quadrant was thrown at three to five spots at random, with 15 meter intervals along the section. Each 1 m^2 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;

$$\% \, Severity\ Index = \frac{D1}{Y1} * \frac{D2}{Y2} * 100$$

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 (Table1), 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 measurement	Quantitative levels	Qualitative measurement	Quantitative levels	Qualitative measurement	Quantitative levels
One time	1	Low	1	Flowering	1
Two times	2	Medium	2	Milking	2
Three times	3	High	3	Dough	3
Four times	4	Very high	4	Maturity	4

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		

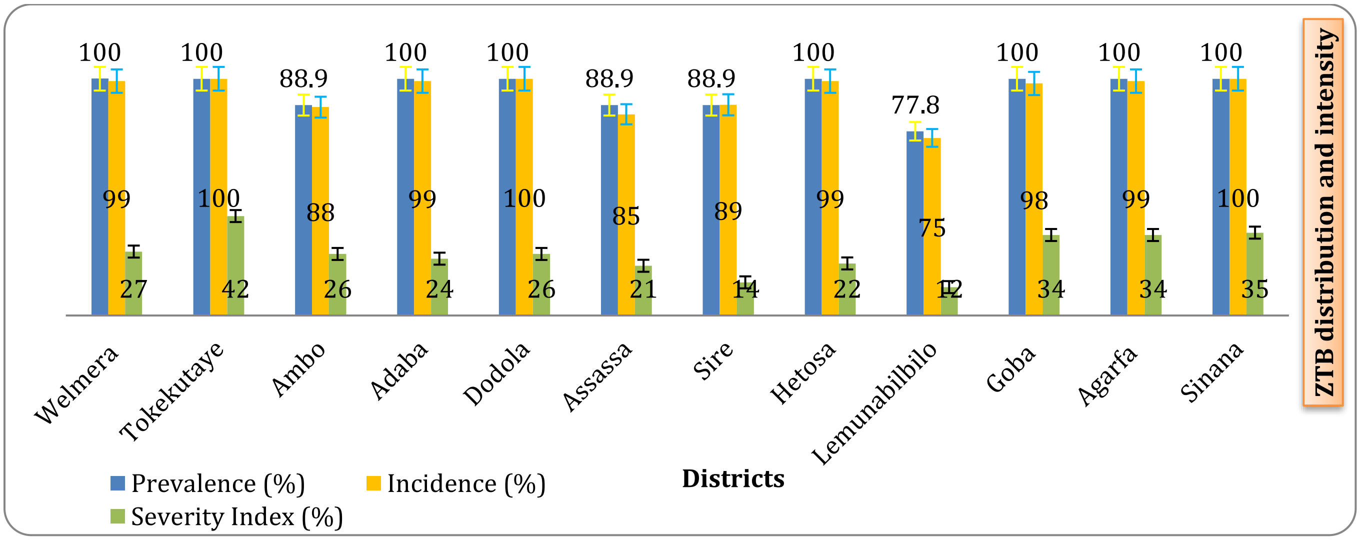

Figure 3. Disease distribution and intensity in 2019 in Oromia, Ethiopia.

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^{ab}
West Arsi	23.5^{ab}
Arsi	15.64^{b}
Bale	34.57^{a}
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	Parameter estimate	
	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 $R^2 = 0.22$; 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 $R^2 = 0.74$; WIL-Weed infestation level, PF-Plowing frequency, ALT - Altitude, and GS - Growth stage; Ns indicates non-significant

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).

