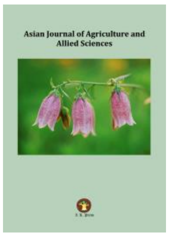

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Stem Rust (*Puccinia graminis* f.sp tritici) Management in Wheat through Fungicide Spray Frequencies in Central Ethiopia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Among various wheat diseases, stem rust caused by *Puccinia graminis* f. sp. *Tritici* is the most striking, contributing to large yield losses in Ethiopia. The effect of fungicide spray frequency on yield components of bread wheat was studied under natural infection conditions at Mareko and Dalocha during 2021 growing season. Two different fungicides, namely, Nativo SC 300 (trifloxystrobin 100 g/l + tebuconazole 200 g/l) at 0.75 l/ha and Tilt® 250EC (propiconazole) at 0.5 l/ha, were compared in field at three spraying frequencies. Experiment was in a randomized complete block design with three replications. Unsprayed controls were also included for comparison. The disease resulted in relative grain loss up to 53.5% at Dalocha and 41.9% at

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Mareko in control plots. However, terminal rust severity, average coefficient of infection and AUDPC values were significantly ($p<0.05$) reduced due to frequent fungicide application. Compared with those the untreated plots, thousand kernel weight and grain yield increased ($p \leq 0.05$) in fungicide-treated plots. Moreover, Nativo SC 300 provided the greatest increase in yield and was found effective in decreasing stem rust across both locations. Significant negative correlations were observed between TRS, ACI and AUDPC and between grain yield, kernel per spike and TKW, with correlation coefficients ranging from r=-0.85** to r=-0.33**. Cost-benefit analysis revealed, the combined use of Nativo SC 300 three times at ten-day intervals provides the greatest net benefit and is the best management strategy.

Keywords: AUDPC; fungicide; grain yield; severity; stem rust.

1. INTRODUCTION

“Wheat (*Triticum* spp.) is one of the most productive and important crop worldwide in the 21st century” (Curtis and Halford, 2014). “It is one of the key staples food and second most important cereal crop worldwide after rice” (Pant et al., 2020). “Bread wheat (*Triticum aestivum* L.) is major food security crop in Africa. Ethiopia is the first country in sub-Saharan Africa to produce large amounts of wheat” (FAOSTAT, 2022). “There are approximately 4.57 million farm households that are directly dependent on wheat farming as a major source of food and cash in Ethiopia” (CSA, 2022). “The crop ranks second in terms of total production next to maize (*Zea mays* L.)” (CSA, 2022). “Ethiopia's government is currently paying special attention to target crops in the strategic goal of achieving food self-sufficiency as well as export commodities. The crop covers an area of 1.89 million hectares, with a total production of 5.78 million tons” (CSA, 2022). However, the productivity of wheat remains low at 3.04 t/ha (CSA, 2022), which is significantly lower than the average yield productivity in Africa and globally. This is most likely due to a number of diseases attacking and reducing the quantity and quality of the produce. Among these diseases, wheat stem rust, also known as black rust, is caused by the fungus *Puccinia graminis* f. sp. *tritici* Ericks and Henn (*Pgt*) and is an economically important disease that occurs in most wheat-growing areas of Ethiopia.

“Wheat stem rust (black rust) causes enormous yield losses due to its frequent occurrence in many areas of the world. During epidemics, stem rust can cause up to 100% yield loss in susceptible wheat varieties” (Bechere et al., 2000). “Due to the evolution of existing races of stem rust or the movement of new aggressive strains across different agro ecosystems, this disease is challenging for most Ethiopian wheat varieties. Among the currently known stem rust races prevailing in Ethiopia, the TKTTF race is the most predominant and destructive race among several popular bread wheat varieties that significantly halts several resistance genes. The lack of an adequate genetic diversity background of cultivars and the presence of a single Sr-resistance gene deployed in most commercial cultivars contributed to failure resistance and led to great yield losses. Warmer temperatures and humid weather promote stem rust, which can result in significant yield losses” (Haldore et al., 1982, Roelfs et al., 1992). “The persistence of rust as a serious disease in wheat can be attributed to the specific characteristics of the rust fungi that make wheat stem rust difficult to treat. These characteristics include the capacity to produce large numbers of spores that can be disseminated through the wind over long distances and infect wheat under favourable environmental conditions and the ability to genetically modify the spores, thereby producing new races with increased aggressiveness in resistant wheat cultivars” (Stephen et al., 2022, Meyer et al., 2021).

