Journal of Science and Development

Volume 13 No. 2, 2025

HAWASSA UNIVERSITY

Journal of Science and Development

Volume 13, No. 2 2025

ISSN (Online): 2789-2123; (Print): 2222-5722

Contents

Journal of Science and Development, JSD

Original Research Article||

Climatic Trend analysis based on rainfall and temperature in Southern Ethiopia

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Abstract

Reports show that the average global temperature has risen by roughly 0.6°C in the last century and projected to increase by 1.8°C to 4°C by 2100. This global trend is also influencing regional temperature changes in Africa and elsewhere. This study was conducted to provide up-to-date information of climatic trends for the better management of climate change impacts in the southern Ethiopia. The analysis is based on the annual rainfall, maximum, minimum and mean temperature differences across lowland, midland and highland agro-ecologies. The overall objective of the study was to investigate the trend of annual rainfall, maximum, minimum and mean temperature and to see difference between agro-ecologies. The Mann-Kendall trend test and Sen's slope estimate were employed to find the nature of the climate change trend and significance level. The data was analyzed using Microsoft Exel and Mann-Kendall Excel software. The results showed a decreasing trend of total annual rainfall by -2.56 mm, -4.66 mm, and -3.29 mm per year in the midland, lowland, and highland agro-ecologies respectively. The mean temperature has been increased by 0.014°C, 0.032°C, and 0.066°C per year for the midland, lowland and highland agro-ecologies, respectively. The maximum and minimum temperatures also have shown an increasing trend over the observed period. The negative Sen's slope values for the annual rainfall in the three agro-ecologies indicate the declining trends, and the positive Sen's slope values for the maximum, minimum and mean temperature in each of the three agro-ecologies indicate a rising trend over time. Therefore, it is recommended that the climate variability in southern Ethiopia needs further monitoring technique, and there is a need to consider the climate change trend to minimize its effects on agricultural production and overall livelihood of smallholders in the study area.

Keywords: Climate change, climate variability, Mann-Kendall trend test, Sen's slope estimate, Trend analysis

Original submission: February 23, 2025; **Revised submission**: May 08, 2025; **Published online**: October 15, 2025
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INTRODUCTION

Changes in the mean state of temperature and rainfall at global, regional, national and local levels are the key variables as a proxy for the prevalence of climate change. The effects of climate change, such as rising sea levels, melting polar ice caps, wild bushfires, severe droughts, etc., are felt all over the world and have made it one of the most important issues in the field of sustainable development (Ali et al., 2013; Dioha and Kumar 2020). Over the last century, the average global temperature has risen by roughly 0.6°C (IPCC, 2014). The Intergovernmental Panel on Climate Change's Fourth Assessment Report projects that by 2100, the average global surface temperature will increase by 1.8°C to 4°C. Africa has been identified as one of the continents that is most susceptible to the effects of climate change, with the most sensitive economic sector, agriculture (Serdeczny et al., 2017).

Climate change primarily affects developing countries, with Ethiopia being one of the most vulnerable (Cherie and Fentaw 2015). Ethiopia has historically struggled with climate variability, which has an impact on food security, economic growth, and agricultural production (Conway and Schipper, 2011; Tigchelaar et al., 2018). Climate change poses a threat to Ethiopia's agriculture sector which is heavily reliant on rainfall resulting in very poor productivity (Deressa et al., 2011). This is due to the fact that temperature and precipitation are impacted by climate change as expressed by extreme events such as drought and flooding (Muleta et al., 2019). Smallholder farmers in Ethiopia are challenged by increased rainfall variability, rising temperatures, and a high frequency of extreme events as well as the increasing occurrences of pests and diseases (Tesfaye et al., 2016). Indeed, these climatic variations could decrease agricultural production and productivity causing an impact on smallholders' standard of living (Yalew et al., 2017). Consequently, smallholder farmers' status in terms of food security has suffered to a greater extent (Alemu and Mengistu, 2019).

Due to their limited adaptive capacity to climate change impacts and reliance on steady agricultural output from year to year, subsistence farmers are especially susceptible to climate unpredictability (Schlenker and Lobell, 2010; Mekasha et al., 2014; Asfaw et al., 2018). Ethiopia's rainfall and temperature variability are predicted to worsen due to climate change, which could expose farmers to more climate-related risks (Ayanlade et al., 2018; Samy et al., 2019) and the resulting food insecurity (Simane et al., 2016). Ethiopia's rainfall is extremely variable, exhibiting a broad range of patterns with no discernible trend toward change (Conway and Schipper, 2011). Unprecedented extreme occurrences may result from changes in the frequency, severity, spatial expanse, and time scale of extreme climate conditions brought on by climate change (Wagaye and Endalew, 2020).

Ethiopia, like any other low-income and vulnerable country, relies mostly on rain-fed agriculture, which is particularly vulnerable to and has been most negatively impacted by climate change and variability (Moges and Bhat, 2021). Temperature and rainfall trend analysis at the national level is prevalent, but there is very limited study on the local level dealing with this issue. Therefore, the objective of this study was to examine the state of temperature and rainfall trends and their significance level in southern Ethiopia.

MATERIALS AND METHODS

Description of the Study Area

This study was conducted in Dilla-zuria and Bule districts of the Gedeo zone and Abaya district West Guji Zone in the southern part of Ethiopia. Dilla-zuria district is located between 6º15′05″ N-6º26′35″N latitude and 38º 15′ 55″ - 38º 24′ 02″ E longitudes. The altitudinal range of Dilla-Zuria district ranges from 1350 m to 2550 m a.s.l. The mean monthly rainfall of the district ranges from 83.7-310 mm with an average of 172.9 mm. The rainfall is bimodal occurring between March up to June and September to October with the highest amount of rainfall occurring between May and September and the lower between October and February. The mean monthly temperature ranges from 15.4°C to 17.9°C. Based on traditional agro-ecological zones classification, the district is mainly categorized under mid-land agro-ecology which lies between 1500–2300 m.a.s.l. (Gorfu & Ahmed, 2012). Communities rely on agroforestry elements including trees, coffee, enset, fruits and annual and perennial crops.

Bule district is located between 60 04' 16"-60 23' 50" North latitude and 38^0 16' 20"-38^0 26' 11" East longitudes. The mean annual rainfall of Bule district ranges from 1,200 mm - 1,800mm and the mean annual temperature is between 15.1°C and 22.5°C. The district has two rainy seasons namely, the short rainy season (from March to May) and the long rainy season (from July to December). Bule district is categorized under highland agro-ecology. Rain-fed annual crop cultivation, including barley, wheat, maize, and pulse crops like beans and peas, dominates the district's primary land use types overall.

Abaya district is located between latitudes 6°10′ N and 6°20′ N and longitudes 38°00′ E and 38°10′ E. The altitude of the district ranges from 1200-2060 m.a.s.l. It has an estimated average annual rainfall of about 1223 mm and the average annual temperature ranges from 16°C–28°C. Maize, groundnuts, barley, "teff," sorghum, haricot beans, wheat, field peas, and faba beans were the main crops grown. The midland and lowland agro-ecologies share 70% and 30% of the total area of the district respectively.

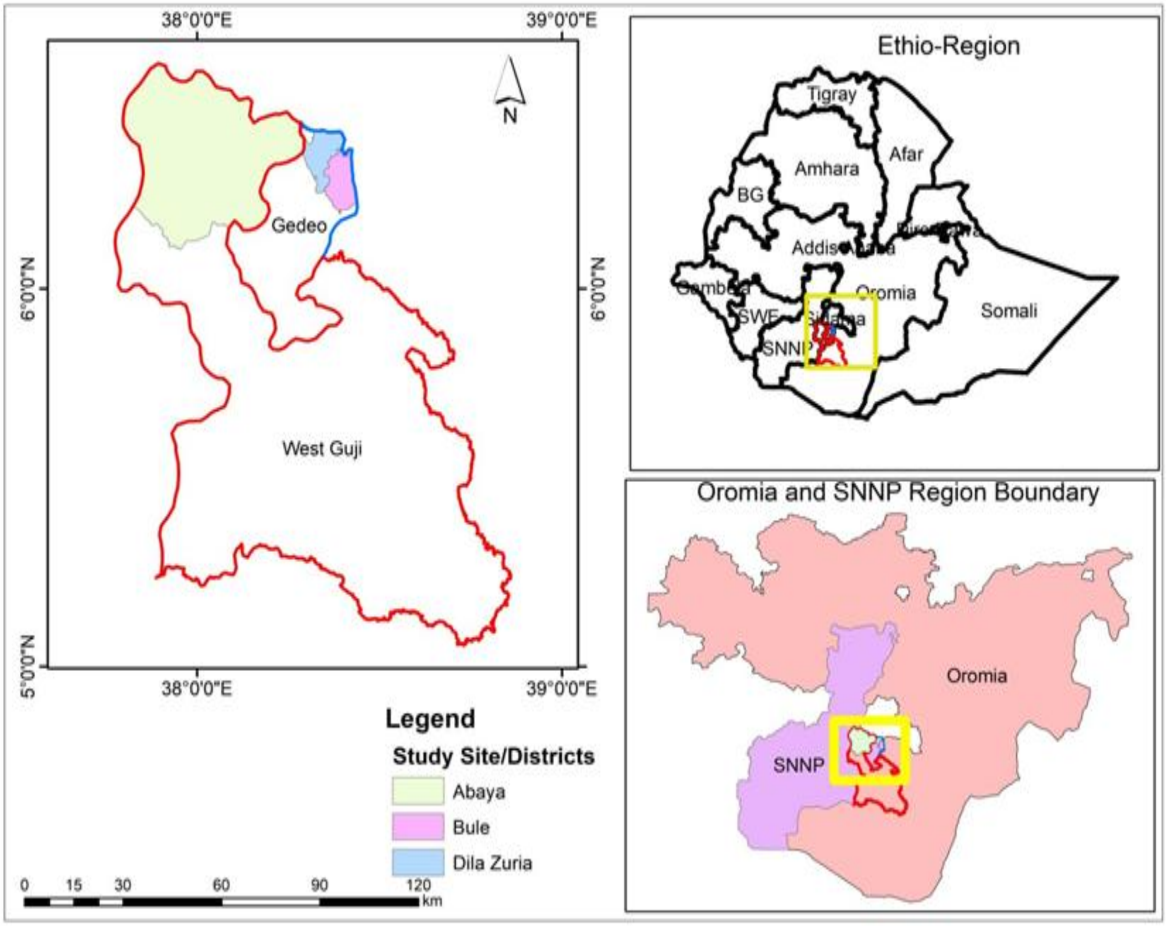

Figure 1. Map of the study area

Data Collection The daily climatic data for rainfall, maximum, minimum and mean temperatures were obtained from the National Meteorological institute (NMI). The historic data of for rainfall, maximum, minimum and mean temperatures was collected from 1981 to 2018. The overall research data for this study were collected based on secondary data sources to address the goals of the study. The data was used to analyses the climate change trend and variability in the study area.

Data Quality Control

The quality of the data was visually and statistically assessed. Visually, the data were checked and detected for outlier and missing data to avoid erroneous/typing error data that can cause changes in the final result. Whereas, the MK test method was checked and tested statistically with the trend free pre-whiting process and the variance correction approaches before applying the test. The trend free pre-whiting process was proposed to remove the serial correlation from the data before applying the trend test (Yue et al., 2002; Hamed, 2009). Likewise, to overcome the limitation of the occurrence of serial autocorrelation in time series, the variance correction procedure was applied as proposed by Hamed and Rao (1998).

Data Analysis

The long-term change of climate factors over time can be studied using a variety of experiments.

Several methods, such as the Standardized Rainfall Anomaly Index to assess the frequency and intensity, were used to measure the variations in observed climatic trends and variability across time. The Mann-Kennel (MK) trend test was used to identify climate trends in time series data, the Standard Precipitation Index (SPI) was used to measure the rainfall deficit of the observed time in the study area, and the coefficient of variation (CV) was used to estimate the seasonal and annual variation of climate change patterns.

Descriptive Statistics

The binning input variables are summarized in the descriptive statistics table. In a single table, the descriptive approach presents univariate summary statistics for many variables. The sample size (number of observations), mean, minimum, maximum, variance, standard deviation, and number of instances with valid values are all examples of statistics. The factor variable's lowest and greatest categories are represented by the values for Minimum and Maximum. On the other hand, the mean is calculated by dividing the sample size (n) by the total of all data values (Xi)

$$Mean(\bar{x}) = \sum_{i=1}^{n} \frac{xi}{n} \quad (1)$$

When Xi is the sum of all data values, n is the sample size

Coefficient of Variation (CV)

During the observation period, CV was used to identify climate variability. Climate variability is divided into three categories: low (CV < 20), moderate (20 < CV > 30), and high (CV > 30). Therefore, the more variable the climate in the study area, the greater the CV value, and vice versa. Equation (2) can be used to get the value of CV.

$$\frac{\delta}{\mu} x100 \quad (2)$$

Where CV is the coefficient of variation, δ is the standard deviation and μ is the mean precipitation of the recording period.

Standard Anomaly Index (SAI)

The SAI was used to calculate the negative and positive anomalies of climate change in the study area. It helps to identify the drought period by determining the dry and wet years of the recording period for precipitation and the hot and cold years of the period for temperature. This index is calculated using the equation given below:

$$z = \frac{xi - \bar{x}i}{s} \quad (3)$$

Where Z is standardized climate anomaly; xi is the annual climate for the historical record; $\bar{x}i$ is the mean of annual rainfall and s is the standard deviation of the annual climate for the historical observation of the time series.

Mann- Kendall (MK)

One popular technique for identifying climatic patterns in time series data is the MK trend test. The MK test is used to identify seasonal and annual trends of climate parameters that are monotonically growing or decreasing. The Mann–Kendall (MK) (non-parametric) test is usually used to detect an upward trend or downward (i.e. monotonic trends) in a series of hydrological data (climate data) and environmental data. The null hypothesis for this test indicates no trend, whereas the alternative hypothesis indicates a trend in the two-sided test or a one-sided test as an upward trend or downward trend (Pohlert, 2016). Climate outliers have less of an impact on the MK test's ability to detect annual and seasonal trend changes. However, if autocorrelation is present in the time series data, the MK test result might contain some mistake. This issue was resolved by doing the pre-whitening technique without making any changes, and there was no discernible amount of serial autocorrelation at any lags.

Following the serial autocorrelation test, the MK test from the Z value and trend from Sen's slope (β) estimation was computed based on monthly, seasonal, and annual rainfall data from 1981–2018 in the study area.

The MK test statistic (S) is calculated as follows:

$$s = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} sign \ (xj - xi) \quad (4)$$

$$\text{Sign (x)} = \begin{cases} 1 \ if \ x > 0 \\ 0 \ if \ x = 0 \\ -1 \ if \ x < 0 \end{cases} \quad (5)$$

If S>0, then later observations in the time series tend to be larger than those that appear earlier in the time series and it is an indicator of an increasing trend, while the reverse is true if S<0 and this indicates a decreasing trend.

Sen's estimator is another non-parametric test used to identify a trend in a series as well as the magnitude of the trend. The Sen's slop estimate requires at least 10 values in a time series. This test computes both the slope (i.e. linear rate of change) and intercepts according to Sen's method (Sen 1968). Likewise, as Drápela and Drápelová (2011) described the linear model can be calculated as follows:

$$f(x) = Qx + B \quad (6)$$

Where Q is the slope, B is constant.

RESULT AND DISCUSSIONS

Analysis of Rainfall Variability

The results of the research showed that all the three agro-ecologies exhibited rainfall variability. Bule district (the highland agro-ecology) had highest rainfall variability with the highest CV (16.2) as compared to the two districts, while Dilla-zuria (the midland agro-ecology) had the lowest variability with lowest coefficient of variation (13.3) (Table 1). The highest rainfall variability in Bule district implies that there is unpredictable highest and lowest rainfall occurrence between years which will affects farmers' decision to set planting time of their crop. The rainfall variability can also be explained by occurrence of erratic rain which will also have negative impact on the farming activity in the area. During the focus group discussion, the farmers in the area has witnessed their occurrence of erratic rainfall is becoming the most important problem for the agricultural practices in the area. The farmers also added that the erratic nature of rain had negatively affected their agricultural farm. According to the famers in the study area, the problem even worse in annual cropping than perennial crop farming. Farmers in Abaya district have also informed as high yield loss has been recorded particularly on maize and teff crop. Farmers in Bule district have also told us as they are in fear of land slayed due to erratic rainfall in the district.

Table 1. Rainfall Summary of the three study districts for the years 1981-2018

District	N	Minimum	Maximum	Mean	Std.Deviation	CV
Dilla	38	858.4	1461.1	1185.9	157.7	13.3
Abaya	38	780.7	1489.9	1175.3	173.0	14.7
Bule	38	816.5	1696.4	1232.9	199.2	16.2

Analysis of Rainfall Trend

The findings indicated a downward tendency and implied a declining trend over time, with the Mann–Kendall statistic values for the annual total rainfall for Dilla-zuria, Abaya, and Bule district being -1.11, -1.68, and -0.93 respectively (Table 2). The findings also showed that, there was downward trend of rainfall across all agro-ecologies. This implies that the amount of total annual rainfall is declining. For Dilla-zuria, Abaya, and Bule district, the Sen's slope estimate for the annual total rainfall was -2.56, -4.66, and -3.29, respectively. These values indicate a downward tendency and a declining trend over time. Both Sen's slope estimates and the Mann-Kendall statistic indicated a declining trend in the annual total rainfall for each of the three agro-ecologies.

Table 2. Mann-Kendall trend and Sen's slope estimate of annual rainfall for the years 1981-2018

District	N	Test Z	Sig.	Q
Dilla	38	-1.11		-2.56
Abaya	38	-1.68	+	-4.66
Bule	38	-0.93		-3.29

Figure 2 shows the calculated yearly rainfall anomalies in the study area from 1981 to 2018. Both positive and negative anomalies were found in the computed standard rainfall anomalies result, suggesting that the observed time series had rainfall variability between districts and years. The year 2010 had the highest positive anomaly (+1.75) and the year 2016 had the highest negative anomaly (−2.08) for the Dilla-zuria district. The year 2010 saw the most positive anomaly (+1.82) for the Abaya district, while the year 2016 saw the highest negative anomaly (−2.28) (Figure 2).

The year 2010 saw the highest positive anomaly (+2.33) for Bule district, while the year 2016 exhibited the highest negative anomaly (−2.09). Based on the results, we can say that 2016 was the driest year for the districts of Dilla-zuria and Abaya. In 2015, these two districts also received a little rain. This is probability due to the fact that Ethiopia endured its first drought in history in 2015 as a result of El Niño that struck several regions of the nation. In contrast to Dilla-zuria and Abaya districts, Bule (highland), still has negative anomalies, but the value does not significantly differ from the mean value. This implies that the highland district is less affected by El Niño as compared to Dilla- zuria and Abaya district. In other word the two districts; Dilla-zuria (the mid-land) and Abaya (the low-land) are more affected by El Niño.

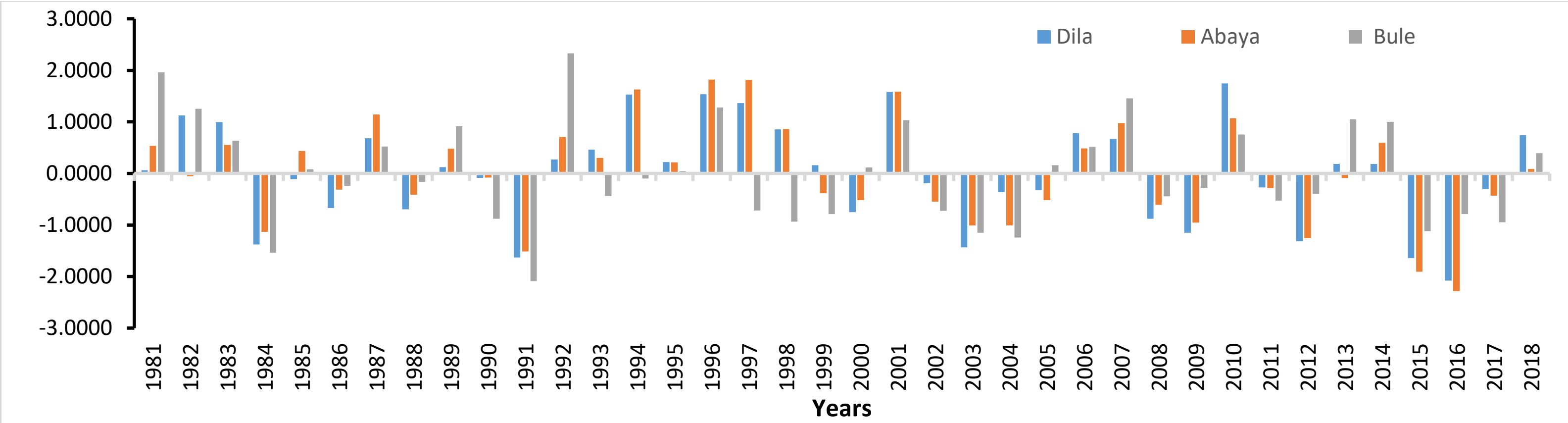

Figure 2. Standard anomalies of Total annual rainfall 1981-2018

Analysis of temperature variability

Table 3 illustrates how the three districts' mean, minimum, and maximum temperatures varied between 1981 and 2018. The highest variability for maximum temperature is was recorded in Abaya district (the lowland agro-ecology) with the coefficient of variation of 3.90%. The highest variability for minimum and maximum temperature was recorded in Dilla-zuria district (the midland agro-ecology) with the coefficient of variation of 23.33 and 14.97% respectively (table 3).

Table 3. Statistics of annual temperature for years 1981-2018

District	Temperature	N	Max.	Min.	Mean	Std.Dev	CV
Dilla-zuria	TMax	38	26.6	22.7	24.4	0.8	3.33
	TMin	38	12.5	0.7	9.0	2.1	23.33
	Tmean	38	18.9	12.9	16.7	2.5	14.97
Abaya	TMax	38	30.3	23.3	28.2	1.1	3.90
	TMin	38	13.8	2.4	11.5	2.2	19.13
	Tmean	38	21.9	15.6	19.9	1.2	6.03
Bule	TMax	38	23.8	20.0	22.3	0.8	3.58
	TMin	38	11.6	8.5	10.2	0.9	8.82
	Tmean	38	17.5	14.8	16.2	0.7	4.32

Analysis of Temperature Trends

Maximum Temperature

According to the results, the maximum temperatures for the three districts had positive Mann–Kendall values. These values indicate that there is an increasing trend and suggest that the temperatures will continue to rise over time. The findings also showed that, although the increases in Dilla-zuria and Abaya were not significant, the trend of the maximum temperature increase in Bule district was significant at a 99.9% confidence level ($p < 0.001$) (Table 4). In this research, the Sen's estimate for the maximum temperature was calculated. The result showed that there was positive Sen's estimate's value indicating that the maximum temperature in each of the three districts has been rising over time. Sen's slope estimates and the Mann-Kendall statistic both reveal a rising trend in the mean maximum temperature across all three districts; therefore, the results are consistent. Figure 3 shows the calculated mean Maximum Temperature anomalies in the research area from 1981 to 2018. The results of the computation of standard rainfall anomalies showed both positive and negative anomalies, suggesting that the observed time series had fluctuation in mean maximum temperature between districts and years. The biggest negative anomaly (-2.00) and highest positive anomaly (+2.58) for the Dilla-zuria district were recorded in 2010 and 2015, respectively. The year 2015 saw the most positive anomaly (+1.81) for the Abaya district, while the year 2010 saw the highest negative anomaly (-4.36). The year 2015 saw the most positive anomaly (+1.71) for Bule district, while the year 2010 saw the highest negative anomaly (−2.74). Given that all three districts had the highest negative anomalies, we can infer from the results that 2010 was the coldest year overall. Since the district as a whole had the largest positive anomalies, 2015 was the hottest year for all three districts. Ethiopia suffered from a catastrophic drought in 2015 as a result of El Niño that struck at various locations throughout the nation.

Minimum Temperature

According to the results, the minimum temperatures for all the three districts had positive Mann-Kendall statistic. The positive values indicate an upward trend and suggest that the temperatures will continue to rise over time. As per Table 4, the results also showed that the minimum temperature increment trend in Bule district is significant at 99.9% confidence level ($p < 0.001$), and in Dilla-zuria, it is significant at 99% confidence level ($p < 0.01$). Meanwhile, the minimum temperature increment trend in Abaya district is significant at 95% confidence level ($p < 0.05$). In this research, the Sen's estimate for the minimum temperature was also calculated. For all the three districts, Sen's estimate for the maximum temperatures had positive value. The positive Sen's estimate's value indicates that the maximum temperature in each of the three districts has been rising over time. Both Sen's slope estimate and the Mann-Kendall statistic reveal an increasing trend in the mean maximum temperature across all three districts, therefore the results are consistent.

Figure 4 shows the calculated mean Minimum Temperature anomalies in the research area from 1981 to 2018. The results of the computation of standard rainfall anomalies showed both positive and negative anomalies, suggesting that the observed time series had fluctuation in mean minimum temperature between districts and years. The year 1993 had the highest positive anomaly (+1.59) and the year 1983 had the highest negative anomaly (-3.80) for the Dilla-zuria district. The highest positive anomaly (+1.021) and negative anomaly (-4.04) were observed in 1993 and 1986, respectively, for the Abaya district. In the Bule district, the largest negative anomaly (−1.73) was recorded in 1989, while the highest positive anomaly (+1.62) was recorded in 2009. The results show that every district experienced negative anomalies between 1984 and 1988. Although the largest negative anomaly for Bule district was recorded in 1989, the highest negative anomalies for Dilla-zuria and Bule districts were also recorded during this time period. Abaya and Bule districts saw positive anomalies from 2010 to 2018, meaning that the lowest temperatures recorded in each of the aforementioned years were higher than the average minimum temperatures over the 1981–2018 time frame. The period from 2010 until 2018 is a period during which Dilla-zuria district also had the majority of positive anomalies, with the exception of 2015 and 2016, during which Dilla-zuria district experienced negative abnormalities.

Annual Mean Temperature

According to the results, the average temperatures for Dilla-zuria, Abaya, and Bule districts had positive Mann-Kendall statistic values. The positive values indicate an increasing trend and suggest that the temperatures will continue to rise over time. Additionally, the results showed that the minimum temperature increment trend in Bule district is significant at 99.9% confidence level ($p < 0.001$) and in Dilla-zuria, it is significant at 95% confidence level ($p < 0.05$). In contrast, the minimum temperature increment trend in Abaya district is non-significant ($p < 0.05$) (Table 4). The results of this study concur with those of the study of Asfaw et al., (2018); Roy and Das (2013), Tabari and Talaee (2011), Conway et al., (2005) and Stafford et al., (2000). In this study, the Sen's estimate for the annual mean temperature was also calculated. For the all the three districts Sen's estimate's value was positive indicating that the maximum temperature in each of the three districts has been rising over time. Both Sen's slope estimate and the Mann-Kendall statistic reveal a rising trend in the mean maximum temperature across all three districts, therefore the results are consistent.

Figure 5 shows the calculated mean annual temperature anomalies in the research area from 1981 to 2018. The calculated standard mean annual temperature anomalies result showed both positive and negative anomalies, suggesting that the observed time series had mean annual temperature fluctuation between districts and years. The maximum positive anomaly (+1.9248) and the highest negative anomaly (-3.46) for the Dilla-zuria district were recorded in 1993 and 1986, respectively. The maximum negative anomaly (-3.54) and highest positive anomaly (+1.63) for the Abaya district were recorded in 1986 and 1993, respectively. Bule district's had greatest positive anomaly. The largest negative anomaly (−1.97) was recorded in 1989, while the highest positive anomaly (+1.68) was recorded in 2009. The results show that every district experienced negative anomalies between 1984 and 1990. During this period, the three districts' highest negative anomalies were also noted. However, from 2011 to 2018, all three districts saw positive anomalies, meaning that the lowest temperature recorded in each of the aforementioned years was higher than the average minimum temperature throughout the 1981–2018 time frames. According to the results, the average temperatures for Dilla-zuria, Abaya, and Bule districts had Mann-Kendall statistic (Z) values of 0.53, 2.16, and 5.10, respectively. These values indicate an upward trend and suggest that the temperatures will continue to rise over time. Additionally, the results showed that the minimum temperature increment trend in Bule district is significant at 99.9% confidence level ($p < 0.001$) and in Dilla-zuria, it is significant at 95% confidence level ($p < 0.05$). In contrast, the minimum temperature increment trend in Abaya district is non-significant ($p < 0.05$) (Table 4). The results of this study concur with those of the study of Asfaw et al., 2018; Roy and Das (2013), Tabari and Talaee (2011), Conway et al., (2004), Daniel et al., (2014), and Stafford et al., (2000). In this study, the Sen's estimate (Q value) for the yearly mean temperature was also calculated. For the districts of Dilla-zuria, Abaya, and Bule, the Sen's estimate (Q value) for the mean temperatures was 0.014, 0.032, and 0.066, respectively. Sen's estimate's positive value (Q value) indicates that the maximum temperature in each of the three districts has been rising over time. Sen's slope estimate (Q) and the Mann-Kendall statistic (Z) both reveal a rising trend in the mean maximum temperature across all three districts; therefore, the results are consistent. Figure 5 shows the calculated mean annual temperature anomalies in the research area from 1981 to 2018. The calculated standard mean annual temperature anomalies result showed both positive and negative anomalies, suggesting that the observed time series had mean annual temperature fluctuation between districts and years. The highest positive anomaly (+1.9248) and the highest negative anomaly (-3.46) for the Dilla-zuria district were recorded in 1993 and 1986, respectively. The highest negative anomaly (-3.54) and highest positive anomaly (+1.63) for the Abaya district were recorded in 1986 and 1993, respectively. Bule district has highest positive anomaly. The highest negative anomaly (−1.97) was recorded in 1989, while the highest positive anomaly (+1.68) was recorded in 2009. The results show that every district experienced negative anomalies between 1984 and 1990. During this period, the three districts' highest negative anomalies were also noted. However, from 2011 to 2018, all three districts saw positive anomalies, meaning that the lowest temperature recorded in each of the aforementioned years was higher than the average minimum temperature throughout the years1981–2018.

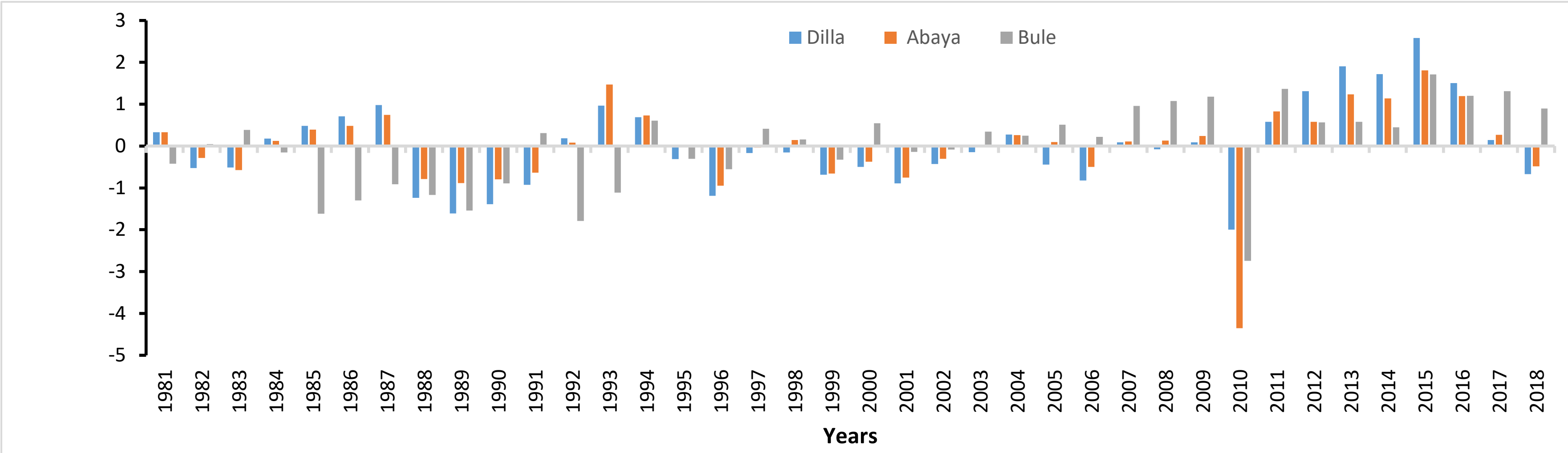

Figure 3. Standard anomalies of maximum Temperature 1981-2018

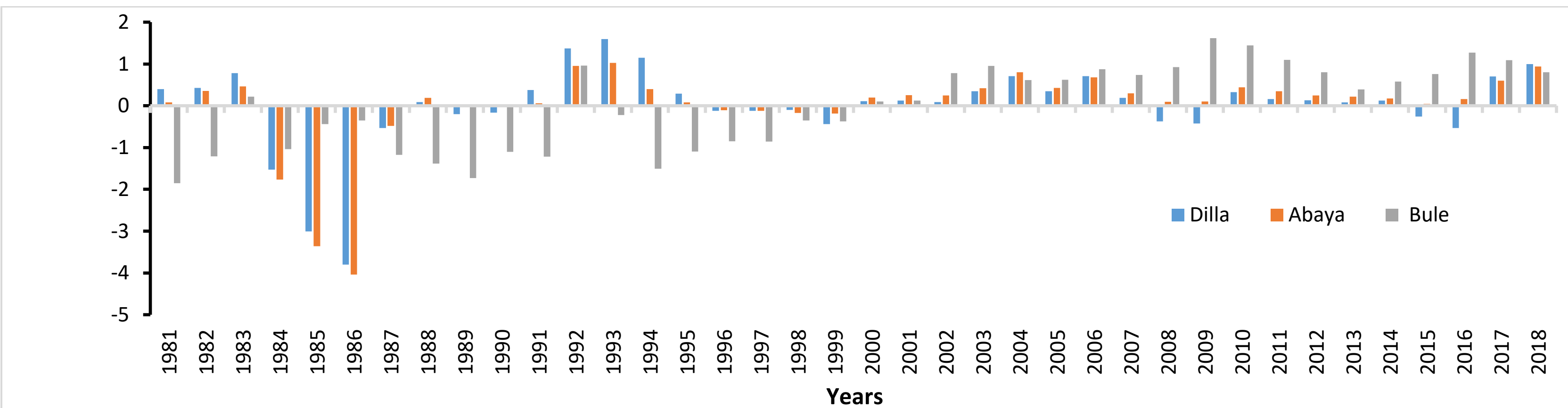

Figure 4. Standard anomalies of minimum Temperature 1981-2018

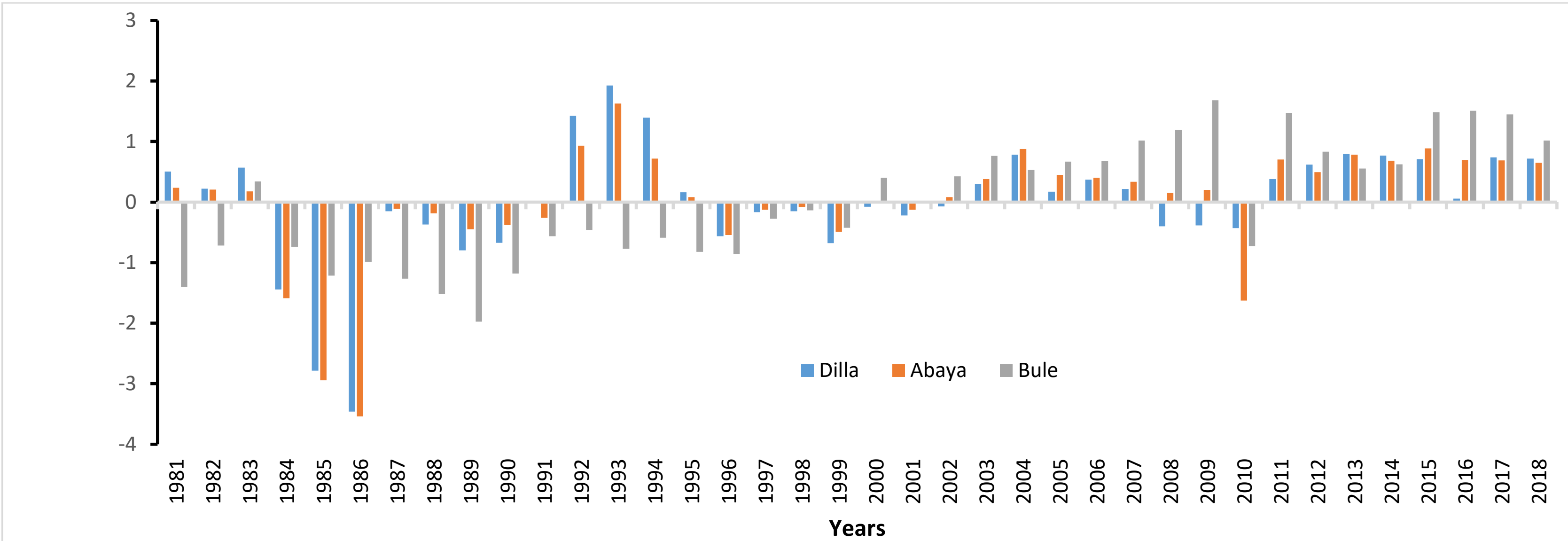

Figure 5. Standard anomalies of mean Temperature 1981-2018

Table 4. Mann-Kendall trend and Sen's slope estimate of maximum, minimum and mean Temperature Dilla-zuria, Abaya and Bule district 1981-2018

Variable	District											
	Dilla-zurial				Abaya				Bule			
	N	Test Z	Sig.	Q	N	Test Z	Sig.	Q	N	Test Z	Sig.	Q
T max	38	1.73		0.022	38	1.84		0.023	38	4.60	***	0.052
T min	38	2.34	*	0.03	38	3.21	**	0.04	38	5.85	***	0.05
T mean	38	0.53		0.014	38	2.16	*	0.032	38	5.10	***	0.066

CONCLUSIONS

In this study, variability and trend analysis of annual rainfall, minimum temperature, and mean temperature has been conducted for highland, midland and lowland agro-ecologies in southern Ethiopia. The result showed that highest rainfall variability was recorded in highland agro-ecology there was downward trend of rainfall in all agro-ecologies.

