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Mastitis in Lactating Cows, Camels and Goats in Borana, Southern Ethiopia

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Abstract

Mastitis is a complex and multi-factorial disease, the occurrence and severity of which depends on factors related to the animal, the environment and pathogens involved. Mastitis reduces the quality and quantity of milk, and is a disease of great economic and public health importance. A cross-sectional study was conducted to estimate the prevalence of mastitis and identify the associated risk factors in cows, camels and goats in Yabello district of Borana zone, Southern Ethiopia. A total of 980 lactating animals (400 cows, 370 camels and 210 goats) were sampled and examined clinically for mastitis presence. Milk samples were further tested by California Mastitis Test (CMT) to detect subclinical mastitis. The study results revealed that 49.75% of the cows, 35.41% of camels and 24.76% of goats had udder infections. Cows had significantly higher mastitis prevalence than camels and goats. Quarter/halve level prevalence was 24.94% in cows, 12.64% in camels and 15.71% in does while 76 teats (63 in cows and 13 in camels) were found to be blind. Risk factors identified for cows were age above five years (OR=5.75), parity of 3 and above (OR=3.13), early lactation stage (OR=0.49), animals having teat lesions (OR=1.50) and teat affected by ticks (OR=2.3). Similarly, early lactation stages, udder tick infestations and teat lesion were the risk factors for camels. Mastitis prevalence was higher in multifarious goats, does above five years, and those with udder ticks and teat lesions. In conclusion, mastitis was widely prevalent in lactating cows, camels and goats in the pastoral area, where milk is the major staple food. Hence, proper control strategies and awareness of livestock keepers are needed to reduce mastitis prevalence and its negative impacts on milk production in the study area.

Key words: Cattle; Camels; Goats, Mastitis, Risk factors, Ethiopia.

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INTRODUCTION

Ethiopia holds large potential for dairy development due to its large livestock population and suitable agro-ecology of the country. In Ethiopia, milk production is traditionally from indigenous breeds, whose milk yield per animal/lactation is affected by multifaceted constraints (Felleke and Geda, 2001). Data of central statistical authority of Ethiopia (CSA, 2009) showed that cattle have the largest contribution (81.2%) of the total national annual milk output in Ethiopia, followed by goats (7.9%) and camels (6.3%). In spite of the substantial potential, the dairy sector is not developed to the expected level. Per head productivity of lactating animals has always been sub-optimal given the low genetic potential of the animals, poor nutrition and prevailing diseases.

Mastitis is a multi-etiological and complex disease, which can be defined as inflammation of the parenchyma of mammary glands featured by physical, chemical and pathological changes taking place in glandular tissues. The occurrence of disease is an outcome of interplay between infectious agents, host and environmental factors (Radosttits et al., 2007; FAO, 2014). Mastitis is one of the most important economically devastating diseases of dairy animals affecting particularly farmers in developing world, with different levels of economic losses (Hogeveen et al., 2011). It results in severe economic losses from reduced milk production, value of discarded milk, treatment and labor costs, and milk withdrawn following treatment in addition to causing premature culling. In view of the degree of inflammation, mastitis can be classified as clinical and sub clinical. Depending on the organisms involved, mastitis is classified as contagious or environmental (Quinn et al., 2004). Various risk factors have been identified for mastitis in dairy animals such as breed, milk production level, hygiene, milking practices, age, parity and stage of lactation (Radostitset al., 2007). The diagnosis is performed by clinical examination (inspection and palpation) for clinical mastitis, California Mastitis Test (CMT) for subclinical form of mastitis, and bacterial isolation for confirmatory diagnosis (Quinn et al. 2004).

Although some authors reported the prevalence of mastitis from different animals in pastoralist systems in Ethiopia (Megersa et al., 2010; Adane et al., 2012; Regassa et al., 2013), there is still dearth of compiled research reports regarding the magnitude and associated risk factors of mastitis among lactating cows, camels and goats kept under pastoral system in the same eco-zone. Therefore, the objectives of the study were to estimate prevalence of clinical and sub clinical mastitis among lactating cows, camels and goats and factors associated with mastitis occurrence in the study area.

MATERIALS AND METHODS

General description of the study area

The study was conducted in Yabello district, Borana zone, Oromia Regional State, southern Ethiopia. Yabello district is geographically located at 5° 23′ 49″ N, 39° 31′ 52″ E, and at a distance of 564 km South of Addis Ababa. The Borana area is characterized by arid and semi-arid conditions and by a pastoral/agro-pastoral production system.Cattle (232 949) are dominating animal species followed by goats (98 781), sheep (39 073) and camels (22 972) (Coppock, 1994).

Study population, study design and sampling procedures

Lactating cows, camels and goats which were managed under extensive husbandry systems in the selected pastoral associations (PAs') of the study area constituted the targeted population. A cross-sectional study was conducted from August 2016 June 2017 to estimate prevalence of mastitis and determine associated risk factors among lactating cows, camels and goats mastitis. Four administration kebeles or pastoral associations (PAs), namely, Darito, Dida-Yabello, Gagna and Surupawere randomly selected out of 18 Kebeles of the district, and included in the present study. Households from selected PAs were included in the study based on at least two species ownership and their willingness to take part in the study. Subsequentlylactating cows, camels and goats from households were sampled and examined for mastitis and individual milk samples were tested for subclinical mastitis using California Mastitis Test.

Sample size determination

The sample size was determined using the formula given by Thrusfield, (2005) by taking the species specific prevalence from the previous studies in same area such as 59.1% (Adane et al., 2012), 44.8% (Regassa et al., 2013) and 15.5% (Megersa et al., 2010) for cows, camels and goats, respectively. Likewise, confidence level of 95% and desired absolute precision 5% were used based on the following formula:

$$n = \frac{1.96^2 * (P * (1 - P))}{d^2}$$

Where n=required sample size,
P=expected prevalence,
d=desired precision

An attempt was made to increase the sample size by 5% to compensate any loss during sample collection and processing. Eventually, a total of 980 dairy animals (400 cows, 370 camels and 210 goats) were sampled and examined or tested for mastitis. The calculated sample size was proportionally allocated to the respective kebeles based on the estimated total target population.

Data collections

Variables considered as risk factors were age, parity, lactation stage, presence of ticks and lesions on udder/teat. Age of the study animals were determined by the information from the owner and dentition characteristics (Abegaz and Awgichew, 2009; Andrews, 2015). Three level groupings were made for age, parity and stage of lactation respective to the three species whereas tick infestation and udder/teat lesion were recorded as dummy variable i.e. present or absent of the case. Tick infestation was considered as tick positive when one or more ticks were present on teat/udder.

Udder examinations

The udder was inspected and palpated to detect fibrosis, inflammatory swellings, visible injury and swelling of supra mammary lymph nodes. The size and consistency of teats were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness and blindness. Tick infestation and presence of teat lesion were also noted. Physical appearance of milk including color, consistency, and viscosity, presence of clots, flakes, blood and watery secretions were used to check for clinical mastitis (Radostits et al., 2007).

California mastitis test (CMT)
Milk samples were examined for visible abnormalities and screened by CMT according to Quinn et al. (2004). A squirt of milk, about 2 ml from each teat was placed in each of shallow cups in the CMT paddle after discarding the first few streams of milk from each teat. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The interpretation was in such a way that CMT score of 0 was taken as negative, while CMT scores of trace, 1+, 2++ and 3+++, were considered positive. Cows, camels and goats were considered positive for subclinical mastitis, when at least one teat of an animal was positive in the CMT.

Statistical analysis
The collected data were checked for completeness and consistency, coded and entered to Excel spread sheet. Different variables were descriptively summarized as proportion or mean, using STATA software version 12 (College Station, TX, USA). Associations between the occurrence of mastitis and potential risk factors were analyzed by univariable logistic regression. Finally, variables with P-value <0.25 were subjected to multivariable logistic regression analysis. We checked multicollinearity among the predictor variables, and those with correlation coefficient below 0.6 were included in the model. Finally, post estimation was evaluated using Hosmer Lemeshow goodness of fit test. The strength of association between the risk factors and the prevalence of mastitis was analyzed using the odds ratio and for all the analysis performed, $p<0.05$ was taken as statistically significant.

RESULTS

Table 1 shows results of mastitis prevalence at animal and quarter levels stratified by animal species. Accordingly, out of a total 400 lactating cows examined, 199 cows had udder infections (49.75%, 95% CL: 44.7-54.8%). The prevalence of clinical and subclinical mastitis were 7.5% and 42.25%, respectively. Out of 1600 cow quarters examined, 63 (3.94%) and 44 (2.75%) of quarters were blind or clinically positive, respectively.

The overall mastitis prevalence in camel was 35.41%, 95% CL: 30.5-40.5) out of which, 3.51% and 31.89% were clinical and sub-clinical cases, respectively. From a total of 1480 camel quarters examined 13(0.89%) of the teats were blind, while 22 (1.49%) and 165 (11.15%) of the quarters were positive for clinical and subclinical mastitis, respectively.

Lactating does had a lower mastitis prevalence of 24.76% (95% CL: 19.1-31.2%) with clinical and subclinical cases being 2.38% and 22.38%, respectively. Studied does had no blind teats unlike other animals. The overall udder halves level prevalence of mastitis was 15.7% (95% CL: 12.4-19.6). Overall, significantly higher mastitis prevalence was observed in cows than camels and goats, but no difference was observed between the later groups.

Table 1. Prevalence of clinical and subclinical mastitis at animal and quarter/halve levels

Species	Animals (N)	Blindteat N(%)	Clinical N(%)	Subclinical N(%)	Total N(%)	95% CI
Animal level						
Cows	400	51 (12.8)	30 (7.5)	169 (42.3)	199 (49.8)	44.7-54.8[a]
Camels	370	11 (2.9)	13 (3.5)	118 (31.9)	131 (35.4)	30.5-40.5[b]
Goats	210	0	5 (2.4	47 (22.4)	52 (24.8)	19.1-31.2[b]
Quarter level						
Cattle	1600	63 (3.9)	44 (2.8)	355 (22.2)	399 (24.9)	22.8-27.1 [a]
Camels	1480	13 (0.9)	22 (1.5)	165 (11.2)	187 (12.6)	11.0-14.4 [b]
Goats	420	0	6 (1.4)	60 (14.3)	66 (15.7)	12.4-19.6[ab]

[ab] Prevalence with different superscriptions differ significantly

Risk factors associated with mastitis prevalence in different animals are shown below for cows (Table 2), camels (Table 3) goats (Table 4). Risk factors analysis revealed that mastitis was significantly associated with tick infestation and teat lesion in all three species. The odds of udder infections were respectively two folds, 10 and 38 times more in lactating cattle, camels and goats with udder tick infestations compared to animals with tick free udders. Likewise, lactating cows, camels and goats with udder or teat lesion had 12, 1.5 and 3.2 times higher odds of mastitis occurrence than their counterparts.

Table 2. Multivariable logistic regression of risk factors associated with cow mastitis

Risk factors	Animals (N)	Positives (N)	Prevalence (%)	Odds Ratio	P-value
Kebeles					
Darito	90	43	47.8		
Dida-Yabello	87	52	59.8	1.93	0.046
Gagna	94	41	43.6	0.88	0.690
Surupa	129	62	48.1	0.87	0.650
Age (yrs)					
3-4	201	75	37.3		
≥ 5	199	123	61.8	5.75	0.000
Parity					
1st	92	39	42.4		
2nd	104	45	44.2	1.64	0.128
3rd	99	51	51.5	3.13	0.010
4thand above	105	64	60.0	2.94	0.028
Lactation(month)					
Early (1 to 3)	119	69	58.0		
Mid (4 to 7)	140	54	38.6	0.49	0.008
Late (≥ 8)	141	75	53.2	0.83	0.499
Teat tick					
Present	98	63	64.3	2.30	0.002
Absent	302	135	44.7		
Teat lesion					
Present	132	78	59.1	1.50	0.044
Absent	268	120	44.8		

Mastitis occurrences have significantly increased with age and parity of the animals in cows and goats, while no difference was observed for camels. The likelihood of udder infection was 5.8 and two times more in cows and does in age group of five and above years. When Kebeles were compared, prevalence was higher in Didayabello than other kebeles for cattle, but the differences were not significant for camels and goats. Prevalence was higher in early lactation stage for both cattle and camels.

Table 3. Multivariable logistic regression of risk factors associated with camel mastitis

Risk Factors	**Animals (N)**	**Positives (N)**	**Prevalence (%)**	**Odds Ratio**	**P-value**
Kebeles					
Darito	116	39	33.6		
Didayabello	84	25	29.8	0.95	0.884
Gagna	77	32	41.6	1.93	0.080
Surupa	93	35	37.6	1.31	0.463
Age (years)					
4 to 5	135	41	30.4		
6 to 7	117	42	35.9	1.77	0.317
≥ 8	118	48	40.7	1.82	0.407
Parity (number)					
1	110	35	31.8		
2	107	34	31.8	1.37	0.586
3and above	153	62	40.5	1.33	0.694
Lactation (month)					
Early(1 to 3)	132	72	54.6		
Mid (4 to 7)	118	35	29.7	0.45	0.014
Late (≥ 8)	120	24	20.0	0.28	0.000
Teat ticks					
Present	52	44	84.5	9.87	0.000
Absent	318	87	27.4		
Teat lesion					
Present	56	48	85.7	12.37	0.000
Absent	314	83	26.4		

Table 4. Multivariable logistic regression of risk factors associated with goat mastitis

Risk factors	Animals (N)	Positives (N)	Prevalence (%)	Odds Ratio	P-value
Kebeles					
Daritu	54	15	27.8		
Didayabello	52	12	23.1	1.18	0.759
Gagna	53	12	22.6	0.66	0.483
Surupa	51	13	25.5	1.33	0.606
Age (years)					
2 to 4	112	21	18.8		
5-above	98	31	31.6	2.04	0.041
Parity (number)					
1st and 2nd	74	11	14.9		
3rd and 4th	69	13	18.8	1.13	0.835
5thand above	67	28	41.8	18.64	0.003
Lactation (month)					
Early (1 to 2)	73	23	31.5		
Mid (3 to 4)	58	12	20.7	0.68	0.441
Late (≥ 5)	79	17	21.5	0.66	0.362
Teats Ticks					
Present	19	17	89.5	37.88	0.000
Absent	191	35	18.3		
Teats lesion					
Present	23	14	60.9	3.32	0.040
Absent	187	38	20.3		

DISCUSSION

The study showed a high overall cow level mastitis prevalence of 49.8% (95%CI: 44.7%-54.8%), with higher subclinical than clinical mastitis (42.3% vs7.50%). These results are comparable to the 50% prevalence reported from North Shewa and Borana zones of Ethiopia (Belina et al., 2016). The prevalence is higher than some of the reports from Ethiopia such as 34.3% by Megersa et al., (2012) from Hawassa town and 42% by Serda and Dame, (2015) in Arsi Zone. But a higher prevalence as 59.1% (272/460) was also reported by Adane et al., (2012) from Borana area. Observed higher subclinical mastitis (42.25%) than clinical mastitis (7.5%) is what was anticipated and in line with other study reports (Mekibib et al., 2010;Adane et al., 2012; Moges et al., 2012). Such a greater proportion of subclinical mastitis occurrence compared to clinical is attributable to the defense mechanism of the udder that makes it unnoticeably undergoes pathological changes in the mammary gland, while negatively affecting milk production (Radostits et al., 2007). The sub-clinical form can be as large as 15 to 40 times more prevalent compared to clinical form, and usually precedes the clinical form and is of long duration (FAO, 2014). This makes clinical mastitis is the 'tip of the iceberg' whileSubclinical mastitis, invisibly large proportion analogous to the submerged ice, is by far the more costly disease in the dairy of herds. Thus, it can silently result in reduction of milk yield and substantial economic losses without being noticed by the livestock keepers. Herders have no chance of detecting the disease until it develops to clinical forms that can be treated and cured. Consequently, sub-clinical cases remain a continuing source of infection for susceptible animal in the herd results in accumulation of cases as spontaneous self-clearance is very low. High level of subclinical cases generally suggests absence of herd health monitoring and testing in tradition husbandry system which otherwise would allow early detection of cases and implementation treatments (Karimuriboet al., 2006). Lack of awareness on mastitis control and prevention strategies (e.g. poor hygienic practices and poor milking practices) as well as misconceptions among pastoral herders also contribute to constant transmission of infections among susceptible animals (Amenu et al., 2017).

High prevalence of mastitis among lactating animals, especially in dominant livestock species such as cattle suggests its plausible impact on pastoral livelihood through reduction of milk for family consumption and household income from dairy sale. Intra mammary infection, at subclinical levels, has been reported to affect milk production negatively, mainly due to physical damage to the mammary parenchyma of the affected mammary gland (Radostits et al., 2007). According to FAO (2014), in areas with poor animal health service and lack of treatment means at traditional farming, mastitis occurrence increases causing decrease in milk yield of up to 33% per infected quarter, and poses a public health risk due to consumption of unsafe milk. Though there is no study that quantify the economic impacts mastitis in pastoral areas, reports from other region showed the economic losses due to mastitis to range from 61 to 198 Euro per cow per year (Hogeveen et al., 2011). In addition to reducing milk production, mastitis causing pathogens or their toxins can pose health risk to humans. Thus the high level of mastitis prevalence in pastoral area implicate its paramount importance in food security and public health perspective as the pastoralists are highly dependent on dairy consumption (Amenu et al., 2017).

The prevalence of camel mastitis (35.41%, 95% CI: 30.5%-40.5%)is comparable with the findings of Jemal et al. (2017) from Eastern Ethiopia and Wubishet et al. (2016) from Borana area who found an overall prevalence of 31% and 34.7%, respectively. In contrast, another study reported a higher proportion (44.8%) of mastitis cases in camels in Borana areas (Regassa et al., 2013) while Husein et al. (2013) reported a lower figure of 30.2%fromJijiga.Similarly, observed occurrences udder infection of goats (24.76%, 95% CI: 19.1-31.2%)is higher than previous reports of15.5% (Megersa et al., 2010) from Borana, 18% (Gebrewahid et al., 2012) from Tigrai and 17.8% (Wazha, 2015) from Botswana. The difference in prevalence may be due to concurrent disease involvement (tick infestation), animal health care, agro-ecology, climate, production system that vary from place to place. As mastitis is a complex disease involving interactions of various factors such as management and husbandry, environmental conditions, animal risk factors and causative agents, its prevalence varies considerably (Radostitset al., 2007).

Association of mastitis with increasing age is in line with reports from elsewhere (Adane et al., 2012; Zeryehunet al., 2013; Serda and Dame, 2015; Qayyum et al., 2016). The increasing prevalence of udder infection with an increase age is due to reduced physiological defense mechanism of the udder and relaxed sphincter with advancing age to overcome bacterial pathogens, so that pathogenic organisms get access to the glandular tissue and cause inflammation of mammary glands (Radostitset al., 2007). As animals get older their teats become enlarged and have more relaxed sphincter muscles being subjected to constant suckling by a number of calves, which increase the accessibility and entrance of infectious agent into the teat canal. Similar to age, animals with higher parity were at increased risk of infection which is in line with the finding of Zeryehun et al. (2013). Moreover, occurrence of more cases with age and parity could be due to the fact that mastitis case are not well treated in pastoral areas, so that the disease becomes chronic with the possibility of carryover of infection from one parity to the next (Abdurahman 2006;Megersa et al., 2010).

Early lactation stage had higher relative prevalence (58%) than mid (38%) lactation stage similar to reports elsewhere (Adane et al., 2012; Zeryehun et al., 2013). Absence of dry cow therapy regime can reduce capacity of the quarter to provide phagocytic and bactericidal activity during early lactation and could possibly attributable to high prevalence of mastitis. Higher prevalence during early stages of lactation could be linked to several factors such as high milk production and more favorable conditions for infection occurrences in addition to low immune response related to calving stresses. Most new infection occurs during the early part of dry period and in the first two months of lactation, especially with environmental pathogens (Radostits et al., 2007).