“There are several management options available to manage wheat stem rust disease. Resistance breeding is the most important technique for wheat rust prevention. Stem rust epidemics are effectively controlled through the deployment of effective stem rust resistance genes in wheat varieties” (Singh et al., 2006, Jin et al., 2006). In the last few decades, several rust-resistant wheat varieties have been developed and released in Ethiopia. However, due to the evolution of new races, these resistant varieties, such as Ogolcho and Hidase, which were released in 2012 and 2012, respectively, have become susceptible to stem rust after years of adaptation, resulting in severe damage. “In many stem rust hotspot areas of Ethiopia, it is no longer possible to produce wheat without foliar fungicide spray. The development of new pathotypes of stem rust fungus reduces the resistance of released bread wheat varieties, making fungicide intervention necessary. The timely application of fungicides effectively prevents rust and will be profitable for farmers. Large-scale commercial wheat growers in Ethiopia extensively use fungicides to control rust" (Ayele et al., 2008, Badebo, 2002, Hailu and Fininsa, 2009). The Siltie and Gurage zones of central Ethiopia have agro-ecological potential for growing wheat. Farmers in these areas produce different bread wheat varieties; Ogoloch, in particular, is the most commonly preferred cultivated variety. In the presence of wheat varieties with high yield potential in these areas, stem rust disease threatens wheat production. Therefore, the present study aimed to evaluate the response of wheat stem rust management to different fungicide spray frequencies.

2. MATERIALS AND METHODS

2.1 Description of the Experimental Area

The present research was conducted in the experimental areas of the Mareko and Dalocha districts during 2021 season. Mareko is found in the Gurage zone, whereas Dalocha is found in the Silte zone, and both are situated under midland agro-ecologies. Geographically, Mareko is located at 08°05'67''N, 38°18'18''E and 1824 m.a.s.l. The mean annual rainfall in the area is 675 mm, while the mean average air temperature is 27.5 °C. The soil type is dominated by clay soil (Cromic Luvisole and Haplic phaeozems). Dalocha is located at 07°83'62''N, 38°22'12''E and 1925 m.a.s.l. It receives a mean annual rainfall of 750 mm with a mean annual average air temperature of 22.7°C. The soil type is dominated by clay loam soil (cromic vertisole). Both locations are hot spots for stem rust disease. The major rainfall characteristics of the areas include a short rainy season from March to May and a main rainy season from June to September.

2.2 Experimental Design and Treatments

The field experiment used was a randomized complete block design (RCBD) with three replications. The fungicide treatments used were Nativo SC 300 and Tilt® 250EC. There were seven treatment combinations: three fungicide spray frequency combinations from each fungicide type and one unsprayed control. Fungicides were sprayed on runoff at the initial stem rust symptom using a manual Knapsack sprayer in 250 lha^{-1} water when disease severity reached 20% in the tested variety (How did you measure 20%). A total of three consecutive sprays were used at both locations. Plastic sheets were used to protect adjacent plots from effects of fungicidal drift. Artificial inoculation was not carried out due to the high stem rust pressure, and highly hot-spotted experimental sites were used naturally. The tested bread wheat variety namely, Hidasse was sown in experimental plots of 1.2 × 2.5m or ($3m^2$) each containing 6 rows spaced 0.2 m apart. The spaces between plots and blocks were 0.5 and 1 m, respectively. The wheat variety was planted at the recommended seed rate of 125 $kgha^{-1}$. The plots were sown manually in rows at appropriate times based on the crop calendar of the areas. The recommended rate of NPS fertilizer (100 $kgha^{-1}$) at planting and urea (41 kgN/46 kg P_2O_5 ha^{-1}) with split application were applied as sources of fertilizer. Hand weeding was performed three times to ensure that the plots were weed free.

2.3 Disease Assessment

Stem rust disease data were obtained by estimating the approximate percentage of leaf area affected at 10-day intervals using the modified Cobb scale (Peterson et al., 1948), which is a combination of disease severity and severity. The severity of stem rust was scored prior to each spray of fungicide. A total of four consecutive disease assessments were made, and each observation was converted to a coefficient of infection (CI) by the methods outlined by (Roelfs, 1985) in which the values of severity were multiplied by the constant number of host responses given, i.e., immunity = 0, R= 0.2, MR = 0.4, MS = 0.8 and S = 1. Disease severity and the average coefficient of infection were calculated using the formula described below.

2.4 Disease Severity

$$\text{Disease severity} = \left(\frac{\text{Area of plant tissue affected}}{\text{Total area of plant tissue examined}}\right) * 100 \quad 1$$

$$\text{ACI} = \left(\frac{\text{Disease Severity} * \text{constant host responcs value}}{\text{Total number of observation recorded}}\right. \quad 2$$

2.5 Analysis of Disease Progress

Area under disease progress curve (AUDPC): calculated using the CI values from the original rust severity data by using the following formula as suggested by (Arama et al., 2000).

$$AUDPC = \sum_{i=1}^{n-1} \left(\left(\frac{x_i + x_i + 1}{2} \right) (t_i + 1 - t_i) \right) \quad 3$$

where xi = the average coefficient of infection of the ith record, X_{i+1} = the average coefficient of infection of the i+1th record, t_{i+1} - t_i = the number of days between the ith record and the i+1th record, and n = the number of observations.

