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Original Research Article||

Variability through crossing and subsequent selection towards a commercial cultivar development in summer flower *Hypericum androsaemum* L.

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Abstract

Hypericum androsaemum L., a popular summer flowering plant, faces significant challenges in commercial cultivation due to susceptibility to leaf rust diseases. These diseases affect marketability, forcing reliance on fungicides that increase costs and pose environmental concerns. This study aimed to enhance the commercial potential of *H. androsaemum* by introducing rust resistance gene through crossing a rust-susceptible commercial cultivar with a rust-resistant weedy genotype. The crossing was conducted at Milko Flower Farm, central Ethiopia, in 2021, resulting in viable F1 progenies. From the F1 population, five progenies were selected based on their desirable traits, such as tall stems, deep green rust-free leaves, and large deep red berries. Subsequent trials in 2022 using a completely randomized block design revealed that Progeny One exhibited the most favorable traits, including uniformity, distinctiveness, and reproducibility compared to the commercial cultivar used as a parent in crossing. This progeny was named *H. androsaemum* 'Ruby Excess,' registered and now grown commercially for export. The study demonstrates that breeding programs integrating rust resistance with ornamental traits can reduce fungicide use, enhance marketability, and mitigate environmental impacts. Furthermore, the methodology employed in this study could be adapted to introduce rust resistance into other commercial cultivars of *H. androsaemum*. However, the viability of F1 seeds and proximity of weedy populations to flower farms highlight the need for measures to prevent unintended spread and potential invasiveness. Therefore, implementing measures to prevent unintended spread is essential.

Key words: Crossing, *Hypericum androsaemum*, New cultivar, Ruby Excess, Rust resistance, Weedy genotype.

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INTRODUCTION

Floriculture is a vital component of global agricultural trade, with Ethiopia emerging as Africa's second-largest flower exporter, following Kenya (Dirriba and Mihretu, 2024). The sector contributes significantly to the national economy by creating employment opportunities and generating foreign exchange. Flowers have become Ethiopia's second most important export commodity after coffee, representing 14.1% of total export earnings. Furthermore, floriculture accounts for an impressive 80% of Ethiopia's horticultural revenue, showcasing its role as a key driver of agricultural development (Abas and Dame, 2015; Dirriba and Mihretu, 2024). Among the various flower species cultivated in Ethiopia, *H. androsaemum*, commonly known as tutsan, is particularly notable for its dual value: ornamental appeal and potential pharmacological benefits (Dias, 2003; Chavez and Lyrene, 2009).

H. androsaemum is a tetraploid species (2n = 40) well-known for its bright yellow blooms and striking berries, enhancing its appeal in horticultural markets (Dias, 2003; Chung et al., 2010). In contrast, other taxa in different sections exhibit a chromosome number of 2n = 20 (Robson and Adams, 1968). However, inheritance studies conducted by Olsen et al. (2006) demonstrated that the segregation patterns for foliage traits in *H. androsaemum* align with a diploid model. It is part of the Hypericum genus, which includes approximately 400 species of trees, shrubs, and herbs. The features that set *H. androsaemum* apart from other species in the Androsaemum section are its shorter styles and unique black berries, which stay soft even after ripening (Huxley et al., 1992). The species originates from temperate areas of Europe and Western Asia (Bailey, 1949) but has expanded beyond its native habitat because it is grown for decorative purposes. It has been naturalized in areas like Australia, New Zealand, and Chile through horticultural trade (Heenan, 2008).

In Australia, New Zealand, and the northwestern United States, it is considered an invasive species (Weber, 2003; Olsen et al., 2006). In Ethiopia, *H. androsaemum* was introduced during the expansion of the cut flower industry. While its cultivation has boosted the local horticultural trade, it has also raised ecological concerns. Weedy populations of the species are often found growing near flower farms, where they are cultivated for commercial purposes. Observations suggest that these weedy populations could potentially become invasive, as seen in other parts of the world. For instance, in Australia, *H. androsaemum* has been classified as an invasive species, with studies showing that its increasing abundance negatively impacts native species richness (Carey, 2007).

Hypericum androsaemum (tutsan) reproduces both sexually, through insect pollination, and vegetatively, enhancing its adaptability for natural propagation and breeding programs. The backcross breeding method involves mating a hybrid offspring with a recurrent parent lacking a desired resistance gene. This gradually incorporates the rust resistance gene from the donor parent into the recurrent parent, which retains its ornamental traits despite rust susceptibility. This strategy effectively transfers beneficial genes while preserving aesthetic qualities. The primary production regions for *Hypericum androsaemum* in Ethiopia, including Wolkite, Mehal Amba, Woliso, Holeta, Sebeta, and Ginchi, face significant challenges from leaf rust disease caused by the fungal pathogen *Melampsora hypericorum*. *Melampsora hypericorum* is an orange rust fungus that attacks Hypericum species, particularly Tutsan (*Hypericum androsaemum*). It forms yellow aecia on the leaf undersides, resulting in corresponding yellow or reddish patches on the upper surfaces. Rust infections render plants unmarketable, forcing growers to rely heavily on fungicides, which increase production costs and pose environmental risks. Identified in Australia in 1991, *M. hypericorum* has caused significant declines in *H. androsaemum* populations in certain areas, although some populations exhibit natural resistance. Breeding programs that combine rust resistance with desirable ornamental traits offer a sustainable and cost-effective alternative to fungicides, reducing reliance on chemical inputs while maintaining plant quality and market value. Dias (2003) and Carey (2007) highlight the potential of such breeding strategies, while Wise et al. (2004) emphasize the broader economic and regulatory challenges posed by ornamental rusts, including reduced plant value, unmarketable products, and increased costs. These studies stress the urgent need for research into rust-resistant cultivars and advanced diagnostic tools for effective disease management.

The success of any crop improvement program depends heavily on the availability of genetic variability and heritability of the traits of interest. In the case of *H. androsaemum*, genetic variability can be harnessed through conventional breeding techniques such as germplasm introduction, plant selection, and hybridization, as well as through modern biotechnological tools (Bekele and Gedebo, 2020; Belay et al., 2024). These methods enable the introduction of desirable traits, such as rust resistance, larger berries, deep green foliage, and vibrant colors, into commercial cultivars. Crossing genotypes with complementary traits generates genetic variability, which can then be exploited to develop superior cultivars through subsequent selection and propagation. Long before the advent of molecular genetics, Biffen (1905) demonstrated that rust resistance in some crops followed Mendel's laws of inheritance. This foundational work laid the groundwork for modern rust-resistance breeding efforts. Leus (2018) discusses breeding strategies for disease resistance in ornamental plants, highlighting the importance of reducing chemical treatments and improving plant health, serving as a model for similar work in *H. androsaemum.*

Trueblood et al. (2010) conducted cross-breeding of *H. androsaemum* to develop triploid clones with traits such as attractive foliage and reduced invasiveness. Although these clones exhibited the desired qualities, they were infertile. In contrast, other forms of *H. androsaemum* were fertile, highlighting the challenge of breeding non-invasive cultivars that also maintain fertility. Inspired by this, we observed healthy, rust-free weedy Hypericum genotypes growing naturally near Milko Flower Farm in Central Ethiopia. These weedy plants exhibited favorable traits, such as deep red berries and rust-free leaves, but had some limitations, like smaller berries and lighter green leaves. On the other hand, the commercial cultivar featured larger berries and green leaves, but had undesirable traits like light red berries and rust-prone leaves. The goal of this study was to develop new cultivars that combine the rust resistance of weedy genotypes with the ornamental appeal of commercial varieties.

MATERIALS AND METHODS

The Study Area

The study was conducted at Milko Flower Farm in Central Ethiopia, located 136 kilometers from Addis Ababa, within the Gurage Zone, specifically in Gedebano Gutazer Welene District, Desa kebele (14° 56' 52.33" N, 39° 9' 15.72" E). The farm is approximately six kilometers from Mehal Amba town and sits at an altitude of 2,300 meters above sea level. The research took place in an open field rather than in a greenhouse. The soil type is clay loam, characterized by a high water-holding capacity. The area experiences an annual rainfall of 151 mm, with a distinct seasonal pattern. The peak rainfall occurs during August and September, coinciding with the region's main rainy season. In contrast, the driest period is observed between December and February, with minimal precipitation. The temperature in the area also shows moderate variation, with a recorded minimum temperature of 9°C and a maximum temperature of 24°C, reflecting a relatively mild climate conducive to agricultural and ecological activities. These climatic characteristics play a significant role in determining the area's vegetation, water availability, and agricultural potential.

Plant Materials

The study began with the identification of two parent genotypes of *Hypericum androsaemum.* Milko Flower Farm provided the first parent genotype *Hypericum androsaemum* 'Shiney Romance' (P1) a commercial cultivar that was rust susceptible, while having lovely decorative qualities including large, light red berries and deep green leaves. The second parent genotype (P2) of the same species was obtained growing weedy at the farm edges of Milko Flower Farm. Notably, the plants found at the farm's edges produced rust free light green leaves and small sized red berries. It is believed that these plants may have originated from other Hypericum cultivars that grew at the farm in previous years.

One hundred plants of each parent were established as a mother stock from stem cuttings in 2020. The two parent stocks were established on two separate beds of $10m^2$ (1m width x 10m length) each with 50cm spacing between beds. The genotypes were planted with a spacing of 50 cm between rows and 20 cm between plants within a row. The plant nutrients commonly used for commercial cultivars that thrive in the same nutrient-depleted plots for multiple years were considered not applicable to the current study. Since this study was conducted on plots where Hypericum has not previously been grown, and the soil is not depleted, only standard fertilizer nutrients were utilized. Therefore, Nitrogen fertilizer in the form of urea 100 kg/ha was side dressed in split immediately after planting and 30 days after planting. The blended fertilizer NPSB 50 Kg/ha was side dressed at planting. The plots were watered twice a day, in the morning and in the afternoon, to a field capacity.

Breeding Procedures

The breeding procedures involved synchronizing flowering stages of parent plants, inducing flower initiation, cross-pollination, and selecting progeny. These steps are outlined as follows.

Synchronizing Flowering

In 2021 the stems of the two plots were cut back uniformly at height of 10 cm above ground after 60 days from planting. Twenty days after cutback, a new flush began to emerge, and forty days after the cutback, the stems were thinned back to one stem per plant. Once the plants grew to a height of 40 cm, we provided additional light using a compact fluorescent (CFL) bulb with an intensity of 150 lux and a wavelength of 610-700 nm. This bulb was positioned three meters above the canopy of the plants and was used for six hours each day, from 6 PM to midnight, for a duration of 30 days. Since Hypericum is a long-day plant, the extra light was needed for achieving uniform flower induction.

Cross Pollination

At flowering, before anthesis, the anthers of 25 female parent plants (P1) were removed to emasculate the flower, and they remained covered with a protective bag to prevent contamination or unintended pollination. The anthers of the male parent (P2), however, were not emasculated. At anthesis, the mature anthers from P2, which contained pollen grains, were carefully collected and used to dust the stigma of P1. The stigma of P1 was then kept covered for 15 days to avoid contamination until fruit formation was completed, after which the protective cover was removed. When the berries reached maturity and turned black, the seeds from P1 were collected.

Progeny Selection

On first of January 2021, F1 seeds were sown in a bed measuring 10 m² (10 m in length and 1 m in width). The spacing between rows was set at 30 cm, and within each row, seeds were planted 20 cm apart, with two seeds per hill. When the seedlings grew to a height of 40 cm additional light was given to initiate flowering using compact fluorescent (CFL) bulb with an intensity of 150 lux and a wavelength of 610-700 nm for six hours each day, from 6 PM to midnight, for a duration of 30 days.

At flowering, the plants produced berries that varied in size and color intensity, and produced leaves varied in greenness, as well as resistance to rust. Since there was a noticeable incidence of rust at the farm, additional rust inoculation was not necessary. Among the plants evaluated, those that produced relatively larger and deep red berries and deep green leaves, with no rust spots, were selected. A total of 300 progenies were assessed, and five top-ranking progenies were chosen. These five F1 progenies were considered to be five potential cultivars. On first of July 2021 these five progenies and two parent genotypes were planted on seven separate plots each $5m^2$ (5m length and 1m width) using stem cuttings as a planting material to establish mother stock.

Experimental Design

On May 1, 2022, stem cuttings from five progenies and two parental controls (P1 and P2) were planted in seven separate beds, each measuring 5 m² (5 m in length and 1 m in width), with three replications. The experimental design utilized was a Randomized Complete Block Design (RCBD). The spacing between rows was set at 30 cm, while the spacing between plants within each row was 20 cm, resulting in three rows in each bed. At the height of 30 cm all the plants were cut back to the height of 10 cm from ground, to put all the progenies at the same starting point for subsequent evaluation. Several stems sprout from the cut point. These were thinned back to one stem per plant. Sixty days after cutback, additional light was provided as usual to achieve uniform flower induction. The same rates of standard fertilizers were applied as described under the Planting materials section.

The field evaluation was carried out from May to the end of November, during which the weather in the experimental area was predominantly rainy. The humid and wet conditions of this season promote fungal growth, making it an ideal time to select plants that are resistant to rust.

Data Collection

Data collection was conducted by experienced supervisors at Milko Flower Farm. The supervisors' skill was valuable as the size and color of the berries, along with the color were assessed through visual evaluation. Berry size and color, as well as leaf color, were determined at the stage of full berry growth, which coincides with the time when flowers are typically harvested for sale. However, the incidence of leaf rust was evaluated throughout the growth period.

The progenies with their parents were assessed based on several characteristics: berry color (deep red and light red), relative berry size (small and large), leaf color (light green and deep green), stem length was measured for 10 plants per plot at flowering and resistance to rust was recorded as the percent leaf cover by the rust.

Data Analysis

The analysis of variance was done for days to flowering, stem length and leaf rust intensity (percent of leaf coverage by the rust) using MINITAB version 19. The mean separation was conducted using the Least Significant Difference (LSD) method at a significance level of 5%.

RESULTS AND DISCUSSION

Seed Viability and Invasiveness

In this study, cross-pollination between a commercial cultivar and a weedy genotype of *Hypericum androsaemum* produced viable seeds in the F1 generation, demonstrating the species' invasive potential. The presence of weedy plants at farm edges further underscores its ecological significance as a weed, a characteristic also observed in regions like New Zealand and Australia (Heenan, 2008). In contrast, Trueblood et al. (2010) found that triploid clones of *H. androsaemum* lack female fertility, suggesting their non-invasive nature. This duality highlights the importance of controlled production and management to balance the ornamental value of the plant with its invasive risks. In Ethiopia, strategies such as composting plant materials from flower farms and utilizing triploid clones could help minimize the spread of *H. androsaemum*. Additionally, harvesting before seed maturity, when berries are fully developed but not yet dispersed, could further reduce the plant's invasiveness.

Resistance to Leaf Rust

In the current study, the progenies of *Tutsan* exhibited significantly varying responses to rust fungus ($P < 0.001$, Table 4), with Progeny One showing rust-free leaves (Table 5). This aligns with Casonato et al. (1999), who found that some *Tutsan* populations have natural resistance to rust. However, McLaren et al. (1997) observed that *Melampsora hypericorum* caused varying damage to *Tutsan* and was even used as a biological control to manage its invasiveness. Previous research has identified other strains of the rust fungus in Australia (Baker, 1955; Whatman, 1967), but these strains were ineffective in controlling *Tutsan* (Groenteman, 2009). The reasons for these discrepancies are not well understood and may involve factors such as environmental conditions, genetic susceptibility, or differences in the pathogenicity of the rust.

While the rust resistance of *Tutsan* complicates efforts to eradicate it as a weed, this characteristic also presents an opportunity. Given its status as a high-value commercial plant, *Tutsan*'s leaf rust disease resistance allows for the cultivation of healthy plants without the need for costly and environmentally harmful fungicides.

Berry and Leaf Characteristics

In the current study, Parent 2 exhibited rust-free leaves (Table 5), making it a promising candidate for rust resistance. However, this cultivar lacks other desirable ornamental traits, such as larger, deeper-colored berries and aesthetically appealing leaf characteristics. To address this limitation, crossbreeding is essential to combine the rust-resistance traits from Parent 2 with the ornamental traits found in other cultivars. In this study, we examine two parental genotypes: the commercial cultivar, which has larger berries and deep green leaves but is susceptible to rust with light red berries, and the weedy genotype, which has smaller berries and light green leaves but exhibits resistance to rust and deep red berries, which are preferred for ornamental purposes. A cross between these two genotypes resulted in progeny displaying various combinations of these four traits (Table 1 and 5).

Among the progeny, Progeny One exhibited the ideal combination of traits: larger, deep red berries and deep green, rust-free leaves (Table 1 and 5). This combination enhances both the ornamental appeal and rust resistance, which is the desired outcome of the breeding program. We chose to vegetatively propagate this progeny through stem cuttings to preserve the desired traits without introducing genetic variability that could arise from sexual reproduction. Vegetative propagation ensures the offspring remain genetically identical to Progeny One, thus maintaining the combination of deep red berries, larger berry size, deep green leaves, and rust resistance. Furthermore, this method avoids the risk of undesirable traits reappearing, which can happen when seeds are used for propagation (Trueblood et al., 2010; Beeresha et al., 2024).

Table 1. Genotypes of *Hypericum androsaemum* grown at the Milko Flower Farm experimental field in the Gurage Zone of Central Ethiopia during the year 2022 in two cycles January to May and June to September and evaluated for, berry color, berry size, and leaf color.

No	Genotypes	Berry color	Berry size	Leaf color
1	Progeny 1	Deep red	Large	Deep Green
2	Progeny 2	Deep red	Large	Deep Green
3	Progeny 3	Light red	Large	Deep Green
4	Progeny 4	Light red	Small	Deep Green
5	Progeny 5	Light red	Small	Deep Green
6	Commercial cultivar (Parent 1)	Light red	Large	Deep Green
7	Weedy genotype (Parent 2)	Deep red	Small	Light Green

Days to Flowering and Stem Length (cm)

The genotypes exhibited very highly significant differences in the number of days to flowering ($P < 0.001$, Table 2). This variation is expected because the genotypes are heterogeneous, meaning they consist of different genetic makeups. Both parent plants flowered significantly earlier than all progenies, except for Progeny 4 (Table 5). Progenies 1 and 2 exhibited significantly later flowering compared to all other genotypes. Additionally, highly significant differences in stem length were observed ($P < 0.001$, Table 3). Progenies 1 and 2 had significantly longer stems than the other genotypes, although the difference in stem length between these two progenies was not significant (Table 5). While earlier flowering is generally preferred, it is often associated with shorter and less vigorous plants compared to those that flower later. Genotype 1, which flowers later and has a longer stem, appears to be a promising candidate for cultivation. Longer-stemmed plants tend to have higher market value, making Genotype 1 potentially more profitable than the earlier-flowering, shorter-stemmed genotypes. When such heterogeneous genotypes are propagated by seed rather than through vegetative propagation, the offspring can exhibit a wider range of traits, including flowering time. This phenomenon is consistent with findings by Trueblood et al. (2010), which highlight how seed propagation can lead to greater variability in traits.

Table 2. Analysis of Variance for days to flowering

Source of variation	DF	SS	MS	P-Value
Genotypes	6	254.6	42.4	0.000
Block	2	2.0	1.0	0.397
Error	12	12.0	1.0	
Total	20	268.6		

Table 3. Analysis of Variance for stem length

Source of Variation	DF	SS	MS	P-Value
Genotypes	6	124.6	20.8	0.000
Bock	2	0.7	0.3	0.619
Error	12	8.0	0.7	
Total	20	133.2		

Table 4. Analysis of Variance for leaf rust intensity (%)

Source of Variation	DF	SS	MS	P-Value
Genotypes	6	18271.6	3045.3	0.000
Bock	2	103.2	51.6	0.271
Error	12	424.1	35.34	
Total	20	18799.0		

Table 5. Mean of days to flowering, stem length and leaf rust intensity (percent leaf coverage by rust) of the studied Genotypes of *H. androsaemum* grown at the Milko Flower Farm experimental field in the Gurage Zone of Central Ethiopia during the year 2022 in two cycles January to May and June to September

No	Genotypes	Days to flowering	Stem length	Rust intensity %
1	Progeny 1	59a	41.0a	91.7a
2	Progeny 2	60a	38.7ab	63.3b
3	Progeny 3	56c	38.0b	45.0c
4	Progeny 4	51d	35.3c	38.3c
5	Progeny 5	57bc	36.3bc	36.7c
6	Parent 1	51d	35.3c	2.7d
7	Parent 2	51d	34.3c	2.0d
	Mean	55	37	40
	$LSD_{5\%}$	1.8	1.5	10.8

The m*ean followed by the same letter (s) in the same column are not significantly different at the 5% level of significance*

CONCLUSIONS

The cross-pollination between the leaf rust-susceptible commercial cultivar and the resistant weedy genotype of *Hypericum androsaemum* resulted in the production of viable F1 seeds. The viability of these seeds, combined with the presence of weedy plants near flower farms where this species is not native, raises concerns about the potential for invasive growth. This underscores the need for measures to prevent the unintended spread of this cultivar. One of the F1 progenies exhibited several desirable ornamental traits, including rust resistance and deep red berries inherited from the weedy parent, as well as larger berries and deep green leaves from the commercial variety. Additionally, the progeny displayed longer stems than either parent. The progeny consistently exhibited distinct, uniform characteristics, making it suitable for commercial use as a new cultivar. This cultivar has been named *H. androsaemum* ‘Ruby Excess’, registered, and assigned a product code by Floricode. It is currently being cultivated on a commercial scale for export. The introduction of this rust-resistant cultivar offers the potential to reduce fungicide use, thus lowering associated costs and mitigating the environmental impact of fungicide application. Furthermore, the methodology used in this study provides a framework for introducing leaf rust resistance into other commercial varieties of *H. androsaemum*.

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Original Research Article||

Demand and supply side enablers and barriers of periconceptional folic acid supplementation among pregnant women in southern Ethiopia: A phenomenological qualitative study

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Abstract

Background: Folic acid deficiency during conception is linked to neural tube defects, small for gestational age, and low birth weight. Adequate periconceptional folic acid supplementation (PFAS) has been shown in most studies to significantly reduce the risk of neural tube defects. Thus, the aim of this study was to investigate the demand and supply side enablers and barriers of periconceptional folic acid supplementation among pregnant women in southern Ethiopia.
Methods: A phenomenological qualitative study design was implemented among 35 purposively sampled pregnant women and 18 health professionals. We collected qualitative data through focus group discussions, in-depth interviews, and key informant interview techniques. Each of the collected data were transcribed verbatim and translated into the English language. The data were then entered into OpenCode software, which was used to manage the entire coding process and analyzed thematically.
Results: The study identified women's better health-seeking behavior and positive attitudes toward future PFAS use as enablers for service users. Service provider-side enablers include creating community awareness, health education, good perceptions of health professionals, integration with current health services, charge-free distribution of FAS, and application of the national supply management system. The main service user barriers were a lack of preconception care, an unplanned pregnancy, a late initiation of ANC follow-up, a lack of knowledge about PFAS, misconception of women, and financial constraints. We identified several service provider side barriers, including insufficient knowledge of health professionals, insufficient allocation of folic acid supplements, insufficient access, and ineffective supply stock management.
Conclusion: We must provide nutrition education about PFAS and its benefits for NTDs prevention, as well as engage in community activism and raise public awareness through mass media communication. Furthermore, PFAS should be integrated into health facilities and communities' current health care services across the country.

Key words: Enablers, barriers, periconceptional folic acid supplementation, neural tube defects, pregnant women, Southern Ethiopia

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INTRODUCTION

Folate is a very vital vitamin for gene expression, and it has a fundamental role in the synthesis and metabolism of proteins. It also acts as a cofactor in reactions that are determinants of cell division and cell maintenance through epigenetic mechanisms (Burdge and Lillycrop, 2012, WHO, 2015).

Folic acid deficiency during conception is linked with neural tube defects (Agopian et al., 2013, Detrait et al., 2005),small for gestational age and low birth weight (Hodgetts et al., 2015, Li et al., 2017). Likewise, folic acid deficiency relates to adverse pregnancy outcomes in the mothers, such as spontaneous abortion, preterm birth, and pre-eclampsia (Gaskins et al., 2014, Li et al., 2019, Kim et al., 2014, Wen et al., 2016).

Neural tube defects are multifactorial and preventable anomalies of brain and spinal cord neurulation that develop in humans between 21 and 28 days after pregnancy(World Health Organization, 2020, Pitkin, 2007). Every year, approximately 300,000 newborns worldwide are born with neural tube defects (NTDs), and the prevalence in Africa averages 11.7 and ranges (5.2–75.4) per 10,000 newborns (Zaganjor et al., 2016). In Ethiopia, the prevalence of NTDs ranges from 28.6 to 130.9 per 10,000 births(Mekonnen et al., 2021, Berihu et al., 2018).

Periconceptional folic acid supplementation(PFAS) is an intake of the folic acid supplement of 400 μg/d from preconception (4–12 weeks) until the end of the first trimester of pregnancy (8–12 weeks) (World Health Organization, 2006, Gomes et al., 2016). In most nations throughout the world, PFAS has been demonstrated to significantly reduce the incidence of pregnancies with neural tube defects (Dean et al., 2014, Liu et al., 2018).

Furthermore, a study revealed that adequate folate levels are difficult to attain only through diet. As a result, pregnant women must take five to ten times more folate than non-pregnant women (Wang et al., 2016). Even though, periconceptional use of FAS would be a simple and useful approach; this opportunity is frequently missed(Czeizel et al., 2013).

Multifaceted factors are identified as a challenge on PFAS including lack of awareness of women, unplanned pregnancies, late pregnancy diagnosis and higher number of pregnancies (Bitzer et al., 2013, Viegi and Bertini, 2018, Wegner et al., 2020, da Rosa et al., 2019). A study in conducted in Adama Ethiopia showed that maternal age, early ANC registration, consultation about preconception, previous unsuccessful pregnancies and level of awareness were factors significantly associated with folic acid use for the prevention of NTDs(Dessie et al., 2017). Currently, iron folic acid tablets are being delivered in Ethiopia through the ANC platform of the health facilities. The main focus of IFA is the prevention of anemia among pregnant women (Molla et al., 2019, Gebremichael and Welesamuel, 2020, Federal Ministry of Health of Ethiopia, 2016). However, the effort to provide periconceptional folic acid supplementation to prevent NTDs is a recent development in Ethiopia. Moreover, there is paucity of research evidence on the operational enablers and barriers of PFAS in Ethiopia. Thus, this study explored the enablers and barriers of PFAS among pregnant women in Southern Ethiopia.

MATERIALS AND METHODS

Study Setting

The study was conducted at six hospitals in the Sidama Regional State (SRS) and Central and South Ethiopia Regions (former South Nation Nationality People Regional State) of Ethiopia.

Study Design and Period

A phenomenological qualitative design was applied to explore pregnant women's and health professionals' perceptions of demand and supply-side enablers and barriers to periconceptional folic acid supplementation. This study was part of the Ph.D. research project that ran from April 14, 2022 to March 27, 2023.

Recruitment and Sampling

In the study, we purposefully selected six hospitals from three regions, including Hawassa University Comprehensive Specialized Hospital located in Hawassa City, Arbaminch General Hospital, Jinka General Hospital, Halaba Kulito General Hospital, Konso Karat Primary Hospital, and Worabe Comprehensive Specialized Hospital. In the selection, we tried to include primary, general, and specialized hospitals that serve both urban and rural populations.

Participants were purposefully selected. We recruited "service user women" from the client waiting areas of pregnancy follow-up clinics. Health care workers (medical doctors, midwives, and pharmacy professionals) and health decision-makers were recruited from selected hospitals and Sidama Regional State (SRS) and former South Nation Nationality People Region (SNNPR) health bureaus. Similarly, the Ethiopia pharmaceutical supply agency (EPSA) representative was recruited from Hawassa branch office.

From the 34 FGD participants approached, 29 agreed to participate in the FGDs and IDIs, but five were unable to participate because of a time shortage and busy home duties. In this qualitative study, we performed four focus group discussions, and a total of 29 participants were involved, with 6–9 participants in each FGD. Six (6) in-depth interviews among pregnant women, one per selected hospital were conducted. In the current study, twelve (12) in-depth interviews were done with health care workers (six antenatal care service heads and six pharmacy heads), and three (3) KIIs were conducted with medical directors representing the selected hospitals. Additionally, three (3) KIIs were conducted among health decision-makers, two from the maternal, child, and nutrition directorate SRS and former SNNPR health bureaus, and one from the supplier (EPSA-Hawassa branch).

Data Collection Procedure

We collected qualitative data using three different data collection techniques, which include focus group discussions (FGDs), in-depth interviews (IDIs) and key informant interviews (KIIs). Among pregnant women, the focus group discussions lasted 60 to 80 minutes, and interviews lasted 20 to 30 minutes. The focus group discussions were done using a semi-structured discussion guide, and for IDIs and KIIs, we used an interview guide as a tool of data collection, which was adopted after reviewing different literature (Tong et al., 2007, O'Brien et al., 2014). The data was collected by the principal investigator and two trained research assistants with MSc degrees in maternity and reproductive health who had experience in qualitative research.

All interview questions were written in Amharic, and one health worker from Konso Primary Hospital served as a local language translator on-site. To recruit study participants, the data collection team contacted pregnant women in the ANC follow-up clinic waiting area and requested their agreement to participate in IDIs and FGDs. The study purpose and approaches were explained during recruitment, and then informed, verbal, and written consent to participate in the study was obtained from all the participants. The interviews were conducted in one of the consultation rooms at the MCH to minimize interruptions and enhance the privacy of the participants. Each interview was audiotaped after securing permission from each participant. These audio recordings were complemented by field notes. Interviews were continued until all categories were well defined and thoroughly researched.

Interviews with health professionals, decision-makers, and suppliers were also done using a semi-structured interview guide by the principal investigator. After receiving written consent, health care workers (HCWs), decision-makers, and supplier were interviewed in a private room, mostly at their offices. A standard for reporting qualitative research was employed.

Trustworthiness

In this qualitative study we maintained trustworthiness criteria of credibility, transferability, dependability, and conformability (Korstjens and Moser, 2018). To maintain the study's credibility, discussions were made with the principal investigator and study participants to establish confidence. We spoke with managers and hospital-level facilitators. Using the participants' words in the final report boosted credibility. The researchers assured the study participants that they were not involved in the provision or management of health care services. The study's dependability was ensured by rigorously reviewing the transcribed data against the audio recorded files. More information regarding the concerns was obtained through probing and elaboration. Independent assistant and lead researcher discussed the coded results, and any conflicts in interpretation were debated and resolved.

To ensure data transferability, the study involves both pregnant women and health professionals, allowing for insight and rich information from both service users and providers. To maintain the finding's confirmability, we did peer debriefing and member checking to ensure that the findings were objective and unaffected by any biases or preferences of the researchers.

Data Analysis
The audio files of the interview data were transcribed verbatim and then translated into English by the principal investigator. The translated text was imported into OpenCode 4.02 software to facilitate the coding process. Coding was carried out by picking pertinent elements of the study participants' statements as they emerged. First, principal investigator and two research teams read each transcript multiple times to become acquainted, and the technique of inductive coding was employed.
Themes and sub-themes were created by looking for linkages between categories based on frequency of occurrence. We included critical and unexpected information provided by participants that appears in many papers, as well as any additional remarks relevant to this study. The data derived from the original transcripts was then organized into charts with headings and subheadings and built into a thematic framework (Green and Thorogood, 2018).

Operational Definitions
Periconception Period: is a time that lasts 4-12 weeks before conception and extends from the last menstrual period to 12 weeks of pregnancy.

Periconceptional Folic Acid Supplementation: is the practice of taking folic acid supplements 4-12 weeks before conception and from the last menstrual period until 12 weeks of pregnancy to reduce the risk of neural tube defects (NTDs).

RESULTS

Socio-demographic Characteristics of Pregnant Women

A total of 35 pregnant women (29 in FGDs and 6 in IDIs) participated in this study. The majority of pregnant women were between the ages of 25 and 34, with 20 of the research participants being urban women.

The majority of the women who participated had at least a secondary education, and housewives made up more than half of the women's occupations. A total of twenty-one pregnant women who participated in the study had pregnancies three or more times, and the majority of the women had less than three children (Table 1)

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Table 1:- Socio-demographic characteristics of pregnant women who participated in the study, Southern Ethiopia, 2023.