In addition to the above mentioned factors, tick infestation and udder/teat lesions were significantly associated with increased udder infections in all lactating animals examined, which is reasonable given the lack of primary defense barrier to reduce bacterial colonization and infections. Udder or teats of animals in pastoral area are prone to thorny bush injury or inflicted by ticks that are responsible cause of lesions and predisposing udder to bacterial invasion. Additionally, use of anti-suckling device, fibers from plants or strip of cloth are tied to the teat to prevent the calf from sucking camel is common in pastoral areas that may damage the teat (Abera et al., 2010). According to Viguier et al. (2009), injuries and lesions affecting the teat end frequently result in increased mastitis problems because of interference with the protective effect of the teat orifice, which is a major barrier preventing bacteria from entering the gland. Teat injuries provide a medium for the growth of the pathogenic bacteria and secondary infections which may induce mastitis and or delay healing of mastitis.

CONCLUSIONS

The study revealed high prevalence of mastitis in lactating cows, camels and goats in the Borana, southern Ethiopia, and confirms that the subclinical form is the most prevalent compared to clinical cases in all species studied. The high prevalence of mastitis in a pastoral region implies its negative effect on milk production and consumption where dairy is a major staple food. Observed association of risk factors with prevalence of mastitis in lactating cows, camels and goats, suggest the need to consider them mastitis control program. Therefore, to reduce the occurrence of udder infections among lactating animals in the study areas, appropriate control measures targeting improved animal health service, improved management and milking practices, tick control, and the setting up of monitoring for subclinical mastitis through CMT are needed.

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Surface Energy Balance Algorithm for Land (SEBAL) based Evapotranspiration Estimation in Lower Gilgel Abay Catchment Lake Tana Sub-Basin, Ethiopia

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Abstract

On land, Evapotranspiration (ET) plays an important role in the water cycle and is an important parameter in water resources management. Remote sensing is one of the important sources of data and techniques to estimate many climate elements including evapotranspiration. The estimation based on remote sensing is vital for the management of water resources in the catchment. This study estimated the spatio-temporal variation of evapotranspiration in the lower Gilgel Abay catchment, Lake Tana sub-basin from January to March 2016. The evapotranspiration was quantified using the Surface Energy Balance (SEBAL) algorithm and Landsat 8 imagery with climate data. For this analysis, ASTER GDEM, GRASS-python & reference weather parameters from Bahir Dar weather station were used. Parameters including surface radiance, surface reflectance, surface albedo, NDVI, LAI, surface emissivity, surface temperature, net radiation, soil heat flux, sensible heat flux, and latent heat flux were computed. Consequently, the hourly, daily, monthly and seasonal evapotranspiration in the study area were calculated with SEBAL python. The pixel wise calculation shows that the values of the spatial variation of mean ET varied from 0 mm/day to 7.39 mm/day with a mean value of 4.78 mm/day for 23 December 2016. The computed ET values for the months December to March, the maximum estimated ET over the whole catchment ranged from 6.51 mm/day to 7.82 mm/day. The mean ET ranged from 4.37 mm/day to 4.78 mm/day while the seasonal ET was 539.92 mm for 2016. ET values were computed for different conventional methods using REF-ET software. The value of Standard P-M for the weather station was used as a reference to compare the values obtained from other conventional methods as well as SEBAL method. The mean value of the study area from SEBAL calculation approached the point values of CIMIS penman, standard P-M and Priestly Taylor methods. The analyses are vital from the perspective of water resources management on various surfaces of the earth that need to be understood to achieve sustainable development of water resources in the basin and recommended to apply in the remaining sub-basins in the region.

Keywords: Evapotranspiration, Gilgel Abay, GRASS-GIS, Landsat-8, RS, SEBAL.

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INTRODUCTION

The ability to predict evapotranspiration (ET) levels is a valuable asset to manage water resources. ET is a good indicator of the effectiveness of irrigation and the total water consumption of vegetation. Evapotranspiration information is useful for irrigation supply planning, regulation of water rights, and watershed studies (Waters, et al., 2002). ET is important in predicting soil water availability, flood forecasting, rainfall forecasting, and projecting changes in occurring heat waves and droughts (Merlin, et al., 2014). With the use of remote sensing, it is possible to directly derive the water consumed as ET without the need to quantify other complex hydrological processes (Trezza, et al., 2013).

Accurate estimation of ET plays an important role in the quantification of the water balance at the level of basin, river basin, and regional scale for better planning and management of water resources. Accurate quantification of ET in irrigated agriculture is crucial to optimize crop production, water distribution planning, irrigation management, assessment of the effects of land-use change on water yields, development of best practices of management to minimize the degradation of surface and groundwater and assessment of land use and practices of water resources management and environmental quality (Irmak, et al., 2006a; 2006b). Several methods are available to estimate ET: Bowen Ratio energy balance (BREBS) systems, weighing lysimeters, and water balance techniques offer powerful alternatives for measuring ET and

other energy flows on the surface. Despite the elegance, high precision, and theoretical attractions of these techniques for measuring ET, their practical use in large areas could be limited.
Agriculture is the backbone of the Ethiopian economy. Therefore, to maximize agricultural production for domestic use and to complement the industry's leading economy, irrigation is the non-alternative option. Hence, wise use of limited water resources, reliable maps of surface energy fluxes for assessing surface-atmosphere interaction and knowledge, and proper quantification of evapotranspiration are important to maximize production with better irrigation management practices.

The evaporation flux was commonly estimated through hydrological models. ET is highly variable in both space and time due to the wide spatial variability of precipitation, hydraulic characteristics of soils, and vegetation types and densities. It is variable in time due to the variability of climate. Satellite images provide an excellent means for determining and mapping the spatial and temporal structure of ET. The estimation of evapotranspiration using remote sensing-based approaches is largely unexplored research in the study area.

Therefore, to overcome this problem, satellite-based evapotranspiration estimation was employed in the lower part of Gilgel Abay catchment, Lake Tana sub-basin. The catchment contributes much of the water into the Lake Tana. Consequently, the evapotranspiration estimation is vital from the perspective of accurate water balance of the Lake Tana and water resources planning in the catchment. The main objective of this study was, therefore, to estimate evapotranspiration using Surface Energy Balance Algorithm for Land /SEBAL and landsat-8 data using SEBAL-python and GRASS-GIS. Consequently, it enables us to know the amount of water used by the evapotranspiration process over specified months in the catchment.

STUDY AREA

The lower part of Gilgel Abay catchment is located in the Northwest highlands of Ethiopia between 259026.33 m to 311468.19 m N latitude and 1256107.36 m to 1306929.16 m E longitudes (
Figure 1). The study area covers a total area of 1,743.47 km^2 upstream of Lake Tana. The Gilgel Abay River is contributing about 60% of the inflow into Lake Tana. (Tessema, 2006).

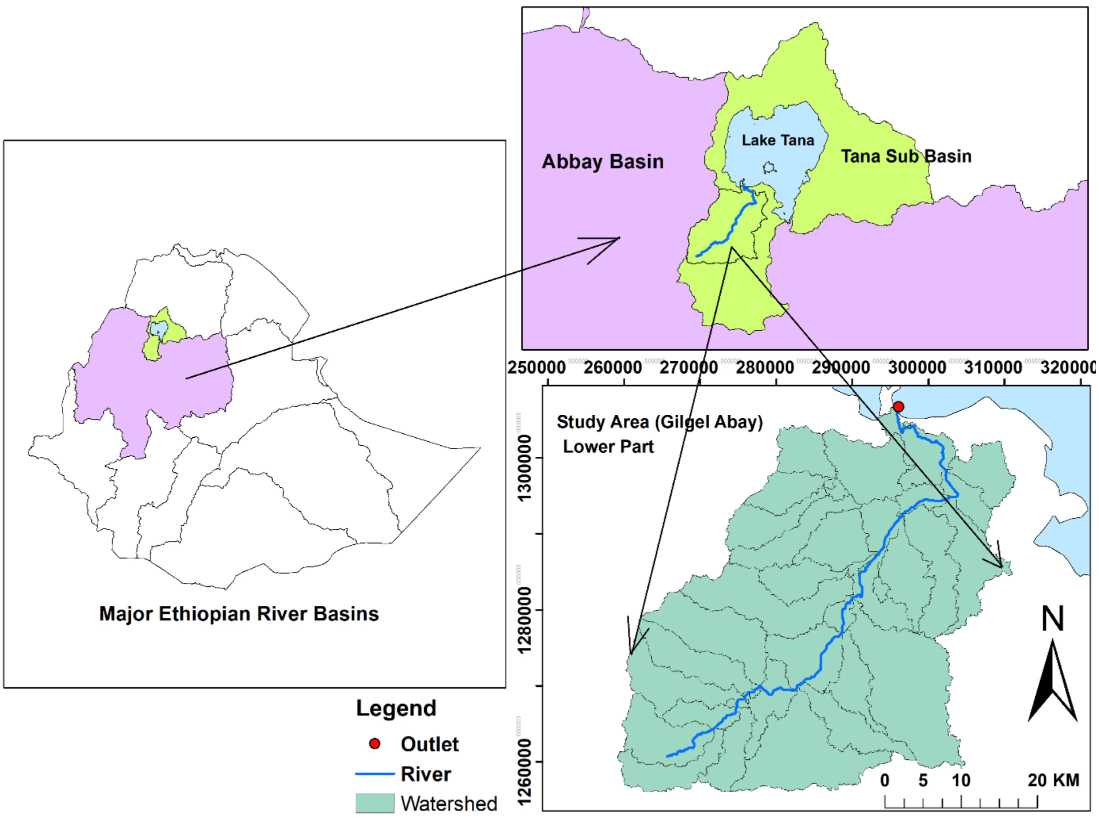

Figure 1. Location Map of the Study area

The elevation of Lower Gilgel Abay catchment varies from 1699 to 2667 m a.s.l. The slope map of the study area was derived from DEM and varies from 0 to 50%. In general, the annual average temperature of the Gilgel Abay basin falls in the range of 16 ^{0}C to 20 ^{0}C (Kebede, 2009). Based on BCEOM (1998) the dominant soil type is Halpic Luvisol which covers 37.52%.

MATERIALS AND METHODS

To estimate ET cloud-free Landsat 8 scene (LS8 - OLI/TIRS) including MTL file for Dec 23, Jan 22, Feb 07, and Mar 10/2016 with Path: 170 and Row: 52 and reflectance of each band, Digital Elevation Model (DEM) from ASTER UTM format, Re projected the DEM and renamed to MDT_Sebal.TIF, shape file of the study area and SEBAL_GRASS-Master file all were organized in the same datasets to run the algorithm. The land use/cover classification was derived from www.geoportal.rcmrd.org and nine land-use types were identified for the study area. Annual cropland use types occupy the largest percentage of the study area (more than 88%). The accuracy of the classified map was validated by different techniques like ERDAS Imagine with google earth, QGIS with open layer plugins (google satellite, google hybrid, and Google Street), and google earth tools. The SEBAL algorithm (Bastiaanssen, et al, 1998; 2005) was used to compute the evapotranspiration.

Atmospheric correction models to calculate reflectance, surface albedo, emissivity, surface temperature and elements of the solar radiation balance, including net radiation, soil heat flux, sensitive heat flow, latent heat flow, and finally calculate instantaneous and daily evapotranspiration values were calculated using GRASS-GIS software with the help of GRASS-python file developed by Wagner (2017). The methodological framework used in the research is provided in Fig. 2.

SEBAL MODEL

In the SEBAL model, ET was computed from satellite images and weather data using the surface energy balance equation. Since the satellite image provides information for the overpass time only, SEBAL computes an instantaneous ET flux for this overpass time. The ET flux was, then, calculated for each of the pixels of the image as a “residual” of the surface energy budget equation:

$$\lambda ET = R_n - G - H \text{ ------------------------------ (1)}$$

Where: λ ET is an instantaneous value (latent heat flux) for the time of the satellite overpass (W/m^2). Rn is the net radiation flux at the surface (W/m^2), G is the soil heat flux (W/m^2), and H is the sensible heat flux to the air (W/m^2).

The net radiation flux at the surface (Rn) represents the actual radiant energy available at the surface. It was computed by subtracting all outgoing radiant fluxes from all incoming radiant fluxes. This is given in the surface radiation balance equation:

$$Rn = RS\downarrow - \alpha\, RS\downarrow + RL\downarrow - RL\uparrow - (1-\varepsilon o)\, RL\downarrow \text{------(2)}$$

RS↓ is the incoming shortwave radiation (W/m2), α is the surface albedo (dimensionless), RL↓ is the incoming longwave radiation (W/m^2), RL↑ is the outgoing longwave radiation (W/m^2), and εois the surface thermal emissivity (dimensionless).

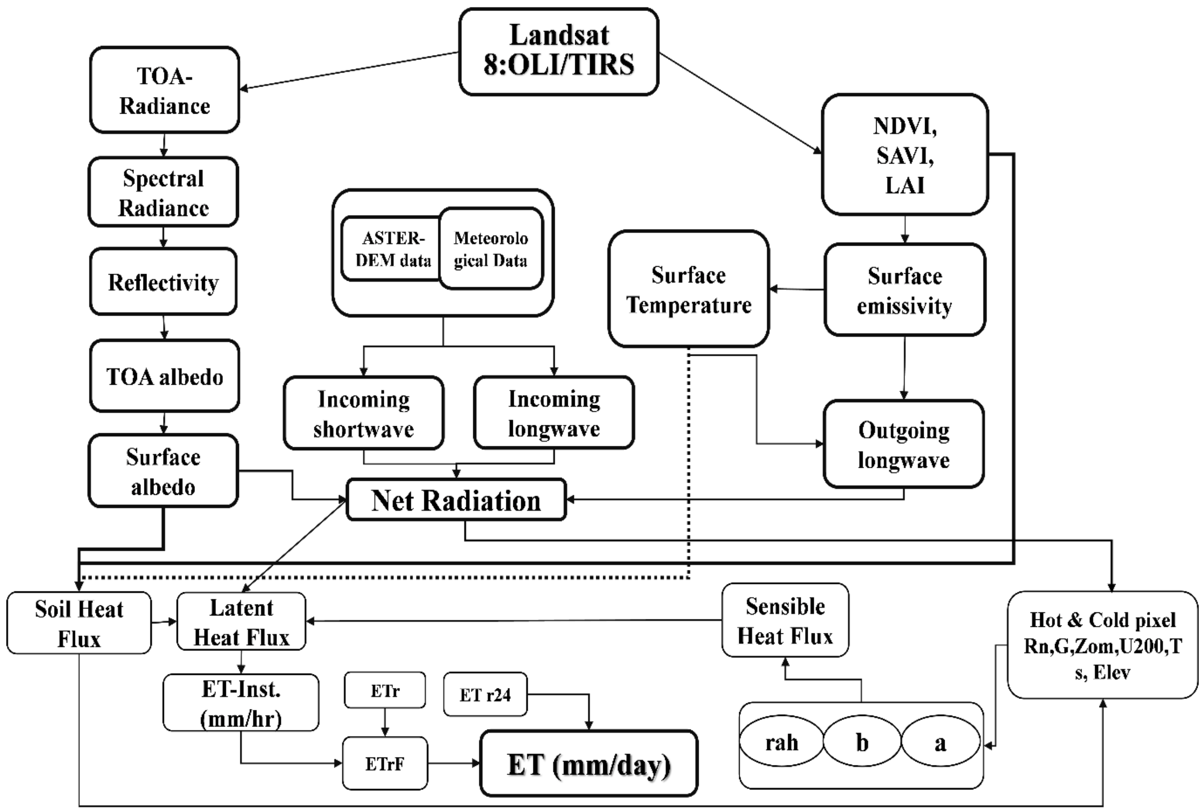

Figure 2. General methodological flow chart

Calculation of Surface Radiation elements

The net surface radiation flux (Rn) was computed using the surface radiation balance equation. Atmospheric correction for solar radiation elements is important in remote sensing analysis; its necessity depends on the objectives of the analysis. The analysis of this study was carried out using semi-automatic classification plugin /SCP/ in QGIS software.

Surface albedo was computed by the equations mentioned in Tasumi, et al. (2008) and shown below.

$$\alpha = \frac{\alpha_{TOA} - \alpha_{Path\_radiance}}{\tau_{sw}^{2}} \text{ --------------------(3)}$$

Where; *αpath_radiance* is the average portion of the incoming solar radiation across all bands that is backscattered to the satellite before it reaches the earth's surface, and ranged from 0.025 to 0.04 in SEBAL and 0.03 is recommended.

τsw is shortwave atmospheric transmissivity and calculated using the equation

$$\tau_{sw} = 0.75 + 2\times10^{-5} \times z \text{ --------------------------- (4)}$$

Where z is an elevation of an area defined by ASTER GDEM Data (MDT_Sebal).

Vegetation indices

Three commonly used vegetation indices (NDVI, LAI, and SAVI) were computed using the reflectivity values in GRASS-SEBAL. Those indices were used to calculate the outgoing longwave radiation through a function of surface temperature and land surface emissivity.

Normalized Difference Vegetation Index (NDVI), and Leaf Area Index (LAI) were calculated by visible and near-infrared bands (Waters, et al., 2002; Lillesand, et al., 2004). For Landsat-8, NDVI is a sensitive indicator of the amount and condition of green vegetation. Values for NDVI range between -1 and +1. Green surfaces have NDVI between 0 and 1 and water and cloud are usually less than zero.

$$NDVI = \left(\frac{NIR - RED}{NIR + RED}\right) \text{ ---------------------------(5)}$$

Soil adjustment Vegetation Index (SAVI) is an index that attempts to "subtract" the effects of background soil from NDVI so that impacts of soil wetness are reduced in the index. It is computed as (Waters, et al, 2002).

$$SAVI = \left(\frac{(1+L)(NIR - RED)}{L + NIR + RED} \right) \text{----------------- (6)}$$

Where; L is a constant for SAVI. If L is zero, SAVI becomes equal to NDVI. A value of 0.5 frequently appears in the literature for L (Waters, et al, 2002).
Leaf Area Index (LAI), the ratio of the total area of all leaves on a plant to the ground area represented by the plant, was computed following the empirical equation by Weng, et al. (2004).

$$LAI = -\ln \frac{\left(\frac{0.69 - SAVI}{0.59} \right)}{0.91} \text{----------------------- (7)}$$

Emissivity and land surface temperature

Both surface emissivity (broadband and narrowband) were used for the calculation of the total longwave radiation emitted from the surface and surface temperature respectively.

The values of emissivity were estimated from NDVI and LAI as follows:

$$\varepsilon = 1.009 + 0.047 \times \ln(NDVI) \text{--------------(8)}$$

Where NDVI > 0, otherwise, emissivity is assumed zero (e.g. water)
Narrow band emissivity was computed using the following empirical equation.

$$\varepsilon NB = 0.97 + 0.0033 \times LAI \text{ ----------------------- (9)}$$

ε0 = 0.95 + 0.01 x LAI ------------------------------ (10)

Where LAI < 3,
When LAI >= 3; ε (emissivity) = 0.98 (Kosa, 2011; Opoku et al., 2008).
For water, NDVI < 0 and surface albedo < 0.47, ε0 = 0.985 and εNB = 0.99 (Waters, et al., 2002)
For snow, NDVI < 0 and surface albedo >= 0.47, ε0 = 0.985 and εNB = 0.99 (Waters, et al., 2002)

For this calculation, NDVI, LAI, and surface albedo are input parameters to compute emissivity in both cases. Hence, computation is done using GRASS-GIS SEBAL python.