2.6 Yield Parameter Assessment

Agronomic data were recorded on four central rows in an experimental unit. The details of the agronomic parameters are:

Spike length (SL) (cm) average length of 5 spikes, randomly selected in four central rows of each plot.

Kernels per spike (KPS) measured from main tillers of five randomly selected plants and average of five plants was used for data analysis.

Thousand kernel weight (TKW) (g) counting grains by adjusting the moisture content to 12.5%.

Grain yield (GY) was measured from the four central rows with moisture adjusting to 12.5% and converting it to kg/ha^{-1}.

2.7 Cost Benefit Analysis and Yield Loss Estimations

Cost-benefit analysis is a prerequisite for determining the economic feasibility of fungicide application in relation to grain yield loss. The net return from wheat production was calculated by considering the type of fungicide used and the application cost to control stem rust. Fungicide and application costs were used to calculate the net returns from applying fungicides to control wheat stem rust. The average wheat price in the local market was used for the CB analysis. The average prices for fungicides ($ha^{-1}) were obtained from local chemical distributing agencies and from three chemical companies. Detailed descriptions of the price of wheat, the fungicides used and the cost of application are presented in Table 1. Information on the cost of fungicide application was obtained from four large-scale commercial farm chemical applicators in an area. The net return from fungicide application was calculated as follows:

$$Rn = YiP - (Fc + Ac) \quad (1)$$

where Rn is the net return from fungicide application ($ ha^{-1}); Yi is the yield increase from fungicide application (kg ha^{-1}), calculated by subtracting the yield in the control treatment from the yield in the fungicide treatments; P represents the wheat price ($Kg^{-1}); Fc represents the fungicide cost ($ ha^{-1}); and Ac represents the fungicide application cost ($ ha^{-1}).

The relative losses in yield and yield component of each variety were determined as a percentage of that of the protected plots of the respective variety.

$$RL(\%) = \left(\frac{Y1 - Y2}{Y1} \right) * 100$$

where RL = relative loss (reduction in the parameters of grain yield and TKW), Y1 = the mean of the respective parameters in protected plots (plots with maximum protection) and Y2 = the mean of the respective parameters in unprotected plots (i.e., unsprayed plots or sprayed plots with varying levels of disease).

2.8 Statistical Analysis

Due to the heterogeneity of the data across locations, the analyses were performed separately using the general linear model procedure of the SAS software package version 9.3. Comparison of treatment means was performed using the least significant difference test (LSD) at the 5% probability level. The relationships between epidemiological parameters and yield and yield components were correlated using Proc-Corr Pearson's correlation. Linear regression of the pooled AUDPC data for stem rust was used to predict grain yield losses.

Table 1. The fungicide costs, fungicide application costs and wheat prices used for cost-benefits

Fungicides	Fungicides cost FC ($ha^{-1})	Fungicide application cost (AC) Cost (ha^{-1})	FC + AC ($ha^{-1})	Wheat price (ha^{-1})
Nativo SC 300	40	11.2	55.1	77.4
Tilt 250 EC	33.7	11.2	51.2	77.4

Source: Survey data from Makamba plc, General Chemical & Trading Pvt. Co., and Makubu Plc

3. RESULTS AND DISCUSSION

3.1 Disease severity (%)

Substantial stem rust pressure occurred at both testing sites. The present study revealed that terminal rust severity (TRS) was significantly affected (P<0.05) by individual treatments and their combinations at both locations (Tables 2 and 3). At Mareko, the highest TRS of 93.3% was recorded in the untreated plot, whereas the lowest TRS was recorded in the plot sprayed three times and treated with Nativo 300 SC (Table 2). On the other hand, at Mareko, the highest TRS of 78% was recorded in the unsprayed plots, while the lowest TRS was recorded in the plot sprayed three times with the Nativo 300 SC fungicide (Table 3). The first symptoms of stem rust appeared 38 DAP at Mareko and 51 DAP at Dalocha. Stem rust decreased after the first and second spraying frequencies. However, an obvious reduction occurred during the third frequency**.** These factors resulted in a variation in resistance level among plants due to fungicidal activity. “Significant variability was not existed across the two locations interims of disease pressure. This might be because of the agro ecological similarity between the two locations for the epidemic of the disease. However, the longer epidemic duration at Mareko resulted in the highest mean terminal rust severity compared to that at Dalocha. The growth and development of wheat are adversely affected by stem rust due to the warm and moist environmental conditions that prevail. The maximum severity in both environments could be due to the inherent susceptibility of the host to particular pathogens. The expression of stem rust occurred much earlier in relation to the host growth stage at both locations, which influences its epidemiology. Similarly, the date of disease onset is directly related to the development of an epidemic” (Roelfs, 1985). “The disease was severe on all leaves of the plant, including the flag leaves. This was indicated by necrotic lesions on the leaves and stems of the plant. There was also a reduction and destruction of green leaf tissues and loss of photosynthetic area on infected leaves, withering of leaves and severe defoliation in unsprayed plots. This study was in line with the findings” (Hepperly, 1990) who reported that early disruption of the plant’s photosynthetic capacity and competition with reproductive structures by the pathogen leave less assimilates available for grain fill than in situations where rust infection occurs later. Inversely supplemented fungicides reduced disease infestation to the lowest level through the responsible active ingredients in the formulation. Therefore, stem rust infections are most effectively controlled by the application of fungicides close to or just after head emergence.