Characteristics	FGD (N=29)	IDI (N=6)
Age group (Yrs.|)		
<25	12	1
25-34	17	5
Resident		
Rural	13	2
Urban	16	4
Educational status		
Cannot read and write	7	0
Primary school	4	1
Secondary school	8	2
Higher education	10	3
Occupational status		
Housewife	17	3
Government employee	7	2
Self-employee	3	1
Student	2	0
Gravidity		
Less than three	13	1
Three and more	16	5
Children Alive		
None	7	1
Less than three	14	4
Three and more	8	1

Socio-demographic Characteristics of Health Service Providers

In this qualitative study, 18 health professionals (12 in IDIs and 6 in KIIs) participated. The study included 14 health professionals aged 25 to 34.

Whereas about half of IDI and KII professionals have diplomas and MSc/MPH degrees, respectively. Half of the study participants had at least seven years of experience providing health services (Table 2).

Table 2:- Sociodemographic characteristics of health service providers who participated in the study, Southern Ethiopia, 2023.

Participants	Age	Gender	Educational status	Profession	Work experience (Yr.)
Health professionals					
ANCH-1	30	Female	BSc, Degree	Midwife	3
ANCH-2	27	Female	Diploma	Midwife	4.5
ANCH-3	30	Female	BSc, Degree	Midwife	10
ANCH-4	28	Female	Diploma	Midwife	3
ANCH-5	28	Female	BSc, Degree	Midwife	8
ANCH-6	26	Male	BSc, Degree	Midwife	6
PSH-1	40	Female	Diploma	Druggist	9
PSH-2	25	Male	Diploma	Druggist	4.5
PSH-3	32	Male	Diploma	Druggist	12
PSH-4	28	Male	Diploma	Druggist	6
PSH-5	30	Male	BSc. Degree	Pharmacist	8
PSH-6	35	Male	BSc, MPh	Pharmacist	8
MD-1	32	Male	Medical doctor	General practitioner	3
MD-2	27	Male	Medical doctor	General practitioner	3
MD-3	27	Male	Medical doctor	General practitioner	3.6
Health Decision Makers and Supplier					
SRS-HB	42	Male	BSc, MPh	MCHN, Directorate	20
SNNPRS-HB	38	Female	BSc, MPh	MCHN, Directorate	15
EPSA-H	32	Male	BSc, MPh	Branch Representative	8

ANCH: Antenatal care head; PSH: Pharmacy service head; MD: Medical director; SRS-HB: Sidama Regional State-Health Bureau; SNNPRS-HB: South Nation Nationality Peoples Regional State-Health Bureau; EPSA-H: Ethiopia Pharmaceutical Supply Agency Hawassa; MCHN: Maternal Child Health and Nutrition

Folic Acid Supplement Types and Forms Delivered at the Facility

The types and forms of folic acid supplements delivered by the health facilities, as confirmed by the service providers, are iron-folic acid tablets and folic acid-only tablets. However, since the current supplementation focuses on iron-deficiency anemia, most people receive ferrous sulfate tablets without folic acid. In the focus group discussions, the majority of participants said that they took "iron" or “key kinine” to represent iron tablets or a combination of folic acid and iron. Likewise, there were fewer women who reported to have consumed folic acid from multiple micronutrient supplements with the name "Prenatal," which is expensive, and only those women who could afford to buy it from private pharmacies consumed it during their pregnancies.

Pregnant women reported:

> *“I took anemia correction tablets during my current pregnancy; the tablet is red and round-shaped.” (FGD, 25 years old, rural woman).*
>
> *"During my current pregnancy, I took red and round tablets in a plastic cup from the hospital" (IDI, a 25-year-old mother of a child).*
>
> *“I am taking iron tablets, and I thought the name was prenatal." (IDI, 25 years old, merchant).*

In addition, the service providers who were respondents to IDIs and KIIs indicated that, at their facilities, a combination of iron-folic acid tablets and non-enteric-coated forms are predominantly available, and women are receiving these supplements. For instance, one respondent said,

> *“In our facility pharmacy, we have both folic acid and iron combination oral tablets, commonly known as Fifol, and folic acid alone tablets.”* (IDI, PSH-2)

Another participant added,

"Folic acid is available in a combined form with iron (iron-folate tablets) and non-enteric-coated tablets." (IDI, ANCH-4)

Practice of Periconceptional Folic Acid Supplementation (PFAS)

It is internationally recommended that women receive folic acid supplementation from the moment they are trying to conceive until 12 weeks of pregnancy, which is the recommended period to prevent congenital anomalies, including neural tube defects. However, this study found that almost all pregnant women had not received FAS during the periconceptional period, and that regional health facilities do not implement periconceptional folic acid supplementation. Most of the women who participated in the FGDs and in the IDIs started to consume folic acid after the third month or within the second trimester, which is outside of the PFAS period.

A pregnant women stated,

"After confirming my pregnancy, I began taking tablets in the fourth month of my current pregnancy." (IDI, 30 years old, housewife)

The service providers working in the facility and decision makers affirmed that,

"Most of pregnant woman didn't took folic acid within periconceptional period." (IDI, ANCH-4)

"Folic acid supplementation is not being given in a timely manner; if it is taken during this period, it is good for the fetus and the mother." (IDI, PSH-1)

"Provision of supplementation for women within the internationally accepted and recommended intake period for folic acid tablets is not being implemented in our facility." (KII, MD-2)

"Based on our regional current situation, we are delivering folic acid during the pregnancy period, and it is not much before pregnancy." (KII, SNNPRS-HB)

"Mothers take folic acid pills after confirmation of their pregnancy status, not before." (KII, SRS-HB)

Thematic Analysis Findings

The study's findings were thematized, with nine sub-themes identified as enablers and thirteen as barriers to periconceptional FA supplementation (Table 3).

User side Enablers for Periconceptional Folic Acid Supplementation

Better Health-seeking Behavior

A few women with better health-seeking behaviors received advice and a prescription to take folic acid before pregnancy or in the earlier months within the first trimester. Since they have had better health-seeking behavior, they started taking folic acid tablets within the recommended period. For instance, a woman reported that,

"I started taking with the first month of pregnancy because I received an instruction from a health worker. But I don't have any knowledge about the standard period" (FGD-3, a 23-year-old government employee).

On top of that, two IDI participant women stated,

"I visited a hospital in a nearby town and received information from health workers on the advantages of taking "werja tekelakay kinine," or abortion protective tablets, before pregnancy. I then received the tablets and continued taking them today" (IDI, a 27-year-old housewife).

*"I started taking the tablets during the second month of my current pregnancy after receiving health care providers' advice. In addition, I went to the facility because my husband and I were planning to have a child, and the pregnancy was planned" (*IDI, 32 years old, mother of two children).

Positive Perception of Women

The majority of the pregnant women who participated in the FGDs and IDIs indicated they were willing to receive FA during the recommended period by the health professionals, which could be before pregnancy or within the first 3 months of their pregnancy. Almost all FGDs affirmed the possibility of receiving FA within the recommended time frame if information is provided by HPs. Participants in the FGD and IDIs assert,

"I think it is much better to take within 3 months before and after conception I will take for the future because folic acid will help for unborn

baby health" (FGD-3, a 27-year-old government employee woman).

"I'm not sure about this period, but if the health workers tell me to obey, I'll take whatever pills they give me as long as it doesn't harm my unborn baby" (IDI, a 25-year-old mother of three children)

"I think it is possible to begin and finish an iron tablet during this time period, as recommended by health workers. Therefore, it is possible for all women, not just those affected by "werja" abortion frequently" (IDI, a 27-year-old housewife)

Table 3:- Thematic analysis of demand and supply side enablers and barriers of periconceptional folic acid supplementation among pregnant women in southern Ethiopia, 2023.

Major Themes	Sub-themes	Categories
Enablers for PFAS	User (demand) side	- Better health seeking behavior - Positive perception of women to use PFAS in the future
	Provider (supply) side	- Creating awareness in the community - Health/nutrition education - Good perception of HPs on PFAS - Post-miscarriage service delivery experience - Integration with current health services - Charge-free distribution of supplement - Use of national supply management system
Barriers for PFAS	User (demand) side	- Low preconception care service use - Unplanned pregnancy - Late initiation of ANC follow-up - Lack of knowledge about PFAS among women - Misconception of women about FAS benefits - Non-compliance of women to FAS intake - Financial constraints of women to buy FA tablets
	Provider (supply) side	- Inadequate knowledge of HPs on PFAS - Unavailability of PFAS guidelines - Procuring non-enteric FAS - Insufficient access to FAS supply at HFs - Ineffective stock management of supply at HFs - Improper allocation of FA supply by RHB and EPSA

Provider Side Enablers for Periconceptional Folic Acid Supplementation

In this thematic analysis, creating awareness in the community, health education, good perception of HPs, FAS service delivery experience after miscarriage, integration with current health services, charge-free distribution of FAS, and use of the national supply management system were the identified service provider enablers.

Good Perception of Health Professionals

Most health professionals working in health facilities have a positive perception and attitude toward the effectiveness of implementing PFAS. The health facility workers and an EPSA representative who took part in the IDIs and KIIs reported on the issue that,

"I think it is possible to implement PFAS, and definitely if we work on this recommendation, we will achieve a lot because I am observing and hearing an increase in the birth of infants with cases of congenital anomalies, including NTDs, in recent years in the health facility" (IDI, ANCH-6)

> *"In our hospital, the folic acid pill was not being given in the internationally accepted time frame. But I think there is a possibility if there is a sufficient supply of folic acid pills"* (IDI, PSH-4)

> *"The internationally accepted and World Health Organization (WHO) recommended intake period for folic acid tablets is not being implemented. Thus, until now, we have not worked with dedication to deliver supplements at this time frame, but it is good to work on it in the future"* (KII, MD-2)

> *"It is possible to be implemented, and in the case of folic acid tablet distribution at the time recommended by the WHO, we can say it is possible. It is better to fully include them in the report and requisition form (RRF) format so that they can take the health plan as per their needs"* (KII, EPSA-HB).

Creating Awareness in the Community

The findings of this study indicated that awareness creation about PFAS in the community was one of the enablers for most health professionals and regional health decision-makers who participated in both the IDIs and KIIs. The main issues raised include awareness about folic acid and its prevention benefits for congenital anomalies through communication media with extensive advocacy, and sensitization by involving couples and the community at large. Four health workers who participated in the IDIs and KIIs reported that,

> *"All healthcare professionals must take part in raising awareness about folic acid. Congenital abnormalities and inadequate folic acid intake are issues that need intervention in our society. For a mother to deliver a healthy baby at nine months of pregnancy, she needs to be aware of the advantages of taking folic acid" (IDI, PSH-1).*

> *"It is better if women are made aware of pre-pregnancy and taking folic acid through communication media, because mothers in cities that are close to health professionals and those who use social media may have awareness, but most rural mothers may not have awareness. Therefore, it is good if awareness is created through mass media" (IDI, ANCH-5).*

> *"Periconceptional folic acid supplementation can be applicable if awareness creation is implemented well using campaigns to give folic-acid pills at the international standard and by involving the community starting from health posts, health centers and to the hospitals"* (KII, MD-1).

> *"It is necessary to remember that it is crucial to involve the users of folic acid pills as a couple (husband and wife) in the awareness-creation activities that will serve as a reminder and help to avoid commitment diverting factors*" (KII, MD-3).

In addition, regional decision maker stated,

> *"There should be extensive advocacy and sensitization activity, focusing on taking the right folic acid supplement during the recommended time window"* (KII, SRS-HB).

Health/Nutrition Education

The service providers who participated in this study pointed out that providing health/nutrition education on PFAS focusing on reproductive-age women who planned to be pregnant encouraged them to start taking it timely for the prevention of NTDs. The main approach should be to use visual teaching aids and to educate the women with practical examples to enhance their consumption of PFAS. Health workers from the facilities that participated in IDIs and KII asserted, *"We should give health education for all reproductive-age women to take folic acid supplementation before pregnancy if they planned it and to encourage women who suspect they are pregnant to start taking folic acid tablets within the recommended period." (IDI, ANCH-2)*

"Health education is a key to bringing behavioral changes among pregnant women. So we should provide health education about the health benefits of folic-acid supplementation and what congenital anomalies, including NTDs, mean both at the community level by health extension workers and by midwifes and nurses at the facility level" (IDI, ANCH-6)

"During health education sessions, we have to use the child with NTDs as an example and explain to the mothers why the child has this issue. We should clarify that the mother's failure to take folic acid prior to conception and during her pregnancy is what caused the baby's problem. We must so warn them that they will also experience this issue if they refuse to take it" (KII, MD-3).

Post-miscarriage Service Delivery Experience

Health professionals revealed that health facilities are providing FAS after a woman miscarries or gives birth to a fetus with congenital anomalies in the facility. A pregnant woman who participates in IDI also witnessed receiving the service after fetal loss. For instance, a pregnant woman from IDI stated that,

"I started and completed taking tablets three months before this pregnancy and am still taking iron tablets received from a health worker at the hospital after traveling to a nearby town." (She cried and then continued narration). "I lost my fetus two times previously, and I want to thank the health workers because, currently, both I and my fetus are doing well" (IDI, 27-year-old woman, housewife).

Additionally, two ANC health workers from IDIs affirmed that,

"Rarely, women who have a planned pregnancy, who are aware of the benefits of folic acid, and those who have experienced a miscarriage take the pills prior to pregnancy" (IDI, ANCH-3)

"Those pregnant mothers who gave birth in our hospital and encountered congenital anomalies, as well as women who had a miscarriage, take the iron-folate tables for 3 months after the incident" (IDI, ANCH-4).

Integration with Current Health Services

In this qualitative study, some of the health professionals and regional decision-makers indicated that to promote effective implementation of PFAS, it is better to use the family planning service as a contact area, implement projects, and also use existing community-based health intervention approaches. Health workers who participated in the IDIs and a KII participant regional decision maker stated that,

"We should carry out awareness-raising activities mainly among reproductive-age women by giving family planning service provision as the main starting point for the initiation of folic acid supplementation when they remove their contraceptive methods at the facility level. However, many women get pregnant without planning it" (IDI, ANCH-1)

"I believe the family planning service division should start by advising the use of folic acid pills when clients come to receive family planning services. This is because early pregnancy initiation is a problem" (IDI, PSH-1)

"To address preconception folic acid supplementation using women in one to five groups to identify and reach those women who have a pregnancy plan in the community. Also, we have to engage the HEWs and health development army" (KII, SNNPR-HB).

Charge-free Distribution of Supplement

In the health facilities, folic acid, either in iron-folic acid or folic acid-only supplements, is delivered charge-free to pregnant women. This practice may help as one of the facilitators for the success of periconceptional folic acid supplementation. Health professionals who participated in the IDIs affirmed that the delivery of FAS for women during ANC follow-up is free of charge if sufficient supplies are available at the health facilities. A pregnant woman who participated in IDI witnessed that, *"I received the iron-folate tablets from the public hospital for free"* (IDI, 32 years old, housewife).

In addition, a midwife and pharmacy professional who participated in the IDIs confirmed the issue that,

"*When there are adequate folic acid tablets available at our hospital pharmacy, we offer free folic acid tablets to all pregnant women. However, sometimes there is a lack of supplementation in the middle of the service provision. Thus, if iron –folic acid tablets were not available in the institution, women were forced to obtain them after buying them from a private pharmacy"* (IDI, ANCH-5).

"Folic-acid pills are available free of charge from the pharmacy while supplies are available. Currently, women are given a prescription to purchase folic acid, which they then purchase from a private pharmacy" (IDI, PSH-4).

Use of the National Supply Management System

Nationally, Ethiopia uses a national drug and supply management system with a RRF filling process across the nation, including folic acid supplements. Regional decision-makers who participated in the KIIs, reported that FAS is delivered based on requests from the health facilities. A regional decision maker stated that,

"Folic acid tablets and other medical supplies are requested from the Drug Fund and Supply Agency in accordance with what we call the RRF format. Thus, the distributions to the health facilities are done on a request basis by the Ethiopia Pharmaceutical Supply Agency (EPSA). That is why we see it as having a strong and positive side" (KII, SNNPR-HB).

User Side Barriers for Periconceptional Folic Acid Supplementation

Service users' barriers were one of the sub-themes. In the discussions and interviews, lack of preconception care service use, unplanned pregnancy, late initiation of ANC follow-up, lack of knowledge about PFAS among women, misconception of women about FAS benefits, non-compliance of women to FAS intake, and financial constraints of women to buy FA tablets were the main barriers identified by users.

Lack of Preconception Care

Lack of preconception care (PCC) use is one of the barriers to the effectiveness of periconceptional folic acid supplementation. The majority of the pregnant women in the FGDs and IDIs indicated they had never accessed health care services before their current pregnancy. Two pregnant women who took part in the IDIs affirmed that,

"I didn't use pre-pregnancy health services concerning pregnancy preparation" (IDI, 25 years old, mother of a child).

"I didn't use health services to learn about pregnancy preparedness before becoming pregnant" (IDI, 30 years old, housewife).

Unplanned Pregnancy

In the study, one of the barriers mentioned was having an unplanned pregnancy. A midwife who participated in an IDI reported that,

"I believe it is possible to use folic acid during the periconceptional period if we implemented it correctly; however, the main issue is that most pregnancies are unplanned" (IDI, ANCH-1).

Late Initiation of ANC Follow-up

Late antenatal care initiation during pregnancy was one of the hindering factors for periconceptional supplementation. As most of the women witnessed, they started their first follow-ups between the third and fourth months of their pregnancy. The women's late beginning of pregnancy follow-up was confirmed by the health professionals involved in this study.

A pregnant woman who participated in an IDI reported that,

"I started using the pregnancy follow-up service in the fourth month of my pregnancy, and today is my third follow-up visit. I am now in the eighth month of my pregnancy" (IDI, 25 years old, mother of a child).

Also, HCWs who took part in an IDI and KII affirmed that,

"I think the main problem is that almost all women come to the ANC service late or after the recommended period" (IDI, ANCH-4).

"Most women visit our hospital in the second three months of pregnancy; before that, they get services at the health center and first-level hospital. They come to us mainly when they

suspect that there may be a problem related to their pregnancy" (KII, MD-3).

Lack of Knowledge

Lack of knowledge about PFAS is an important barrier among service users. All of the women didn't have any knowledge or information about preconception folic acid supplementation. Health professionals affirmed that most women are not aware of the benefits of folic acid and about PFAS. For instance, a woman who participated in one of the FGDs stated that,

> *"I didn't take folic acid tablets at the indicated time since I didn't have any information about the period because health workers are responsible for guiding us"* (FGD-1, a 26-year-old housewife).

Furthermore, a midwife and pharmacy professionals that participated in IDIs confirmed that,

> *"Most women don't have adequate knowledge about the recommended period and the periconceptional folic acid supplementation service"* (IDI, ANCH-5).
>
> *"Folic acid supplementation is not being given in a timely manner; if women receive FA tablets during this period, it is good for the fetus and the mother, but usually none is taken before pregnancy due to a lack of awareness" (IDI, PSH-1).*

Non-compliance of Women

We discovered that non-compliance of women with taking the prescribed dose of FAS is one of the hindering factors of PFAS. The health professionals who participated in the IDIs affirmed that some women started FA before pregnancy but didn't complete taking it, and some women complained about physical illness for the non-compliance as indicated during the FGD. In addition, a pharmacy head reported women's fear of harm to the fetus and herself when taking drugs for a longer period of time.

For instance, a pregnant woman who participated in a FGD reported that,

> *"I faced problems often because of gastric irritation, and still, I am taking folic acid and iron tablets" (FGD-4, a 30-year-old mother of two children).*

Additionally, health workers who participated in IDIs stated that,

> *"Most of the women come after conception, so we provide for them during the pregnancy period. We usually provide them with Iron-Folic Acid (IFA) tablets after 4 months of their pregnancy because they complain about gastric illness and hyperemesis; as a result, they refuse to take the supplements earlier than 4 months"* (IDI, ANCH-6)
>
> *"Among pregnant women, the inability to take the prescribed amount of folic acid pills is one of the problems. To name a few of the issues that keep away women from taking folic acid, including nausea and stomach irritation after taking the pill, we advise them to take the medicine with food"* (IDI-PSH-3).

Misconceptions among Women

One of the barriers mentioned by some of the women was the misconception of women in the community about the effects of taking iron with folic acid tablets during pregnancy. Women taking iron folate tablets will increase blood pressure, lower fetal movement, make our child thinner, and delayed growth are some of the misconceptions raised by women from FGDs. Furthermore, health workers saw several misconceptions, such as the belief that ingesting FAS will make our child thinner and hinder growth, as well as neglect and some traditional community beliefs. Pregnant women who participated in the FGDs stated on the matter that,

> *"I experienced low fetal movement within a few days after taking the tablets, then a health worker told me it returned to normal and became well. I wonder if many women, like me, ask what the benefits of taking tablets during their pregnancies are because our moms and grandmothers didn't use them, but they gave birth to normal babies." (FGD-1, a 24-year-old housewife)*
>
> *"Since a pregnant woman gains weight as a result of pregnancy, taking a tablet may increase the amount of blood, and blood pressure will increase too. Due to these reasons, there are some women who are not willing to take it in the community." (FGD-1, a 23-year-old high school student)*

On top of that, a midwife and medical doctor who took part in IDI and KII, respectively, reported that, *"Among women, the misconceptions reported during our counselling session include that taking the pill can increase blood pressure, make our child thinner, and delay his growth"* (IDI, ANCH-1).

"Factors preventing pregnant mothers from taking folic acid pills include negligence, some negative traditional perceptions in the community about taking medicine during this time, and a belief that it is not useful for pregnant mothers" (KII, MD-2).

Financial Constraints

Most of the women witnessed that they were unable to purchase the FAS from a private pharmacy because they had financial difficulties; as a result, they were unable to take or continue FAS. Women participating in FGDs and IDIs, and service providers reported that women were forced to buy tablets or supplements when there was an unavailability of supply at the health facility pharmacy. Pregnant women who participated in FGD and IDIs stated that,

"I have children to take care of, and I don't have enough money to buy folic acid pills from a private pharmacy, so I couldn't take them" (FGD-2, a 29-year-old housewife).

"I received only a month of tablets free of charge, but the other two months doses I purchased from a private pharmacy. Because of financial difficulties, I faced challenges in completing the full dose" (FGD-3, a 23-year-old government employee).

"The tablets were not available in the facility; as a result, I bought the tablets from a private pharmacy for 350 Ethiopian Birr for 30 tablets. It was expensive" (IDI, 22 years old, merchant woman).

In addition, a midwife who participated in an IDI reported that,

"Folic acid capsules are often not available at the hospital pharmacy; thus, a client receives a prescription to purchase from a private pharmacy, and the cost is expensive. Because of this, even if we prescribe it to them, they will not be able to afford to buy it" (IDI, ANCH-4).

Provider Side Barriers for Periconceptional Folic Acid Supplementation

Service providers play a crucial role in the success of PFAS. However, the main barriers were inadequate knowledge of HPs on PFAS, lack of national guidelines and protocols, procuring low-efficacy FAS by HFs, insufficient access to FAS supply, improper stock management of supply at HFs, and inadequate allocation of FA supply by RHB and EPSA.

Inadequate Knowledge of HPs

Health professionals having poor knowledge of the importance of implementing periconceptional FAS was one of the main barriers from the service provider side. Some service providers indicated that low knowledge about taking folic acid within the periconceptional period is one of the barriers to effective PFAS. A regional decision maker and pharmacy professional affirmed that,

"I think the health professionals themselves need to be knowledgeable and must understand the protocol well. Previously, health workers advised using only iron-folate supplements when pregnant. The community's view and habit of solely taking folic acid tablets during pregnancy, health professionals' lack of awareness on the topic, and timely folic acid tablet administration are among the challenges" (KII, SRS-HB).

"Folic acid supplementation is not being given in a timely manner; there is a lack of proper knowledge regarding the timing and benefits of folic acid intake among health professionals" (IDI, PSH-1).

Unavailability of PFAS guidelines

One of the barriers is the lack of national PFAS implementation guidelines. A regional decision maker who took part in a KII commented about the matter,

"Based on our regional current situation, we are delivering folic acid during the pregnancy period, and it is not much before pregnancy. A guideline for PFAS is not available, and currently it is being prepared and endorsed at the federal ministry of health (FMOH) level" (KII, SNNPR-HB).

Procuring Non-enteric FAS

The form of folic acid supplement, which is a non-enteric-coated tablet with increased side effects, is one of the service provider's barriers. In addition, a

HP who participated in the IDIs indicated that because enteric-coated FAS with better efficacy were not available in the HF pharmacy, those who complained of side effects were encouraged to buy from a private pharmacy. A pregnant woman who took part in an IDI stated that,

> *"The tablets that are delivered from the hospitals have caused gastric irritation and discomfort. It is preferable to have the hospital provide us with the safest tablets so we can consume the prescribed dose completely"* (IDI, 32 years old, housewife).

In addition, a pharmacy head, a regional health decision maker, and an EPSA representative who were involved in this interview reported that,

> *"Some pregnant women didn't take all of the folic acid pills that are recommended for them. Uncomfortable feelings and gastric irritability are a few of the complications that keep women from taking folic acid. We advise pregnant women to buy enteric-coated folic acid tablets from private pharmacies since they are expected to finish all the prescribed folic acid tablets"* (IDI, PSH-1).

> *"Enteric-coated folic acid tablets or iron folate tablets would be better if distributed instead of the non-coated pill... It is preferable to deliver enteric-coated folic acid tablets to all health facilities in place of the non-enteric-coated pills that were previously distributed to some facilities for a brief period before being discontinued"* (KII, EPSA-HB).

Insufficient Supply

In this study, insufficient FAS supply at the health facilities was identified as one of the barriers. Women who participated in the IDIs and KIIs indicated that they faced lack of supply or poor access to the tablet, shortage and inconsistency of the FAS tablets from the facility pharmacy. The supply scarcity of folic acid pills was evidenced by health workers in the facility and regional health decision-makers who participated in IDI and KIIs, respectively, saying,

> *"There is a lack of sufficient supply of folic acid pills in the middle of service provision, supply loss (stock-out), and interruption of supply in our hospital"* (IDI, PSH-6).

> *"Folic acid pills are being given at the hospital, and supplies are available as much as possible. Since there are users of the service in the hospital from the neighboring zone, when there is a large number of users, the supply from the EPSA is not sufficient for our hospital and is not commensurate with our needs"* (KII, MD-2).

> *"It's hard to argue there is a real supply scarcity; more accurately, there is an artificial supply shortage. There may occasionally be a shortage in some portions of a zone or district, even though there is an adequate supply of tablets in some locations"* (KII, SNNPR-HB).

Ineffective Stock Management

Ineffective stock management is one of the barriers to effective PFAS. The providers of the service, including health professionals, regional decision-makers, and EPSA, witnessed the facilities' improper use of folic acid supplements. The national supplier EPSA recommended that facilities should make sure that supplies are delivered to consumers promptly. A pharmacy head who participated in IDI indicated that,

> *"Sometimes the folic acid supplements are available in the pharmacy store of a facility, but without proper stock management they may expire. Additionally, some facilities may have an overstock of the folic-acid tablets, so the responsible bodies should make redistribution to the nearby health facilities to solve the supply problem"* (IDI, PSH-6).

In addition, the regional health decision maker and EPSA representative who participated in the KIIs stated that,

> *"Folic acid supplements are being misused in some medical facilities, which causes them to expire. If folic acid tablets are widely available in a healthcare facility in large quantities, it is advisable to distribute them to other facilities that are in need because this will be more beneficial to society at large. Likewise, there is a shortage of supplement supply occasionally in some facilities as a result of inappropriate requests or improper RRF filling."* (KII, SNNPR-HB)

> *"After the tablets reach the health facilities, it is not necessary to keep them in the store; rather, it is necessary to distribute the supplements. Furthermore, it is mandatory to ask the mothers what they want, give advice, and motivate the women to take the supplements. This is necessary so that the folic acid pill does not expire"* (KII, EPSA-HB).

Improper Supply Allocation

Mismatching of the folic acid supplement service users demand against supply distribution to health facilities was also another barrier. A gap in the supply distribution against MOH-approved quotas to HFs is one of the barriers from the EPSA side. A pharmacy head and medical director who participated in an IDI and a KII stated that, *"We asked EPSA for the supply of iron-folic acid pills but could not find them. It's possible that folic acid pills are hardly available at their national drug store due to a supply shortage. But there was no problem before. In fact, it used to expire because no one was interested in taking the tablets, but now it disappears when users start to take the tablets"* (IDI, PSH-4).

> *"At the national level, there are discrepancies in the EPSA side's supply distribution. We faced an inadequate supply compared to demand because the supply is allocated to users who reside beyond the hospital's catchment area"* (KII, MD-2).

On top of that, a regional health decision maker who took part in a KII reported that,

> *"In the case of the supply of folic acid tablets, there is no problem with supply at the moment, but in the middle of service provision, there are shortages of supply and sometimes the distribution of expired folic acid tablets. As a result, we were experiencing supply shortages"* (KII, SRS-HB).

DISCUSSION

Folic acid supplementation during the periconceptional period plays a pivotal role in the prevention of neural tube defects (Silva et al., 2017). However, in the study, we discovered that periconceptional folic acid supplementation was not frequently provided in health facilities, despite the fact that this is the recommended period to avoid neural tube defects. Almost all pregnant women did not receive FAS at the recommended time. Women's better health-seeking behavior and positive perceptions of future PFAS use were identified as facilitators among service users. This finding, supported by other research, found that women's health-seeking behavior was substantially related to maternal health care (Vincent et al., 2017, Machira and Palamuleni, 2018).

Women who exhibited strong health-seeking behavior received guidance and were given a prescription to take folic acid prior to pregnancy or throughout the first trimester. Women's positive perceptions of utilizing PFAS in the future were potential facilitators, most women were prepared to take folic acid supplements prior to or during the first three months of their pregnancy, as advised by health professionals.

In contrast, one of the barriers to periconceptional folic acid supplementation cited by service users was a lack of preconception care services. The majority of the pregnant women who took part in the study had never received preconception care services before their current pregnancy. Similarly, research conducted in Ethiopia revealed that women's awareness and usage of preconception care were significantly low (Wegene et al., 2022, Ayele et al., 2021).

As reported by health professionals, one of the most challenging aspects of consuming PFAS during the periconceptional period is that the majority of pregnancies are unplanned. Another study from Italy found that women who do not plan their pregnancy use the least preconception FAS (Nilsen et al., 2016). Similarly, late antenatal care initiation by women was one of the barriers to PFAS. Most participants reported that they began their initial ANC visits between the third and fourth months of their pregnancies. The results are in line with research conducted in Southwest Ethiopia, which showed that 66% of pregnant women initiated their first antenatal care late (Tesfaye et al., 2017).

Another barrier that women faced was a lack of knowledge regarding PFAS. The majority of women who participated in the FGDs and IDIs in the present study found that they had no knowledge or information on folic acid intake before or during the first trimester of pregnancy. Likewise, studies in Germany (Wegner et al., 2020) and Japanese women (Obara et al., 2017) indicated that a lack of understanding of the necessity of folic acid for good pregnancy outcomes is associated with a failure to use periconceptional FA supplements. Furthermore, our study identified another barrier: women's misconceptions regarding the effects of iron-folate tablets during pregnancy. Some women and service providers suggested that taking iron-folate tablets causes elevated blood pressure, lowers fetal movement, makes infants thinner, and delays growth. Misconceptions about FAS have been reported in other countries as well. For instance Nigerian women said that taking iron-folate tablets endangers both the mother and the child, causes newborns to grow too big, and makes childbirth difficult for mothers (Siekmans et al., 2018).

We also investigated women's noncompliance with the prescribed dose of FA as one of the barriers. According to health professionals, some women started taking FA prior to pregnancy but stopped, while others complained about gastrointestinal discomforts and hyperemesis as a reason for noncompliance. Moreover, women were concerned about the danger to the fetus and themselves if they used drugs for an extended period of time. A similar result from South Africa indicated that because women were not experiencing physical disease, they were demotivated to adhere to supplements and considered they did not need them (Silubonde et al., 2022). Further, the present exploratory study revealed that the majority of the study's female participants were unable to obtain the FAS from a private pharmacy due to financial limitations. As a result, they were unable to use FAS or discontinued them.