Both Sen's slope estimates and the Mann-Kendall statistic indicated a declining trend in the annual total rainfall for all the three agro-ecologies. Sen's slope estimates and the Mann-Kendall statistic both reveal increasing trend in the maximum, minimum and mean temperature across all the three agro-ecologies. The result also showed that there is significant increment on minimum temperature in midland agro-ecology. There was also significant increment on minimum and mean temperature in midland agro-ecology. In highland agro-ecology, there was strong significant increment in maximum, minimum and mean temperature in highland agro-ecology. From the result we can conclude that the result of both Sen's slope estimate and the Mann-Kendall statistic are consistent. Thus, the decreasing trend of rainfall and increasing trend of temperature due to climate change and other factors can lead to weather extremes in the study area.

REFERENCES

Alemu, T., & Mengistu, A. 2019. Impacts of climate change on food security in Ethiopia: adaptation and mitigation options: a review. Climate change-resilient agriculture and agroforestry: Ecosystem services and sustainability, 397-412. [Scholar Google]

Ali, M. A., Hoque, M. A., & Kim, P. J. 2013. Mitigating global warming potentials of methane and nitrous oxide gases from rice paddies under different irrigation regimes. Ambio, 42: 357-368. [Scholar Google]

Asfaw, A., Simane, B., Hassen, A., & Bantider, A. 2018. Variability and time series trend analysis of rainfall and temperature in north central Ethiopia: A case study in Woleka sub-basin. Weather and climate extremes, 19: 29-41. [Scholar Google]

Ayanlade, A., Radeny, M., Morton, J. F., & Muchaba, T. 2018. Rainfall variability and drought characteristics in two agro-climatic zones: An assessment of climate change challenges in Africa. Science of the Total Environment, 630: 728-737. [Scholar Google]

Bayable, G., Amare, G., Alemu, G., & Gashaw, T. 2021. Spatiotemporal variability and trends of rainfall and its association with Pacific Ocean Sea surface temperature in West Harerge Zone, Eastern Ethiopia. Environmental Systems Research, 10: 1-21. [Scholar Google]

Cherie, G.G. & Fentaw, A. 2015. Climate change impact assessment of dire dam water supply. AAUCED HES, Ethiopia.

Conway, D. 2005. From headwater tributaries to international river: Observing and adapting to climate variability and change in the Nile basin. Global Environmental Change, 15(2): 99-114. [Scholar Google]

Conway, D., & Schipper, E. L. F. 2011. Adaptation to climate change in Africa: Challenges and opportunities identified from Ethiopia. Global environmental change, 21(1): 227-237. [Scholar Google]

Deressa, T. T., Hassan, R. M., & Ringler, C. 2011. Perception of and adaptation to climate change by farmers in the Nile basin of Ethiopia. The Journal of Agricultural Science, 149(1): 23-31. [Scholar Google]

Dioha, M. O., & Kumar, A. 2020. Exploring greenhouse gas mitigation strategies for agriculture in Africa: the case of Nigeria. Ambio, 49(9): 1549-1566. [Scholar Google]

Drápela, K., & Drápelová, I. 2011. Application of Mann-Kendall test and the Sen's slope estimates for trend detection in deposition data from Bílý Kříž (Beskydy Mts., the Czech Republic) 1997-2010.

Gorfu, D., & Ahmed, E. 2012. Crops and agro-ecological zones of Ethiopia. Ethiopian Institute of Agricultural Research.

Hamed K. and Rao A. 1998. A modified mann-kendall trend test for autocorrelated data. J Hydrol., 204(1–4): 182–196. [Scholar Google]

Hamed K. 2009. Enhancing the effectiveness of prewhitening in trend analysis of hydrologic data. J Hydrol., 368(1-4): 143–155. [Scholar Google]

I. P. C. C. 2014. Climate change 2014 synthesis report. IPCC: Geneva, Szwitzerland, 1059-1072.

Mekasha, A., Tesfaye, K., & Duncan, A. J. 2014. Trends in daily observed temperature and precipitation extremes over three Ethiopian eco-environments. International Journal of Climatology, 34(6). [Scholar Google]

Moges, D. M., & Bhat, H. G. 2021. Climate change and its implications for rainfed agriculture in Ethiopia. journal of Water and Climate Change, 12(4): 1229-1244. [Scholar Google]

Muleta, B. G., Mummed, Y. Y., & Kurtu, M. Y. 2019. Assessment of beef cattle production and marketing practice in Eastern Oromia, Ethiopia. Effect of Climate Change on Agricultural Production and Community Response in Daro Lebu & Mieso District, West Hararghe Zone, Oromia Region National State, Ethiopia. Food Science and Quality Management, 85: 25-36. [Scholar Google]

Pohlert, T. 2016. Non-parametric trend tests and change-point detection. CC BY-ND, 4: 1-18.

Roy, T. D., & Das, K. K. 2013. Temperature trends at four stations of Assam during the period 1981-2010. Int. J. Sci. Res. Publ., 3(6): 1-3. [Scholar Google]

Samy, A., G. Ibrahim, M., Mahmod, W. E., Fujii, M., Eltawil, A., & Daoud, W. 2019. Statistical assessment of rainfall characteristics in upper Blue Nile basin over the period from 1953 to 2014. Water, 11(3): 468. [Scholar Google]

Schlenker, W., & Lobell, D. B. 2010. Robust negative impacts of climate change on African agriculture. Environmental Research Letters, 5(1): 014010. [Scholar Google]

Sen, P. K. 1968. Estimates of the regression coefficient based on Kendall's tau. Journal of the American statistical association, 63(324): 1379-1389. [Scholar Google]

Serdeczny, O., Adams, S., Baarsch, F., Coumou, D., Robinson, A., Hare, W., ... & Reinhardt, J. 2017. Climate change impacts in Sub-Saharan Africa: from physical changes to their social repercussions. Regional Environmental Change, 17: 1585-1600. [Scholar Google]

Simane, B., Zaitchik, B. F., & Foltz, J. D. 2016. Agroecosystem specific climate vulnerability analysis: application of the livelihood vulnerability index to a tropical highland region. Mitigation and Adaptation Strategies for Global Change, 21: 39-65. [Scholar Google]

Stafford, J. M., Wendler, G., & Curtis, J. 2000. Temperature and precipitation of Alaska: 50 year trend analysis. Theoretical and Applied Climatology, 67: 33-44. [Scholar Google]

Tabari, H., & Talaee, P. H. 2011. Analysis of trends in temperature data in arid and semi-arid regions of Iran. Global and Planetary Change, 79(1-2): 1-10. [Scholar Google]

Tesfaye, K., Seid, J., Getnet, M., & Mamo, G. 2016. Agriculture under a changing climate in Ethiopia: challenges and opportunities for research. Ethiopian J. Agric. Sci., 67-86. [Scholar Google]

Tigchelaar, M., Battisti, D. S., Naylor, R. L., & Ray, D. K. 2018. Future warming increases probability of globally synchronized maize production shocks. Proceedings of the National Academy of Sciences, 115(26): 6644-6649. [Scholar Google]

Wagaye. B. and Endalew A. 2020. "Climatology & Weather Forecasting Temperature and Rainfall Trends in North Eastern Ethiopia." Climatology & Weather Forecasting OPEN Vol. 8(3): 1–6. [Scholar Google]

Worku, M. A., Feyisa, G. L., & Beketie, K. T. 2022. Climate trend analysis for a semi-arid Borana zone in southern Ethiopia during 1981–2018. Environmental Systems Research, 11(1): 2. https://doi.org/10.1186/s40068-022-00247-7 [Scholar Google]

Yalew, A. W., Hirte, G., Lotze-Campen, H., & Tscharaktschiew, S. 2017. Economic effects of climate change in developing countries:

Economy-wide and regional analysis for Ethiopia (No. 10/17). CEPIE Working Paper.

Yue S, Pilon P, Phinney B, Cavadias G. 2002. The influence of autocorrelation on the ability to detect trend in hydrological series. Hydrol Process, 16(9): 1807–1829. [Scholar Google]

Original Research Article||

Bio-Organic Amendments Enhanced Growth, Nodulation, and Nutrient Uptake of Faba Bean in Acidic Soils of Sidama Region, Ethiopia

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Abstract

Faba bean (*Vicia faba* L.) production and productivity in the highlands of Ethiopia are considerably low due to acidic soil conditions. Enhancing soil fertility and increasing nutrient accessibility through optimized agronomic practices is essential for improving productivity. Hence, this study was conducted under controlled conditions in a lath house to examine the effects of coffee husk biochar and *Rhizobium* inoculation on the agrosymbiotic and nutrient uptake of various faba bean varieties. Four varieties (Local, Dosho, Gebelcho, and Numan), four inoculation treatments (uninoculated and inoculated with strains FB-EAR-15, TAL-1035, and EAL-110), and three rates of biochar (0, 5, and 10 t ha^{-1}) were tested on acidic soils collected from the Goriche and Hagere Selam districts. The treatments were arranged in a completely randomized factorial design with three replications. The findings indicated that the varieties, inoculation, and biochar significantly influenced ($p < 0.05$) the majority of the parameters measured. Furthermore, the three-way interaction among these treatments markedly influenced nodule dry weight. Notably, the Gebelch variety combined with strain EAL-110 and 10 t ha^{-1} of biochar led to a 22.3% increase in nodule dry weight compared to the Local variety that did not receive inoculation or biochar. The use of the Dosha variety, strain EAL-110, along with a biochar application rate of 10 t ha^{-1}, hugely improved the growth, nodulation, and nutrient uptake of faba beans in acidic soil. Therefore, it is critical to evaluate these agronomic practices in real field conditions in Ethiopia to enhance the productivity of faba beans in these areas.

Key words: Biochar, Coffee husk, Faba bean, Nodules, Rhizobium

Original submission: December 16, 2024; **Revised submission**: July 01, 2025; **Published online**: December 31, 2025

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INTRODUCTION

Among legumes, faba bean (*Vicia faba* L.), commonly known as broad bean, is one of the oldest cultivated crops globally (Mínguez & Rubiales, 2021). Its production is estimated at approximately 4.8 million tons per year, with China being the largest producer (1.8 million tons per year), followed by Ethiopia, Australia, and the United Kingdom with 0.9, 0.4, and 0.3 million tons per year, respectively (FAOSTAT, 2021). Being a legume, the faba bean fixes atmospheric nitrogen (N) through symbiotic association with rhizobia. It contributes to sustainable agriculture by maintaining and improving soil fertility, which plays a key role in crop rotation (Ghorbi et al., 2023).

Ethiopia is considered a significant center of secondary diversity for faba beans, which thrive in highland regions at altitudes ranging from 1800 to 3000 meters above sea level. It is one of the leading legumes in the country, valued for its ecological and nutritional benefits. However, the average yield across the nation is unfortunately low. This is primarily due to the fragmentation of arable land and a decline in soil fertility, which results from agricultural practices that often involve minimal or no fertilization (Kermah et al., 2018). Furthermore, soil acidity plays a crucial role in limiting production in legume farming (Fekadu et al., 2018; Tadele et al., 2019). It can significantly impact the availability of essential nutrients, hinder root development, and reduce overall plant health, ultimately leading to lower crop yields (Warke &

Wakgari, 2024). As a result, smallholder farmers typically achieve an average crop yield of no more than 2.1 t ha^{-1} (CSA, 2022).

A range of agricultural strategies has been suggested to address the challenges associated with acidic soils and enhance crop yields (MoA, 2020). These strategies encompass the cultivation of acid-tolerant crops, the application of lime, and the use of organic fertilizers (Tusar et al., 2023). Among these options, the combination of lime and organic fertilizers is frequently considered the most effective method (Enesi et al., 2023). However, the high costs and limited availability of these organic fertilizers and lime have sparked increased interest in the use of locally sourced, cost-effective organic materials and mineral fertilizers in a coordinated approach (Golla, 2019). It has been suggested that organic materials such as crop residues, manure, compost, and biochar could be viable alternatives to lime for remediating acidic soils (Tazebew et al., 2024).

The use of effective rhizobial strains has been shown to enhance various growth parameters in soybean varieties. For instance, Beruk et al. (2024) highlighted improvements in nodulation, dry biomass production, and plant height. Similarly, Samago et al. (2018) demonstrated that inoculating common beans with effective rhizobia strains led to significant increases in plant height, shoot dry weight, the number of nodules, and the dry weight of those nodules. Additionally, Kebede (2021) found that inoculating soybeans with rhizobial strains significantly increased root dry weight. Furthermore, Allito et al. (2020) found that inoculating faba beans with rhizobia enhanced nutrient availability, emphasizing the positive effects of microbial inoculants on soil health and fertility.

Adding biochar to soil offers a strategy for sequestering carbon, managing waste, immobilizing pollutants, enhancing fertility, and reducing nitrous oxide (N_2O) emissions from degraded soils (Jeffery et al., 2015; Afshar & Mofatteh, 2024). Numerous studies have demonstrated the beneficial effects of biochar on acidic soils (Zhang et al., 2019; Premalatha et al., 2023). The mechanism of soil fertility improvement by biochar can be explained by reduced soil acidity (Bedassa, 2020; Nepal et al., 2023; Bekchanova et al., 2024), improved soil structure and nutrient transport (Kapoor et al., 2022), and increased water and nutrient retention (Alkharabsheh et al., 2021; Yadav et al., 2023; Khan et al., 2024). Given the benefits of these agronomic practices, this study investigated the effect of coffee husk biochar and *Rhizobium* inoculation on faba bean agrosymbiotic performance and nutrient uptake in acidic soils from two districts in the region. It also aims to provide insights into sustainable agricultural practices that can increase crop yields and improve soil health in acidic environments. The findings could be extremely beneficial to farmers in the region, providing practical solutions to increase productivity while promoting environmental sustainability.

MATERIALS AND METHODS

Description of Soil Collection Sites

The pot experiment was conducted from July 10 to September 7, 2021, in the lath house at Hawassa University College of Agriculture. Soil samples were collected from the Hagere Selam and Goriche districts in the Sidama Region of Ethiopia. Hager Selam is located approximately 350 kilometers south of Addis Ababa, at coordinates 38°29′17″ E and 6°31′42″ N, with an elevation of 2,749 meters above sea level (m.a.s.l.). In comparison, Goriche is located 317 km from Addis Ababa and 44 km from Hawassa, the regional capital, at a latitude of 38°35′22″ E and a longitude of 06°50′39″ N, with an elevation of 2,813 m.a.s.l. Both areas experience bimodal rainfall patterns, featuring long rainy seasons and receiving over 1,500 millimeters of precipitation annually (Tadesse et al., 2025).

Sources of Biochar, Faba Bean and *Rhizobium* Strains

Coffee husks intended for biochar production were sourced from coffee processing facilities located in the Dale district of the Sidama region, an area known for its rich coffee cultivation. These husks, which are the outer shells of coffee beans, are typically considered agricultural waste but have gained attention for their potential use in biochar production due to their high carbon content and ability to improve soil health. *Rhizobium* strains (FB-EAR-15, TAL-1035, and EAL-110), were obtained from the Holeta Agricultural Research Center in Ethiopia. The performance of these strains for cultivating faba beans in Ethiopia has been well-documented in field conditions (Mitiku & Mnalku, 2019; Tadesse et al., 2025). The details of the faba bean varieties utilized in the experiment are presented in Table 1.

Table 1. Description of faba bean varieties tested in this experiment

Varieties	Seed source	Year of release	Days to physiological maturity	Altitude	Yield (quintal ha⁻¹) on	
					Research	Farmers field
Dosha	Holeta ARC	2009	120-165	2050-2800	25-62	23-39
Gebelcho	Holeta ARC	2006	103-167	1900-3000	25-44	20-30
Numan	Kulumsa ARC	2016	137-148	1800-3000	36	22-38
Framer's variety				1900- 3000	22	12

ARC = Agricultural Research Center

Biochar Preparation

Biochar was produced at the Akaki Metal Engineering Company in Addis Ababa using a controlled low-oxygen process in an electric furnace pyrolysis reactor. This method optimizes the conversion of organic materials into biochar, a stable carbon form that improves soil quality and sequesters carbon. The production began with selecting shell material as feedstock, which was then heated to 500°C for three hours (Dume et al., 2015). This high temperature facilitates the thermal decomposition of organic material into biochar while minimizing volatile compound release. The low-oxygen environment prevents complete combustion, promoting carbon-rich biochar formation.

Treatments, Experimental Design, and Procedures

The factors studied were four faba bean varieties [Local, Dosho, Gebelcho, and Numan], four *Rhizobium leguminosarum* biovar *viciae* strains [uninoculated and inoculated with strains FB-EAR-15, TAL-1035, and EAL-110], and three biochar rates from coffee husks [0, 5, and 10 t ha^{-1}]. Treatments were arranged in a factorial combination using a completely randomized design with three replicates. Plastic pots with a base diameter of 19.2 cm, a top diameter of 23 cm, and a height of 19.5 cm were used for growing faba beans. The biochar-soil mixtures incorporated two different rates of biochar: 5 t ha^{-1} (equivalent to 6.1 g of biochar pot^{-1}) and 10 t ha^{-1} (12.2 g of biochar pot^{-1}). Each mixture, along with a control soil containing no biochar, was allocated three kilograms and placed into experimental pots, with three replicates for each treatment. These pots were then placed in a controlled environment within a lath house and regularly irrigated to maintain optimal soil moisture. After a two-week incubation period, the seeds of each faba bean variety were inoculated with specific *Rhizobium* strains using a peat-based inoculant, following the recommended application rate of 10 g per kg of seed. *Rhizobial* inoculation was conducted in a shaded area to maintain the viability of the strains. Subsequently, four seeds from each variety and treatment were planted in separate pots. Once germination took place, the seedlings were thinned to keep two healthy plants per pot. Throughout the growing season, the pots were watered to ensure optimal moisture levels for the developing seedlings.

Physicochemical Analysis of the Soil and Biochar

Soil sampling and analysis

The soil sampling and analysis were performed before the implementation of the treatments. To evaluate the initial values at each experimental site, representative random soil samples were collected from a depth of 0 to 20 cm, following the standard soil sampling protocol. After physically homogenizing the samples, two representative composite subsamples were prepared from each site for physicochemical analysis. The collected soil samples were air-dried at room temperature and sieved through a 2 mm mesh wire for selected physicochemical analyses. The sample soil particle size distribution was determined using the hydrometer method (Dewis & Freitas, 1970). The soil pH was measured using a digital pH meter from the supernatant suspension of 1:2.5 H_2O, as described by Van Reeuwijk (2002). The bulk density of the soil was measured using undisturbed soil samples. The total nitrogen (N) content was determined using the Kjeldahl method, while the measurement of soil organic carbon (OC) followed the protocols established by Walkley & Black (1934). The available P was assessed using the Bray-II method (Bray & Kurtz, 1945). The exchangeable bases, along with the cation exchange capacity (CEC), were evaluated by saturating the soil with neutral 1 M ammonium acetate. The ammonium ions (NH_4^+) adsorbed on the surface were displaced by applying a 1 M potassium chloride (KCl) solution. Following this, the CEC of the sample was estimated using the Kjeldahl distillation method (Polemio & Rhoades, 1977). Exchangeable Ca^{2+} and Mg^{2+} in ammonium acetate were measured by an atomic absorption spectrophotometer (AAS), and exchangeable K^+ and Na^+ were measured by a flame photometer (Anderson & Ingram, 1994). The exchangeable acidity was determined by saturating the soil samples with potassium chloride solution and titrating them with sodium hydroxide, as described by McLean (1965).

Biochar Sampling and Analysis

The coffee husk biochar sample underwent analysis for chemical properties, including pH, total N, OC, available P, exchangeable bases and CEC. The pH was measured in distilled water at a 1:10 biochar: water mass ratio after shaking for 30 minutes (ASTM, 2009). The OC was determined using the Walkley-Black method, and the total N was determined using the Kjeldahl method (Sanvong & Nathewet, 2014). Available P was determined using the Olsen extraction method (Olsen & Sommers, 1982). The exchangeable bases and CEC were measured at pH 7.0 after displacement using the 1 N ammonium acetate method and then estimated by titration and distillation of the ammonium that was displaced by sodium (Gaskin et al., 2008).

Data Collection

Growth, Photosynthesis and Nodulation Parameters

Plant height was measured at the mid-flowering stage during nodulation and shoot biomass assessments, while chlorophyll content (greenness) was measured on the 36th day before flowering, just at mid-flowering, and at the 56th day after planting by taking three leaves (youngest fully expanded leaves) per plant using a chlorophyll meter (Model Minolta SPAD 502). Nodulation assessment was conducted at the mid-flowering stage (50%) to prevent the detachment of roots along with nodules during the uprooting process, utilizing the third replicated pot that housed three plants. Given the interlocking nature of the roots and the challenges associated with separating nodules from individual plants without causing damage, we carefully uprooted all three plants simultaneously. The plants were then carefully divided into shoots, roots, and nodules. The soil that clung to the roots was gently shaken off. The nodules from each plant were picked and spread on the sieve to wash and drain water from their surface. Then, they were counted, and their average was taken for pots as nodule number plant^{-1}. These nodules were oven-dried at 70°C for 48 hours for the determination of their dry weight. The shoot dry matter of the plants was determined at the mid-flowering stage from plants sampled for nodulation. The plant sample was placed in labeled perforated paper bags and oven-dried at 70°C for 48 hours to determine the shoot dry matter yield.

Nutrient Concentration and Uptake Analysis

Shoots and roots of harvested faba bean plants were oven-dried at 70°C until a constant weight was recorded. The oven-dried samples were then ground in a simple grinder with a stainless-steel blade and stored in polyethylene bags for analysis. The %N in the sample was estimated through the Kjeldahl method; 5 mL of an aliquot was taken into the distillation flask, and 10 mL of 40% sodium hydroxide (NaOH) was added to it. The flask was then connected to the distillation setup with a 100-

mL conical flask containing 25 mL of boric acid solution. The distillate was cooled for a few minutes and titrated with 0.01 N standard sulphuric acid. Then, the %N was calculated as follows:

$$\% \mathbf{N} = \frac{(\mathbf{T\ X\ N\ X\ 1.4})}{\mathbf{wt\ of\ sample}}$$

Where T = volume of acid used for titration (mL), N = normality of acid = 0.01 N, and sample weight = 1 g.

N uptake = %N concentration in shoot x shoot dry matter (g plant^{-1})

The concentration of P in the shoot sample was determined according to Ryan et al. (2001). Plant tissue samples (1.0 g) were heated in porcelain crucibles at 500°C for 5 hours. The ash was transferred into a 200-ml Erlenmeyer flask with 20 ml of 20% HNO_3, and the sample was filtered through a No. 1 filter paper into a 100-ml volumetric flask. The contents were then washed until 90 ml of the filtrate was collected. The filtrates were analyzed with a spectrophotometer at 460 nm.

P uptake = %P concentration in shoot x shoot dry matter (g plant^{-1})

Data Analysis

The normality of the data was checked in advance using the Shapiro–Wilk normality test. The homogeneity of variance between the two soil sites was tested using Bartlett's test, and the results were found to be valid for conducting a combined analysis. The combined analysis of variance was performed to assess the variation among treatments (*Rhizobium* strains, faba bean varieties, biochar rates, and interactions among all four factors) using the general linear model (GLM) of the Statistical Analysis System (SAS) software version 9.4. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 5% probability level. For the sake of this manuscript, only a three-way interaction of variety, *Rhizobium*, and biochar was considered. Correlation analysis was performed using Pearson's simple correlation coefficients for the intended parameters.

RESULTS AND DISCUSSION

Physicochemical properties of soils and biochar

Table 2 details the physicochemical properties of the soils and the biochar utilized in this experiment. The result indicates that the textural class of the soil taken from Gorche was clay loam with a pH of 5.2, while the soil from Hager Salam was clay with a pH value of 5.0, categorized as strongly acidic (Tadesse et al., 1991). The results showed that the soil from both sites was acidic and needed some kind of reclamation for the nutrients to be available for the plant. The OC contents of the sites were medium (2.1%) and low (1.4%) for Gorche and Hagere Selam, respectively (Debele, 1982). The total N and available P contents of the sites were low (Ethio, SIS, 2014), while the exchangeable bases were categorized as medium and low (Debele, 1982). The CEC of the soils from both sites falls between the ranges of medium (Hazelton & Murphy, 2016). However, the available Fe (40.6 and 43.2 mg kg^{-1}) and Mn (44.4 and 50.8 mg kg^{-1}) contents of the soils were more than the optimal requirements of the plants (Lindsay & Norvell, 1978). On the other hand, the pH value and OC content of the biochar were 10.5 and 21.1%, respectively (Table 2). Overall, the results showed that biochar had superior chemical properties to soils from both sites. Furthermore, biochar demonstrated a higher CEC, which measures the soil's ability to hold positively charged ions. This property is important for soil health because it helps retain essential nutrients and reduces leaching, promoting sustainable agricultural practices. The alkaline nature of biochar also contributed to an increase in soil pH, which can be beneficial in acidic soils and help to create a better environment for plant growth.

Table 2. Physicochemical properties of soils and biochar

Sites	Particle size distribution (%)			Textural class	pH (H_2O)	Bulk density (g cm^{-3})	Total N	Organic C	Available P	Exchangeable cations							
										Fe	Mn	Zn	Ca	Mg	K	Na	CEC
							(%)		(mg kg^{-1})				(cmol$_c$ kg^{-1})				
	Clay	Silt	Sand														
Soil parameters																	
Goriche	41.2	15.0	43.8	Clay loam	5.2	1.23	0.15	2.1	4.91	40.6	44.4	1.5	6.3	1.4	0.7	1.2	20.5
Hager Salam	37.2	25.0	37.8	Clay	5.0	1.22	0.12	1.4	3.43	43.2	50.8	2.0	5.2	1.2	0.4	1.2	19.5
Biochar parameters																	
	-	-	-	-	10.5	-	0.54	21.1	14.3	16.2	0.8	7.2	52.5	9.8	2.5	4.2	52.5

Effect of Treatments on Growth, Photosynthesis and Nodulation

Plant Height and Chlorophyll Content

The averaged data from the two sites revealed that plant height and chlorophyll content were significantly ($p< 0.05$) affected by varieties and *Rhizobium* inoculation. However, biochar rates did not affect plant height or chlorophyll content of the faba bean (Table 3). The Numan and Dosha varieties demonstrated the tallest plant heights and the highest chlorophyll content, respectively. This indicates that these two varieties possess superior traits that may contribute to their overall vigor and photosynthetic efficiency. Conversely, the Local variety had the shortest plant heights and the lowest chlorophyll content. These differences in plant height and chlorophyll levels among the different varieties can likely be attributed to genetic variations (Zhu et al., 2024). Additionally, Theeuwen et al. (2022) emphasized that genetic factors greatly influence plant morphology and physiological traits, such as growth rates and chlorophyll production.

Likewise, the tallest plant height and highest chlorophyll content were recorded from plants inoculated with strains FB-EAR-15 and EAL-110, indicating their unique growth-enhancing properties. Plants treated with the FB-1035 strain also showed higher leaf chlorophyll content, though less than the others. In contrast, uninoculated plants had the lowest height and chlorophyll levels, emphasizing the role of these beneficial bacteria in plant health. The improved growth observed in strains EAR-15 and EAL-110 inoculated plants can likely be attributed to enhanced access to N, a critical nutrient for plant development (Adissie et al., 2018; Samago et al., 2018). *Rhizobium* bacteria are known for their ability to fix atmospheric N, converting it into a form that plants can readily absorb and utilize. This increased N availability may not only support greater overall growth but could also positively influence other growth-related traits, such as total leaf chlorophyll content. Chlorophyll is essential for photosynthesis, and higher levels of this pigment can lead to improved energy capture and utilization, further contributing to the plant's vigor and productivity (Fathi, 2022). In line with these results, Kandil & Özdamar (2023) reported higher chlorophyll content in plants in response to *Rhizobium* inoculation.

Table 3. Growth, chlorophyll content, and nodulation as affected by main effects of faba bean varieties, inoculation and biochar application

Treatments	Plant height (cm)	Chlorophyll content (%)	Nodule number ($plant^{-1}$)	Shoot dry weight ($g\ plant^{-1}$)	Root dry weight ($g\ plant^{-1}$)
Varieties					
Dosha	70.1^{b}	37.7^{a}	103.8^{a}	5.8^{a}	4.6^{a}
Gebelecho	69.3^{b}	37.4^{ab}	99.6^{b}	5.6^{ab}	4.3^{b}
Numan	72.7^{a}	37.6^{ab}	102.5^{a}	5.7^{ab}	4.3^{b}
Local	68.2^{b}	37.0^{b}	98.5^{b}	5.22^{b}	3.9^{c}
LSD	**2.0**	**0.63**	**3.96**	**0.46**	**0.22**
Rhizobium					
EAL-110	71.9^{a}	37.8^{a}	104.5^{a}	5.9^{a}	4.5^{a}
FB-EAR-15	71.5^{a}	38.1^{a}	104.7^{a}	5.8^{a}	4.4^{a}
FB-1035	68.5^{b}	37.2^{ab}	99.1^{b}	5.4^{b}	4.2^{a}
Un inoculated	68.0^{b}	36.6^{b}	87.3^{b}	5.2^{c}	3.9^{b}
LSD	**2.4**	**0.99**	**4.8**	**0.17**	**0.23**
Biochar ($t\ ha^{-1}$)					
0	63.6	36.0	90.75^{c}	4.8^{c}	3.8^{c}
5	70.7	37.7	102.0^{b}	5.7^{b}	4.3^{b}
10	75.9	38.7	111.5^{a}	6.3^{a}	4.85^{a}
$LSD_{0.05\%}$	**NS**	**NS**	**2.84**	**0.29**	**0.39**
$CV_{(\%)}$	**10.1**	**3.75**	**6.72**	**6.1**	**9.1**

Values followed by dissimilar letters in the column are statistically significant; NS = non-significant.

Nodule number and dry weights

The averaged data from the two sites revealed that nodule number, shoot and root dry weights were significantly ($p < 0.05$) affected by varieties, *Rhizobium* inoculation and biochar applications (Table 3). Likewise, the three-way interaction effect was significant on nodule dry weight of the faba bean (Table 4). Among the varieties, Dosha and Numan produced more nodules and shoot biomass compared to Gebelecho and the local varieties (Table 3). This marked increase in nodule formation indicates a more effective symbiotic relationship with N_2-fixing bacteria, allowing these varieties to utilize atmospheric N more efficiently. As a result, this enhanced nodulation ability plays a crucial role in their overall biomass production. This observation is consistent with findings from several researchers who have reported notable differences among the legume varieties studied. For example, Biruk et al. (2024) identified similar trends in their research, highlighting that certain legume varieties consistently perform better in nodule formation and biomass growth. Similarly, Tadewos et al. (2022) supported these conclusions, stressing the importance of selecting appropriate legume varieties to improve soil health and boost crop yields in agricultural practices. In addition, the ability of legumes to form nodules and produce biomass is largely influenced by a combination of soil characteristics and biological factors, including levels of available P, soil pH, type and vigor of legumes, rhizobial population, and their effectiveness (Gopalakrishnan et al., 2015). Moreover, different legume varieties exhibit varying capacities for nodule formation and biomass generation, which can be attributed to their genetic makeup and adaptability to specific environmental conditions (Yohannes et al., 2024).

The results revealed that plants inoculated with *Rhizobium* strains FB-EAR-15 and EAL-110 exhibited a greater number of nodules and higher biomass yields compared to those inoculated with strain FB-1035 or the uninoculated control (Table 3). The formation of more nodules is indicative of a more effective symbiotic relationship, as these nodules are the sites where N_2 fixation occurs. Consequently, the legumes that formed more nodules were able to access more fixed N, which is essential for their growth and development. The enhanced growth and dry matter accumulation observed in the plants inoculated with the more effective *Rhizobium* strains can primarily be attributed to their ability to supply fixed N directly to the legumes. This N is crucial for various physiological processes, including protein synthesis, enzyme function, and overall plant metabolism. As noted by Allito et al. (2020), the presence of compatible rhizobial strains not only boosts N_2 fixation but also contributes to the overall health and productivity of legume crops. Accordingly, the compatibility between legume species and the particular rhizobial strains present in the soil is vital for the success of their symbiotic association. When the right rhizobia are present, they significantly enhance legumes' ability to absorb N from the atmosphere, thus fostering their overall growth and biomass accumulation.