3.2 Average Coefficient of Infection (%)

A considerable gap existed among the treatment combinations in terms of their average coefficient infections at both locations (Tables 2 and 3). At Mareko, the highest ACI of 86.3% was recorded in unsprayed plots; conversely, the lowest ACI of 17.3% was recorded in plots sprayed three times with the fungicide Na tivo SC 300 (Table 2). At Dalocha, the highest ACI of 80% was recorded in the unsprayed plot. On the other hand, the lowest ACI of 6.3 was recorded for the plot sprayed three times with the Nativo SC 300 fungicide (Table 3). The pattern of stem rust pressure starting from initial symptoms to terminal severities showed the suppression of diseases due to fungicidal treatment. “The maximum stem rust severity at both locations showed sufficient rust pressure across locations with a maximum ACI value. The widespread occurrence of stem rust throughout the season could be due to climatic conditions, the amount of pathogen source, and the duration of infection. Climatic variations between the two locations influence the interaction between stem rust and wheat. The disease pressure in each environment influences the performance of wheat. Both fungicides reduced the extent of stem rust disease; however, Nativo SC 300 offered better rust pressure control compared to Tilt 250 EC. This is because of differences in their active ingredients and their effectiveness in combating stem rust. However, a single application of Nativo SC 300 was relatively more effective than two applications of Tilt 250 EC in reducing grain yield loss. This uneven effectiveness might be due to the continuous use of Tilt 250 EC over several years, which has contributed to the development of resistance in certain pathogens. Likewise, continued and unwise use of fungicides has led to the development of resistance by the pathogen” (Green et al., 1990, Haldore et al., 1982). The difference in the effectiveness of fungicides can also be due to different active ingredients in the product formulation and the pathogens themselves being resistant to the active substances.

3.3 Area under Disease Progress Curve (% days)

The rate of stem rust disease progress significantly differed (p<0.05) among the treatment combinations at Mareko and Dalocha (Tables 2 and 3). At Mareko, the highest AUDPC value of 1645 % days was recorded for the unsprayed plot, whereas the lowest AUDPC of 478.3% days was recorded for the plot sprayed three times with Nativo SC 300 (Table 2). At Dalocha, the highest AUDPC of 1245.2% was recorded in the unsprayed plot. On the other hand, the lowest AUDPC of 245.1% days was recorded for the plot sprayed three times with the Nativo SC 300 fungicide (Table 3). The area under the disease progress curve is a true disease parameter because it is directly related to yield loss. The AUDPC for Marko was slightly greater in the unsprayed plots (Fig. 1). The disease progression rate of the pathogen is primarily influenced by environmental variability, which enhances vast epidemic development across locations. On the other hand, variability of the pathogen might also be another prominent factor affecting the rate of stem rust development. Both fungicides significantly reduced the AUDPC to the lowest level, and the untreated plot had a greater AUDPC. The results of this study are in line with the findings of (Meyer, 2021). who reported a greater AUDPC in untreated plots than in treated plots. Similarly, (Pant, 2020, Peterson et al., 1948). suggested that “appropriate fungicide application during the onset of disease decreases subsequent disease progression on plants and is effective at achieving the maximum grain yield”. Fig. (1) shows the regression analysis of the epidemiological parameter AUDPC and grain yield during the 2020 growing season.