Folic acid, either alone or in combination with other micronutrients, lowers the occurrence of neural tube abnormalities. Similarly, a study found that folic acid supplementation reduced the likelihood of pregnancy termination due to fetal abnormalities (De-Regil et al., 2015). The current study found that raising community awareness regarding PFAS was one of the facilitators. The emphasis should be on increasing public awareness of folic acid and its benefits in reducing congenital abnormalities, particularly NTDs, through advocacy and communication channels, as well as sensitization through the engagement of couples and society at large.

The service providers in this study emphasized the criticality of providing health education for women of reproductive age about PFAS, particularly those who intend to become pregnant; this can motivate them to begin taking it on time in order to prevent NTDs. To improve women's consumption of PFAS, we should include visual aids and practical scenarios in the sessions. Health professionals and regional decision-makers have identified integration of PFAS with current health services as a very crucial enabler. In order to properly implement PFAS, the service providers suggested that we use the family planning service as a point of contact, to carry out projects, and to make use of already-existing community-based health intervention services.

In this study, we found that delivering folic acid supplements following miscarriage or fetal loss with congenital malformations (CMs) is one of the enablers from the care provider's perspective. A study on the effects of folic acid supplementation on abortion risk found that consuming more folate from supplements was linked with a reduced incidence of spontaneous abortion (Gaskins et al., 2014).

In addition, the majority of health professionals working in healthcare facilities suggested that administering PFAS within the time frame given is advantageous. Thus, the majority of HPs are optimistic about the feasibility of periconceptional FA supplementation. Despite supply issues in many health facilities, iron-folic acid or folic acid-only supplements, may be available to pregnant women free of charge in the pharmacy. This practice may be a facilitator for the success of periconceptional folic acid supplementation. All participants and service providers agreed to deliver free FAS to women during ANC follow-up if adequate supplies were available at the health facilities.

The broad use of uniform medication, medical logistics, and reporting tools is crucial for effective healthcare supply chain management. Within the findings, a regional health decision maker stated that the EPSA distributes folic acid tablets and other medical supplies to health facilities nationwide on a request basis using the RRF format. This delivery method is seen as a strong and positive experience. A qualitative study in Bahir- Dar confirmed that almost all respondents felt that a credible and detailed RRF report is crucial for accurate forecasting and optimal distribution performance when supplied on time (Tilahun, 2022). The folic acid supplementation program is included within the Ethiopian antenatal care standards, which indicate that every pregnant woman should consume 60 mg of elemental iron (ferrous sulfate, ferrous fumarate, or ferrous gluconate) and 0.4 mg of folic acid per day for six months (180 tabs) (Ministry of Health Ethiopia, 2022). If she did not finish and, the entire dose was not consumed throughout pregnancy; the remainder was completed after delivery. If the area is prone to anemia, iron supplements may be continued for an extra three months. As a result, the emphasis is mostly on preventing maternal iron deficiency anemia during pregnancy but fails to provide periconceptional folic acid supplementation, which prevents neural tube defects and other congenital anomalies.

One of the most significant concerns identified in our qualitative study was the lack of national-endorsed guidelines or working protocols for PFAS, despite efforts to develop them. This study revealed that there are substantial policy gaps in administering PFAS. Another key barrier to appropriate PFAS was the inadequate knowledge of health professionals about PFAS. A study conducted in China supports the finding that health professionals' knowledge regarding periconceptional folic acid consumption was insufficient in certain areas (Cui et al., 2021). Similarly, research in Northern Ethiopia found that approximately 50% of health workers lacked adequate knowledge of folic acid during the periconception period (Demilew and Asres, 2017). Enteric coating is a typical process used in the creation of oral medicinal dosage forms. The primary benefit of enteric coating is that it protects the drug from acidic pH and enzymatic destruction in the stomach, as well as mitigating the negative effects of certain drugs (Maderuelo et al., 2019).

One of the challenges noted for HFs in our study was receiving FA supplements that are non-enteric coated and which have high side effects. A study in India showed that among iron-folic acid supplements, enteric-coated or delayed-release ferrous sulfate preparations can trigger less nausea and lower adverse gastrointestinal effects(Iqbal et al., 2015). Some health professionals who participated in IDIs stated that because enteric-coated FA tablets were not provided at the HF pharmacies, pregnant women were advised to purchase from private pharmacies. Improper allocation of FA supply by RHB and EPSA was identified in this investigation as another significant barrier. The majority of service providers stated in their response that there is an imbalance between the supply of folic acid to healthcare facilities and the demand for supplements. The supply distribution disparity between MOH-approved quotas for health facilities and the verified EPSA-related mismatch was also confirmed by specific healthcare specialists and regional health decision-makers.

A study in northwestern Tanzania revealed that women who received an iron-folate supplement at each appointment were more likely to continue using IFA supplements than women who did not access supplements at each visit (Lyoba et al., 2020). However, in this qualitative study, insufficient access to FA tablet supply at HFs was one of the main challenges explained as a barrier to PFAS.

The majority of participants indicated the absence, shortage, and supply inconsistencies of the FAS tablets from the facility pharmacy. Studies in Pakistan(Nisar et al., 2014) and Uganda (Kiwanuka et al., 2017) reported that women with inadequate access to iron and folic acid supplements had lower adherence to the doses. Inventory management contributes to maintaining a consistent supply for patients and preventing product stock outs for essential medical supplies (Kefale and Shebo, 2019).

Overall our study found that poor stock management and misuse of FAS at the health facility level are challenges to successful PFAS. Key informants, including regional health decision-makers and an EPSA official, confirmed that folic acid supplements are overstocked in some health facilities, causing them to expire. Furthermore, EPSA, who are the FAS suppliers, urged that health facilities should distribute supplies to users as soon as possible.

The study's strengths and limitations include the lack of evidence from qualitative studies on the barriers and facilitators of PFAS in Ethiopia; as a result, the findings will assist programmers and policymakers in developing an effective strategy for reducing neural tube defects and other birth defects that can be prevented with folic acid supplements. The study attempted to include the perspectives of medical experts and pregnant mothers. The study's limitations include its focus on only six healthcare facilities, nearly all of which are general hospitals or higher, as well as its failure to include inputs from health centers and health posts. More research is needed to address some of the issues that this study failed to consider.

CONCLUSIONS

Periconceptional folic acid supplementation guidelines and protocols should be implemented as a priority to prevent neural tube defects and other adverse birth outcomes across the country. Nutrition education on PFAS and its benefits for NTD prevention needs to be delivered targeting women of reproductive age, adolescent girls, and newlywed couples. Although we must engage in community activism and attempt to raise public awareness through mass media communication. Furthermore, PFAS should be integrated into the current health care services provided by healthcare facilities and communities. In addition, substantial donor support is required to maintain consistent access to safe and high-quality folic acid supplements.

ETHICAL CONSIDERATIONS

The Institutional Review Board of Hawassa University granted ethical permission for the study (Ref No. IRB/025/14). We then obtained a permission letter from the college as well as approval letters from the medical directors and administrators at each study facility. We received both oral and written consent from the study participants. All gathered information and medical records were kept private and confidential.

CONFLICTS OF INTEREST

The authors declare that they have no conflicting interests.

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Original Research Article||

Characterization of *Colletotrichum sublineolum* Isolates and Screening of Sorghum Cultivars for their Reaction against Sorghum Anthracnose in East Hararghe

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Abstract

Anthracnose, caused by *Colletotrichum sublineolum* is an important disease of sorghum in Ethiopia. Assessing variability in a pathogen population and screening host genotypes against a particular disease is an important step in devising a sound disease management strategy. This study was aimed to characterize *C. sublineolum* isolates from three selected sorghum-growing districts (Haramaya, Kersa, and Girawa) of East Hararghe and evaluate sorghum cultivars' reaction against the disease. Infected leaf samples were collected from the three districts during 2022 cropping season. A total of 31 *Colletotrichum sublineolum* isolates were examined for their morphological and cultural traits; 11, 10, and 10 isolates were from Girawa, Kersa and Haramaya districts, respectively. Cultural and morphological characteristics of *C. sublineolum* isolates were studied by growing them on potato dextrose agar at 25 ºC. Virulence of the 31 isolates was determined in three reference sets of sorghum genotypes with a detached leaf assay following a standard procedure. Twenty released sorghum cultivars and three rating reference sets of sorghum genotypes were screened in net house and laboratory conditions. The *C. sublineolum* isolates showed variation in cultural and morphological characteristics. The isolates had largely gray to light gray colony color on the upper side. The colony growth ranged from 14.50 mm to 42.33 mm. The conidial length and width ranged from 4.97 to 25.74 µm and 2.44 to 4.07 µm, respectively, with oval and falcate shapes. The virulence level varied significantly among the isolates tested. Isolates HA7A, HA3A, HA5A, HA6B, KD8C, KD8D and GM9B had higher virulence, while isolates GM2A HA1A HA4A, and KD4D had lower virulence levels on reference sorhum genotypes. The sorghum cultivars tested had significantly different responses to *C. sublineolum*. Cultivars Dekeba, Tilahun, Abshir, and Argity were identified as resistant to anthracnose disease. The finding of this study might be useful for breeding sorghum varieties for anthracnose resistance.

Key words: Anthracnose, *Colletotrichum sublineolum*, Characterize, Resistance, Sorghum bicolor, Virulence

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INTRODUCTION

Sorghum (*Sorghum bicolar*) is a drought tolerant cereal crop grown in semi-arid areas. It is used as a source of food, forage and biofuel (Girard and Awika, 2018). Sorghum serves as a source of income for the smallholder farmers producing the crop. It contains carbohydrates, iron, protein, and vitamin B3 (Palavecino et al., 2019). Sorghum is the fifth most important cereal crop in the world, following wheat, rice, maize, and barley. The global production of sorghum was 58 million tons in 2022 (FAO, 2023). In Ethiopia, four million tons of Sorghum was produced on 1.48 ha of land during 2023 (FAOSTAT, 2025). The crop accounts for 15.92% of grain production in the country (CSA, 2019). The average yield in actual, on-farm, and on-station trials was 2.4 t/ha, 3.3 t/ha, and 4.2 t/ha, respectively (Belachew et al., 2022). The production of sorghum in Ethiopia is more concentrated in the north-central, western, northwestern, and eastern mid-altitude regions (Wortmann et al., 2013). Oromia region is the major sorghum- growing region of Ethiopia contributing 42% of the country's sorghum production (USDA, 2025). Sorghum is the most important cereal crop grown in East Hararghe Zone of Oromia region.

Production and productivity of Sorghum in Ethiopia is constrained by biotic and abiotic factors. Diseases, weeds, and insects are the biotic factors limiting sorghum production in the country (Netsanet and Bewket, 2022). Anthracnose, caused by *Colletotrichum sublineolum*, is among the important production constraints of sorghum in Ethiopia (Rooney et al., 2002; Chala et al., 2010; Netsanet and Bewket, 2022). The pathogen can attack root, stalk, leaf and sheath as well as grains of sorghum (Koima et al., 2023). The disease is favored by extended periods of cloudy, warm, humid and wet weather conditions (Thakur et al., 2007). The optimum temperature range for conidia infection, germination and sporulation of *C. sublineolum* is 20-32 ^{o}C, 10-30 ^{o}C, 25-30 ^{o}C, respectively (Saloti et al., 2022). The conidia germinate on the leaf surface in the presence of water, producing germ tubes and appresoria which enables the fungus to penetrate into the host cell. (Thakur et al., 2007). The fungus survives as mycelium (on crop residue, weeds and seeds) and conidia (on seeds) (Koima et al., 2023). It may cause yield losses of 20-80% (Marley, 2004). Yield losses with a range of 26 to 35% war reported on susceptible cultivar from eastern Ethiopia (Girma et al., 2009).

Colletotrichum sublineolum is a diverse and heterogeneous fungal species (Costa et al., 2003). The first report on the presence of *C. sublineolum* races was made in 1967 and then, between 1967 and 1991, 44 different Colletotrichum spp. races or pathotypes were reported (Thakur and Mathur, 2000). Variability in *Colletotrichum sublineolum* populations in terms of morphological and cultural characteristics as well as virulence has been reported previously by different scholars (Thomas and Frederiksen, 1995; Costa et al., 2003; Souza-Paccola et al., 2003; Zanette et al., 2009; Chala et al., 2011; Tsedaley et al., 2021; and Mekonen et al., 2024). Wang et al. (2006) reported more than 40 pathotypes of *C. sublineolum* that attacked resistant sorghum genotypes over time.

Different cultural practices including, using resistant varieties, crop rotation and removal of crop residue can be used for managing sorghum anthracnose (Netsanet and Bewket, 2022). Foliar sprays of carbendazin and maneb and mancozeb and seed treatment with Apron-plus (a mixture of methalaxyl, carboxin and furathiocarpas) were effective to control the disease (Akpa et al., 1992). Among the different anthracnose management strategies available, the use of anthracnose resistant sorghum varieties is cost effective, most efficient and environmentally friendly way to control sorghum anthracnose (Abreha et al., 2021; Netsanet and Bewket, 2022). Breeding for anthracnose resistance has been a topic of research in different parts of the world including Ethiopia (Xu et al., 2020; Ridzuan et al., 2018; Mengistu et al., 2018.). Aragaw and Terefe (2024) reported promising sorghum genotypes that can be used as sources of resistance against anthracnose disease. However, the existence of variability in *C. sublineolum* population and the change in the virulence pattern of the pathogen through time makes the use of resistant varieties for anthracnose management difficult (Prom et al., 2009). Hence, continuous studies on the variability of the pathogen population along with periodic screening of local and improved sorghum genotypes to identify new sources of *C. sublineolum* resistance, are essential for devising sustainable and effective management strategies and for resistance breeding for sorghum anthracnose disease. This study was, therefore, designed to study the variability of *Colletotrichum sublineolum* isolates collected from Eastern Hararghe and evaluate the reaction of sorghum cultivars to the pathogen.

MATERIALS AND METHODS

Description of Sample Collection Areas

The diseased plant samples collection was carried out in three districts (Haramaya, Kersa, and Girawa) which are located in East Hararghe zone, Eastern Ethiopia, during 2022 growing season. The GPS coordinates and climatic data of the districts is shown in Table 1. The map of the study districts is presented in Figure 1. .

Table 1. The GPS coordinates and climatic data of the districts from which diseased plant samples were collected

Distric	Altitude (masl)	Latitude (N)	Longitude(E)	Mean annual rainfall (mm)	Mean annual Temp.(ºC)
Haramaya	2008 - 2114	9°23'123" - 9°24'972"	41°55'99" - 42°02'215"	600- 1260	9.9- 24.2
Kersa	2088 - 2044	9°22'92" - 10°21'814"	41°55'134" -42°54'843"	410 - 1200	18-26
Girawa	1923 - 2020	9°20'621" - 9°24'88"	41°53'499" - 41°53'884"	550-1100	20-27

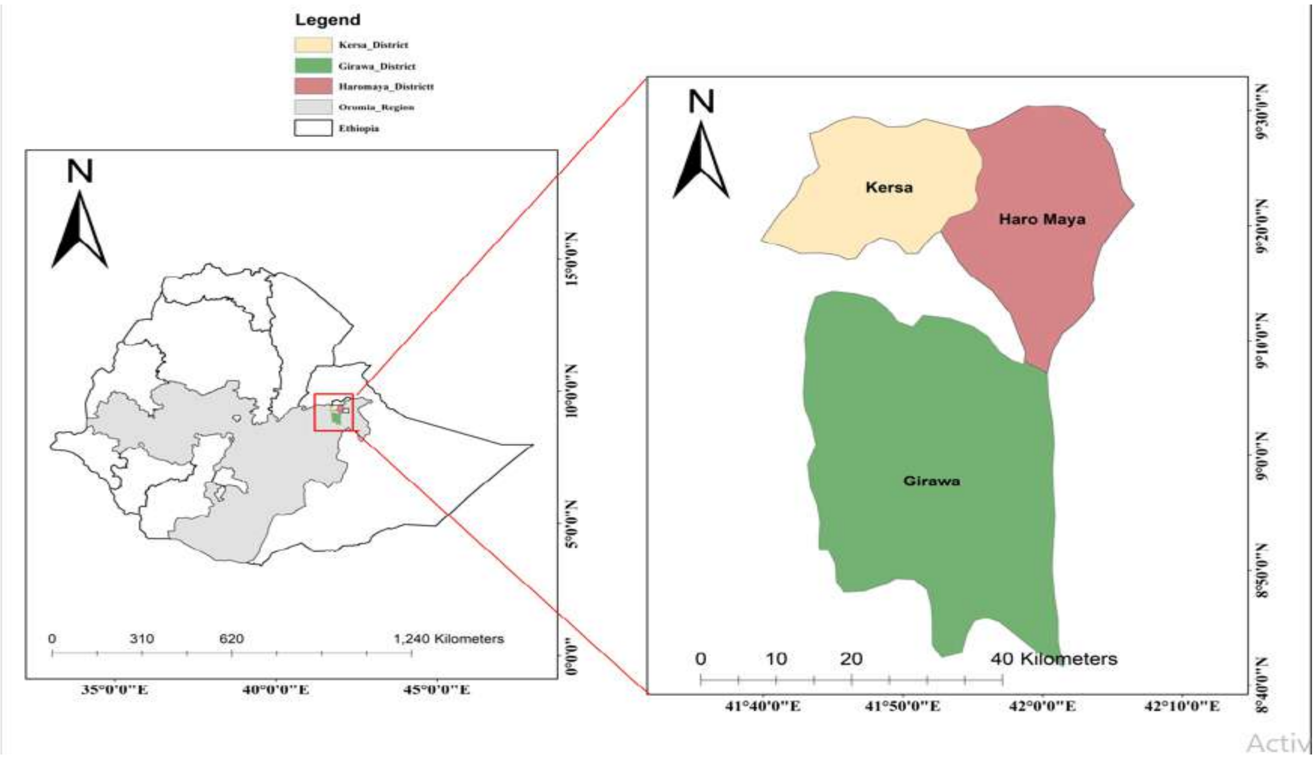

Figure 1. Map showing sample collection districts in Eastern Hararghe, Ethiopia.

Sample Collection

Sorghum fields were randomly selected at intervals ranging from 4-5km. From each district 15 to 19 sorghum samples showing typical anthracnose symptoms were collected. A total of 53 sorghum fields were visited during sample collection. Samples were kept in paper bags, and labeled with relevant information and taken to

Melkassa Agricultural Research Center Plant Pathology Laboratory for analysis.

Isolation and Identification of Sorghum Anthracnose Pathogen

Anthracnose infected Sorghum leave samples were cut into small pieces. The sample pieces were surface sterilized within 5% sodium hypochlorite solution for 3 minutes. The sterilized pieces were then rinsed three times in sterile distilled water and dried under a laminar flow hood. The dried samples were transferred into Potato Dextrose Agar (PDA) culture media and kept at 25°C for three to five days. The cultures were sub-cultured into new PDA medium to get pure cultures. Pure cultures of single spore isolates were kept at 4°C for further analysis.

Characterization of *Colletotrichum sublineolum* Isolates

For characterization study, a total of 31 *C. sublineolum* representative isolates were selected from the isolates collected from the three districts. Fungal culture of each isolate with 6mm diameter was taken from the growing margin using cork borer and placed at the center of new PDA plate. The PDA plates were then kept at 25°C in the Laboratory using CRD design with three replications for each isolate. The colony radial growth was measured five times at 48 hrs. interval. The radial growth was measured at two perpendicular angles of the reverse side of the culture plates. The other cultural and morphological characteristics studied were colony color, shape, elevation, margins, conidial size and conidial shape. Colony color was determined using the RGB (red, green, and blue) color chart (Anonymous, 2003). Width and length of conidia were measured using an Olympus-SC50 digital compound microscope. The conidial shape of the isolates was also studied by observing under a compound microscope with 10X and 40X magnifications.

Determination of the Virulence of *C. sublineolum* Isolates Using Detached Leaf Assay

The virulence study was conducted using rating reference sets (RRS) of sorghum genotypes (ETSL101173- a resistant reference set; Bonsa - a moderately resistance reference set; and BTX-623 - a susceptible reference set for *C. sublineolum* isolates). These three RRS sorghum genotypes were planted in pots filled with sterilized potting media composed of black soil, sand, and FYM mixture at a 3:2:2 ratio and kept in the net house. The description of sorghum genotypes used for the virulence study is presented in Table 2.

For preparation of inoculum for the experiment, the representative isolates were cultured on PDA medium at 25 °C for 7-10 days. The suspension of conidia for each isolate was prepared by suspending culture scraped from 7-10-day-old cultures in sterile distilled water, stirring vigorously for 90 seconds, and then filtering through two layers of cheesecloth. Before inoculation, the concentration of conidia suspension was adjusted to 1×10^6 mL^{-1} by using a hemocytometer. Healthy sorghum leaves from a 30-day-old plant were collected from the net house. The leaves were cut, surface sterilized using Clorox, and rinsed three times in sterile distilled water. The sterilized leaves were placed in Petri dishes lined with sterilized and moistened tissue papers. Individual genotypes leaves were drop-inoculated by placing 10 µl spore suspensions of each isolate and incubated on the laboratory bench until anthracnose symptoms were observed. The treatments were arranged in a factorial CRD design with three replications. The isolates virulence was determined by image analysis and a visual rating using a 1-9 scale (Thakur et al., 2007). Disease severity was recorded at 4, 7, and 10 days after inoculation (DAI). The lesion area (in square centimeters) was determined from the images using the free software ImageJ.

Table 2. **Descriptions of sorghum genotypes used for the virulence study of *Colletotrichum sublineolum* isolates.**

Genotypes	Resistancelevel	Source	Remark
BTX-623	Susceptible	MARC	Released
Bonsa	Medium resistance	MARC	Released
ETSL101173	Resistance	MARC	Advanced

Evaluation of Sorghum Cultivars' Reaction against *C. sublineolum*

Twenty released sorghum cultivars with three RRS genotypes were evaluated for their reaction against *C. sublineolum* under net house and laboratory conditions. The *C. sublineolum* isolates used for inoculation were chosen based on the result of pathogenicity test. Accordingly, the highly virulent isolates, HA3A, HA7A, HA5A, GM9B, and KD8C were mixed together to inoculate the cultivars tested. The isolates were mixed to have more aggressive *C. sublineolum* population and maximize the chance of getting promising resistant sorghum cultivars which may have many genes effective against several races of the *C. sublineolum*. Completely randomized design (CRD) with three replication was used for both detached leaf and intact leaf experiments.

Detached Leaf Assay

The most five highly virulent *C. sublineolum* isolates were mixed together to inoculate detached leaves of the test cultivars and RRS lines. Other inoculation procedure, data collection methods, and data type were mostly similar, as mentioned in the section on determination of virulence.

Intact Leaf Assay

The mixtures of the isolates with a 1×10^6 mL^{-1} concentration of conidia suspension was sprayed on 4-week-old sorghum plants until runoff. Inoculated plants were regularly examined for the development of *C. sublineolum* symptoms. Disease severity was evaluated by visual observation using a 1-9 scale (Table 3) (Thakur et al., 2007). The severity was assessed for five weeks at every seven-day intervals following inoculation. The Area under Disease Progress Curve (AUDPC) was calculated from the severity data using the formula below (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{Xi + Xi + 1}{2}(ti + 1 - ti)$$

Where, xi is the cumulative disease severity expressed as a proportion at the ith observation, where ti is the time (days after sowing) at the ith observation and n is a total number of observations.

Table 3. Disease severity scoring scale of anthracnose on sorghum

S. No	Disease severity scoring scale (1-9 scale)*	Disease reaction class
1	0 to <1% leaf area covered with hypersensitive reaction with mild yellow flecks	HR
2	1-5% leaf area covered with hypersensitive lesions without acervuli	R
3	6-10% leaf area covered with hypersensitive lesions without acervuli	R
4	11-20% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	MR
5	21-30% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	MR
6	31-40% leaf area covered with coalescing necrotic lesions with acervuli	S
7	41-50% leaf area covered with coalescing necrotic lesions with acervuli	S
8	51-75% leaf area covered with coalescing necrotic lesions with acervuli	HS
9	76-100% leaf area covered with coalescing	HS

*Disease severity score (Thakur et al, 2007).

Data Analysis

Colony growth, conidia size, disease severity, and, AUDPS data were subjected to an analysis of variance (ANOVA) using SAS software (version 9.2). Tukey's honestly significant difference test was used to separate the treatment means.

RESULTS

Cultural and Morphological Characteristics of *Colletotrichum sublineolum* Isolates

The 31 *C. sublineolum* isolates collected from Eastern Hararghe showed differences in colony color, margin, and colony elevations (Table 4 and Figure 2). Most isolates had a flat colony elevation whereas the rest had raised colony elevation. The majority of *C. sublineolum* isolates had circular colony margins, with some having irregular colony margins. The isolates formed varied colony color on the upper side and reverse side on PDA. After 10 days of growth, five types colony color on the upper side of *Colletotrichum sublineolum* isolates were observed. The colony colors formed were dirty white (6), gray (11), salmon white (5), light gray (6), and white (3). Seven different types of colony colors were recorded on reverse side as dirty white (7), dim gray (5), burlywood (6), light yellow (2), sand brown (2), light goldenrod (3), and light gray (6).

Table 4. **Colony characteristics of *Colletotrichum sublineolum* isolates cultured on PDA at 25°C for ten days.**

Isolate*	Colony color Upper	Reverse	Colony Elevation[a]	Colony Margins[a]
GM1E	Light gray	Light gray	Flat	Irregular
GM2A	Light gray	Light gray	Raised	Circular
GM3A	Salmon white	Burly wood	Raised	Circular
GM4B	Gray	Dim gray	Raised	Circular
GM5A	Light gray	Light goldenrod	Raised	Circular
GM6B	Salmon white	Light gray	Raised	Irregular
GM7A	Light gray	light gray	Raised	Circular
GM8A	Gray	Dim gray	Raised	Irregular
GM9B	Gray	Dim gray	Flat	Irregular
GM10A	Light gray	Light gray	Flat	Circular
GM11E	Gray	Dirty white	Flat	Circular
HA1A	Light gray	Burly wood	Flat	Circular
HA2B	Gray	Burly wood	Flat	Circular
HA3A	Gray	Dim gray	Raised	Circular
HA4A	Gray	Dim gray	Flat	Circular
HA5A	Dirty white	Dirty white	Flat	Irregular
HA6B	Dirty white	Dirty white	Flat	Circular
HA7A	Dirty white	Dirty white	Flat	Circular
HA8A	Dirty white	Dirty white	Flat	Circular
HA9B	Gray	Light yellow	Flat	Irregular
HA10C	Gray	Light yellow	Flat	Irregular
KD1B	Salmon white	Light goldenrod	Raised	Circular
KD2A	Salmon white	Light goldenrod	Raised	Circular
KD3E	White	Light Gray	Flat	Circular
KD4D	Salmon white	Burly wood	Raised	Circular
KD5A	White	Sandy brown	Flat	Circular
KD6A	Dirty white	Dirty white	Flat	Circular
KD7C	Gray	Burly wood	Raised	Irregular
KD8D	Dirty white	Dirty white	Flat	Irregular
KD9B	White	Sandy brown	Flat	Circular
KD10C	Gray	Burly wood	Raised	Irregular

* GM1A-GM11E isolates were obtained from Girawa, HA1A-HA10C from Haramaya, and KD1B-KD10C from Kersa

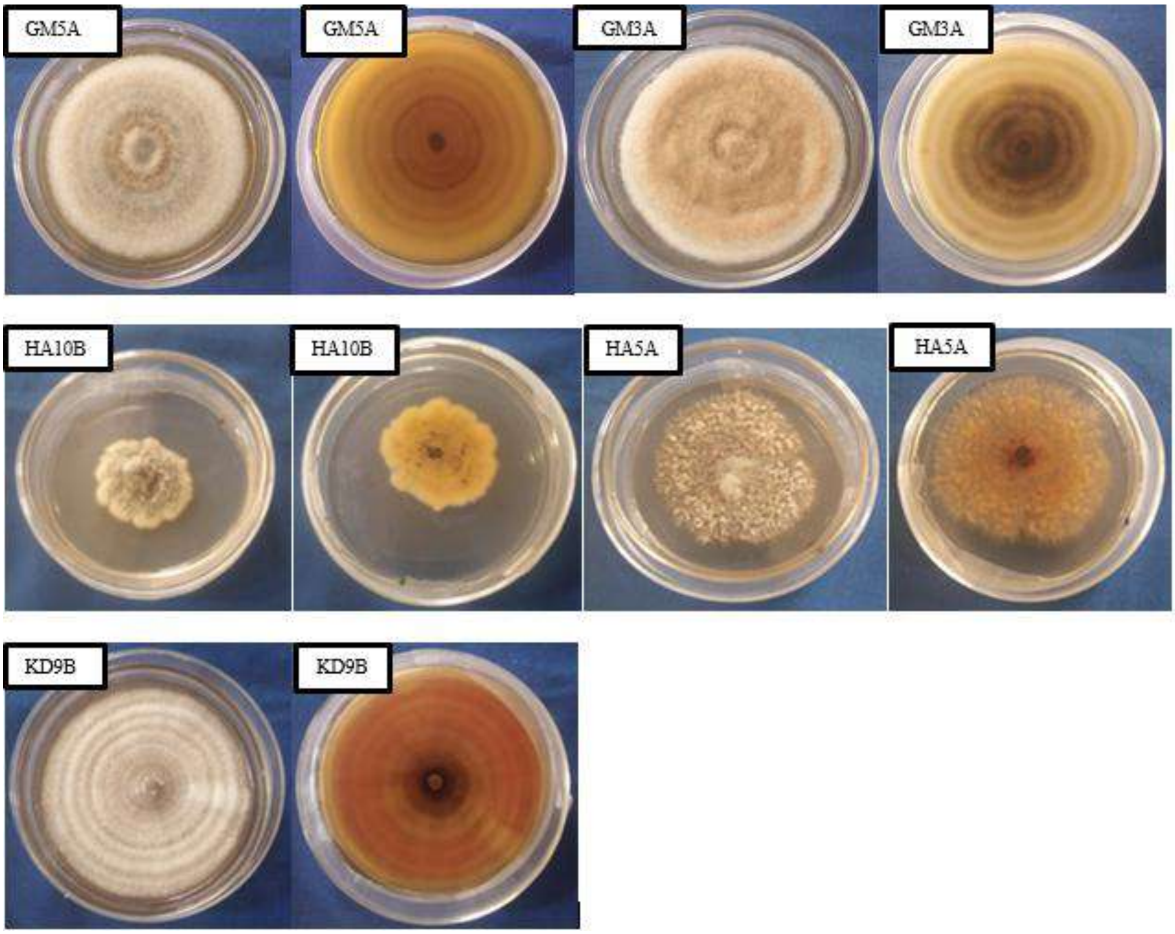

Figure 2. **Cultural difference among *Colletotrichum sublineolum* isolates obtained from Eastern Hararghe after culturing on PDA at 25ºC for ten days.**
Note: GM5A and-GM3A isolates from Girawa; HA10B and HA5A from Haramaya; and KD9B from Kersa.

There was significant ($p<0.0001$) variation in colony radial growth of some *C. sublineolum* isolates (Table 5). The radial growth of the isolates ranged from 14.50 mm to 42.33 mm at 10 days of incubation on PDA. Isolate KD5A had the highest mean radial colony growth (42.33 mm) at 10th day although non significantly different from the radial growth of isolates GM5A, HA1A, HA1B, KD1B, KD2A,KD9B and KD10C. Whereas, HA10C isolate had the lowest radial growth of colony (14.50 mm) at 10th day although not significantly different from the radial growth of isolates GM1E, GM8A, GM10A, HA4A).