The use of biochar resulted in higher nodule formation and biomass than the control treatments (Table 3). This increase in the number of nodules can likely be attributed to the rise in soil pH, which creates a more conducive environment for beneficial soil bacteria. These bacteria play a crucial role in the N_2-fixing process, which is essential for the growth and development of leguminous plants. The elevated pH levels not only promote the proliferation of these beneficial microorganisms but also boost enzyme activity, which is vital for various biochemical processes in the soil. Furthermore, biochar contributes to the availability of essential nutrients, such as N, P, and K that support overall plant growth and health. Supporting this observation, Sisay & Abebawe (2021) found that faba beans grown in acidic soils showed the highest nodule counts when 20 t ha^{-1} of biochar was applied along with the recommended P. This finding underscores the importance of biochar in improving soil conditions, particularly in environments where soil acidity can limit plant performance. The synergistic effect of biochar and P not only enhances nodule formation but also promotes better nutrient uptake, leading to healthier and more productive plants. Additionally, Iijima et al. (2015) reported that the application of biochar led to improved growth, increased nodulation, and enhanced yields in bean crops. Their research highlights the multifaceted benefits of biochar, which include not only the physical and chemical improvements to soil structure but also the biological enhancements that facilitate plant growth. The increased nodulation observed in their studies suggests that biochar may help create a more favorable habitat for rhizobia, the N_2-fixing bacteria that form symbiotic relationships with leguminous plants.

The interaction between variety, inoculation, and biochar application revealed that the Gebelcho variety had the highest nodule dry weight when inoculated with strain EAL-110 and treated with 10 t ha^{-1} of biochar. However, when neither biochar application nor inoculation was used, the local variety showed the lowest nodule dry weight (Table 4). The average dry weight of nodules did not show marked differences among the three strains and various biochar rates. However, there were 29.8%, 24.2%, and 21.7% increments of the dry weight for the Gebelcho, Numan, and Dosha varieties, respectively, when inoculated with EAL-110 and treated with 10 t ha^{-1} of biochar, compared to the uninoculated control without biochar. This finding emphasizes the challenges faced by rhizobial species in acidic soils, which can decrease their viability and functionality, disrupting their symbiotic relationships with leguminous plants and hindering N_2 fixation. Acidic conditions can lead to nutrient deficiencies and decreased microbial activity. Conversely, biochar enhances soil structure, increases water retention, and boosts nutrient availability. Additionally, it stabilizes soil pH, creating a more favorable environment for the growth of rhizobia. These improvements encourage the proliferation and activity of *Rhizobium* species, strengthening their symbiotic relationship with leguminous plants, which is crucial for effective N_2 fixation and enhanced nutrient uptake. The observed differences confirm that nodulation depends on the interactions of the legume variety, the *Rhizobium* strain, and the biophysical environment of the soil (Giller et al., 2013). The present result was consistent with the findings of Allito et al. (2020), who reported a significant interaction of host varieties with rhizobial strains and location on symbiotic traits of faba bean varieties. Moreover, to optimize the benefits of symbiotic N_2 fixation, the inoculant strain must be efficient and match the desired variety in a growing agroecological zone (Beltayef et al., 2018).

Table 4. Three-way interaction effect of variety, inoculation and biochar on the nodule dry weight of faba bean

		Nodule dry weight (mg plant^{-1})		
		Biochar (t ha^{-1})		
Varieties	***Rhizobium***	**0**	**5**	**10**
Dosha	EAL-110	209.9 c-m	216.0 a-h	221.3 a-c
	FB-EAR-15	202.5 j-o	207.6 d-n	213.1 b-k
	FB-1035	199.8 l-p	211.9 b-l	217.7 a-e
	Uninoculated	207.47d-n	207.1 e-n	219.6 a-d
Gebelcho	EAL-110	201.2 k-p	217.2 a-f	225.8 a
	FB-EAR-15	203.5 i-o	212.3 b-k	216.4 a-g
	FB-1035	198.6 m-p	209.6 c-m	217.9 a-d
	Uninoculated	196.9 n-p	202.7 j-o	205.2 f-n
Numan	EAL-110	198.2 m-p	207.2 e-n	223.0 ab
	FB-EAR-15	196.4 n-p	215.4 a-i	220.1 a-c
	FB-1035	198.6 m-p	202.9 j-o	215.9 a-i
	Uninoculated	199.0 m-p	203.7 h-o	204.1 g-n
Local	EAL-110	189.4 pq	207.3 d-n	213.6 a-j
	FB-EAR-15	181.6 qr	191.3 o-q	209.6 c-m
	FB-1035	181.4 qr	207.4 d-n	211.6 b-l
	Uninoculated	175.5 r	206.3 e-n	207.7 d-n
LSD$_{0.05\%}$			12.4	
CV $_{(\%)}$			**9.9**	

Values followed by dissimilar letters in the column are statistically significant.

Effect of Treatments on Nutrient Concentration and Uptake

The combined data from the two sites revealed significant differences in nutrient concentration and uptake between the different faba bean varieties. The Dosha variety had the highest N and P concentrations, as well as the greatest nutrient uptake. The Gebelecho and Numan varieties closely followed, displaying impressive nutrient levels as well. In contrast, the local variety had the lowest concentrations and uptake of these nutrients (Table 5). The variations observed among different faba bean varieties provide an important foundation for the development of new cultivars with higher mineral content. In line with these findings, Etemadi et al. (2018) noticed differences in nutrient concentration and absorption among faba bean varieties. Similarly, Hacisalihoglu (2024) identified a group of faba beans with superior nutrient composition, which could be beneficial for research focused on genetic improvement and tackling future global food security issues.

The inoculation of *Rhizobium* significantly increased N and P concentrations, as well as improved faba bean uptake compared to the control treatment (Table 5). This marked increase in nutrient levels and absorption indicates that the *Rhizobium* strain used in this study has greater competitive advantages and effectiveness than the native rhizobia typically found in the soil. The increased N levels can be attributed to the symbiotic relationship formed between *Rhizobium* bacteria and the faba bean roots, which aids in converting atmospheric N into a form readily available for plant utilization. Similarly, the increase in P levels is vital, as P is an essential nutrient that plays a key role in various physiological processes, including energy transfer, photosynthesis, and the synthesis of nucleic acids. The improved P uptake observed in the faba beans treated with *Rhizobium* indicates that the introduced strain may also enhance the plant's ability to access this critical nutrient, potentially through mechanisms such as the secretion of root exudates that solubilize P or the formation of more extensive root systems (Janati et al., 2021). In line with this finding, Beyene & Tuji (2024) reported that inoculation resulted in enhanced plant growth and consequently increased biological activity associated with the roots of the host plant due to increased root exudation and organic matter decomposition, which in turn improved nutrient availability. Likewise, Khoso et al. (2023) found that plant growth-promoting rhizobacteria influences the overall physiology of plants, leading to improved nutrient absorption and enhanced root activity.

The interaction between variety, inoculation, and biochar application revealed that the Gebelcho variety had the highest nodule dry weight when inoculated with strain EAL-110 and treated with 10 t ha^{-1} of biochar. However, when neither biochar application nor inoculation was used, the local variety showed the lowest nodule dry weight (Table 4). The average dry weight of nodules did not show marked differences among the three strains and various biochar rates. However, there were 29.8%, 24.2%, and 21.7% increments of the dry weight for the Gebelcho, Numan, and Dosha varieties, respectively, when inoculated with EAL-110 and treated with 10 t ha^{-1} of biochar, compared to the uninoculated control without biochar. The application of biochar has been shown to remarkably enhance the concentrations and uptake of N and P compared to control treatments (Table 4). The increased N levels and plant uptake can be attributed to a variety of factors, including improved nodulation and root growth in faba beans following the addition of biochar to the soil. Improved nodulation is critical because it strengthens the symbiotic relationship between faba beans and N_2-fixing bacteria, resulting in greater N availability for the plants. Similarly, the increase in P uptake can be largely attributed to changes in soil pH caused by biochar's liming effects. When biochar is incorporated into the soil, the pH increases, allowing phosphate ions to be released more easily. In many soils, P is bound to Al and Fe, making it less available to plants. However, the higher pH levels caused by biochar application help to liberate these phosphate ions, increasing their availability for plant uptake. Furthermore, biochar not only provides essential nutrients but also helps faba beans absorb P more effectively. Its porous structure enhances soil aeration and water retention, promoting root growth and nutrient uptake. It is also high in organic carbon and minerals, which help to replenish the soil's nutrient levels. This enrichment not only increases the availability of nutrients for plant uptake, but it also promotes improved nutritional status and plant growth (Egamberdieva et al., 2021).

Table 5. Nutrient concentrations and uptake as affected by main effects of faba bean varieties, inoculation and biochar application

Treatments	N concentration (%)	N-uptake (mg g^{-1})	P concentration (%)	P-uptake (mg g^{-1})
Varieties				
Dosha	4.3^{a}	25.7^{a}	0.17^{a}	10.0^{a}
Gebelecho	4.3^{a}	24.6^{a}	0.17^{ab}	9.5^{ab}
Numan	4.2^{a}	24.6^{a}	0.17^{ab}	9.5^{ab}
Local	3.9^{b}	21.04^{b}	0.16^{b}	8.6^{b}
LSD	**0.19**	**2.26**	**0.005**	**0.96**
Rhizobium				
EAL-110	4.6^{a}	27.4^{a}	0.17^{a}	10.1^{ab}
FB-EAR-15	4.4^{b}	25.7^{b}	0.17^{a}	10.3^{a}
FB-1035	4.1^{c}	22.7^{c}	0.16^{ab}	9.00^{bc}
Uninoculated	3.7^{d}	19.8^{d}	0.15^{b}	8.2^{c}
LSD	**0.19**	**1.28**	**0.01**	**1.2**
Biochar (t ha⁻¹)				
0	3.6^{c}	17.4^{c}	0.18^{a}	11.3^{a}
5	4.3^{b}	24.6^{b}	0.17^{b}	9.6b
10	4.7^{a}	29.7^{a}	0.15^{c}	11.3^{a}
$LSD_{0.05\%}$	**0.29**	**2.19**	**0.01**	**1.1**
$CV_{(\%)}$	**8.4**	**10.3**	**6.74**	**9.7**

Values followed by dissimilar letters in the column are statistically significant; NS = non-significant.

Correlation Analysis

A significant positive correlation was found between shoot dry matter and various factors, including chlorophyll content (r = 0.49), nodule number (r = 0.78), root dry weight (r = 0.72), N and P concentration (r = 0.78 and r = 0.65), and uptake (r = 0.93 and r = 0.94), respectively (Table 6). This suggests that faba bean varieties with higher shoot dry weight would have better growth, nodulation, and higher nutrient concentration and uptake, which in turn increases productivity. In addition, the positive relationship between shoot dry weight and root nodulation indicates improved N_2 fixation, which, in turn, leads to higher crop growth and dry matter production. In line with this finding, Oono & Denison (2010) suggested that shoot dry weight and nodulation were good indicators of symbiotic N_2 fixation efficiency in legumes. Moreover, Mothapo et al. (2013) indicated that plants that formed more nodules produced higher shoot and root dry matter. The positive association between shoot dry weight and leaf chlorophyll content (r = 0.49) further suggests enhanced rates of photosynthetic and carbohydrate accumulation in faba beans through improved plant N content. The stronger association of shoot dry weight with root dry weight could be due to the stronger association of root dry weight with that of N and P concentration that ultimately enhances the growth and development of below- and aboveground parts of the faba bean plant.

Table 6. Correlation (r) between shoot dry weight and nodulation, growth, and nutrient concentration and uptake of faba bean varieties

Parameters	Pearson's correlation coefficient (r)	P value
Shoot dry weight versus		
chlorophyll content	0.49	**
nodule number	0.78	***
root dry weight	0.73	***
N concentration	0.78	***
P concentration	0.65	***
N uptake	0.93	***
P uptake	0.94	***

N = nitrogen, P = phosphorous, **, ***, significant at the p≤0.01 and p≤0.001 levels, respectively.

CONCLUSIONS

Soil acidity presents a significant challenge for the successful cultivation of faba beans, impacting factors such as plant growth, nodulation, and nutrient absorption. This research demonstrates that incorporating coffee husk biochar and inoculating faba bean seeds with different strains of rhizobial bacteria resulted in notable improvements in the soil's physicochemical properties. These improvements led to better nutrient uptake, increased growth rates, and enhanced nodulation in faba bean plants. Thus, the strategic use of efficient inoculants and biochar has been critical in increasing the agrosymbiotic and nutrient use efficiency of faba bean varieties. Nevertheless, it is essential to conduct field tests on these amendments before offering reliable recommendations to our farmers, thereby assisting them in boosting production and ensuring their household food security.

Data Availability

The data used to support the results of this study are included within the manuscript and any further information is available from the corresponding author upon request.

Disclosure statement

The authors declare that they have no conflicts of interest.

REFERENCES

Adissie, S., Gedamu, E., Tsegaye, A., & Feyisa, T. 2018. Effect of rhizobial inoculants on yield and yield components of faba bean (*Vicia fabae* L.) on vertisols of were Illu District, south Wollo, Ethiopia. *CABI Agriculture and Bioscience*, *2*. [Scholar Google]

Afshar, M., & Mofatteh, S. 2024. Biochar for a sustainable future: Environmentally friendly production and diverse applications. *Results in Engineering*, 23: 102433. https://doi.org/10.1016/j.rineng.2024.102433. [Scholar Google]

Alkharabsheh, H. M., Seleiman, M. F., Battaglia, M. L., Shami, A., Jalal, R. S., Alhammad, B. A., ... & Al-Saif, A. M. 2021. Biochar and its broad impacts in soil quality and fertility, nutrient leaching and crop productivity: A review. *Agronomy*, *11*(5), 993. https://doi.org/10.3390/agronomy11050993. [Scholar Google]

Allito, B. B., Ewusi-Mensah, N., & Logah, V. 2020. Legume-rhizobium strain specificity enhances nutrition and nitrogen fixation in faba bean (*Vicia faba* L.). *Agronomy*, *10*(6): 826. https://doi.org/10.3390/agronomy10060826. [Scholar Google]

Anderson, J. M., & Ingram, J. S. 1994. Tropical soil biology and fertility: a handbook of methods. *Soil Science*, *157*(4): 265. [Scholar Google]

ASTM. 2009. Standard Test Method for Chemical Analysis of Wood Charcoal." American Society for Testing and Materials, Conshohocken, PA, USA.

Bedassa, M. 2020. Soil acid management using biochar: Review. *International Journal of Agricultural Science and Food Technology*, *6*: 211-217. https://dx.doi.org/10.17352/ijasft. [Scholar Google]

Bekchanova, M., Campion, L., Bruns, S., Kuppens, T., Lehmann, J., Jozefczak, M., ... & Malina, R. 2024. Biochar improves the nutrient cycle in sandy-textured soils and increases crop yield: a systematic review. *Environmental evidence*, *13*(1). https://doi.org/10.1186/s13750-024-00326-5. [Scholar Google]

Beltayef, H., Melki, M., Saidi, W., Samaali, S., Muscolo, A., Cruz, C., & Garoui, T. 2018.

Betterment of biological nitrogen fixation in snap bean under Mediterranean semi-arid conditions. *Bulgarian Journal of Agricultural Science*, *24*(2): 244-251. [Scholar Google]

Beruk, H., Yoseph, T., & Ayalew, T. 2024. Unlocking the potential of inoculation with *Bradyrhizobium* for enhanced growth and symbiotic responses in soybean varieties under controlled conditions. *Agronomy*, *14*(6). https://doi.org/10.3390/agronomy14061280. [Scholar Google]

Beyene, B. B., & Tuji, F. A. 2024. Inoculation of Erythrina brucei with plant-beneficial microbial consortia enhanced its growth and improved soil nitrogen and phosphorous status when applied as green manure. *Heliyon*, *10*(9): e30484. https://doi.org/10.1016/j.heliyon.2024.e30484. [Scholar Google]

Bray, R. H., & Kurtz, L. T. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, *59*(1): 39-46. [Scholar Google]

CSA. 2022. Federal Democratic Republic of Ethiopia, Agricultural Sample Survey, Report on Area and Production of Major Crops Statistical Bulletin 584, Addis Ababa, Ethiopia," 1: 1-121.

Debele, B. 1982. The physical criteria and their rating proposed for land evaluation in the highland regions of Ethiopia [soil potentiality]. *World Soil Resources Reports (FAO)*, (54).

Dewis, J., & Freitas, F. 1970. Physical and chemical methods of soil and water analysis. 10: 275.

Dume, B., Berecha, G., & Tulu, S. 2015. Characterization of biochar produced at different temperatures and its effect on acidic nitosol of Jimma, Southwest Ethiopia. *International Journal of Soil Science*, *10*(2): 63. [Scholar Google]

Egamberdieva, D., Ma, H., Alaylar, B., Zoghi, Z., Kistaubayeva, A., Wirth, S., & Bellingrath-Kimura, S. D. 2021. Biochar amendments improve licorice (*Glycyrrhiza uralensis* Fisch.) growth and nutrient uptake under salt stress. *Plants*, *10*(10): 2135. https://doi.org/10.3390/plants10102135. [Scholar Google]

Enesi, R. O., Dyck, M., Chang, S., Thilakarathna, M. S., Fan, X., Strelkov, S., & Gorim, L. Y. 2023. Liming remediates soil acidity and improves crop yield and profitability-a meta-analysis. *Frontiers in Agronomy*, *5*: 1194896. https://doi.org/10.3389/fagro.2023.1194896. [Scholar Google]

Etemadi, F., Barker, A. V., Hashemi, M., Zandvakili, O. R., & Park, Y. 2018. Nutrient accumulation in faba bean varieties. *Communications in Soil Science and Plant Analysis*, *49*(16): 2064-2073. https://doi.org/10.1080/00103624.2018.1495729. [Scholar Google]

Ethio, SIS. 2014. Soil fertility and fertilizer recommendation atlas of Tigray region. *Ministry of Agriculture (MOA) and Agricultural Transformation Agency (ATA)*.

FAOSTAT. 2021. Food and Agriculture Organization Corporate Statistical Database. http://www.fao.org/faostat/en/#data/QC

Fathi, A. 2022. Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: A. *Agrisost*, *28*: 1-8. https://doi.org/10.5281/zenodo.7143588. [Scholar Google]

Fekadu, E., Kibret, K., Melese, A., & Bedadi, B. 2018. Yield of faba bean (*Vicia faba* L.) as affected by lime, mineral P, farmyard manure, compost and rhizobium in acid soil of Lay Gayint District, northwestern highlands of Ethiopia. *Agriculture & Food Security*, *7*: 1-11. https://doi.org/10.1186/s40066-018-0168-2. [Scholar Google]

Gaskin, J. W., Steiner, C., Harris, K., Das, K. C., & Bibens, B. 2008. Effect of low-temperature pyrolysis conditions on biochar for agricultural use. *Transactions of the ASABE*, *51*(6): 2061-2069. https://doi.org/10.13031/2013.25409. [Scholar Google]

Ghorbi, S., Ebadi, A., Parmoon, G., Siller, A., & Hashemi, M. 2023. The use of faba bean cover crop to enhance the sustainability and resiliency of no-till corn silage production and soil characteristics. *Agronomy*, *13*(8): 2082. https://doi.org/10.3390/agronomy13082082. [Scholar Google]

Giller, K. E., Franke, A. C., Abaidoo, R., Baijukya, F., Bala, A., Boahen, S., ... & Vanlauwe, B. 2013. N2Africa: putting nitrogen fixation to work for smallholder farmers in Africa. In *Agro-ecological intensification of agricultural systems in the African highlands*, 156-174. [Scholar Google]

Golla, A. S. 2019. Soil acidity and its management options in Ethiopia: A review. *International Journal of Scientific Research and Management*, *7*(11): 1429-1440. https://doi.org/10.18535/ijsrm/v7i11.em01.

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L., & Krishnamurthy, L. 2015. Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech*, *5*: 355-377. https://doi.org/10.1007/s13205-014-0241-x. [Scholar Google]

Hacisalihoglu, G. 2024. Analysis of Nutritional Traits: Natural Variation within 90 Diverse Faba Bean (*Vicia faba*) Genotypes and Daily Value Contribution. *Crops*, *4*(3): 440-446. https://doi.org/10.3390/crops4030031. [Scholar Google]

Hazelton, P., & Murphy, B. 2016. *Interpreting soil test results: What do all the numbers mean?*. CSIRO publishing.

Iijima, M., Yamane, K., Izumi, Y., Daimon, H., & Motonaga, T. 2015. Continuous application of biochar inoculated with root nodule bacteria to subsoil enhances yield of soybean by the nodulation control using crack fertilization technique. *Plant Production Science*, *18*(2): 197-208. https://doi.org/10.1626/pps.18.197. [Scholar Google]

Janati, W., Benmrid, B., Elhaissoufi, W., Zeroual, Y., Nasielski, J., & Bargaz, A. 2021. Will phosphate bio-solubilization stimulate biological nitrogen fixation in grain legumes?. *Frontiers in Agronomy*, *3*: 637196. https://doi.org/10.3389/fagro.2021.637196. [Scholar Google]

Jeffery, S., Bezemer, T. M., Cornelissen, G., Kuyper, T. W., Lehmann, J., Mommer, L., ... & van Groenigen, J. W. 2015. The way forward in biochar research: targeting trade-offs between the potential wins. *Gcb Bioenergy*, *7*(1): 1-13. https://doi.org/10.1111/gcbb.12132. [Scholar Google]

Kandil, A. E., & Özdamar Ünlü, H. 2023. Effect of rhizobium inoculation on yield and some quality properties of fresh cowpea. *Cogent Food & Agriculture*, *9*(2): 2275410. https://doi.org/10.1080/23311932.2023.2275410. [Scholar Google]

Kapoor, A., Sharma, R., Kumar, A., & Sepehya, S. 2022. Biochar as a means to improve soil fertility and crop productivity: a review. *Journal of Plant Nutrition*, *45*(15): 2380-2388. https://doi.org/10.1080/01904167.2022.2027980. [Scholar Google]

Kebede, E. 2021. Competency of rhizobial inoculation in sustainable agricultural production and biocontrol of plant diseases. *Frontiers in Sustainable Food Systems*, *5*: 728014. https://doi.org/10.3389/fsufs.2021.728014. [Scholar Google]

Kermah, M., Franke, A. C., Adjei-Nsiah, S., Ahiabor, B. D. K., Abaidoo, R. C., & Giller, K. E. 2018. N2-fixation and N contribution by grain legumes under different soil fertility status and cropping systems in the Guinea savanna of northern Ghana. *Agriculture, Ecosystems & Environment*, *261*: 201-210. https://doi.org/10.1016/j.agee.2017.08.028. [Scholar Google]

Khan, S., Irshad, S., Mehmood, K., Hasnain, Z., Nawaz, M., Rais, A., ... & Ibrar, D. 2024. Biochar production and characteristics, its impacts on soil health, crop production, and yield enhancement: A review. *Plants*, *13*(2): 166. https://doi.org/10.3390/plants13020166. [Scholar Google]

Khoso, M. A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., ... & Liu, F. 2023. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. *Plant Stress*, 11: 100341. https://doi.org/10.1016/j.stress.2023.100341. [Scholar Google]

Lindsay, W. L., & Norvell, W. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil science society of America journal*, *42*(3): 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x. [Scholar Google]

McLean, E. O. 1965. *Aluminum. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 9: 978-998.* https://doi.org/10.2134/agronmonogr9.2.c16.

Mínguez, M. I., & Rubiales, D. 2021. Faba bean. In *Crop physiology case histories for major crops*, 452-481. Academic Press. https://doi.org/10.1016/B978-0-12-819194-1.00015-3.

Mitiku, G., & Mnalku, A. 2019. Faba Bean (*Vicia faba* L.) Yield and yield components as influenced by inoculation with indigenous rhizobial isolates under acidic soil condition of the central highlands of Ethiopia. *Ethiopian Journal of Agricultural Sciences*, *29*(3): 49-61. [Scholar Google]

MoA. 2020. Sustainable use of rehabilitated land for economic development (SURED) programmeTechnical manual. Soil fertility management Second edition https://www.moa.gov.et/.

Mothapo, N. V., Grossman, J. M., Sooksa-Nguan, T., Maul, J., Bräuer, S. L., & Shi, W. 2013. Cropping history affects nodulation and symbiotic efficiency of distinct hairy vetch (*Vicia villosa* Roth.) genotypes with resident soil rhizobia. *Biology and Fertility of Soils*, *49*: 871-879. https://doi.org/10.1007/s00374-013-0781-y. [Scholar Google]

Nepal, J., Ahmad, W., Munsif, F., Khan, A., & Zou, Z. 2023. Advances and prospects of biochar in improving soil fertility, biochemical quality, and environmental applications. *Frontiers in Environmental Science*, *11*: 1114752. https://doi.org/10.3389/fenvs.2023.1114752. [Scholar Google]

Olsen, S. R., & Sommers, L. E. 1982. Phosphorus. Page AL, Miller RH, Keeney DR (eds). Methods of soil analysis, Part 2. American Society of Agronomy Inc. *Soil Science Society of America Inc., Madison, Wisconsin USA*.

Oono, R., & Denison, R. F. 2010. Comparing symbiotic efficiency between swollen versus nonswollen rhizobial bacteroids. *Plant Physiology*, *154*(3): 1541-1548. https://doi.org/10.1104/pp.110.163436. [Scholar Google]

Polemio, M., & Rhoades, J. D. 1977. Determining cation exchange capacity: A new procedure for calcareous and gypsiferous soils. *Soil Science Society of America Journal*, *41*(3): 524-528. https://doi.org/10.2136/sssaj1977.03615995004100030018x. [Scholar Google]

Premalatha, R. P., Poorna Bindu, J., Nivetha, E., Malarvizhi, P., Manorama, K., Parameswari, E., & Davamani, V. 2023. A review on biochar's effect on soil properties and crop growth. *Frontiers in Energy Research*, *11*: 1092637. https://doi.org/10.3389/fenrg.2023.1092637. [Scholar Google]

Ryan, J., Estefan, G., & Rashid, A. 2001. Soil and plant analysis laboratory manual, International Centre for Agricultural Research in the Dry Areas (ICARDA). *Aleppo and National Agricultural Research Centre (NARC), Islamabad, Pakistan*, *172*.

Samago, T. Y., Anniye, E. W., & Dakora, F. D. 2018. Grain yield of common bean (*Phaseolus vulgaris* L.) varieties is markedly increased by rhizobial inoculation and phosphorus application in Ethiopia. *Symbiosis*, *75*: 245-255. https://doi.org/10.1007/s13199-017-0529-9. [Scholar Google]

Sanvong, C., & Nathewet, P. 2014. A Comparative Study of Pelleted Broiler Litter Biochar Derived from Lab-Scale Pyrolysis Reactor with that Resulted from 200-Liter-Oil Drum Kiln to Ameliorate the Relations between Physicochemical Properties of Soil with Lower Organic Matter Soil and Soybean Yield. *Environment Asia*, *7*(1): 95-103. [Scholar Google]

Sisay, A., & Abebawe, A. 2021. Response of Coffee Husk Biochar on Growth of Faba Bean on Nitisol and Soil Nutrients. *International Journal of Energy and Environmental Science*, *6*(4): 78-85. https://doi.org/10.11648/j.ijees.20210604.12. [Scholar Google]

Tadele, M., Mohammed, W., & Jarso, M. 2019. Genetic variability on grain yield and related agronomic traits of faba bean (*Vicia faba* L.) genotypes under soil acidity stress in the central highlands of Ethiopia. *Chemical and Biomolecular Engineering*, *4*(4): 52-58. https://doi.org/10.11648/j.cbe.20190404.12. [Scholar Google]

Tadesse, N., Yoseph, T., & Demrew, Z. 2025. Agronomic Performances of Faba Bean (*Vicia faba* L.) Varieties to Rhizobial Inoculation, Biochar and Lime Applications under Acidic Soil Conditions in Ethiopia. *Food and Energy Security*, *14*(3): e70091. https://doi.org/10.1002/fes3.70091. [Scholar Google]

Tadesse, N., Yoseph, T., Demrew, Z., & Nebiyu, A. 2025. Improving faba bean (*Vicia faba* L.) productivity and nutrient availability through organic amendments and bio-inoculants in acidic soils stress. *Agrosystems, Geosciences & Environment*, *8*(1): e70073.

https://doi.org/10.1002/agg2.70073. [Scholar Google]

Tadesse, T., Haque, I., & Aduayi, E. A. 1991. Soil, plant, water, fertilizer, animal manure and compost analysis. Working Document No. 13. *International Livestock Research Center for Africa*.

Tadewos, T., Ayalew, T., & Yoseph, T. 2022. Effect of biological and chemical fertilizers combination on yield of Mung Bean (*Vigna radiate* L. Wilczek) at Hawassa Southern Ethiopia. *Plant*, *10(4)*: 97-104. https://doi.org/10.11648/j.plant.20221004.12. [Scholar Google]

Tazebew, E., Addisu, S., Bekele, E., Alemu, A., Belay, B., & Sato, S. 2024. Sustainable soil health and agricultural productivity with biochar-based indigenous organic fertilizers in acidic soils: insights from Northwestern Highlands of Ethiopia. *Discover Sustainability*, *5*(1): 205. https://doi.org/10.1007/s43621-024-00380-6. [Scholar Google]

Theeuwen, T. P., Logie, L. L., Harbinson, J., & Aarts, M. G. 2022. Genetics as a key to improving crop photosynthesis. *Journal of Experimental Botany*, *73*(10): 3122-3137. https://doi.org/10.1093/jxb/erac076. [Scholar Google]

Tusar, H. M., Uddin, M. K., Mia, S., Suhi, A. A., Wahid, S. B. A., Kasim, S., ... & Anwar, F. 2023. Biochar-acid soil interactions—A review. *Sustainability*, *15*(18): 13366. https://doi.org/10.3390/su151813366. [Scholar Google]

Van Reeuwijk, L. P. 2002. Procedures for soil analysis: Wageningen, International Soil Reference and Information Centre. 6th.

Walkley, A., & Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, *37*(1): 29-38. [Scholar Google]

Warke, A. T., & Wakgari, T. 2024. A Review on the Impact of Soil Acidification on Plant Nutrient Availability, Crop Productivity, and Management Options in the Ethiopian Highlands. *Agriculture, Forestry and Fisheries 13(2):* 31-45. https://doi.org/10.11648/j.aff.20241302.13. [Scholar Google]

Yadav, S. P. S., Bhandari, S., Bhatta, D., Poudel, A., Bhattarai, S., Yadav, P., ... & Oli, B. 2023. Biochar application: A sustainable approach to improve soil health. *Journal of Agriculture and Food Research*, *11*: 100498. https://doi.org/10.1016/j.jafr.2023.100498. [Scholar Google]

Yohannes, L., Yoseph, T., & Ayalew, T. 2024. *Bradyrhizobium* Inoculation Improved Agrosymbiotic Performances of Cowpea [*Vigna unguiculata* (L) Walp] Varieties at Two Sites in Ethiopia. *International Journal of Agronomy*, *2024*(1): 6582068. https://doi.org/10.1155/2024/6582068. [Scholar Google]

Zhang, M., Riaz, M., Zhang, L., El-Desouki, Z., & Jiang, C. 2019. Biochar induces changes to basic soil properties and bacterial communities of different soils to varying degrees at 25 mm rainfall: More effective on acidic soils. *Frontiers in Microbiology*, *10*: 1321. https://doi.org/10.3389/fmicb.2019.01321. [Scholar Google]

Zhu, P. K., Zeng, M. Y., Lin, Y. H., Tang, Y., He, T. Y., Zheng, Y. S., & Chen, L. Y. 2024. Variability in leaf color induced by chlorophyll deficiency: Transcriptional changes in bamboo leaves. *Current Issues in Molecular Biology*, *46*(2): 1503-1515. https://doi.org/10.3390/cimb46020097. [Scholar Google]

Original Research Article||

Prevalence and Predictors of Undernutrition Among Women of Reproductive Age Receiving Antiretroviral therapy (ART) at Yirgalem Hospital: Evidence from a Cross-Sectional Analysis

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Abstract

Background: Human Immunodeficiency Virus (HIV) and malnutrition are interconnected in a vicious cycle, where each condition can independently cause progressive damage to the immune system. Recent, site-specific evidence on the prevalence and predictors of undernutrition among reproductive-age (15–49 years) women on ART at Yirgalem Hospital is lacking, as most Ethiopian studies focus on broader adult ART populations or other groups, leaving a gap for targeted, facility-level analysis. Assessing nutritional status in ART patients is vital, as it directly affects treatment effectiveness and outcomes, underscoring the need for integrated care.

Method: A cross-sectional study was conducted with randomly sampled Reproductive Age Women (15-49 yrs) on ART (n=268), to examine the prevalence of undernutrition and its correlates from February to May 2023. Data on socio-economic and demographic characteristics, nutrition-related factors, and health indicators—including CD4 count, WHO clinical stage, and opportunistic infections—were collected. Descriptive statistics such as frequencies, percentages, means, and standard deviations were calculated. Associations between socio-demographic and predictor variables were assessed using binary and multivariable logistic regression, with statistical significance set at $p < 0.05$ and 95% confidence intervals.

Result: Nearly 39% of reproductive age women on ART were undernourished (BMI < 18.5 kg/m2). Women with no formal education (AOR=3.10(1.63, 7.60), advanced WHO Clinical stages (AOR=3.30(1.53, 7.11)), poor adherence to ART in past six months (AOR=3.43(1.78, 6.61), and food insecurity (AOR=2.10(1.25, 4.34) were significantly associated with undernutrition. Conclusion: Undernutrition remains a significant public health issue among reproductive-age women on ART. Key factors such as basic education for mothers, improving ART adherence, and addressing food insecurity are critical in tackling this problem. A longitudinal study is recommended to better understand these factors and develop evidence-based interventions aimed at improving the nutritional and health outcomes of women on ART.

Keywords: Antiretroviral Therapy, Adherence, Educational status, Food insecurity, Undernutrition

Original submission: November 14, 2024; **Revised submission**: December 14, 2025; **Published online**: December 31, 2025

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INTRODUCTION

Human Immunodeficiency Virus (HIV) is a major public health problem and is closely linked to undernutrition, as it increases the risk of nutrient deficiencies and weight loss among affected individuals (Gona et al., 2020; Woldemariam et al., 2015). According to the Joint United Nations Program on HIV/AIDS (UNAIDS), an estimated 38.4 million people are living with HIV worldwide. Of these, 67% are in the African Region, with 1.5 million new infections and 650,000 HIV-related deaths reported globally (UNAIDS, 2022). Furthermore, 53% of people living with HIV/AIDS were women and girls (WHO, 2022), and 46% of all new HIV infections were among women and girls (all ages) in 2022. In sub-Saharan Africa, women and girls (all ages) accounted for 63% of all new HIV infections (UNAIDS, 2023).

In sub-Saharan Africa, women of reproductive age account for over 60% of adults living with HIV

(Ntlansana et al., 2019; Westreich et al., 2011). In Ethiopia, more than half a million people were living with HIV/AIDS in 2017, with women of childbearing age representing the majority (FMOH, 2018; Ayele et al., 2018). Although prevalence varies across regions, the national adult HIV prevalence (ages 15–49) was 0.93% in 2019, with women comprising 61% of infections (FHAPCO, 2023). Women often demand to initiate ART earlier and utilize healthcare services more than men but may face incomplete adherence due to childcare responsibilities, economic pressures, and lack of partner support (El-Khatib et al., 2011). Pregnancy can further compromise treatment outcomes, potentially due to physiological changes such as increased blood volume and body mass, which may result in under-dosing of ART (Westreich et al., 2011; Momper et al., 2021; Saliya MS, 2018).