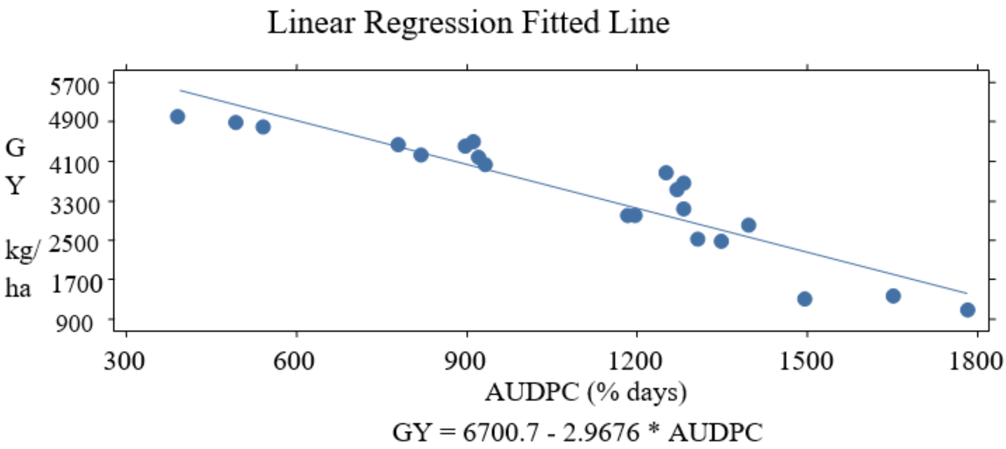

Fig. 1. Linear regression between grain yield and rAUDPC at Mareko

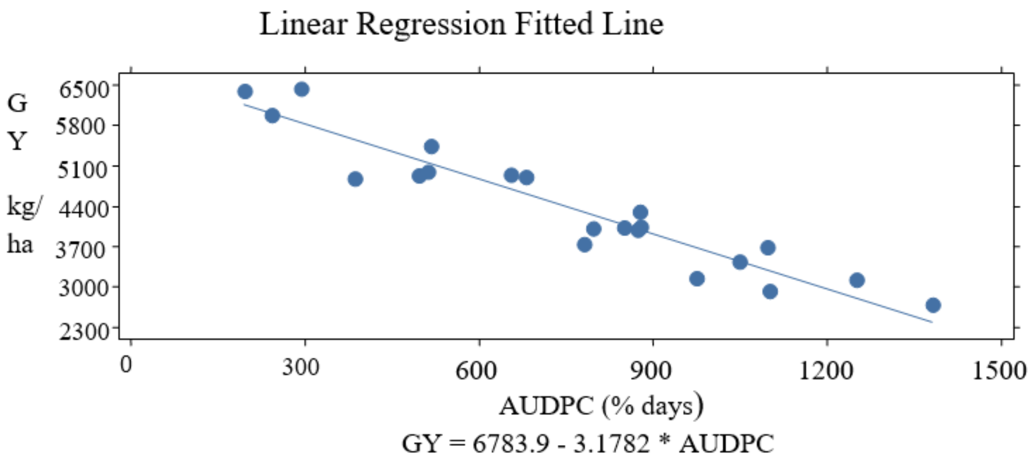

Fig. 2. Linear regression between grain yield and rAUDPC at Dalocha

The regression curve predicted grain yield loss in wheat production due to disease (Figs. 1 and 2). There was a negative linear relationship between wheat yield and AUDPC at both sites. Accordingly, as the AUDPC increased, the grain yield decreased. The points around the line (Figs. 1 and 2) indicate a stronger association between stem rust and grain yield loss. A higher AUDPC indicates less effectiveness of fungicide and a lower grain yield, and a lower AUDPC indicates greater effectiveness of the fungicide, resulting in higher grain yields.

3.4 Spike Length (cm)

Statistical analysis revealed a significant ($P < 0.05$) effect of the treatment combination on spike length at both testing locations (Tables 1 and 2). At Mareko, the greatest spike length of 8.46 cm was obtained from the plot treated three times with Nativo SC 300, followed by the plot sprayed two times with the same fungicide (7.76). On the other hand, the shortest spike lengths of 6.1 for Mareko and 6.02 for Dalocha were obtained from the untreated plots (Tables 1 and 2). Spike length is considered an important yield-contributing trait. “The reductions in spike length due to stem rust contributed to the lowest grain yield. However, significant increases in spike length were obtained due to the application of fungicide. The data presented in Tables 1 and 2 also show that the spike length of the protected plots was greater than that of the infected/unprotected plots during the study season. The application of fungicides suppresses disease development and protects the crop canopy, which is vital for dry matter accumulation and yield” (Viljanen-Rollinson and Marroni, 2006).

3.5 Kernel Per Spike

The current study revealed that fungicide application frequency and combination had a significant (P 0.05) effect on the number of kernels per spike at both locations (Tables 2 and 3). The highest numbers of kernels per spike, 63.3 in Mareko and 60.7 in Dalocha, were obtained from three times treated plot with Nativo SC 300 at both locations, while the lowest numbers of kernels per spike, 39.5 in Mareko and 29.6 in Dalocha, were obtained from the untreated plot. The number of kernels per spike is one of the most important yield parameters (Shah et al., 2007, Fonseca and Patterson, 1968). “Compared with that in Dalocha, spike infection in Mareko occurred relatively early. Stem rust attacks the glumes and awns of wheat, leading to the accumulation of spores on the florets and on the surface of the developing grain. These situations contributed to the minimum number of kernels of the variety tested. A reduction in grain number and size reduces the dry matter content of wheat grains by affecting the sugar supply to developing seeds, which directly results in a large yield loss” (Subba-Rao et al., 1989, Marsalis and Goldberg, 2006, Khushboo et al., 2001). However, healthy and robust grains resulting from the application of foliar fungicides contribute to overall grain yield. Fungicide applications protect the flag leaf from infection until after the kernels have filled. Three times spraying of fungicide increased the number of kernels per spike by reducing the disease pressure to the lowest level.