Table 5. Colony radial growth of *C. sublineolum* isolates cultured on PDA at 25ºC for ten days

Isolate*	Colony radial growth (mm)[a]				
	2 Days	4 Days	6 Days	8 Days	10 Days
GM1E	8.67[abcde]	17.00[abcd]	21.83[f]	28.17[g]	33.83[fg]
GM2A	7.83[bcde]	16.83[abcd]	21.00[f]	27.67[g]	32.83[g]
GM3A	9.17[abc]	19.00[a]	29.50[a]	37.83[ab]	41.42[ab]
GM4B	4.00[hi]	5.67[h]	9.17[ij]	16.00[jk]	19.00[ijk]
GM5A	9.17[abc]	18.67[a]	27.83[ab]	37.92 [ab]	41.50[a]
GM6B	8.33[abcde]	17.67[abc]	25.83[bcd]	33.67[de]	37.83[bcd]
GM7A	7.50[de]	15.50[cd]	21.92[ef]	28.58[g]	33.25[g]
GM8A	7.83[bcde]	17.33[abcd]	25.58[bcd]	32.42[e]	36.92[def]
GM9B	3.67[i]	10.83[e]	14.83[g]	17.83[hij]	21.17[hij]
GM10A	7.58[cde]	15.50[cd]	23.33[def]	31.50[ef]	34.17[efg]
GM11E	3.58[i]	6.00[gh]	10.00[ij]	15.75[jk]	18.75[jk]
HA1A	9.50[a]	18.42[a]	27.50[ab]	35.50[bcd]	39.50[abcd]
HA2B	9.00[abcd]	18.00[ab]	27.25[abc]	34.00[cde]	39.17[abcd]
HA3A	5.16[ghi]	9.83[ef]	15.00[g]	20.17[h]	24.50[h]
HA4A	5.33[gh]	15.00[d]	20.83[f]	28.83[gf]	36.33[defg]
HA5A	5.75[fg]	9.83[ef]	13.67[gh]	18.83[hi]	24.00[h]
HA6B	5.17[ghi]	8.25[fg]	11.67[hi]	16.58[ij]	22.50[hi]
HA7A	4.83[ghi]	7.08[gh]	9.58[ij]	13.00[lm]	17.50[kl]
HA8A	0.45[ghi]	7.41[fgh]	10.42[ij]	13.83[kl]	16.58[kl]
HA9B	4.16[ghi]	6.75[gh]	9.17[ij]	11.83[lm]	15.42[kl]
HA10C	4.16[ghi]	6.33[gh]	7.83[j]	10.50[m]	14.50[l]
KD1B	8.83[abcd]	18.00[ab]	26.50[bc]	3.40[cde]	39.00[abcd]
KD2A	9.42[ab]	18.16[ab]	26.92[abc]	34.17[cde]	39.00[abcd]
KD3E	8.75[abcde]	15.75[bcd]	21.92[ef]	28.17[g]	32.83[g]
KD4D	8.33[abcde]	18.00[ab]	27.33[abc]	36.00[abcd]	41.00[abcd]
KD5A	7.83[bcde]	17.83[abc]	27.67[ab]	38.33[a]	42.33[a]
KD6A	5.00[ghi]	11.50[e]	15.67[g]	18.92[hi]	23.83[h]
KD7C	8.83[abcd]	17.67[abc]	26.33[bc]	33.67[de]	37.50[cde]
KD8D	5.00[ghi]	12.00[e]	16.18[g]	18.92[hi]	23.83[h]
KD9B	7.19[ef]	17.58[abc]	27.50[ab]	36.58[abc]	41.42[ab]
KD10C	7.67[cde]	17.16[abcd]	24.67[cde]	33.75[de]	39.08[abcd]
CV (%)	7.35	5.45	4.32	3.15	3.62
LSD	1.62	2.44	2.80	2.69	3.62

*GM1E -GM11E isolates from Girawa; HA1A -HA10C isolates from Haramaya; and KD1B - KD10C isolates from kersa. CV (%) is the coefficient of variation. LSD= Least Significant Difference. Mean values with the same letter within a column do not differ significantly at 5% level of significance.

[a] Colony radial growth was measured at 2 days interval, .

There was a highly significant ($p<0.0001$) variation in conidia size between isolates of *C. sublineolum* (Table 6). The mean conidia length and width ranged from 4.97 to 25.74µm and 2.44 to 4.07 µm, respectively. Isolate HA3A had the highest mean conidial length (25.74 µm) and width (4.07 µm), while isolate KD7C had the lowest mean conidial length (4.97 µm) and width (2.44 µm) although not significantly different from the conidial length and width of some of isolates. The *C. sublineolum* isolates produced two types of conidia; namely, falcate and oval shapes (Table 6 and Figure 3). The falcate conidia shape is larger than the oval type. However, there was no significant variation in conidia length among the falcate conidia shapes of the different isolates except for GM9B. The oval conidia shape was of small conidia size and no significant variation among the isolates that produced oval conidia shape. The majority of *C. sublineolum* isolates (20) that infected sorghum had oval conidia shape while the remaining 11 isolates had a falcate conidia shape.

Table 6. The conidial characteristics of *Colletotrichum sublineolum* isolates from eastern Hararghe after ten days of incubation.

Isolate*	Conidia shape	Conidia size (µm)[a]	
		Length	Width
GM1E	Oval	5.03[c]	2.44[d]
GM2A	Oval	5.39[c]	2.76[d]
GM3A	Oval	5.67[c]	2.56[d]
GM4B	Falcate	25.53[a]	3.76[abc]
GM5A	Oval	5.54[c]	2.58[d]
GM6B	Oval	5.40[c]	2.64[d]
GM7A	Oval	5.76[c]	2.68[d]
GM8A	Oval	5.28[c]	2.50[d]
GM9B	Falcate	19.74[b]	3.83[abc]
GM10A	Oval	5.13[c]	2.51[d]
GM11E	Falcate	25.65[a]	3.76[abc]
HA1A	Oval	5.50[c]	2. 46[d]
HA2B	Oval	5.62[c]	2.71[d]
HA3A	Falcate	25.74[a]	4.07[a]
HA4A	Oval	5.14[c]	2.60[d]
HA5A	Falcate	25.24[a]	3.91[ab]
HA6B	Falcate	24.67[a]	3.54[bc]
HA7A	Falcate	25.39[a]	3.70[abc]
HA8A	Falcate	23.56[a]	3.38[c]
HA9B	Falcate	24.42[a]	3.78[abc]
HA10C	Falcate	23.73[a]	3.86[abc]
KD1B	Oval	5.40[c]	2.55[d]
KD2A	Oval	5.31[c]	2.49[d]
KD3E	Oval	5.59[c]	2.50[d]
KD4D	Oval	5.67[c]	2.72[d]
KD5A	Oval	5.87[c]	2.63[d]
KD6A	Falcate	24.72[a]	3.67[abc]
KD7C	Oval	4.97[c]	2.44[d]
KD8D	Falcate	23.95[a]	3.66[abc]
KD9B	Oval	5.31[c]	24.47[d]
KD10C	Oval	7.20[c]	2.78[d]
CV (%)		**6.28**	**5.10**
LSD		**2.59**	**0.49**

*GM1E-GM11E isolates from Girawa; HA1A-HA10C isolates from Haramaya; and KD1B-KD10C isolates from Kersa. LSD= Least Significant Difference. CV (%) is the coefficient of variation.
[a]Means followed by the same letter within a column is not significantly different from each other.

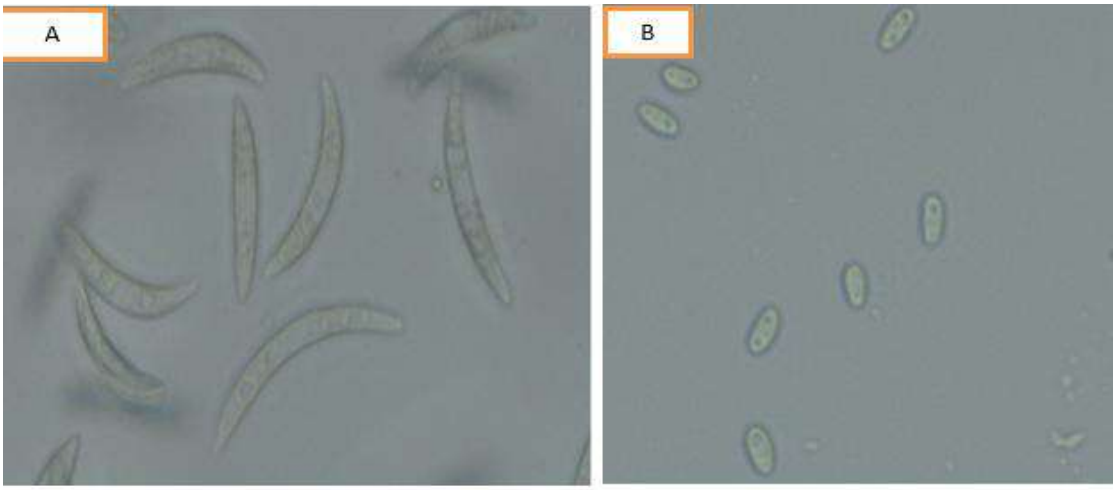

Figure 3. Morphological variation in the conidia of *Colletotrichum sublineolum* isolates after ten days of incubation. A=falcate conidia (HA3A); B=oval conidia (HA7C).

Determination of the Virulence of *Colletotrichum sublineolum* Isolates on Detached Sorghum Leaves

The result of this study demonstrated that the isolates from various locations of Eastern Hararghe had varying virulence levels on the three sorghum rating reference sets tested on detached leaves (Table 7, Figure 4). The mean area of lesion on the sorghum genotypes due to the disease varied from 0 cm^2 to 6.09 cm^2 after 10 days of inoculation. HA7A isolate had the largest mean lesion area (6.09 cm^2), while isolates GM2A and KD4D had the smallest mean lesion area (0.00 cm^2) in all tested sorghum genotypes. The disease severity score was between 1.0 and 7.33 after 10 days of inoculation. Isolate HA7A inoculated to genotype BTX-623 resulted in the highest mean severity score (7.33), while GM2A and KD4D isolates had a low mean severity score (1.00) in all sorghum genotypes tested. The genotype ETSL101173 had resistant to moderately resistant reaction to all the isolates. In this study, high virulence was detected in the interaction of all three genotypes with the isolates HA7A, HA3A, HA5A, and KD8C. Low virulence was the results of all genotypes interacting with GM2A, HA1A, HA10C and KD4D isolates. The genotypes BTX-623 and Bonsa showed susceptible reaction to isolates, GM9B, HA3A, HA5A, HA6B, HA7A, HA8B, KD7C and KD8D. All the three genotypes tested showed resistant reaction to isolates, GM2A, HA1A, HA4A, and KD4D. Overall, most of isolates were highly virulent on BTX-623 genotype compared to the other two genotypes.

Table 7. Interaction of *Colletotrichum sublineolum* isolates with Sorghum Genotypes in the development of Sorghum Anthracnose on detached leaves

Isolates[a]	BTX-623			BONSA			ETSL101173		
	Lesion Area	Severity Scale	Disease reaction	Lesion Area	Severity Scale	Disease reaction	Lesion Area	Severity Score	Reaction type
GM1E	3.37	4.67	MR	0.12	1.33	HR	0.00	1.00	HR
GM2A	0.00	1.00	HR	0.00	1.00	HR	0.00	1.00	HR
GM3A	1.00	3.00	R	2.72	4.67	MR	1.53	4.00	MR
GM4B	4.26	6.00	S	2.11	4.33	MR	0.98	2.33	R
GM5A	4.22	6.33	S	3.21	4.67	MR	2.64	4.00	MR
GM6B	4.25	6.33	S	3.53	5.67	MR	3.41	5.67	MR
GM7A	0.43	2.33	R	3.93	5.67	MR	0.00	1.00	HR
GM8A	2.94	4.33	MR	2.12	4.33	MR	0.00	1.00	HR
GM9B	5.47	6.67	S	4.80	6.33	S	3.67	5.33	MR
GM10A	2.36	4.33	MR	1.59	4.00	MR	1.60	4.00	MR
GM11E	4.86	6.33	S	2.30	4.33	MR	1.40	3.33	R
HA1A	1.27	3.67	R	0.98	3.00	R	0.78	2.67	R
HA2B	2.69	4.33	MR	0.48	2.00	R	0.00	1.00	HR
HA3A	5.62	7.00	S	4.69	6.33	S	3.77	5.67	MR
HA4A	0.50	2.33	R	1.15	3.00	R	0.00	1.00	HR
HA5A	5.54	7.00	S	4.90	6.33	S	3.89	5.33	MR
HA6B	4.38	6.33	S	4.55	6.00	S	1.27	3.33	R
HA7A	6.09	7.33	S	5.47	6.67	S	4.02	5.33	MR
HA8B	4.46	6.33	S	4.41	6.00	S	1.35	3.33	R
HA9A	4.04	5.67	MR	1.72	4.00	MR	1.60	4.00	MR
HA10C	1.40	3.67	R	1.57	4.00	MR	2.22	4.33	MR
KD1B	3.15	5.33	MR	1.38	3.67	R	0.00	1.00	HR
KD2A	3.07	4.33	MR	1.83	4.00	MR	3.16	5.33	MR
KD3E	2.74	4.67	MR	0.00	1.00	HR	1.31	3.67	R
KD4D	0.00	1.00	HR	0.00	1.00	HR	0.00	1.00	HR
KD5A	4.37	6.00	S	3.99	5.67	MR	1.38	3.67	R
KD6A	4.70	6.33	S	1.60	4.00	MR	1.31	3.67	R
KD7C	4.36	6.00	S	4.25	6.00	S	1.22	3.67	R
KD8D	5.58	7.00	S	5.07	6.67	S	3.65	5.67	MR
KD9B	2.54	4.33	MR	2.70	4.67	MR	1.13	3.33	R
KD10C	3.21	4.33	MR	3.31	4.00	MR	0.00	1.00	HR
CV of severity score =13.59; CV of lesion area = 14.88									

[a]GM1E-GM11E isolates from Girawa; HA1A-HA10B isolates from Haramaya; and KD1B-KD10C isolates from Kersa.

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.

CV (%) = coefficient of variation;

Mean values with the same letter within a column are not significantly different from each other.

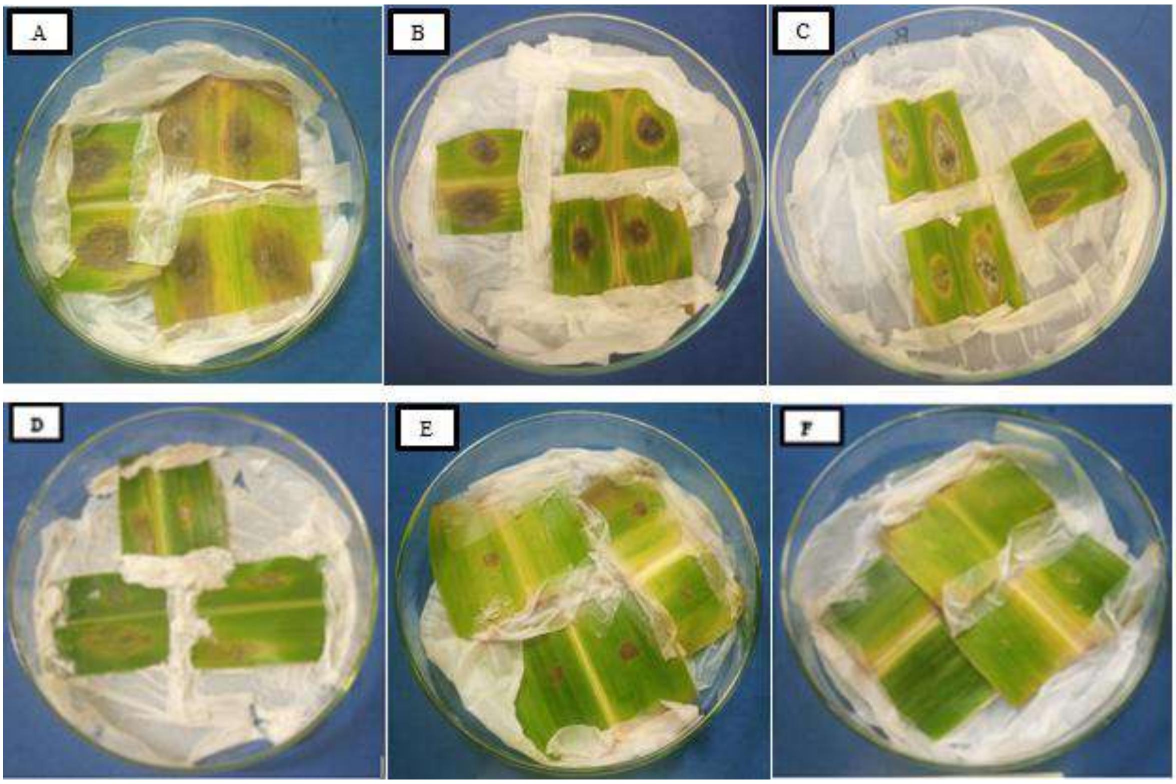

Figure 4. **Interaction of *Colletotrichum sublineolum* isolates with Sorghum rating reference sets.**
A= HA7A isolate with BTX-623; B = HA7A isolate with Bonsa; C = HA3A isolate with BTX623; D= GM10A isolate with BTX-623; E = HA1A isolate with ETSL101173; and F = KD4D with ETLSL101173.

Responses of Sorghum Cultivars to *Colletotrichum sublineolum* Isolates

Detached Leaf Assay

The result of this study showed significant ($p<0.0001$) variation among sorghum cultivars reaction against *Colletotrichum sublineolum* isolates (Table 8). The reaction of sorghum cultivars against *Colletotrichum sublineolum* isolates in the detached leaf test is presented in Figure 5**.** The lesion area ranged from zero to 7.21 cm^2. The cultivar Abshir had the lowest mean lesion area, while Adelle had the highest mean lesion area. On the other hand, the disease severity score ranged from one to 8.67. The mean disease severity score of the Abshir cultivar was the lowest (1), while that of Adelle was the highest (8.67). Based on the finding of the detached leaf assay, Sorghum cultivars, Adelle, Dibaba, Jiru, Chiro, Dagim, Seredo, Gambella-1107, BTX-623 and Bonsa cutivars were susceptible to the disease. Among these, Adelle and Dibaba cultivars showed highly susceptible reactions and had disease severity scores of 8.67 and 8.00, respectively. The resistant checks, ETSL101173 and nine other sorghum cultivars displayed moderate resistant reaction against *Colletotrichum sublineolum*. Abshir, Tilahun, Argity, and Dekeba cultivars with disease severity scores of 1.00, 2.33, 3.33, and 3.67, respectively showed reactions ranging from resistant to highly resistant against the pathogen.

Table 8. Sorghum cultivars' reaction to *Colletotrichum sublineolum* in the detached leaf test.

Cultivar	Lension area (cm^2)*	Severity score*	Disease reaction type
Abshir	0.00^{j}	1.00^{j}	HR
Adelle	7.21^{a}	8.67^{a}	HS
Argity	1.37^{ij}	3.33^{hi}	R
Baji	3.09^{efgh}	4.33^{fghi}	MR
Berhan	4.09^{def}	5.67^{cdefg}	MR
Birmash	2.33^{ghi}	4.33^{fghi}	MR
Bonsa	5.61^{bcd}	6.66^{abcde}	S
BTX-623	6.70^{ab}	7.67^{abc}	S
Chiro	5.46^{bcd}	6.67^{abcde}	S
Dagim	4.35^{cde}	6.00^{bcdef}	S
Dibaba	6.89^{ab}	8.00^{ab}	HS
Dekeba	1.32^{ij}	3.67^{ghi}	R
ETSL101173	4.08^{def}	5.67^{cdefg}	MR
Gambella-1107	5.86^{abc}	7.33^{abcd}	S
Gobiye	2.50^{ghi}	4.67^{efgh}	MR
Jiru	5.71^{abc}	7.00^{abcd}	S
Macia	4.11^{def}	5.67^{cdefg}	MR
Meko	3.31^{efg}	5.33^{defgh}	MR
Melkam	2.73^{efgh}	4.67^{efgh}	MR
Seredo	6.70^{ab}	7.33^{abcd}	S
Teshale	2.94^{efgh}	4.00^{fghi}	MR
Tilahun	0.62^{j}	2.33^{ij}	R
76Ti#23	1.56^{hij}	4.00^{fghi}	MR
CV (%)	13.04	12.27	
LSD	1.57	2.07	

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.
CV (%) = coefficient of variation; LSD=Least Significant Difference
Mean values with the same letter within a column are not significantly different from each other.

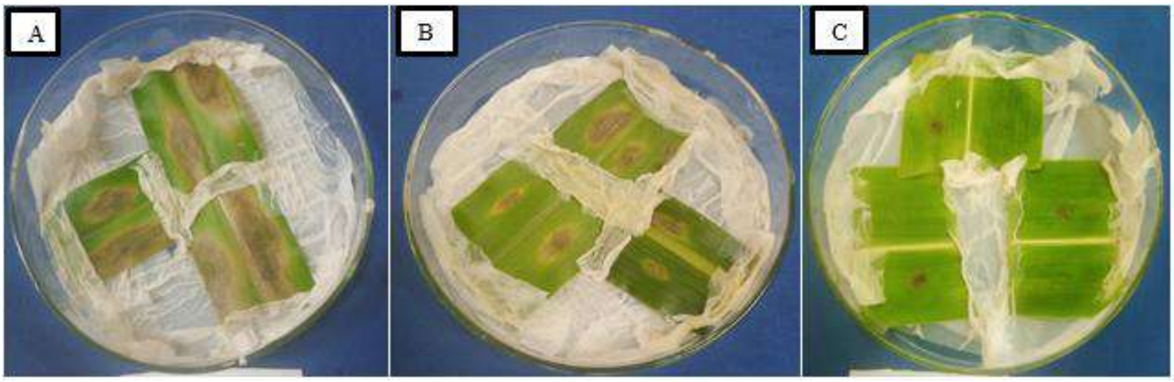

Figure 5. Reaction of sorghum cultivars against *Colletotrichum sublineolum* in the laboratory. A = highly susceptible (Adelle). B=moderately resistant (Melkam); C=resistant (Tilahun)

Intact Leaf Assay

Host Response to Sorghum Anthracnose Disease under Net House Conditions

Based on the finding of this study, significant ($p<0.0001$) differences were observed among sorghum cultivars response to *Colletotrichum sublineolum* isolates under net house conditions (Table 9). The sorghum cultivars were divided into different disease reaction types based on their disease severity score which ranged from 1.00 to 8.33. The reaction of sorghum cultivars against a mixture of *Colletotrichum sublineolum* isolates under net house conditions is presented in Figure 6. Anthracnose symptoms were observed in all cultivars except Tilahun and Abshir that were highly resistant to the disease. Three cultivars, namely Seredo, Dibaba, and Adelle, with disease severity scores of 8.33, 8.11, and 8.00, respectively were classified as highly susceptible. Around seven cultivars, including the universally susceptible check (BTX-623), were found to be susceptible to the disease. Eight cultivars, including the resistance check (ETSL101173) and the moderate resistant check (Bonsa), had moderately resistant reactions. On the other hand, Gobiye, Dekeba, and Argity cultivars with disease severity scores of 3.89, 3.89, and 2.89, respectively were resistant to anthracnose disease.

Table 9. Average disease severity scores and types of disease reactions on some sorghum cultivars against *Colletotrichum sublineolum* under net house conditions

Cultivar	Severity (1-9)	Reaction type*
Abshir	1.00^{j}	HR
Adelle	8.00^{abc}	HS
Argity	2.89^{i}	R
Baji	4.22^{hi}	MR
Berhan	6.33^{bcdef}	S
Birmash	5.11^{efgh}	MR
Bonsa	5.56^{defgh}	MR
BTX-623	6.56^{abcde}	S
Chiro	6.78^{abcd}	S
Dagim	6.11^{defg}	S
Dibaba	8.11^{ab}	HS
Dekeba	3.89^{hi}	R
ETSL101173	4.67^{fghi}	MR
Gambella-1107	6.22^{cdef}	S
Gobiye	3.89^{hi}	R
Jiru	7.67^{abcde}	S
Macia	4.56^{fghi}	MR
Meko	6.11^{defg}	S
Melkam	4.33^{ghi}	MR
Seredo	8.33^{a}	HS
Teshale	5.22^{efgh}	MR
Tilahun	1.00^{j}	HR
76Ti#23	4.11^{hi}	MR
CV (%)	11.26	
LSD	1.86	

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.

CV (%) = coefficient of variation; LSD=Least Significant Difference

Mean values with the same letter within a column are not significantly different from each other.

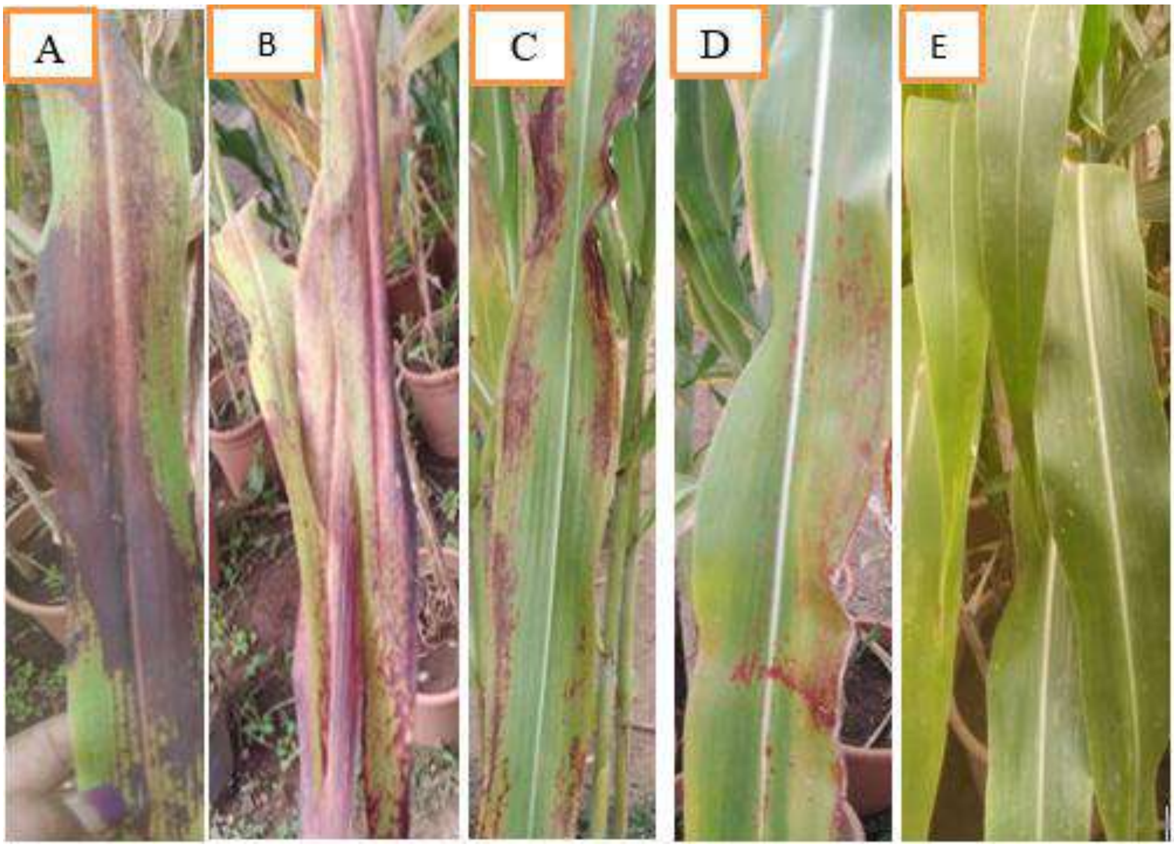

Figure 6. **Reaction of sorghum cultivars against a mixture of *Colletotrichum sublineolum* isolates under net house conditions. A and B = highly susceptible; C=susceptible; D= moderately resistant; and E=resistant**

Percent severity index and area under disease progress curve (AUDPC) of Anthracnose

The percent severity index and area under disease progress curve of anthracnose disease on sorghum cultivars grown under net house conditions is presented in Table 10. There was significant variations ($p<0.0001$) in percent severity index within the sorghum cultivars tested. The final percent severity index (FPSI) value for anthracnose disease ranged from 11.11% to 92.59%. The cultivar Seredo had the highest FPSI value (92.59%) although not significantly different from FPSI values of Adelle, BTX-623, Chiro, Dibaba, Jiru and Seredo. On the other hand, cultivars Tilahun and Abshir had significantly ($P<0.0001$) lower FPSI value (11.11%) compared to FPSI values of other genotypes.

The area under the disease progress curve showed a highly significant ($p<0.0001$) difference between the tested sorghum genotypes. The AUDPC values of the cultivars tested ranged from 311.11%-days to 1597.77 %-days. The highest AUDPC value (1598.77 %-days) was computed for cultivar Dibaba, though not significantly different from AUDPC values of Adelle, BTX623, Chiro, Jiru, and Seredo cultivars. On the other hand, significantly lower AUDPC value (311.11%-day) was observed on cultivars Tilahun and Abshir compared to the values of the other sorghum cultivars (Table 8).

Table 10. **The mean percent severity index (PSI) and area under progress curve (AUDPC) of *Colletotrichum sublineolum* on sorghum cultivars under net house conditions.**

Cultivar	IPSI (%)*	FPSI (%)*	AUDPC (%-days)*
Abshir	11.11[c]	11.11[j]	311.11[i]
Adelle	24.69[a]	88.89[abc]	1564.20[a]
Argity	11.11c	32.10[i]	566.05[hi]
Baji	16.05[bc]	46.91[hi]	980.86[defg]
Berhan	18.52[abc]	70.37[bcdef]	1175.61[bcde]
Birmash	11.11[c]	56.79[efgh]	946.30[defg]
Bonsa	20.99[ab]	61.73[efgh]	1162.75[bcdef]
BTX-623	18.52 [abc]	72.84[abcde]	1270.37[abcd]
Chiro	24.69[a]	75.31[abcde]	1421.60[ab]
Dagim	13.58[bc]	67.90[defg]	1158.02[bcdef]
Dibaba	20.99[ab]	90.12[ab]	1598.77[a]
Dekeba	11.11[c]	43.21[hi]	795.06[gh]
ETSL101173	14.81[bc]	51.85[fghi]	959.26[defg]
Gambella-1107	13.58[bc]	69.14[cdef]	1153.70[bcdef]
Gobiye	11.11[c]	43.21[hi]	786.42[gh]
Jiru	17.28[abc]	85.19[abcd]	1326.54[abc]
Macia	14.81[bc]	50.62[fghi]	989.51[cdefg]
Meko	11.11[c]	67.90[defg]	1088.89[bcdefg]
Melkam	11.11[c]	48.15[ghi]	829.63[fgh]
Seredo	18.52[abc]	92.59[a]	1529.63[a]
Teshale	13.58[bc]	58.03[efgh]	993.83[cdefg]
Tilahun	11.11[c]	11.11[j]	311.11[i]
76Ti#23	17.29[abc]	45.68[hi]	885.80[efgh]
CV (%)	**15.79**	**11.26**	**10.45**
LSD	**7.70**	**20.62**	**339.92**

AUDPC= Area Under Disease Progress Curve; IPSI=initial percent severity index, FPSI=Final percent severity index; CV (%) =coefficient of variation. LSD= Least Significant Difference.
*The mean values in a column that have the same letters are not significantly different from one another.

DISCUSSION

Sorghum is an important cereal crop supporting the livelihood of millions of smallholder farmers in Ethiopia. However, its productivity is constrained by biotic and abiotic factors. Sorghum anthracnose (*Colletotrichum sublineolum*) is one of the most serious diseases affecting sorghum production in Ethiopia in general and East Hararghe in particular. Due to the variable nature of the pathogen population, it is critical to periodically study variability in the pathogen population and screen cultivars against the pathogen to devise a sound management strategy for the disease. The result of this study revealed that there was variation in cultural and morphological characteristics of *Colletotrichum sublineolum* isolates collected from East Hararghe. The isolates showed variation in colony colors, margins, and elevations.

Most of the isolates had gray to light gray colony color on the upper surface. In general, seven different reverse side colony colors of *C. sublineolum* isolates were observed in this study. Similarly, Aragaw et al. (2023) reported differences in cultural and morphological characteristics of *C. sublineolum* isolates obtained from Eastern Ethiopia. Tsedaley et al. (2016) also reported variability in the colony characteristics of *C. sublineolum* isolates collected from south western and western Ethiopia. The colonies had whitish gray and yellow to purple gray color on the upper sideds of the petri dishes and goldenrod, brownish, purple grayish, and whitish colors on the reverse side of the petri dishes. In another study by Were and Ochuodho (2012), The mycelial color of all *C. sublineolum* isolates was gray, but with various color intensities on upper and rever side of culture plate.

The mean colony radial growth of *Colletotrichum sublineolum* isolates obtained in this study varied significantly and ranged from 14.50 mm to 42.33 mm. Significant differences in radial growth of *C. sublineolum* isolates was also reported by Chala et al. (2011). Zanette et al. (2009) reported *C. sublineolum* isolates with varied radial growth. The variations in radial colony growth of the *C. sublineolum* isolates could be due to the pathogen's genetic diversity.