A significant proportion of individuals who require Antiretroviral Therapy (ART) are malnourished because of low energy intake combined with increased energy demands due to HIV and other related infections (Seid et al., 2023). Undernutrition constitutes an important threat to the success of HIV programs in sub-Saharan Africa (Fuseini et al., 2021). HIV affects nutrition by reducing food consumption, impairing digestion and nutrient absorption; causing changes in metabolism, the virus also directly attacks and destroys the cells of the immune system (Kotler, 2000). HIV/AIDS incidentally occurs in populations where malnutrition is already an epidemic. In Africa, early evidence suggested that HIV infection has a direct effect on nutritional status (Koethe et al., 2010).

A low body mass index (BMI) is a well-established indicator of poor nutritional status and has been consistently linked to reduced immune recovery, advanced HIV disease, and increased mortality among people on ART (Fuseini et al., 2021; Steinhart, 2001; Crum-Cianflone et al., 2011). Evidence from Southern Ethiopia, including Hawassa and surrounding areas, shows that women and adolescents on ART frequently experience poor dietary diversity and high levels of undernutrition, largely due to persistent food insecurity and limited access to nutrient-rich foods (Shiferaw and Gebremedhin, 2020; Markos et al., 2020). Despite national and regional initiatives to incorporate nutrition services into HIV care in Ethiopia (FMOH, 2018) and the availability of regional studies documenting the burden of undernutrition among individuals on ART (Gebremichael, 2018; Gebru et al., 2020), there remains a notable gap in facility-level assessments that evaluate the effectiveness of targeted interventions aimed at mitigating undernutrition among adults receiving ART. In response to these challenges, this study was designed to assess the prevalence and associated factors of undernutrition among reproductive-age women receiving ART at Yirgalem General Hospital.

MATERIALS AND METHODS

Study Design and Period

An institution-based cross-sectional study was carried out at Yirgalem General Hospital in the Sidama region of Ethiopia from February to May 2023. The hospital, situated in Yirgalem town, is located 47 km southeast of the regional capital, Hawassa, and 260 km from Addis Ababa, the capital of Ethiopia at a latitude of 6°45′N, longitude 38°25′E, and an elevation of 1,725 meters above sea level (SZBoFED, 2007). During the study period, 700 HIV-positive women of reproductive age were receiving ART services at the hospital.

Source and Study Population

The source populations were all HIV-positive women from 15 to 49 years old were attending at ART clinic of the Yirgalem General Hospital. The study populations were randomly selected HIV-positive women from 15-49 years old fulfilling the inclusion criteria and attending ART clinic of the Yirgalem General Hospital during the study period.

Inclusion and Exclusion Criteria

All HIV-positive women aged 15–49 years on ART at least for six months and visited the ART clinic at Yirgalem General Hospital during the study period were considered eligible for inclusion in the study. Pregnant and lactating women, as well as those who were seriously ill and unable to participate, were excluded to avoid confounding factors related to pregnancy, lactation, or severe illness.

Sample size Determination

We calculated the sample size separately for the two objectives, estimating prevalence and identifying associated factors—using the single population proportion formula and selected the larger estimate.

For the first objective, the sample size was computed using the single population proportion formula, a 95% confidence level ($Z\alpha/2 = 1.96$), a 5% margin of error ($d = 0.05$), and the prevalence of undernutrition reported from Humera Hospital, Ethiopia (Hadgu et al., 2013), with an additional 10% for non-response. Given that approximately 700 HIV-positive reproductive-age women attended ART services at Yirgalem General Hospital during the study period, a population correction formula was applied, yielding a final sample size of 268.

For the second objective, the sample size was determined using the double population proportion formula in Epi Info 7.2, based on findings from a study conducted in public health facilities in West Shewa Zone, Central Ethiopia on food insecurity and nutritional status among people living with HIV/AIDS (Gebremichael et al., 2018). Using a 95% confidence level, 80% power, a 1:1 ratio of exposed to unexposed, the estimated proportion of outcome among the unexposed, and a 10% non-response rate, the calculated sample size was 172. After comparing the results from both objectives, the larger sample size—268—was selected as the final sample size for this study.

Table 1. Sample size estimation for identifying associated factors using the single population proportion formula

Variables	Assumption	Percent unexposed with outcome	Sample size	Final sample size after adding for non response rate
WHO clinical stage III/V	Power 80, CI 95, unexposed to exposed ratio 1, OR=3.3	71	76	86
Food security status	Power 80, CI 95, unexposed to exposed ratio 1, OR=5.3	47	152	172

Sampling technique

Participants were selected at random from reproductive-age women (15–49 years) attending ART follow-up care at Yirgalem General Hospital between February and May 2023, using predefined inclusion criteria to ensure that every eligible woman had an equal chance of being included rather than being chosen based on convenience or personal judgment. After identifying eligible patient medical records, relevant clinical, laboratory, and demographic information was collected using a data abstraction form designed to capture these details. The data abstraction form was developed based on the ART entry and follow-up forms used in the ART clinic at Yirgalem General Hospital and by the Ministry of Health of Ethiopia. A pretest was conducted to gain insights into the data abstraction process, assess how information was extracted from medical records, and refine the form before finalizing it for the study.

Data Collection

Data were collected using a pre-tested, structured questionnaire administered through face-to-face interviews to capture socio-demographic, behavioral, and anthropometric information. Nutritional status was assessed using BMI, calculated from weight and height measurements, with a BMI <18.5 kg/m^2 indicating undernutrition (Gibson and Meredith-Jones, 2024). Household food insecurity was measured using the nine-item Household Food Insecurity Access Scale (HFIAS) (Coates et al., 2007; MOH, 2018), while dietary intake was evaluated via a 24-hour recall determining dietary diversity and meal frequency (FAO, 2016). ART adherence was categorized as good (≥95%) or poor (≤94%) based on documented missed doses (Gebru et al., 2020). Two trained nurses from Yirgalem General Hospital collected primary data under the supervision of a senior health professional, with training provided on study objectives, data collection principles, and ethical considerations. Primary data were obtained directly from participants through interviews and anthropometric measurements, while secondary data were extracted from hospital records such as patient charts.

Variables

The main outcome of this study was undernutrition, assessed using body mass index (BMI), calculated as weight in kilograms divided by height in meters squared. The study's independent variables encompassed various socio-demographic and economic factors, including age, sex, ethnicity, religion, marital status, education level, monthly income, and occupation Additionally, factors like internalized stigma, living conditions, and social support were considered, as these variables may influence the respondents' experiences and perceptions related to food insecurity or financial constraints. Nutritional-related factors (nutritional counseling) Medical factors (CD4 count, HIV/AIDS stages, HIV-related disease)

Data Quality Procedures

To ensure data quality, the principal investigator closely supervised all stages of data collection, monitoring completeness, accuracy, and reliability. The questionnaire was initially prepared in English, translated into Amharic, and back translated into English to ensure consistency. It was pre-tested on 5% of participants in a similar setting to assess clarity, sensitivity, and completeness, with modifications made as needed. Data collectors, including nurses, health officers, and human nutrition professionals, received two days of training on questionnaire and anthropometric measurements. Weight and height were measured to the nearest 10 g and 0.1 cm, respectively. Daily feedback and corrections were provided by the supervisor to maintain high data quality throughout the collection process.

Data Analysis

Data was entered into EpiData version 3.1 and analyzed using SPSS version 26. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used to summarize participant characteristics. Associations between undernutrition and potential predictors were assessed using bivariate and multivariable logistic regression, with adjusted odds ratios (AOR) and 95% confidence intervals (CI) reported. Statistical significance was set at $p < 0.05$, and multicollinearity was checked using variance inflation factors (VIFs). To ensure data quality, the principal investigator provided daily supervision and feedback to data collectors, and all completed questionnaires and abstraction forms were regularly checked for completeness, accuracy, and consistency, with any discrepancies addressed promptly.

RESULTS AND DISCUSSION

Socio-economic and Demographic Characteristics of Respondents

All 268 selected women participated in the study, yielding a 100% response rate. The respondents had a mean age of 37.7 (±7.03) years. About one-third (33.6%) were of Sidama ethnicity, and most participants (71.3%) resided in urban areas. Nearly 72% reported a monthly income of less than 700 ETB. (Table 1).

Clinical and Health-related Characteristics of the Respondents

The majority, 79.1% of the study participants were on ART at least for one year, and 20.9% of clients reported having gastrointestinal symptoms in the past six months. Only 18.3% of respondents experienced eating difficulty in the past six months. Nearly 31.4% of the respondents were in WHO clinical stage I, and the majority, 72.8% of the clients had good adherence to ART in the past six months (Table 2).

Nutritional, Food Security, and Behavioral Characteristics of the Participants

The dietary assessment showed that most participants reported consuming cereals, legumes, and dark green vegetables within the 24 hours preceding the survey. In contrast, the intake of animal-source foods, including meat/fish, milk and milk products, and eggs—was notably low (Figure 1). Nearly three-quarters of the respondents (74%) consumed three or more meals during the same period. Despite this, approximately 35% of households experienced food insecurity, and the prevalence of undernutrition (BMI < 18.5 kg/m²) among the women was high at 39.2%. Moreover, the vast majority of participants (83.6%) reported no current alcohol consumption (Table 3).

Factors Associated with Undernutrition among Respondents

Of the eight variables with a p-value <0.25 in the bivariate analysis, only four—educational level, WHO clinical stage, ART adherence in the past six months, and household food security status—remained significant predictors of undernutrition in the multivariable logistic regression model (Table 4). Women with no formal education were over three times more likely to be undernourished compared to those with formal education (AOR = 3.10; 95% CI: 1.62–7.60). Similarly, participants at WHO clinical stage four had a 3.3-fold higher likelihood of undernutrition than those at stage one (AOR = 3.30; 95% CI: 1.53–7.11). Poor adherence to ART in the past six months increased the odds of undernutrition by more than three times compared to good adherence (AOR = 3.43; 95% CI: 1.78–6.61). Additionally, food insecurity was associated with more than double the risk of undernutrition compared to food-secure households (AOR = 2.10; 95% CI: 1.25–4.34).

DISCUSSION

In this study, the prevalence of undernutrition among adult women on ART was 39.2%, which is lower than figures reported in earlier studies by Mulu et al. (2016) and Hadgu et al. (2013), which documented prevalences of 46.8% and 42.3%, respectively. However, this finding is higher than reports from other parts of Ethiopia, including studies by Kenea et al. (2015) 27%, Alebel et al. (2020) 26%, Gemede et al. (2021) 31.2%, Regassa and Gudeta (2022) 16%, and Gedle et al. (2015) 25%. These discrepancies may reflect differences in socioeconomic conditions, dietary patterns, and regional variations in food security and access to healthcare services.

Educational status emerged as a significant predictor of nutritional status among women on ART in this study. Women with no formal education were more likely to be undernourished compared to those who had received formal schooling, a finding that is consistent with reports from previous studies (Nanewortor et al., 2021; Thapa et al., 2015; Anlay et al., 2016). This association may be explained by the fact that education enhances awareness of proper nutrition, health-seeking behavior, and self-care practices, all of which contribute positively to maintaining good nutritional status. These findings suggest that improving women's educational opportunities should be considered an important component of comprehensive HIV care.

The WHO clinical stage was also found to be significantly associated with undernutrition in this study. Women in WHO clinical stage II and above had a higher likelihood of being undernourished compared with those in stage I. This result aligns with findings from studies conducted in southwest Ethiopia, Tigray, and Asella, as well as national and regional meta-analyses (Hadgu et al., 2013; Teklu et al., 2020; Tesfa et al., 2021; Regassa and Gudeta, 2022; Seid et al., 2023). Advanced clinical stages reflect compromised immunity and increased susceptibility to opportunistic infections, which can impair food intake and nutrient absorption. In addition, the combined effects of infections, antibiotic use, and ART-related side effects may reduce appetite and further exacerbate undernutrition.

In this study, poor adherence to ART over the previous six months was significantly associated with undernutrition. Women with poor adherence were more than three times as likely to be undernourished compared with those who maintained good adherence, a finding consistent with reports from Bench Sheko Zone, southwest Ethiopia, and Arba Minch (Shifera et al., 2022; Kalil et al., 2020; Negussie and Sultan, 2020). Poor adherence may promote viral replication and CD4 cell depletion, leading to weakened immunity and disease progression, which in turn can impair dietary intake and nutrient absorption, ultimately contributing to undernutrition.

Food insecurity is another factor predicting undernutrition in the present study. Our result showed that food-insecure women on ART were more than twice as likely to be undernourished. This finding was similar to other studies in Ethiopia and elsewhere (Tolasa et al., 2015; Gedle et al., 2015; Oluma et al., 2020; Schaible & Kaufmann, 2007; Nnyepi, 2009). This was clearly due to a lack of access to sufficient food to meet dietary needs resulting in undernutrition. Evidence indicates that HIV/AIDS exacerbates existing food insecurity, adversely affecting the nutritional status of individuals on ART and contributing to weight loss and wasting. These findings underscore the impact of food insecurity on undernutrition and call for comprehensive interventions by ART program implementers and nutrition stakeholders. Integrated strategies combining nutrition support, food security initiatives, and educational programs for women on ART are needed. Policies that promote nutritional counseling, strengthen ART adherence, and address socioeconomic vulnerabilities could help mitigate undernutrition and improve overall health outcomes in this population.

Strength and limitations

The present study used anthropometric measurement and dietary assessment to evaluate under-nutrition among women on ART. However, there might be a memory lapse, and recall bias on the ART adherence data. Moreover, potential social desirability may bias the food insecurity assessment responses. The nature of the cross-sectional study design couldn't establish cause and effect relationship between dependent and independent variables.

CONCLUSIONS and RECOMMENDATIONS

This study found a substantial burden of undernutrition among women of reproductive age receiving ART, indicating a serious public health concern in the study setting. Undernutrition was significantly associated with lack of formal education, advanced WHO clinical stage, poor ART adherence in the preceding six months, and household food insecurity. These findings emphasize the need for integrated and targeted interventions that strengthen food security, promote sustained ART adherence, and improve women's access to education and nutritional counseling. Furthermore, future longitudinal studies are recommended to better clarify causal pathways and to support the design of more effective, evidence-based nutritional interventions for women on ART.

AKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the study participants, data collectors, and healthcare workers at Yirgalem Hospital for their invaluable contributions. This research was not funded by any organization. H.G. and Z.T. were responsible for research design, data acquisition, analysis, and interpretation. Z.T. prepared the draft manuscript, while H.G. critically reviewed and edited it. All authors reviewed and approved the final manuscript.

Disclosure statement

The authors declare that they have no conflicts of interest.

Data Availability

All necessary data used for this study is available from the corresponding author upon a reasonable request.

REFERENCES

Alebel A, Kibret GD, Petrucka P, Tesema C, Moges NA, Wagnew F, Asmare G, Kumera G, Bitew ZW, Ketema DB, Tiruneh T, Melkamu MW, Hibstie YT, Temesgen B, Eshetie S. 2020. Undernutrition among Ethiopian adults living with HIV: a meta-analysis. BMC Nutr., 6(1): 10. doi: 10.1186/s40795-020-00334-x. [Scholar Google]

Anlay, D.Z., Alemayehu, Z.A., Dachew, B.A. 2016. Rate of initial highly active anti-retroviral therapy regimen change and its predictors among adult HIV patients at University of Gondar Referral Hospital, Northwest Ethiopia: a retrospective follow up study. AIDS Res Ther., 13(1): 10. https://doi.org/10.1186/s12981-016-0095-x. [Scholar Google]

Ayele G, Tessema B, Amsalu A, Ferede G & Yismaw G. 2018. Prevalence and associated factors of treatment failure among HIV/AIDS patients on

HAART attending University of Gondar Referral Hospital Northwest Ethiopia. BMC Immunol., 19(1):1–13. doi:10.1186/s12865-018-0278-4. [Scholar Google]

Coates J., Swindale A., Bilinsky P. 2007. Household Food Insecurity ccess Scale (HFIAS) for Measurement of Food Access: Indicator Guide V 3 Food and Nutrition Technical Assistance Project (FANTA) Washington, DC.

Crum-Cianflone NF, Roediger M, Eberly LE, Ganesan A, Weintrob A, Johnson E, Agan BK. 2011. Infectious Disease Clinical Research Program HIV Working Group. Impact of weight on immune cell counts among HIV-infected persons. Clin Vaccine Immunol., 18(6): 940-6. doi: 10.1128/CVI.00020-11. [Scholar Google]

El-Khatib Z, Ekstrom AM, Coovadia A, EJ, Petzold M, Katzenstein D, Morris L, Kuhn L. 2011. Adherence and virologic suppression during the first 24 weeks on antiretroviral therapy among women in Johannesburg, South Africa - A prospective cohort study. BMC Public Health, 11. doi:10.1186/1471-2458-11-88. [Scholar Google]

FAO. 2010. Guidelines for Measuring Household and Individual Dietary Diversity. Food and Agriculture Organization of the United Nations. Rome.

FHAPCO. HIV/AIDS National Strategic Plan (NSP) for Ethiopia 2021-2025. Available online https://www.prepwatch.org/wp-content/uploads/2022/07/Ethiopia-HIVAIDS-National-Strategic-Plan-2021-25.pdf. (Accessed on Dec 2023)

Fuseini H, Gyan BA, Kyei GB, Heimburger DC, Koethe JR. 2021. Undernutrition and HIV Infection in Sub-Saharan Africa: Health Outcomes and Therapeutic Interventions. Curr HIV/AIDS Rep., 18(2): 87-97. doi: 10.1007/s11904-021-00541-6.

FMOH. 2018. Ethiopia. National Consolidated Guidelines for Comprehensive HIV Prevention, Care and Treatment. FMOH. 1–238.

Gebremichael DY, Hadush KT, Kebede EM, Zegeye RT. 2018. Food Insecurity, Nutritional Status, and Factors Associated with Malnutrition among People Living with HIV/AIDS Attending Antiretroviral Therapy at Public Health Facilities in West Shewa Zone, Central Ethiopia. Biomed Res Int. 18: 1913534. doi: 10.1155/2018/1913534. [Scholar Google]

Gebru TH, Mekonen HH, Kiros KG. 2020. Undernutrition and associated factors among adult HIV/AIDS patients receiving antiretroviral therapy in eastern zone of Tigray, Northern Ethiopia: a cross-sectional study. Archives of Public Health, 78(1): 1–8. https://doi.org/10.1186/s13690-020-00486-z PMID: 33072319. [Scholar Google]

Gedle D, Gelaw B, Muluye D, Messele M. 2015. Prevalence of malnutrition and its associated factors among adult people living with HIV/AIDS receiving antiretroviral therapy at Butajira Hospital, southern Ethiopia. BMC Nutri., 1: 5. doi: 10.1186/2055-0928-1-5. [Scholar Google]

Gedle, G. Mekuria, G. Kumera, Eshete T, Feyera F,Feyera F, Ewnetu T. 2015. Food Insecurity and its Associated Factors among People Living with HIV/AIDS Receiving Anti-Retroviral Therapy at Butajira Hospital, Southern Ethiopia, Journal of Nutrition & Food Sciences, 5(2). doi:10.4172/2155-9600.1000347. [Scholar Google]

Gibson RS and Meredith-Jones K. 2024. Principles of Nutritional Assessment: Body Size. https://nutritionalassessment.org/bodysize /.3rd Edition,

Gemede HF, Kaba F, Dufera M. 2021. Nutritional Knowledge, Practices, Nutritional Status and the Associated Factors Among HIV Positive Mothers On Antiretroviral Therapy: Evidence from Cross Sectional Survey in Abay Choman Health Centers, Western Ethiopia. Research Square; doi: 10.21203/rs.3.rs-968599/v1.

Gona, P.N., Gona, C.M., Ballout, S., Rao, S.R., Kimokoti, R., Mapoma, C.C. and Mokdad, A.H. 2020.'Burden and changes in HIV/AIDS morbidity and mortality in Southern Africa Development Community countries, 1990–2017', BMC Public Health, 20. doi: 10.1186/s12889-020-08988-9. [Scholar Google]

Hadgu TH, Worku W, Tetemke D, Berhe H. 2013. Undernutrition among HIV positive women in Humera hospital, Tigray, Ethiopia: antiretroviral therapy alone is not enough, cross sectional study. BMC Public Health, 9. doi: 10.1186/1471-2458-13-943. [Scholar Google]

Kalil FS, Kabeta T, Jarso H, Hasen M, Ahmed J, Kabeta S. 2020. Determinants of Undernutrition Among Adult People on Antiretroviral Therapy in Goba Hospital, Southeast Ethiopia: A Case–Control Study. Nutrition and Dietary Supplements, 12: 223-236.

doi.org/10.2147/NDS.S276311. [Scholar Google]

Kenea MA, Garoma S, Gemede HF. 2015. Assessment of adult nutritional status and associated factorsamong ART users in Nekemte referral hospital and health center, east Wollega zone, Ethiopia. Journal of Food and Nutrition Sciences, 3(2): 56-63. doi: 10.11648/j.jfns.20150302.15.

Koethe JR, Limbada MI, Giganti MJ, Nyirenda CK, Mulenga L, Wester CW, Chi BH, Stringer JS. 2010. Early immunologic response and subsequent survival among malnourished adults receiving antiretroviral therapy in Urban Zambia. AIDS, 24(13): 2117-2121. doi: 10.1097/QAD.0b013e32833b784a. [Scholar Google]

Kotler DP. 2000. Body composition studies in HIV-infected individuals. Ann N Y Acad. Sci.; 904(1): 546-552. doi: 10.1111/j.1749-6632.2000.tb06514.x. PMID: 10865803. [Scholar Google]

MOH. 2018. National Comprehensive HIV Prevention Care and Treatment Training for Health care. Ministry of Health Ethiopia.

Markos M, Lolaso T, Mengistu A, Tariku Z. 2020. Dietary Diversity and Associated Factors Among Adult HIV Positive Patients on Anti-Retroviral Therapy in Public Hospitals of Kembata Tembaro Zone, Southern Ethiopia. HIV AIDS – Research and Palliative Care, 12: 859-868. doi: 10.2147/HIV.S278855. [Scholar Google]

Momper JD, Wang J, Stek A, Shapiro DE, Scott GB, Paul ME, Febo IL, Burchett S, Smith E, Chakhtoura N, Denson K, Rungruengthanakit K, George K, Yang DZ, Capparelli EV, Mirochnick M, Best BM; IMPAACT P1026s Protocol Team. 2021. Pharmacokinetics of darunavir and cobicistat in pregnant and postpartum women with HIV. AIDS, 35(8): 1191-1199. doi: 10.1097/QAD.0000000000002857. [Scholar Google]

Mulu H, Hamza L, Alemseged F. 2016. Prevalence of Malnutrition and Associated Factors among Hospitalized Patients with Acquired Immunodeficiency Syndrome in Jimma University Specialized Hospital, Ethiopia. Ethiop. J. Health Sci., 26(3): 217–226. https://doi.org/10.4314/ejhs.v26i3.4 PMID: 27358542. [Scholar Google]

Ntlantsana V, Hift RJ, Mphatswe WP. 2019. HIV viraemia during pregnancy in women receiving preconception antiretroviral therapy in KwaDukuza, KwaZulu-Natal. South Afr J HIV Med. 20(1):1–8. doi:10.4102/sajhivmed.v20i1.847. [Scholar Google]

Nanewortor BM, Saah FI, Appiah PK, Amu H, Kissah-Korsah K. 2021. Nutritional status and associated factors among people living with HIV/AIDS in Ghana: cross-sectional study of highly active antiretroviral therapy clients. BMC Nutr., 7(1). doi: 10.1186/s40795-021-00418-2. [Scholar Google]

Negussie BS & Sultan H. 2020. Nutritional Status and Its Effect on Treatment Outcome among HIV-Infected Children Receiving First-Line Antiretroviral Therapy in Arba Minch General Hospital and Arba Minch Health Center, Gamo Zone, S," Chapters, in: Nancy Dumais (ed.), Nutrition and HIV/AIDS - Implication for Treatment, Prevention and Cure, Intech Open. doi: 10.5772/intechopen.85851

Nnyepi, Ms. 2009. The risk of developing malnutrition in people living with HIV/AIDS: Observations from six support groups in Botswana. South African Journal of Clinical Nutrition, 22(2): 89–93. doi.org/10.1080/16070658.2009.11734224. [Scholar Google]

Ntlantsana V, Hift RJ, Mphatswe WP. 2019. HIV viraemia during pregnancy in women receiving preconception antiretroviral therapy in KwaDukuza, KwaZulu-Natal. South Afr. J. HIV Med., 20(1): 1–8. doi:10.4102/sajhivmed.v20i1.847. [Scholar Google]

Oluma A, Abadiga M, Mosisa G, Etafa W, Fekadu G. 2020. Food Insecurity among People Living with HIV/AIDS on ART Follower at Public Hospitals of Western Ethiopia. Int J Food Sci.:8825453. doi: 10.1155/2020/8825453. [Scholar Google]

Regassa TM, Gudeta TA. 2022. Levels of undernutrition and associated factors among adults receiving highly active anti-retroviral therapy in health institutions in Bench Maji Zone, Southwest Ethiopia. Front Nutr., 9. doi: 10.3389/fnut.2022.814494. [Scholar Google]

Saliya, M. S., Azale, T., Alamirew, Tesfaye D. 2018. Assessment of nutritional status and its associated factors among people affected by human immune deficiency virus on antiretroviral therapy: A cross sectional study in

Siltie zone, South Ethiopia. Healthcare in Low-Resource Settings, 6(1). https://doi.org/10.4081/hls.2018.6361. [Scholar Google]

Schaible UE, Kaufmann SH. 2007. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med; 4(5): e115. doi: 10.1371/journal.pmed.0040115. [Scholar Google]

Seid A, Seid O, Workineh Y, Dessie G, Bitew ZW. 2023. Prevalence of undernutrition and associated factors among adults taking antiretroviral therapy in sub-Saharan Africa: A systematic review and meta-analysis. PLoS ONE, 18(3): e0283502. https://doi.org/10.1371/journal.pone.0283502. [Scholar Google]

Shifera N, Yosef T, Matiyas R, Kassie A, Assefa A, Molla A. 2022. Undernutrition and Associated Risk Factors among Adult HIV/AIDS Patients Attending Antiretroviral Therapy at Public Hospitals of Bench Sheko Zone, Southwest Ethiopia. J. Int. Assoc Provid AIDS Care. 21:23259582221079154. doi: 10.1177/23259582221079154.[Scholar Google]

Shiferaw H, Gebremedhin S. 2020. Undernutrition Among HIV-Positive Adolescents on Antiretroviral Therapy in Southern Ethiopia. Adolesc. Health Med. Ther., 20: 101-111. doi: 10.2147/AHMT.S264311. [Scholar Google]

Steinhart CR. 2001). HIV-associated wasting in the era of HAART: a practice-based approach to diagnosis and treatment. AIDS Read. 11(11):557 – 60, 566-9. PMID: 11789018.

SZBoFED. 2007. Sidama Zone Socio-Economic Profile. Sidama Zone Finance and Economic Development Department; SZBoFED: Hawassa, Ethiopia, Available online: https://www.scirp.org/reference/referencespapers

Teklu T, Chauhan NM, Lemessa F, Teshome G. 2020. Assessment of Prevalence of Malnutrition and Its Associated Factors among AIDS Patients from Asella, Oromia, Ethiopia. Biomed Res Int. doi: 10.1155/2020/7360190. [Scholar Google]

Tesfa Mengie, Demeke Dejen, Temesgen Muche, Getacher L. 2021. Under Nutrition and Its Determinants Among Adults Receiving Antiretroviral Therapy in Ethiopia: A Systematic Review and Meta-analysis. International Journal of Homeopathy & Natural Medicines. 7(1): 1-6. doi: 10.11648/j.ijhnm.20210701.11. [Scholar Google]

Thapa R, Amatya A, Pahari DP, Bam K, Newman MS. 2015. Nutritional status and its association with quality of life among people living with HIV attending public anti-retroviral therapy sites of Kathmandu Valley, Nepal. AIDS Res Ther., 12. doi: 10.1186/s12981-015-0056-9. [Scholar Google]

Tolasa, B. D. Demisse, D., Tesfaye T, Belachew T. 2015. Food Insecurity and Associated Factors among People Living with HIV Attending ART Clinic in Fitche Zonal Hospital , Ethiopia, Journal of Pharmacy and Alternative Medicine, 8: 8–17. [Scholar Google]

UNAIDS Fact Sheet. 2022. Available online: www.unaids.org/sites/default/files/media_asset/UNAIDS_Fact Sheet_en.pdf. (Accessed on 23 Sept 2023).

UNAIDS. Epidemiological estimates. 2023. Available online: https://www.unaids.org/en/resources/documents/2023/2023_unaids_data. (Accessed on April 2023).

Westreich D, Cole SR, Nagar S, Maskew M, Van der Horst C, Ian Sanne I. 2011. Pregnancy and virologic response to antiretroviral therapy in South Africa. PLoS One, 6(8): e22778. doi:10.1371/journal.pone.0022778. [Scholar Google]

WHO HIV/AIDS Data and Statistics. 2022. Available online: https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information /hiv-data-and-statistics. (Accessed on 23 Sept 2023).

Woldemariam, A., Yusuf, M., Beyene, and Yenit, M.K. 2015 . 'Factors associated with dietary diversity among HIV positive adults over 18 years attending ART clinic at Mettema Hospital, Northwest Ethiopia: a cross-sectional study', Journal of AIDS and Clinical Research, 6(2). doi: 10.4172/2155-6113.1000490. [Scholar Google]

Table 1. Socioeconomic and demographic characteristics of 15-49yrs old women on ART at Yirgalem General Hospital, Sidama region, Ethiopia, 2023 (n=268)

Variables	Frequency(n)	Percentage (%)	Mean (SD)
Age, y			33.7(7.1)
Ethnicity			
Wolaita	90	33.6	
Gurage	80	29.9	
Sidama	29	10.8	
Oromo	25	9.3	
Amhara	14	5.2	
Other	30	11.2	
Marital status			
Not married	76	28.4	
Married/living together	125	46.6	
Divorced/Widowed/separated	67	25	
Residence			
Urban	191	71.3	
Rural	77	28.7	
Educational Status			
Secondary school and above	129	48.4	
Primary school(1-8)	71	26.49	
No formal education	68	25.37	
Occupation			
Government employed	60	22.4	
Merchant	51	19	
Daily laborer	89	33.2	
Farmer	48	17.9	
Student	20	7.5	
Average monthly income (ETB)			
<700	194	72.39	
701-1500	47	17.54	
>1500	27	10.07	

1. 1USD=59ETB

Table 2. Clinical stage and Health related characteristics of 15-49yrs old women on ART at Yirgalem General Hospital, Sidama region, Ethiopia, 2023 (n=268)

Variables	Frequency	Percentage
Duration on ART		
<12 months	56	20.9
≥12 months	212	79.1
Had Cotrimoxazole prophylaxis		
Yes	219	81.7
No	49	18.3
Gastro intestinal symptoms in past six month		
Yes	56	20.9
No	212	79.1
Disclosed HIV status		
Yes	224	83.6
No	44	16.4
Eating difficulty in last six months		
Yes	49	18.3
No	219	81.7
History of TB in the last six months		
Yes	7	2.6
No	261	97.4
WHO clinical stage		
Stage I	84	31.4
Stage II	65	24.3
Stage III	53	19.8
Stage IV	66	24.5
Recent CD4 count		
≥500 cells/mm3	51	19.0
200-499 cells/mm3	190	70.9
<200 cells/mm3	27	10.1
Adherence to HAART in past six month		
Good adherence	195	72.8
Poor adherence	73	27.2
Opportunistic infection in last six month		
Yes	41	15.3
No	227	84.7

3.

Table 3. Nutritional, Dietary, and Behavioral Characteristics of 5-49yrs old women on ART at Yirgalem General Hospital, Sidama region, Ethiopia, 2023 (n=268)

Variables	Frequency	Percentage
Number of meal/24 hr		
≥3	199	74.3
<3	69	25.7
Change in feeding after tested positive for HIV		
Yes	160	59.7
No	108	40.3
Types of change in feeding style		
Quality feeding	45	28.3
Frequency	69	27.5
Feeding cooked food	16	6.0
Quantity	29	10.8
Had nutritional counseling		
Yes	226	84.3
No	42	15.7
Food security status		
Food secure	175	65.3
Food insecure	93	34.7
Nutritional status		
Not undernourished	160	60.8
Undernourished	105	39.2
History of cigarette smoking		
Yes	15	5.6
No	253	94.4
History of alcohol intake		
Yes	44	16.4
No	224	83.6

Table 4. Bivariate and Multivariable Analysis of Factors Associated with Undernutrition Among 15-49yrs old Women on ART, Yirgalem Hospital, 2023 (n=268)

Variables	Undernutrition Yes	Undernutrition No	COR 95% CI	AOR 95% CI	P value
Educational status					
College/University and above [a]	19	35	1	1	
Secondary school	30	45	1.22(0.59,2.53)	0.50(0.62,3.62)	
Primary school	16	55	0.43(0.24,1.17)	0.59(0.23,1.51)	
No formal education	40	28	2.63(1.25,5.50) *	3.10(1.63,7.60) *	0.013
WHO clinical stage					
I [a]	34	50	1	1	
II	12	53	0.33(0.15,0.71) *	0.33(0.14,0.75)	
III	11	42	0.38(0.17,0.85) *	0.31(0.13,0.75)	
IV	48	18	3.92(1.95,7.85) **	3.30(1.53,7.11) **	0.002
Age in years					
20-29	27	56	0.97(0.54-1.72)	0.96(0.51-1.80)	
30-39	52	62	1.97(1.02-3.71)*	1.89(0.93-3.83)	
40-49	26	45	1	1	
Residence					
Urban	73	118	0.87 (0.50-1.49)	1.15(0.62-2.14)	0.61
Rural	32	45	1	1	
Adherence to ART in the last six months					
Good adherence [a]	62	133	1	1	
Poor adherence	43	30	3.07(1.76,5.34) **	3.43(1.78,6.61) **	0.000
Eating difficulity					
Yes	15	34	0.63(0.32-1.22)	1.40(0.68-2.84)	
No	90	129	1	1	
Food security status					
Secure [a]	57	118	1	1	
Insecure	48	45	2.32(1.31,3.70) **	2.10(1.25,4.34) **	0.008
Meal frequency					
≥3 meals/day	79	121	1	1	
<3 meals/day	26	41	1.02(0.58-1.81)	1.35(0.02-5.60)	

Abbreviations: AOR, adjusted odd ratio; COR, crude odd ratio. * Statistically significant $P < .05$; ** Statistically significant $P < .001$ [a] Reference categories;

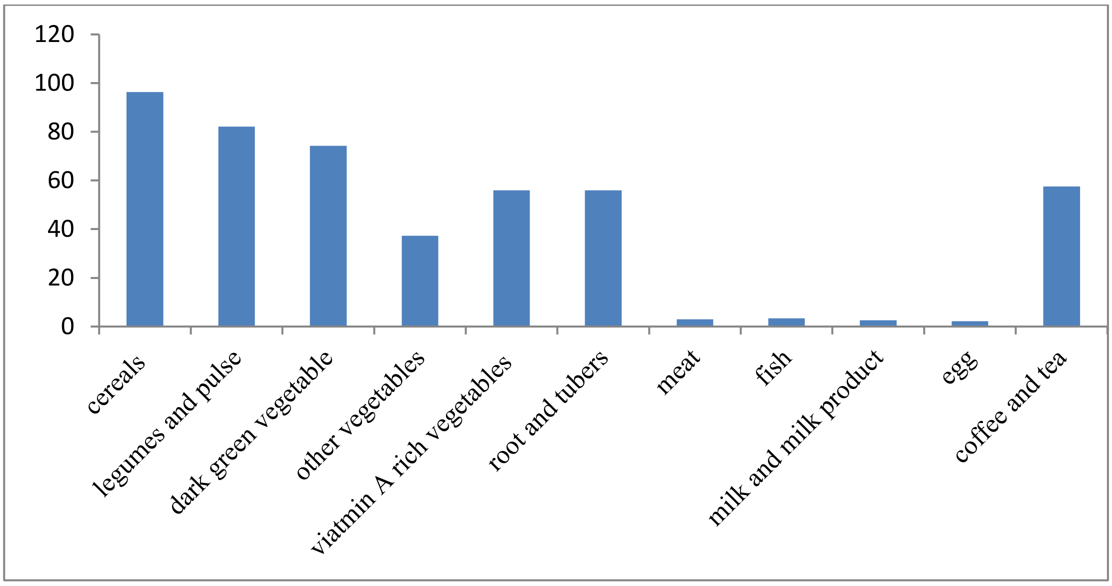

Figure 1. Consumption patterns of food-groups among 15-49yrs old women on ART at Yirgalem, General, Hospital, Sidama region, Ethiopia, 2023 (n=268)

Original Research Article||

Isolation and Pathogenic diversity among Fusarium oxysporium f.sp. capsici isolates in southern Ethiopia and evaluation of Biocontrol agents against the Pathogen

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Abstract

Fusarium wilt is one of the major biotic factors affecting hot pepper production in Ethiopia. Therefore, this study was designed with the objectives of analyzing the pathogenic diversity of Fusarium oxysporium f.sp. capsici isolates and to evaluate the biocontrol potential of some antagonists for the management of the disease. For this purpose, twenty nine isolates of Fusarium oxysporium f.sp. capsici were identified from visibly diseased plant samples collected from the 80 surveyed fields based on macroscopic and microscopic characteristics. Pathogenicity test was done for the 29 Fusarium oxysporium f.sp. capsici isolates and based on diseases severity index value, isolates were classified as highly pathogenic, moderately pathogenic, and weak. Isolate LWS7 was found to be the most pathogenic isolate that induced disease and was used for the studies involving biocontrol agents. The in vitro and in vivo efficacy trial of six Trichoderma spp. and Bacillus subtilis was investigated under dual culture assays in the lab and in a greenhouse, respectively. The highest (81.8%) and lowest (48%) mycelial growth inhibition were estimated from Trichoderma harzianum and Trichoderma hamatum, respectively. Lower wilt incidence (31%) was recorded on pepper plants following seedling root dip inoculation by Trichoderma viride. In addition to their significant effect on mycelia growth in-vitro, the bioagents evaluated in the current experiment were also found to result in significant reduction of the disease and promotion of growth of hot pepper in soil application and seedling root dip tests. This finding indicated that bioagents are an important development direction for their role in the sustainable development of agriculture. The efficacy and economic feasibility of hot pepper management through biocontrol agents may need additional investigation to come up with conclusive results.