3.6 Thousand Kernel Weight (g)

The performance of the bread wheat variety in terms of the thousand-kernel weight (TKW) significantly differed ($P < 0.05$) due to the effects of fungicide treatments at the two locations. At Mareko, the maximum TKW of 37.8 g was obtained from the plot treated three times with Nativo SC 300 fungicide, followed by the plot sprayed twice with the same fungicide (35.6 g). At the other location (Dalocha), the maximum TKW of 36.7 g was obtained from the plot treated with Nativo SC 300 with three spray frequencies followed by two spray frequencies with the same fungicide (Table 2). The disease resulted in relative TKW losses of up to 29.4% at Dalocha and 30.1% at Mareko on unsprayed plots. The variability in thousand kernel weight was more prominent among the treatment combinations at both locations (Tables 1 and 2). This negatively reduced the kernel quantity and quality of the produce. This trend was consistent with the findings of (Everts et al., 2001). who suggested that the shrivelling of wheat kernels reduces flour yield. Nutrients mainly produced in flag leaves are used by the fungus instead of being transported to the grain, resulting in Shrivelling kernels (Seck et al., 1988, Subba-Rao et al., 1989, Hasan et al., 2012). However, fungicide application significantly increased the amount of TKW. Similarly, (Tadesse *et al.,* 2010) reported a significant increase in TKW as a result of Tilt treatments.

Table 2. Effect of fungicide application frequency on disease incidence and yield components in Mareko during the 2021 cropping season

Treatments		**TS**	**ACI**	**AUDPC**	**SL**	**KPS**	**TKW**		**GY**	
Chemical	**Frequency**	**(%)**	**(%)**	**(% days)**	**(cm)**		**(g)**	**Loss (%)**	**(kg/ha)**	**Loss (%)**
Tilt	1x	65.0^{b}	60.3^{b}	1353.3^{b}	6.46^{e}	44.0^{de}	28.9^{e}	23.4	3454.7^{e}	27.6
	2x	53.3^{c}	46.6^{cd}	1238.3^{c}	7.23^{bc}	51.4^{b-d}	31.3^{d}	17.2	3805.0^{d}	20.2
	3x	43.3^{d}	37.3^{d}	863.3^{d}	7.06^{cd}	53.8^{bc}	33.7^{c}	10.8	4446.1^{b}	6.76
Nativo	1x	60.0^{bc}	52.3^{bc}	1251.7^{c}	6.60^{de}	46.5^{c-e}	32.2^{d}	14.8	3549.4^{e}	25.6
	2x	40.0^{d}	23.3^{e}	891.7^{d}	8.46^{a}	59.1^{ab}	35.6^{b}	5.82	4123.2^{c}	13.5
	3x	35.0^{d}	17.3^{e}	478.3^{e}	7.76^{b}	63.6^{a}	37.8^{a}	0.00	4768.9^{a}	0.00
Control		93.3^{a}	86.3^{a}	1645^{a}	6.10^{e}	39.5^{e}	26.4^{f}	30.1	2767.4^{f}	41.9
CV%		9.8	13.1	15.3	4.4	8.08	1.2		17.5	
$LSD_{0.05}$		11.2	15.6	63.8	0.55	8.8	3.21		334.5	

Note: Means in the same column followed by the same letters are not significantly different.

Abbreviations: LSD $_{0.05,}$ List significant difference at 5%; CV (%), Coefficient of variation at (%); TS, terminal rust severity; ACI, average coefficient of infection; AUDPC, area under disease progress curve; KPS, kernel per spike; TKW, thousand kernel weight; GY, grain yield

Table 3. Effect of fungicide application frequency on disease incidence and yield components at Dalocha in the 2021 cropping season

Treatments		**TS**	**ACI**	**AUDPC**	**SL**	**KPS**	**TKW**		**GY**	
Chemical	**Frequency**	**(%)**	**(%)**	**(% days)**	**(cm)**		**(g)**	**Loss (%)**	**(kg/ha)**	**Loss (%)**
Tilt	1x	61.6^{bbb}	61.3^{b}	1041.8^{bb}	6.51^{cd}	53.3a^{ab}	29.9^{bc}	18.5	3398.3^{d}	45.8
	2x	46.6^{c}	46.6^{c}	838.3^{c}	6.72^{bc}	46.1^{bc}	28.3^{c}	22.8	3989.2^{c}	36.3
	3x	40.0^{cd}	34.8^{d}	467.2^{c}	6.98^{b}	53.0^{ab}	34.6^{ab}	5.71	4908.8^{b}	21.7
Nativo	1x	56.1^{b}	56.6^{bc}	851.7^{c}	6.69^{bc}	49.7^{ab}	29.5^{bc}	19.6	3993.3^{c}	36.3
	2x	31.6^{d}	22.0^{e}	618.6^{d}	7.31a	53.0^{ab}	36.4^{a}	0.86	5092.2^{b}	18.8
	3x	13.3^{e}	6.3^{f}	245.1^{f}	7.5^{a}	60.7^{a}	36.7^{a}	0.00	6270.6^{a}	0.00
Control		78.3^{a}	80.0^{a}	1245.2^{a}	6.02^{e}	29.6^{c}	25.9^{c}	29.4	2915.5^{e}	53.5
CV%		10.1	13.3	11.9	5.71	10.9	9.95		8.9	
$LSD_{0.05}$		8.42	10.4	147.1	0.49	9.09	5.59		446.7	