The conidial size of *C. sublineolum* isolates varied significantly and the mean conidial length and width ranged from 4.97 to 25.74 µm and 2.44 to 4.07 µm, respectively. Significant variations among the *C. sublineolum* isolates in terms of conidia width and length was reported by Mekonen et al. (2024). Two different types of conidial shape, falcate and oval were produced by *C. sublineolum* isolates in the present study with the majority of isolates having oval-shaped conidia. Falcate and oval type of conidia of *C. sublineolum* was also reported by Souza-Paccola et al. (2003) and Thomas and Frederiksen (1995). Chala et al. (2011) also reported morphological differences in the conidial shape of *C. sublineolum* isolates. Zanette et al. (2009) reported variation in conidial morphology of *C. sublineolum* isolates and explained that the variation might be due to pathogen's attempt to overcome panicle resistance to infection. The variations in cultural and morphological characteristics of *C. sublineolum* isolates in the present study might be related to the pathogen's diversity, environment and sorghum genotypes.

The result of the virulence test showed variation in virulence of *C. sublineolum* isolates tested on reference sorghum sets. This implies the presence of different pathotypes of *C. sublineolum* infecting sorghum. Based on the finding, isolates HA7A, HA3A, HA5A, KD8C, and GM9B had higher levels of virulence. Overall, the isolates collected from sorghum growing fields in Haramaya district had higher virulence response than those isolates from the other locations in East Hararghe. High anthracnose severity from Haramaya district was also reported previously (Aragaw et al., 2019). Differences in virulence and aggressiveness in *C. sublineolum* isolates was also previously reported from Ethiopia (Mekonen et al., 2024; Tsedaley et al., 2021). The variations in virulence among *C. sublineolum* isolates could be due to the genetic variation in the pathogen population, genotypes used for the test and the difference in environmental variables in the areas from which the isolates were collected.

Although different management options including resistant varieties, crop rotation, removal of crop residue and chemicals are available for managing sorghum anthracnose, the use of resistant varieties is cost effective, and environmental friendly strategy for the management of sorghum anthracnose (Abreha et al., 2021). Hence, screening of sorghum germplasm against anthracnose disease is crucial to look for source of resistance for the disease. The result of the screening test in this study revealed that there was significant variation among sorghum cultivars' reaction to *C. sublineolum.* Variation in sorghum genotypes reaction to anthracnose disease was also reported previously (Chala and Tronsmo, 2012; Prom et al., 2012); Cuevas et al., 2014 and Cuevas et al., 2016; Koima et al., 2023). Disease severity and the area under disease progress curve varied amongst the tested sorghum cultivars. The majority of the cultivars screened were either susceptible or moderately resistant to the disease with relatively few cultivars showing resistant responses. Different responses to the anthracnose disease were observed under both net house and laboratory conditions in five cultivars (Berhan, Gobiye, Meko, Seredo, and Tilahun) and the moderately resistant check Bonsa. However, Dekeba, Tilahun, Abshir, and Argity showed a highly resistant response to anthracnose disease under both conditions. Erpelding (2010) documented very susceptible, susceptible, and resistant responses of sorghum accessions to *C. sublineolum* isolates. Prom et al. (2016) found that all sorghum lines except one were susceptible when inoculated with a mixture of *C. sublineolum* isolates in detached leaf assay. The variation in responses of the sorghum genotypes tested for anthracnose disease in the present study might indicate the presence of genetic variation for host resistance.

CONCLUSIONS

There was variation in the morphological and cultural characteristics as well as the virulence level of the *C. sublinelum* isolates collected from East Hararghe. Sorghum genotypes screened in this study varied in their reaction to anthracnose disease. Four sorghum cultivars, namely Dekeba, Tilahun, Abshir, and Argity were resistant to *C. sublineolum* under net house and laboratory conditions. The finding of this study would serve as baseline information for the national breeding program for breeding for sorghum anthracnose resistance. Conducting variability studies of pathogen populations with molecular tools would be important to overcome limitations associated with morphological pathogen variability studies. To come up with a more concrete conclusions about the reactions of the genotypes tested for anthracnose disease, repeating the experiment under field condition would be of paramount importance.

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Original Research Article||

Pesticide use practice and implications on farmer's health: the case of Shebedino district, Sidama Region, Ethiopia

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Abstract

The current need to produce marketable vegetables entails increasing pressure to use agricultural pesticides. Shebedino district of Sidama region is known for its agricultural crop production and farmers use agrochemicals, mainly for the control of pests. However, information on pesticide usage practice and human health problems associated with inappropriate applications of pesticides were lacking. The present study, was therefore, aimed to assess the major types of pesticides used in the study area, examine farmer's attitudes about pesticides, and evaluate the health problems associated with pesticide use in Shebedino district of Sidama region, Ethiopia. A community-based cross-sectional survey was conducted to assess farmers' pesticide handling practice and associated risks in the study area. Farmers who use pesticides were purposively selected for the interview. The result showed that the extent of pesticide utilization in the study area has been increasing, but farmers' knowledge on pesticide handling and the risk awareness were very low. The results revealed that none of the interviewed farmers in the district received training on the proper use and storage of pesticides. A total of seventeen different pesticide were used by the farmers, of which mancozeb was the dominant fungicide used by all farmers. Nearly all the farmers apply pesticides through spraying, however, except some head covering and handkerchiefs, farmers/ applicators never use pesticides protective equipment while spraying. Headache (87.65%), skin rash (49.38%) dizziness (48.15%) and blurred vision (43.21%) were among the health problems reported by pesticide users. The finding showed that there is high risk of farmer's pesticide exposure in the study area, the pesticide residues may accumulate in the food products (vegetables) and cause a wider public health risks. Intervention through awareness creation and training on appropriate pesticide handling measures are required to reduce the potential health and environmental hazards in the district.

Key words: Health problems, Knowledge, Pesticide handling, Toxicity symptoms, Unsafe practices

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INTRODUCTION

Pesticides are considered as a vital component of modern farming, playing a major role in maintaining high agricultural productivity (Srivastav, 2020). If applied carefully, pesticides can contribute to increased agricultural productivity. Without pesticide application, the crop yield will be reduced significantly due to insect damage, weed infestations and plant diseases (Jansen and Dubois, 2014). The report by Tudi et al. (2021) showed that pesticide utilization allows farmers to avoid 78, 54 and 32% fruits, vegetables and cereal yield losses, respectively. Pesticides are easy to apply, reduce the cost of production and are sometimes the only option for pest management (Chala, 2022). However, extensive applications of pesticide like in the sub-Sahara African region, including Ethiopia, may lead to a serious environmental degradation and various health problems (Loha et al., 2018).

Agricultural pesticides are introduced to Ethiopia in the 1960's when various types of agrochemicals were imported by private and public companies and since then, their use has been increasing rapidly (MOA, 2013). Currently, the need to feed the growing population of the country and the growing interest to produce exportable volumes of agricultural product to access the global market entail an increasing pressure to intensify agriculture and use chemical pesticides. For instance, during the 2020/21 cropping season, about 6.85 million smallholders applied pesticides on an estimated crop area of 4,456,912 ha (MOA, 2021). The report showed only the treated area, but not the frequency of pesticide application which would be high, especially in vegetable growing areas. The growth of the agricultural sector in Ethiopia, partly associated with intensive application of pesticides. In our country, there is increasing trends in the use of pesticides; however, there is lack of regulatory mechanisms that reduce the side effects on human health and the environment (FDRE, 2018). Selection of the appropriate pesticide type and amount, mixing and application time are vital to enhance the efficiency and effectives of the pesticide used, and minimize environmental risks (Van et al., 2020). However, several reports showed the misuse of pesticides, and inappropriate storage and disposal of empty pesticide containers in Ethiopia (Mengistie et al., 2017; Mergia et al., 2021). Insufficient knowledge regarding safe and proper pesticide handling by farmers in Ethiopia has led to a serious health and environmental problems (Mergia et al., 2021). Indiscriminate and inappropriate use of pesticides results in illness and poisoning of individuals, as well as contamination of the environment (Mengistie et al., 2017). About 17 different pesticide residues including six banned ones were reported from the study area (Feleke and Belete, 2025). The report showed the potential risks of river and underground water contaminations by the pesticide residues. Recent studies on pesticide residues in Ethiopia also revealed the existence of different health and environmental problems associated with mismanagement of pesticides (Abaineh et al., 2024; Loha et al., 2024).

Vegetable production in Ethiopia, including in Sidama region, is highly dependent on the application of pesticides, and the presence of pesticide residues in fruits, vegetables, and groundwater has been reported in these areas (Mutengwe et al., 2016; Loha et al., 2020). Recent study around Hawassa city, Sidama region, indicates that farmers lack knowledge about proper application and storage of pesticides (Alshalati, 2021). Only few researches on the subject have been reported from the region so far (Gesesew et al., 2016 and Alshalati, 2021). Further studies on the extent of pesticide use, protective methods and pesticide use associated health problems are needed to design more effective methods that reduce the exposure to pesticide, and enhance farmers to adopt sustainable agricultural practices. The present study was therefore, conducted to assess the major types of pesticides used, farmers' attitude and awareness about pesticide management, identify and evaluate the farmers' health problems related to pesticide use in Shebedino district of Sidama region, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in Shebedino, one of vegetable producing districts of Sidama region, Ethiopia. Shebedino is found at 6^0 46′ to 7^0 45′ N and 39^0 34′ to 39^0 53′ E, 30 km from Hawassa city, capital of the region. Land features of the district include plains and plateaus with varying hills and altitudes. The area has annual temperature, elevation and rainfall ranging from 16 to 25 ° C, 1500 to 3000 m and 800 to 1600 mm, respectively (BoFED, 2015). Farmers in the district produce different types of vegetables such as tomato, cabbage, potato, and pepper, to supply to many market centers in the region. Site map of the study area is shown in Figure 1.

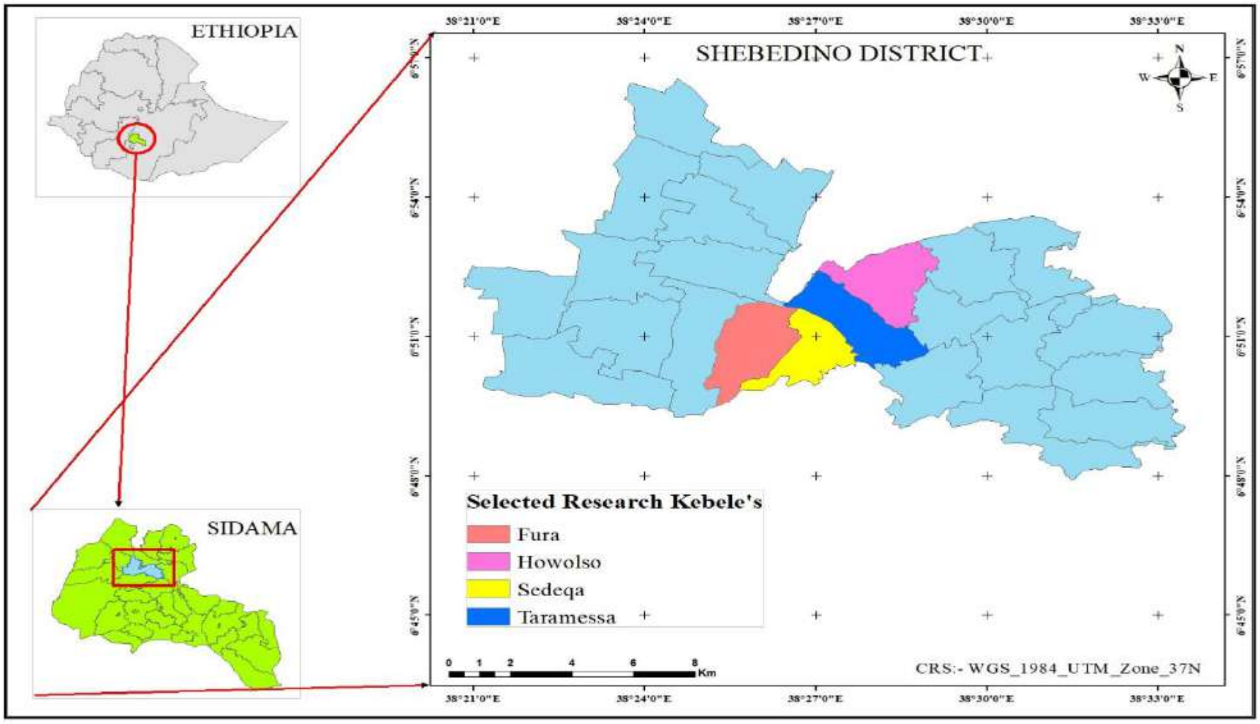

Figure 1. Location map of the study area

Study Methodology

Purposive sampling technique was employed to identify sample households for the present study. Four intensive vegetable producing kebeles (Fura, Howolso, Sedeqa and Taramessa) and 30 households from each kebele, a total of 120 sample households were selected intentionally. Farmland size, type of crop produce and management practices was the major selection criteria. The selected households were those having more than at least 0.5 ha of farmland, cultivated the farm for more than two years and produce mainly vegetables with the use of chemical pesticides. It was assumed that an increase in the frequency of cultivation increased the frequency of pesticide use per unit area per year (Maurya et al., 2019). The study was conducted from March to June, 2023.

The required information was collected using semi structured and pretested questionnaire through face-to-face interviews using the Kobo Toolbox application. Prior to data collection, information about the aim of the study and the confidentiality of the data gathered were communicated to the respondents and their agreement was confirmed. All vegetable producing farmers included in the study were those who have irrigation facilities and had more than two years of experience in pesticide utilization. The data collected includes farm size, income, land tenure situation, type of vegetable they produce and extent of pesticides application, vegetable production experience, etc. Basic information on frequently used pesticides and their source, methods of pesticide application and follow up of instructions, pesticide storage and empty container disposal mechanism, safety measures, perception on pesticide use, awareness of farmers on the effect of improper pesticide application and associated risks were collected. Furthermore, focus group discussions were conducted with development agents and key informants such as district agriculture bureau officers, pesticide retailers and environmental health officers. Interviews were also conducted with randomly selected pesticide applicators who have been hired by farmers at the time of pesticide application.

Data Analysis

The collected data were cleared and entered in the MS-Excel spreadsheet and analyzed using version 29 of SPSS (2022). The results were presented in descriptive statistics such as frequencies, and percentages for specific variables, and as means for continuous variables.

RESULTS AND DISCUSSION

Characteristics of the Farmer Households

The basic socio-demographic information of selected households was presented under table 1. Most of the farmers (96.67%) involved in the present study were males (Table 1). The dominance of males over females might be associated with the culture and nature of work as men are usually involved in pesticide handling than women (Mrema et al., 2017). The gender ratio presented in this study was in agreement with the previous report by Zyoud et al. (2010). It was revealed that most of the respondents, (86.67%) were in the age between 24–64 years. The decrease in the number of farmers with younger and older age might be due to lack of interest and labor demanding nature of agricultural practices. The tendency to migrate in to the nearby cities for better job and payment might be the other reasons for limited participation of youth in vegetable production practices (May et al., 2018).

Table 1. Socio-demographic characteristics of the sample farmers in the study area

Socioeconomic indicators	Categories	Frequency	Percentage (%)
Sex	Male	116	96.67
	Female	4	3.33
Marital status	Married	111	92.50
	Single	9	7.50
	widowed	-	-
	Divorced		
Educational status	Illiterate	6	5.00
	Formal education	79	65.83
	Primary	28	23.33
	Secondary	7	5.83
	Certificate	-	-
	Diploma/Degree	-	-
Age	≤15		
	15-24	12	10.00
	24-64	104	86.67
	≥64	4	3.33
Farm Size	<1 ha	110	91.67
	>1ha	10	8.33
Pesticide use (last 5 years)	Increasing	120	100
	constant		
Farming Experience	2-4	24	20.00
	5-10	89	74.17
	>10	7	5.83
Professional training	Yes	-	
	No	120	100
Source of income	Agriculture	113	94.17
	Other	7	5.83

Regarding the education of the farmers, 65.83% of them had basic education out of which 23.33% primary and 5.83% secondary education. Majority (74.17%) of the farmers who participated in the assessment had 5-10 years of vegetable production experience and agriculture was the only means of income for 94.17% of the farmers (Table 1). Vegetables were the most commonly grown agricultural products in the study area. Among them, tomatoes and cabbage were the most common ones followed by potatoes. Most of the farmers produce both tomatoes and cabbage for consumption as well as for local markets.

The present study showed that the extent of chemical pesticide utilization in the district has been increasing for the last five years, but none of the farmers received training on the use and safe handling of pesticides during the same period. This might be due to unavailability of a relatively vigilant institution like the Ethiopian Horticultural Producer and Exporters Association (EHPEA) and others that provide training to farmers and agriculture extension workers. Previous studies on pesticide utilization in Ethiopia showed that more than 80% of individuals who spray pesticides had not received any training on pesticide utilization and management practice (Negatu et al., 2016; Agmas and Adugna, 2020). The present finding showed an increase in pesticide use, as compared to previous estimates in the various farming systems in Ethiopia. It appeared to be 13-fold higher than in case for small scale individual farmers in Ethiopia (Pretty and Pervez, 2015). The increased use of pesticides in combination with the generally poor pesticide handling and management practices in this study could potentially lead to serious human and environmental risks.

Knowledge, Attitudes and Understanding towards Pesticide among Farmers

The farmers' level of knowledge on pesticide, routes of exposure, impact on the environment, health effects of pesticide and awareness of pesticide policy and code of practice are summarized in table 2. Regarding protective equipment while they were spraying pesticides, 40 (33.33%) used normal clothes, 46 (38.33%) used cotton overalls. Only a small proportion of the surveyed farmers, farm workers and pesticide applicators utilized pesticide protective Equipment (PPE). Except for the use of some sort of head covering and handkerchiefs, there was no complete PPE used by any of applicators in the study area, mostly exposing their face, hands, palms and their fingers (Table 2).

Previous surveys in Ethiopia indicated that personal protection was not always provided and not always commonly used during application of pesticides (Mergia et al, 2021). Alshalati (2021) reported that, in Sidama region Tulla kebele, almost all the study participants (93.15%) did not use any means of pesticide protective equipment while mixing and applying pesticides and 6.85% of the respondents reported the use of gloves only. Even if 49 (40.83%) of the respondents indicated that they could read and understand labels on pesticide containers, only 21(17.50%) could understand and follow instructions. Some of them also revealed that they usually buy pesticides which have no labels on the containers. Reading the label and using scaled equipment are important as to adhere to the recommended amount of pesticides, which can result in very high exposures if used over the recommended amount or might result in pesticide resistance if used at rates below the recommended utilization. Other studies showed similar figures with only 27 and 31% of respondents in the central eastern and Zeway part of Ethiopia respectively read pesticide labels (Negatu et al., 2016; Mergia et al., 2021*)*. It has been observed that a lever-operated knapsack sprayer was the only type of sprayer used by small-holder farmers in the district. The present finding showed that 67.50% of vegetable farmers in the area mix the different pesticides while 32.50% of them never use mixed pesticides (Table 2).

Table 2. Farmers' Knowledge, Attitude, and Understanding of Pesticides Use (n = 120)

Variable	Frequency	Percentage (%)
Do you wear protective clothing when applying pesticides?		
Yes	46	38.33
No	40	33.33
Yes Sometimes	34	28.33
At what time during the day you apply the pesticides?		
Morning	37	30.83
Middle day	25	20.83
Afternoon	56	46.67
Evening	2	1.67
Do you read and understand pesticides label		
Yes sometimes	49	40.83
Yes always	43	35.83
No	28	23.33
Which basic information do you check during pesticide purchase?		
Name of pesticide	27	22.50
Expiry date	24	20.00
Price	21	17.50
Type pest control/kill	21	17.50
Rate of application	21	17.50
Did you follow the instruction that you read on the pesticide container in application?		
Yes always	21	17.50
Yes some time follow	34	28.33
No	65	54.17
Do you mix pesticides?		
Yes	81	67.50
No	39	32.50
Do you rotate pesticides?		
Yes	102	85.00
No	18	15.00

As explained by the farmers during the group discussion, those farmers who mix the different pesticides spray their farms during the production time less frequently as compared to those who never mix the pesticides. This might be due to the different mode of action and target pests of the pesticides that enable the farmers for a better control of the pests. In agreement with the present study, Van et al. (2020) explained that selecting appropriate sprayer and application time of pesticide plays a key role for effective control of the pests; minimize the adverse effects on crops, the environment and human health from pesticide residues.

Information for efficient utilization pesticides are important, particularly for small-holder farmers, however, none of pesticide importers employed technical employees at district or farm level for such purposes. As shown in figure 2, farmers in the district get information on use and pesticide storage from development agents, neighbors and retailers who have limited knowledge on agro-chemical utilization. Previous report revealed the existence of big difference between experts and beginners in the way they perceive, remember and express their observations through the language they use (Kim et al., 2011). This finding was in agreement with the finding of

Brhane et al. (2017) who reported that 57.2% of the farmers seek information from woreda agricultural extension officers.

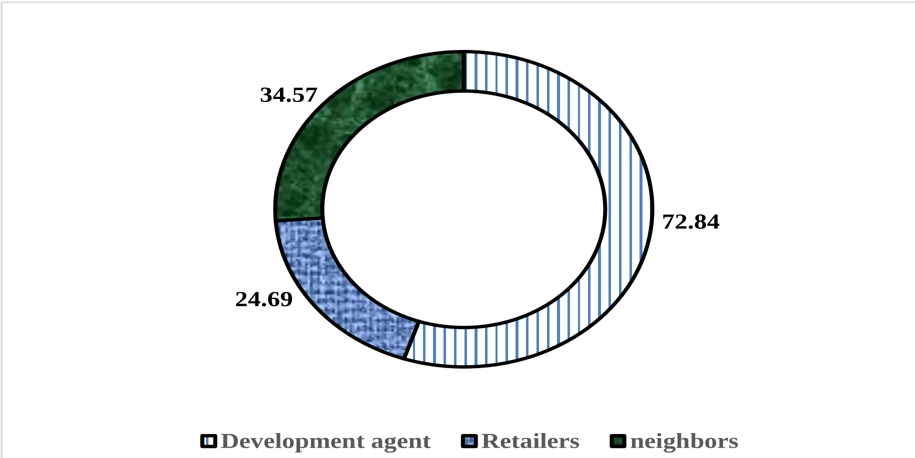

Figure 2. Source of information about use and storage of pesticide

Hazardous Impacts of Pesticides on Human Health

The present study regarding health problems after pesticide application showed that the majority of the pesticide applicators feel discomfort after spraying the pesticide. The feeling of headache (87.50%), skin rashes (49.17%), dizziness (48.33%), blurred vision (43.33%) and difficulty in breathing (30.00%) were also reported as major health problem symptoms by the applicators in the district (Figure 3). Similar studies on pesticide associated health problems revealed that 68% pesticide user farmers in Ethiopia reported some sort of unidentified illness, the rest felt head ache (37%), skin irritation (28%) vomiting (8%) and other symptoms after applying pesticides (Mojo and Zemedu, 2022). A study conducted elsewhere in Ethiopia also showed an increased risk of respiratory system diseases related to young workers with increased exposure to pesticides (Negatu et al., 2016). Moreover, regular deaths of farmers and blurred vision because of unintentional exposure or misuse of pesticides were also reported in Ethiopia and Ghana (Loha et al., 2018). The study of Abaineh et al. (2024) confirmed that pesticides are among the top ten human health problems in the northern part of, Ethiopia, and pesticide-related cases are reported to the Zonal and the Regional Health offices monthly.

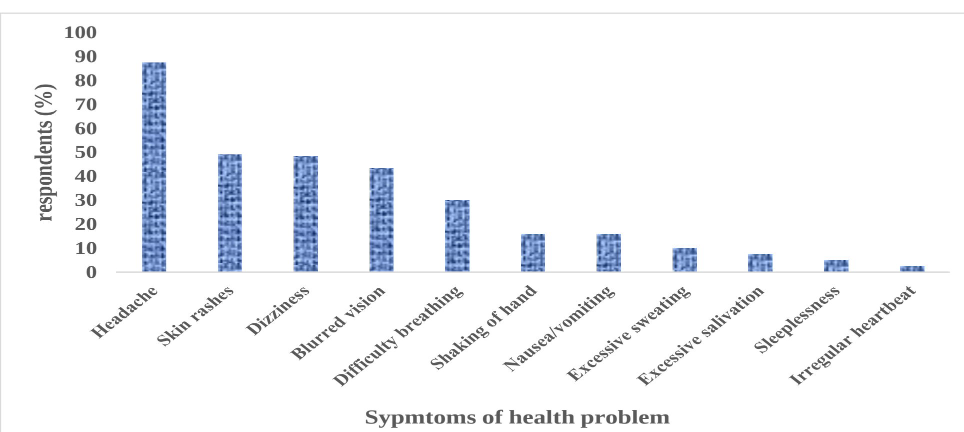

Figure 3. Symptoms of pesticide exposure reported by farmers in the district (multiple answers were possible)

Pesticides Used by Small-holding Vegetable Producers

The present investigation showed that different pesticides are used by vegetable producers in the district. A total of 17 pesticides were found to be used in the district. The pesticides are presented in table 3 based on the world health organization (WHO), acute toxicity hazard class (WHO, 2010). Most of the pesticides used in the study area were belongs to the WHO toxicity Class II (moderately hazardous), followed by Class-U (unlikely to present acute hazards in normal use) and Class III (slightly hazardous). Extremely hazardous (Ia) or highly hazardous (Ib) classified pesticides were not reported by any of the farmers. The primary WHO Class II pesticides reported by small-scale farmers in the study area included metalaxyl, profenofos, dimethoate and Chlorpyrifos. Among Class-U pesticides, mancozeb were the most reported pesticides. The use of herbicides such as propanil and 2, 4-D were also reported by some farmers. Insecticides and fungicides have been used by 95 and 90% of farmers, respectively, in the district. In related to this study, Abaineh et al. (2024), reported that farmers have been using various types of pesticides to control weeds and pests of crops with different spraying frequencies in Ethiopia.

Out of the pesticides applied on vegetable crops of the study area, Metalaxyl and Dimethoate will persist up to 30 days, and Chloropyrifos and Diazinon will remain in the environment for a range of 30–100 days (Rahman et al., 2020). Since the vegetables are consumed by the farmers or presented to the market a few days after spraying, there will be a high chance of the pesticide to be consumed by the consumers. Similar studies in Fogera District, Ethiopia confirmed that all the farmers supplied their cultivated crops from the first day after pesticide spray (Abaineh et al., 2024).

Table 3. Pesticide use in surveyed farms

No	Common name	Chemical class	WHO	Type
1	Metalaxyl	Phenyl amide	II	F
2	Karate	Synthetic pyrethroid	III	I
3	Dimethoate	Organophosphate	II	I
4	Mancozeb	Dithiocarbamate	U	F
5	Copperoxichloride	Inorganic	II	F
6	Ethioprofos	Organophosphate ester	II	I & N
7	Dimethomorph	Morpholine	III	F
8	Profenofos	Organophosphate	II	I
9	Malathion	Organophosphate	III	I
10	Cymoxanil	Cyano acetamide oxime	II	F
11	Chlorpyrifos	Organophosphate	II	I
12	Abamectin	Bio-origin	NL	I
13	Propiconazole	Triazole	II	F
14	Sulfur	Inorganic	III	F
15	Kocide	Oxime + inorganic	II	F
16	Diazinon	Organophosphate	II	I
17	Copper	Inorganic	III	F

NL = not listed; II = moderately hazardous, III = slightly hazardous, U = unlikely to present acute hazard, I=Insecticides, F= Fungicides, N=Nematicides.

Pesticides Use, Storage and Disposal

With regard to pesticide use practices, storage and disposal mechanisms of empty containers; the finding revealed that most farmers in the study area had inappropriate practices. Figure 4 showed that most respondents were not concerned about over-dosing; 41.98% of them applied the leftover solution to crops repeatedly. Others disposed of the solution in the field and water bodies which could be another source of environmental pollution. Similarly, Agmas and Adugna (2020) reported that, 72% of pesticide users responded that they apply the leftover solution to crops repeatedly. Others (28%) disposed of the solution in the field and in the water body.

Regarding farmers pesticide storage in the district, our investigation revealed that a significant number of respondents (44.4%) kept pesticides in their residential house (Figure 5). None of the respondents reported direct use of pesticides immediately after purchase. Our finding was similar with the report from farmers around Lake Ziway watershed, Ethiopia (Mergia et al., 2021). The majority of vegetable producing farmers (60.9%) in the North-Western part of Ethiopia reported that they stored pesticides in the living houses (Agmas and Adugna, 2020). The finding showed that even children and mothers who have no direct involvement in vegetable production practice may have direct exposure to the pesticides.

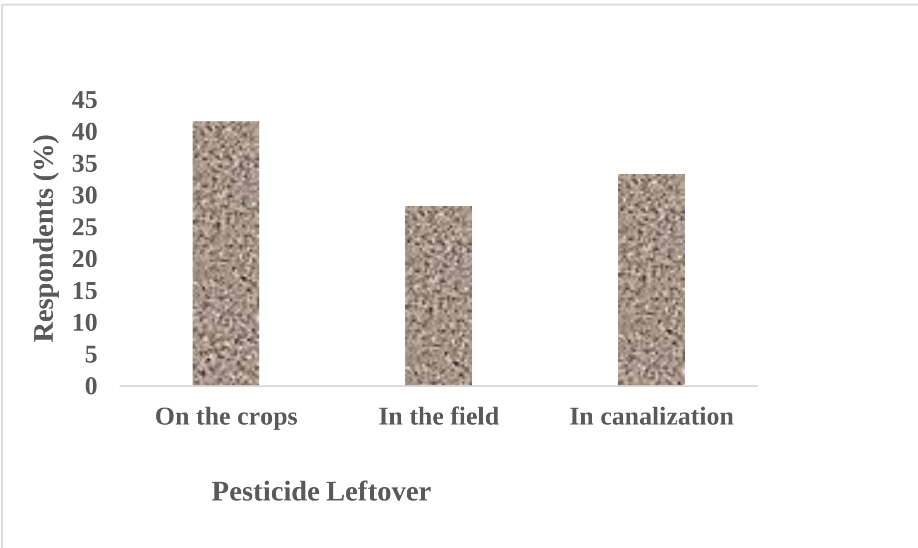

Figure 4. Pesticide leftover disposal practice in the study area

Unsafe pesticide containers disposal was also another pesticide management problem in the district. The finding showed that 42% of the households disposed the containers through burning, while more than 49% of the farmers threw the containers either on the open field or in the garbage. This indicates that, in addition to contaminating the soil and water bodies, the containers have a chance to harm the health of young children and livestock.

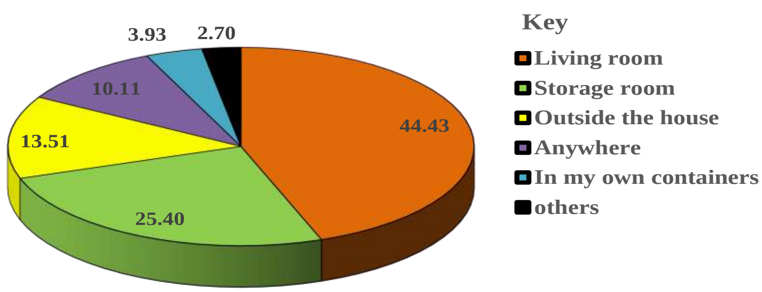

Figure 5. Pesticide Storage Practices

Likewise, Ligani (2016) reported that about 65% of the respondents hang empty pesticide containers near the farm, while Ocho et al. (2016) described that 32% of the households re-use pesticide containers for other purposes. In all cases, the expired pesticides, after spray leftovers and containers have not been properly disposed of and have inevitable environmental pollution, and human and livestock health effects. The disposal of empty pesticide containers on farmland itself and mixing the pesticides in sensitive areas were also observed. Farmer's pesticide use practices in the study area that cause human and environmental risks are presented under table 4. The practice could expose farmers and the ecosystems in general to the hazardous effects of pesticide residues (Negatu et al., 2016; Agmas and Adugna, 2020). A recent report from the study area showed the presence of ten different pesticide residues in soils taken from vegetable growing farm lands, out of which six were banned by different organizations (Feleke and Belete, 2025). However, farmers were found to underestimate the effects of highly toxic pesticides that enter into soil, water bodies during mixing and spraying, and the residues on supplied vegetables, which are the primary risk factors for consumers.