Key words: Fusarium wilt, Biological control, Pathogenicity, Trichoderma spp

Original submission: December 16, 2024; **Revised submission**: July 01, 2025; **Published online**: December 30, 2025

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INTRODUCTION

Hot pepper (Capsicum annuum L.) is an important vegetable crop in tropical areas worldwide. It belongs to the Solanaceae family and in the genus Capsicum (Dias et al., 2013). Capsicum is the second most important genus in family Solanaceae next to Solanum (Berhanu et al., 2011). Hot pepper is a high-value spice crop, which is consumed as vegetable, spice, or condiment in its fresh, dried, or processed forms. Hot pepper is very important vegetable and spice crop in Ethiopia, but its production is constrained by many diseases including Fusarium wilt (Fusarium oxysporium f.sp. capsici) (Assefa et al., 2015). Despite its importance as commercial spice and vegetable crop, the hot pepper acreage, production, and productivity are falling (Fekadu and Dendena, 2006). Hot pepper is attacked by several bacterial, viral and fungal diseases as well as insect pests. Among the known hot pepper diseases, those caused by Fusarium spp., Phytophthora spp., Leveillula taurica, Verticilllum spp., Rhizoctonia solani, Colletotrichum spp. and Cercospora capsici (Mekonen and Chala, 2014); bacterial diseases caused by Xanthomonas campestris pv. visicatoria, Ralstonia solanacearum and Erwinia carotovora Kassahun et al., 2016); and viral disease such as pepper mottle virus (Tameru et al., 2003) have been reported in Ethiopia.

Fusariums wilt disease, caused by the soil-borne fungus Fusarium oxysporum f.sp. capsici, is the most serious disease affecting pepper plants, reducing growth, fruit yield, and pepper quality (Wongpia and Lomthaisong 2010). Fusarium wilt of pepper has become increasingly common on open-field-grown peppers in recent years. The fungus can infiltrate a plant with its sporangial germ tube or mycelium via penetrating plant roots, through wounds, or straight through the root tip or at the point of lateral root development (Koste and Bart, 2013).

Fusarium wilt is a disease that affects hot peppers all around the world. Ethiopia is one of a few African countries that produce capsaicin and oleoresin for the export market from locally selected materials that contributed substantially to the national economy. Among biotic factors that affect pepper production in Ethiopia, Fusarium wilt that is caused by Fusarium oxysporium is one of the most economically important diseases, and it accounts for yield losses of up to 80% (Aklilu et al., 2007). In recent years, the importance of the disease has been increasing and is given considerable attention by hot pepper producers and other stakeholders. Fusarium wilt from untreated Marako-fana resulted in a relative yield reduction of 68-71% in Ethiopia (Teshome et al., 2012).
Fusarium wilt symptoms begin with modest vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older lower leaves. The disease is distinguished by a slight yellowing of the lower leaves and withering of the upper leaves, followed by permanent drooping of the entire plant and browning of the vascular tissue (Black and Rivelli, 1990). The most prevalent symptoms of F. oxysporium infection were leaf chlorosis, vascular discolouration and plant wilting. Fusarium oxysporium f.sp. capsici is a soil borne fungal pathogen and thrives in conditions with high soil moisture (20%) and temperature (25-30°C) (Sekhon and Singh, 2007).
Fusarium wilt disease is soil-borne in nature, making the application of fungicides impractical due to their environmental and health hazards to applicators and consumers, as well as their non-selective nature that also affects beneficial organisms. As a sustainable and ecofriendly alternative, biological control using biocontrol agents such as species from the genus Trichoderma has been recognized (Sabalpara et al., 2009) as a promising approach for managing soil-borne plant pathogens. These Trichoderma species suppress pathogen populations either by directly parasitizing them or by competing for essential nutrients, thereby limiting their growth. Biological control strategies are considered environmentally safe, economical, and effective. They operate through various mechanisms, including competition for space and nutrients, stimulating host plant defenses to induce tolerance or resistance, and producing antibiosis through the synthesis of harmful fungal chemicals or enzymes (Matar et al., 2009). Overall, Trichoderma-based biocontrol agents offer a targeted and sustainable method for managing fungal plant diseases in agricultural systems (Abada and Eid, 2014). In Integrated disease management program, biological control is crucial because the Fusarium pathogen is soil-borne and can survive for years, making single control methods (like crop rotation or some fungicides) less effective on their own. BCAs provide long-term, consistent disease control by establishing a beneficial microbial balance in the plant root system (rhizosphere).

Furthermore, due to the pathogen's nature (it survives in the soil) control measures such as fungicide use are limited. Yet, biological control agents with an eco-friendly approach are increasingly required in crop protection strategies to combat the indiscriminate use of chemicals. Many investigations on biocontrol agents have revealed antifungal activity against Fusarium oxysporium f.sp. capsici (Heydari & Pessarakli, 2010; Endriyas et al., 2020). As there is a lack of knowledge on the potential of biological control agents for pepper wilt disease management in Southern Ethiopia, evaluation of different biocontrol agents is important. Therefore, this study was designed with the objectives of analyzing the pathogenic diversity of Fusarium oxysporium f.sp. capsici isolates and to evaluate the biocontrol potential of some antagonists for the management of the disease.

MATERIALS AND METHODS

Characterization and Pathogenicity Test of the Fusarium oxysporium f.sp. capsici Isolates

Diseased Sample Collection, Isolation and Identification

Disease plant samples were collected from three administrative zones (Gurage, Silte and Halaba) and five pepper growing districts (Abeshge, Meskan Mareko, Lanfro and Wera) of the southern region of Ethiopia during 2022 main cropping season. Stem/root samples from diseased pepper plants showing the typical symptoms of Fusarium wilt (lesions on the stem and root, brown vascular discoloration, leaf epinasty and yellowing, wilting and plant death) were taken from each field. The samples were placed in plastic bags and labeled with relevant information and brought to Hawassa University Plant Protection Laboratory and preserved at 4ºC until isolation and identification of the pathogen.

The infected root/stem samples were cut into smaller pieces using sterile scissors and washed under running tap water in the laboratory. The segments were surface sterilized with 2% sodium hypochlorite solution for 3 min, washed three times with sterile distilled water, and dried on sterile blotting paper. Five patches of the plant specimens were then plated onto potato dextrose agar (PDA) medium supplemented with Antibiotics (streptomycin 30 µg/l, chlortetracycline 10mg/lt), to avoid bacterial contaminations and incubated at 25±1ºC for 7 days. Seven days after incubation, the colonies were transferred and sub-cultured on to fresh PDA plates to obtain pure cultures. Then spores of the fungi were subsequently identified according to their cultural and morphological features (Leslie and Summerell, 2006; Booth, 1971).

Cultural and Morphological Characterization

The cultural (macroscopic) and morphological (microscopic) features of *F.oxysporum* isolates were characterized according to Leslie and Summerell (2006) and Booth (1971). Accordingly, the colony characters: color (upper and reverse), colony shape, margin, elevation and radial growth, days to pigmentation and colony surface texture were visually examined in 7 days old pure cultures. Fungal colonies (single spore) were transferred into fresh SNA media (Spezieller Nährstoffarmer Agar) (1L of distilled water, 1g KH2PO4, 1g KNO3, 0.5g MgSO4·7H2O, 0.5g KCl, 0.2g Glucose, 0.2g Sucrose, 20g Agar) medium (Leslie and Summerell 2006) to get conidia. Conidia characteristics: presence or absence of microconidia and chlamydospore, number of septa in macroconidia, shape of macroconidia, and shape of microconidia were examined.

Pathogenicity Test

Pathogenicity test of 29 isolates was conducted in Southern Agricultural Research Institute (SARI) under greenhouse conditions at a temperature of 28ºC using the susceptible variety Mareko Fana (Kassahun et al., 2016). Inoculum of the individual isolates was prepared on PDA. From 10 days old cultures, conidia were harvested to 15ml beaker by adding 10 ml of sterile distilled water (SDW) in each Petri plate. To remove mycelial masses, the suspension was filtered through sterile cheese cloth. From the filtered culture, conidia were resuspended in SDW and the final conidial concentration was adjusted to $1x10^6$ spore/ml using a haemocytometer.

Inoculation was performed following the standard cut-root dip inoculation technique (Herman and Perl-Treves, 2007; Karimi et al., 2010). Accordingly, roots of 4 weeks old seedling of hot pepper, raised in the greenhouse at 28ºC in a pot filled with oven sterilized (at 65ºC for 72 hours) soil, compost and sand mixture in the ratio of 1:0.5:0.5, respectively, were trimmed with a sterile scissor and submerged into tubes containing 9 ml of the spore suspension for 30 minute.

Roots of control plants were similarly cut and dipped in SDW for the same period of time. Then, inoculated seedlings were transplanted into 3litre capacity pots filled with oven sterilized soil (at 65ºC for 72 hours) , compost and sand in the ratio of 1:0.5:0.5, respectively (Demissie et al., 2021) arranged in a Completely Randomized Design

(CRD) and grown in the greenhouse under conditions described above. For each isolate, a total of 9 seedlings (3 seedlings/pot with 3 replications) were used. Similar numbers of plants per replication were used as control. Koch's postulate was tested by the isolation of the pathogen from diseased plants and re-inoculating the isolated *F. oxysporum* isolates onto new and healthy plants. Observation of similar symptom and re-isolation and characterization of morphological features were done.

Evaluation of Biocontrol Agents Against the Pathogenic Isolates

***In Vitro* Test**

Trichoderma spp. i.e. *Trichoderma harzianum, T. viride, T. longibrachiatum, T. hamatum, T. asperellum* and *T.atroviride*), and a bacterium (*Bacillus subtilis)* obtained from the stock culture of Ambo Plant Protection Research Center were tested for their antagonistic activity against *Fusarium oxysporium* f.sp. *capsici* by dual culture technique.

From five days old culture with the help of sterilized corkborer, 5mm mycelial discs of antagonist as well as pathogenic isolates was taken from the tip of young hyphal growth both for antagonist as well as pathogenic isolates and placed on Potato dextrose agar (PDA) at the opposite side of each other (dual culture) incubated at 28ºC for one week. A distance of 5cm between the inoculation point of *Fusarium oxysporium* isolates and the antagonists (*Trichoderma* spp.) was kept for formation of inhibition zone. In case of evaluation of bacterial antagonist, 24 hr. old culture of *Bacillus subtilis* was streaked with inoculating loop at one end of the Petri plates and mycelial disc (5mm) of the test fungus (*Fusarium oxysporum* f.sp. *capsici*) was placed at the other end.

For each BCA-FOC combination, three replicates were used and arranged in CRD. Mycelial growth inhibition of FOC due to the BCAs was measured as radius of FOC colony towards the BCA colony and in the corresponding control Petri dishes starting from three days post incubation (DPI) and continued until seven DPI. For control, three replicates were used and arranged in CRD, then the radius of the colony was measured from center to the edge of the plate. Finally, percent radial mycelial growth inhibition was calculated by comparing FOC colony growth in BCA plates with that of the control plates and expressed in percent using the formula described below (Rini and Sulochana, 2006).

$$I = \frac{C-T}{C} \times 100$$

Where, I= Percent of inhibition in growth of the *Foc*

C= Radial growth of the *Foc* (cm) in control plate

T= Radial growth of the *Foc* (cm) in the antagonists.

In Vivo Test

Mass Production and Preparation of Biocontrol Agents and Fusarium Inoculum

Mass productions of *Trichoderma* spp. were prepared by using sorghum grain. In each pot 3kg of soil was added and each antagonist was applied in three replication. 3kg soil in each pot was inoculated with 60g of inocula of each antagonist (thus a total of 180g substrate was used for three replicates in each antagonist (Rini and Sulochana, 2006). The samples of moistened substrates were transferred to 500 ml sized conical flask and autoclaved twice at 15 psi at 121ºC for 30 minutes. The flasks were allowed to cool down under room temperature before inoculation of antagonists. The culture of *Trichoderma* spp. were prepared by harvesting the spore from one week old culture of *Trichoderma* spp. and a concentration of 10^6 spore/ml was injected into the autoclaved flask with sterile water and incubated at 25±2ºC for two weeks (Singleton et al., 1992). Finally after two weeks the antagonists were grown in the substrate and air dried at room temperature before applied to the soil. For *Bacillus subtilis*, inoculum was prepared according to Zhang et al. (2009) (with minor modification). Bacterial culture was grown in nutrient agar for 72 h and with shaking at 150 rpm and adjusted to a concentration of 10^8 CFU ml^{-1} for soil treatments and seedling root dip method (Zhang et al., 2009).

For soil treatments, peat (compost) was used as the bacterial carrier or delivery substrate in soil treatments. Sterile peat was drenched with bacterial broth cultures at the rate of 50 ml of bacterial broth to 50g of peat. Peat was air dried at room temperature for 48 h and then processed into a powder form (Zhang et al., 2009). However, the controls were dipped in sterilized water and transplanted as the same as other treatments.

The isolate identified as the most aggressive was used for the evaluation of biocontrol agents. Mass production of Fusarium inoculum was prepared by using sand-barley (1:1, w: w and 40% water) mixture. The samples of moistened mixture of sand-barley with water (substrates) were transferred to 500 ml sized conical flask and autoclaved twice at 15 psi at 121ºC for 30 minutes. The flasks were allowed to cool down under room temperature before inoculation. The autoclaved medium was then inoculated with a 5-mm disk of more aggressive *Fusarium oxysporium* f.sp. *capsici* test pathogen and incubated at 25±2ºC for two weeks (Singleton et al., 1992). After two weeks *Fusarium oxysporium f.*sp. *capsici* inoculum was grown in the substrate and air dried at room temperature before it was applied to the soil.

Inoculation Methods

Soil application (SA), antagonists were applied to the soil at the rate of 20g/kg of soil a week prior to transplanting, and also Fusarium inoculum was mixed to the soil at the rate of 20g/kg in to the pot one day before sowing (Rini and Sulochana, 2006). The control treatment was inoculated only with Fusarium inoculum one day before sowing. Seedling root dip application, *Fusarium oxysporium* f.sp. *capsici* inoculum were mixed to the soil at the rate of 20g/kg in to the pot one day before sowing and solutions of all Trichoderma used as a treatment were prepared at the concentration of $1x10^6$ spore/ml. Hot pepper seedlings (Mareko fana) were carefully uprooted and their roots were washed with sterilized water and dipped in a solution of biocontrol agent for 30 minute. Tween-20 was used as sticker during seedling root dip (SRD) after which the seedlings were transplanted into prepared pots in green house.

Experimental Treatments and Design

The experiment was laid out in a CRD. Fifteen (15) treatments were used for the experiment with three replications and a total of 45 pots were used (Table 1). In each pot three plants were used, thus a total of 135 plants.

Table 1. Treatments used for the *in vivo* experiment

Treat. No	Treatment
1	*Trichoderma viride* (SA) + *Foc* (SA)
2	*Trichoderma harzianum* (SA) + *Foc* (SA)
3	*Trichoderma asperellum* (SA) + *Foc* (SA)
4	*Trichoderma atroviride* (SA) + *Foc* (SA)
5	*Trichoderma longibrachiatum* (SA) + *Foc* (SA)
6	*Trichoderma hamatum* (SA) + *Foc* (SA)
7	*Bacillus subtilis* (SA) + *Foc* (SA)
8	*Trichoderma viride* (SRD) + *Foc* (SA)
9	*Trichoderma harzianum* (SRD) + *Foc* (SA)
10	*Trichoderma asperellum* (SRD) + Foc (SA)
11	*Trichoderma atroviride* (SRD) + Foc (SA)
12	*Trichoderma longibrachiatum* (SRD) + Foc (SA)
13	*Trichoderma hamatum* (SRD) + Foc (SA)
14	*Bacillus subtilis* (SRD) + Foc (SA)
15	Control + Foc (SA)

Where, SA =Soil application, SRD = seedling root dip

Disease Assessment

Disease data (severity) were collected at 7, 14, 21, 28, 35, 42 and 49 days after inoculation (Wongpia and Lomthaisong 2010; Demissie et al., 2021). To determine the disease severity 0-5 scale was used based on the percentage of affected plant part (Table 2). Disease severity index (DSI) was calculated following (McKinney, 1923).

$$DSI = \frac{PxQ}{MxN}\ x\,100$$

Where **P** = Number of plants in each disease severity class

Q = Numerical value of each severity class

M = Maximum disease score (the highest possible rating)

N = Total number of plants assessed

The area under the disease progress curve (AUDPC) was also calculated as follows.

$$\% - AUDPC = \sum_{n=1}^{n}\left(\frac{Yi+1+yi)}{2}(\mathrm{ti}+1 - \mathrm{ti}\right)$$

Where n=total number of observations, Yi = initial DSI at the i^{th} observation, ti= time of the i^{th} assessment in days, and n= total number of observation, t=day after inoculation, the unit for y in the sample data is % of development stage unit. The value of DSI and AUDPC were used to classify the level of pathogenicity amongst the different Fusarium isolates (Demissie et al., 2021). Moreover, plant fresh and dry weight was taken.

Table 2. Assessment scale for pepper Fusarium wilt severity.

Scale(0-5)	Description
0	No symptoms
1	Initial symptoms or 1–10% chlorosis of leaves
2	10–20% chlorosis of leaves
3	20–50% chlorosis of leaves
4	>50% chlorosis of leaves and initial symptoms flaccidity of the top leave
5	Completely or the major part of the plant wilted or death

To evaluate the *in vitro* efficacy of antagonists, diameter of inhibition zone (mm) was measured. Diameter of inhibition zone (mm) was measured by taking the average diameter of inhibition zone observed in three petridish for each antagonist and calculated using the following formula (Rini and Sulochana, 2007).

$\frac{C-T}{C}\ x\,100$ Where, I= Percent of inhibition in growth of the *Foc*

C= Radial growth of the FOC (cm) in control plate

T= Radial growth of the FOC (cm) in biological control in *Trichoderma* spp.

To evaluate *in vivo* efficacy of antagonists, wilt incidence, plant height, number of leaves, root length, fresh and dry weight of shoot and root was taken.

Data Analysis

All experimental data such as disease and plant data were subjected to analysis of variance using one way ANOVA by SAS software version 9.3 (SAS, 2017). Least Significant Difference (LSD) at 0.05 probability level was used for mean separation.

RESULTS AND DISCUSSION

Characterization and pathogenicity test of Fusarium oxysporium f.sp. capsici isolates

Macroscopic characterization

In this study from eighty samples, twenty nine isolates were identified as *Fusarium oxysporium* f.sp. *capsici* (Appendix 1). Seventeen (58.6%) of the isolates had pink colony color while nine isolates (31%) had white colony color and the remaining three isolates (10.4%) had creamy white colors on PDA plates (Fig. 1 and Appendix 1. Regarding the colony pigmentation, seven isolates (24%) had light reddish purple pigmentation, 13 isolates (45%) had intense reddish purple and the remaining nine isolates (31%) had pale yellow (Dull white) pigmentation.

Thirteen isolates (44.8%) had irregular shapes, whereas 14 isolates (48.3%) had circular and two isolates (6.9%) had filamentous shapes. The elevations of the colony were flat type in 19 (65.5%) isolates and raised in 10 (34.5%) isolates. The colony margins of the isolates were entire in 17 (58.6%) isolates undulate in 10 (34.5%) isolates and Filliform in two isolates (6.9 %). The colony growth, 18 (62%) isolates had fluffy growth, 10 (34.5%) isolates had suppressed growth and only one isolate (3.5%) had scanty fibrous growth (Fig 1 and Appendix 1). Regarding the colony surface texture, 16 (55.2%) isolates had smooth, eight (27.6%) isolates had rough and five isolates (17.2%) had rugose colony surface texture. Considering the radial growth of isolates, isolates were grouped as slow growing (2.4-2.9 cm), medium growing (3-3.5 cm), and fast growing (3.6-4.5 cm) according to Gabrekiristos *et al.* (2020). Accordingly, four (13.8%) isolates were slow growing, eight (27.6%) isolates were medium growing and the remaining 17 (58.6%) isolates were fast growing. The current results agree with the finding of Kaushal (2016) who reported colony color variation (from pink to white) in FOC isolates of hot pepper and also similar result were reported by Gabrekiristos *et al.* (2020). Jaywant (2016) also reported the production of white, cream white and creamy-pink to pink colony colors by *Fusarium oxysporum* isolates. The differential colour of the *Fusarium oxysporum* isolates may be due to the presence of specific pigment produced as by-product and involved in the enzymatic activities.

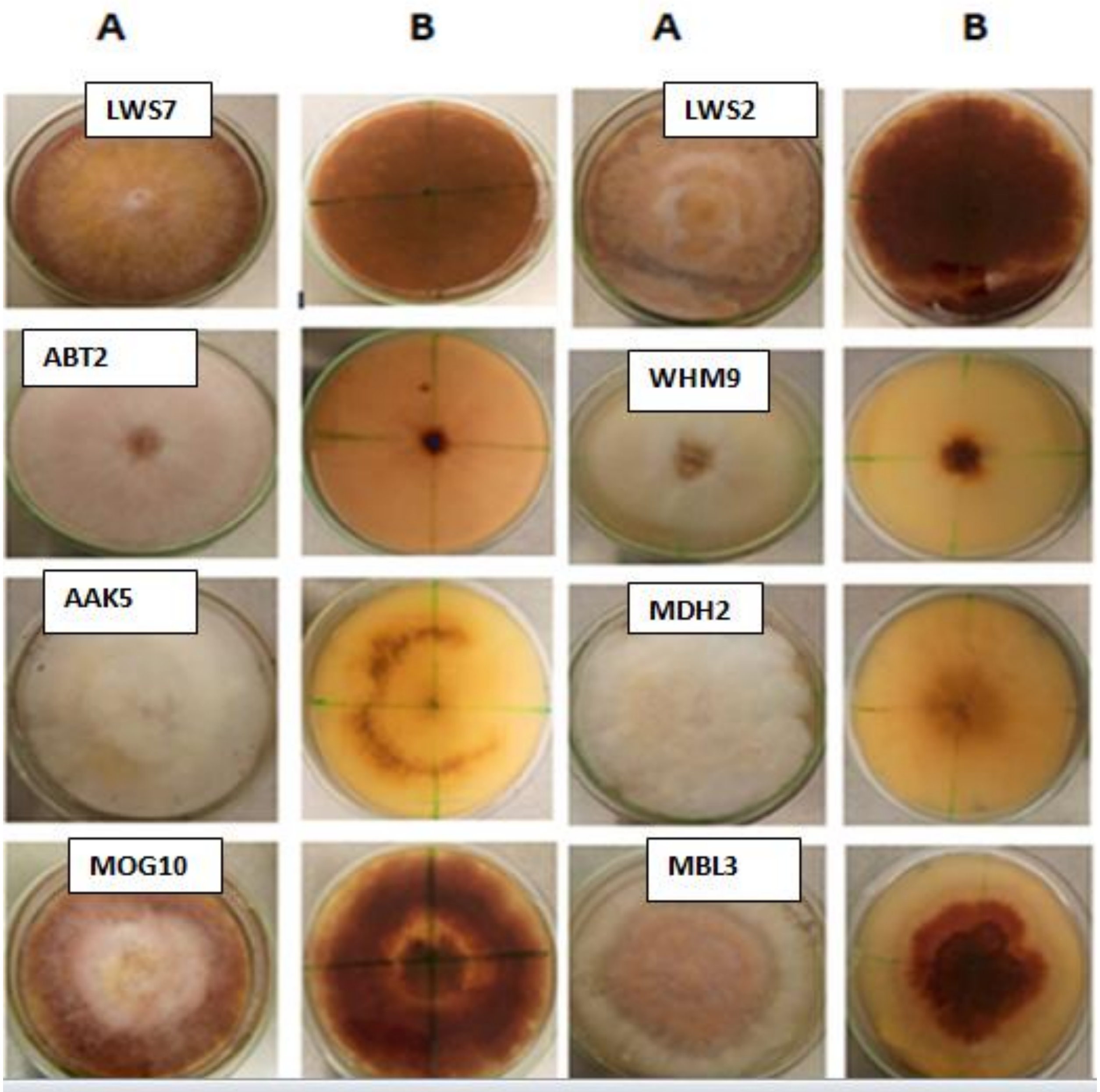

Figure 1. Pure cultures of *Fusarium oxysporium* f.sp. *capsici* isolates surface view (A) and reverse view (B) on PDA plates across a column respectively.
Note: AAKS and **ABT2** are isolates from Abeshge, **LWS2** and **LWS7** from Lanfro, **MOG10** and **MBL3** from Meskan, **WHM9** from Wera and **MDH2** from Mareko district.

Microscopic Characterization
Among 29 FOC isolates, sixteen of these isolates had straight shape, and 13 isolates had sickle shaped macro-conidia Fig 2 and Table 3. Variations in the number of septations in the macroconidia were also observed. The most frequent macroconidia septations were in the range of 2 to 3 and less frequently in between 2 and 5 conidial

septations were observed. Twenty six isolates had microconidia without septation; among them 15 isolates had oval shape, and 11 isolates had eliptical shape and the other three isolates had no microconidia at all. All the 29 isolates also produced chlamydospore in single and in pairs (Fig 2 and Table 3). Results from the present work agree with those from Jaywant (2016), Ferniah *et al.* (2014) and Kaushal (2016) that reported the ability of the pathogen to produce macroconidia, microconidia, hyphae with septation and chlamydospores. In the current study, three isolates namely MOG10, WAT6 and LST6 did not produce microconidia, however the macroconidia characteristics along with the colony feature were sufficient to identify them as isolate of *Fusarium oxysporium* in this regards.

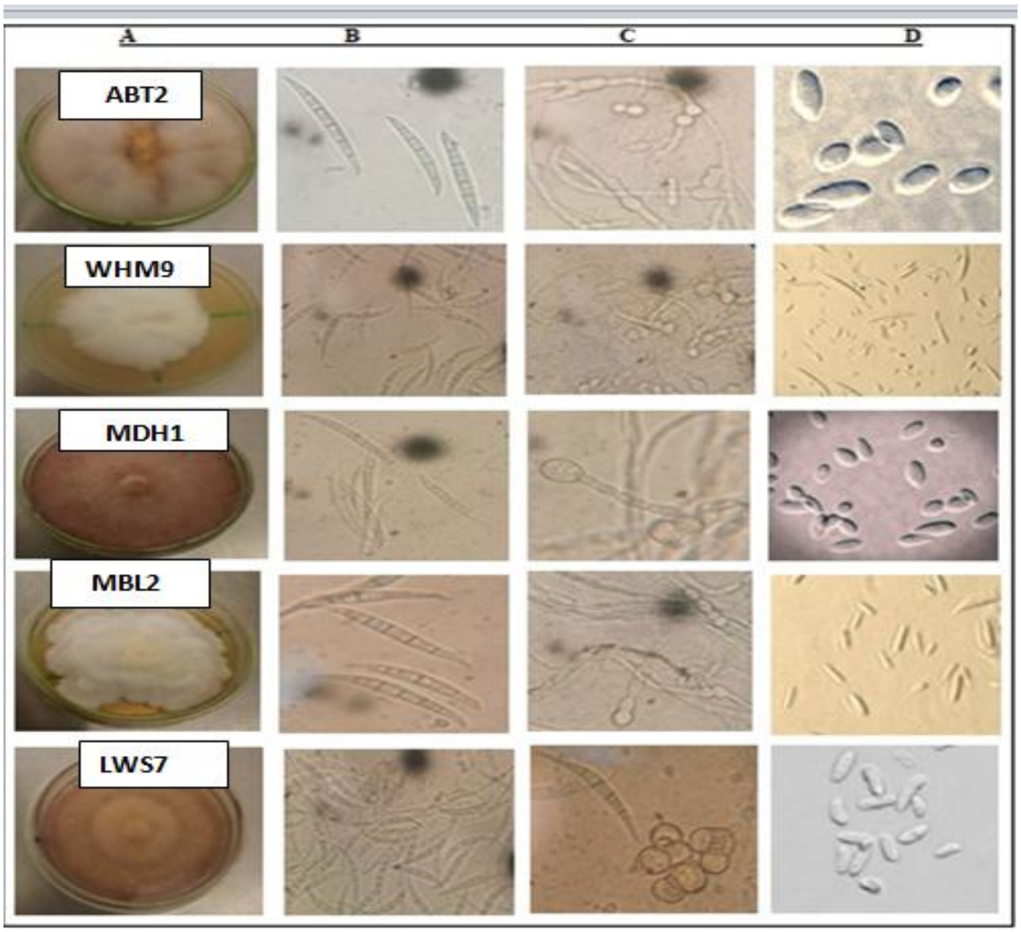

Figure 2. Pure cultures of *Fusarium oxysporium f.sp. capsici* isolate growing on PDA plates and their corresponding conidia observed under microscope (40x).
Where, A= Pure culture of the isolate of *Fusarium oxysporium f.sp. capsici* , B= Macroconidia, C= Chlamydospore, D=Microconidia across a column respectively.
Note: **LW57** is isolate from Lanfro, **ABT2** from Abeshge, **MDH1** from Mareko, and **MBL2** from Meskan and **WHM9** from Wera district

Table 3. Microscopic features of *Fusarium oxysporium* f.sp. *capsici* isolates from major pepper growing districts of southern Ethiopia during 2022 main cropping season

SN.	Isolate code	Conidia				Chlamydospore (After 15 days)
		Shape of macro conidia	Number of septa.	Micro conidia	Shape of microconidia	
1	**AAK1**	Straight	3-4	+	Oval	+
2	**AAK2**	Sickle shaped	3-5	+	Eliptical	+
5	**AAK5**	Sickle shaped	3-5	+	Oval	+
7	**AAK7**	Straight	3-5	+	Oval	+
9	**ABT2**	Straight	4-5	+	Oval	+
12	**ABT5**	Sickle shaped	2-3	+	Oval	+
15	**ABT8**	Straight	2-4	+	Eliptical	+
19	**MOG3**	Sickle shaped	3-5	+	Oval	+
25	**MOG9**	Sickle shaped	2-4	+	Eliptical	+
26	**MOG10**	Straight	2-4	-	-	+
28	**MBL2**	Sickle shaped	2-5	+	Eliptical	+
29	**MBL3**	Straight	2-4	+	Oval	+
32	**MDH1**	Straight	2-3	+	Oval	+
33	**MDH2**	Sickle shaped	2-4	+	Oval	+
39	**MDH8**	Sickle shaped	2-5	+	Eliptical	+
40	**MDH9**	Straight	3-5	+	Eliptical	+
42	**MDM2**	Straight	3-4	+	Oval	+
43	**MDM3**	Straight	2-4	+	Oval	+
51	**WHM3**	Straight	4-5	+	Oval	+
55	**WHM7**	Sickle shaped	3-4	+	Eliptical	+
57	**WHM9**	Sickle shaped	2-5	+	Oval	+
62	**WAT5**	Straight	2-3	+	Eliptical	+
63	**WAT6**	Straight	2-3	-	-	+
65	**LST1**	Straight	2-3	+	Eliptical	+
68	**LST4**	Sickle shaped	2-3	+	Oval	+
70	**LST6**	Sickle shaped	2-5	-	-	+
73	**LST9**	Sickle shaped	2-5	+	Oval	+
75	**LWS2**	Straight	3-5	+	Eliptical	+
80	**LWS7**	Straight	2-5	+	Eliptical	+

Where, "+" sign indicates presence and "-" sign indicates absence.

Pathogenicity Test

The results of pathogenicity test indicated that Fusarium isolates from the current study were able to infect pepper plants leading to symptoms such as stunted growth, leaf shading and yellowing, curling, wilting, flaccidity and or plant death while no symptoms were found on control plants. Symptoms were observed starting from the second week of post-inoculation whilst no symptoms were found on control plants (Fig 3). The specific symptoms and disease severity varied depending on the virulence level of the specific isolate of *Fusarium oxysporum* f.sp. *capsici*. Furthermore, re-isolation of the pathogen from diseased plants and re-inoculating the isolated fungi onto new and healthy plants was done and all the 29 isolates were pathogenic to pepper. Therefore, Koch's postulates were fulfilled.

Fusarium oxysporum f.sp. *capsici* isolates were grouped into three based on DSI value (disease severity index). Isolate with DSI value between 41–100% was considered as highly pathogenic: between 21–40% moderately pathogenic and between 1-20% weak pathogen) according to Demissie et al. (2021). In the current study, one isolate was identified as highly pathogenic, 27 isolates were moderately pathogenic, and one isolate was weakly pathogenic (Table 4). The highest (116.7) and the lowest (23.7) AUDPC values were recorded in isolates LWS7 and AAK2, respectively, and this confirms highly significant variability between the isolates. Moreover, fresh and dry weight of root and stem were measured and results also revealed significance variability among the isolates (Table 4).