Note: Means in the same column followed by the same letters are not significantly different

Abbreviations: LSD $_{0.05}$, List Significant Difference at 5%, CV (%), Coefficient of Variation at (%); TS, Terminal Rust Severity; ACI, Average Coefficient Infections; AUDPC, Area Under Disease Progress Curve; KPS, Kernel Per Spike; TKW, Thousand Kernel Weight; GY, Grain Yield

3.7 Grain Yield (kg)

Analysis of variance clearly revealed a significant ($P < 0.05$) effect of fungicide application frequency on grain yield at both experimental sites (Tables 1 and 2). At Mareko, the maximum grain yield of 4768.9 kg/ha^{-1} was obtained from three times treated with the Nativo SC 300 fungicide, followed by the three time sprayed with the Tilt 250 EC fungicide (4461.6 kg/ha^{-1}). At the other location (Dalocha), the maximum grain yield of 6270.6 kg/ha^{-1} was obtained from the Nativo SC 300 treated plot at three time spraying frequencies, followed by the 5092.2 kg/ha^{-1} treated by the same fungicide with two spraying frequencies. The lowest grain yields of 2767.4 kg/ha^{-1} and 2915.5 kg/ha^{-1} were obtained from the untreated plots at Mareko and Dalocha, respectively. Grain yield differences and losses were greatest at both sites due to severe disease pressure. Similarly, (Ali et al., 2007) suggested that "variability in yield depends on the extent of disease pressure. An inverse relationship was found between disease severity and grain yield, implying that stem rust directly affects kernel quality, causing wheat grains to shrink. Both tested fungicides outperformed the untreated control in terms of grain yield". The use of Tilt 250 EC reduced disease severity while increasing grain yield. Similarly, (Asmmawy et al., 2013) reported that Tilt-protected plots remained almost free of stem rust. However, the fungicides differed in their effectiveness, with Native SC 300 providing better control of stem rust than Tilt 250 EC.

3.8 Correlation Analysis between Disease and Agronomic Variables

Correlations between stem rust and other traits were computed. Pearson correlation coefficient analysis revealed a significant positive correlation between grain yield and kernel per spike (r=0.61**) and between grain yield and thousand kernel weight (r=0.86**) (Tables 4 and 5). These values are true indicators of the contributions of yield components to grain yield. On the other hand, grain yield, TKW and kernel per spike were significantly negatively correlated with terminal severity (r=-0.87**, r=-0.78**, and r=-0.39*, respectively) (Tables 4 and 5). The

Table 4. Correlation between disease parameters and yield and yield components at Mareko

	TRS	ACI	AUDPC	SL	KPS	TKW	GY
TRS							
ACI	0.85**						
AUDPC	0.84**	0.91**					
SL	-0.31*	-0.28*	-0.22^{ns}				
KPS	-0.31*	-0.10ns	-0.07^{ns}	0.26*			
TKW	-0.78**	-0.69**	-0.57**	0.42*	0.45**		
GY	-0.87**	-0.83**	-0.77**	0.49**	0.59**	0.86**	

*Note: **, refers to mean values significant at @=0.01; *, refers to mean square values significant at @=0.05; ns, refers to mean square values not significant at @ = 0.05.*
Abbreviations: TRS, Terminal Rust Severity; AUDPC, Area Under Disease curve; ACI, Average Coefficient of Infection; SL, Spike Length; KPS, Kernel Per Spike; PH, Plant Height; TKW, Thousand Kernel Weight; GY, Grain Yield

Table 5. Correlations between disease parameters and yield and yield components at Dalocha

	TRS	ACI	AUDPC	SL	KPS	TKW	GY
TRS							
ACI	0.81**						
AUDPC	0.87**	0.89**					
SL	-0.29*	-0.24*	-0.18^{ns}				
KPS	-0.39*	-0.16^{ns}	-0.06^{ns}	0.28*			
TKW	-0.75**	-0.63**	-0.54**	0.33*	0.38*		
GY	-0.82**	-0.71**	-0.64**	0.40*	0.61**	0.79**	

*Note: **, refers to mean values significant at @=0.01; *, refers to mean square values significant at @=0.05; ns, refers to mean square values not significant at @ = 0.05.*
Abbreviations: TRS, terminal rust severity; AUDPC, area under disease curve; ACI, average coefficient of infection; SL, spike length; KPS, kernel per spike; PH, plant height; TKW, thousand kernel weight; GY, grain yield.