The black body temperature was corrected with respect to the surface emissivity (ε) values to compute the land surface temperature (Ts) using the formula given by Weng, et al., (2004).

$$Ts = \frac{Tb}{1 + (\lambda \times Tb / \gamma) \times \ln \varepsilon NB} \text{------------------ (11)}$$

Where: **λ** is the average of limiting wavelengths of band 10 of Landsat8-TIRS (10.8)

$$\gamma = h \times c / a(0.01438 m.K) \text{ ------------ (12)}$$

γ = 14380
a = Boltzmann constant (1.38x10-23j.k)
h = Plank's constant (6.626x10-34 J.s)
c = velocity of light (2.998 x 108 m/s)

Calculation of Solar Radiations Elements and ET
The solar radiation and ET were computed following the procedure set by (Bastiaanssen, et al., 1998).
Incoming shortwave radiation (RS↓) (W/m^2) was calculated using the available climatic parameters (sunshine hours, relative humidity, minimum and maximum temperature cloud cover, and geographic location), the solar constant, the solar incidence angle, a relative earth-sun distance, and the calculated atmospheric transmissivity. It is calculated, assuming clear sky conditions, as a constant for the image time (Waters, et al., 2002; Chiemeka, 2008) using:

$$Rs \downarrow = G_{Sc} \times \cos\theta \times d_r \times \tau_{sw} \text{ ---------------- (13)}$$

Where GScis solar constant (1,367 W/m^2), θ is the solar incidence angle, = sun elevation angle, given in the header data file of the landsat-8 imagery, *dr*= the inverse squared relative earth-sun distance, and is the atmospheric transmissivity.

The outgoing longwave radiation (RL↑) was computed at each pixel using the Stefan-Boltzmann equation (W/m^2) (Eq. 14) with a calculated surface emissivity and surface temperature (Waters, et al., 2002; Opoku Duah, et al., 2008). This calculation is done using GRASS-GIS SEBAL Python.

$$RL \uparrow = \varepsilon 0 \times \sigma \times Ts^4 \text{ -------------------- (14)}$$

Where **ε0** is the broadband surface emissivity, **σ** is Stefan-Boltzmann constant (5.67 x 10-8 W/ m^2/ K4) and Ts is surface temperature in K.

The incoming longwave radiation (RL↓), the downward thermal radiation flux from the atmosphere (W/m^2), was computed using a modified Stefan-Boltzmann equation with atmospheric transmissivity and a selected surface reference temperature. To compute this radiation (*RL*↓), the hot and cold pixels were selected as anchor pixels and are located in the area of interest. The "cold" pixel was selected as a wet, well-irrigated crop surface having full ground cover by vegetation whereas the "hot" pixel was selected as a dry, bare agricultural field where ET is assumed to be zero.

Using surface albedo (α), outgoing longwave radiation (RL↑), and broadband surface emissivity (ε0) along with the incoming shortwave radiation (RS↓) and the incoming longwave radiation (RL↓), net radiation flux was calculated.

Calculation of surface energy budget elements

Soil heat flux (G) and sensible heat flux (H) were computed in SEBAL using the surface energy budget equation. Soil heat flux (G) is the rate of heat storage in the soil because of the temperature gradient between the soil surface and the underlying upper layers of the soil (W/m^2) (Opoku Duah, et al., 2008; Waters, et al., 2002; Tasumi, et al., 2003). It is calculated using the equation given below:

$$G / R_n = T_s / \alpha(0.0038\alpha + 0.0074\alpha^2)(1 - 0.98NDVI \quad \text{----(15)}$$

Where;
Ts is the surface temperature (^{0}C),
α is the surface albedo, and
NDVI is the Normalized Difference Vegetation Index.
G is then readily calculated by multiplying G/Rn by the value for Rn computed in the above equation.

The ratio of G/Rn was assigned equal to 0.5 for the condition of NDVI < 0; Ts< 4 ^{0}C and α > 0.45. The resulting image was converted to soil heat flux (G) in W/m2 by multiplying the ratio G/Rn by Rn. (Waters, et al., 2002).

Sensible Heat Flux (H) is the rate of heat loss to the air by convection and conduction, due to temperature gradients (Morse, et al., 2001) and (Oberg and Meless, 2006). It was computed using the following equation:

$$H = (\rho \times c_p \times dT) / r_{ah} \quad \text{------------------------- (16)}$$

Where: **H** is sensible heat flux, **ρ** is air density (kg/m^3), *C*p is air specific heat (1004 J/kg/K), dT is near surface temperature differences (T_1-T_2) in K between two heights (Z_1 & Z_2) above the zero plane displacement, and ***rah*** is aerodynamic resistance to heat transport (s/m).

Latent heat flux is the rate of latent heat loss from the surface due to evapotranspiration (Waters, et al., 2002). It was computed for each pixel using Equation (17)

$$\lambda ET = R_n - G - H \quad \text{---------------------------- (17)}$$

Where: λ ET is an instantaneous value for the time of the satellite overpass (W/m^2).
Instantaneous value of ET in equivalent evaporation depth was computed as:

$$ET_{inst} = 3600 \times \frac{\lambda ET}{\lambda} \quad \text{--------------------------- (18)}$$

Where: ET*inst* is the instantaneous ET (mm/hr), 3600 is the time conversion from seconds to hours, and λ is the latent heat of vaporization or the heat absorbed when a kilogram of water evaporates (J/kg).

After calculating latent heat flux (λ), ETinst (mm/hr) and reference ET fraction (ETrF) were calculated simultaneously at each pixel level for the image time (ETr & U @10:52:11 local time). Once the Instantaneous ET (ETinst) and Reference ET Fraction (ETrF) were developed, daily evapotranspiration calculation was made using Equation (19).

$$ETrF = \frac{ET_{inst}}{ET_r} \quad \text{------------------------------- (19)}$$

Where, ETinst is instantaneous ET (mm/hr) and ETr is the reference ET at the time of the image from the REF-ET software (mm/hr) developed by the University of Idaho (Allen, 2000). ETrF is similar to the well-known crop coefficient; Kc. ETrF was used to extrapolate ET from image time to periods of 24 hours or more.

ETr was used in SEBAL to estimate the ET at the "cold" pixel and to calculate the reference ET fraction (ETrF) and ETr was computed for a given weather station using the REF-ET software (Allen. 2000).

Daily values of ET (ET24) were often more useful than instantaneous ET. SEBAL computes the ET24 by assuming that the instantaneous ETrF computed in the model is the same as the 24-hour average. Finally, the ET24 (mm/day) was computed as:

$$ET_{24} = ETrF \times ET_{r\_24} \text{ ------------------------ (20)}$$

Where, ETr-24 is the cumulative 24-hour ETr for the day of the image (Waters, et al., 2002). This is calculated by adding the hourly ETr values over the day of the image.

$$ET_{r\_24} = \sum_{h}^{24} ET_{r-h} \text{ --------------------------- (21)}$$

The evapotranspiration map that covers a season/month of full growth was derived from the 24-hour evapotranspiration data by extrapolating the ET24 proportionally to the reference evapotranspiration (ETr).

RESULTS AND DISCUSSION

The majority of the study area (more than 88%) came under annual cropland use types (Figure 3).

The surface albedo value was lower for the water body having an average value (0.106) recognized due to high absorption properties (Fig. 4), while the settlement with the highest average value (0.194) was recognized because of the low absorption and scattering from such land cover types. According to Ayad et al., (2016), surface albedo having high absorption properties (forest, grass, and water body) are recognized with low average values of 0.05 to 0.08 while, surface albedo having low absorption properties (bare land and rock outcrop) are recognized with highest average values of 0.18 and 0.17, respectively.

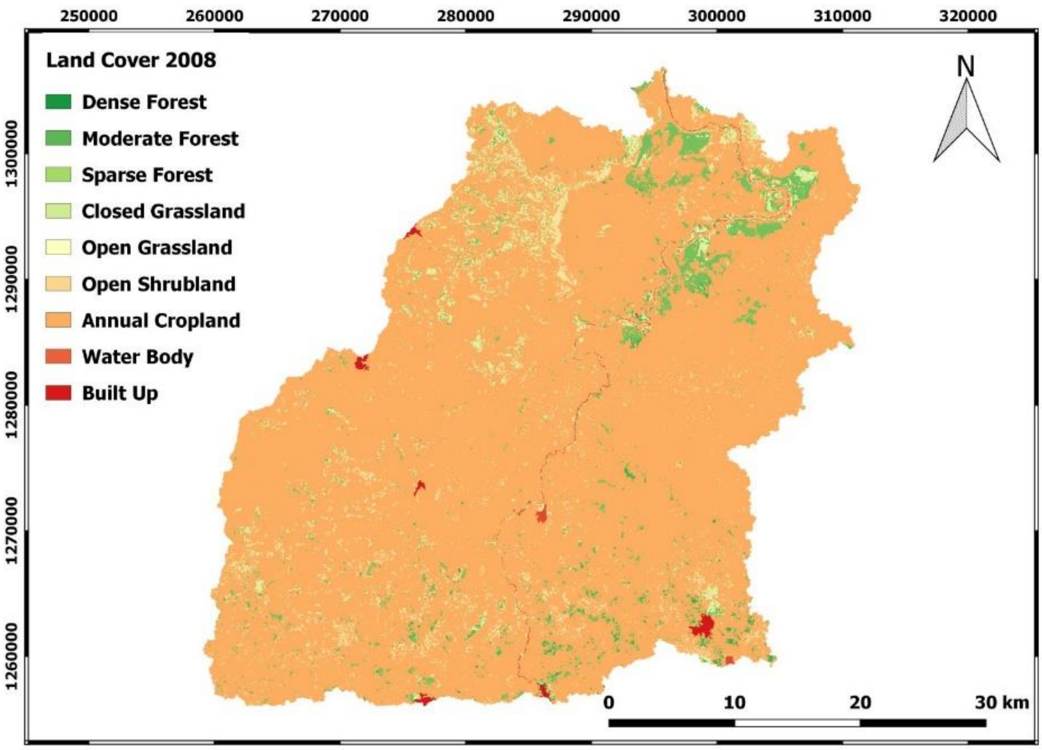

Figure 3. Land Use Map of the study area (Source: RCMRD (2015))

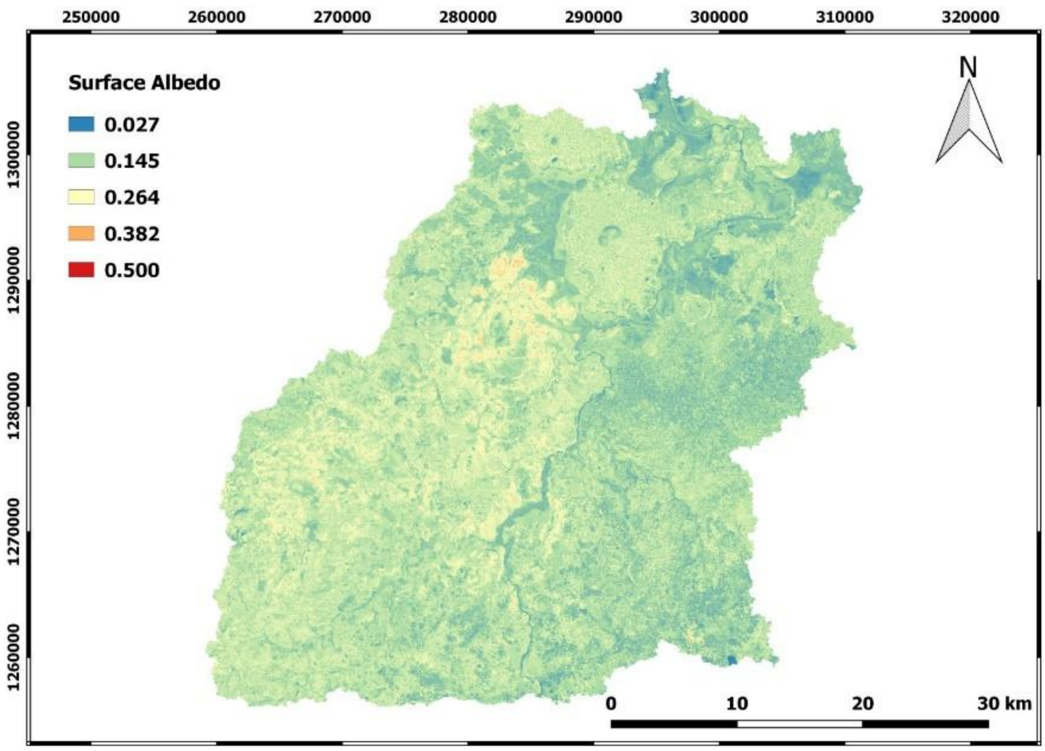

Figure 4. Map of Surface albedo

The NDVI values of the computed spatial variation ranged from -0.90 to 0.85 (Fig. 5). The NDVI mean value of the water body was low (0.12), whereas 4.37% of the area coverage (forest) showed high NDVI with the mean value of 0.61. The areas along with the closed grassland represent low LAI (Fig. 6) of the mean value 0.12, whereas 4.37% of the area coverage (forest) showed high LAI with the mean value of 0.68. According to Jana, et al., (2016) the areas along with the river (water body) represent a low vegetation index of ranges from 0.14 to 0.22 whereas, forest areas showing high vegetation density recognized by higher average values of NDVI ranges from 0.6 to 0.65.

Using the radiant temperature and the computed value of surface emissivity (narrowband surface emissivity εNB), the surface temperature (K) map was derived. Figure 7 clearly showed the dependence of the Surface Temperature estimation on the surface albedo and the vegetation. Variations in the mean temperature values were low and ranged from 28.23 ^{0}C in forest area and water body due to high absorption properties (lower surface albedo and higher NDVI) to 33.28 ^{0}C for the other land cover types (Fig. 8).

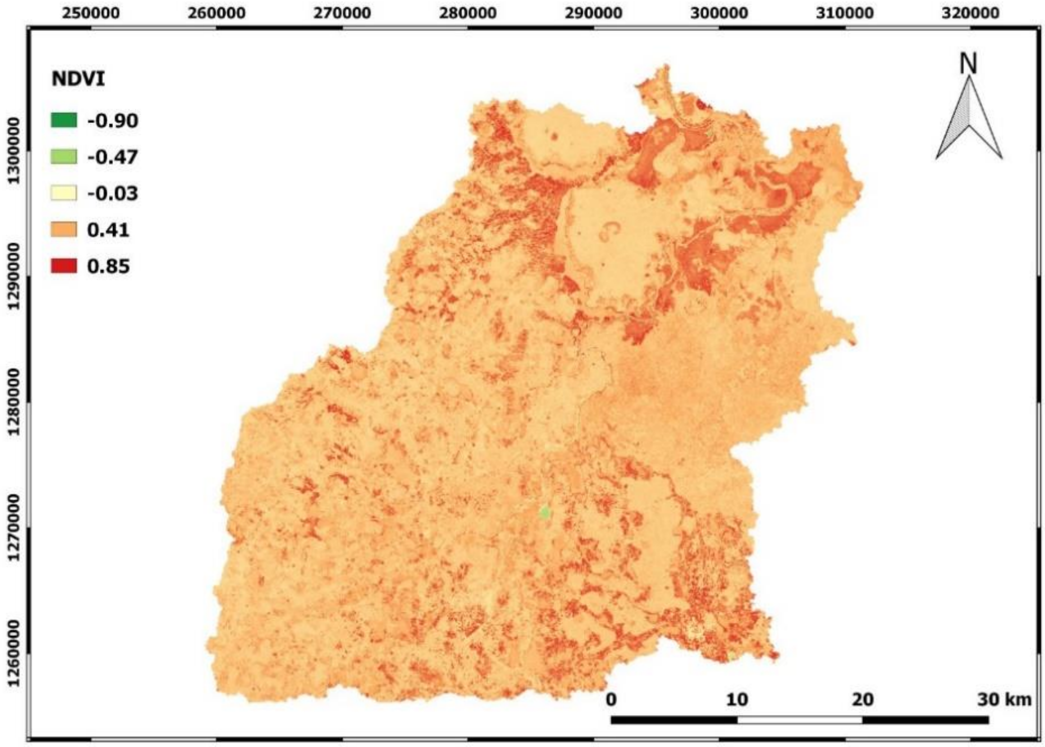

Figure 5 Map of Normalized Difference Vegetation Index

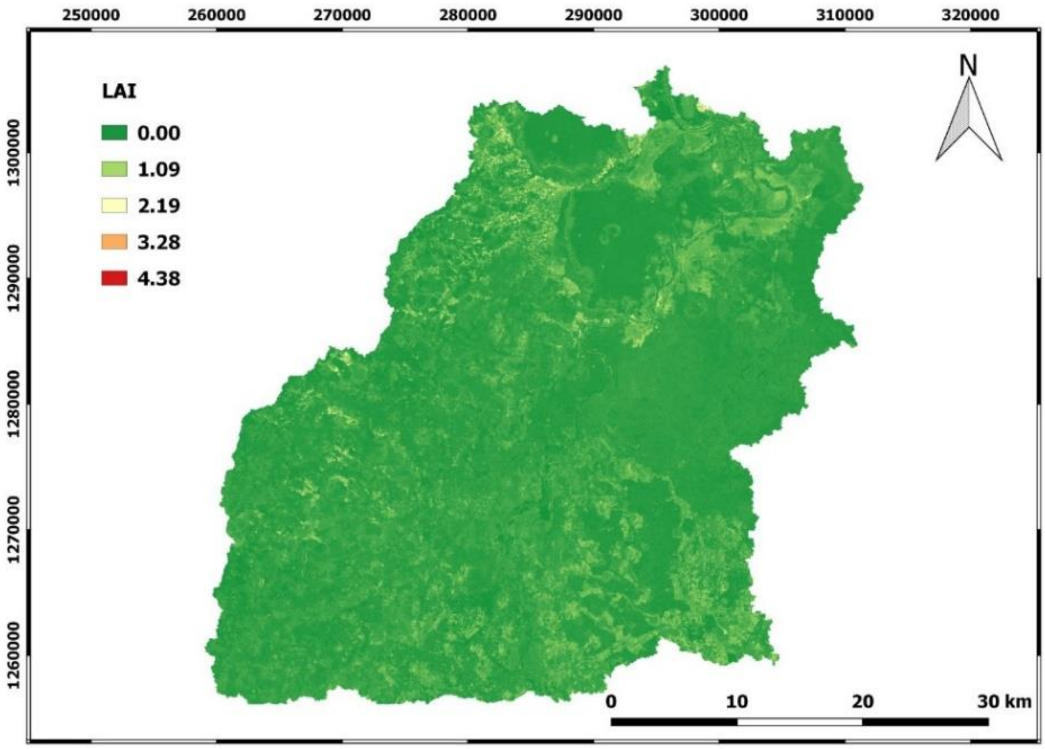

Figure 6. Map of Leaf Area Index

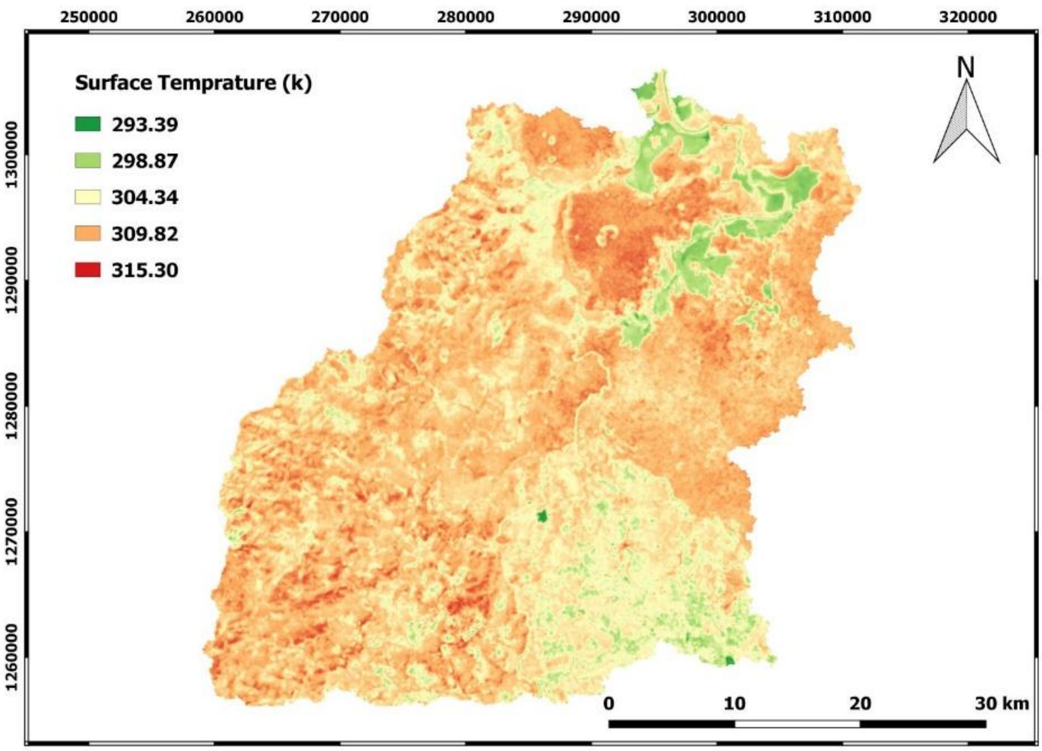

Figure 7. Land surface temperature (^{0}K)

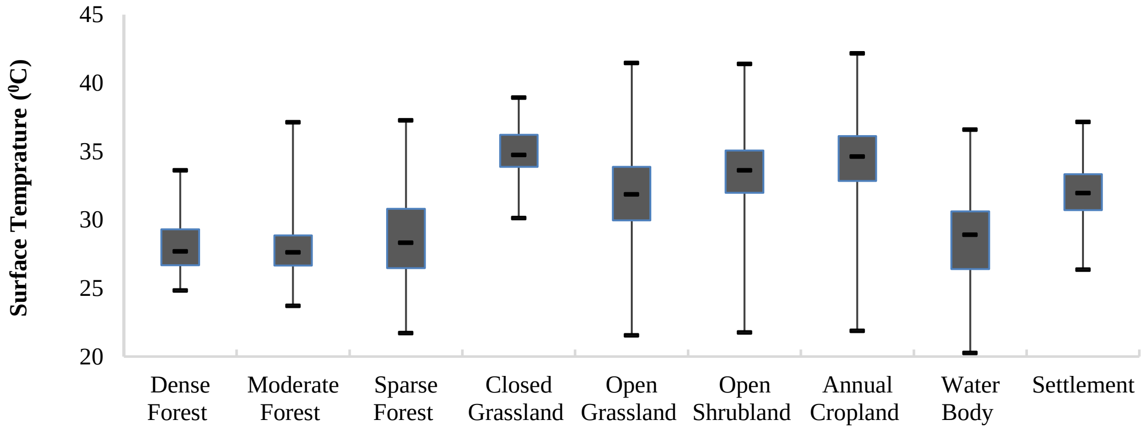

Figure 8. Mean surface temperature for land covers.