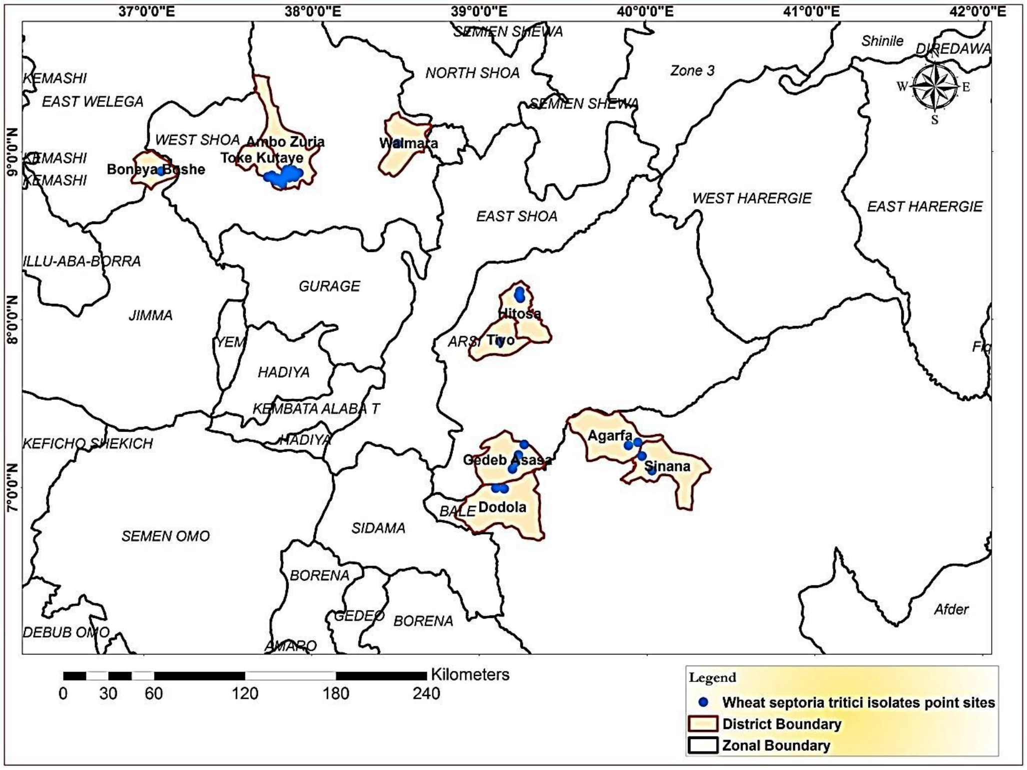

Figure 6. Map showing the geographic locations of the *Zymoseptoria tritici* isolates in 2019 in Oromia Ethiopia.

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

Sr. No	Isolate code	Geographical source			Varieties source
		Zone	District	Kebele	Names
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	Digalu
4	EtAm-4	West Shoa	Ambo	Bojibilo	Danda'a
5	EtAm-5	West Shoa	Ambo	Yaechebo	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	Yaechebo	Danda'a
15	EtAm-20	West Shoa	Tokekutaye	Malkedera	
16	EtAm-21	West Shoa	Tokekutaye	Maruf	Hidase

17	EtAm-22	West Shoa	Tokekutaye	Maruf	Digalu
18	EtAm-23	West Shoa	Tokekutaye	Maruf	Huluka
19	EtAm-26	West Shoa	Tokekutaye	Gorobiyo	Gololcha
20	EtAm-27	West Shoa	Tokekutaye	Adersabila	Hidase
21	EtAm-28	West Shoa	Tokekutaye	Adersabila	Danda'a
22	EtAm-29	West Shoa	Tokekutaye	Adersabila	Hidase
23	EtAm-30	West Shoa	Tokekutaye	Adersabila	Hidase
24	EtB-1	Bale	Goba	Sinja	Hidase
25	EtB-2	Bale	Sinana	Shalo	Ogolcho
26	EtB-3	Bale	Agarfa		
27	EtB-4	Bale	Goba	Sinja	Candidate
28	EtB-5	Bale	Agarfa	Ilani	Ogolcho
29	EtB-6	Bale	Sinana	Amalama	Ogolcho
30	EtB-7	Bale	Sinana	Robearea	Ogolcho
31	EtB-8	Bale	Gasera	Wute	
32	EtB-10	Bale	Goba	Misira	Ogolcho
33	EtA-3	Arsi	Hetosa	Hatehandode	Ogolcho
34	EtA-4	Arsi	Hetosa	Hatehandode	Kubsa
35	EtA-7	Arsi	Hetosa	Seruanketo	Ogolcho
36	EtA-8	Arsi	Lemunabilbilo	Kulumsa agricultural research center in sb-station	
37	EtA-11	Arsi	Hetosa	Hatehandode	Kubsa
38	EtA-19	Arsi	Tiyo	Dosha	Danda'a
39	EtSh-1	West Arsi	Assassa	Debara	Ogolcho
40	EtSh-2	West Arsi	Dodola	Bekola	Paven-76
41	EtSh-4	West Arsi	Dodola	Kechamachare	Ogolcho
42	EtSh-5	West Arsi	Assassa	Edobelo	Kubsa
43	EtSh-6	West Arsi	Assassa	Tuse	Kubsa
44	EtSh-7	West Arsi	Assassa	Kulumsa agricultural research center in sb-station	

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 *Parastgnospora nodurum* 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 *Parastgnospora nodurum* 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 it 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.

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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.