Based on current results, isolate LWS7 was found as the most pathogenic isolate that induced disease and was used as inoculum for the studies involving biocontrol agents. The difference in virulence among the isolates may be due to differences in genes responsible for pathogenicity and virulence

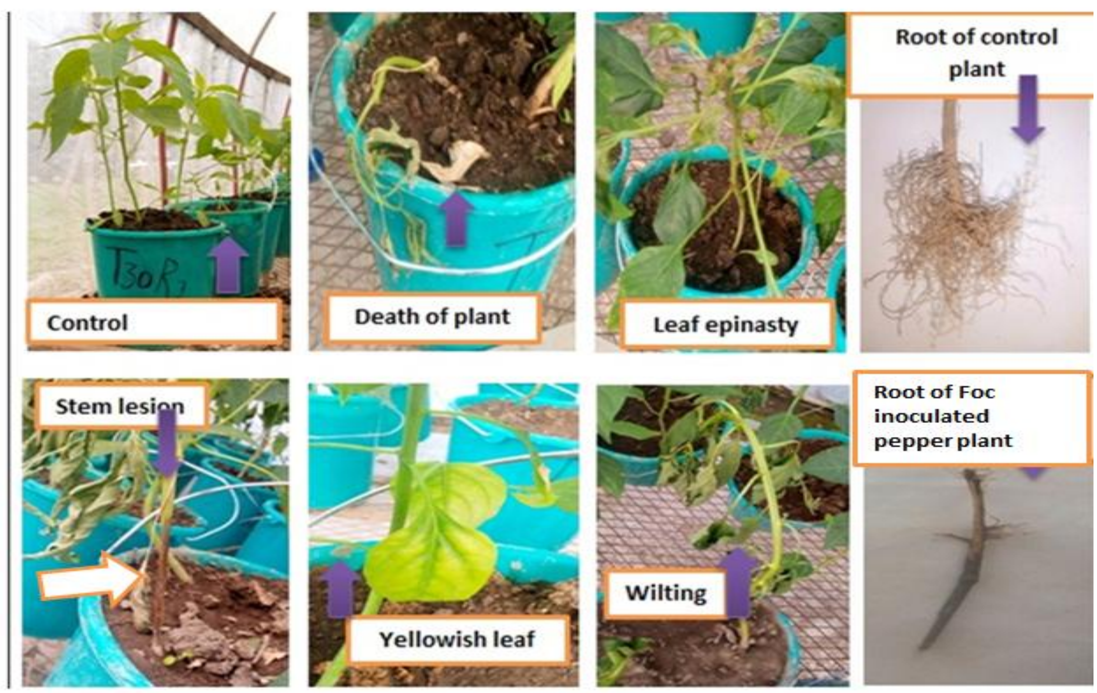

Figure 3. Picture of different symptoms on Foc treated (Inoculated) and untreated (Control) pepper plants)

Table 4. Pathogenicity of *Fusarium oxysporium* f.sp. *capsici* isolates on susceptible pepper variety Mareko fana

Isolates	AUDPC (%-day)	DSI (%)	RFW (g)	SFW (g)	RDW (g)	SDW (g)	Pathogenicity
LWS7	116.70	44.79	8.80	24.80	1.23	4.63	Highly Pathogenic
MDH1	67.30	37.49	9.33	36.60	2.10	17.16	Moderately pathogenic
MDH8	68.89	33.68	9.30	35.50	2.63	17.40	Moderately pathogenic
MDH2	69.65	33.00	9.67	44.00	2.56	22.50	Moderately pathogenic
LST9	39.00	29.50	9.66	48.56	2.30	23.00	Moderately pathogenic
LWS2	44.00	28.30	10.3	47.00	3.20	18.66	Moderately pathogenic
LST1	46.70	27.90	9.66	33.30	1.60	8.66	Moderately pathogenic
MOG3	37.74	27.00	9.66	47.90	2.40	15.26	Moderately pathogenic
ABT8	36.95	26.67	9.83	46.60	2.90	24.80	Moderately pathogenic
ABT5	37.74	26.00	9.50	59.16	2.80	24.50	Moderately pathogenic
AAK7	24.46	26.00	9.33	59.56	1.50	29.00	Moderately pathogenic
LST4	26.00	25.70	9.67	60.00	1.67	13.56	Moderately pathogenic
WHM9	51.80	25.40	9.50	54.70	3.00	21.66	Moderately pathogenic
MOG10	43.90	25.00	10.33	58.90	2.96	23.70	Moderately pathogenic
ABT2	34.95	25.00	9.66	51.70	2.16	20.20	Moderately pathogenic
MDH9	29.20	24.40	9.50	46.10	1.50	15.00	Moderately pathogenic
AAK5	68.50	24.00	9.66	35.60	1.40	12.30	Moderately pathogenic
WHM3	50.97	24.00	9.30	35.80	2.03	15.76	Moderately pathogenic
AAK1	42.37	24.00	9.30	41.80	1.76	15.50	Moderately pathogenic
AAK2	23.70	24.00	9.30	52.56	2.60	21.80	Moderately pathogenic
MDM2	64.97	23.80	9.67	36.80	1.56	18.00	Moderately pathogenic
MDM3	49.00	23.50	9.33	36.60	1.70	12.00	Moderately pathogenic
WHM7	43.94	23.50	9.30	39.30	2.90	19.43	Moderately pathogenic
MOG9	32.26	23.50	10.33	60.90	3.10	20.30	Moderately pathogenic
WAT6	29.17	22.86	9.33	35.90	2.96	15.43	Moderately pathogenic
MBL3	29.00	22.00	10.33	43.90	3.20	16.90	Moderately pathogenic
MBL2	46.26	21.58	10.00	45.40	3.50	17.30	Moderately pathogenic
LST6	28.00	21.30	9.16	60.26	2.90	22.86	Moderately pathogenic
WAT5	35.37	19.68	9.50	40.90	2.26	21.00	Weakly pathogenic
Control	—	—	12.16	88.30	3.23	46.33	—
CV (%)	6	6.6	6.4	1.7	5	4.68	—
P value	<0.0001	<0.0001	0.0003	<.0001	<.0001	<.0001	—

Where, RFW=Root fresh weight, RDW= Root dry weight, SFW=Shoot fresh weight, SDW=Shoot dry weight. Highly Pathogenic: DSI =41–100%, moderately pathogenic: DSI =21–40%, weak: DSI = 1–20%. (Demissie *et al.,* 2021), (Aklilu *et al*., 2018) Where, DSI= Disease severity index. % - AUDPC values were also used to classify the level of pathogenicity among the different Fusarium isolates (Demissie et *al*., 2021).

Evaluation of Biocontrol agents against the pathogenic isolates

***In vitro* test**

All seven biocontrol agents (BCAs) significantly (P < 0.0001) reduced the radial growth of *Fusarium oxysporum* f.sp. *capsici in vitro* compared to the control treatment. The highest (81.8%) and the lowest (48%) mycelial growth inhibition were obtained from *Trichoderma harzianum* and *Trichoderma hamatum*, respectively, (Fig 4 and Table 5). The significant reduction in mycelia growth by biocontrol agents might be the result of the antagonistic organisms action adopting numerous types of mechanisms such as antibiosis, parasitism, induced resistance, and competition for nutrients and lytic enzymes to inhibit the growth of *Fusarium oxysporium* f.sp.

capsici (Suprapta, 2012; Segarra et al., 2013). Endriyas et al. (2020) reported that the fungal BCAs, the *Trichoderma* spp. were the most effective and provided up to 85.2% mycelial growth inhibition which agrees with results of the current study. *T. viride* and *T. harzianum* as the most significant antagonistic organisms due to secretion of extracellular lytic enzymes and other compounds like harzianien and viridian which enhance their antagonistic activity against Fusarium wilt of pepper (Ozbay and Newman, 2004). *Trichoderma* spp. hinder pathogenic invasion through the release of organic metabolite such as chitinase, pachybasin and volatile inhibitory compounds *i.e*. acetaldehyde (Bunker and Kusum, 2001).

Bacillus subtilis caused a very highly significant (P<.0001) reduction in the radial growth of *Fusarium oxysporium* f.sp. *capsici* compared with control treatment. The efficacy of *Bacillus subtilis* was significantly higher than two of *Trichoderma* species (*T.atroviride and T.hamatum)* and significantly lowers than the other four species of *Trichoderma*. The current results were in agreement with the findings of Abada and Ahmed (2014), and Endriyas et al. (2020) who reported significant reduction to the radial growth of *Fusarium oxysporium* f.sp. *capsici* due to *Bacillus subtilis*.

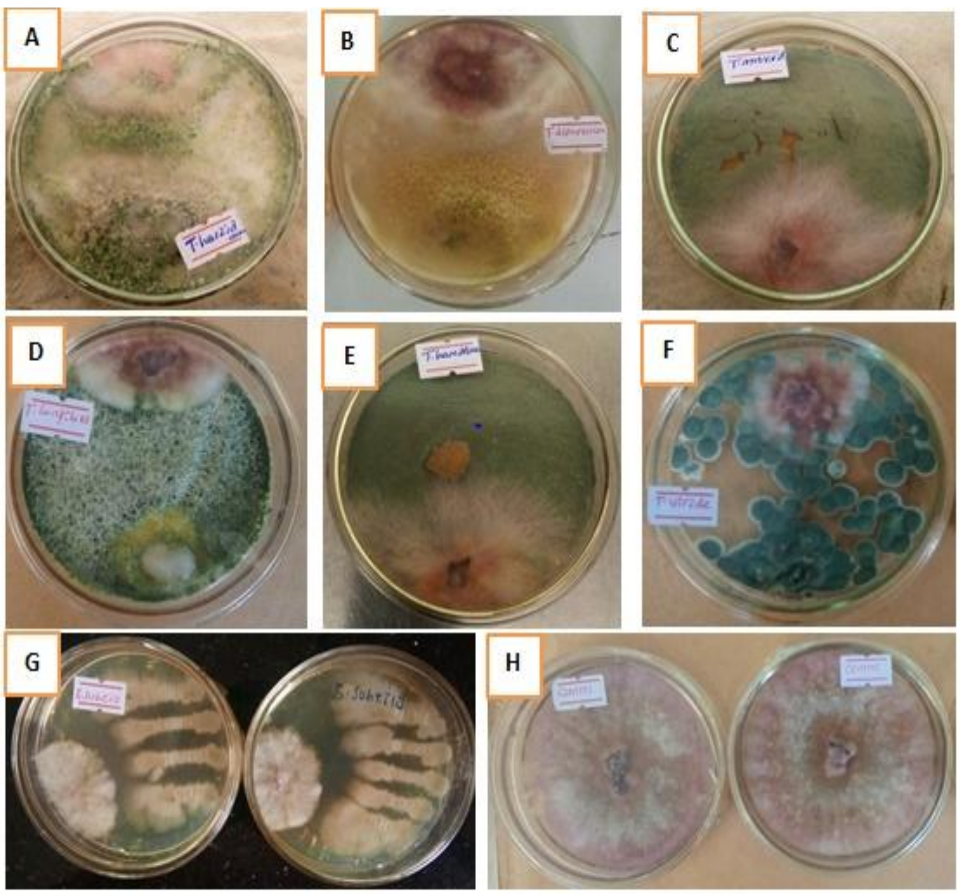

Figure 4. Dual culture assay on potato dextrose agar showing mycelial growth inhibition of *Fusarium oxysporium* f.sp.*capsici* (LWS7). Where, A=*T.harzianum,* B=*T.asperellum,* C=*T.atroviride,* D=*T.longibrachiatum,* E=*T.hamatum,* F= *T.viride,* G=*Bacillus subtilis,* H=*Control*

Table 5. Effect of biocontrol agents on *Fusarium oxysporium* f.sp. *capsici* mycelium growth

Antagonists	Mean radial growth of Foc (mm) ±SE	Inhibition (%)	Scale of antagonistic activity
Trichoderma harzianum	16.33±0.27	81.8[a]	****
Trichoderma viride	25.00±0.27	72.2[b]	***
Trichoderma asperellum	26.33±0.47	70.7[b]	***
Trichoderma longibrachiatum	27.00±0.47	70.0[b]	***
Bacillus subtilis	34.00±0.47	62.0[c]	***
Trichoderma atroviride	45.67±0.54	49.3[d]	*
Trichoderma hamatum	46.67±2.72	48.0[d]	*
Control (Without antagonist)	90.00	0.0[e]	
LSD(0.05)	4.55		
CV (%)	4		
P-value	**<.0001**		

Means with the same letter in a column are not significantly different at P≤0.05.
*The effectiveness of Trichoderma isolates was determined using the following inhibitory scale (Soytong, 1988)
**** = Very high antagonistic activity (above 75%)
*** = High antagonistic activity (61-75%)
** = Moderate antagonistic activity (51-60%)
* = Low antagonistic activity (<50%)
– = No antagonistic activity

In Vivo test

Effect of biocontrol agents on Fusarium wilt disease.

Soil application (SA)

All the seven biocontrol agents tested in the current experiment significantly decreased disease incidence as compared to the control treatment. The lowest (37.8 %) and the highest (53.3%) disease incidence were recorded with *Trichoderma harzianum* and *Trichoderma hamatum* treatment respectively (Table 6). In the current study, *Trichoderma viride* controlled the disease up to 60%. Results are in line with Anjum et al. (2020), where *Trichoderma* treatments applied in soil significantly decreased fusarium wilt chili disease. Sharma et al. (2004) also reported that soil application of *Trichoderma viride* controlled root rot of *Prosopis cineraria* caused by *Fusarium solani* by up to 56%.

The reduced disease incidence may be due to *Trichoderma* spp. acting as biocontrol agents through mycoparasitism, disrupting pathogen cell walls and absorbing nutrients and by enhancing plant resistance via root architecture improvement during pathogen interaction (Yao et al., 2023). The saprophytic nature, and fast mycelial growth and strong environmental adaptability of *Trichoderma*, seizes the invasive part of the pathogenic fungi in the root of a plant, thus hindering the invasion of the pathogen fungi. Additionally, it might be possible that *Trichoderma* rapidly absorb the nutrients required for the growth of the pathogenic fungi, resulting in nutrient deficiency and inhibiting the growth and reproduction of the pathogenic fungi (Halifu et al., 2020; Bazghaleh et al., 2020).

A wilt incidence of 46.7% was recorded in soil inoculated with *Bacillus subtilis*, while the maximum disease incidence (86.7%) was recorded in the control treatment. Zhang et al. (2009) reported that soil treatments of *Bacillus subtilis* provided better protection against Fusarium root rot of Soybean caused by *Fusarium oxysporum* and *Fusarium graminearum* pathogens. Ghonim (1999) and Amer and Utkhede (2000) reported *Bacillus subtilis* protected tomato plants against *Fusarium oxysporum* f.sp. *lycopersici* and cucumber and lettuce from root rot against *Pythium aphanidermatum* diseases. This might be due to the fungistatic and fungitoxic modes of *Bacillus subtilis* to disrupt of hyphae following contact with the fungal pathogen at the leaf surface. Moreover colonization of plant roots by *Bacillus subtilis* can induce a distinct broad spectrum resistance response in both below and above ground parts of the plant.

Seedling Root Dip (SRD)

The current result revealed that all the inoculated biocontrol agents significantly decreased disease incidence in comparison with control treatment in a seedling root dip test. Results are more or less in line with that of the soil inoculation test. The lowest (31%) and highest (46.67%) disease incidence were recorded with *Trichoderma viride* and *Trichoderma hamatum,* respectively,(Table 7). Forty percent disease incidence was recorded in seedlings inoculated with *Bacillus subtilis* while the maximum disease incidence (86.7%) was recorded in the control treatment.

The current results are in agreement with those of Ankita et al. (2018) and Nazneen et al. (2021), where seedling treatment with *Trichoderma viride* and *Trichoderma harzianum* were found to have highly significant effect in managing wilt disease and exhibit lesser incidence in hot pepper under field and glass house conditions. This might be due to increasing of sporulation of Trichoderma around the hyphae of Fusarium that decreases the pathogen population (Sharma et al. 2011). Ahmed et al. (2003) also reported that *Trichoderma harzianum* was effective against root rot of Pepper caused by *Ralstonia solani* when they were used as suspensions for root drenching. This might be due to the ability of Trichoderma to quickly adsorb to the roots of crops for propagation, and the hyphae quickly wrap the roots of crops to form a protective layer, protect the roots of crops from the invasion of pathogens, and kill the nearby pathogens (Halifu et al. 2020).

Kloepper et al. (2004) and Szczech and Shoda (2006) reported application of *Bacillus subtilis* to seedlings has been found effective for suppressing soil borne diseases and has successfully induced systemic resistance in the treated plants. Additionally, Ahmed et al. (2003) and Lee et al. (2008) reported the effectiveness of *Bacillus subtilis* against *Ralstonia solani* and *Phytophthora capsici* in pepper plants. This might be due to *Bacillus subtilis* rapidly grows, outcompeting pathogens for space and preventing spore infection (Cazorla et al. 2007).

Table 6. Effect of biocontrol agents on the Fusarium wilt disease under greenhouse condition

Mode of treatment application	Treatment	Incidence (%) Mean±SE
Soil application (SA)	*T.harzianum*	37.78±0.36^{efg}
	T.viride	40.00±0.31^{def}
	T.asperellum	44.44±0.92^{cde}
	T.longibrachiatum	46.67±0.54^{bcd}
	T.atroviride	51.00±0.92^{bc}
	T.hamatum	53.33±0.72^{b}
	B.subtilis	46.67±0.54^{bcd}
Seedling root dip (SRD)	*T.harzianum*	33.33±0.27^{fg}
	T.viride	31.00±0.90^{g}
	T.asperellum	37.78±0.64^{efg}
	T.longibrachiatum	40.00±0.47^{def}
	T.atroviride	42.20±0.55^{de}
	T.hamatum	46.67±0.54^{bcd}
	B.subtilis	40.00±0.47^{def}
	Control	**86.67±0.27^{a}**
CV (%)		**10**
LSD (0.05)		**7.59**

Means with the same letter in a column are not significantly different at P≤0.05.

Effect of Biocontrol Agents on Hot Pepper Growth

The biocontrol treatments had significant ($p<0.05$) impact on pepper growth parameters including plant height, number of leaves, root length, and fresh and dry weight of roots and shoots of hot pepper (Table 7).

The height of pepper plants in the current experiment varied from 32cm in the negative control plants to 63cm in plants treated with *Trichoderma harzianum*. The number of pepper leaves ranged from 17 in control plants to 47 in *Trichoderma viride* treated plants (SA).

The maximum (10.83cm) root length was recorded with *Trichoderma viride* (SRD) and minimum (6cm) root length were recorded with soil application of *Trichoderma atroviride* and *Trichoderma hamatum* followed by *Trichoderma asperellum* (SA) and *Trichoderma hamatum* (SRD) that resulted in 7cm root length. The lowest root length (4.8cm) was obtained in control plants. The maximum (45.67g) and minimum (36.67g) shoot fresh weight were recorded with *Trichoderma harzianum* (SRD) and *Trichoderma hamatum* (SA) respectively; however, the lowest shoot fresh weight (24.33g) was obtained in control plants. The maximum (23.76g) and minimum (17.76g) shoot dry weight were recorded with *Trichoderma harzianum* (SRD) and *Trichoderma hamatum* (SA) respectively; however, the lowest shoot dry weight (8g) was obtained in control plants. The maximum (8.47g) and minimum (6.16g) root fresh weight were recorded with *Trichoderma harzianum* (SRD) and *Trichoderma hamatum* (SA) respectively, however; the lowest root fresh weight (3.13g) was obtained in control plants. The maximum (3.97g) and minimum (2.6g) root dry weight were recorded with *Trichoderma harzianum* (SRD) and *Trichoderma atroviride* (SA) respectively, however the lowest root dry weight (1.63g) was obtained in control plants.

The current results are in agreement with those of Marra et al. (2006) and Anjum et al. (2020) reports, where *Trichoderma* spp. have been shown to improve the growth of lettuce, tomato, and pepper plants. It is also in line with the findings that *Trichoderma* spp. augmented plant growth in crops such as tomato, pepper, cucumber, cacao and beans (Macias et al., 2018). This may be related to the fact that *Trichoderma* spp. colonizes the plant roots and secretes chemical stimulants acting as endophytic symbionts beneficial for the plants (Harman et., al 2004). Similar results were also reported by those of Sharma et al. (2012), Sharma (2018), Stewart and Hill (2014) where increased growth of various crops with the use of antagonist *Trichoderma* spp. This indicates species of Trichoderma are unique groups of rhizospheric microorganisms associated with certain beneficial effects to enhance plant growth and development (Oszust et., al 2020, Panchalingam et al., 2022, Mohiddin et al., 2021).

Results of the current study also revealed that *Bacillus subtilis* may have promoted growth of hot pepper as compared with control treatment. This coincides with Xing et al. (2003) and Ryder (1998), who reported that *Bacillus subtilis* promoted plants. This might be due to that *Bacillus* grow very fast and occupies the court of infection and preventing pathogen spores to reach susceptible tissues in competition for spaces (Wolk and Sarkar, 1994). Additionally, Abada and Ahmed (2014) reported that plants grown in soil infested with Bacillus strains were of high values of plant height than that grown in the control (uninfected soil). The possible reason to this might be due to the fact that *Bacillus subtilis* is soil inhabitant and survive under a broad spectrum of environmental conditions.

Table 7. Effect of biocontrol agents on the growth parameters of hot pepper under greenhouse condition

Treatment application mode	Treatment	No. of Leaves Mean±SE	Plant height(cm) Mean±SE	Root length(cm) Mean±SE	Shoot fresh weight(g) Mean±SE	Shoot dry weight(g) Mean±SE	Root fresh weight(g) Mean±SE	Root dry weight(g) Mean±SE
Soil application	*T.harzianum*	45.7 ±1.2^{a}	57.7±1.2^{bc}	9.00±0.14^{c}	42.67±0.98^{b}	22.33±0.54^{ab}	7.33±0.13^{bcd}	3.53±0.05^{bc}
	T.viride	47.3 ±0.7^{a}	59.0±0.7^{b}	8.00±0.24^{de}	40.67±0.27^{cd}	20.33±0.72^{cde}	7.40±0.09^{bc}	3.67±0.05^{b}
	T.asperellum	35.7 ±1.2^{de}	40.0±0.7^{fg}	7.00±0.24^{fg}	39.67±0.72^{def}	19.23±0.05^{def}	6.67±0.27^{defg}	3.27±0.02^{de}
	T.longibrachiatum	36.0 ±1.1^{d}	42.7±1.2^{ef}	6.67±0.36^{gh}	38.00±0.47^{fg}	18.77±0.47^{ef}	6.46±0.25^{efg}	2.90±0.08^{f}
	T.atroviride	35.0 ±0.3^{def}	40.0±0.5^{fgh}	6.00±0.24^{h}	38.30±0.72^{efg}	17.83±0.13^{f}	6.30±0.16^{fg}	2.60±0.09^{g}
	T.hamatum	34.0±0.3^{defg}	39.0±0.5^{gh}	6.00±0.24^{h}	36.67±0.72^{g}	17.76±0.11^{f}	6.16±0.07^{g}	2.63±0.05^{g}
	B.subtilis	36.0±0.5^{de}	40.0±0.9^{fgh}	6.67±0.14^{gh}	39.33±0.72^{def}	18.80±0.36^{ef}	6.67±0.12^{defg}	3.06±0.11^{ef}
Seedling Root Dip	*T.harzianum*	42.0±0.5^{b}	63.0±0.5^{a}	9.67±0.27^{b}	45.67±0.54^{a}	23.76±0.35^{a}	8.47±0.28^{a}	3.97±0.09^{a}
	T.viride	39.0±0.7^{c}	56.0±0.9^{c}	10.83±0.27^{a}	43.06±0.05^{b}	22.00±0.73^{abc}	8.00±0.09^{ba}	3.77±0.02^{ab}
	T.asperellum	34.0±0.5^{defg}	46.7±0.5^{d}	8.33±0.27^{cd}	41.67±0.27^{bc}	20.90±0.63^{bcd}	7.40±0.32^{bc}	3.40±0.09^{cd}
	T.longibrachiatum	32.0 ±0.3^{g}	38.0±0.7^{gh}	7.33±0.27^{efg}	40.00±0.47^{cde}	19.86±0.51^{de}	6.90±0.23^{cdef}	3.23±0.02^{de}
	T.atroviride	33.7±0.3^{efg}	38.0±0.7^{gh}	7.33±0.27^{efg}	39.33±0.27^{def}	19.50±0.59^{def}	7.03±0.11^{cde}	3.17±0.07^{de}
	T.hamatum	32.0 ±0.3^{g}	37.0±0.9^{h}	7.00±0.50^{fg}	39.33±0.72^{def}	18.86±0.38^{ef}	6.67±0.11^{defg}	2.90±0.05^{f}
	B.subtilis	33.0 ±0.5^{fg}	45.0±0.5^{de}	7.67±0.3^{def}	40.33±0.27^{cd}	19.67±0.98^{def}	6.96±0.09^{cdef}	3.23±0.11^{de}
	Control	17.0±0.7^{h}	32.0±0.5^{i}	4.76±0.12^{i}	24.33±0.27^{h}	8.000±0.36^{g}	3.13±0.19^{h}	1.63±0.07^{h}
	LSD (0.05)	**2.4**	**2.7**	**0.9**	**1.97**	**1.9**	**0.67**	**0.26**
	CV	**4**	**3.6**	**7.5**	**3**	**5.97**	**5.97**	**5**

Means with the same letter in a column are not significantly different at P≤0.05.

CONCLUSIONS

Hot pepper (Capsicum annuum L.) is an important vegetable and spice crop in tropical areas worldwide including Ethiopia. Among the biotic factors that affect pepper production in Ethiopia, Fusarium wilt caused by Fusarium oxysporium f.sp capsici is one of the most economically important diseases as it accounts for yield losses of up to 80%. In the current work, twenty nine isolates of Fusarium oxysporium f.sp. capsici were identified from 80 samples. The pathogenicity of the 29 isolates on pepper was confirmed in a greenhouse experiment and isolate LWS7 was identified as the most virulent isolate. As a result, it was used to evaluate the efficacy of biocontrol agents. Results of the in vitro test revealed significant effect of the bioagents and the level of mycelial growth significantly varied among the antagonists tested with the highest (81.8%) and lowest (48%) inhibition caused by Trichoderma harzianum and Trichoderma hamatum, respectively. In addition to their significant effect on mycelia growth in-vitro, the biocontrol agents evaluated in the current experiment were also found to result in significant reduction in the disease and promote of the growth of hot pepper in soil application and seedling root dip tests.

From the current result it can be concluded that the Fusarium wilt of hot pepper can be managed by using biocontrol agents especially Trichoderma harzianum and Trichoderma viride. The present study also confirmed the variability of the Foc isolates and efficacy of biocontrol agents. Molecular identification Fusarium oxysporium f.sp. capsici of should be done to better characterize identified isolates in the country. The efficacy of more biocontrol agents against Fusarium wilt of pepper should be evaluated under greenhouse and field conditions on a large scale.

ACKNOWLEDGEMENT

Wolkite University is gratefully acknowledged for the scholarship provided to the author. The authors are also thankful for the EWA-BELT project for the finical support.

DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

Abada K.A, Ahmed M.A. 2014. Management of Fusarium wilts of sweet pepper by Bacillus strains. American Journal of Life Sciences, 2(6-2):19-25. https://doi.org/10.11648/j.ajls.s.2014020602.13. [Scholar Google]

Abada K.A, Eid K.E. 2014. A Protocol suggested for management of canta-loupe downy mildew. American Journal of Life Sciences, 2(6-2):1-10. https://doi.org/doi/10.11648/j.ajls.s.2014020602.11.

Ahmed A.S, Ezziyyani M, Sanchez C.P, Candela M.E. 2003. Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. European Journal of Plant Pathology, 109(6): 633-637. https://doi.org/10.1023/A:1024734216814. [Scholar Google]

Aklilu S, Ayana G, Abebie B, Abdissa T. 2018. Screening for Resistance Sources in Local and Exotic Hot Pepper Genotypes to *Fusarium* Wilt (*Fusarium oxysporium*) and Associated Quality Traits in Ethiopia. Journal of Advance Crop Science Technology, 6(3): 367-376. https://doi.org/10.4172/2329-8863.1000367. [Scholar Google]

Aklilu, S. Berhanu B, Bekele K. 2007. Survey report on current pepper production constraints in major pepper growing areas of Ethiopia. EIAR Addis Abeba, Ethiopia.

Amer G.A, Utkhede R.S. 2000. Development of formulations of biological agents for management of root rot of lettuce and cucumber. Canadian journal of microbiology, 46(9): 809-816. https://doi.org/10.1139/w00-063. [Scholar Google]

Anjum N, Shahid A, Iftikhar S, Mubeen M, Ahmad M, Jamil Y, Rehan M, Aziz A, Iqbal S, Abbas A. 2020. Evaluations of Trichoderma isolates for biological control of *Fusarium* wilt of chili. Plant Cell Biotechnology and Molecular Biology, 21(59): 42-57. [Scholar Google]

Ankita S, Harshita R.S, Ankur V. 2018. Bioefficacy of *Trichoderma harzianum* and *Trichoderma viride* against *Fusarium oxysporum* f.sp. *capsici* causing wilt disease in chilli. Journal of Pharmacognosy and Phytochemistry, 7(5): 965-966. [Scholar Google]

Assefa M, Dawit W, Lencho A, Hunduma T. 2015. Assessment of wilt intensity and identification of causal fungal and bacterial pathogens on hot pepper (*Capsicum annuum* L.) in Bako Tibbe and Nonno districts of West Shewa Zone, Ethiopia. International Journal of Phytopathology, 4 (1):21-

28. https://doi.org/10.33687/phytopath.004.01.0972. [Scholar Google]

Bazghaleh N, Prashar P, Woo S, Vandenberg A. 2020. Effects of lentil genotype on the colonization of beneficial *Trichoderma* species and biocontrol of Aphanomyces root rot. Journal of Microorganisms, 8(9): 1290-1292. https://doi.org/10.3390/microorganisms8091290. [Scholar Google]

Berhanu, Y., B. Derbew, G. Wosene, and M. Fekadu. 2011. Variability, Heritability and Genetic Advance in Hot Pepper (*Capsicum annum* L.) Genotypes in West Shoa, Ethiopia. Journal of Agriculture and Environment, 10(4): 587–592. [Scholar Google]

Black, L.L. and Rivelli, V. 1990. June. *Fusarium* wilt of pepper in Louisiana. In Proceeding 10th National Pepper Conference (pp. 25-27).

Booth, C. 1971. The genus Fusarium. Kew, UK, Common wealth Mycological Institute, pp 237.

Bunker R.N, Kusum M. 2001. Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. Journal of Mycology and Plant Pathology, 31(1): 50-53. [Scholar Google]

Cazorla, F.M., Romero, D., Pérez-García, A., Lugtenberg, B.J.J., Vicente, A.D. and Bloemberg, G. 2007. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. Journal of applied microbiology, 103(5): 1950-1959. [Scholar Google]

Demissie S, Megersa G, Meressa B.H, Muleta D. 2021. Resistance levels of Ethiopian hot pepper (*Capsicum* spp.) varieties to a pathogenic *Fusarium* spp. and in vitro antagonistic effect of *Trichoderma* spp. Archives of Phytopathology and Plant Protection, 54(11-12): 647-663. https://doi.org/10.1080/03235408.2020.1853494. [Scholar Google]

Dias, G. B., V. M. Gomes, T. M. Moraes, U. P. Zottich, G. R. Rabelo, A. O. Carvalho, M. Moulin, L. S. A. Gonçalves, R. Rodrigues, and M. Da Cunha. 2013. Characterization of *Capsicum* Species Using Anatomical and Molecular Data. Genetics and Molecular Research, 12 (4): 6488–6501. [Scholar Google]

Endriyas G., Daniel T., Getachew A. 2020. Prospects of host resistance and biocontrol agent for the management of hot pepper *Fusarium* wilt (*Fusarium oxysporum* f.sp. *capsici*) in the Central Rift valley of Ethiopia, Academic Research Journal of Agricultural Science Research, 8(3): 156-163. [Scholar Google]

Fekadu, M. and Dandena, G. 2006. Status of Vegetables Crops in Ethiopia. Ugandan Journal of Agriculture, 12(2): 26-30.

Ferniah, R.S., Daryono, B.S., Kasiamdari, R.S. and Priyatmojo, A. 2014. Characterization and pathogenicity of *Fusarium oxysporum* as the causal agent of *Fusarium* wilt in chilli (*Capsicum annuum* L.). Microbiology Indonesia, 8(3): 121-126. [Scholar Google]

Gabrekiristos E, Teshome D, Ayana G. 2020. Distribution and Relative Importance of Hot Pepper *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *capsici)* and Associated Agronomic Factors in the Central Rift Valley of Ethiopia. Journal of Advanced Crop Science Technology, 8(1): 1000437. [Scholar Google]

Ghonim, M. I. 1999. Induction of systemic resistance against *Fusarium* wilts in tomato with the biocontrol agent *Bacillus subtilis*. Bulletin of Faculty of Agriculture, University of Cairo, 50(2): 313-328. [Scholar Google]

Halifu S, Deng X, Song X, Song R, Liang X. 2020. Inhibitory mechanism of *Trichoderma virens* ZT05 on *Rhizoctonia solani*. Journal of Plants, 9(7): 7-8. https://doi.org/10.3390/plants9070912. [Scholar Google]

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species - Opportunistic, Avirulent Plant Symbionts. Nature Reveiws, 2: 43-56. [Scholar Google]

Herman R, Perl-Treves R. 2007. Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *melonis* Race 1.2 in Cucumis. Journal of Plant Disease, 91(9): 1180-1186. [Scholar Google]

Heydari A, Pessarakli M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of biological sciences, 10(4): 273-290. https://doi.org/10.3923/jbs.2010.273.290. [Scholar Google]

Jaywant, 2016. Pathogenic Variability and Management of *Fusarium* wilt of Chilli (*Capsicum annuum* L.), PhD Thesis in plant pathology in College of Agriculture CCS Haryana Agricultural University Hisar.

Karimi R, Owuoche JO, Silim SN. 2010. Inheritance of *Fusarium* wilts resistance in pigeon pea (*Cajanus cajan* (L.) Millspaugh). Indian Journal of Genetics and plant Breeding, 70(3): 271-276. [Scholar Google]

Kassahun S, Tariku H, Mekonnen A. 2016. Characterization and evaluation of hot pepper (*Capsicum annuum* L.) cultivars against bacterial wilt disease (*Ralstonia solanacearum*). Pyrex Journal of Microbiology and Biotechnology Research, 2(3): 22-29.

Kloepper J.W, Ryu C.M, Zhang S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Journal of Phytopathology, 94(11): 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259. [Scholar Google]

Koste A. Yadeta and Bart P.H.J. T. 2013. The xylem as battle ground for plant hosts and vascular wilt pathogens. Frontiers in Plant science, 4(10): 97-98. [Scholar Google]

Lee K.J, Kamala-Kannan S, Sub H.S, Seong C.K, Lee G.W. 2008. Biological control of *Phytophthora* blight in red pepper (*Capsicum annuum* L.) using *Bacillus subtilis*. World journal of microbiology and biotechnology, 24(7): 1139-1145. https://doi.org/10.1007/s11274-007-9585-2. [Scholar Google]

Leslie, J. F., and B. A. Summerell. 2006. The *Fusarium* Laboratory Manual. 388. New Jersey: Wiley-Blackwell.