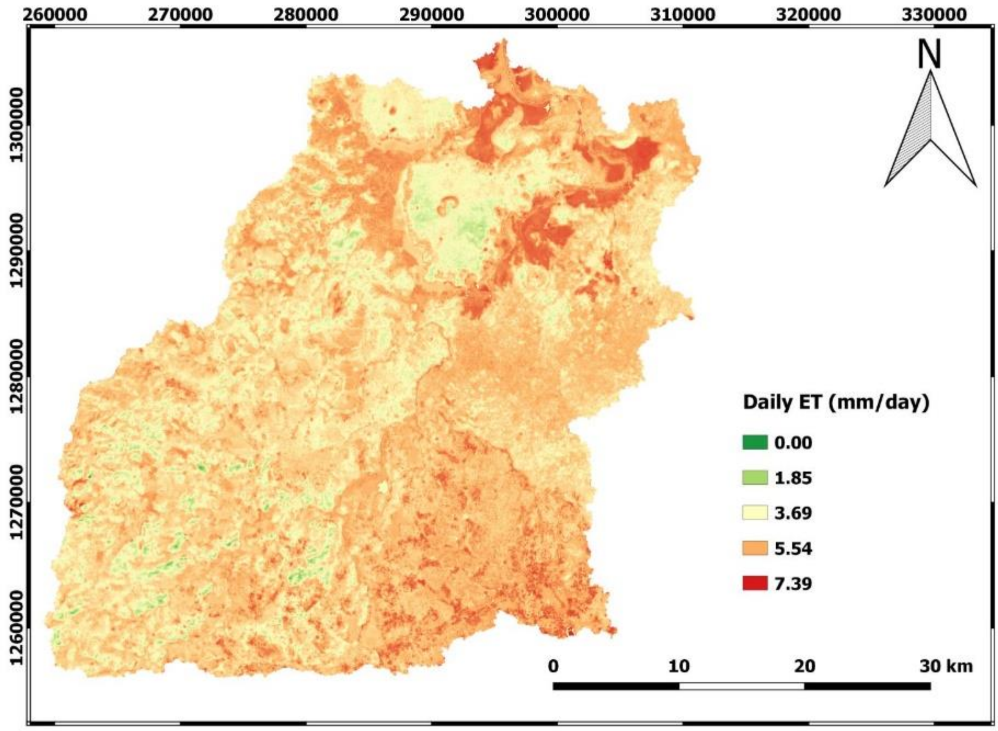

Figure 9. Map of Daily Evapotranspiration

Table 1: Daily evapotranspiration (mm/day) distribution for different land cover type

Land Cover Type	Area (km^2)	%	Daily ET (mm/day)				
			Min	Max	Range	Mean	STD
Dense Forest	0.22	0.01	4.82	7.00	2.18	6.25	0.38
Moderate Forest	6.33	0.37	0.64	7.09	6.45	6.23	0.46
Sparse Forest	68.41	3.99	1.14	7.00	5.86	5.97	0.50
Closed Grassland	0.51	0.03	2.51	5.89	3.38	4.42	0.50
Open Grassland	49.43	2.88	0.63	7.17	6.55	5.42	0.77
Open Shrub land	61.67	3.60	0.27	6.81	6.54	4.96	0.62
Annual Cropland	1,516.57	88.49	0.00	7.09	7.09	4.69	0.73
Water Body	5.99	0.35	3.25	7.39	4.14	5.13	1.15
Settlement	4.72	0.28	3.17	6.69	3.52	5.20	0.51

The daily evapotranspiration value ranged from 0 to 7.39 mm/day, and a mean value of 4.78 mm/day (Fig. 9). The computed daily ET values for each land use type showed that forest land use exhibit the largest ET with a mean value of 6.25 mm/d as compared to the grassland with a mean value of 4.42 mm/d (Table 1). These findings are in line with studies conducted in the Rift Valley Lakes basin and Lake Tana sub-basin (Ayenew, 2003; Temesgen, 2009; Muhammed, 2012; Mulugeta et al., 2017).

During satellite overpass time, the variation of ET in mm/day in relation to NDVI and surface temperature in ^{0}C, water body had a high surface temperature of about 28.10 ^{0}C and the estimated ET from the same land use was mainly contributed from evaporation, which was about 5.13 mm/day (Figure **10**.10). On the other hand, the forest area showed an average surface temperature of 28.27 ^{0}C with high vegetation index (0.61) and a mean ET value of 6.15 mm/day.

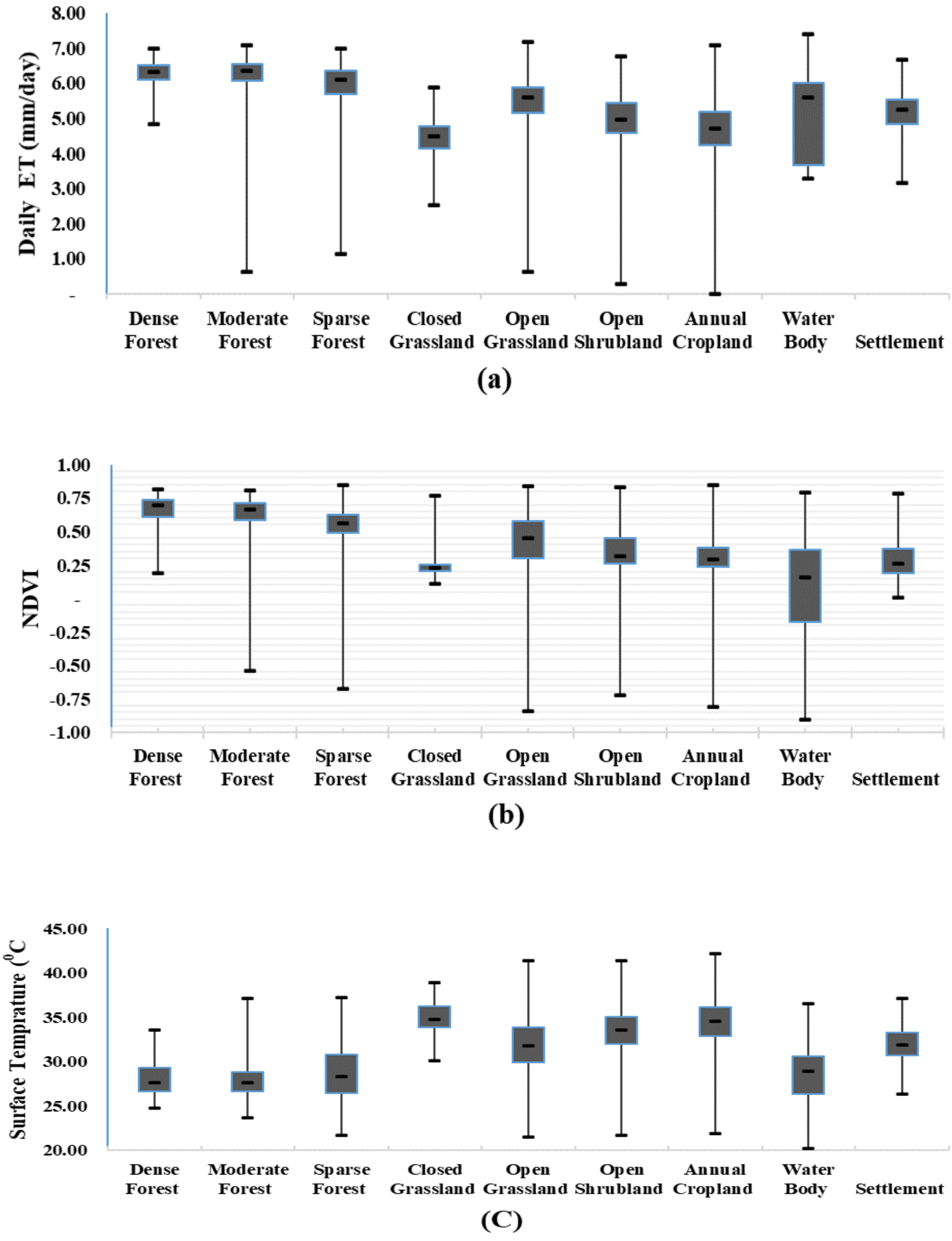

Figure 10. Comparison between ET (a), NDVI (b) ST (c) during satellite overpass time.

The maximum estimated actual evapotranspiration over the whole catchment ranged from 6.51 mm/day (Jan) to 7.82 mm/day (Mar) (Figure 11). The mean actual evapotranspiration ranged from 4.37 mm/day (Feb) to 4.78 mm/day (Dec). Daily ET was nearly the same as the average ET in annual cropland (88.49%), higher in the forestland cover types and lower in closed grassland cover type (Figure 11).

Figure12 showed the trend of daily ET for each month in different land cover types. Forestland cover types higher in December and January than in February and March due to higher NDVI and lower surface temperature during those months.

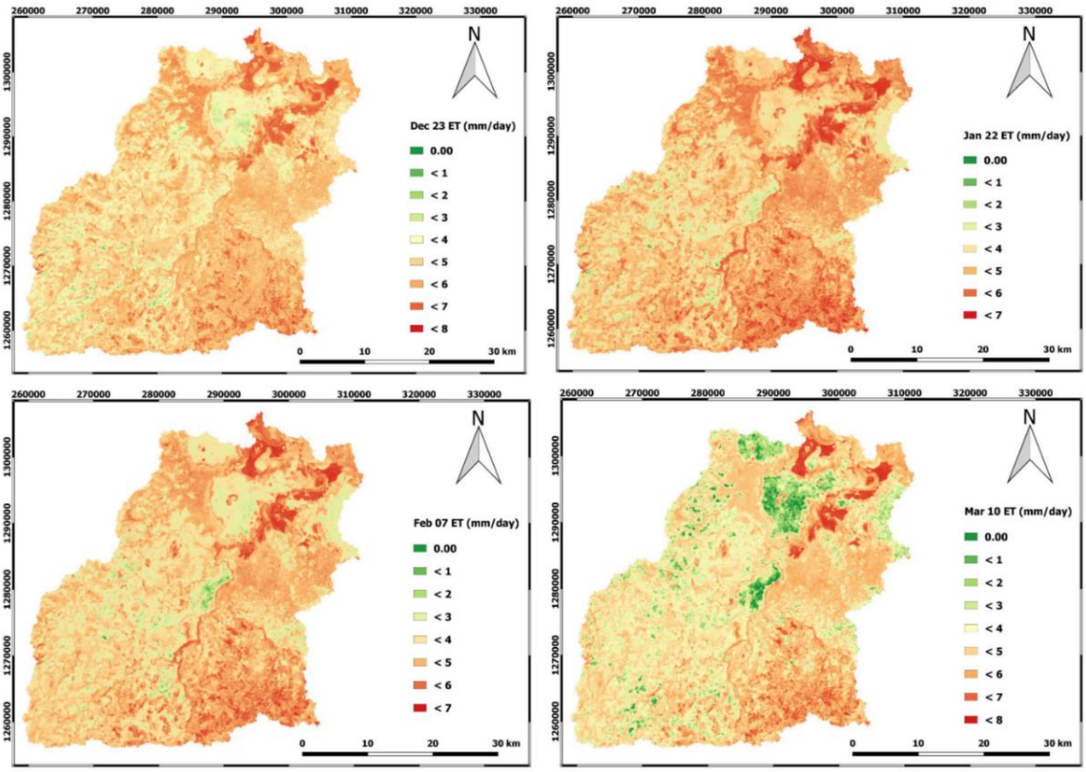

Figure 11. Map of Twenty-Four-hour evapotranspiration for four months (Dec to March 2016)

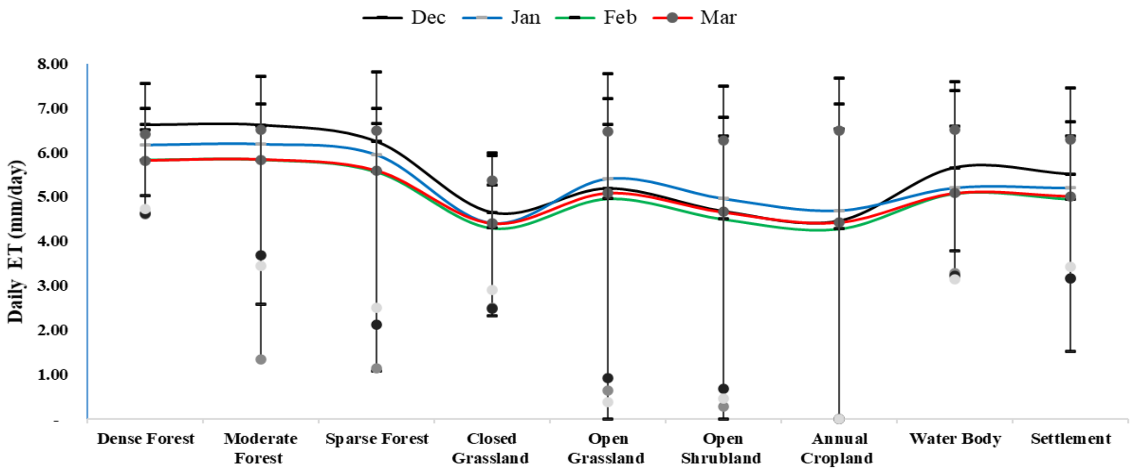

Figure 12. ET (mm/day) distribution for different land cover types in selected month.

A seasonal ET map of the study area for selected months (December to March 2016) was prepared (Fig. 13). The mean maximum ET (mm) was in forestland cover type (Table 2), but this covered a low proportion of the total study area (4.37%). The highest area coverage (88.49 km^2) produced the minimum ET (528.08 mm). The mean value of the study area was 537.92 mm for this selected four months in 2016.

The seosnal ET variation for different land cover types are presented in Figure14. ...

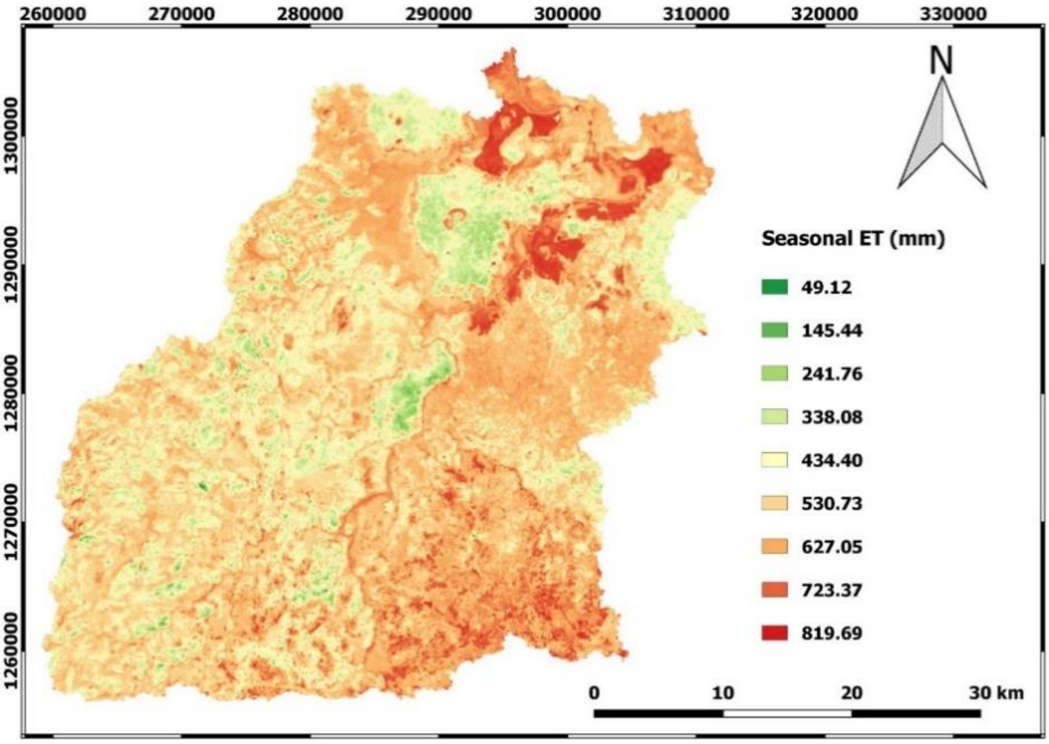

Figure 13. The combination map of ET for the selected period (Dec to Mar 2016) of the study area.

Table 2. Seasonal ET distribution and its area coverage for each land use type.