Table 4. Factors that expose environment and humans to effects of pesticide residues

No.	Variables	Frequency	Percentage (%)
1	Entry of pesticide residues into water bodies at time of washing the pesticide spraying equipment near water bodies.	53	44.17
2	Entry of pesticide residues into home at time of washing the pesticide spraying equipment near to home and bath room in the house.	30	25.00
3	Entry of pesticide residues into surrounding farm lands at time of washing the pesticide spraying equipment at yard.	62	51.67
4	Spraying with wind direction	46	38.33
5	Spraying pesticide on harvested vegetables and supplying them to consumer	116	96.67
6	Using hazardous pesticide to control pests	107	89.17
7	Knowledge gaps on side effect of pesticide among frames	104	86.42
8	Used pesticide container throwing in to open field and trash	59	49.17
9	Used pesticide container burning	51	42.50

Mixing Mancozeb with Dimethoate for cabbage and Mancozeb with Karate for tomato were the common practices in the study area (table 5). Farmers mix pesticides to save their time, and believed that mixed pesticides have a higher efficacy in pest's control. However, farmers have no information on the compatibility of differently formulated pesticides such as emulsifiers and wetting agents. Furthermore, farmers reported that they never practice the use of special tools to mix and apply pesticides on their farms. Hand-mixing increases the risk of pesticide exposure through dermal absorption or ingestion as farmers can easily carry traces of pesticides from their hands to their mouth. The present finding was in agreement with the report by Mengistie et al. (2017). A report by Ngowi et al. (2007) showed that interactions between the different pesticides or with water minerals can reduce its efficiency or make it become more toxic. Place of mixing on the other hand seemed to play a role for environmental pollution. Most farmers mix pesticides near water bodies, or in the yard, which can easily contaminate the ecosystem.

Table 5. Pesticide mixtures by small-holder farmers in the study area

No.	Pesticides	Types	Crop
1	Mancozeb + Dimethoate	Fungicides + insecticide	On Cabbage
2	Mancozeb + Abamectin	Fungicides + insecticide	On Tomato
3	Mancozeb + Karate	Fungicides + insecticide	On Cabbage
4	Boss + Abamectin	Insecticide + Insecticide	On Tomato
5	Dimethoate + Helerate	Insecticide + Insecticide	On Cabbage
6	Redomil + Tutan	Fungicide + Insecticide	On Tomato
7	Sulfur + Best	Organic + Insecticide	On Tomato
8	Redomil + Karate	Fungicides + insecticide	On Tomato
9	Boss + Karate	Insecticide + Insecticide	On Tomato
10	Kocide + Dimethoate	Fungicides + insecticide	On Cabbage
11	Mancoze + Copper	Fungicide + Fungicide	On Cabbage
12	Karate + Kocide	Insecticide + Fungicide	On Tomato
13	Coppeerchloroxide + Tutan	Inorganic compound + Insecticide	On Cabbage
14	Mancozeb + Helerate	Fungicide + Insecticide	On Tomato
15	Copper chloroxide + Helerate	Inorganic compound + Insecticide	On Cabbage

CONCLUSIONS

The pesticide use practice and its effect on farmer's health in Shebedino district of Sidama region has been assessed. The result showed that various types of pesticides are used indiscriminately by vegetable produces in the district. More than 17 different pesticides have been used in the study area, among which mancozeb was the predominant one. Improper mixing, storage and disposal mechanism of empty containers were among the major problems that expose farmers for various health hazards. Headache, skin rash, dizziness, and blurred vision were some of the symptoms of health problems associated with pesticide use in study area. Farmer's lack of awareness about proper use of pesticide and inability to read and understand the pesticides labels were observed. Absence of legal control over pesticide and knowledge gap of the farmers were also the other reasons for inappropriate management of pesticides in the study area. Pesticide applications should be restricted to trained personals, and concerned authorities need to intervene to insure proper pesticide management. The present finding is believed to serve as first-hand information for further studies on contamination of vegetables and groundwater by pesticide residues in Sidama region and beyond.

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Original Research Article||

Evaluation of Ethiopian Cassava Varieties for Quality of Cookies from Cassava-Chickpea Composite Flour

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Abstract

The use of cassava powder in bakery products, including cookies, is gaining attention in Ethiopia. However, cassava varieties grown in Ethiopia vary widely in their characteristics, which may influence their food applications that require investigation. This study aimed to evaluate the effects of cassava varieties and chickpea proportion on the quality of cookies from cassava-chickpea composite flour. The composite flour was prepared by blending powders from four cassava varieties (*Kello, Qulle, Hawassa-4, and Chichu*) with chickpea flour at 40%, 50%, and 60% composition. The findings indicated that the functional characteristics and physical properties of cookies were significantly affected by cassava varieties and chickpea proportion. Cookies formulated from Hawassa-4 cassava variety exhibited a high spread ratio. A cookie from 40% Hawassa-4 cassava and 60% chickpea was found to have better spread ratio and sensory qualities (taste, texture, flavor, and crispiness), high protein (12.85%) and fiber (5.90%) contents. Mineral analysis revealed the highest calcium content (75.90 mg/100 g) in cookies made with 40% Qulle, and the highest zinc content (2.23 mg/100 g) in those with 40% Kello. The phytate-to-calcium molar ratio (0.05–0.08) and phytate-to-zinc ratio (0.66–5.88) in all cassava-chickpea composite cookies were within acceptable limits, ensuring bioavailability of these minerals. However, the phytate-to-iron ratio exceeded the threshold of 1 in most formulations, highlighting potential challenges with iron absorption. These findings highlight the potential of cassava-chickpea composite cookies, particularly those made with 40% Hawassa-4 cassava, as a nutritionally valuable option to address protein-energy malnutrition in Ethiopia.

Key words: Cassava varieties, Chickpea proportion, Cookie, Physicochemical properties, Bioavailability of minerals

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INTRODUCTION

The utilization of locally available raw materials offers a strategic pathway to enhance food security and nutritional quality, particularly in developing countries such as Ethiopia (Owasa & Fall, 2024). This approach not only addresses immediate dietary needs but also promotes agricultural sustainability and resilience (Bozsik et al., 2022). Cookies, widely consumed across all age groups, are conventionally made from wheat flour (Ikuomola et al., 2017). However, increasing concerns over food insecurity and dietary-related health issues underscore the need for more nutritious and locally sourced alternatives. In response, this study investigates the potential of composite flours made from cassava (Manihot esculenta) and chickpea (Cicer arietinum) to develop wheat-free cookies. Beyond meeting the growing demand for gluten-free bakery products, this formulation aims to diversify food options while improving their nutritional value.

Several studies have investigated the use of cassava and chickpea flours in baked products. For instance, cassava-chickpea composite flours have been used in bread and cake formulations to enhance protein content and improve functional properties (Adebayo & Olatunde, 2020; Santos, *et al.,* 2018). Other studies have explored cassava-legume blends for snack production, revealing improved nutritional composition and acceptable sensory properties (Alamu, *et al.,* 2020; Maziya-Dixon et al., 2017). However, limited studies have specifically focused on the use of Ethiopian cassava varieties in combination with chickpea flour for cookie production. As such, this study lies in evaluating the effect of local cassava varieties and chickpea blending ratios on the quality attributes of gluten free cookies a product not yet thoroughly explored in this context.

Cassava is a root crop widely cultivated in the southern part of Ethiopia and plays an important role in local diets (Tadesse et al., 2013). The application of cassava flour is increasingly recognized in bakery products, including cookies (Bogale et al., 2022; Kebede et al., 2012). According to Senanayake *et al.* (2024), cassava powder holds significant potential as an alternative to wheat flour in baked goods due to its cost-effectiveness and local availability. Cassava powder was prepared following the method of Kebede et al. (2012) with slight modifications. The roots were peeled, sliced, sun-dried, and milled to produce cassava flour for use in the composite formulations. However, cassava varieties grown in Ethiopia exhibit distinct characteristics, influencing their suitability for various food products (Akonor *et al*., 2023). These varietal differences may affect the functional, nutritional, and sensory attributes of the final product, making it essential to evaluate their impact on cookie quality.

Chickpea, the second most important legume crop after faba beans in Ethiopia, plays a vital role in the country's agriculture and nutrition (Asfaw & Shiferaw, 2010). As a major pulse crop, it supports food security while providing a valuable source of protein (Fikre and Bekele, 2020). It plays a significant role in the country's agriculture, contributing to nutrition security and providing a valuable source of protein (Fikre and Bekele, 2020). Chickpea flour has been traditionally used in Ethiopian cuisine for dishes like *shiro* (a spiced stew), and it is also consumed as a roasted snack (Ashenafi et al., 2023). The high protein content of chickpea makes it an ideal complement to carbohydrate-rich root crops like cassava in composite flour formulations (Garske et al., 2023). Unlike traditional cookies made from wheat flour, this study explores the use of composite flours from cassava and chickpea to create wheat-free cookies, addressing the rising demand for gluten-free bakery products. Therefore, this study aims to evaluate the functional, physicochemical, and sensory properties of cookies made from composite flours of Ethiopian cassava varieties and chickpea.

MATERIALS AND METHODS

Materials

Four distinct varieties of cassava grown in Ethiopia, *Kello*, *Qulle*, *Hawassa-4*, and *Chichu*, were obtained from the Hawassa Agricultural Research Centre, Ethiopia. The cassava roots utilized were 18 months post-planting. Chickpea samples (Kabuli type, Arerti variety) were procured from the Debre Zeit Agricultural Research Centre. Other necessary ingredients for cookie preparation, including refined palm oil (Tena sunflower oil), granulated white sugar, table salt, and wheat flour (Eshet) were obtained from local supermarkets in Hawassa city, Ethiopia.

Preparation of Cassava Floor

Following the method of Kebede *et al.* (2012), cassava powder was prepared with a slight modification to the drying time, which was extended to 15 hours. Fresh cassava roots were peeled, washed, and sliced into approximately 3-5 cm thicknesses with a diameter of 10-15 mm. Then exposed to sunlight until it achieved a moisture content of 10–12%. The dried pieces were milled into flour and sieved through a 500 µm sieve and finally stored in polyethylene bags at 4°C until use.

Preparation of Chicken Floor

The chickpea flour was prepared according to Olika *et al.* (2019). The seed was manually cleaned of all foreign matter, broken grains, and other impurities. The cleaned seed was soaked for 2 hours by immersing the seeds in water (Seed: water (1:3 V/V). The soaked seeds were then dried under sunlight for 10 hours. Milling was done to dhal the chickpea partially, then the dehulled whole grains were then cleaned. Then ground into flour and sieved through sieve a 500 µm sieve size. The resulting flour was packed into polyethylene plastic bags and stored at 4 °C until required for further processing.

Formulation of Composite Flour

The composite flour was prepared by blending cassava and chickpea flours at ratios of 40:60, 50:50, and 60:40 (cassava: chickpea) for each of the four cassava varieties. These blends were designated as C1, C2, and C3, respectively. A reference control (C0) containing 100% wheat flour was also prepared for comparison, as shown in Table 1. The cassava powder and chickpea flour were weighed separately with the aid of a digital electronic balance.

Table 1. Formulation of cookie from cassava-chickpea composite flour

Sample Code	Cassava powder (%)	Chickpea flour (%)	Wheat flour (%)
C1	40	60	0
C2	50	50	0
C3	60	40	0
C0	0	0	100

Determination of Functional Properties of Cassava-chickpea Composite Flour

Determination of Swelling Power of Composite Flour

The swelling power characteristics of the cassava-chickpea composite flour were investigated following the method outlined by Kusumayanti *et al.* (2015). The swelling power (g/g) was calculated by dividing the mass of the sediment (Wsd) by the original sample weight (Ws).

Swelling (g/g) = (Wsd/Ws dry basis) Eq1

where Wsd is dried sediment mass and Ws is original sample weight.

Determination of Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

The water and oil holding capacities of cassava-chickpea composite flours and wheat flour were determined following the method outlined in reference (Aremu *et al.,* 2007).

WAC or OAC(g/mL) = [((W3-W2))/(W1)] ……………………………………… Eq2

Where: W1 is weight of sample (db), W2 is weight of test tube, and W3 is weight of test tube and sediment.

Bulk Density

Bulk density of composite flour was determined according to Oladele and Aina (2007) method. About 50 g of composite flour was put in to a 100 mL measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density was calculated as weight of the cassava-chickpea composite flour (g) divided by its volume (cm^3)

Cookie Preparation

The cookies were prepared as described by Jemziya and Mahendran (2015). The composite flour used for cookie preparation was based on cassava and chickpea flours, following the formulations described in section 2.3. Cookie dough was prepared by mixing 100 g of composite flour with 50 g of powdered sugar, 40 g of oil, 3 g of baking powder, and 0.8 g of salt. The dough was kneaded to a firm consistency, rolled out to a thickness of 5 mm, and cut into circular shapes (7.4 cm diameter) using a cookie cutter. The cookies were placed on a greased aluminum baking tray and baked in a pre-heated electric convection oven (UNOX XF043 model) at 200°C for 10 minutes. After baking, the cookies were cooled to room temperature and packed in polyethylene bag until sensory evaluation. A portion of the cookies was oven-dried at 40°C and ground into cookie powder for subsequent proximate and functional analysis.

Determination of Spread Ratio of Cookies

The cookies were selected randomly for physical analyses or spread ratios. The weight of the dough for each cookie should be consistent, 25 grams, 1 cm thickness in a baking tray, and cut into circles having 7.4 cm diameter. Baking conditions such as time, temperature, and tray placement were remaining constant, and cookies should cool for the same duration before measurement. The diameter and thickness were accurately measured by using a ruler, and the spread ratio was calculated by dividing the diameter by the thickness post-baking.

To determine the diameter of cookies, six samples were placed next to another, and the total diameter was measured accurately by using calipers. All of the cookies were rotated at an angle of 90°, and the new diameter was measured (for replication). The average of diameter was recorded. The thickness of the cookies was measured by four cookies stacking above the others and restacking four times. The average thickness was recorded. The spread ratio was calculated by dividing the diameter of cookies by the thickness of cookies (AACC, 2000).

Determination of Proximate Composition of Cookies

Moisture content

The moisture content of the cookie flour was determined according to the method of the Association of Official Analytical Chemists (AOAC, 2000). The moisture content of the cookies was calculated as follows:

$$MC\ (\%) = \left[\frac{W1-(W3-W2)}{W1}\right] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ Eq3}$$

Where: MC = moisture content, db), W1 = the weight of sample (g) before drying, W2 = the weight of the aluminum cup, and W3 = the weight of the aluminum cup plus the weight of the sample after drying.

Crude Fat

The fat content was determined using soxlet ether extraction techniques according to the standard expressed in AOAC (2000). method No. 920.39. The fat content was calculated using the formula. A five-gram sample was used for extraction.

Crud Fat (db) %= [[(W2-W1)/W] ∗100] ……………………………………….. Eq 4

Where: W1 is weight of the extraction flask (g), W2 is weight of the extraction flask + the extracted crude fat (g), and W is weight of the sample (g).

Ash Content

The sample ash content was determined according to AOAC (2000). method 923.03 by taking about 3.0 g of sample after carbonization and ignition at 500 °C for 6 h in the muffle furnace (Model 2-525, J. M. Ney furnace, Yucaipa, USA).

Ash% = ((W3-W2)/W1) * 100 …………Eq 5

Where W1 is the weight of dried cookie flour before ashing, W3 is the weight of dried cookie flour after ashing with the weight of the crucible, and W2 is the weight of the crucible.

Crude Fiber

The acid and alkaline digestion procedure was used to assess the crude fiber content in accordance with the standard method in AOAC (2000). No. 962.09. About 1.5 g of the sample was used for the determination of crude fiber.

Crude fiber % (db) = [(W2-W3)/W1]∗100..Eq 6

Where: W1 is weight of samples (g); W2 is weight of crucible and residue after drying (g).

W3 is the weight of the crucible and ash after incarnations (g).

Crude Protein

The protein content was determined following the Kjeldhl method in accordance with the standard method in AOAC (2000). About 1 g of samples were used for crude protein determination. The borate ion was titrated with a standardized 0.1N hydrochloric acid solution using Kjeldahl analyzer distillation until a light pink color was observed.

Nitrogen% = [(Vs-Vb)/10W]∗NHCl*14.01g.…Eqn (7)

Where: Vs is volume of HCl consumed during titration of sample (mL), Vb is volume of HCl consumed during titration of blank (mL), N is normality of HCl used (0.1), W is weight of sample (g), 14 is molecular weight of nitrogen

The percent of nitrogen was converted to the protein percentage as follows:

Crude protein % = %N* F ………… Eq (8)

Where: the conversion factor is 6.25

Determination Mineral Analysis (Calcium, Zinc, and Iron) of Cookies

The mineral contents of the cookie product were determined by the procedure of AOAC (2005). The extracted minerals Fe, Ca, and Zn were analyzed by atomic absorption spectrophotometer after removal of organic material by dry ashing. The residue was dissolved in dilute acid. The solution was sprayed into the flame of the atomic absorption spectrophotometer (Buck. Scientific model 210GP, UAS), and the absorption of the metal to be analyzed was measured at a specific wavelength. The stock standard solutions of minerals (Ca, Fe, and Zn) were diluted with 0.3 N HCl to concentrations that fall within the working range (0.0-00.6, mg/kg for calcium analysis; 0.0-00.6, mg/kg for zinc analysis; 0.0- 18.0 mg/kg for iron analysis).

Mineral determination: The minerals were obtained from dried ash. The ash was wetted completely with 5 mL of 6N HCl and dried on a low-temperature hot plate. A 7 mL of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boiled. The ash solution was cooled to room temperature in open air in a hood and filtered through a filter paper (What Man No. 1) into a 50-mL graduated flask. A 5 mL of 3 N HCl was added into each crucible dish and heated until the solution just boiled, cooled, and filtered into the flask. The crucible dishes were again washed three times with deionized water; the washings were filtered into the flask. A 2.5 mL of 10% lanthanum chloride solution was added into each graduated flask. Then the solution was cooled and diluted to the mark (50 mL) with deionized water. A blank was prepared by taking the same procedure as the sample.

$$\text{Mineral content (\%)} = ([(a-b) \times V])/W \times 100 \quad \text{……………………………Eq (9)}$$

Where: W = weight (g) of samples; V = volume (mL) of extract; a = concentration (μg/mL) of sample solution; b = concentration (μg/mL) of blank solution.

Determination of Anti-nutrient Analysis Contents of Cookies

HCN Contents Determination of Cookie Products

The quantification of hydrogen cyanide (HCN) levels in cookie products was conducted using Konzo Kits Protocol B2, following the methodology outlined by Bradbury *et al.* (1999). To initiate the process, a 100 mg of the sample was carefully weighed and placed on top of a buffer/enzyme paper disc, identifiable by a black spot, within a flat-bottomed plastic bottle. Next, 1.0 mL of distilled water was gently added with a plastic pipette and then thoroughly mixed. A yellow indicator paper attached to a plastic strip was introduced into the bottle, which was then sealed with a screw-capped lid to facilitate reaction. For each sample, subsequent analysis involved the careful removal of the plastic backing sheet from the indicator paper. The indicator paper was then transferred to a test tube, into which 5.0 mL of distilled water was added. The test tube was allowed to stand at room temperature for approximately 30 minutes, with occasional gentle stirring to ensure uniform distribution. The solution's absorbance was measured at 510 nm using a spectrophotometer.

The total cyanide content, expressed in parts per million (ppm), was calculated using the formula: total cyanide content (ppm) = 396 × absorbance. To ensure the reliability of the results, both positive and negative controls were included for each experimental set. The negative control involved the preparation of a solution without the inclusion of any sample, while the positive control utilized A buffer/enzyme paper disc, with a known cyanide concentration (approximately 10 ppm on a pink standard paper), to ensure accuracy and consistency in detection.

Condensed Tannins

Condensed tannin was analyzed by vanillin-HCI method of Price *et al*. (1978). The Vanilli-HCI reagent was prepared by mixing equal volume of 8% concentrated HCI in methanol and 1% Vanilli in methanol. The solution of the reagent was mixed just before use. A 0.2 g of the ground sample was placed in small conical flask. Then 10 ml of 1% concentrated HCI in methanol was added. The conical flask was capped and continuously shaken for 20 min and the content then is centrifuged at

2500 rpm for 5 minutes. About 1 ml of the supernatant was pipette in to a test tube containing 5ml of Vanillin-HCI reagent. Absorbance at 450 nm was read on spectrophotometer after 20 minutes' incubation at 30°C. A blank sample was also analyzed and its absorbance was subtracted from sample absorbance. A standard curve was prepared for catechin (0-1.2 mg/ml). Tannins content was expressed as catechin equivalent as follows:

Tannin (%) = (c×10×100)/Sw……….. Eq (10)

Where: C = concentration corresponding to the optical density, 10 = Volume of the extract (ml), Sw = Sample weight (mg)*2.10* 2.7.1 Condensed tannins

Phytate

The phytate content of the samples was determined by following the procedure mentioned by Vaintraub and Lapteva (1988). The 0.4 g of the dried sample were extracted with 10 milliliters of 0.2N HCl for one hour at ambient temperature. The mixture was then centrifuged at 3000 rpm for 30 minutes. The clear supernatant was collected for phytate estimation. To 3 milliliters of the supernatant, 2 milliliters of Wade reagent were added. The solution was then homogenized and centrifuged again at 3000 rpm for 10 minutes. The absorbance of the solution was measured at 500 nm using a UV-Vis spectrophotometer. The phytate concentration was calculated by comparing the absorbance of the sample with that of a blank, which consisted of 3 milliliters of 0.2N HCl and 2 milliliters of Wade reagent. The amount of phytic acid was determined using a phytic acid standard curve, and the results were expressed as phytic acid in µg/ml of fresh weight.

Phytic acid in µg/g = ((A_s-A_b)-Intercept)/ Slope x W ……………………………Eq (11)

Where A_s= sample absorbance, A_b= blank absorbance, W= weight of sample

A stock solution was prepared by dissolving 0.1814 grams of phytic acid in 100 milliliters of 0.2N HCl. A series of standard solutions containing 4-40 µg/ml phytic acid in 0.2N HCl were prepared from this stock solution. Three milliliters of each standard solution were pipetted into 15 milliliter centrifuge tubes, with 3 milliliters of 0.2N HCl used as a zero-level blank. To each tube, 2 milliliters of Wade reagent were added, and the solutions were mixed on a vortex mixer for 5 seconds. The mixtures were then centrifuged at 3000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 500 nm using 0.2N HCl to zero the spectrophotometer. A calibration curve (absorbance vs. concentration) was plotted using Excel, and the slope and intercept were determined from this curve.

Sensory Analysis

A hedonic sensory evaluation was conducted to assess the consumer acceptability of the cookie samples. Sensory evaluation was conducted on formulations using the Hawassa-4 cassava variety at 40%, 50%, and 60% chickpea composition. This variety demonstrated superior physical (spread ratio) and functional properties (WHC and OHC). In addition, selecting a single high-performing variety like Hawassa-4 allowed us to reduce the number of samples for sensory evaluation while still ensuring the reliability and relevance of the results. The evaluation was carried out by a panel of 50 semi-trained members drawn from the College of Agriculture, Hawassa University. Panelists were selected based on their regular consumption of cookies and their willingness to participate, ensuring they had sufficient familiarity with the product to provide informed evaluations.
The sensory test employed a nine-point hedonic scale, a standard method in consumer preference studies due to its simplicity and effectiveness in capturing degrees of liking (Addo-Preko *et al*., 2023). The scale ranged from 1 = "dislike extremely" to 9 = "like extremely," allowing panelists to express a wide range of preferences for each sensory attribute.

Samples were served three hours after baking to ensure consistency in temperature and texture. Each cookie was presented in a randomized order on plates coded with three-digit numbers to minimize bias. The formulations tested during the sensory evaluation were prepared as described in Section 2.3 and baked following the procedure outlined in Section 2.5. A cookie made from 100% wheat flour was included as the control sample for comparative purposes.

Data Analysis
Data were analyzed using one-way analysis of variance (ANOVA) in JMP Pro 14 software to determine the significance of factors at a 95% confidence level. Sensory evaluation scores from the hedonic scale were used to calculate the mean sensory scores for each sample. Differences among means were separated using Tukey's Honest Significant Difference (HSD) test. For graphical representation, data were imported and visualized using Microsoft Excel. Results were expressed as mean values ± standard deviation.

RESULTS AND DISCUSSION

Functional Properties of Cassava-Chickpea Composite Flour

The functional properties of the cassava-chickpea composite flour are presented in Table 2. The bulk density ranged from 0.405 g/cm³ to 0.505 g/cm³, with the highest recorded in the 40% *Chichu* formulation. The lowest bulk density (0.405 g/cm³) was observed for 60% *Chichu*. Bulk density affects the compactness of the dough. Higher chickpea content led to increased bulk density, indicating a potential for denser cookie dough. This aligns with findings by Apotiola and Fashakin (2013), who observed similar trends with soybean-cassava blends. This indicates that heavier weight (high bulk density) might have an impact on the final texture and chewiness of cookies.

WHC was highest in 40% Kello (2.98 g/mL) and lowest in 60% Qulle (2.56 g/mL). WHC of control sample (2.92) is resembles to the WHC of formulation with higher chick pea proportion (Table 2). Higher WHC is associated with higher protein content, as proteins have strong water-binding capabilities (Makinde & Adebile, 2018). Moisture retention is essential for achieving softer cookies, as reported by Aljobair (2022). Therefore, the enhanced WHC observed in chickpea-containing blends could improve the texture and freshness of cookies, aligning with consumer preferences for soft, moist products (Aljobair, 2022).

For oil holding capacity (OHC), 40% Qulle formulation scored the highest (2.12 g/mL) and the lowest OHC was seen in 60% Kello (1.82 g/g). OHC of control sample (2.05) is resembles to the OHC of formulation with higher chick pea proportion (Table 2). The oil holding capacity in cookie making is important as it contributes to flavor retention, mouth feel, and a richer texture (Aremu *et al*., 2007). As chickpea content increased, OHC also tended to increase, a trend consistent with findings by Akubor and Ukwuru (2003) in cassava-soybean composite flour. Flours with higher OHC are advantageous in cookie formulations because they help in flavor delivery and create a more indulgent eating experience, potentially boosting consumer appeal.

The swelling power of the control sample (100% wheat flour) was the highest (7.3), resulting in a more expanded and airy texture compared to all cassava-chickpea blends. This indicates that cassava-chickpea blends may produce denser, less aerated cookies due to the lower swelling capacity of cassava starch compared to wheat starch. Swelling power indicates the ability of starch molecules to retain water through hydrogen bonding, which influences the final cookie volume, structure, and lightness (Dat, 2018). These differences might impact sensory attributes such as crumb structure, crispiness, and visual appeal.

Table 2. Functional properties of composite flour blends of cassava and chickpea at different ratios

Variety-Composition	Bulk Density	WHC (mL/g)	OHC (mL/g)	Swelling power (mL/g)
40% Chichu	0.505±0.5 [a]	2.97±0.71[a]	2.04±0.51[cd]	3.42 ±0.30[h]
50% Chichu	0.455±0.11[cd]	2.73±0.41[bc]	2.005±0.61[de]	4.02 ±0.5[fg]
60% Chichu	0.405±0.01[e]	2.58±0.61[ef]	1.95±0.21[fg]	4.56±0.40[d]
40% Hawassa-4	0.505±0.21[a]	2.94±0.06[a]	2.09±0.41[ab]	4.32±0.52[e]
50% Hawassa-4	0.49±0.30[ab]	2.795±0.31[b]	2.01 ±0.61[ce]	4.98±0.80[c]
60% Hawassa-4	0.485±0.31[ab]	2.72 ±0.27[bd]	1.985±0.41[ef]	5.53±0.72[b]
40% Kello	0.475±0.31[bc]	2.98±0.5 [a]	2.04±0.50[cd]	3.83±0.38[g]
50% Kello	0.455±0.21[cd]	2.65 ±0.41[cf]	1.975 ±0.31[eg]	4.05±0.50[f]
60% Kello	0.445±0.4 [d]	2.6 ±0.34[d f]	1.82[h]±0.21	4.62±0.41[d]
40% Qulle	0.495±0.07[ab]	2.69 ±0.21[be]	2.12±0.21[a]	2.93±0.61[i]
50% Qulle	0.475±0.41[bc]	2.61 ±0.11[df]	2.0±0.3[de]	3.45±0.31[h]
60% Qulle	0.455±0.21[cd]	2.56±0.6[f]	1.93±0.21[g]	3.88±0.26[fg]
Control	0.495±0.31[ab]	2.92±0.81[a]	2.05±0.32[bc]	7.3 ±0.14[a]

Where WHC = water holding capacity, OHC = oil holding capacity, 40% = 40% cassava + 60% chickpea, 50% = 50% cassava + 50% chickpea, and 60% = 60% cassava + 40% chickpea, Values are mean ±SD. Each value with the same column followed by different letters is significantly different, and the same letters are not significantly different at the level of 0.05.

Spread Ratio of Cookies from Cassava-Chickpea Composite Flour

The spread ratio is a key measurement of how much a cookie spreads or flattens out during baking. The highest spread ratio (5.74) was recorded for cookies made from the 40% Hawassa-4 formulations (Figure 1). The lowest spread ratio (4) was recorded for cookies made from 60% Chichu formulation. A higher spread ratio often results in thinner, crisper cookies, which some consumers prefer for their crunchiness (Boz, 2019; Thorat and Lande, 2017). Cookies from Hawassa-4 variety ranged from 4.80 – 5.74, falls nearly within the ideal range of 5–6, supporting both crispness and structural integrity (Ashwath and Sudha, 2021). The addition of chickpea to cassava flour resulted in a significant ($p < 0.05$) increase in the spread ratio of the cookies from cassava-chickpea composite flour. A similar finding was observed by Leticia *et al.* (2022), who reported that the spread ratio of cookies from cassava-mung bean composite flour increased with the higher the proportion of mung bean flour (Akubor & Ukwuru, 2003). The higher the spared ratio, the reduced hardness, and subsequently the taste preference level increased (Leticia *et al.,* 2022).

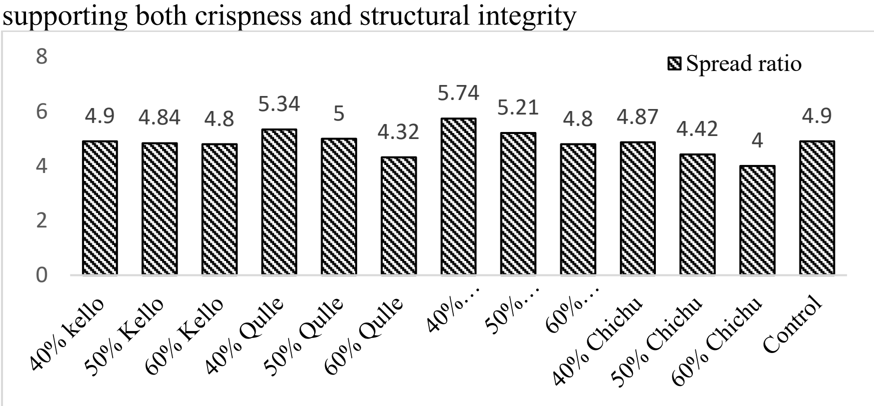

Figure 1. Spread ratio record of cookies from chickpea-cassava composite flour

Where 40% = 40% cassava + 60% chickpea, 50% = 50% cassava + 50% chickpea and 60% = 60% cassava + 40% chickpea

Proximate Composition of Cookies Made from Cassava-Chickpea Composite Flour

The proximate composition of cookies made from cassava-chickpea composite flour, as shown in Table 3, highlights significant variations in moisture, ash, fat, fiber, and protein content across different varieties and compositions. The variation in proximate composition influences cookie texture, flavor, and nutritional profile. The highest moisture content (4.95%) was observed in 40% Chichu, while the lowest moisture level (3.40%) was observed for 60% Hawassa-4. The higher moisture content of the cookie was seen in the cookie from higher chickpea flour, which might be because of the higher fiber content in chickpea. A similar finding was observed by Leticia *et al*. (2022), who reported that higher moisture was observed for cookies from high fiber content. Fiber types such as cellulose have hydrophilic properties (Célino *et al.,* 2014). The moisture content of the cookies was less than 10% to reduce the chances of spoilage by microorganisms and consequently guarantee good storage stability (Ayo *et al*., 2007). Ash content was highest (4.19%) in 40% Hawassa-4 while the lowest ash content (2.05%) was scored for the control sample (100% wheat flour). The varying ash content levels across cassava-chickpea formulations imply differences in mineral content, which can impact the nutritional profile. Ash content is an indication of the mineral content of the product (Czaja *et al.,* 2020). Fat content was higher (25.3%) for 40% Chichu, while the control sample had the lowest (19.95%) fat content. Fat content in cookies contributes to a richer mouth feel and potentially more flavorful cookies (Ikuomola *et al.,* 2017).