Macias R.L, Guzman G.A, García J.P. Contreras C.H.A. 2018. *Trichoderma atroviride* promotes tomato development and alters the root exudation of carbohydrates, which stimulates fungal growth and the biocontrol of the phytopathogen *Phytophthora cinnamomi* in a tripartite interaction system. FEMS Microbiology Ecology, 94(9): 137-139. https://doi.org/10.1093/femsec/fiy137. [Scholar Google]

Marra R, Ambrosino P, Carbone V, Vinale F, Woo S.L, Ruocco M, Ciliento R, Lanzuise S, Ferraioli S, Soriente I, Gigante S. 2006. Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Journal of Current genetics, 50: 307-321. https://doi.org/10.1007/s00294-006-0091-0. [Scholar Google]

Matar S. M., E, Kazzaz S. A., Wagih E. E., E, Diwany A. I., Moustafa H. E., Abo - Zaid G. A., Abd-Elsalam H. E., Hafez E. E. 2009. Antagonistic and inhibitory effect of *Bacillus subtilis* against certain plant pathogenic fungi. International Biotechnology, 8(1): 53-61. [Scholar Google]

McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Research, 26(5): 195–217. [Scholar Google]

Mekonen, S. and Chala, A. 2014. Assessment of hot pepper (*Capsicum* sp.) diseases in southern Ethiopia. International Journal of Science and Research, 3(3): 91-95 [Scholar Google].

Mohiddin, F.A., Padder, S.A., Bhat, A.H., Ahanger, M.A., Shikari, A.B., Wani, S.H., Bhat, F.A., Nabi, S.U., Hamid, A., Bhat, N.A. and Sofi, N.R. 2021. Phylogeny and optimization of *Trichoderma harzianum* for chitinase production: evaluation of their antifungal behaviour against the prominent soil borne phyto-pathogens of temperate India. Journal of Microorganisms, 9(9): 1962-1965. [Scholar Google]

Nazneen H, Patar S, Das R. 2021. Bio-Efficacy of *Trichoderma viride* 1.15 % WP (Bio Cure-F) Against Wilt Disease of Chilli Caused by *Fusarium oxysporum* f.sp. *capsici* under field Condition. International Journal of biological forum, 13(1): 507-511. [Scholar Google]

Ozbay N, Newman S.E. 2004. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. Pakistan Journal of Biological Sciences, 7(4): 478-484. [Scholar Google]

Oszust, K., Cybulska, J. and Frąc, M. 2020. How do *Trichoderma* genus fungi win a nutritional competition battle against soft fruit pathogens. A report on niche overlap nutritional potentiates. International Journal of Molecular Sciences, 21(12): 4235. [Scholar Google]

Panchalingam, H., Powell, D., Adra, C., Foster, K., Tomlin, R., Quigley, B.L., Nyari, S., Hayes, R.A., Shapcott, A. and Kurtböke, D.İ. 2022. Assessing the various antagonistic mechanisms of *Trichoderma* strains against the brown root rot pathogen *Pyrrhoderma noxium* infecting heritage fig trees. Journal of Fungi, 8(10): 1105. [Scholar Google]

Rini C.R, Sulochana K.K. 2006. Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent pseudomonads *(Pseudomonas fluorescens)*. Journal of Tropical Agriculture, 44(1-2): 79-82. https://jtropag.kau.in/index.php/ojs2/article/view/159. [Scholar Google]

Ryder M.H, Yan Z, Terrace T.E, Rovira A.D, Tang W, Correll R.L. 1998. Use of strains of Bacillus isolated in China to suppress take-all and rhizoctonia root rot, and promote seedling growth of glasshouse-grown wheat in Australian soils. Journal of Soil Biology and Biochemistry, 31(1): 19-29.

https://doi.org/10.1016/S0038-0717(98)00095-9. [Scholar Google]

Sabalpara A.N, Priya J, Waghunde R.R, Pandya J.P. 2009. Antagonism of *Trichoderma* against sugarcane wilt pathogen (*Fusarium moniliformae*). American-Eurasian Journal of Sustainable Agriculture, 3(4): 637-638. [Scholar Google]

SAS (Statistical Systems Analysis). 2017. SAS User's Guide. Version 9.3. Cary (NC): SAS Institute Inc.

Segarra G, Aviles M, Casanova E, Borrero C, and Trillas I. 2013. Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34. Phytopathological Mediterranian, 52(1): 77−83. [Scholar Google]

Sekhon R.K, Singh P. 2007. Influence of edaphic factors and cultural practices on the development of *Fusarium* wilt of muskmelon. Research Punjab Agricultural University, 44 (1): 50-54.

Sharma S, Pal R, Gupta P.P, Kaushik J.C. 2004. Management studies on root rot of *Prosopis cineraria* caused by *Fusarium solani.* Journal of Tropical Forest Science, 16(1): 71-77. https://www.jstor.org/stable/23616388. [Scholar Google]

Sharma K.K. 2018. *Trichoderma* in Agriculture: An Overview of Global Scenario on Research and its Application. International Journal of Current Microbiology and Applied Science, 7(8): 1922-1933. https://doi.org/10.20546/ijcmas.2018.708.221. [Scholar Google]

Sharma P, Patel A.N, Saini M.K, Swati D. 2012. Field Demonstration of *Trichoderma harzianum* as a Plant Growth Promoter in Wheat (*Triticum aestivum* L). Journal of Agricultural Science, 4(8): 65-73. https://doi.org/10.5539/jas.v4n8p65. [Scholar Google]

Sharma P, Kumar V, & Sharma RK. 2011. Complexity of *Trichoderma-Fusarium* interaction and its effect on the growth and sporulation of the test organisms. Australian Journal of Crop Science, 5(8): 1027-1038. [Scholar Google]

Singleton L.L, Mihail J, Rush C. 1992. Methods for research on soil-borne phytopathogenic fungi. Journal of American Phytopathological Society, 6: 265-268. https://www.cabidigitallibrary.org/doi/full/10.5555/19932339041.

Soytong K. 1988. Identification of species of *Chaetomium* in the Philippines and screening for their Biocontrol properties against seed Born Fungi of rice. PhD. Thesis Department of Plant pathology, ULPB College, Laguna, Philippines.

Stewart A, Hill R. 2014. Applications of *Trichoderma* in plant growth promotion. Journal of Biotechnology and biology of Trichoderma: Elsevier, 415-428. https://doi.org/10.1016/B978-0-444-59576-8.00031-X. [Scholar Google]

Suprapta DN. 2012. Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. Journal of ISSAAS, 18(2): 1-8. [Scholar Google]

Szczech M, Shoda M. 2006. The effect of mode of application of *Bacillus subtilis* RB14-C on its efficacy as a biocontrol agent against *Rhizoctonia solani*. Journal of Phytopathology, 154(6): 370-377. https://doi.org/10.1111/j.1439-0434.2006.01107.x. [Scholar Google]

Tameru, A. Hamacher, J. and Dehne, H.W. 2003. The increase in importance of Ethiopian Pepper mottle Virus (EPMV) in the rift valley part of Ethiopia; time to create Awareness among researchers an extension workers, Paper presented at Deutsches Tropentage, October 18-2-2003. Gottingen, Germany.

Teshome B, S, Chemeda F. and Dereje G. 2012. Integrated Approach and Plant Extract Management Options against Pepper Wilt (*Fusarium oxysporum* var. *vasinfectum*) at Bako, Western Ethiopia, MSc. Thesis in Plant Pathology, Haramya University, Ethiopia.

Wolk, M. and S. Sarkar. 1994. Antagonism *in vitro* of *Bacillus* sp., against *Rhizoctonia solani* and *Pythium* spp. Journal of Pest Science and Plant Protection, 67(1): 1-5. [Scholar Google]

Wongpia A, Lomthaisong K. 2010. Changes in the 2DE protein profiles of chilli pepper (*Capsicum annuum*) leaves in response to *Fusarium oxysporum* infection. Journal of Science Asia, 36(4):259-270. https://doi.org/10.2306/scienceasia1513-1874.2010.36.259. [Scholar Google]

Xing L, Ding Z, Wenxiang Y, Li D, Daqun L. 2003. A study on the effect of *Bacillus* on downy mildew of cucumber. Journal of Plant Protection, 29(4): 25-27. https://doi/full/10.5555/20043054917. [Scholar Google]

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J. and Chen, J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Frontiers in microbiology, 14.

https://doi.org/10.3389/fmicb.2023.1160551. [Scholar Google]

Zhang J.X, Xue A.G, Tambong J.T. 2009. Evaluation of seed and soil treatments with novel *Bacillus subtilis* strains for control of soybean root rot caused by *Fusarium oxysporum* and *Fusarium graminearum*. Journal of plant Disease, 93(12): 1317-1323. https://doi.org/10.1094/PDIS-93-12-1317. [Scholar Google]

Original Research Article||

Monitoring Milk Yield and Composition Traits in Ethiopian Zebu x Holstein Friesian Crosses: Influence of Genotype, Location, Lactation Stage, and Parity in Urban Milk Production System of Southern Ethiopia

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Abstract

This study evaluated the effects of genotype (G), parity (P), lactation stage (LS), and location (L) on milk yield and composition traits of Holstein Friesian crossbred dairy cows in the Shashemene-Dilla milkshed. A total of 117 lactating cows, 39 per location (Shashemene, Hawassa, and Dilla), with 13 cows from each genotype group (50%, 75%, and 87.5% HF) were assessed. Milk yield traits, including peak yield (PY), total yield (TY), lactation length (LL), peak day (PD), and average daily Milk yield (ADMY), were analysed. Milk composition traits fat (F), protein (P), lactose (L), milk density (MD), salt (S), pH, freezing point (Fpt.), and total solids (TS) were analyzed using the Milkoscan FT2. Data on milk yield and composition were processed using a linear mixed-effect model in R 4.3.3. GraphPad Prism 10.4.0 was used to visualize overall milk production trends, while Origin software was used to plot changes in ADMY across the lactation stages for each genotype. The results revealed that the 87.5% HF crosses outperformed 75% and 50% HF groups in ADMY, TY, PY, LL, and PD. Conversely, 50% HF crosses showed higher MD, TS, P, Fpt., and S., while 75% HF cows had superior fat content over both 50% and 87.5%HF. Location significantly influenced MD, Fpt., and S ($P < 0.05$), and genotype × location interactions were significant for MD, TS, P, and SNF. Lactation stage significantly ($P < 0.001$) affected F, MD, TS, P, and S, while parity influenced all yield traits except PD. ADMY showed a significant negative correlation with fat percentage ($r = -0.22$) and total solids ($r = -0.22$). Among the compositional traits, protein was strongly correlated with density ($r = 0.86$) and lactose ($r = 0.63$), while solids-not-fat also demonstrated a strong association with density ($r = 0.76$). Overall, the findings indicate that higher Holstein-Friesian inheritance enhances milk yield, it is also strongly influenced by location effect. Therefore, raising awareness on milk production potential evaluation to use strategic selection based on recorded performance and gradual improvement of management practices is recommended to maximize the productivity of crossbred dairy cows under smallholder production system.

Key words: Genotype, Milk yield, Milk constituents, Lactation Length

Original submission: September 07, 2025; **Revised submission**: December 30, 2025; **Published online**: December 31, 2025

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INTRODUCTION

Ethiopia is expecting a doubling of the number of middle-class consumers by the year 2030, with fast growth in terms of population (now exceeding 100 million), which will lead to higher demand for livestock products, including milk (Ndambi et al., 2017). In response to this growing demand, the Ethiopian government has set an ambitious goal of quadrupling national milk production by 2031 through targeted interventions aimed at improving the productivity of dairy cattle, camels, and goats (Leggesse et al., 2023).

Ethiopian cattle populations, known for their considerable genetic diversity and varying degrees of admixture, represent a valuable genetic resource for the development of context-specific dairy genotypes. This diversity holds promise for genetic improvement programs tailored to the country's wide range of agroecological zones (Goshme and Dadi, 2024). To capitalize on this potential, various breed improvement initiatives have been implemented, including the distribution of crossbred heifers, the provision of improved dairy stocks, and the expansion of artificial insemination (AI) and bull services (Kumar et al., 2014). In urban and peri-urban areas, dairy farmers typically raise Holstein–Zebu and Jersey–Zebu crossbred cows under zero- or semi-zero-grazing systems with minimal access to pasture (Deneke et al., 2022).

Genetic improvement efforts have led to the development of crossbred cattle, which are generally more productive than indigenous breeds (Gizaw et al., 2017). However, the success of these programs relies heavily on continuous performance monitoring under prevailing farm management conditions (Guadu and Demissie, 2016). Several studies have assessed the productive performance of crossbred and indigenous cattle, often focusing on exotic blood levels within research stations or government-owned farms, as well as selected urban and peri-urban dairies (Gizaw et al., 2017). Nonetheless, Ethiopia's dairy supply chain remains underdeveloped in terms of quality assurance, safety protocols, and organizational structure, with limited routine evaluations of herd performance and farm capacity (Feyisa et al., 2024).

Urban dairy systems in cities such as Hawassa, Shashemene, and Dilla play a critical role in bridging the national demand-supply gap. These systems maintain both improved (crossbred or high-grade) and indigenous cattle genotypes, contributing substantially to household income up to 43.6–79.7% of the gross annual income in the Shashemene–Dilla milkshed (Tegegne et al., 2013; Mengistie, 2016). Although several studies have examined the use of Holstein Friesian, crossbred dairy cows in Ethiopia, regarding region-specific insights into how blood level, environmental conditions, and cow-side factors affect milk production for On-farm periodical evaluation remains limited (Getahun et al., 2020; Beneberu and Alem, 2025).

Therefore, this study aims to address this gap by conducting a year-long, on-farm evaluation of the productive performance of crossbred dairy cows kept in farms across the Shashemene–Dilla milkshed. The study focuses on identifying both genetic and non-genetic factors influencing milk yield and assessing the physico-chemical properties of raw milk under real farm conditions.

MATERIALS AND METHODS

Study Area Description and Source of Sample

Data for this study were collected from 3 study locations (Shashemene, Hawassa, and Dilla) found in the Shashemene-Dilla milkshed, Ethiopia. The geographical location ranges from 6°24′30″N to 7º 12’ N latitude and 38°28″E to 38º 36’ E longitude (Figure 1). All selected sites are in the East African Great Rift Valley. According to Yigrem et al. (2008), two major dairy production systems were distinguished in the study milkshed: the mixed crop-livestock system characteristic of rural and peri-urban environments and the urban dairy system situated within urban centers. Study areas were selected based on the availability of Holstein Friesian crosses lactating dairy cows with the desired genotype (50%HF, 75%HF, 87.5% HF).

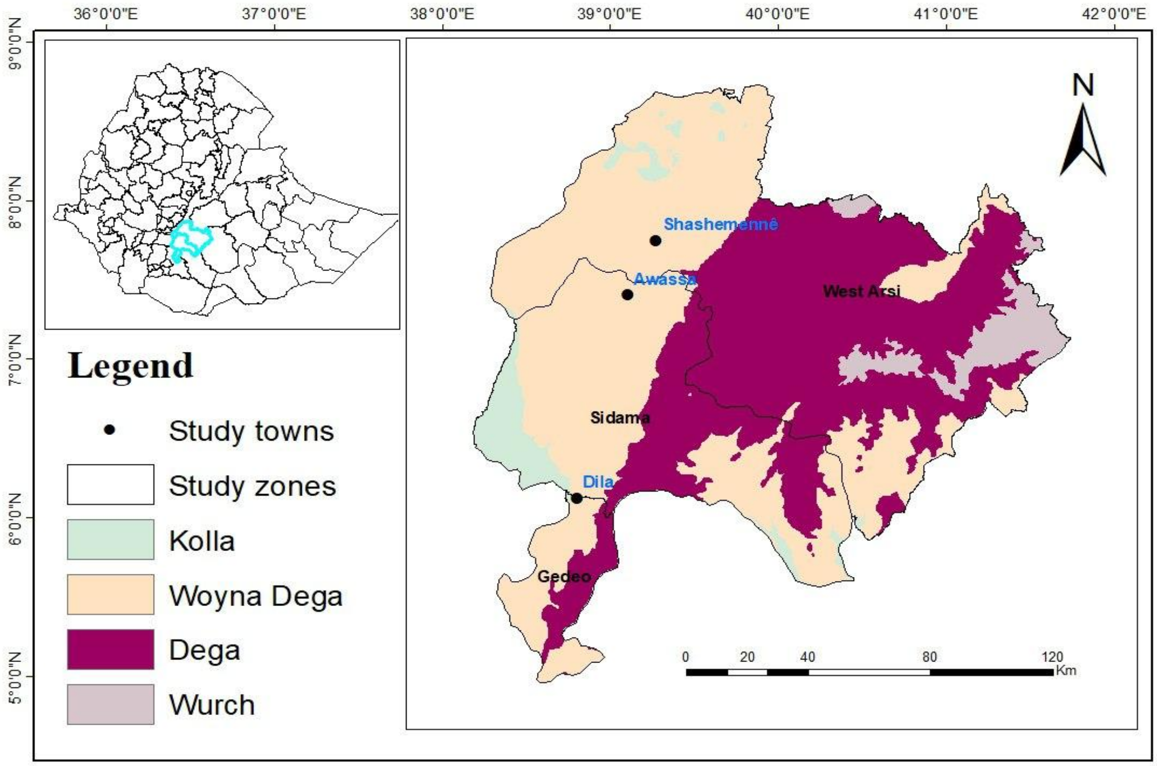

Figure 1. Map illustrating the three-milkshed locations where the study has been conducted

Agro-ecological conditions of milkshed locations: Kolla = lowland, Woynadega =Middle altitude; Dega = Temperate; Wurch = Frost

Sampling and Source of Milk Samples

Three study locations, namely Shashemene, Hawassa, and Dilla (SHA, HAW, and DIL), and nine farms, of which three from each location, were purposively selected based on the availability of the Ethiopian Zebu x HF crossbred dairy cattle (50%HF, 75%HF, and 87.5%HF) in the respective farms. The recorded data sheet from selected farms was used to determine the desired genotype, level of parity, and lactation stage. A total of 117 HF crosses (39 from each location) were used for on-farm evaluation of genetic and non-genetic factors affecting milk yield and composition traits. For each genotype (50%HF, 75%HF, and 87.5%HF), 13 Animals were selected from each location. Again, individual lactating cows were purposively selected at their 1-month postpartum (early lactation) and were grouped into three parities: primiparous (P), multiparous in the second (M2), and in the third (M3). Raw milk samples for milk constituents' determination were collected periodically from a total of 117 cows (13 cows from 9 farms) in the study locations during their early, mid, and late lactation stages.

All genotype groups within each participating farm were maintained under similar management practices, including feeding, housing, and milking routines. Management differences were applied only according to physiological needs such as lactation stage, pregnancy, or age class rather than genotype category.

Milk Yield Characteristics Data Collection

Milk yield data, of 117 lactating cows (grouped under primiparous and multiparous in the second and third lactation) were collected from the dairy producers' milk record cards for the whole lactation period once in a week manner. The milk yield of these dairy cows was monitored for the whole lactation period starting at the first week of December 2022-2023.

Raw Milk Composition Determination
The Raw milk sample for composition and quality analysis was aseptically collected from each cow during the three lactation stages and immediately taken to the HU (Hawassa University) dairy technology laboratory. The raw milk collection was done after discarding the first three to five milk strands, and about 40ml of morning or evening milk was aseptically stored in sterile sample bottles. The milk composition traits (fat, total protein, lactose, milk density, salt, pH, freezing point, and total solids) of the raw milk samples were determined using a Milkoscan FT2 (Foss Electric at HU dairy technology laboratory) apparatus. The measurement was done following the manufacturer's protocol for the consecutive three lactation stages (Souhassou *et al.,* 2018).

Data Management and Analysis
Data were processed using a linear mixed-effect model in R 4.3.3 by taking genotype (50%HF, 75%HF, and 87.5%HF), parity (primiparous, second, and third parity), lactation stage (early, mid, and late), and location (Shashemene, Hawassa, and Dilla) as fixed effects and the cows' ID as random effect. Means between fixed effects were separated using Tukey's range test. The effect of class variables was expressed as Least Squares Means (LSM). The milk production trends and illustrating the pattern of changes across lactation stage varying by genotype on the other hand were visualized with GraphPad Prism 10.4.0 and Origin software, respectively. SAS 9.4 was used for milk production traits correlation analysis.

A linear mixed-effects model was fitted including two interaction terms to investigate whether the effect of Genotype differs across Location, and the effect of the lactation stages parity across the lactation stages. The model was specified as:

$$Y_{ijklm}=\mu + L_i + G_j + (L \times G)_{ij} + P_k + LS + (P \times LS)_{kl} + u_m + \varepsilon_{ijklm}$$

Where:

- Y_{ijklm} is the response variable (fat percentage, Milk density, lactose percentage, total solid percentage, protein percentage, freezing point (° C), salt percentage, solid not fat percentage, Average daily milk yield (litters/day)), total yield, lactation length, peak yield, peak day.
- μ is the overall intercept,
- L_i is the fixed effect of the i[th] location,
- G_j is the fixed effect of the j[th] genotype category,
- $(L\times G)_{ij}$ is the fixed interaction effect between location and genotype,
- P_k is the fixed effect of parity category,
- LS_l is the fixed effect of lactation stage,
- $(P \times L)_{kl}$ is the fixed interaction effect between Parity and, Lactation Stage
- u_m is the random intercept for cow identity m, ($u_m \sim N(0,\sigma^2_u)$ accounting for correlation among repeated measurements from the same animal
- $\varepsilon ijklm$ is the residual error ($\varepsilon ijklm \sim N(0,\sigma 2)$).

RESULTS

The range of the coefficient of variation for milk production traits was from 1.42% to 68.30%. The most variable trait was total yield (TY), while pH was the least variable**.** The mean values for the milk composition traits were milk fat (F) 4.17, density(D) 30.98, lactose(L) 4.64, total solid (TS) 10.73, protein(P) 3.27, freezing point (Fpt.) 0.54, salt (S) 0.70, pH 6.58, and solid not fat (SNF) 6.56. Whereas average daily milk yield (ADMY) 6.21, total yield (TY) 1448.73liters, lactation length (LL) 246.95 days, peak yield (PY) 11.01liters, and peak day (PD) 87.03 days were the mean values for milk yield characteristics.

Effect of Genotype, Parity, Location, and Lactation Stage on Milk Yield Characteristics
The crossbred dairy cows with 87.5% HF genotype had the highest average daily milk yield (9.97 liters/day/cow), Total Yield 2571 liters, and Peak Yield (PY) =16.64 liters/day/cow with longer Lactation Length (LL)=292days and Peak Day=93.63[th]day (Table 1). The 50% HF cows, on the other hand, were inferior for LL but had comparable TY and PY with 75% HF crosses and PD with 87.5% HF crosses.

The HF crossbred cows at the second(M2) and third (M3) parity had higher TY and PY, yet the longest lactation length was recorded for cows at the second parity as compared to the primiparous and the third parity cows. TY and LL, on the other hand, were affected significantly (P<0.05) by location, while PY and PD were the ones not affected by location. The HF crosses at Hawassa, Shashemene, and Dilla had higher, intermediate, and lower recorded TY, respectively (Table 1).

Table 1. Milk Yield Characteristics (LSM ± SE) as Affected by Genotype, Parity, and Location

Variables	TY	LL	PY	PD
Genotype (G)				
HF50%	821.58± 3.38^{b}	207.86±3.20^{c}	7.81±0.29^{b}	87.16±3.57^{ab}
HF75%	951.61±39.85^{b}	236.88±3.61 ^{b}	8.47±0.25^{b}	80.70±3.41^{b}
HF87.5%	2571.99±155.60^{a}	292.35±6.75^{a}	16.64±0.64^{a}	93.63±3.34^{a}
Parity (P)				
P	1034.35±74.29^{b}	225.59±4.42^{c}	8.59±0.44^{b}	82.82±3.24^{b}
M2	1540.24±159.04^{a}	253.91±8.45^{a}	11.87±0.82^{a}	85.32±3.86^{ab}
M3	1770.59±193.52^{a}	257.60± 7.89^{b}	12.47±0.86^{a}	93.35±3.21^{a}
Location (L)				
SHA	1440.96±146.35^{ab}	254.68±7.81^{a}	10.84±0.83ab	86.66±3.77
HAW	1565.68±128.28^{a}	239.74±8.12^{b}	11.41±0.87^{a}	88.95±3.16
DIL	1338.54±128.29^{b}	242.67± 6.56^{b}	10.67±0.67^{b}	85.87±3.62
Source of variation				
G	<0.00	<0.00	<0.00	0.04
P	<0.00	<0.00	<0.00	0.10
L	0.00	0.01	0.03	0.77

^{abc} means with different superscripts are significantly different (*P<0.05); **P<0.01; ***P<0.001)
Abbreviation: TY = total yield; LL= lactation length; PY= peak yield, PD = peak day

The interaction effect of genotype and lactation stage on the average daily milk yield of the HF crosses in the studied milkshed is presented in Figure 2. The 87.5% HF cows exhibited superiority over the remaining two genotypes (50% and 75%) regarding average daily milk yield throughout the three lactation stages. The average daily milk yield of the 87.5%, 75%, and 50% HF genotype cows in the current study was 5.54, 6.95, and 12.2 liters for early lactation, 4.86, 4.97, and 11.8 for mid-lactation, and 1.91, 2.28, and 5.87 liters for late lactation, respectively (Figure 2).

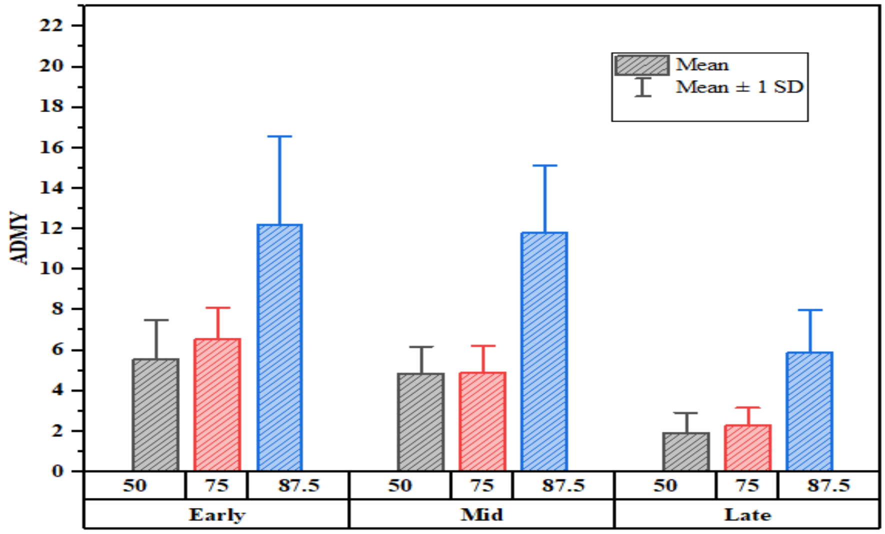

Figure 2. Effect of genotype and lactation stage on average daily milk yield of HF crosses

Milk Yield Patterns by Genotype, Location, and Parity

Graphs plotted using mean and standard error of average daily milk yield for the three genotypes, locations, and parity represented as a milk production graph in figures 3, 4, and 5. As presented in Figures 3, 4, and 5 genotype, location, and parity affected the average daily milk yield of HF crosses at the early, peak, mid, and late lactation. Average daily milk yield, milk yield peak, and lactation length were higher for HF 87.5% and lowest for HF 50% (Figure **3**). Holstein Friesian crosses at Hawassa had a higher peak and average daily milk yield than crossbreds at Shashemene and Dilla (***Figure*** **4**).

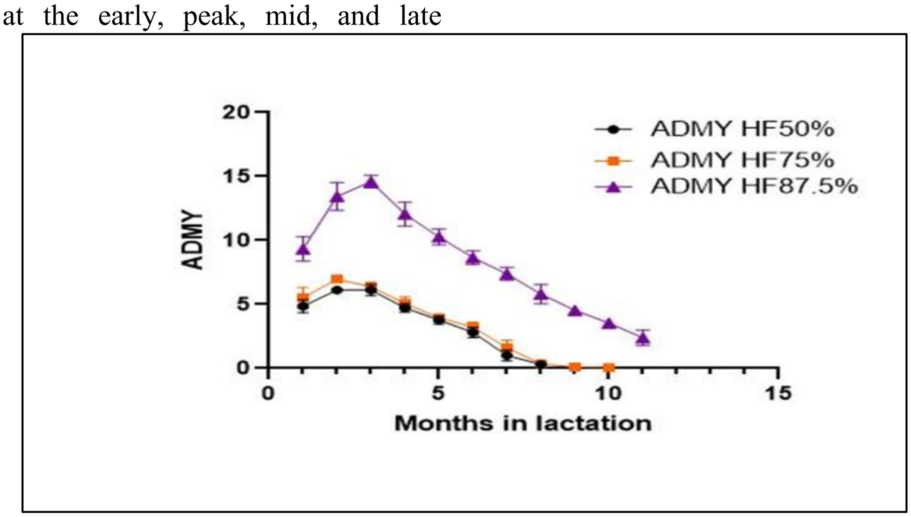

Figure 3. The mean and standard error for the average daily milk yield of three different genotypes of HF crosses

Abbreviation: ADMYHF50% = average daily milk yield of 50% Holstein Friesian crosses; ADMYHF75% = average daily milk yield of 75% Holstein Friesian crosses; ADMYHF75% = average daily milk yield of 87.5% Holstein Friesian crosses;

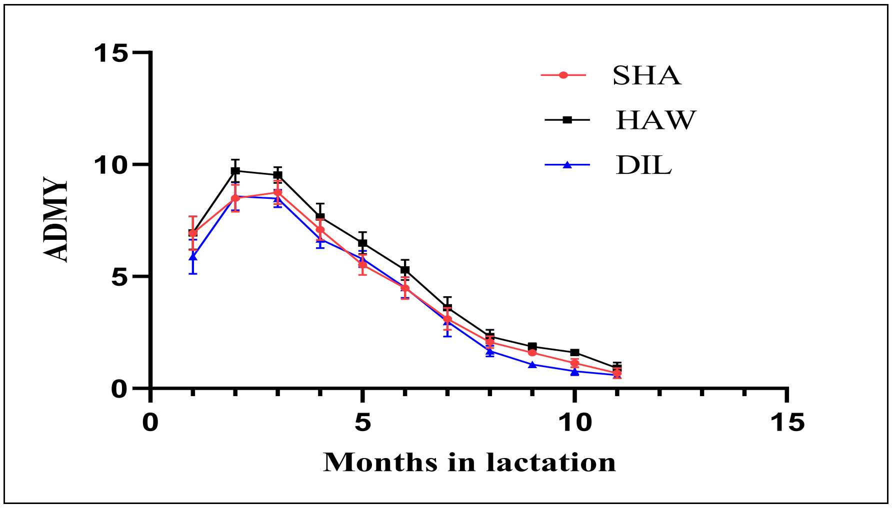

Figure 4. Mean and standard error for average daily milk yield of HF crosses at the three different location throughout lactation

Abbreviation: SHA = Shashemene; HAW = Hawassa; DIL= Dilla

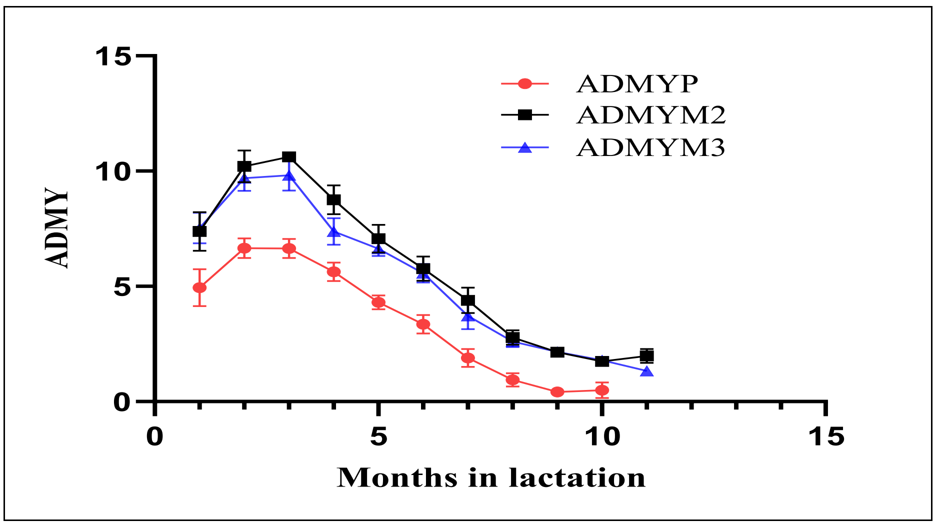

Figure 5. Mean and standard error for average daily milk yield of HF crosses grouped in three different numbers of parity throughout lactation

Abbreviation: ADMYP = average daily milk yield of the primiparous cows; ADMYM2 = average daily milk yield of multiparous cows in the second parity; ADMYM3 = average daily milk yield of multiparous cows in the third parity

The effect of Genotype and Location on milk composition traits

Genotype (genotype) affected almost all milk composition traits except L (Table 2). Crossbreds with 50% HF genotype have shown superiority over the other two genotypes for MD (32.16), TS (11.04), P (3.35), Fpt. (0.55), S (0.71), and SNF (6.84).

The 75% HF crosses also showed superiority for fat content (4.35) over the other genotypes and intermediate for milk pH value (6.58). The milk quality trait recorded higher for 87.5% HF crosses was milk pH value (6.60), and MY (Average daily milk yield) was also high for this genotype. Among the tested milk composition traits, L was not affected by genotype.

From the tested milk quality traits, Milk-density (MD), Freezing Point (Fpt.), and Salt (S) were affected significantly (P<0.05) by the studied locations. The interaction effect of genotype and location was also significant for MD, TS, P, T, and SNF.

Table 2. Least squares mean of milk composition traits and average daily milk yield as affected by location and genotype

Variables	F	MD	L	TS	P	Fpt.	S	pH	SNF	ADMY
Location (L)										
Shashemene	4.11	31.6^{a}	4.68	10.8	3.32	0.55^{a}	0.70^{ab}	6.58	6.69	5.98
Hawassa	4.30	31.3^{ab}	4.64	10.8	3.27	0.55^{ab}	0.70^{a}	6.58	6.48	6.71
Dilla	4.10	30.1^{b}	4.60	10.6	3.23	0.53^{b}	0.68^{b}	6.60	6.50	5.94
Genotype (G)										
HF 50%	4.20^{ab}	32.2^{a}	4.69	11.0^{a}	3.35^{a}	0.55^{a}	0.71^{a}	6.57^{b}	6.84^{a}	4.10^{b}
HF 75%	4.35^{a}	30.1^{b}	4.59	10.6^{b}	3.19^{b}	0.53^{b}	0.69^{b}	6.58^{ab}	6.20^{b}	4.56^{b}
HF 87.5%	3.96^{b}	30.7^{b}	4.64	10.6^{b}	3.29 ^{ab}	0.54^{ab}	0.68^{b}	6.60^{a}	6.63^{a}	9.97^{a}
Sources of variation										
L	0.20	0.01	0.28	0.37	0.11	0.02	0.03	0.11	0.31	0.10
G	0.01	0.00	0.14	0.00	0.00	0.03	0.00	0.03	<0.00	<0.00
L x G	0.67	0.00	0.15	0.05	0.00	0.19	0.60	0.20	0.00	0.12

$^{a\ b\ c}$ means that with different superscripts are significantly different (*P<0.05); (**P<0.01): (***P<0.001)

Abbreviation: F = fat percentage; MD = Milk density; L = lactose percentage; TS = total solid percentage; P = protein percentage; FP = freezing point (^{o} C); S = salt percentage; SNF = solid not fat percentage; ADMY = Average daily milk yield (litters/day); HF =Holstein Friesian.