Land Cover Type	Area (km2)	%	Min ET (mm)	Max ET (mm)	Range	Mean ET (mm)	STD
Dense Forest	0.22	0.01	587.52	812.40	224.88	732.88	43.97
Moderate Forest	6.33	0.37	424.14	812.58	388.44	729.33	48.57
Sparse Forest	68.41	3.99	411.27	817.96	406.69	693.85	68.77
Closed Grassland	0.51	0.03	308.07	639.72	331.65	527.66	48.88
Open Grassland	49.43	2.88	114.77	819.69	704.92	612.09	90.21
Open Shrub land	61.67	3.60	152.77	781.97	629.19	556.12	78.25
Annual Cropland	1,516.57	88.49	49.12	804.21	755.09	528.08	95.65
Water Body	5.99	0.35	401.29	802.78	401.48	618.61	104.84
Settlement	4.72	0.28	393.08	785.80	392.71	612.76	60.42

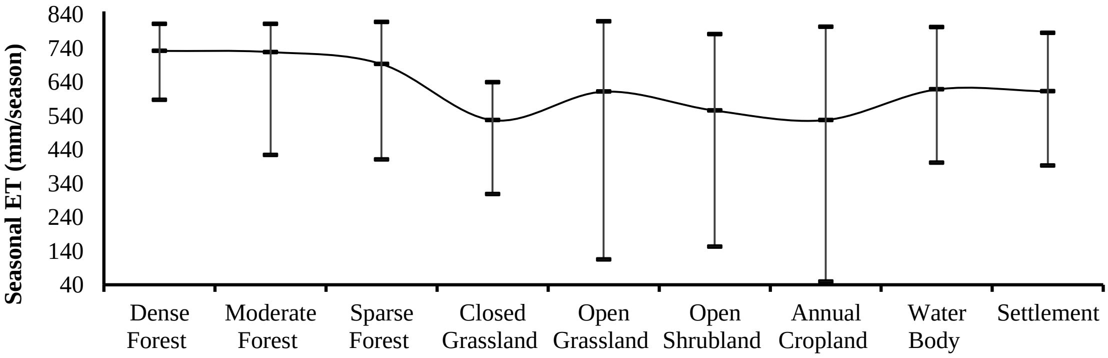

Figure 14. Seasonal ET (mm) distribution for different land cover types.

CONCLUSION

The study examined the SEBAL technique for mapping spatial variation of actual evapotranspiration over the lower part of Gilgel Abay catchment. The estimated result is based on the SEBAL method and Landsat-8 imagery at the satellite overpass time.

The majority of the land cover which mainly controlled the solar radiation and ET regime was represented by annual cropland with 88.49% of the total area, followed by sparse forest that covered about 4%, and the remaining land cover types occupied 11.51% of the total area. The daily ET map indicated that daily ET was nearly the same as the average ET in annual cropland (4.69 mm/day), higher in the forestland cover types (6.25 mm/day) and lower in closed grassland cover type (4.42 mm/day). The least range of variation in ET values was from the area characterized by the absence of vegetation cover. Hence, the variation of daily ET varied from 0 to 6.26 mm/day at the same land cover classes. The study showed a clear relation between land use/land cover and solar radiation elements and the impact of vegetation cover on the ET values in the pixel domain.

The study suggested that the SEBAL model could be applied in similar data-scarce areas at local, regional, and global scales to quantify the actual ET over various land uses and from irrigated agriculture. The spatial and temporal variation ET is a good indicator of irrigation effectiveness and total water consumption from vegetation. Evapotranspiration information from satellite imagery is useful for irrigation development and enables to improve irrigation management. It is necessary for the calculation of soil water balance, an important component of the water cycle, water rights regulation, and river basin hydrologic studies.

Among several remote sensing methods of estimating ET, the SEBAL model could be useful for irrigation water use related studies and practically applicable in the absence of ground data by providing accurate information, determine remotely over a large spatial scale, aggregated over space and time. It is user-friendly and could be widely used across a variety of climates and ecosystems. Hence, the SEBAL model is a useful tool for irrigation water use related studies.

The ET estimation using SEBAL through the use of remote sensing data is one way of estimating the evapotranspiration in a data-scare region whereby the estimation is difficult through water balance models. Moreover; with the availability of streamflow discharge measurement and with limited climate data the ET estimated using this approach could be used as a proxy in hydrological models to overcome climate data limitation in an ungauged catchment. However, the method has its own limitations including subjective specifications of representative hot/dry and wet/cool pixel selection within the image, availability of cloud-free images, lower temporal frequency but high spatial resolution imagery provided by the satellite, estimating sensible heat flux (H) which is greatly affected by the errors in surface-air temperature differences or surface temperatures measurements, and suitable to estimate ET on an hourly basis and not suitable on daily basis which could be due to the use of ETrF in extrapolating instantaneous ET to daily values. The extrapolation assumes that the ETrF at the time of satellite overpass remains constant throughout the day.

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Morphological Responses of *Moringa* (*Moringa stenopetala* L.) Accessions Seedlings to Irrigation interval under Greenhouse Conditions

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Abstract

Moringa (*Moringa stenopetala* L.) has a wide range of adaptability and various uses for human life. However, the plant is not well researched under Ethiopian conditions to maximize its benefit and exploit its potential. Therefore, a pot experiment was conducted to investigate the morphological responses of *M. stenopetala* accessions seedlings to irrigation intervals under greenhouse conditions at Hawassa, Ethiopia. The experiment was conducted using three accessions (Konso, Arbaminch Zuria, and Humbo) and four irrigation intervals (daily, 5 days interval, 10 days interval, and 15 days interval watering) assigned in a completely randomized design with three replications. The accessions significantly differed for most of the morphological traits studied in this experiment. The accession Arbaminch Zuria was higher in seedling height, stem diameter, leaf area, root length, root fresh weight, root dry weight, total seedling fresh weight, and total seedling dry weight than the other accessions. There were no significant differences between Arbaminch Zuria and Konso accessions in leaf number and root diameter. Regarding the irrigation interval, the highest seedling height, stem diameter, leaf area, leaf number, root length, root diameter, root fresh weight, root dry weight, total seedling fresh weight and total seedling dry weight were obtained from the daily watering interval. In contrast, the lowest growth parameters were from the 15 days interval watering. The 10 and 15 days interval watering were statistically similar for seedling height and leaf number. Additionally, fresh and dry leaf weights were significantly influenced by the interaction effect of accession and irrigation interval. The Arbaminch Zuria accession grown under daily watering had the highest fresh and dry leaf weights compared to the other accessions. Shorter interval of irrigation, if possible daily, is recommended to produce quality Moringa seedlings in a shorter period.

Keywords: Drought, Plant height.

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INTRODUCTION

The high demand for food, nutrition and medicine is putting intense deficit in the world (Jane, 2015; FAO, 2018). Therefore, focusing on nutritious, medicinal, multi-purpose and drought tolerant plants is necessary. *Moringa (Moringa stenopetala)* is one of the world's most valuable plants. Indeed, it is considered as a super food and storehouse of nutrition in addition to being remedy for more than 300 diseases (Daba, 2016; Mall and Tripathi, 2017). It is also used for water purification material, and oil and biogas source. Besides, *Moringa* is fast growing plant under wide range of agro-ecologies (Badran *et al*., 2016; Daba and Adisu, 2017).

Moringa stenopetala is domesticated in the east African lowlands and is native to southern Ethiopia (Eyassu, 2014). Several ecotypes and accessions of *M. stenopetala* exist in Ethiopia. *Moringa stenopetala* is often called "cabbage tree" and is an important indigenous vegetable in south western Ethiopia, where it is cultivated as food crop (Eyassu, 2014). It has been adapted in southern Ethiopia, particularly around Konso, Gamo, Goffa, Burji, and South Omo areas for a century (Yohannes and Teshale, 2016). Nowadays, it is hardly possible to find a household without a *Moringa* tree in Konso, Gamo and Goffa homesteads. Hence, *M. stenopetala* is a favorite and main component of the daily meal of the people at the area (Personal observation). *Moringa stenopetala* is particularly important as human food because the leaves have high nutritional value, appear towards the end of the dry season when other sources of green vegetables are limited (Abuye *et al*., 2003).

Nevertheless, biotic and abiotic factors such as pests, soil, temperature, and water deficit can affect and reduce its potential growth, development and functions (Palada and Chang, 2003). Among numerous factors, water deficit is a significant factor in dryland areas and it is critical to seedling growth and development (Awodol and Nwoboshi, 1993). *Moringa* does not have a preference towards a specific habitat but it performs better in hot and humid environments, and thus found throughout a range of eco-zones from dry savanna to rainforests (ref???). It generally prefers altitudes below 600 meter above sea level, although it can grow at elevations of up to 2000 meters in protected tropical zones (Anonymous, 2006). Because of the tuberous taproot, *Moringa* trees are capable of enduring anything between as little as 250 mm to 1500 mm of annual rainfall. Even though *Moringa* is drought tolerant, longer irrigation intervals reduce cell expansion, enlargement, growth and development of plants, especially during early stages (Kamara *et al.*, 2003; Shao *et al*., 2008; Jaleel *et al.*, 2009; Badran, 2016).

Moringa is highly heterozygous because it is highly cross pollinated in nature and has a wide variability in morphological traits (Amoatey *et al*., 2012). Several studies revealed that morphological traits of *Moringa* (seedling height, stem collar girth, root length and diameter, leaf number and area, fresh and dry weights) varied under different irrigation intervals (Wafa, 2006; Kenneth, 2012; Baiyeri *et al*., 2015*;* Jane *et al.* 2015; Sale *et al.*, 2015; Jacob, 2016). However, in Ethiopia, research on seedling performance of *M. stenepetala* accessions under different water management schemes has not been conducted and documented (Eyassu, 2014; Ashenafi, 2014; Sreepada and Vijayalaxmi, 2013). Instead, previous researches have focused mainly on field surveys to assess the traditional uses of *M. stenopetala* based on information from *Moringa* growers. Furthermore, research has been focused on its potential values for human nutrition, water purification, livestock feed, dyes, herbal medicine, oil production, and climate mitigation (Mall and Tripathi, 2017). Therefore, this study investigated morphological responses of *M. stenopetala* accessions seedlings to irrigation intervals.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted under greenhouse condition during October 2018 – January 2018 at Hawassa, Ethiopia. The site is located at 7°05′55" N latitude and 38°47'45" E longitudes, and at an altitudinal of 1689 m.a.s.l. According to 11 years (2007-2018) data, the average annual rainfall and temperature (maximum and minimum) of the area is 971.9 mm, and 27.9 °c and 13.8 °c, respectively, (NMA, 2019).

Description of the greenhouse and its condition

The greenhouse used for the experiment was metal framed protected with metal wire from all sides. The top of the greenhouse was covered by polyacrylic. The daily minimum and maximum air temperatures in the greenhouse were recorded on randomly selected locations for 45 days using mini data logger during the experimental period from October to January 2018. Data logger was placed inside an open bucket to avoid direct sunlight and hanged close to the seedling canopy. The data logger recorded the climate data every hour for 45 days. The average value of 45 days measurements is presented in Fig 1.

Mean minimum and maximum air temperature in the greenhouse were 12.6 ^{0}C and 28.9 ºC, respectively (Fig. 1). Therefore, the greenhouse condition was in the range of optimum temperature (20 to 35 ºC) for raising *Moringa* seedling as described by Alatar, (2011) and Muhl *et al.* (2011).

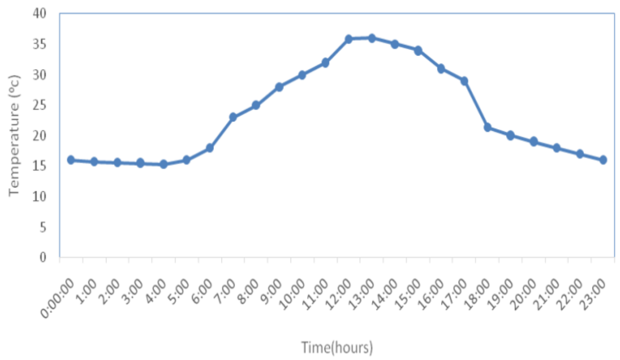

Figure 1. Average daily greenhouse temperature during experimental period (October to January 2018).

Experimental Materials and Procedures

Three *M. stenopetala* accessions seeds were obtained from Arbaminch Agricultural Research Center (AARC), Ethiopia (Table 1). Seeds were planted in perforated black polyethylene tube with 22 cm length and 16 cm wide filled with a soils mixture of top soils, compost and river sand at a ratio of 3:2:1 following the recommendation by Ede *et al*. (2015).

Soil particle size distribution was determined by the Boycouos hydrometric method (Van Reeuwijk, 1992) after destroying OM using hydrogen peroxide (H_2O_2) and dispersing the soils with sodium hexameta phosphate ($NaPO_3$).

Table 1. Description of *Moringa stenopetala* accessions used for the experiment.

Accession code	Origin of accession	Growing altitude	Source

Arbaminch Zuria	Arbaminch Zuria	1222 masl	AARC
Humbo	Humbo	1432masl	AARC
Konso	Konso	1320 masl	AARC

Source: Arbaminch Agricultural Research Center (Personal Communication)

Soil bulk density was determined after drying the soil samples in an oven at 105 ºC to constant weights, while particle density was measured by the pycnometer method (Black, 1965). The pH of the soils was measured in water and potassium chloride (1M KCl) suspension in a 1:2.5 (soil: liquid ratio) potentiometrically using a glass-calomel combination electrode (Van Reeuwijk, 1992). The electrical conductivity (EC) of soils was measured from a soil water ratio of 1:2.5 socked for one hour by electrical conductivity method as described by Sahlemdhin and Taye (2000). The Walkley and Black (1934) wet digestion method was used to determine soil carbon content and percent soil organic matter. Total N was analyzed using the Kjeldahl digestion, distillation and titration method as described by Black (1965) by oxidizing the OM in a concentrated sulfuric acid solution (0.1N H_2SO_4). The soil moisture content at field capacity was determined using pressure plate apparatus (FAO, 2006) (Table 2). Then, the perforated black polyethylene tubes filled with media were arranged under a greenhouse in a factorial scheme of 4 x 3, that was, four irrigation intervals [daily (Control), five day interval, ten days interval and fifteen days interval] and three *M. stenopetala* accessions (Konso, Arbaminch Zuria and Humbo). The 12 treatment combinations were arranged in completely randomized design with three replications. The amount of water required to apply in the perforated black polythene tubes to field capacity was calculated by the formula recommended by FAO (2006) as follows:

$$\text{Amount of water to be applied} = (FC - AMC) x\ D\ x\ A$$

Where, FC = Volumetric moisture content at FC and AMC = Actual volumetric moisture content determined by moisture tester, D = Depth of the perforated black polythene tubes (cm), A= Perforated black polythene tubes area (cm^2).

Table 2. Physical and chemical properties of the experimental media

Properties	Determined values
Soil textural class	Sandy loam
Sand (%)	84.68
Silt (%)	8
Clay (%)	7.32
Total nitrogen (%)	0.4538
Organic matter (%)	9.076
Organic carbon (%)	5.265
pH of media	7.3
Electro conductivity(ms cm^{-1})	0.52
Bulk density(g/cm^2)	1.02
Moisture content at FC (v/v %)	31.9

Two seeds of each *M. stenopetala* accessions were sown on 09 October 2018 at depth of 2 cm and then thinned after germination. All Experimental units were watered to field capacity until the commencements of the treatments (up to 13 days after emergence of seedlings). Then the treatments were applied until the seedlings reached first transplantation. Thereafter, the seedlings were subjected to watering interval treatments, and the seedlings were maintained in those irrigation intervals for two months while subsequent measurement and observations were made on their morphological attributes.

Data Collection

Seedling height and stem diameter (cm) were measured using the method by Ede *et al*. (2015). Seedling height was measured from the surface of the seedling above the media up to tip of the seedling. Seedling stem diameter was measured with digital caliper on the collar of the seedling.

Root length and root diameter (cm) were measured using the method by Jane *et al*. (2015). Three seedlings were randomly selected, and the perforated polyethylene tube was removed with sharp scissors, thereafter, to protect breaking of roots to adhered soil, exactly blow stem- root junction of the seedling was inserted in to the large pot water until the soil adhered with root has been washed. Then, it was measured using a 30 - centimeter ruler placed along-side from underground surface to the root tip of the seedling root. Root diameter was measured using a digital caliper (precision in mm).

Leaf area was measured using the method by Jane *et al*. (2015). The equipment used was LI- 3100C Area meter (precision Square cm), LI-COR. Bio science area meter, Australia.

Total Seedling fresh weight (g) and Leaf fresh weight **(g)** were measured using the method by Ede *et al*.

(2015). After destructive sampling (two months after emergence), seedling fresh weight was recorded by placing whole seedling on sensitive balance. Thereafter, leaves were removed (separated) from selected seedlings. Finally, leaf fresh weight was recorded separately.

Total seedling dry weight (g) and Leaf dry weight **(g)** were measured using the method by Badran *et al*. (2016). After fresh weights of the samples were recorded (at two month), samples were placed in paper bags and then oven dried for 48 hours at 70 °C temperature. Whole seedling and leaf dry weights were measured by electronic sensitive balance.
Fresh and dry weights (g) of root were measured using the method by Wafa and Adam (2006). Three seedlings were selected randomly (at two month). In order to protect breakage of roots in to the adhered soil, part of the seedling exactly below the stem- root junction was inserted in to large pot water until the soil adhered with root was washed. Afterward, the above ground part of the seedlings were separated by using sharp scissor, and then the below ground part sample was rolled by plastic and brought to laboratory. Fresh weight was recorded immediately after harvest using electronic sensitive balance. Finally, roots were placed in paper bag and oven dried with adjusted temperature at 70 °C for 48 hours and then the root dry weight was recorded.

Data Analysis
All data collected were subjected to analysis of variance (ANOVA) using proc GLM procedure of SAS version 9.3 (SAS Institute, 2009). Tukey's HSD test was used to separate means at the 5% level of significance.

RESULTS AND DISCUSSION

Morphological Response of *Moringa stenopetala* Accessions Seedlings to irrigation interval

Seedling stem and leaf traits
The seedling height, stem diameter, leaf number and leaf area of *Moringa* were significantly (P<0.001) influenced by accession and irrigation interval (Table 3). The interaction effect of accession and irrigation interval significantly affected leaf fresh and dry weights. However, all other morphological parameters mentioned above were not significantly (P>0.05) influenced by the interaction of the accessions and irrigation interval (Table 3).

Current results indicated that Arbaminch Zuria accession had the tallest seedlings, the largest stem diameter and wider leaf area compared to the other accessions. On the other hand, accession Humbo was the shortest seedling and had the smallest stem diameter, the least number of leaves and the smallest leaf area than the remaining accessions (Table 4). Accessions Arbaminch Zuria and Konso were not significantly different (P>0.05) in the number of leaves produced. These results are in agreement with Baiyeri *et al.* (2015), who reported significant variations among the accessions of *M. oleifera* seedlings in height, stem diameter, leaf number and area. This might be due to the genetic variability and differences in growth environment of the accessions.

Table 3. Mean square values of seedling height (SH), stem Diameter (SD), leaf number (LN), leaf area (LA), root fresh weight (RFW), leaf fresh weight (LFW) and leaf dry weight (LDW) of *Moringa stenopetala* accessions under different irrigation interval.

Source of variation	Df	Mean squares						
		SH	SD	LN	LA	RFW	LFW	LDW
Accession(A)	2	30.749***	0.042***	137.00***	8091.27***	78.55***	0.45***	0.680***
Irrigation Interval(II)	3	152.945***	0.407***	805.18***	41837.94***	3743.64***	21.31***	7.309***
A* II	6	0.464ns	0.0018ns	13.46 ns	417.22 ns	1.057ns	0.070**	0.105***
Error	24	0.432	0.0013	7.851	179.71	3.614	0.020	0.0026
CV (%)		**4.25**	**5.21**	**12.64**	**10.84**	**8.2**	**5.43**	**3.92**

*Where, ns=indicates non-significant difference at P≤5%,**significant difference at P<0.01% and ***significant difference at P<0.001% probability levels, respectively. df = degree of freedom*

Moreover, seedling height had strong positive association (r=0.94***) with leaf number (Table 5). Furthermore, the largest leaf area produced might be due to the wider leaves produced by accession Arbaminch Zuria. Regarding irrigation interval, the tallest seedling, the largest stem diameter, higher number of leaves and wider leaf area were produced on seedlings grown under daily watering condition. Whereas, seedlings grown under 15 days interval watering produced the smallest stem diameter, and narrow leaf area. Seedling height and leaf number of the seedlings grown under 10 and 15 days interval were not significantly different (P>0.05). Thus, this study confirmed that the seedling height, stem collar girth, leaf number and area of the *M. stenopetala* accessions decreased as watering interval increased. Similar results have been reported by Sale *et al.* (2015), who reported significant decrease in seedling height, stem diameter, leaf number and leaf area of *Moringa* as watering interval increased. Likewise, Daba and Adisu (2017) also reported that plant height significantly increased as watering interval decreased. At the third month after treatment application, Baiyeri *et al.* (2015) also reported significant difference among watering interval in seedling height and stem collar girth of *Moringa*. In other plants like citrus seedlings (Wue *et al*., 2008), and *Abelmoschus esculentus* (Sankar*et al.*, 2008) studies also indicated that increased water stress reduced seedling height and girth of the different cultivars. In general, the inhibition of cell expansion and cell growth is mainly due to low turgor pressure under water deficit conditions, which leads to reduced seedling girth (Shao *et al.*, 2008). Increased irrigation interval was also found to lead to inhibition of cell expansion, cell growth, and developmental process of the plant depending on the accession (Majiken, 2005).