Fiber content varied across samples, with 40% Hawassa-4 containing the highest fiber levels (5.90%) while the 60% Qulle sample was found to have the lowest fiber content (4.01%). The presence of high fiber in food products is essential owing to its ability to facilitate bowel movement (peristalsis), bulk addition to food, and prevention of many gastrointestinal diseases (Satinder *et al.,* 2011). Protein content was highest in 40% Hawassa-4 (12.85%) whereas lower protein content (7.54%) was observed in 60% Chichu formulation. Previous findings confirm that addition of legumes could enhance the protein content of cookies (Apotiola and Fashakin, 2013; Adekunle and Mary, 2014). Overall, these proximate composition differences come primarily due to the difference in cassava-chickpea proportion among products of cookies. As chickpea is a good source of protein, fat, fiber, and ash content Yegrem *et al*. (2022) found that it enhances the nutritional profile of cookies made from cassava.

Table 3. Proximate composition of cookies prepared from cassava-chickpea composite flours at varying formulation levels

Variety and composition	Moisture%	Ash%	Fat%	Fiber%	Protein%
40% Chichu	4.95±0.21[a]	3.29±0.10[de]	23.95±0.21[abc]	5.07 ±0.21[ad]	10.38±0.33[b]
50% Chichu	4.20±0.14[ab]	2.86±0.11[f]	23.15 ±0.14[bd]	4.51 ±0. 2[bd]	10.69±2.14[ab]
60% Chichu	4.34±0.37[ab]	2.78±0.13[f]	22.2±0.35[cd]	4.11±0.13 [cd]	7.54±0.14[c]
40% Hawassa-4	4.60±0.11[ab]	4.19±0.06[a]	23.60 ±0.14[bd]	5.90±0.08[a]	12.85±0.07[a]
50% Hawassa-4	4.12±0.12[ab]	4.02±0.08[ab]	23.1 ±0.07[bd]	5.69±0.05[ab]	9.08±0.06[bc]
60% Hawassa-4	3.40±0.14[b]	3.78±0.09[bc]	26.70±0.28[g]	5.13 ±0.62[ad]	7.35±0.16[c]
40% Kello	4.56±0.77[ab]	3.29±0.42[de]	25.3±0.35[a]	5.17 ±0.06[ad]	10.64±0.01[ab]
50% Kello	4.20±0.28[ab]	3.02±0.13[ef]	33.10±0.28[ab]	4.68 ±0.06[ad]	9.21±0.04[bc]
60% Kello	4.86±0.22[a]	2.99±0.07[ef]	24.25±0.07[ab]	4.32±0.15[cd]	7.72±0.07[c]
40% Qulle	4.05±0.35[ab]	3.80±0.01[bc]	24.2 ± 0.28[abc]	5.42 ±0.14[ac]	10.78±0.15[ab]
50% Qulle	4.80±0.71[ab]	3.28±0.04[de]	23.1 ±0.07[bd]	5.12 ±0.13[ad]	9.23±0.13[bc]
60% Qulle	4.50±0.14[ab]	3.45±0.07[cd]	22.20 ±0.28[bd]	4.01±1.00[d]	7.66±0.23[c]
Control	4.25±0.07[ab]	2.05±0.08[g]	19.95±0.78[e]	0.89±0.03[e]	7.30±0.28[c]

Where, Chichu, Hawassa-4, Kello and Qulle are cassava varieties. 40% = 40% cassava variety+ 60% chickpea, 50% = 50% cassava variety + 50% chickpea and 60% = 60% cassava variety+ 40% chickpea. Values are mean ±SD. Each value with the same column followed by different letters are significantly different and same letters is not significantly different at level of 0.05

Mineral and Anti-Nutrient Composition of Cookie from Cassava-Chickpea Composite Flour

The mineral and anti-nutrient composition of cookies made from cassava-chickpea composite flour varied notably among different varieties and compositions (Table 4). The cookies from cassava-chickpea composite flour demonstrated an enhanced mineral profile, particularly in calcium, zinc, and iron, compared to the control, indicating their potential to serve as a nutrient-dense snack. Calcium content was highest (76.90 mg/100g) in the cookie from 40% Qulle formulation. The lowest calcium content was recorded for 60% Qulle formulation. In contrast, the control cookie (100% wheat) exhibited the lowest calcium content at 14.00 mg/100 g, indicating a lower mineral density compared to the composite flour cookies. Calcium (Ca) is an important bone-related micro element in human nutrition (Ciosek *et al.,* 2021). Zinc levels were highest in 40% Kello (2.23 mg/100g), 50% Qulle (2.21 mg/100g), and the control (2.25 mg/100g) formulations. The lowest zinc level (1.19 mg/100 g) was observed in 60% Chichu formulation. Adequate zinc in cookies supports immunity and growth, especially where zinc deficiency is common. Iron content was highest 4.20 mg/100g in 50% Qulle formulation, whereas 60% Chichu had the lowest iron content (1.80 mg/100g). Phytate content was highest (92 mg/100 g) in 40% Hawassa-4 and lowest in the control cookie (15 mg/100 g). Phytic acid is the primary storage compound of phosphorus in cereals, legumes, nuts, and oilseeds (Lott et al., 2009). The tannins reduced the availability of minerals, proteins, and starch (Barros et al., 2012). However, tannins have antioxidant properties beneficial in small amounts, although higher concentrations may reduce iron absorption. Overall, the enhanced mineral profile, particularly in calcium, zinc, and iron was observed for cookies from cassava-chickpea than the control sample. Therefore, cookies from cassava-chickpea can be considered as nutrient-dense alternative to traditional wheat cookies. The highest HCN content (4.35 mg/100 g) was recorded for the 60% Qulle formulation. The lowest HCN content (1.82 mg/100 g) was recorded for the 40% Chichu formulation. The tolerable limit for HCN in cassava products varies by regulatory guidelines but is generally recommended to be below 10 mg/100 g in ready-to-eat products, according to FAO (1998). The values in this table suggest that while all samples fall below the 10 mg/100 g limit.

Table 4. Mineral and anti-nutrient content of Cookie from Cassava-chickpea Composite Flour

Variety-Composition	Ca mg/100g	Zn mg/100g	Fe mg/100g	Phytate mg/100g	Tannin mg/100g	HCN mg/100g
40% Chichu	75.90±1.84[a]	1.63±0.06[bc]	3.30±0.14[abc]	77.5±0.09[ab]	0.05±0.02[a]	1.82±0.26[c]
50% Chichu	61.00±0.28[bc]	1.40±0.12[bc]	1.95±0.21[d]	70 ±0.08[a-c]	0.07±04[a]	2.37±0.54[bc]
60% Chichu	54.65±0.64[de]	1.19±0.09[c]	1.80±0.14[d]	61±0.06[bc]	0.08±05[a]	3.08 ±0.60[a-c]
40% Hawassa-4	76.65±0.64[a]	1.55±0.14[bc]	3.60±0.42[ab]	92±0.04[a]	0.06±02[a]	2.12±0.40[bc]
50% Hawassa-4	61.55±0.21[b]	1.40±0.07[bc]	2.35 ±0.35[b-d]	80±0.06[ab]	0.08±0.01[a]	2.84 ±0.58[a-c]
60% Hawassa-4	57.15±0.92[cd]	1.23±0.08[c]	2.30 ±0.28[b-d]	72 ±0.06[a-c]	0.09±0.01[a]	3.76±0.48[ab]
40% Kello	73.90±0.42[a]	2.23±0.17[a]	2.60 ±0.28[b-d]	70.5 ±0.08[a-c]	0.07±0.01[a]	3.76±0.49[ab]
50% Kello	63.0±0.99[b]	1.53±0.14[bc]	2.52 ±0.40[b-d]	61±0.04[bc]	0.08±0.01[a]	2.93 ±0.45[a-c]
60% Kello	51.95±2.05[ef]	1.89±0.07[ab]	2.25±0.21[cd]	56.5±0.09[bc]	0.11±0.01[a]	3.76±0.49[ab]
40% Qulle	76.9±0.57[a]	1.78±0.06[ab]	3.95±0.78[a]	63.5 ±0.06[bc]	0.18±0.43[a]	2.30 ±0.42[bc]
50% Qulle	59.7±0.85[bc]	2.21±0.28[a]	4.20±0.14[a]	56±0.08[bc]	0.09±0.01[a]	2.44 ±0.45[a-c]
60% Qulle	48.50±0.57[f]	1.56±0.08[bc]	1.90±0.14[d]	49.5±0.05[c]	0.12±0.01[a]	4.35±0.50[a]
Control	14.0±0.99[g]	2.25±0.21[a]	3.55 ±0.21[a-c]	14.5±0.01[d]	0.01±03[a]	______

Table 5 showed the molar ratios of phytate to minerals (Ca, Zn, and Fe) in cookies made with various cassava-chickpea formulations and a control made of 100% wheat flour. The chelating properties of phytic acid released during food processing or in the gut also bind minerals and make them unavailable as nutritional factors. Understanding these molar ratios is important because high phytate-to-mineral ratios can inhibit the absorption of these essential minerals in the body. Across the different formulations, the phytate-to-calcium ratio ranges from 0.05054 (40% Qulle) to 0.07964 (50% Hawassa-4). Generally, a phytate/Ca molar ratio above 0.17 is considered inhibitory to calcium absorption (Ulrich *et al.,* 2007), so these values suggest minimal interference with calcium absorption. The phytate-to-zinc ratio shows significant variation, with the lowest (0.6604) in the control sample and the highest (5.87973) in 40% *Hawassa-4* formulation. Ratios above 10 are known to inhibit zinc absorption (Gharib *et al.,* 2006), so while these values are relatively low, they are within a tolerable range. The phytate-to-iron ratio ranges from 0.35755 (control) to 2.99429 (50% Chichu). Ratios above 1 are generally considered to impair iron absorption (Ulrich et al., 2007). Formulations from cassava-chickpea have values exceeding this threshold, suggesting possible challenges for iron bioavailability, especially in the Chichu and Hawassa-4 samples.

Table 5. Molar ratio of Phytate-mineral ratio of cookies from cassava –chickpea composite flour

Formulation of Cookies	Molar ratio phytate/Ca	Molar ratio phytate/Zn	Molar ratio phytate/Fe	Molar ratio phytate/Ca
40% Chichu	0.06241	4.74032	2.00014	0.06241
50% Chichu	0.06869	4.88227	2.99429	0.06869
60% Chichu	0.06785	3.18037	2.86772	0.06785
40% Hawassa-4	0.07294	5.87973	2.16254	0.07294
50% Hawassa-4	0.07964	5.66061	2.88072	0.07964
60% Hawassa-4	0.07657	5.79867	2.64901	0.07657
40% Kello	0.05834	3.15395	2.31081	0.05834
50% Kello	0.05880	3.94948	2.04837	0.05880
60% Kello	0.06669	2.98754	2.14374	0.06669
40% Qulle	0.05054	3.56173	1.37108	0.05054
50% Qulle	0.05696	2.51013	1.12828	0.05696
60% Qulle	0.06261	3.17502	2.22687	0.06261
Control	0.06506	0.66040	0.35755	0.06506

Where 40% = 40% cassava + 60% Chickpea, 50% = 50% cassava + 50% Chickpea and 60% = 60% cassava + 40% Chickpea

Sensory Acceptance of Cookies from Cassava-Chickpea Composite Flour

Table 6 shows the sensory acceptance scores for cookies made from cassava-chickpea composite flour at 40%, 50%, and 60% cassava proportion and 100% wheat (control). The 50% blend had the highest color score (7.77), while the control had the lowest color acceptance (5.08). Sensory evaluation was conducted solely on formulations using the Hawassa-4 cassava variety: chick pea proportions such as 40:60, 50:50, and 60:40. This is due to as this variety demonstrated superior physical (spread ration) and functional properties (WHC and OHC). The above figure suggests that 50% blend is the most visually appealing among the formulations. The chickpea-incorporated cookies had a golden-brown color, which is widely accepted as desirable for cookies (Ikuomola *et al.,* 2017). The golden-brown color of chickpea-enriched cookies is likely due to Maillard browning, which occurs during baking when reducing sugars react with proteins (Ikuomola *et al*., 2017). This desirable color was most pronounced in the 50% cassava blend (score = 7.77), surpassing the control (7.08).

The control sample (7.08) and the 40% cassava-chickpea formulation (6.77) scored highest in taste score. The 50% and 60% cassava formulations received lower taste scores. The decrease in taste score for higher cassava levels may be due to the earthy flavor of cassava. This finding is consistent with Akubor & Ukwuru (2003).

The 40% cassava- 60% chickpea formulation scored the highest (7.15) in favorable profile. The 60% cassava blend had the lowest flavor score (5.17). The 40% cassava formulation is closest to control sample in consumer preferences in terms of flavor, which might be highlighting the potential of formulation for cookies making.

The 40% cassava blend achieved a texture score of 7.92, close to the control (8.00). This result could be attributed to the flour's higher spread ratio, water-holding capacity (WHC), and oil-holding capacity (OHC), which improve moisture retention and contribute to the familiar mouth feel of traditional cookies (Makinde & Adebile, 2018).

The control sample, prepared from 100% wheat flour which is the standard and most commonly used ingredient in conventional cookie production was included as a baseline to benchmark the sensory quality of the composite flour formulations. It received the highest score for crispiness (7.33), indicating it was the most preferred in terms of crisp texture. This highlights the familiarity and acceptability of wheat-based cookies among consumers and provides a reference point to evaluate the performance of cassava-chickpea blends, which are being explored as alternative, locally available, and gluten-free options.

Cookies from 40% Hawassa-4 formulation had recorded highest in crispiness (7.21) while cookies from 60% cassava formulation had the lowest crispiness score (6.27). The crispiness of cookies from control sample was higher than that of cassava-chickpea composite cookies. This can be attributed to a lower swelling capacity of cassava-chickpea composite flour than wheat starch, leading to a denser, less crispy texture aligned with the finding by Adekunle & Mary (2014).

The 40% cassava blend had an overall acceptance score of 7.21, slightly lower than the control (7.67) but still within a favorable range (Figure 2). This indicates that cookies made with 40% cassava and 60% chickpea can be a viable alternative to wheat-based cookies, especially for consumers seeking gluten-free or alternative baked products. Therefore, 40% Hawassa-4 cassava and 60% chickpea blend stands out for its balance in taste, flavor, texture, test, and overall acceptance, making it a suitable alternative to wheat flour cookies.

Table 6. Sensory Evaluation Results for Cookies Made from Cassava-Chickpea Composite Flour

Formulation	Color	Taste	Flavor	Texture	Crispiness	Overall acceptance
40% Hawassa-4	6.38±1.71^{b}	6.77±0.98^{a}	7.15±1.24^{a}	8.00±0.79^{a}	7.31±1.39^{a}	7.21±0.94^{ab}
50% Hawassa-4	7.77±0.90^{a}	5.31±1.65^{b}	6.48±1.00^{b}	5.92±2.08^{c}	6.69±1.34^{ab}	7.15±1.04^{ab}
60% Hawassa-4	7.40±1.16^{a}	5.67±0.86^{b}	5.17±1.08^{c}	6.83±1.34^{b}	6.27±0.87^{b}	7.00±0.92^{b}
Control	5.08±1.46^{c}	7.08±1.87^{a}	7.00±1.17^{ab}	7.92±0.96^{a}	7.33±1.81^{a}	7.67±1.51^{a}

Where 40% = 40% cassava + 60% Chickpea, 50% = 50% cassava + 50% Chickpea and 60% = 60% cassava + 40% Chickpea, Values are mean ±SD. Each value with the same column followed by different letters are significantly different and same letters is not significantly different at level of 0.05

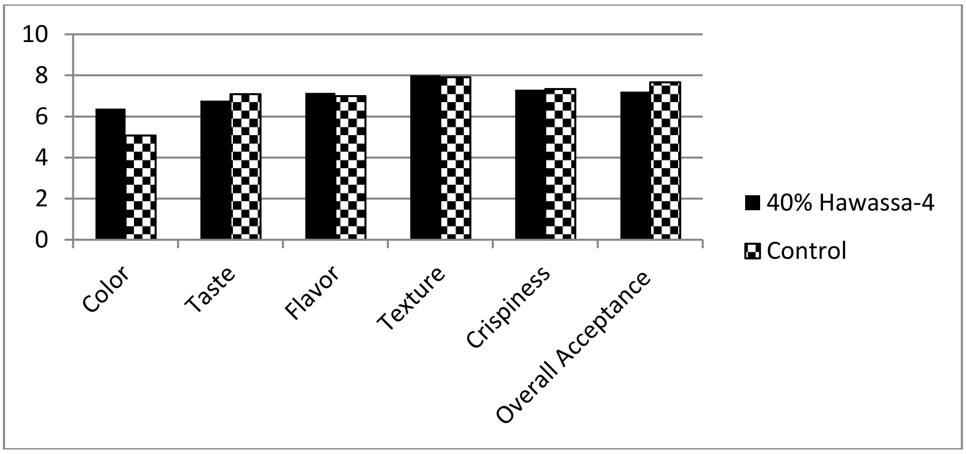

Figure 2. Sensory evaluation result of the 40% Hawassa-4 formulation and control sample

CONCLUSIONS

The results of this study indicated that the functional characteristics and physical properties of cookies were significantly influenced by both cassava variety and chickpea proportion. Among the different formulations, the 40% cassava-60% chickpea blend using the Hawassa-4 variety emerged as the optimal formulation. These cookies achieved the highest spread ratio and exhibited desirable sensory qualities, particularly in terms of texture, flavor, crispiness, taste, and overall acceptance, which are crucial for consumer acceptance. In addition to this the cassava-chick pea cookies had good protein, fiber and micro nutrient content than the control sample. This study shows the commercial potential of using local crops like cassava and chickpea to produce nutritious, acceptable cookies, supporting import substitution, value addition, and economic growth in producing regions. By leveraging locally available raw materials, the approach also offers a sustainable alternative to wheat-based products, with potential to influence both industrial practices and consumer choices in areas where cassava and chickpeas are abundant.

By promoting the use of locally available cassava and chickpea flours in cookie production, this study contributes to the development of sustainable food systems and supports Ethiopia's efforts toward improved food security and reduced reliance on imported wheat. Future research should focus on optimizing processing parameters such as baking temperature and time to further enhance product quality. It is also recommended to test cassava-chickpea flour blends in other baked goods, such as breads, muffins, or cakes, to assess their broader applicability.

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CONFLICTS OF INTEREST

Authors declare that they have no conflict of interest regarding the publication of this paper.

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Original Research Article||

Groundwater Recharge and Urban Water Balance of Hawassa City, Ethiopia

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Abstract

In urban areas groundwater recharge is not only from rainfall, but also from wastewater generated from different water users and water supply leakages. In this study different natural and urban recharge components that exists in the recharge process of Hawassa city, southern Ethiopia was estimated. The natural recharge was evaluated based on primary and secondary data using water balance equation. Runoff was estimated using Soil Conservation Service – Curve Number (SCS-CN) and evapotranspiration using Simplified Surface Energy Balance Index (SSEBI) against annual average precipitation. The urban recharge was evaluated from all water supplied by providers and in-situ groundwater sources, consumptive uses, supply leakages and onsite wastewater. The average rainfall was 1163mm/yr, of which the average runoff depth throughout Hawassa City was 88.63mm/yr. However, the average runoff depth in the developed part of the city was 108.4 mm/yr and in the rural area 76.5 mm/yr. The average actual evapotranspiration in Hawassa city was estimated to be 841.33 mm/yr. Yet, for the developed city of Hawassa it was estimated to be 683.06 mm/yr and for the undeveloped area 933.94 mm/yr. The high rate of evapotranspiration in the rural area is due to high evapotranspiration from wetlands and agricultural land cover types. Therefore, the average natural recharge and urban recharge of the study area were 233.04 mm/yr and 52.7 mm/yr respectively. The specific urban recharge in the developed urban area was estimated to be 142.78 mm/yr, which is a quality degraded water recirculating in the urban aquifers. This result can not only indicate the depth of water recharge, but also the nature of groundwater quality in Hawassa City, and inspire actions to mitigate water quality deterioration and increase storage.

Key words: Curve number, Evapotranspiration, infiltration, Natural recharge, Urban recharge

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INTRODUCTION

Aquifers are the world's largest reserves of liquid freshwater (Mishra, 2023). The process of urbanization largely alters the hydrological cycle (McGrane, 2016) that might exist in natural environments, which also affects surface and groundwater resources under urban areas. Wakode et al. (2018) identified four impacts of urbanization on the hydrological cycle, including flooding, water scarcity, changes in surface and groundwater regimes, and water pollution. These effects are a result of surface impermeability, increased water demand from various users, surface and subsurface alteration by infrastructure, and water pollution from point and non-point sources.

Rapid urban growth requires the provision of large quantities of water for various uses from surface or groundwater sources (McDonald et al., 2014). This in turn results in high levels of surface or subsurface wastewater discharge, which also pollutes surface or groundwater. This has a major impact on the water balance of urban areas (Foster, 1990).

When assessing the water balance of urban areas, natural and urban aquifer recharge, in-situ and imported/exported water sources, natural and anthropogenic effects on evapotranspiration need to be considered (Lerner, 2003). And all these factors require intensive data, which may not be available. As a result, calculating the water balance of urban areas is complicated and subject to high uncertainties. With rapid urban growth, soil sealing reduces the natural recharge to aquifers, but high levels of water supply from groundwater and/or surface water, with associated leakage from the supply system, industrial effluent and on-site sanitation effluent, will increase urban (indirect) recharge (Morries et al., 2006; Lerner, 2003). Groundwater management in urban areas therefore requires detailed knowledge of the hydrogeological system and adequate methodology for predicting groundwater recharge inputs and source water quality evaluations.

Hawassa City is the capital of the Sidama Regional State and one of the fastest growing cities in Ethiopia. The main source of water for the city is groundwater. Potable groundwater is extracted from wells on the outskirts of the city and distributed throughout the city. In addition, many hand-dug and borehole wells provide water for various purposes within the city. Although soil sealing reduces natural recharge in urban areas, on-site sanitation facilities and leakage from water supply pipes increase water recharge to aquifers. Recirculation and reuse of groundwater degrades water quality (Jjemba et al., 2014). This is common in developing cities such as Hawassa. The groundwater quality analysis in Hawassa City indicates this fact (Alemu et al., 2024a and 2024b). Groundwater management in this city is important because the city is expanding into the area of high-quality groundwater source.

The objective of this study is to identify and analyze the components of groundwater recharge systems in Hawassa City and to estimate the groundwater budget, so that the information can be used to anticipate sustainable groundwater use through an effective and efficient groundwater management plan, taking into account groundwater quality.

MATERIALS AND METHODS

Description of the Study Area

Hawassa City is located in the Lake Hawassa Watershed in the Central Rift Valley of Ethiopia. It is located in the region of longitudes $38^0 28'$-$38^0 33'$ E and latitudes $6^0 58'$-$7^0 05'$ N (Figure 1). The terrain of the region is made up of flat plains and a few volcano mountains, with elevations ranging from 1680 to 2027 meters a.s.l.

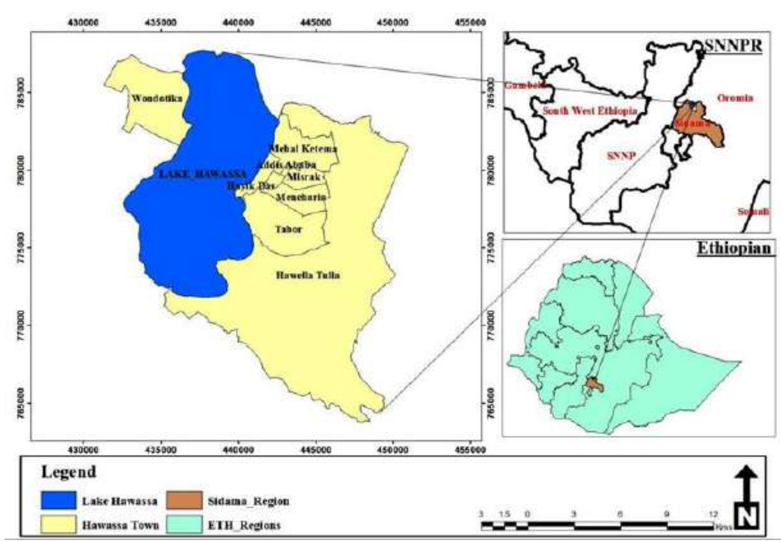

Figure 1. Study Area

The wet and humid months are from April to October. The major rainy season lasts from July to September, and the long-term average annual rainfall is around 954.9 mm (National Meteorological Agency of Ethiopia, 2020). 1,599 mm/year is the potential evapotranspiration (PET). According to NMA, the minimum PET is 102 mm in July and the highest PET is 173 mm in December (NMA). Hawassa City lies on the eastern side of Lake Hawassa. As the lake is the terminal lake of the catchment, the run-off from the city also drains into the lake.

According to Halcrow Group (2009) study, the two main aquifers in the area are the overlaying volcano-lacustrine sediments, and fractured and jointed ignimbrites. Sands, tuff, and pumice are interlayered with clay aquitard in volcano lacustrine sediment aquifers. The lacustrine material is between 40 and 60 meters thick. Boreholes excavated in the lacustrine sediments frequently reveal multiple aquifers divided by clay strata. The most extensive and generally high-quality groundwater aquifer in the main Ethiopian Rift is found beneath faulted tertiary ignimbrites (Hulluka et al., 2023; Halcrow Group, 2009).

Procedures of Groundwater Recharge Analysis

Groundwater recharge in urban areas was estimated by calculating natural recharge from precipitation and urban recharge derived from water supply and leakages from swears networks (Lerner, 2003). Urban groundwater recharge from precipitation could be calculated using water balance equations (Eq.1) such as

$$U_n = P - ET_a - Q_u \qquad \text{(Eq.1)}$$

Where U_n = Natural groundwater recharge, P = Precipitation, ET_a = Actual evapotranspiration and Q_u = Runoff from urban surfaces

Precipitation data was collected from the Hawassa meteorological station for the years (2017-2020), ET_a was calculated from land surface temperature and Penman-Monteith (Filgueiras et al., 2019; Cai et al., 2007) ET_o, and runoff is estimated using the SCS-CN method. The total area of Hawassa city is 154.544 km^2 out of which 55.0737 km^2 is the developed section of the city.

However, urban recharge was calculated from leakage from supply networks, irrigation and sanitation systems. Although it is difficult to find data on leakage because the infrastructure is underground and the processes are not visible, considering the components of the system and analyzing them separately will reduce errors. Lerner (2020) stated that net urban recharge is calculated using (Eq.2).

$$U_u = W_i + GW_a - CU - S_l \quad \ldots\ldots\ldots\text{(Eq.2)}$$

Where U_u = Urban recharge, W_i = imported water, GW_a = Local abstracted groundwater, CU= Consumptive use and S_l = effluent leaving the urban area.

In this calculation, the inputs to the system were imported water and locally abstracted groundwater. These components were actually subdivided into the share of different types of users and water lost in the distribution system and unaccounted for water. The consumptive use, portion of consumed water that is embedded in the body of a process parts or is removed from the system in the form of evaporation/evapotranspiration (Grubert et al., 2020); and wastewater transported outside the urban boundary by various users were considered as an output of the system.

In the water balance analysis of Hawassa City, water sources to the city, end use and outputs information from different sources gathered and used. The Hawassa City Water Supply and Sewerage Services Enterprise provide potable water for residential, institutional, commercial and industrial uses. Additionally, many households, service providers, institutions, industries and irrigation farms use their own water supply from underground sources within their premises. At the time of the water sources survey, even though in-situ water source data is not exhaustive it was observed that the total volume of water consumed from private wells was half the volume supplied by the municipal water supply system. Figure 2 shows the distribution of in-situ water supply wells in Hawassa City.

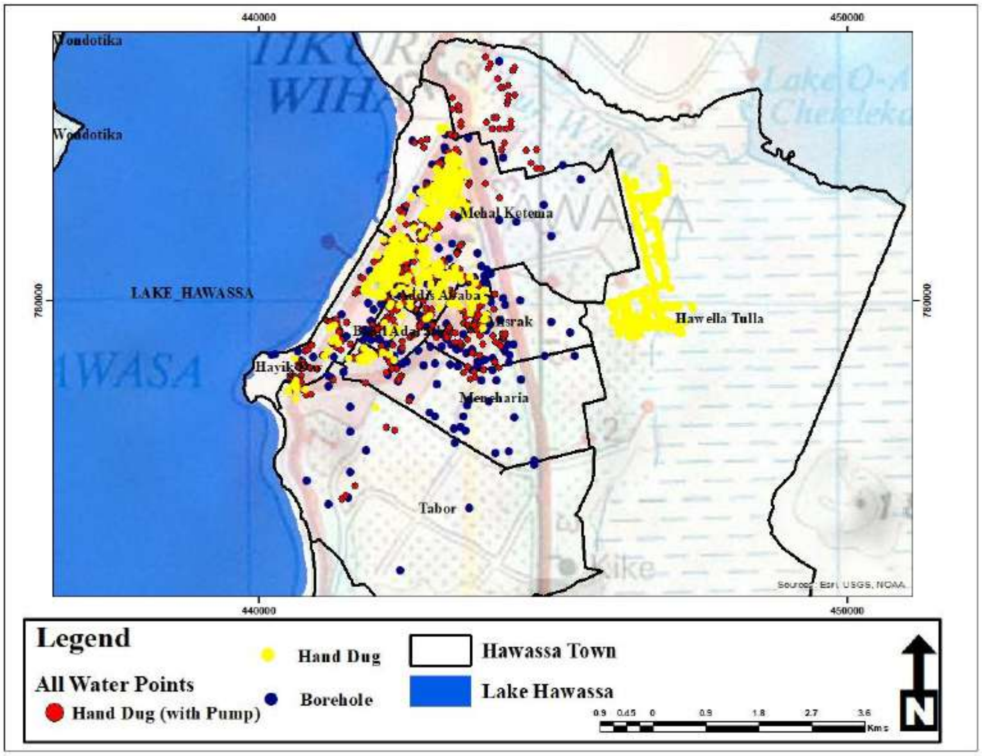

Figure 2. Inventoried in-situ groundwater source points (N = 1579)

Although an inventory was made of the amount of water consumed from onsite water sources, it was not exhaustive, as the inventory depends on the willingness of the well owners. However, the inventory result can indicate the discrepancy between demand and supply of water in the city and is also an important input for the calculation of urban recharge. Since part of the urban population is not supplied with enough water, per capita water consumption cannot indicate the spatial distribution of water supply. Instead, considering the total volume of water supplied to different users and estimating distribution losses will reduce errors (Al-washali et al., 2016). For this calculation, average daily water supply data for different users and expected system losses from the Hawassa Water Supply and Sewerage Service Enterprise collected and used.

The city of Hawassa has no sewerage system and no wastewater treatment plant. Household wastewater includes liquid waste from toilets, baths, showers, kitchens and sinks, which is discharged into a drain. The World Bank (2005) study found that 92% of households reported having toilets that discharged into a tank or pit that was never filled or had to be emptied. This means that the majority of household wastewater is recharging groundwater below the surface. Even the vacuum trucks that collect sewage from septic tanks and latrines dump the waste on sand beds to dewater the sludge for drying on Alamura Mountain, which lies on the outskirts of Hawassa. As the treatment plant is not impermeable and is poorly managed, the potential for aquifer recharge is high. Only a limited number of industries and public institutions have wastewater treatment plants, which fully or partially treat wastewater and discharge it into nearby streams within the city limits. Based on basic assumptions and literature insights, the wastewater discharge and the volume of recharge in Hawassa City aquifers estimated using the following complimentary equations.