The Effect of Lactation Stage and Parity on Milk Composition Traits

The lactation stage was significantly (P<0.001) affecting F, MD, TMS, P, and S of the HF crossbred dairy cows in the studied milk shed (Table 4). Higher F (4.60), MD (31.49), TMS (11.20), and P (3.34) content were recorded at the early and late lactation stages (Table 3). Milk was recorded as higher at early and mid-lactation compared to the late stage of lactation. The milk samples from mid and late lactation had low and intermediate salt content, respectively. The freezing point was high for early, intermediate for mid, and low for late lactation stage, respectively. The interaction effect of lactation stage and parity was also exhibited only on the protein content of the sampled milk.

Table 3. Least squares means of milk production traits as affected by lactation stage and parity

Variables	F	MD	L	TS	P	Fpt.	S	pH	SNF	ADMY
Lactation Stage(LS)										
Early	4.60^{a}	31.5^{a}	4.63	11.2^{a}	3.34^{a}	0.55	0.71^{a}	6.58	6.59	8.10^{a}
Mid	3.20^{b}	29.5^{b}	4.59	9.65^{b}	3.09^{b}	0.54	0.69^{b}	6.58	6.45	7.18^{a}
Late	4.71^{a}	32.0^{a}	4.69	11.4^{a}	3.39^{a}	0.55	0.69^{ab}	6.60	6.64	3.35^{b}
Parity (P)										
Pr.	4.09	31.5	4.66	10.8	3.32^{a}	0.54^{ab}	0.70	6.59	6.69	4.58^{b}
M2	4.28	31.0	4.68	10.8	3.30^{ab}	0.55^{a}	0.70	6.59	6.53	7.21^{a}
M3	4.14	30.5	4.58	10.6	3.21^{b}	0.53^{b}	0.69	6.57	6.46	6.84^{a}
Sources of variation										
LS	<0.00	<0.00	0.10	<0.00	<0.00	0.19	0.03	0.13	0.46	<0.00
P	0.13	0.22	0.12	0.17	0.02	0.02	0.81	0.09	0.33	<0.00
LS x P	0.97	0.98	1.00	0.98	0.98	0.89	0.98	0.73	0.99	0.44

a b c means that with different superscripts within a column are significantly different (*P<0.05); (**P<0.01); (***P<0.001)

Abbreviation: F = fat percentage; MD = Milk density; L = lactose percentage; TS = total solid percentage; P = protein percentage; FP = freezing point (°C); S = salt percentage; SNF = solid not fat percentage; ADMY = Average daily milk yield (litters/day); Pr. = Primiparous; M2 = Multiparous in the second lactation; M3 = multiparous in the third lactation.

Correlation between Milk Production Traits

Figure 6 presents the Pearson correlation coefficients among average daily milk yield (ADMY) and key milk composition traits, including fat percentage (F), protein percentage (P), lactose percentage (L), total solids percentage (TS), solids-not-fat percentage (SNF), density (D), freezing point (Fpt.), salt content (S), and pH (Figure 6). Statistical significance is indicated by $p < 0.05$ (*), $p < 0.01$ (), and $p < 0.001$ (), while NS denotes non-significance.

A notable negative association was observed between ADMY and most compositional parameters, particularly F (r = -0.22, $p < 0.001$), TS (r = -0.22, $p < 0.001$), and D (r = –0.13, $p < 0.05$). Fat percentage exhibited a positive correlation with TS (r = 0.43, $p < 0.001$) and a significant negative correlation with SNF (r = –0.42, $p < 0.001$).

Protein content was highly correlated with L (r = 0.63, $p < 0.001$), D (r = 0.86, $p < 0.001$), Fpt. (r = 0.45, $p < 0.001$), and S (r = 0.48, $p < 0.001$). Lactose content demonstrated strong positive associations with TS (r = 0.52, $p < 0.001$), SNF (r = 0.47, $p < 0.001$), and D (r = 0.62, $p < 0.001$). TS, SNF, and D were closely interrelated, with the strongest correlation observed between SNF and D (r = 0.76, $p < 0.001$).

Freezing point showed moderate positive associations with P (r = 0.45, $p < 0.001$), L (r = 0.56, $p < 0.001$), and S (r = 0.45, $p < 0.001$), while being negatively associated with pH (r = -0.18, $p < 0.01$). Salt content was moderately correlated with P, L, and SNF, and exhibited a significant positive correlation with pH (r = 0.29, $p < 0.001$). In contrast, pH generally showed weak relationships with other compositional parameters (Figure 6).

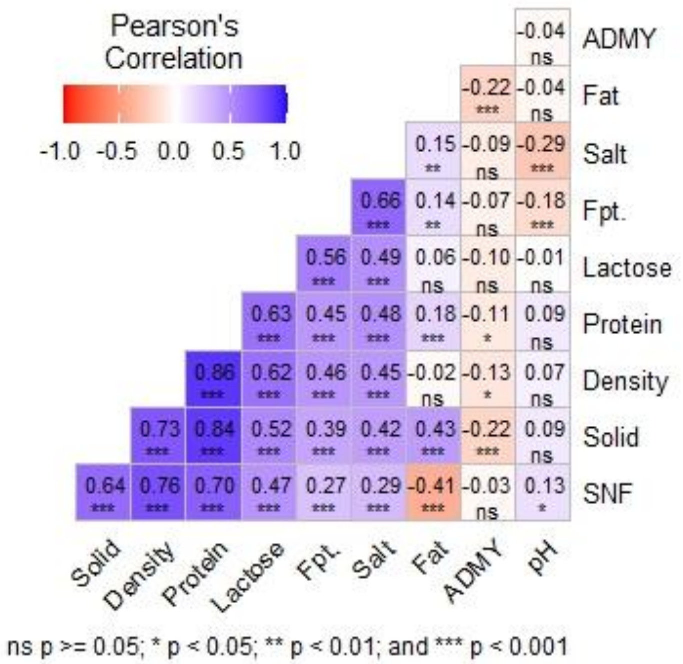

Figure 6. Pearson's correlation coefficient (r) for milk yield traits of Holstein Friesian crosses. The color intensity represents the strength of the correlations, with darker shades indicating a stronger association between traits and lighter shades a weaker association. Each square displays both the correlation coefficient (r) and the corresponding p-value, indicating the statistical significance of the association.

DISCUSSION

Milk production and composition traits showed considerable variation, with coefficients of variation (CV) ranging from 1.47% (pH) to 68.30% (total yield). The reported CVs across the pooled population, reflecting both within and between population differences suggest that these traits are suitable for genetic improvement through selection, aligning with the earlier findings of Alphonsus et al. (2015). The ADMY of the sampled population observed in this study was higher than values reported by Demeke (2020) and Taju, (2018), who found 4.62 ± 0.35 L and 4.73 L/day for crossbred dairy cattle in different regions of Ethiopia. Conversely, it was lower than the results reported by Tegegne et al. (2013) and Getahun et al. (2020). Similarly, TY and LL in the current study were lower than the values reported by Getahun et al. (2020b). Kumar et al. (2014) reported a higher PY (12.15 ± 0.82 L) and mean lactation milk yield (2069.16 ± 78.44 L) for crossbred cows, while Bisrat and Nigussie, (2016) observed lower TY but longer LL compared to the present study. These variations between the current study and results reported by different researchers may be attributed to differences in management, nutrition, parity, age, lactation stage, and season (Zhang et al., 2024). The current study also demonstrated superior values compared to Bekele et al. (2023) and Yoseph et al. (2022) for fat (4.17%), density (30.98 kg/m³), lactose (4.64%), freezing point (-0.54°C), and salt (0.70%). The mean milk fat and protein contents exceeded the Ethiopian Standards (ES) of 3.50% and 3.20%, respectively (Table 1).

Effect of Genotype, Parity, Location, and Lactation Stage on Milk Yield Characteristics

Among the genotypes, cows with 87.5% Holstein-Friesian (HF) genotype produced the highest TY but had the shortest LL compared to Hago, (2020). Their PY was also higher than that reported by Gebreyohannes et al. (2013). The 50% HF crosses, although inferior in LL, had TY and PY comparable to 75% HF crosses, and PD to 87.5% HF crosses (Table 2). Beneberu, (2023) reported on-station lactation yields ranging from 1293.01 ± 23.70 L to 2957.46 ± 72.98 L, and LL ranging from 298.68 ± 5.17 to 374.05 ± 7.24 days, whereas on-farm yields ranged from 631.69 ± 222.98 L to 2705.43 L, and LL from 241.65 ± 26.22 to 310.91 ± 41.83 days. The TY and LL of the genotypes in the present study were lower than most on-station reports but comparable with some on-farm values.

The LL in this study was inconsistent with the standard 305-day lactation except for 87.5%HF inheritance, though extended lactations have been reported elsewhere (Beneberu, 2023). While extended LL may benefit cow health and fertility, low milk yield during this period raises concerns over excessive fattening. This highlights the need for individualized strategies for extended lactation management (Van *et al.,* 2022).

Parity significantly affected all milk yield traits, whereas location did not influence ADMY, PY, or PD. Yield generally increased with parity, consistent with Gebreyohannes *et al.* (2013), Worku *et al.* (2016), and Getahun *et al.* (2020b). TY was comparable between Shashemene and Hawassa, though LL was longer in Shashemene than in Hawassa and Dilla, likely due to environmental differences. Bedada *et al,* (2021) observed similar trends.

Milk Production Patterns by Genotype, Location, and Parity

Higher exotic genotypes were associated with greater ADMY across all lactation stages (Figure 2), consistent with Silva *et al.* (2019). Parity also influenced ADMY: primiparous cows had the lowest yields throughout lactation, likely because nutrients were still being partitioned toward growth (Marumo *et al.,* 2022). Evangelista *et al.* (2024) and Nalla *et al.* (2022) likewise reported lower PY in primiparous cows. Location-wise, Hawassa herds showed higher PY and ADMY than those in Shashemene and Dilla, echoing findings from Oloo *et al.* (2022) on the influence of agroecological variation. Since the farms in the current study exist under the urban production, differences in producer practices, such as feed quality, milking routines, and veterinary care, may have contributed significantly to the observed performance variation.

Effect of Genotype and Location on Milk Composition Traits

Genotype significantly influenced most milk composition traits ($p < 0.05$), except lactose (L) and temperature (T), in agreement with Kebede *et al.* (2018), who also found lactose to be unaffected by genotype. Milk constituent percentages generally declined with increasing exotic genotype. The 50% HF crosses were superior for most traits—milk density (32.16), total solids (11.04%), protein (3.35%), freezing point (0.55°C), salt (0.71%), and solids-not-fat (6.84%) aligning with Cheruiyot *et al.,* (2018) and Bekele *et al.* (2023).

Fat percentage was highest in 75% HF crosses (4.35%), contrasting with Chanda *et al.,* (2022), who found them inferior to 50% HF. The 87.5% HF crosses had the highest yields but the lowest composition values. Higher milk pH in high-yielding cows may be linked to increased rumen pH from greater rumination (Souza *et al.,* 2022). The inverse relationship between yield and constituent concentration has been well documented (Craig *et al.,* 2022; Brito *et al.,* 2021), reflecting selection emphasis on yield at the expense of fertility and health (Oltenacu *et al.,* 2023).

Location significantly affected milk density, freezing point, and salt ($p < 0.05$) but not fat, lactose, total solids, protein, pH, or solids-not-fat. Dilla, the warmest site, had the lowest milk density, salt, and freezing point. Most major milk constituents showed little variation across locations, likely because the sites share similar agroecological zones. Nonetheless, performance advantages in optimal temperature–humidity conditions have been noted (Bernabucci *et al.,* 2014; Zewdu *et al.,* 2014). Genotype × location (G × L) interactions were solids, protein, temperature, and solids-not-fat), reflecting differences in genotype performance across environments (Gebreyohannes *et al.,* 2014; Silva *et al.,* 2024).

Effect of Lactation Stage and Parity on Milk Composition Traits

Lactation stage significantly affected fat, milk density, total solids, protein, temperature, and salt ($p < 0.001$), but not lactose, freezing point, pH, or solids-not-fat. These results are consistent with Connolly *et al.* (2023) and Kumar *et al.* (2021), who found lactose, SNF, and pH to be stable across lactation stages. However, Sabek *et al.* (2021) reported that higher parity and longer days in milk negatively affected udder health and milk quality in tropical cows. Proper adjustment for parity and lactation stage allows more accurate estimation of true genetic potential of the cows and minimizes bias in selection decisions. This aligns with findings by Kumar et al. (2021), who reported strong phenotypic associations between lactation stage, parity, and performance traits. Therefore, recognizing these effects is particularly important in dairy systems where cows differ in reproductive status and stage of production, as failure to account for them could lead to inaccurate ranking and suboptimal genetic progress.

Correlation among Milk Production Traits

The strongest positive correlation was between milk density and protein content (r = 0.86), consistent with Suhendra *et al,* (2020). Protein and total solids were also highly correlated (r = 0.84). Fat content was positively correlated with total solids (r = 0.43), supporting Desye *et al.* (2023), who reported r = 0.88 for similar traits.

Freezing point was positively correlated with all major milk composition traits, reflecting its dependence on solute concentration, a colligative property rather than solute type (Khider *et al.* 2021). This aligns with its use in detecting milk adulteration or dilution, whether accidental or intentional (Kumar *et al.,* 2024). In contrast, pH was negatively correlated with freezing point (r = -0.18), consistent with Rai *et al.* (2022), who reported r = -0.31 for similar relationships in crossbred dairy cows.

CONCLUSIONS

The on-farm monitoring revealed that both genetic and non-genetic factors significantly affect milk yield and composition traits in Holstein Friesian crossbred cows. While higher exotic genotypes enhanced milk volume, 50% HF crosses exhibited superior total solid, milk density and salt content. These findings highlight that increased milk yield tends to reduce component concentrations. Considering the current structure of the Ethiopian dairy sector, where milk pricing is largely volume-based, milk yield continues to be the most economically relevant trait for selection. Although milk composition traits are biologically important, their expression is strongly affected by management, nutrition, and environmental conditions in addition to genetics. Therefore, improving management practices and monitoring quality parameters may provide more immediate benefit, while keeping the option open for future integration of composition-based selection as the industry evolves.

REFERENCES

Alphonsus, C., Essien, I. C., Gn, A., and P. P. Barje. 2011. Factors Influencing Milk Yield Characteristics in Bunaji and Friesian x Bunaji Cows in Northern Nigeria. In *Animal Production*, 13(3): 143-149. [Scholar Google]

Bedada, K. W., Kechero, Y., and G. P. J. Janssens. 2021. Seasonal and agro-ecological associations with feed resource use and milk production of ranging dairy cows in the Southern Ethiopian Rift Valley. *Tropical animal health and production*, *53*: 1-8. [Scholar Google]

Beneberu, N. 2023. Review on Milk Yield Performance of Crossbred Dairy Cows in Ethiopia. *Advances in Bioscience and Bioengineering*, 11(3). doi: 10.11648/j.abb.20231103.11. [Scholar Google]

Beneberu, N., and D. Alem. 2025. Meta-analysis for Milk Production Performance Traits of Jersey cross, 50% HF and 75% HF Cross Dairy Cattle in Ethiopia, *Meta Analysis*,U KR Journal of Agriculture and Veterinary Sciences, *1*(1): 14-25. [Scholar Google]

Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N., and A. Nardone. 2014. The effects of heat stress in Italian Holstein dairy cattle. *Journal of Dairy Science*, *97*(1), 471-486. doi: 10.3168/jds.2013-6611. [Scholar Google]

Bokharaeian, M., Toghdory, A., Ghoorchi, T., Ghassemi N. J., and I. J. Esfahani. 2023. Quantitative associations between season, month, and temperature-humidity index with milk yield, composition, somatic cell counts, and microbial load: A comprehensive study across ten dairy farms over an annual cycle. *Animals*, *13*(20): 3205. doi: 10.3390/ani13203205 [Scholar Google]

Brito, L. F., Bédère, N., Douhard, F., Oliveira, H. R., Arnal, M., Peñagaricano, F., and F. Miglior. 2021. Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal*, *15*: 100292. doi: 10.1016/j.animal.2021.100292. [Scholar Google]

Chanda, T., Khan, M. K. I., Chanda, G. C., and G. K. Debnath. 2022. Effect of farm categories on quality and quantity of milk produced by different crosses of Holstein-Friesian cows. *Agricultural Reviews*, *43*(3): 389-393. doi: 10.18805/ag.RF-214. [Scholar Google]

Cheruiyot, E.K., Bett, R.C., Amimo, J.O., F.D.N. Mujibi. 2018. Milk composition for admixed dairy cattle in Tanzania. *Frontiers Genetics* 9: 142. doi: 10.3389/fgene.2018.00142. [Scholar Google]

Connolly, C., Yin, X., and L. Brennan. 2023. Impact of lactation stage on the metabolite composition of bovine milk. *Molecules*, *28*(18): 6608. doi: 10.3390/molecules28186608. [Scholar Google]

Craig, A. L., Gordon, A. W., Hamill, G., and C. P. Ferris. 2022. Milk composition and production efficiency within feed-to-yield systems on commercial dairy farms in Northern Ireland. *Animals*, *12*(14): 1771. doi: 10.3390/ani12141771. [Scholar Google]

Deneke, T. T., Bekele, A., Moore, H. L., Mamo, T., Almaw, G., Mekonnen, G. A., and S. Berg. 2022. Milk and meat consumption patterns and the potential risk of zoonotic disease transmission among urban and peri-urban dairy farmers in Ethiopia. *BMC Public Health*, *22*(1): 222. doi: 10.1186/s12889-022-12665-4. [Scholar Google]

Desye, B., Bitew, B. D., Amare, D. E., Birhan, T. A., Getaneh, A., and Z. H. Gufue. 2023. Quality assessment of raw and pasteurized milk in Gondar city, Northwest Ethiopia: A laboratory-based cross-sectional study. *Heliyon*, *9*(3): e14202. doi: 10.1016/j.heliyon.2023.e14202. [Scholar Google]

Evangelista, A. F., Martins, R., Valotto, A. A., Dias, L. T., and R. D. A. Teixeira. 2024. Environmental factors on the prediction of the lactation curve of Holstein cows. *Pesquisa Agropecuária Brasileira*, *59*. doi: 10.1590/S1678-3921.pab2024.v59.03366. [Scholar Google]

Feyisa, B. W., Haji, J., and A. Mirzabaev. 2024. Adoption of milk safety practices: evidence from dairy farmers in Ethiopia. *Agriculture and Food Security*, *13*(1). doi: 10.1186/s40066-024-00479-z. [Scholar Google]

Gebreyohannes, G., Koonawootrittriron, S., Elzo, M. A., and T. Suwanasopee. 2013. Variance components and genetic parameters for milk production and lactation pattern in an Ethiopian multi-breed dairy cattle population. *Asian-Australasian Journal of Animal Sciences*, *26*(9): 1237–1246. doi: 10.5713/ajas.2013.13040. [Scholar Google]

Gebreyohannes, G., Koonawootrittriron, S., Elzo, M. A., and T. Suwanasopee. 2014. Genotype by environment interaction effect on lactation pattern and milk production traits in an Ethiopian dairy cattle population. *Agriculture and Natural Resources*, *48*(1): 38-51. [Scholar Google]

Getahun, K., Hundie, D., and Y. Tadesse. 2020. Productive Performance of Crossbred Dairy Cattle. *International Journal of Agricultural. Science,* 30(2): 55-65. [Scholar Google]

Glória, J. R. D., Bergmann, J. A. G., Quirino, C. R., Ruas, J. R. M., Pereira, J. C. C., Reis, R. B., and S. G. Coelho. 2012. Environmental and genetic effects on the lactation curves of four genetic groups of crossbred Holstein-Zebu cows. *Revista Brasileira de Zootecnia*, *41(11)*: 2309-2315. doi: 10.1590/S1516-35982012001100002. [Scholar Google]

Goshme, S., and H. Dadi. 2024. Genetic diversity and footprint of the Ethiopian cattle population, and the application of molecular information on sustainable cattle genetic improvement: Opportunities, challenges, and future directions in Ethiopia. A comprehensive review. *Biological Diversity*, *1*(3-4): 147-157. doi: 10.1002/bod2.12026. [Scholar Google]

Kebede, E. 2018. Effect of cattle breed on milk composition in the same management conditions. *Ethiopian Journal of Agricultural Sciences*, *28*(2): 53-64. [Scholar Google]

Khider, M., Ahmed, N., and W. A. Metry. 2021. Functional ice cream with a coffee-related flavor. *Food and Nutrition Sciences*, *12*(8): 826-847. doi: 10.4236/fns.2021.128062.

Kumar, A., Mandal, R. S., Bhatt, S., and A. Kumar. 2024. Physiological Edema. *Periparturient Diseases of Cattle*, 331-338. doi: 10.1002/9781394204007.ch30. [Scholar Google]

Kumar, N., Tkui, K., Tadesse Tegegne, D., and A. T. Mebratu. 2014. Productive Performance of Crossbred Dairy Cows and constraints faced by dairy farmers in Mekelle, Ethiopia. 7(1). www.iosrjournals.org. [Scholar Google]

Kumar, S., Gupta, I. D., Sharma, N., Deginal, R., Kumar, A., Chauhan, A., and A. Verma. 2021. Effect of season, parity, and stage of lactation on productive performance of Sahiwal cattle. *Indian Journal of Animal Research*, *55*(5): 597-602. [Scholar Google]

Leggesse, G., Gelmesa, U., Jembere, T., Degefa, T., Bediye, S., Teka, T., and S. Chemeda. 2023. Ethiopia National Dairy Development Strategy 2022–2031. https://hdl.handle.net/10568/135703.

Marumo, J. L., Lusseau, D., Speakman, J. R., Mackie, M., and C. Hambly. 2022. Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. *Journal of Dairy Science*, *105*(2): 1225-1241. doi: 10.3168/jds.2021-20698. [Scholar Google]

Nalla, K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M., and A. K. Puniya. 2022. Impact of probiotics on dairy production efficiency. *Frontiers in microbiology*, *13*: 805963. [Scholar Google]

Ndambi, A., van der Lee, J., Endalemaw, T., Yigrem, S., Tefera, T., and K. Andeweg. 2017. Four important facts on opportunities in the Ethiopian dairy sector. *Practice brief DairyBISS project. Wageningen Livestock Research, Wageningen*.

Nyokabi, N. S., Phelan, L., Gemechu, G., Berg, S., Lindahl, J. F., Mihret, A., and H. L. Moore. 2023. From farm to table: exploring food handling and hygiene practices of meat and milk value chain actors in Ethiopia. *BMC Public Health*, *23*(1): 899. doi: 10.1186/s12889-023-15824-3. [Scholar Google]

Oloo, R. D., Ekine-Dzivenu, C. C., Ojango, J. M., Mrode, R. A., Chagunda, M., and A. O. Mwai. 2022. Effects of breed exoticness, agro-ecological zone, and their interaction on production and fertility traits of multibreed dairy cattle in Kenya. *ILRI Research Report*.

Oltenacu, P. A., and D. M. Broom. 2023. The impact of genetic selection for increased milk yield on the welfare of dairy cows. *Animal welfare*, *19*(S1): 39-49. doi: 10.1017/S0962728600002220. [Scholar Google]

Rai, P., and N. Adhikari. 2022. Study of the relationship among milk parameters in crossbred dairy cattle. doi: 10.21203/rs.3.rs-1920222/v1. [Scholar Google]

Sabek, A., Li, C., Du, C., Nan, L., Ni, J., Elgazzar, E., and S. Zhang. 2021. Effects of parity and days in milk on milk composition in correlation with β-hydroxybutyrate in tropic dairy cows. *Tropical Animal Health and Production*, *53*. [Scholar Google]

Shibru, D., Tamir, B., Kasa, F., and G. Goshu. 2019. Effect of season, parity, exotic gene level, and lactation stage in milk yield and composition of Holstein-Friesian crosses in the central highlands of Ethiopia. *European Journal of Experimental Biology*, *9*(4): 15. [Scholar Google]

Silva Neto, J. B., Mota, L. F., Londoño-Gil, M., Schmidt, P. I., Rodrigues, G. R., Ligori, V. A., and F. Baldi. 2024. Genotype-by-environment interactions in beef and dairy cattle populations: A review of methodologies and perspectives on research and applications. *Animal Genetics*, *55*(6): 871-892. doi: 10.1111/age.13483. [Scholar Google]

Silva, M. V. G. B., Carvalheiro, R., Santos, D. J. A., Tonhati, H., Albuquerque, L. G., and C. N. Costa. 2019. Heterosis in the lactation curves of Girolando cows with emphasis on variations of the individual curves. *Journal of Applied Animal Research*, 47(1): 85-95. doi: 10.1080/09712119.2019.1575223. [Scholar Google]

Souza, J. G., Ribeiro, C. V., and K. J. Harvatine. 2022. Meta-analysis of rumination behavior and its relationship with milk and milk fat production, rumen pH, and total-tract digestibility in lactating dairy cows. *Journal of Dairy Science*, *105*(1), 188-200. doi: 10.3168/jds.2021-20535. [Scholar Google]

Standard, E. 2009. Unprocessed whole/raw cow milk specification. *Ethiopian Standard*, *3460*, 2009.

Suhendra, D., Rahman, M. A., and M. Megawati. 2020. Correlation of protein contents and milk temperatures, with milk density of Friesian Holstein (fh) cow in Ngablak district of Magelang regency, Central Java. *Journal of Livestock Science and Production*, *4*(2): 289-294.

Taju H. 2018. Productive and Reproductive Performance of Indigenous Ethiopian Cow under Small Household Management in Dawro Zone, Southern Ethiopia. International Journal of Current Research and Academic Review, 6 (5). doi: 10.20546/ijcrar.2018.605.007.

Toghdory, A., Taghi G., Mohammad A., Mostafa B., Mojtaba N., and GN. Jalil. 2022. "Effects of Environmental Temperature and Humidity on Milk Composition, Microbial Load, and Somatic Cells in Milk of Holstein Dairy Cows in the Northeast Regions of Iran" *Animals* 12(18): 2484. doi: 10.3390/ani12182484. [Scholar Google]

Wondossen, A., Mohammed, A., and E. Negussie. 2015. Milk production performance of Holstein Friesian dairy cows at Holetta Bull Dam farm, Ethiopia.

Yigrem S, Beyene F, Tegegne A, and B. Gebremedhin. 2008. Dairy production, processing and marketing systems of Shashemene-Dilla area, South Ethiopia. IPMS Working Paper. https://cgspace.cgiar.org/items/cb6ec164-6aa9-4169-b774-751753fbfa40.

Zewdu, W., Thombre, B. M., and D. V. Bainwad. 2014. Effect of macroclimatic factors on milk production and reproductive efficiency of Holstein Friesian× Deoni crossbred cows. *Journal of Cell and Animal Biology*, *8*(4): 51-60. doi:10.5897/JCAB2014.0408. [Scholar Google]

Zhang, H., Gao, Q., Wang, A., Wang, Z., Liang, Y., Guo, M., and Y. Wang. 2024. Estimation of Genetic Parameters for Milk Production Rate and Its Stability in Holstein Population. *Animals*, *14*(19): 2761. doi: 10.3390/ani14192761. [Scholar Google]

Journal of Science and Development

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Introduction: A brief background of the subject, statement of the problem and the aims of the paper.

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Conclusions: Describe the contribution of the study to knowledge, and indicate future research needs (if any). The conclusion may also be included in the discussion.

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Authorname_Brief_title.doc, *e.g.* Bloggs_Podocarps_in_southern_Ethiopia.doc, Where Brief_title is the first 4-5 words of the manuscript's title.

Diagrams should be lettered in a sans-serif font (Arial or Helvetica-at least 12-point), for final reduction to single- column (6.9 cm) or double-column (14.3 cm) width. Single column figures are preferred. Black-and-white diagrams should be submitted as uncompressed TIFF (.tif) files or as .jpg files, at a resolution of 300 dpi. Diagrams created in the default mode of Microsoft Excel (frame, colored background, *etc*.) are not acceptable for publication in *JSD.*

Files containing diagrams should be named according to the following convention: Author name _Figure No xxx.tif,
e.g. Bloggs_Figure 006.tif

Photographs should be submitted as high-resolution (at least 600 dpi) greyscale (8-bit).jpg or uncompressed .tif files. The desired final size ('1-col', '2-col' or 'landscape') should be indicated. Always send photographs as separate files, using the same filename convention as above.
Photographs as described above are preferred, but clear, glossy black and white photographs (100×70 mm) on photographic paper may also be submitted. They should be clearly numbered on the back in **soft** pencil.

Tables should be prepared in MS Word's Table Editor, using (as far as possible) 'Simple1' as the model: (Table … Insert … Table … Auto format …Simple 1),
(see JSD_stylesheet.doc for illustration). Tables taken directly from Microsoft Excel are not generally acceptable for publication in *JSD*.

Use Arabic (1, 2, 3 …), not Roman (I, II, III …), numerals for tables. Footnotes in tables should be indicated by superscript letters beginning with 'a' in each table. Descriptive material not designated as a footnote maybe placed under a table as a Note.

Footnotes should be avoided. Wherever possible, incorporate such material in the text, within parentheses.

2. Submission in paper form

Manuscripts may also be submitted on A4paper, subject to the same limits regarding number of words, tables and
figures as above. Separate the manuscript into three sections: (1) **text section**, with figure and table texts at the end;
(2) **figure section** (one figure per page, for reduction to 6.9-cm and 14.3-cmcolumn width); and
(3) **table section** (one table per page). Type the text itself at double line-spacing on one side of the paper only, with top, left and bottom margins set at 2.5 cm. The right margin should, however, be set at 7.5cm, to leave space for reviewers' and editors' comments. Number all pages in sequence, including figures and tables.

The order of headings and sub-headings should be indicated as shown in the style sheet JSD_stylesheet.doc. Keep all levels of heading as short as possible.

Tables, figures and illustrations should be submitted each on a separate page. When a manuscript is submitted in paper form, a CD containing all sections of the paper, including diagrams, is also required. Diskettes (‘floppy disks’) are not admissible.

Conventions
Scientific names must be italicized. At first mention, the author (*e.g.* (L.)) should be given, but must not be italicized.
Use single quotation marks ‘’, unless you are giving a quotation within a quotation, in which case use “‘’”.
Insert … Symbol … Special characters
All data should be given in the metric system, using SI units of measurement.
Use ‘.’ (point) as the decimal symbol. Thousands are shown spaced, thus: 1 000 000.Use a leading zero with all numbers <1, including probability values (*e.g.*, $p< 0.001$).
Numbers from one to nine should be written out in the text, except when used with units or in percentages (*e.g.*, two occasions, 10 samples, 5 seconds, 3.5%). At the beginning of a sentence, always spell out numbers (*e.g.*, ‘Twenty-one
trees were sampled...’).

Use the 24-hour time format, with a colon ‘**:**’ as separator (*e.g.*, 12:15 h). Use day/month/year as the full date format (*e.g.*, 12 August2001, or 12/08/01 for brevity in tables or figures). Give years in full (*e.g.* ‘1994–2001’, never ‘94–01’). Use the form ‘1990s’, not ‘1990’s’ or ‘1990ies’.
Use the en-dash – for ranges, as in ‘1994–2001’
(Insert … Symbol … Special characters En dash).
In stating temperatures, use the degree symbol ‘º’, thus ‘ºC’, **not** a superscript zero ‘0’. (Insert … Symbol …
Normal text),
Define all symbols, abbreviations and acronyms the first time they are used, *e.g.*, diameter at breast height (DBH), meters above sea-level (m asl). In the text, use negative exponents, *e.g.*, g m-2, g m-2 sec-1, m3 ha-1 as appropriate.
Use ‘h’ for hours; do not abbreviate ‘day’.

If possible, format mathematical expressions in their final version (*e.g.,* by means of Equation Editor in MS Word or its equivalent in Word Perfect or Open Office); otherwise, make them understandable enough to be formatted during typesetting (*e.g.*, use underlining for fractions and type the numerator and denominator on different lines).

References
Please inspect the examples below carefully, and adhere to the styles and punctuation shown. Capitalize only proper
names (‘Miocene’, ‘Afar’, ‘The Netherlands’) and the initial letter of the title of papers and books, e.g., write
‘Principles and procedures of statistics’, not ‘Principles and Procedures of Statistics’. Do not italicize Latin abbreviations: write ‘et al.’, **not** ‘*et al.*’

References in the text should use the ‘author-year’ (Harvard) format:
(Darwin and Morgan, 1993) or, if more than two authors, (Anderson et al., 1993)
(Hartman and Kester, 1975; Anderson et al., 1993; Darwin and Morgan, 1994) chronologically.

It is highly recommended that Citations/References Management Software programs such as

Mendeley are used for organizing Citations and Bibliographic lists following the style of Crop Science Journal (alphabetical order) as shown in the following examples:

Journal article

Kalb J.E. 1978. Miocene to Pleistocene deposits in the Afar depression, Ethiopia. *SINET: Ethiop. J. Sci*. 1: 87-98.

Books

Whitmore T.C. 1996. *An introduction to tropical rain forests*. Clarendon Press, Oxford, 226pp.

Steel R.G.D. and Torrie J.H. 1980. *Principles and procedures of statistics*. 2nd ed. McGraw-Hill Book Co., New York. 633 pp.

Contribution as a chapter in books (Book chapter)

Dubin H.J. and Grinkel M. 1991.The status of wheat disease and disease research in warmer areas. In: Lange L.O., Nose1 P.S. and Zeigler H. (eds.) *Encyclopedia of plant physiology. Vol. 2A Physiological plant ecology*. Springer- Verlag, Berlin. pp. 57-107.

Conference/workshop/seminar proceedings

Demel Teketay 2001. Ecological effects of eucalyptus: ground for making wise and informed decision. Proceedings of a national workshop on the eucalyptus dilemma, 15 November 2000, Part II: 1-45, Addis Ababa.

Daniel L.E. and Stubbs R.W. 1992.Virulenceof yellow rust races and types of resistance in wheat cultivars in Kenya. In: Tanner D.G. and Mwangi W. (eds.). Seventh regional wheat workshop for eastern, central and southern Africa. September 16-19, 1991. Nakuru, Kenya: CIMMYT. pp. 165-175.

Publications of organizations

WHO (World Health Organization) 2005. Make every mother and child count: The 2005 World Health Report. WHO, Geneva, Switzerland.

CSA (Central Statistical Authority) 1991. Agricultural Statistics. 1991. Addis Ababa, CTA Publications. 250 pp.

Thesis

Roumen E.C.1991. *Partial resistance to blast and how to select for it*. PhD Thesis. Agricultural University, Wageningen, The Netherlands. 108 pp.

Gatluak Gatkuoth 2008. *Agroforestry potentials of under-exploited multipurpose trees and shrubs (MPTS) in Lare district of Gambella region.* MSc. Thesis, College of Agriculture, Hawassa University, Hawassa. 92 pp.

Publications from websites (URLs)

FAO 2000. Crop and Food Supply Assessment Mission to Ethiopia. FAOIWFP. Rome. (http://www. fao.or~/GIE WS). (Accessed on 21 July 2000).

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ISSN (Online): 2789-2123; (Print): 2222-5722