Table 4. Responses of seedling height (cm), stem diameter (cm), leaf number (count) and leaf area of three *Moringa stenopetala* accessions seedling to irrigation interval under greenhouse condition at Hawassa.

Treatments	Parameters			
Accessions	Seedling height (cm)	Stem diameter (cm)	Leaf number	Leaf area (cm^2/plant)
Konso	15.41± 0.26^{b}	0.69±0.01^{b}	22.88±1.14^{a}	123.81 ±5.47^{b}
Arbaminch Zuria	17.07±0.26^{a}	0.75±0.01^{a}	25.11±1.14^{a}	149.50±5.47^{a}
Humbo	13.87±0.26^{c}	0.63±0.01^{c}	18.47±1.14^{b}	97.56±5.47^{c}
Tukey/HSD	0.67	0.03	2.85	13.66
Irrigation Interval				
Daily (control)	20.39±0.30^{a}	0.98±0.01^{a}	35.37 ±1.32^{a}	215.13 ±6.31^{a}
5 days interval	17.29±0.30^{b}	0.70±0.01^{b}	21.96±1.32^{b}	134.95±6.31^{b}
10 days interval	12.34± 0.30^{c}	0.56±0.01^{c}	17.77± 1.32^{c}	81.06±6.31^{c}
15 days interval	11.78±0.30^{c}	0.51±0.01^{d}	13.52± 1.32^{c}	63.35±6.31^{d}
Tukey/HSD	0.85	0.04	3.64	
CV (%)	4.25	5.21	12.64	

Where, HSD= honestly significant difference, CV= Coefficient of variation, means with different letter in each column of each factor are statistically significant at P≤ 0.05%.

M. stenopetala accessions responded differently when they were exposed to different irrigation intervals (Table 5). The highest leaf fresh and dry weights were measured on accession from Arbaminch Zuria under daily watering conditions. The results further revealed decline in leaf fresh and dry weight variation among the accessions as the stress was intensified. Generally, the Konso accession performed better under 15 days irrigation interval for leaf fresh and dry weights.

The accession Arbaminch Zuria under daily watered conditions significantly differed from others, which indicated that, availability of water is an important factor to express the genetic potential of leaf growth. This indicates that irrigation interval limited the growth potential and narrows down the difference in growth of *Moringa* accessions. The study showed that evaluations of plants under more stressed conditions cannot reflect the true potential of a particular plant or accession. However, the present study indicated that increasing irrigation interval significantly reduced leaf fresh and dry weight of *M. stenopetala* accessions. In line with our study Majken*et al. (*2005) revealed that as plants subjected to different level of irrigation interval, the reduction of leaf fresh and dry weight might be due to the reason that, leaf production and leaf expansion growth are very sensitive to water availability. Furthermore, the leaf production and leaf expansion growth were gradually reduced when plants are subjected to prolonged irrigation interval (Jaleel *et al.*, 2009).The result was also in agreement with works reported by Wafa (2006), Jacob (2013) and Badran *et al*. (2016) who indicated that, leaf fresh and dry weight of *Moringa* are reduced with respect to increased irrigation interval.

Table 5. Interaction effect of accessions and irrigation interval on leaf fresh and dry weight of *Moringa stenpetala* seedlings under greenhouse condition at Hawassa.

	Irrigation interval			
Accessions	Daily	5 Days interval	10 Days interval	15 Days interval
	Leaf fresh weight (gram per plant)			
Konso	4.88±0.11^{b}	2.25±0.11^{cd}	1.93±0.11^{defg}	1.52±0.11^{g}
Arbaminch Zuria	5.33±0.11^{a}	2.48±0.11^{c}	1.96±0.11^{def}	1.63±0.11^{efg}
Humbo	4.56±0.11^{b}	2.03±0.11^{d}	1.70±0.11^{efg}	1.58±0.11^{fg}
	Tukey/HSD = 0.42		CV (%) = 5.43	
	Leaf dry weight (gram per plant)			
Konso	2.56±0.04^{b}	1.84±0.04^{cd}	0.75±0.04^{g}	0.50±0.04^{h}
Arbaminch Zuria	2.80±0.04^{a}	1.70 ±0.04^{d}	0.97±0.04^{f}	0.43±0.04^{hi}
Humbo	1.88±0.04^{c}	1.39± 0.04^{e}	0.55±0.04^{h}	0.32±0.04^{i}
	Tukey/HSD = 0.15……………………CV (%) =3.92			

Where, HSD= honestly significant difference, CV= coefficient of variation, means wit hdifferent letters in the same parameter are statistically significant at $P \leq 0.05$.

Root traits

Root length (cm), collar diameter (cm), fresh and dry weight (gram per seedling)

The longest root length, the highest root diameter, fresh and dry weight was recorded from Arbaminch Zuria accession (Table 6). In contrast, the shortest root length, the thinnest diameter and the smallest root fresh and dry weight was observed in accession Humbo. However, there was no significant (P>0.05) difference in root length among accessions Konso and Humbo. Whereas, there was no significant (P>0.05) difference between Arbaminch Zuria and Konso accessions in root diameter. The variability shown in the present study might be due to the existing genetic variability of the accessions for root related traits. Moreover, the difference among the accessions observed in root biomass could be attributed to longer root length and wider girth of the seedlings.

With regard to irrigation interval, the longest root length, the highest root girth, the largest fresh and dry weight of *M. stenopetala* were recorded from seedlings grown under daily watering. On the other hand, the shortest root length, the lowest root girth, fresh and dry weights were recorded on the seedlings watered in fifteen days interval watering. The present study results also indicated that irrigation intervals significantly influenced all root traits (Table 6). The reduction in root length observed in this study was in agreement with the findings of Badran *et al.* (2016), who observed that the growth declined as watering interval increased. The present result was also confirmed with the study conducted by Daba and Adisu (2017), who reported that root diameter of *M. oleifera* decreased as watering interval increased.

Similarly, root fresh and dry weight of *M. stenopetala* in the present study has been related with patterns reported by Wafa, (2006) and Badran *et al.* (2016), who indicated that, prolonging irrigation interval reduced root fresh weight of *M. oleifera* and *M. peregriana*. Dunford and Vazquez (2005) reported also that, decreased moisture in the soil had a significant reduction in root fresh weight. Additionally, as described above, the reduction of root dry weight might be associated with root length and fresh weight influenced by irrigation interval.

Table 6. Impact of irrigation interval on root length, root diameter, root fresh weight and root dry weight of three *Moringa stenopetala* accessions seedling under greenhouse condition at Hawassa.

Treatments	**Parameters**			
Accessions	**Root length (cm)**	**Root diameter (cm)**	**Root fresh weight (g plant^{-1})**	**Root dry weight (gram plant^{-1})**
Konso	14.23±0.47^{b}	1.71±0.05^{a}	21.41±0.72^{b}	10.29±0.56^{b}
Arbaminch Zuria	16.18±0.47^{a}	1.72±0.05^{a}	24.33±0.72^{a}	13.64±0.56^{a}
Humbo	13.08±0.47^{b}	1.50±0.05^{b}	19.23± 0.72^{c}	7.90±0.56^{c}
Tukey/HSD	**1.19**	**0.12**	**1.81**	**1.41**
Irrigation Interval				
Daily (control)	18.296±0.55^{a}	2.38±0.05^{a}	51.60±0.83^{a}	19.39±0.65^{a}
5 days interval	14.96±0.55^{b}	1.68±0.05^{b}	17.34±0.83^{b}	12.53±0.65^{b}
10 days interval	13.35±0.55^{c}	1.41±0.05^{c}	10.26±0.83^{c}	6.92±0.65^{c}
15 days interval	11.39±0.55^{d}	1.24±0.05^{d}	7.40±0.83^{d}	3.61±0.65^{d}
Tukey/HSD	1.52	0.16	2.31	1.80
CV (%)	8.06	7.45	8.2	13.07

Means with different letter in each column of each factor are statistically significant at P≤0.05.

Total seedling fresh and dry weight

Total seedling fresh weight of the *M. stenopetala* seedling was significantly (P<0.001) influenced by accessions and irrigation interval (Table 7). Also, total seedling dry weight of the *M. stenopetala* seedling was significantly (P<0.001) influenced by both accessions and irrigation interval. However, there was no significant (P>0.05) interaction effect of accessions and irrigation interval on both total seedling fresh and dry weight of the *M. stenopetala* seedlings.

Table 7. Mean square values of total fresh weight (TFW) and total dry weight (TDW) of *Moringa stenopetala* accessions under different irrigation intervals

		Mean squares	
Source of variation	DF	TFW	TDW
Accession(A)	2	175.8***	1046.47***
Irrigation Interval (II)	3	4917.46**	324.47***
A* II	6	7.03 ^{ns}	6.90^{ns}
Error	24	3.614	2.925
CV (%)		**6.76**	**9.91**

*Where, ns, ** and *** indicates non-significant difference at P≤5%, significant difference at P≤1% and P≤ 0.1% probability levels, respectively. df = degree of freedom*

Maximum total seedling fresh and dry weight of the *M. stenopetala* seedling was recorded from the accession Arbaminch Zuria followed by accession Konso and the minimum total seedling fresh weight was recorded from accession Humbo (Table 8). The Maximum seedling fresh and dry weight of the accession Arbaminch Zuria might be associated with its greater production of leaf and root biomass.

With regard to irrigation interval, the highest total seedling fresh and dry weight was recorded on the seedlings grown under daily watering, followed by five days, ten days interval, and the seedlings watered with fifteen days intervals (Table 8). The present experiment revealed that, increased irrigation interval caused in decreased total seedling dry weight of *M. stenopetala* accessions. Similar pattern on *M. oleifera* and *M. peregrine* was reported that increased watering interval (increased drought stress) reduced total seedling fresh and dry weight (Wafa, 2006; Badran *et al*, 2016). Comparable results were also reported by several scholars on many other plants (Anyia and Herzog, 2004; Liuand Stutzel, 2004; Majken *et al.*, 2005; Jacob, 2013). This is because water deficit reduces cell expansion, enlargement, growth and development of plants, especially during early stage. Additionally, the reduction of the total seedling weight with increment of irrigation interval was as a result of a decrease in the correlated parameters such as seedling height, leaf number, leaf area, and root growth

Table 8. Impact of irrigation interval on total seedling fresh weight and total dry weight of three *Moringa stenopetala* accessions seedling under greenhouse condition at Hawassa.

Accessions	Total fresh weight (gram plant^{-1})	Total dry weight (gram plant^{-1})
Konso	27.80±0.77^{b}	15.61±0.69^{b}
Arbaminch Zuria	32.09±0.77^{a}	23.08±0.69^{a}
Humbo	24.45±0.77^{c}	13.07±0.69^{c}
Turkey/HSD	1.93	1.74
Irrigation Interval		
Daily (control)	61.80 ±0.89^{a}	31.05±0.80^{a}
5 days interval	25.63±0.89^{b}	20.00± 0.80^{b}
10 days interval	14.92±0.89^{c}	11.62±0.80^{c}
15 days interval	10.11±0.89^{d}	6.34± 0.80^{d}
Tukey/HSD	2.47	2.22
CV (%)	6.76	9.91

Means with different letter in each column of each factor are statistically significant at P-values ≤ 5%.

CONCLUSION AND RECOMMENDATION

Moringa accessions significantly differed for most of the morphological traits studied in this experiment. The accession Arbaminch Zuria was higher in seedling height, stem diameter, root length, leaf area, root fresh weight, total seedling fresh weight, root dry weight and total seedling dry weight than other accessions. On the other hand, accession Humbo was the lowest in seedling height, stem diameter, root length, leaf number, root diameter, leaf area, root fresh weight, total seedling fresh weight, root dry weight and total seedling dry weight than other accessions.

The experiment showed that *M. stenopetala* had the highest seedling height, stem diameter, root length, root diameter, leaf number, leaf area, root fresh weight, total seedling fresh weight, root dry weight and total seedling dry weight when daily watered, while, watering at 10 and 15 days intervals were statistically similar for seedling height and leaf number. However, the lowest stem diameter, root length, root diameter, leaf area, root fresh weight, total seedling fresh weight, root dry weight and total seedling dry weight were recorded from 15 days interval watering. Additionally, fresh and dry leaf weights were significantly influenced due to the interaction effect of accession and irrigation interval. Therefore, seedling growers can use Arbaminch Zuria accession and can water daily to produce quality *Moringa* seedlings.

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Reproductive disorders of dairy cows managed under intensive and semi-intensive production systems in three selected towns in southern Ethiopia

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Abstract

Reproduction and fertility, mainly affected by several reproductive disorders, are the two pillars of dairy production sustainability and profitability. A cross sectional survey was carried out in dairy farms of Hawassa, Arsi Negelle and Wolaita Sodo towns, southern Ethiopia to estimate the prevalence of reproductive disorders and asses the associated host and management related risk factors. Accordingly, a total of 236 recently calved and pregnant cows were managed in 74 randomly selected dairy farms, which were visited every other week. Besides, record exploration and face-to-face interview of farm owners/attendants were carried out. The results indicated that 82 (34.75%) of the examined cows were affected with one or more reproductive health disorders, which was 49.4% in Wolaita Sodo, 36.54% in Arsi Negelle and 21.78% in Hawassa, respectively. Retained fetal membrane (21.61%), uterine infection (19.49%), dystocia (11.44%) and abortion (7.63%) were the leading reproductive disorders encountered, followed by hypocalcemia, anoestrus, uterine prolapse, repeat breeder and vaginal prolapse. Among the considered host and management related risk factors, number of services per conception (p=0.018), previous history of reproductive disorder (p=0.041), geographic area (p<0.001), farm ownership (p<0.001), ventilation (p=0.001), presence of calving pen (p=0.006) and contact with dogs (p=0.021) were significantly associated with the occurrence of reproductive disorders. The study further indicated that retained fetal membrane, dystocia and abortion had statistically significant (p<0.01) contribution for the occurrence of uterine infection. In general, the problem is prevailing and is among the major bottlenecks for the development of dairy production in the areas. The observed reproductive disorders are highly interdependent with the cause-effect relationship and their occurrence are significantly influenced by several managemental/environmental factors than host factors. Therefore, further research work on the etiological factors of the observed reproductive disorders and enhancing the awareness of the owners/attendants are recommended to improve the management and hygiene of dairy cows and their environment at least during their partum and postpartum period.

Key words: Dairy cows, reproductive disorders, Ethiopia

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INTRODUCTION

The huge livestock resources of Ethiopia have been underutilized because of multi-factorial reasons including widespread infectious and non-infectious diseases, poor management system and unimproved genetic makeup coupled with poor nutrition and absence of well-developed market infrastructure (MOA, 1998). In general, reproduction and fertility are the two key pillars of dairy production profitability (LeBlanc, 2008). The incidence rate for reproductive disorders in dairy herds outnumbered the incidence rates of mastitis and locomotor disease (Gardner et al., 1990; Noakes et al., 2001). These problems, caused by infectious and non-infectious causes, are responsible for reduced milk yield and subsequent reproductive performance of dairy cows (Rajala & Gröhn, 1998; LeBlanc et al., 2002; LeBlanc, 2008) and ultimately cause premature and forced culling of high producing dairy cows (Esslemont & Peeler, 1993).

Studies conducted so far in Ethiopia (Negussie et al., 1998; Shiferaw et al., 2003; Asseged & Birhanu, 2004; Lobago et al., 2006; Amene et al., 2011; Nuraddis et al., 2011; Dinka, 2012) revealed poor reproductive performance of dairy cows because of prevailing reproductive disorders and limited energy intake. For feasible intervention, the poor reproductive performance of dairy cows should warrant investigation on the types and magnitudes of the existing postpartum problems associated with the reproductive issues. In this regard, a couple of researchers (Bitew & Prasad, 2011; Gashaw et al., 2011; Angesom et al., 2013; Getenet et al., 2014; Wujira & Nibret, 2016; Ambaw et al., 2017; Abuna et al., 2018) attempted to address this issue by employing questionnaire survey and observation in some parts of the country. However, the majority of the aforementioned studies were aimed at observing the study animals at a single point in time so that the probability of missing the variable of interest is high. Moreover, they were limited to certain reproductive disorders or on small study area. Perhaps, these limitations have underestimated the type and magnitude of the reproductive disorders in the dairy farms of Ethiopia. In contrast, the present study was based on regular visit of the study animals during the period the event of interest is most likely to occur. Thus, the chance of missing the events is very low. On the other hand, the existing literatures and the scope of reproductive health in the dairy industry demand more intensive investigation. Therefore, the present study was intended to identify and estimate the prevalence of the major clinically manifested postpartum problems and to assess the associated risk factors in dairy cows kept under intensive and semi-intensive dairy farms in selected towns of Southern Ethiopia.

MATERIALS AND METHODS

Study Area

The study was conduct in Hawassa, Wolaita Sodo and Arsi Negelle towns. These towns are known for their high potential in dairy production in Southern Ethiopia and are the major source of milk and other dairy products to the Southern Nations, Nationalities and Peoples Regional State (SNNPRS)as well as to the Sidama and Oromia Regions.

Arsi Negelle town is located in the West Arsi zone of the Oromia regional state at a distance of 232 km from Addis Ababa. The town is situated at an altitude of 2,000 to 2,400 m above sea level, and at a latitude of 7^0 21’ N and longitude of 38^0 42’ E. The average annual temperature of the area varies from 14 to 23 °C while rainfall ranges between 500 and 1000 mm (Mengistu & Solomon, 2015).

Wolaita Sodo town is an administrative capital for the Wolaita zonal administration in SNNPRS. It is located at 380 km south from Addis Ababa. The town is divided in to 3 sub-cities and 11 kebeles (represent the lower administrative units). The town has a census projected population of 111,616 (Amha et al., 2018). Hawassa is the capital city of Sidama region, which is located 275 km south of Addis Ababa along the Addis Ababa - Moyale highway. Hawassa is situated at an altitude of 1750 m above sea level between 6°83' to 7°17' N and 38°24' to 38°72’ E. Hawassa receives an average annual rainfall of 955 mm with mean annual temperature of 20°C (BoFED, 2007).

Study Population and Management Practices

The study population constituted the dairy cows in the dairy farms found in Hawassa city, Wolaita Sodo and Arsi Negelle towns. The majority of dairy farms included in this study were market-oriented smallholder dairy farms with average herd size of ≥5 cows. There were also few governmental and private owned large (commercial) scale dairy farms with herd size of over 50 cows in Hawassa city and Wolaita Sodo town. Most of these farms rear crossbred dairy cows and heifers with a semi-intensive management system. Hay and concentrate (wheat bran, molasses and brewery byproducts) were the common types of feed used almost in all farms although, some owners in Hawassa provided mineral supplements (calcium powder) to their cows to prevent the occurrence of milk fever. The oestrus signs of the cows were mainly detected by the owners and attendants of the farm. Artificial Insemination (AI) was the primary breeding system practiced in the study areas. However, most of the owners opt for natural mating when their cows repeat more than two times following AI. In all the farms, there was no regular vaccination and deworming practices for the common infectious and parasitic diseases, respectively.