Table 6. Effect of fungicides on the net return of bread wheat varieties

Treatment		Mareko					Dalocha			
Chemical	**Frequency**	**FC+AC**	**GY**	**GYI**	**GYIP**	**Rn**	**GY**	**GYI**	**GYIP**	**Rn**
Tilt	1x	51.2	32.5	10.9	843.6	792.5	33.9	4.7	363.8	312.6
	2x	102.4	38.1	16.5	1278.7	1176.3	39.8	10.6	820.4	718
	3x	153.6	45.4	23.8	1842.1	1689.2	49.1	20.1	1555.7	1402.1
Nativo	1x	55.1	35.4	13.8	1068.1	1013.1	39.9	10.7	828.2	765.1
	2x	111	43.2	22	1709.4	1591.8	50.9	21.7	1679.5	1568.5
	3x	166.5	49.7	28.1	2174.9	2008.4	62.7	33.5	2592.9	2426.4
Control		0.00	21.6	0.00	0.00	0.00	29.2	0.00	0.00	0.00

Abbreviations: FC, Fungicide cost ($\$ha^{-1}$); AC, Fungicide application cost; GYI, Grain yield increment from fungicide application ($qtha^{-1}$); P, Wheat price ($\$qt^{-1}$); Rn, Net return from fungicide application ($\$ha^{-1}$)

correlation was relatively strong, indicating the impact of stem rust on the TKW and grain yield reduction. The strong negative correlations of ACI and AUDPC with that of the grain yield (r=-0.97**, r=-0.93**) was the impact of the disease. Similarly, (Ochoa and Parlevliet, 2007) reported the strong negative correlation of grain yield with the area under the disease curve.

3.9 Cost Benefit and Relative Yield Loss Analysis

In the present study, the increase in grain yield was strongly associated with fungicide treatment. At Mareko, the net return from fungicide application varied from 792.5$\$ha^{-1}$ in the one-time Tilt 250 EC-treated plot to 2008.4$\$ha^{-1}$ in the three-time spraying plot with Nativo SC 300 (Table 6). However, at Dalocha, the net return after fungicide application ranged from 312.6 $\$ha^{-1}$ after the plants were sprayed with Tilt 250 EC to 2426.43 $\$ha^{-1}$ after the plants were sprayed three times with Nativo SC 300 (Table 6). The cost-benefit analysis revealed that using Nativo SC 300 with three spray frequencies effectively controlled the disease and yielded a greater economic return. Enormous yield losses due to stem rust were found at both locations. The grain yield losses across locations ranged from 6.79% to 53.5% between the treated and untreated plots, respectively. Varietal susceptibility, time of infection, rate of disease development, crop growth stage and environmental conditions are the major factors affecting grain yield loss (Pretorius, 2004, Chen, 2005). The grain yield and thousand kernel weight losses was the negative effect the disease. Similarly, (El Shamy, 2011) reported "a significant negative correlation between the mean disease severity of leaf rust and percentage loss in thousand kernels and the grain yield of bread wheat. However, relative grain yield losses due to stem rust were reduced in the plots sprayed with both fungicides, and greater grain yield increments were achieved in the treated plots than in the unsprayed controls". "The stem rust was not fully controlled with a single application of fungicides. To reduce disease reinfection, it is advisable to increase the frequency of fungicide application. However, such frequency did not consistently result in an increase in grain yield. Therefore, the application frequency should be based on cost-benefit analysis and the time of onset and early detection of the disease for efficient utilization of fungicides for economic control of the disease" (Roelfs, 1985b, Beard et al., 2004).

4. CONCLUSION AND RECOMMENDATION

Significant reductions in kernel per spike, thousand kernel weight and grain yield were observed due to disease. Stem rust severity exhibited maximum values of the area under the disease progress curve and average coefficient of infection, and these results were in parallel with the observed grain yield losses. The performance of wheat was significantly affected by both environmental and pathogenic variability, resulting in significant differences in disease resistance and susceptibility among treatment combinations. However, this study revealed that the use of foliar fungicides is an important strategy for improving grain yield in the country. The present study suggested that three applications of Nativo SC 300 at the onset of disease symptoms is a cost-effective management strategy for reducing grain yield loss caused by stem rust. With the expectation of a return on investment under high-yield conditions, fungicides should be applied prophylactically and repeatedly to wheat growers. Therefore, the results of the present study suggested that the appropriate use of fungicide frequency for the efficient management of stem rust should be encouraged under natural conditions. However, additional studies in different agro-ecological zones are important for providing more comprehensive recommendations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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