The total urban recharge can be calculated by summing all leakage and effluent entering into underground in the urban boundary (Wakode et al., 2018) as (Eq.3).

$$U_u = L_{ws} + L_{dw} + L_{iw} \quad \text{(Eq.3)}$$

Where U_u = urban recharge, L_{ws} = water supply leakage, L_{dw} = recharge from domestic and commercial facilities waste and L_{iw} = recharge from industrial and institutional effluents.
The value was calculated on the basis of expected supply leakage and in-situ effluent discharge after consumption by different water users and from different sources.

The net urban recharge of Hawassa City was calculated from the available data and assumptions as 90% of wastewater discharge plus water supply leakage using (Eq.4).

$$Uu=0.20*WS+0.9(WW) \ldots\text{(Eq.4)}.$$
$$0.2*WS = L_{ws}, \text{ and } 0.9*WW = L_{dw} + L_{iw}$$

Where Uu is urban recharge, WS is municipal water supply volume and WW is net in-situ wastewater discharge
Net waste water is calculated as the total waste water discharged by all types of water users minus the waste water discharged outside the city boundary. The natural groundwater recharge component required estimation of actual evapotranspiration and surface runoff within the city boundary.

In urban areas, different land use types with contrasting colors exist within limited areas. This results in different surface albedo and evapotranspiration. Currently, it becomes possible to estimate ET based on remotely sensed data. One of the energy balance methods used to calculate evapotranspiration using remotely sensed data is the Simplified Surface Energy Balance Index (SSEBI) (Wróblewski et al., 2021; Kumar et al., 2020; Senay et al., 2007; Sobrino et al., 2007; Bastiaanssen, 2000; Roerink et al., 2000). Here in this research land surface temperature (Li et al., 2004) was calculated from Landsat 8 image and anchor pixels for hot and cold LST selected. Additionally, sample temperature values for each land cover type collected and average temperature for each land cover type calculated. From the anchor pixels temperature and average temperature for each land cover, the Evapotranspiration fraction (Aryalekshmi et al., 2021; Saboori et al., 2021; Li et al., 2019) calculated using (Eq.5)

$$ET_{fLC} = \frac{Th - T(av{-}LC)}{Th{-}Tc} \ldots\ldots\ldots\text{(Eq.5)}$$

Where ET_{fLC} is ET fraction for particular land cover type, T_h anchor hot pixel temperature, T_c anchor cold pixel temperature and $T_{(av\text{-}LC)}$ average temperature for particular land cover type.
ET_{an} for a particular land cover type is calculated using (Eq.6).

$$ET_{an} = ET_{fLC} * ET_o \ldots\ldots\ldots\ldots\ldots\text{(Eq .6)}$$

Where ET_{an} is actual evapotranspiration for each land cover, ET_{fLC} is ET fraction for the particular land cover type and ET_o is Penmam-Monteith (Cai et al., 2007) ET value.

The actual evapotranspiration of the urban area could be estimated by taking into account the land use class weighting. The formula used for the calculation is (Eq .7)

$$ET_a = \sum_{n=1}^{n} (ETan) * \left(\frac{An}{A}\right) \quad \text{(Eq .7)}$$

Where ET_{an} = actual evapotranspiration for each land use type, A_n = area coverage for each land cover type, A = total area in the urban boundary
The second component needed to determine natural recharge is surface runoff. Urbanization reduces infiltration and increases flow velocity due to changes in surface imperviousness and built structures, resulting in increased ponding and runoff. The Soil Conservation Services Runoff Curve Number (SCS-CN) method was used as it is recommended by the US Department of Agriculture for urban hydrology of small watersheds (Cronshey, 1986). The model takes into account landuse/landcover distribution and soil properties. Basically, the SCS-CN method is based on the water balance equation of rainfall within a given rainfall time interval. The formula used to calculate runoff in the SCS-CN method is (Eq .8).

$$Q = \frac{(P-Ia)^\wedge 2}{(P-Ia)+S} = \frac{(P-kS)^\wedge 2}{P+(1-k)S} \quad \text{for } P > kS \text{ and } Q = 0 \text{ for } P <= kS \quad \text{(Eq .8)}$$

Where Q = runoff in mm, P = Precipitation in mm, Ia = Initial Abstraction in mm, S = Maximum soil retention after runoff begins in mm and k = The initial abstraction ratio.

The value of k (initial abstraction ratio) varies from zero to infinity. For the average condition, k is 0.2 and this value has been used by various researchers to calculate runoff, but currently this value is being changed to 0.05 by various researchers (Deshpande and Amit Dhorde, 2023). The Antecedent Moisture Condition (the previous relative moisture of the surface before the rainfall event) was considered by summing the total rainfall that occurred within the previous 5 consecutive days. Three AMC classes were defined based on the total amount of rainfall within the previous 5 days as AMCI (dry), AMCII (normal) and AMCIII (wet) in two (growing and dormant) seasons (USDA, 1986).

The maximum soil retention after runoff starts (S) was calculated and it depends on the hydrological soil group and land cover conditions through the curve number (CN). The curve number has a value between 0 and 100 and the higher the value, the more runoff generation is expected. Four hydrological soil groups A, B, C and D are associated to determine CN, with a soil having a high-water infiltration rate as type A soil and D having the lowest infiltration rate (Kumar et al., 2021). Here gravel and sandy soils are type A and clay soils are type D hydrological soil group. The curve number for each land cover type and hydrological soil group was selected from the look-up table and the weighted CN for the area was calculated from the different LCs. The formula used to convert CN to S is (Eq .9)

$$S = \frac{25400}{CN} - 254 \qquad \text{(Eq .9)}$$

As the curve number shown in the Lookup table is for the normal antecedent moisture condition, it was changed to a CN corresponding to the actual antecedent moisture condition (AMCI or AMC III) and inserted into (Eq .9) above. The formula for converting CNII to CNI or CNIII is (Eq.10) and (Eq .11) respectively.

$$CNI = \frac{CNII}{2.281 - 0.01281 * CNII} \quad \text{or} \qquad \text{(Eq .10)}$$

$$CNIII = \frac{CNII}{0.427 + 0.00573 * CNII} \ldots\ldots \text{(Eq .11)}$$

Finally, with the S-value, daily direct runoff from daily rainfall events was calculated using the runoff estimation equation.

Based on the values of daily precipitation, actual daily evapotranspiration and daily runoff, the natural groundwater recharge was calculated using the water balance equation (Eq.1).

The SCS-CN surface runoff estimation method used by (Deshpande and Amit Dhorde, 2023; Hawkins et al., 2008) was adopted to estimate runoff in this manuscript. Based on Deshpande and Amit Dhorde (2023) the initial soil abstraction (Ia) is proposed to be 0.05 of the potential maximum soil retention (S). Using this relationship, the depth of runoff was calculated using the modified (Eq .12).

$$Q = \frac{(P - 0.05S)^{\wedge}2}{(P + 0.95S)} \qquad \text{(Eq .12)}$$

Components of Urban Water balance of Hawassa City

The water balance provides information on the amount of water flowing into and out of the area under consideration. The water entering Hawassa City from different sources and leaving the city in different forms is considered here. Water entering the city as rainfall, imported water from other catchments as water supply, runoff from upstream of the city boundary, and in situ groundwater extraction are input to the system. Water leaves the city as runoff, wastewater disposal, infiltration into the ground, and evaporation/evapotranspiration. Water balance analysis can help to identify gaps in water demand and supply and the quality of water resources, and to seek possible options to improve gaps and water quality.

Data Sources and Software Used in the Analysis

The data required for this analysis were obtained from, land use/land cover from Wondrade (2023), FAO & IIASA Harmonized World Soil Database version 2.0 (2023), rainfall data from the Meteorological Agency, water supply from Hawassa Town Water Supply and Sewerage Service Enterprise and the researcher's well survey, and various institutional reports and research manuscripts such as Draft Assessment Report" Feasibility Study and Detail Design of Waste Water Management system for Bahir Dar and Hawassa Towns" from Ministry of Water, Irrigation and Electricity and researchers. Google Earth Engine data sources and platform, Arc GIS and EXCEL software were used in the analysis.

RESULTS

Urban recharge and natural recharge were calculated separately, and then the total recharge of Hawassa City was estimated by summing up the two.

Urban Groundwater Recharges Component

Urban groundwater recharge was basically based on the portion of water supply from various sources that enters the urban boundary other than rainfall falling in the area. The sources of water supply to Hawassa City are water supply from Hawassa City Water Supply and Sewerage Service Enterprise, groundwater supply from in situ sources and Lake water supply for Hawassa City greenery by trucks. Although the available data is not exhaustive, the water supply volume shown in the Table 1 used to roughly estimate the recharge volume.

Table 1. **Water Supply types and Volume in Hawassa City (Source: HTWSSSE and Inventory)**

Sr. No.	Source of Water	Volume in m^3/day
1	Municipal water supply	30,000
2	In situ groundwater supply	13,000
3	Truck water supply	1,200

The amount of water supplied is an average and may vary according to the season. Watering for landscaping purposes is applied on a daily basis, especially during the dry seasons of the year. The depth of application is shallow, so that part of the water evaporates or infiltrates into the soil and does not actually reach the groundwater table.

Even the reported amount is far less, as the owners of the groundwater wells were not willing to disclose the amount of water they use; the local groundwater supply is half the amount supplied by the municipal water supply services. In particular, mega water consuming industries in the industrial zone area such as St. Gorge beer factory, Moha beverage factory, textile factory and others were not willing to disclose their water consumption. Out of the collected volume, 3.77%, 11.44% and 84.78% of the water was used by domestic, commercial and services, industrial and public institutions respectively. Industry Park, Hawassa University and Green Herbs Farm were major users of groundwater within their premises. Industry Park has a third stage wastewater treatment plant and a large volume of wastewater is recycled and reused. Hawassa University has an oxidation pond to partially treat wastewater and discharge the water within the campus.

Green Herbs Farm produces vegetables and herbs using drip irrigation in a shaded area. Domestic, and Commercial and service provider in situ groundwater users used the water for non-consumptive purposes and discharge the effluent into septic tanks or soak away pits, which can infiltrate into the groundwater. From the feasibility study report of the Ministry of Water, Irrigation and Energy (MWIE, 2018), 10% of the pits and septic tanks are watertight, as a result, high urban recharge percentage is expected from in situ groundwater users of domestic, commercial and service sectors and public institutions than industries using water recycling and irrigation farms using efficient irrigation methods. The estimation of water consumption and wastewater production was based on these assumptions.

The Hawassa City Water Supply and Sewerage Service Enterprise provided drinking water to households, commercial and service establishments, institutions and some industrial use. Of the total volume supplied, at least 20% (Foster et al., 1999b) is lost through leakage in the transmission, distribution and service connections.

This is the minimum leakage rate for an efficient water supply system. In this analysis minimum industrial and commercial demand was assumed 15% and the public demand was about 5% of the total supply. Therefore, 60% of the water supply is distributed for domestic use. As the current population of Hawassa City is estimated to be about 400,000 based on census data (CSA, 2015), the per capita water consumption per day is about 45 LPCD, which is very low, and hence many in situ water supply facilities are found throughout the city. As the total volume is insufficient, a large proportion of customers are dissatisfied with the services. Therefore, we were forced to take the maximum personal consumption up to 40% of LPCD, and this value was used to estimate the wastewater produced per capita per day.

Hawassa City has no sewerage infrastructure, so sewage and/or fecal sludge is discharged into septic tanks and pit latrines, which are not watertight. According to the feasibility study report (MWIE, 2018), less than 10% of septic tanks or pit latrines are emptied using vacuum trucks. Many service providers such as shower houses, car washes and others use soak away pits to dispose of wastewater. In estimation of volume of wastewater, the following assumption were considered

1. As the volume of water supplied for personal consumption is low consumptive use was calculated at 40% of LPCD.
2. Water supply volume for industry and commercial sectors are basic water needs, and high portion of this water is used for consumptive use. 80% of their share is estimated as consumptive use.
3. 80% of water supply to public institutions is estimated as consumptive use.
4. As the quality of in situ water supply from ground source is not potable, consumptive use of this water is very low for domestic, commercial and service providers. 20% of the volume is estimated as consumptive use.
5. Industries, agricultural farms and public institutions with in situ water supply use the water to produce products. Though there is variation in consumption between industries, public institutions and farm 60% average water consumption is estimated as consumptive use.
6. From MWIE (2018) feasibility study report about Vacuum truck wastewater transportation services in Hawassa City, 12 trucks with at most 10000 litters capacity with 6 trips per day are transporting fecal sludge to treatment plant. This service is provided for all and no clear figures from which sector they transport wastewater most. For this reason, total daily truck capacity is deducted from the total volume of wastewater generated in the City. Table 2 present wastewater estimation and transportation in daily bases for the year 2021.

Table 2. Daily wastewater estimation of Hawassa City

Source type	User Category	Volume of water delivered	Consumptive use	Wastewater generated	Transported wastewater volume	Net wastewater discharge
Public Supply	Domestic	18,000	7,200	10,800	720	17,270.24
	Industry & commercial	4,500	3,600	900		
	Public	1,500	1,200	300		
In situ	Domestic	490.1	98.02	392.08		
	Commercial & service P	1,487.2	297.44	1,189.76		
	Industries & public In.	11,021	6,612.6	4,408.4		

The daily urban recharge is 21,543.216 m^3/day. From this result the annual urban groundwater recharge was estimated at 7,863,274 m^3/yr. Currently, the active urban area of the city is expected to be 55.0737 km^2 of the total expansion area of the city. Although there is a large variation in groundwater recharge in the densely populated urban area and the rural settlement within the urban boundary, the average depth of annual urban recharge in the developed Hawassa City was estimated as 142.78 mm/yr.

Natural Groundwater Recharges Components

Natural groundwater recharge is estimated using the amount of evapotranspiration and runoff from natural precipitation in the area.

Surface Runoff using SCS-CN Method

The Hawassa watershed, where Hawassa City is located, has a bimodal rainy season with the growing season extending from March to October. November, December, January and February are the dormant season months. Antecedent moisture conditions were determined for these two seasons. The LULC with soil data of Hawassa town are presented in Table 3 and Figure 3 presents FAO & IIASA Harmonized Soil Data of Hawassa City.

Table 3. LC and site-specific Soil Data of Hawassa City for SCS CN.

Land use	WR	UR	Fle	LVx	LVh	HSf	TotalArea (Km^2)
Agriculture	0.1944	0.1161	40.3353	9.2259	9.1908	10.5363	69.5988
Forest	0.0225	0.0099	1.5264	1.1817	2.0187	0.3852	5.1444
Woody vegetation	0.054	0.4068	5.1156	0.5418	0.9531	0.7542	7.8255
Scrub land	0.0072	0.0036	0.054	0.0018		0.0162	0.0828
Grass land	0.0909	1.8648	6.3855	0.027	0.0009	0.0279	8.397
Water	0.1863	0.0036	1.1817				1.3716
Built up	0.2322	12.8943	18.4572	0.6327	0.1485	0.7884	33.1533
Bare land	0.0036	0.0432	1.5867	0.2943	0.0279	0.0657	2.0214
Swamp			1.3608			20.2383	21.5991
Masking					5.3505		5.3505
Total Area(Km^2)	0.7911	15.3423	76.0032	11.9052	17.6904	32.8122	154.5444

Based on the hydrological soil group and land cover, the specific land use CN II was selected from the Lookup (USDA, 1986) table and the weighted CN II was calculated accordingly, taking into account the area coverage of the land use. According to the AMC values of the dormant and growing seasons (Chow et al., 2002), the weighted CN II was converted to weighted CN I and CN III using (Eq.10 and 11) for dry and wet conditions respectively. Using the weighted CN values, S was calculated using (Eq.9). Three weighted curve number groups were generated, for the whole urban area, the currently active urban area of the city and the rural part of the city. The weighted CN groups are presented in Table 4.

Table 4. Weighted Curve Number different parts of the City

The Whole area		Developed City		Rural area	
CN I	56	CN I	60	CN I	52
CN II	74	CN II	78	CN II	72
CN III	87	CN III	89	CN III	86

Finally, the daily runoff was calculated considering the condition that Q has a value if P is greater than 0.05S. Four years (2017-2020) of daily rainfall data were used to determine the runoff, and finally the annual average was used to determine the water balance in Hawassa City. The average runoff depth in the whole of Hawassa City was 88.63 mm/yr, which is 7.62% of the average annual rainfall depth. However, the average runoff depth in the developed part of the city was 108.4mm/yr which is 9.3% of the average annual rainfall and in the rural area was 76.5mm/yr which is 6.5% of the average annual rainfall.

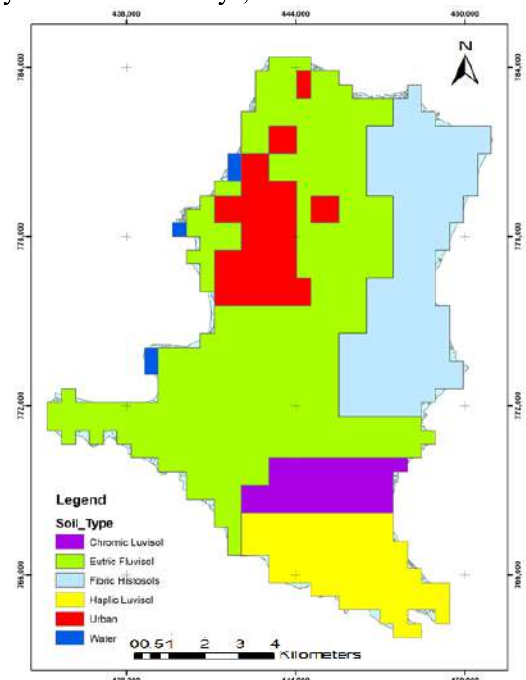

Figure 3. FAO & IIASA Harmonized Soil Data of Hawassa City

Actual Evapotranspiration Estimation

The actual evapotranspiration of a given area depends on the available precipitation. Changes in the depth of precipitation will also change evapotranspiration. With similar climate, soil and topography, higher evapotranspiration is expected in wet conditions than in dry conditions. Land Surface Temperature (LST) was derived from Landsat 8 images by the Google Earth Engine platform (Figure 4) and actual evapotranspiration calculated for different land use types (Figure 5 and Figure 6). The anchor hot (T_h) and cold (T_c) pixels temperature were 43^0C and 24^0C respectively and average ET_o was 1500 mm/year. Based on the average pixels' temperature for each land cover type ET_{fLC} and ET_{an} for each land cover type is calculated using (Eq.5) and (Eq.6). In Table (5) below is presented ET_{fLC} and ET_{an} for each land cover type.

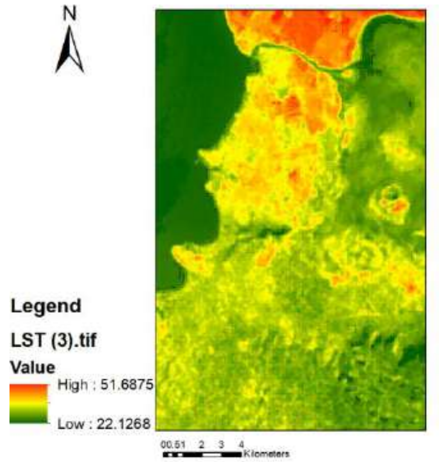

Figure 4. Land surface temperature (^{0}C) of Hawassa Area (June-November, 2020)

Table 5. Evapotranspiration fraction and Evapotranspiration for each LC

	LC	$T_{av\text{-}LC}$ (^{0}C)	ET_{fLC}	Etan (mm/yr)
1	Built up	35.23	0.37	555.00
2	Forest	29.31	0.72	1080.79
3	Woody Vegetation	33.79	0.48	727.11
4	Scrub	36.53	0.34	510.79
5	Grass land	33.58	0.50	743.68
6	Bare land	37.42	0.29	440.53
7	Agricultural land	31.24	0.62	928.42
8	Water	26.15	0.89	1330.26
9	Wetland	29.95	0.69	1030.26

The weighted evapotranspiration for the city of Hawassa was calculated using (Eq.7). Accordingly, the average actual evapotranspiration in Hawassa city was estimated to be 841.33mm/yr and the distribution based on LC is presented in the Table 6.

Table 6. Actual Evapotranspiration of Hawassa City

	LC	ETan(mm/a)	Area(km^2)	Product(mmkm^2)
1	Built up	555.00	33.1533	18400.08
2	Forest	1080.79	5.1444	5560.01
3	Woody Vegetation	727.11	7.8255	5689.96
4	Scrub	510.79	0.0828	42.29
5	Grass land	743.68	8.397	6244.72
6	Bare land	440.53	2.0214	890.48
7	Agricultural land	928.42	69.5988	64616.99
8	Water	1330.26	1.3716	1824.59
9	Wetland	1030.26	21.5991	22252.76
Total			149.1939	125,521.88

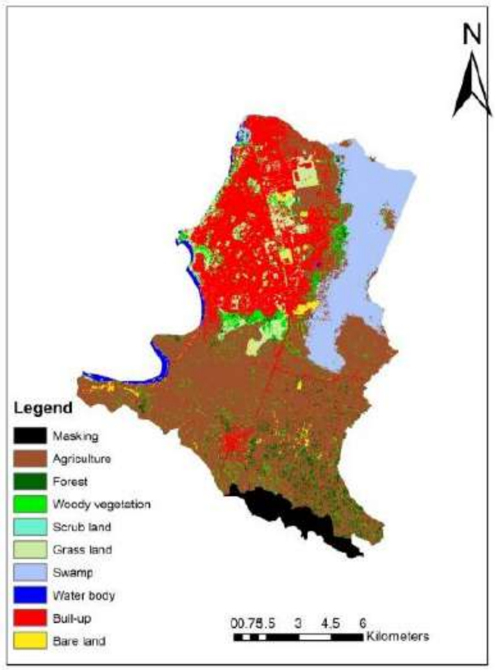

Figure 5. The 2020 LC of Hawassa City (Clipped from Wondrade, 2023)

The actual evapotranspiration of the developed city of Hawassa was estimated as 683.06 mm/yr and the distribution is presented in Table 7.

Table 7. Actual Evapotranspiration of developed part of Hawassa City

	LC	ETan(mm/a)	Area(km^2)	Product(mmkm^2)
1	Built up	555.00	30.6351	17002.48
2	Forest	1080.79	1.0044	1085.54
3	Woody Vegetation	727.11	3.8808	2821.75
4	Scrub	510.79	0.0693	35.40
5	Grass land	743.68	7.3404	5458.94
6	Bare land	440.53	1.0674	470.22
7	Agricultural land	928.42	8.7552	8128.51
8	Water	1330.26	0.7479	994.90
9	Wetland	1030.26	1.5732	1620.81
	Total		55.0737	37,618.55

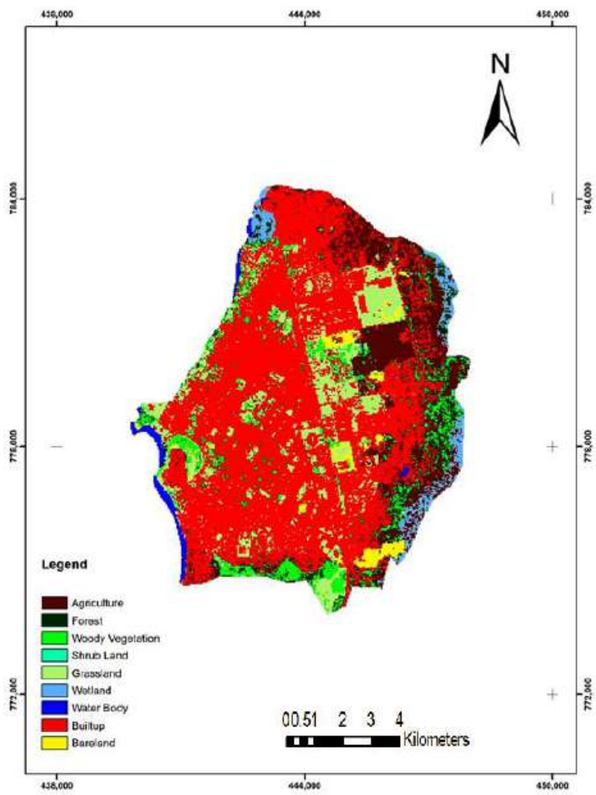

Figure 6. LC of the developed city of Hawassa modified from Wondrade (2023)

The actual evapotranspiration from undeveloped area was estimated 933.94 mm/yr. This value is greater than the value of developed area by 250.88 mm which is a lot of water. Actual evapotranspiration of the rural part of the city is presented in Table 8.

Table 8. Actual Evapotranspiration of rural part of Hawassa City

	LC	ETan(mm/a)	Area(km^2)	Product($mmkm^2$)
1	Built up	555.00	2.5182	1397.6
2	Forest	1080.79	4.14	4474.47
3	Woody Vegetation	727.11	3.9447	2868.21
4	Scrub	510.79	0.0135	6.90
5	Grass land	743.68	1.0566	785.78
6	Bare land	440.53	0.954	420.26
7	Agricultural land	928.42	60.8436	56488.48
8	Water	1330.26	0.6237	829.02
9	Wetland	1030.26	20.0259	20631.95
	Total		94.1202	87,902.67

From the above calculation, it is clear that the actual evapotranspiration from the undeveloped part of the city is greater than the total perimeter of Hawassa City and the developed part of the city because of the large area of wetlands and agricultural land in the undeveloped part of the city, which evaporates at a higher rate.

Natural Recharge of Hawassa City Aquifers

The natural recharge of the aquifer is calculated using (Eq.1). The variables included are precipitation, actual evapotranspiration and surface runoff.

The average rainfall in Hawassa city from Hawassa meteorological station for four years at the time of this research was 1163 mm/yr, and the calculated runoff from the entire city boundary was 88.63 mm/yr, evapotranspiration was 841.33 mm/yr and the natural recharge was 233.04 mm/yr. However, the generated runoff and evapotranspiration from the developed part of the city were 108.4 mm/yr and 683.06 mm/yr respectively, and for the rest of the city were 76.5 mm/yr and 933.94mm/yr respectively. From these values, the natural recharge for the developed part of the city and the rest of the city was 371.54 mm/yr and 152.56 mm/yr respectively. The volume of recharged water was 20.46Mm^3 and 14.36Mm^3/yr for the developed part of the city and the rest of the city respectively. The high evapotranspiration rate of the wetlands and agricultural areas is the reason for the lower recharge and volume.

Total Recharge of Hawassa City Aquifers

The total aquifer recharge in the city is the sum of the natural recharge and the urban recharge components (Eq.4). The average natural recharge component for the whole city was 233.04mm/yr and the urban recharge component was 52.7 mm/yr. The average total recharge for the whole city was 285.74 mm/yr. This value did not indicate the spatial variation of recharge in the developed and rural areas of the city. The variation in recharge was clearly seen when the recharge components in the developed city and the rural area of the city were evaluated separately. The separate analysis of recharge in the developed part of the city and in the rural areas showed that the natural recharge and urban recharge in the developed part of the city were 371.54 mm/yr and 142.78 mm/yr respectively; and the natural recharge in the rural area was 159.36mm/yr and the urban recharge component in this area was not known as there were no water supply data other than water supplied by the municipality. Though the Hawassa City Water Supply and Sewerage Service Enterprise provides drinking water to the rural Kebele settlements from separate wells, and the amount supplied was not greater than the consumptive use volume, this part was not included in this analysis.

The total recharge in the developed part of the city was 514.32 mm/yr. The natural recharge depth in the developed city is greater than the natural recharge in the rural part, which is controversial as urban areas are more impervious than rural areas. The reason for this is that the majority of LC in the rural area is wetlands and agriculture (Table 8), which has the highest evapotranspiration than urban areas (Liu et al., 2010), and the soil type in this area, is predominantly clay at the surface which constrain water infiltration. These two factors are the reason for the difference in recharge between the two areas. This is clearly seen that the evapotranspiration in the rural area is 933.94 mm/yr while in the developed city is 683.06 mm/yr. There is a difference of 250.88 mm/yr between the two evapotranspiration values.

Urban Water Balance of Developed Part of Hawassa City

This section presents the urban water balance of the city of Hawassa. It should be clear that the analysis is based on the city's rainfall and evapotranspiration being averages of different years, but the water supply from the municipal and in-situ sources are the same year values; and the analysis is based only on the available data. Inflow and outflow components are presented in Table 9.

The water inflow to the city of Hawassa includes rainwater, municipal drinking water supply, in-situ water supply from underground sources and truck water supply from the Lake for Urban greenery. The outflow component includes runoff, evapotranspiration, consumptive use volume, truck transported wastewater volume, and urban and natural recharge volume.

Table 9. Water balance of developed part of Hawassa City

Inflow	Symbol	Volume (Mm^3/yr)	Outflows	Symbols	Volume (Mm^3/yr)
Rainfall	P	173.51	Surface runoff	Q	13.223
Municipal water supply	WS	10.95	Evapotranspiration	ETa	125.52
Truck water supply	TR	0.24	Evapotranspiration G	ETg	0.24
In-situ water supply	GW	4.75	Transported Waste water	WW	0.263
			Consumptive use	CU	6.938
			Urban recharge	Uu	7.863
			Natural recharge	Un	34.77
			Waste water loss	WWl	0.630
Total		189.45			189.447

The amount of rainfall in the city was calculated based on the average rainfall depth and the total area of the city in the Lake Hawassa watershed. The mask area shown in Table 3 is part of the Gedabo watershed and the surface flow is not to Lake Hawassa. Municipal water supply and in-situ water supply volumes were calculated based on daily consumption rate and number of days per year. The truck water supply volume was calculated using the daily supply rate and an average of 200 days per year. The sum of all this was 189.45Mm^3/yr and is inflow component. This figure is not exhaustive as many in-situ water supply wells are not included due to lack of data.

Surface runoff (Q) and evapotranspiration (ET_a) volumes were calculated based on average runoff and average evapotranspiration and the area of the entire Hawassa city boundary, excluding the mask area. Evapotranspiration of greenery (ET_g) is the amount of water applied to the city's greenery during dry periods that is expected to be used as transpiration by plants or evaporate from roadside soils or asphalt roads. Consumptive use includes all domestic, industrial, institutional and commercial use from water supply and in-situ water sources. It was calculated from the daily consumptive use of all users and the number of days per year. Total aquifer recharge was calculated as the sum of natural and urban recharge per year and the total area of the urban boundary excluding the mask area. The wastewater transported to the drying bed and 10% of the generated wastewater volume that is expected to evaporate or transpire according to Lerner (2020) as WW and WWl were also included in the calculation. The sum of all these volumes was 189.447Mm^3/yr and considered as the outflow component of the water balance. Like the inflow volume the outflow volume is not exhaustive.

CONCLUSIONS AND RECOMMENDATIONS

In this study, it is observed that the groundwater recharge of the city of Hawassa is different from recharge in rural areas. Large volume of water supplied from different water sources for different water users within a limited area, and the associated leakages from the supply system and the outflow from different users as effluent are the causes of this change. In a city like Hawassa with no sewerage systems and wastewater treatment facilities, low quality water as effluent is recharging the underground. This continuous recirculation of water in the city has incremental effect of contaminating the groundwater. In Hawassa case, the urban groundwater recharge is estimated as 142.78L/m^2 area which is a lot that has a potential to contaminate the major water source for the city. Additionally, as there are water abstractions hotspots at different part of the city there is also expectation of recharge mounds at different locations based on the volume of effluents discharged in to the underground through septic tanks and/or soak away pits. This phenomenon is seen in Condominium residential areas of the city.

In water balance analysis it is clear that estimating evapotranspiration and total recharge taking the whole city boundary as one unit and separating developed part of the city from rural areas shows a great difference which is an important insight in integrated water resource planning. With increasing groundwater abstractions and implementation of the planed Sewerage systems in the developed city will bring increasing static water table depth from the surface and might abandoned shallow water wells in the future.

Therefore, to maintain water availability and increase groundwater quality in the future it is imperative to create awareness in the society about quality and quantity limitations and enforce regulatory measures with planning water resources augmentation measures. In Hawassa City, there are no established monitoring wells for water quality and groundwater level measurements, no inventory of boreholes, and no limits on the amount of water that can be abstracted from the ground. The system needs to be established urgently as it is vivid that the hot spot of high-water abstraction is located in the main water source area of the city.

CONFLICTS OF INTEREST

Authors declare that they have no conflict of interest regarding the publication of this paper.

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Journal of Science and Development
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