The study animals included in this study were reproductively active cows found during their prepartum, partum and postpartum periods. All pregnant cows and heifers at ≥4 months of gestation period found in the selected farms and expected to deliver during the study period were included for the study.

Study Design and Sample Size

A cross sectional study was carried out between November 2017 and May 2018 to identify the major reproductive disorders in dairy cows. Although the selected farms were visited at different points during the study period, the individual cows were examined for a particular outcome event only once. The sampling method employed was systematic random sampling where the study subjects (dairy cows) were selected if they fulfilled the selection criteria. A total of 74 dairy farms, with the herd size of ≥5 cows, were randomly selected from 222 intensive and semi-intensive dairy farms whose records were available in

Hawassa city, Arsi Negelle and Wolaita Sodo towns agricultural bureau (Table 1). From these farms, a total of 236 pregnant cows and heifers (in their first pregnancy) which were at 4th or more months of gestation were selected and registered according to their ear tag numbers.

Table 1. Number of dairy farms selected and surveyed during study period

Geographic areas	No of farms selected	Proportion
Hawassa	25	33.80%
Arsi Negelle	26	35.10%
Wolaita Sodo	23	31.10%
Total	74	100%

Methodology

All selected cows were registered by their ear tag numbers and/or given name for ease of identification. Then, the required information mainly breeds, parity, body conditions, management system, methods of service and the current and previous prepartum and postpartum reproductive health problems were recorded. These factors were then defined and classified for ease of data coding and analysis. The body condition scores were estimated as described by Wildman et al. (1982) and grouped into three categories: poor (scores 1 and 2), medium (score 3) and good (score 4). The breeds of the animals were identified as local, cross breed and exotic (Holstein Friesian and Jersey) based on phenotype, history from owners and the available record. The management system was classified into intensive and semi-intensive. Any reproductive health problem encountered before calving, during calving and after calving were classified and recorded as prepartum, partum and postpartum events, respectively. The study animals and/or farms were visited every other week until the end of study period for any new case or events of interest. Moreover, additional unscheduled visits were made following a call from the farm owners to gather incidental evidences, to make thorough physical examination and hence to timely collect appropriate data on clinical cases. Any abnormality or terminal event that included normal birth, dystocia, still birth, uterine prolapse, metritis, hypocalcaemia and other events were recorded.

After delivery, cows were regularly examined for the presence of retained fetal membrane, any systemic signs, and if any abnormal vaginal discharge occurred during postpartum period. With the exception of 13 cows that delivered on the last months of our study, all the cows were followed for one month post delivery. During each visit, rectal temperature of cows with rough hair coat, depression and reduced appetite was measured by digital veterinary thermometers (Microlife *VT1831*; Measurement accuracy of ± 0.1°*C*). Each postpartum cow was visually inspected for the presence of any discharge on the vulva, perineum or tail. Moreover, after cleaning the vulva, a clean and sterile vaginal speculum was slowly inserted into the vagina of the cows and then the cervix and vagina were thoroughly inspected with illumination from a penlight. The type and nature of the vaginal discharge was differentiated from cows with normal delivery. The nature of discharge was classified as clear mucus, predominantly clear mucus with flecks of pus, muco-purulent (approximately 50% pus and 50% mucus), purulent (>50% pus) but not foul-smelling, or purulent or red-brown and foul smelling according to LeBlanc et al. (2002). Retained fetal membrane was defined as failure to pass the fetal membrane within 24 h postpartum (Khair et al., 2013). Cows or heifers that failed to conceive for three or more consecutive services were considered as repeat breeders (Robert, 1986; Shiferaw et al., 2003). Anoestrus was defined as the failure of the cow to show clinical heat signs for 90 days or more after parturition (Arthur et al., 1992; Bekana et al., 1994). Moreover, abortion was defined as the expulsion of a dead fetus of recognizable size at any stage of gestation (Robert, 1986; Ball & Peters, 2004).

Data Management and Analysis

The data obtained during regular visits were carefully entered into Excel spreadsheet and then coded and analyzed using Stata 14.2 version (Stata Corp, College Station, TX USA). The association of different risk factors such as breed, management system, body condition scores and methods of breeding with overall prevalence of reproductive problems was analysed by using χ^2 (Chi- square) test and values of $p<0.05$ were considered as significant.

RESULTS

Out of the 236 cows examined, 82 (34.75%) of them encountered at least one of the major reproductive health problems. Relatively higher incidence risk of reproductive disorders was recorded from dairy farms in Wolaita Sodo (49.40%), followed by Arsi Negelle (36.54%) and Hawassa City (21.78%) (Table 2).

Retained fetal membrane (21.61%), uterine infection (19.49%), dystocia (11.44%) and abortion (7.63%) were the leading reproductive disorders encountered followed by hypocalcemia, anoestrus, uterine prolapsed, repeat breeder and vaginal prolapse. The study further identified that retained fetal membrane (11.88%), uterine infection (8.91%) and abortion (5.94%) were the leading reproductive disorders in dairy farms of Hawassa City. Whereas, retained fetal membrane (25%), uterine infection (17.31%) and anestrous (13.46%), were the leading reproductive disorders in Arsi Negelle and uterine infection (33.73%), retained fetal membrane (31.32%) and dystocia (21.69%) in Wolaita Sodo towns (Table 2).

Table 2. Major reproductive problems encountered during the 7 months follow-up period

Type of reproductive Problems	Number (%) affected			
	Hawassa (n=101)	Arsi Negelle (n=52)	Wolaita Sodo (n=83)	Total (n=236)
Retained Fetal Membrane	12(11.9)	13(25)	26(31.3)	51(21.6)
Dystocia	4(4.0)	5(9.6)	18(21.7)	27(11.4)
Uterine infection	9 (8.9)	9(17.3)	28(33.7)	46(19.5)
Abortion	6(5.9)	6(11.5)	6(7.2)	18(7.6)
Hypocalcaemia	4 (4.0)	0(0.0)	3(3.6)	7(3.0)
Anestrous	0(0.0)	7(13.5)	0(0.0)	7(3.0)
Uterine prolapsed	4 (4.0)	0(0.0)	1(1.2)	5(2.1)
Repeat breeder	0(0.0)	3(5.8)	0(0.0)	3(1.3)
Vaginal Prolapse	0(0.0)	0(0.0)	2(2.4)	2(0.9)
Over all RPP*	22(21.8)	19(36.5)	41(49.4)	82(34.8)

*At least one of the reproductive problems

The association of different host and management related risk factors with the occurrence of reproductive disorders were assessed. Accordingly, among the host related risk factors, number of services per conception (NSC), breed and history of previous reproductive disorder had statistically significant association ($p<0.05$) with the occurrence of reproductive problems. Moreover, among the considered managemental risk factors, study sites, ownership, ventilation, floor type, presence of calving pen and contact with dogs had statistically significant association ($p<0.05$) with the occurrence of reproductive disorders (Table 3 and 4).

Table 3. Host factors associated with the occurrence of major reproductive problems

Factors	Category	No cows examined	Cows affected, No (%)	χ^2	p value
BCS	Poor	18	5(27.78)	1.68	0.432
	Medium	52	15(28.85)		
	Good	166	62(37.35)		
NSC	One	114	34(29.82)	5.60	0.018
	≥Two	94	43(45.74)		
Parity	One	81	30(37.04)	5.35	0.148
	Two	53	19(35.85)		

	Three	39	10(25.64)		
	≥Four	44	22(50.0)		
Breed	HF	64	10(15.63)	18.96	0.000
	HF cross	137	61(44.53)		
	Jersey	17	8(47.06)		
	Jersey Cross	16	3(18.75)		
History of rep. problem	Yes	41	21(51.22)	4.17	0.041
	No	162	55(33.95)		

BCS=Body condition score, NSC=number of services per conception for the current pregnancy, HF= Holstein Friesian

Table 4. Management Factors associated with the occurrence of major reproductive problems

Factors	Category	No cows examined	Cows affected, No (%)	χ^2	p value
Geographic area	Hawassa	101	22(21.78)	15.42	0.000
	Arsi Negelle	52	19(36.54)		
	Wolaita Sodo	83	41(49.46)		
Source of cows	Raised in the farm	179	63(35.19)	0.07	0.797
	Purchased	57	19(33.33)		
Ownership	Government	43	25(58.14)	21.89	0.000
	Private	133	30(22.56)		
	Cooperative	36	7(19.44)		
Management	Semi- intensive	62	25(40.32)	1.21	0.271
	Intensive	172	56(32.56)		
Ventilation	Poor	71	14(19.72)	14.80	0.001
	Medium	86	42(48.84)		
	Good	77	25(32.47)		
Floor type	Soil	12	1(8.33)	3.86	0.049
	Concrete	222	80(36.04)		
Graze Outside	Yes	47	21(44.68)	2.63	0.109
	No	187	60(32.08)		
Calving pen	Yes	85	39(45.88)	7.49	0.006
	No	149	42(28.19)		
Contact with dogs	Yes	144	58(40.28)	5.3	0.021
	No	90	23(25.55)		
Service Used	Natural	84	30 (35.71)	0.12	0.94
	AI	138	48 (34.78)		
	Both	13	4 (30.77)		

The attempt made to evaluate the association of uterine infection with the other reproductive disorders revealed that retained fetal membrane (RFM), dystocia and abortion had statistically significant association (p <0.05). However, hypocalcemia, uterine and vaginal prolapses were not significantly associated (P>0.05) with uterine infection (Table 5).

Table 5. Association of uterine infection with the other reproductive disorders of cows as a predisposing factor

Predisposing factors	Number of cows	Cows with uterine infection	Cows without uterine infection	χ^2	p-value
RFM	51	35(76.1%)	16(8.4%)	100.1	0.000
Dystocia	27	15(32.6%)	12(6.3%)	25.27	0.000
Abortion	18	8(17.4%)	10(5.3%)	7.73	0.005
Hypocalcemia	7	3(6.5%)	4(2.1%)	2.51	0.113
Uterine prolapse	5	2(4.3%)	3(1.6%)	1.37	0.24
Vaginal prolapse	2	1(2.2%)	1(0.5%)	1.19	0.27

RFM=Retained Fetal Membrane

DISCUSSION

The present study revealed that 34.8% of the cows observed had one or more of the aforementioned reproductive problems. The current prevalence is comparable to the previous reports from central Ethiopia (33.6%) (Angesom et al., 2013), Wolaita Sodo (35.5%) (Wujira & Nibret, 2016) in Ethiopia and a similar study from India (33.85%) (Khan et al., 2016). In contrast, prevalence as low as 9.2% (Ambaw et al., 2017) and as high as 54.6% (Tamirat et al., 2016) were also reported from South Wollo zone and Adea Berga dairy farm (located in West Shewa zone) in Ethiopia, respectively. The difference in the prevalence of the problem among the different towns/city considered in our study and among the current and previous reports could be explained by the variations in the level of owners' awareness and herd management practices among the different farms (Gashaw et al., 2011).

Based on the current study, retained fetal membrane (RFM) was the leading reproductive problem. The prevalence of RFM (21.61%) recorded in the current study is comparable to the reports of Gashaw et al. (2011), who reported 19.2% from Jimma town. However, the current report is considerably higher than other reports from different parts of Ethiopia *viz* 3.7% from Bishoftu town (Abuna et al., 2018), 0.8% from Ada'a district (Esheti & Moges, 2014), 8.6% from Bedelle area (Molalegn & Shiv,2011) and 12.91% from Adama town (Gizaw et al., 2007). In developed countries, RFM affects 5–10% of calvings (LeBlanc, 2008). The variation in the prevalence of RFM among the different studies are majorly attributed to variation in nutritional and management factors which are commonly accepted as predisposing factors for its occurrence. Loss of uterine tone, stress, abortion, late or premature birth, dystocia, twinning, infections, seasonal and hormonal disorders, vitamin and mineral deficiencies have been identified as causes of RFM (Beagley et al., 2010; Bizuayehu & Wale, 2016). Moreover, the difference in the definition of RFM (particularly on the cutoff period) used by the researchers might contribute to the observed difference in the prevalence of RFM among the aforementioned studies.

During the study period, uterine infection, the second most frequently encountered reproductive problems, was detected in 19.5% cows examined in the form of endometritis, metritis or pyometra. According to the existing literatures, the prevalence of uterine infection in different parts of Ethiopia is highly variable and ranges from relatively low (1.2%) (Esheti & Moges, 2014) to higher prevalence (31.6%) (Getenet et al., 2014). In developed countries, uterine infection affects about 20% of lactating dairy cows, with the incidence ranging from 8% to more than 40% (Goshen & Shpigel, 2006; LeBlanc, 2008; Mendonca et al., 2014). The variation in the prevalence of uterine infections among the studies could be associated with the occurrence of other reproductive disorders (Lewis, 1997; LeBlanc et al., 2002). However, the cleanliness of the calving area and hygiene during assisted calvings are generally thought to significantly affect the incidence and outcome of uterine infections (Olson et al., 1986; Youngquist et al., 1988).

Dystocia was the third frequently encountered reproductive problem in the current study with a prevalence of 11.4%. It was widely reported from different parts of Ethiopia with prevalence ranging from 0.79% in Wolaita Sodo (Getenet et al., 2014) to 11.6% in Mekelle (Mekonnin et al., 2015). Results comparable to the current finding were also reported from different countries abroad (Berry et al., 2007; Mee, 2008; Linden et al., 2009; Atashi et al., 2012). Several factors related to genetic, environmental, and management conditions are known to affect the incidence of dystocia (Adamec et al., 2006; Mee, 2008). Dystocia is a leading cause of calf death at or shortly after birth and leads to more retained placentas and uterine infections with the concomitant reduction of milk yield and reproductive performance (Tenhagen et al., 2007; Lopez de Maturana et al., 2007; Atashi et al., 2012). It can also incur huge veterinary costs and in extreme cases, injury to or death of the dam (Dematawewa & Berger, 1997).

The prevalence of abortion (7.63%) recorded in this study is quite higher than the report of Adane et al. (2014) and Tulu & Gebeyehu (2011), who reported 2.56% from Hosanna and 2.9% from Jimma Horro district, respectively. However, it is lower than the 9.05%, 13.9% and 14.6% prevalence reported by Dawite & Ahmed (2013), Molalegn & Shiv (2011) and Hunduma (2013), respectively. The observed difference in the prevalence of abortion among the aforementioned studies could be explained by the difference in the breed composition of the examined herds, prevalence of diseases known to cause abortion, farm management activity specially feeding and sanitation, the study methodology used and the geographical location (Gashaw et al., 2011; Adane et al.,2014). Apart from the presence of several infectious and non-infectious causes (Hutchinson, 2009), the etiologic diagnosis of abortion in cows is difficult and often complicated by numerous factors (Tagesu, 2018).

The present study further indicated that cows with a previous history of any reproductive problems were more likely and significant (p=0.041) to encounter one or more of the reproductive disorders observed. In line with this, cows with four or more parities were more frequently affected by these reproductive problems than cows with fewer parities. These findings are in line with the previous studies (Dinka, 2012; Haile et al., 2014; Bizuayehu & Wale, 2016) and could be explained by the fact that the likelihood of infectious and/or mechanical damages and formation of scar tissue on the uterus and ovaries progressively increase with the subsequent increase in the parities of the cows. Similarly, cows bred two or more times with AI and/or bull service before conception were more significantly (p=0.018) affected with reproductive disorders than those cows with only a single service.

There was a statistically significant association (p<0.00) of reproductive disorders with the study sites and the ownership of the farms considered. The reproductive disorders were more frequent in dairy farms in Wolaita Sodo town (49.46%) and in government owned farms (58.14%). The observed differences could be associated with the differences in the level of awareness among owners and less commitment of the farm attendants and managers in the government owned farms.

The presence of calving pen (p=0.006) and contact with dogs (p=0.021) have statistically significant effect on the occurence of reproductive disorders, the higher prevalence being recorded in cows delivered in a calving pen (45.88%) and in cows that had contact with dogs (40.28%). Pathogens can easily accumulate in and rapidly disseminate in the area when unhygienic calving pen is used and dogs are allowed to roam freely in and around the farms, respectively.

The study further indicated that cows with dystocia, retained placenta and abortion were more likely to develop metritis/uterine infections. Several studies conducted abroad using path analysis and risk assessment indicated consistently that dystocia, retained placenta, nutrition, and metabolic conditions like ketosis and hypocalcemia increased the likelihood that a cow would develop metritis (Lewis, 1997; Földi et al., 2006; Dubuc et al., 2010; Galvao, 2013; Vieira-Neto et al., 2016). In the current study, about 76% of the cows with retained placenta developed uterine infection. This is comparably higher than the studies conducted abroad that found some 25% to 50% of retained placentas having associations with metritis (LeBlanc, 2008; Bonneville-Hébert et al., 2011). The higher infection rate in the current study could be partly explained by the poor hygiene of the calving pen and hands of the assistants and the overall husbandry practices.

Milk fever/hypocalcemia, was not significantly associated (p>0.05) with the occurrence of uterine infection. The absence of association between hypocalcemia and uterine infection could be due to the smaller number of cases encountered and the age (parity) of the study animals. Studies indicated that cows with several parity are quite frequently exposed to hypocalcemia and concomitantly with uterine infections (Lewis, 1997; LeBlanc, 2008). In this regard, over 55% of the cows examined in the current study were in their first or second parity, where calcium absorption and mobilization is not theoretically a problem. Conversely, five out of seven cows with hypocalcaemia were in their third or more parity. Studies also suggest that as parity exceeds three there will be reduced ability to mobilize calcium from bones, a decline in intestinal transport of calcium and the reduced ability to produce calcitriol (Horst, 1986; Chiwome et al., 2017).

The degree of contamination and trauma on the uterine wall increase during the aforementioned predisposing factors and/or reproductive disorders (Silva et al., 2008; Getnet et al., 2018). Following parturition, mainly during associated complications, a wide range of microorganisms (mainly bacteria) ascend from the environment, invade the birth canal and colonize the uterus of dairy cows and concomitantly cause uterine infection. Depending on the species of bacteria involved and severity of damage on the uterine tissue, the consequences of uterine infection range from no detectable effect on any measure of productivity to premature culling and death of the cow (Lewis, 1997).

CONCLUSIONS

The prevalence of reproductive disorders recorded in the current study suggests that the problem is among the major limiting factor on the dairy production in the area with a presumed reduction on the reproductive efficiency, profitability and sustainability of dairy production. Among others, retained fetal membrane and uterine infections were the two leading reproductive disorders detected in all the three towns. The study revealed that number of services per conception, previous history of reproductive disorder, geographic area, ownership, presence of calving pen and contact with dogs had statistically significant effect on the occurrence of the reproductive disorders. Moreover, cows with retained fetal membrane, dystocia and abortion were significantly exposed for uterine infection. Therefore, awareness should be created among the farm owners and/or attendants in order to improve the management and hygiene of dairy cows and their environment at least during their partum and postpartum period with ultimate goal of reducing the impact the aforementioned reproductive disorders on the sustainability of dairy production and profitability. Also, further research on the etiological factors of the observed reproductive disorders is recommended.

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Characteristics of phosphorus sorption under different land use systems at Agarfa, South Eastern Ethiopia

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Abstract

Determination of the Phosphous (P) sorption characteristics of soils is important for a better understanding of P, economic fertilizer application and to recommend appropriate management strategies for high P-fixing soils. The phosphorus adsorption-desorption behavior of soils from four land use patterns of central Ethiopia was studied. The study was conducted to determine the effects of land-use systems on selected soil physicochemical characteristics and soil P sorption capacities. Five major representative land-use systems (forest, grazing, homestead, traditionally cultivated land, and mechanized land) were identified. Composite soil samples were collected at 0-20 and 20-40 cm depth. Sorption data were obtained by equilibrating 3.0 g of each sample with 25 mL of 0.01 M $CaCl_2$ containing various amounts of KH_2PO_4. The resulting data were fitted with Langmuir and Freundlich equations. Sorption maxima (Xm) and The Freundlich coefficient (Kf) values of soils ranged from 104-295mg P kg-1 and 44-70.5 mg P kg-1 based on Langmuir and Freundlich models, respectively. The SPR values ranged between 11 to 22 mg P kg-1 and 14 to 28 mg kg-1 based on Langmuir and Freundlich models, respectively in 0-20 cm depth. In general, P-sorption models can effectively be used to discriminate soils based on P-fixation ability. Moreover, the amount of fertilizer based on EPR estimation is greater than the amount of fertilizer practiced both by traditionally cultivated and mechanized land uses. Thus, it is recommended that this dose of P fertilizer should be revised and the proper amount of fertilizer based on EPR values estimated for each soil be applied.

Key words: Freundlich model, Langmuir Model, P fixation, Standard P requirement

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INTRODUCTION

Soil Phosphorus deficiency is one of the major factors limiting crop yields worldwide (McLaughlin et al., 2011). This deficiency may be due to low P containing parent material from which soil was formed or low inherent P content, high weathering incidence and soil reaction, long term anthropogenic mismanagement through an imbalance between nutrient inputs, and P losses by erosion and surface runoff (Ravikovitch, 1986). To adequately supply P to crops, the addition of P containing fertilizer (or other sources) becomes necessary in most agricultural soils especially in highly weathered tropical acidic soils (McLaughlin et al., 2011).

Langmuir and Freundlich's models are the most widely used models to describe P-sorption characteristics of soils and draw external P-requirements (Freundlich, 1988). These models can discriminate soils based on their ability to sorb/adsorb P from soil solutions. They also give an insight into the strength of P-adsorption on the surface of a particular soil. However, in Ethiopia previous studies of soil P sorption and desorption processes have tended to focus on the P sorption potential of different soil types and the effects of soil physico-chemical properties (Achalu et al., 2013). There is little information on soil P sorption under different crop systems in Ethiopia in general and Agarfa in particular. Moreover, factors affecting the adsorption capacity of the soils and the appropriate amount of P fertilizer required by the soils of the study area have not yet been investigated. Therefore, to resolve the problem studying the sorption capacity of the soils and identifying high and low sorbing soils have important implications for future fertilizer management and recommendation. Thus, the objectives of this study were to investigate P sorption characteristics and physicochemical properties of soils as affected by land-use systems.

MATERIALS AND METHODS

Description of the Study Area and Land Use

This study was conducted at Agarfa ATVET College which is located in Agarfa Woreda of Bale Zone in the Oromia National Regional State, Ethiopia. It lies at an altitude of 2350 m.a.s.l. between 60 58' 40" and 70 20' 0" N, and 390 44' 0" and 400 26' 40" E. The average annual rainfall of the area based on 10-years meteorological data is 799.05 mm, with a bimodal pattern and two growing seasons. The mean annual temperature is 15.3°C while the mean maximum and mean minimum are 15.77 and 15.02°C, respectively. A preliminary survey and field observation was carried out by visual observations to have general information about land use and cultivation history. Accordingly, the five major representative land-use systems (forest, grazing, homestead, traditionally cultivated and mechanized land uses) were identified.

Soil Sampling and Preparation

After the site selection, from the representative fields, ten subsamples per one composite sample (3 composite soil samples per one land use) were collected at the depth of 0-20 and 20-40 cm in a Zigzag sampling scheme using an auger. The soil samples were mixed, air-dried, and passed through a 2 mm sieve for the analysis of selected soil physical and chemical properties.

Physico-Chemical Properties of Soil

Soil particle size distribution was determined by the Bouyoucos hydrometer method (Van Reeuwijk, 1992). The pH of the soils was measured in water suspension in a 1:2.5 (soil: liquid ratio) potentiometrically using a glass-calomel combined electrode (Van Reeuwijk, 1992). Walkley and Black (1934) wet digestion method was used to determine soil carbon content and percent. Available soil P was analyzed according to the standard procedure of the Olsen method (Olsen et al., 1954). Cation Exchange Capacity (CEC) and exchangeable basic cations (Ca, Mg, K, and Na) were determined in the leachate using 1N neutral ammonium acetate adjusted to a pH of 7. Available micronutrients (Fe, Cu, Zn and Mn) were extracted by DTPA and all these micronutrients were measured on AAS, as described by (Lindsay and Norvell, 1978).

P-sorption

Phosphorus sorption characteristics were determined by batch equilibrium methods in which soil samples were agitated with P solutions of known concentrations (Graetz and Nair, 2008). Phosphorus as KH_2PO_4 was dissolved in a 0.01M solution of Calcium chloride in distilled water. According to Graetz and Nair (2000), to study the sorption of P by soils, 3 g air-dried samples of each soil were placed in a 100 mL plastic bottle in which the final volume was adjusted to 25 mL. A stock solution of P for each rate (0, 2, 4, 10, 20, 30, and 40 mg L^{-1} P) was added (Carter and Gregorich, 2008). The P sorption data for the soils were fitted into the following forms of Langmuir and Freundlich models because linear regression is convenient and the best of the data-fitting process.

Langmuir equations:

$C/X = 1/K.Xm + C/Xm$………. (Equation1)

Where C (mg L^{-1}) is the equilibrium concentration, X (mg kg^{-1}) is the amount of P adsorbed per unit mass of adsorbent, K (L mg^{-1}) is a constant related to the energy of sorption, and Xm (mg kg^{-1}) is P sorption maximum.

Freundlich equation:

$X = KCb$ or $\log X = \log kf + b \log C$ …… (Equation 2)

where, K and b (b<1) are constants, X (mg kg^{-1}) is the amount of P adsorbed per unit mass of adsorbent, and C (mg L^{-1}) is the equilibrium concentration.

Data Analysis

By using SAS software (SAS, 1997) P-sorption was described as influenced by land use and cultivation, and Mean comparisons (LSD) were calculated for the different land uses systems to see the relationship between parameters.

RESULTS AND DISCUSSION

Physicochemical Properties of the Soil

The studied land use had OM content ranging from 1.94 to 7.39%, for 0-20 cm depth and 1.82 to 6.02 % for the 20-40 cm depth (Figures 1). Higher total nitrogen (TN) and available phosphorus were observed in forest land followed by that of grassland at both ranges of depth, which could be related to the higher organic matter content in the soils of forest land as reported by Alemayehu and Sheleme (2013) where a significant correlation between organic carbon and total nitrogen were reported at 0-15 cm and 15-30 cm depth, respectively.

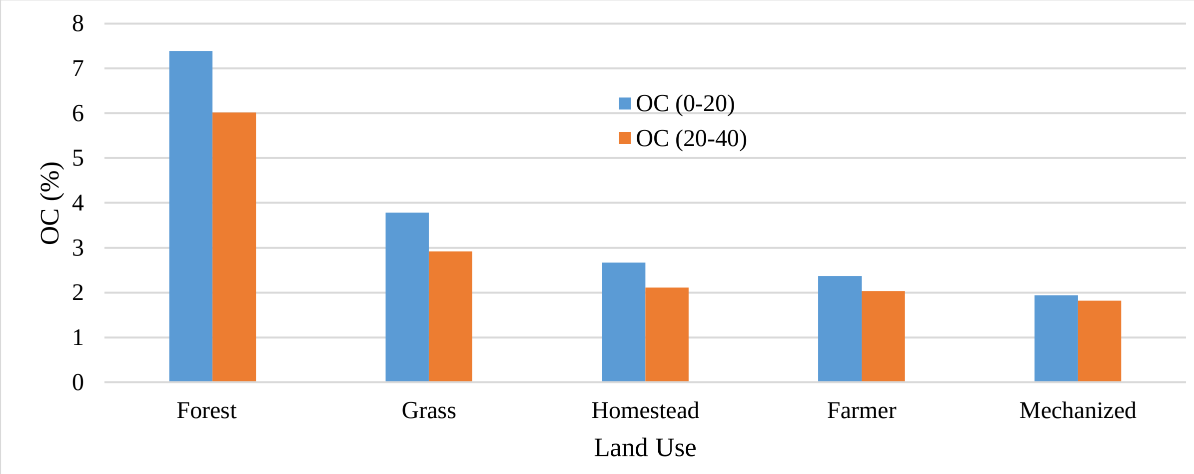

Figure 1: Organic matter content of soils under different land use systems at studied area.

The mean exchangeable cations Ca and Mg content were significantly (P<0.05) different among the land-use systems. The exchangeable calcium (Ca) was highest under homestead land use (Table 1) and lower under grass land in both ranges of depth. Higher concentrations of exchangeable Mg were found under homestead and forest land systems in 0-20 cm and 20-40 cm depths, respectively. The lowest exchangeable Mg were recorded under traditionally cultivated land in both depth (Table 1). Relatively, the highest cation exchange capacity (CEC) values were observed under forest land (52.44 cmol (+) kg^{-1}) followed by that of homestead (51.20 cmol (+) kg^{-1}) at 0-20 cm depth (Table 1) and higher CEC value at 20-40 cm depth were also recorded under the forest land use type.

Table 1. Physical and chemical properties soils at 0-20 and 20-40 cm depths under different land use systems, at Agarfa woreda.

Land use	pH	sand	Silt	Clay	CEC	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
					Cmol (+/kg)				
0-20 cm									
Homestead	7.09a	43a	30b	27	51.20a	23.47a	7.02a	5.59a	4.79
Forest	6.49c	22c	50a	28	52.44a	23.35a	6.27b	3.62c	4.67
Traditional	6.79b	40ab	27b	33	50.66a	23.18a	5.45c	4.91b	4.82
Mechanized	6.63bc	35b	34b	31	41.33b	19.62b	5.94bc	5.53a	4.62
Grassland	6.80b	40ab	30b	30	42.33b	12.94c	5.48c	5.18ab	4.84
LSD (0.5)	0.2	5.1	8.2	NS	3.5	1.7	0.6	0.6	NS
CV (%)	1.2	7.5	12.3	12.2	3.9	4.5	4.8	6.7	3.4
20-40 cm									
Homestead	7.11a	43a	30bc	27	51.25a	23.38a	6.44b	4.61	4.83
Forest	6.30c	23bc	51a	26	52.08a	21.62ab	8.04a	3.53	4.54
Traditional	6.87ab	31b	35b	34	50.13a	22.87a	5.04c	4.51	4.83
Mechanized	6.64b	44a	25c	31	40.43b	19.42b	6.17b	4.86	4.64
Grassland	6.78b	39ab	34b	27	41.60b	12.77c	6.65b	4.87	4.92
LSD (0.05)	0.3	9.7	7.5	NS	5.7	3	0.9	NS	NS
CV (%)	2.4	14	11	15	6.3	7.9	8	15	2.6

Means values within a column followed by the same letter(s) are not significantly different at $p \leq 0.05$.

Significant variations ($p < 0.05$) in available iron (Fe) at both depths were exhibited. Accordingly, the highest available Fe was recorded under forest followed by traditionally cultivated land at both depths, whereas the lowest value was recorded under grassland at both depths. At both depths, available Mn concentrations were higher in forest land soils followed by that of traditionally cultivated system. Moreover, significant variations ($p < 0.05$) in available manganese in both depths were observed among different land-use systems. The highest concentration of available Zinc (Zn) was found in both depths under homestead, whereas the lower concentration was recorded under grass and mechanized land use in 0-20 and 20-40 cm ranges of depth, respectively. Relatively higher available Cu content was observed in forest land (Table 2), which could be due to the relation of copper with organic matter (Alemayehu and Sheleme, 2013).

Table 2. Total nitrogen, available P and extractable micronutrients of soils as influenced by different land uses at different depths, at Agarfa

Land use	TN%	Av. P	Fe	Zn	Cu	Mn
		Mg kg^{-1}				
0-20cm						
Homestead	0.13c	1.70ab	16.24c	1.33a	2.23bc	17.96c
Traditional	0.12cd	1.76a	19.18b	0.63b	3.01ab	23.73b
Forest	0.37a	1.89a	46.04a	0.57b	3.15a	28.96a
Mechanized	0.1d	1.86a	15.83cd	0.56b	2.43b	21.89b
Grass	0.19b	1.45b	11.40d	0.55b	1.64c	1.59d
LSD (0.5)	0.03	0.3	2.9	0.2	0.7	2
CV (%)	8.5	9	7.2	14.4	14	5.8
20-40cm						
Homestead	0.12bc	1.56b	16.95cd	1.41a	2.41b	22. 19c
Traditional	0.1c	1.86a	22.95b	0.68c	2.98a	27.25a
Forest	0.3a	1.75ab	50.95a	1.01b	3.00a	28.73a
Mechanized	0.09c	1.77a	18.57c	0.57c	2.58ab	25.27b
Grass	0.15b	1.40bc	11.29d	0.58c	1.88c	1.88d
LSD (0.05)	0.05	0.3	2.2	0.3	0.5	1.7
CV (%)	15	9.1	4.8	19	10.6	4.3

Means values within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$)

Phosphorous Adsorption Characteristics

P Sorption indices

Sorption behavior was described by the linearized Langmuir sorption model (with regression coefficient of $R^2 > 0.93$) for mechanized, grassland and homestead land-use systems (Table 3). All land uses had sorption maxima ranging from 104 to 295 mg P kg^{-1} with a mean value of 199.5 mg kg^{-1}. The highest sorption maximum was observed under traditionally cultivated land system at 20-40 cm depth range, which might be attributed to the higher clay content of the soil. Furthermore, sorption affinity constant (k), which is the dominant factor showing the bonding energy of the soil to retain P, ranged from 0.32 to 0.74 (L mg^{-1}), with a mean value of 0.53 L mg^{-1}, indicating that the studied soils had variable adsorption energy coefficients.

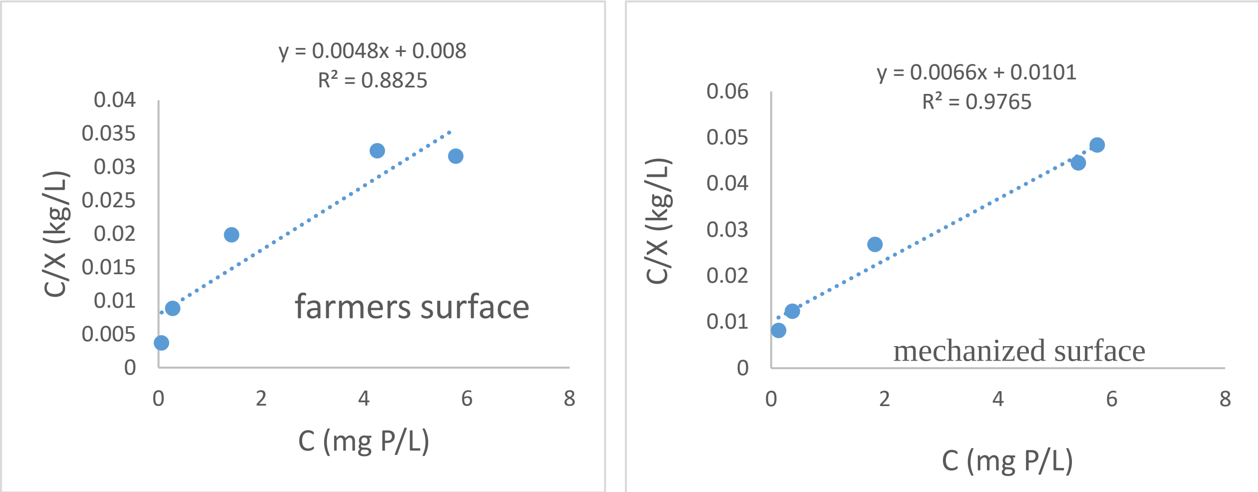

Figure 2: Organic matter content of soils under different land use systems at studied area.

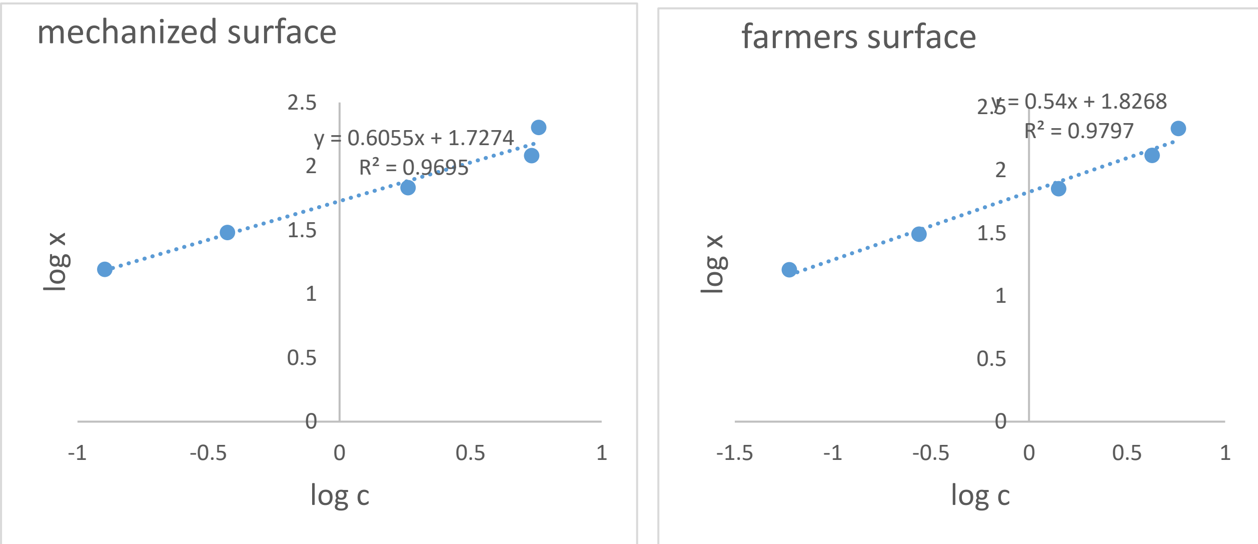

Figure 3: Freundlich P adsorption isotherms of mechanized and traditionally cultivated land use systems.

Table 3. Langmuir and Freundlich sorption parameters and R values of the regression analysis.

Land use	P sorption indices LE				P sorption indices FL			
	Xm (mg kg^{-1})	K (L mg^{-1})	SPRL (0.2) mg kg^{-1}	R^2	b (L kg^{-1})	Kf (mg kg^{-1})	SPRF (0.2) (mg kg^{-1})	R^2
0-20cm								
Forest	104	0.61	11	0.86	0.61	46.6	17.4	0.86
Mechanized	152	0.65	18	0.97	0.61	52.5	19.8	0.96
Grassland	217	0.36	15	0.97	0.72	52	16.4	0.98
Homestead	179	0.4	13	0.93	0.74	47	14	0.90
Traditional	208	0.6	22	0.88	0.54	67.1	28	0.97
20-40cm								

Forest	143	0.74	19	0.87	0.48	53.4	24.6	0.91
Mechanized	213	0.63	24	0.86	0.5	70.5	31.6	0.96
Grassland	175	0.35	12	0.91	0.65	44	15.3	0.95
Homestead	238	0.35	16	0.96	0.69	55.5	18	0.99
Traditional	295	0.32	18	0.90	0.69	61.7	20	0.92

LE= Langmuir equation, FL= Freundlich equation, Xm=Langmuir sorption maximum, K=bonding energy, b= constant of Freundlich equation, SPRL= standard P requirement estimated based on Langmuir model, SPRF= s tandard P requirement estimated based on Freundlich model, Kf=Freundlich surface coverage.

Standard phosphorus fertilizer requirements (SPR)

The soil solution P of 0.2 mg L^{-1} is considered as the amount of available P in the soil solution for optimum plant growth (Bolland et al., 2001). The P required to maintain a soil solution concentration of 0.2 mg P L^{-1} (P 0.2) ranged from 11 to 22 mg P kg^{-1} at the surface depth of the soil in the study soils (Table 3). The amounts of added P required in maintaining the concentration of 0.2 mg P L^{-1} in solution (EPR) were generally lower than the ranges reported in previous studies. For instance, Duffera and Robarge (1999) reported values ranging from 50 to 201 mg P kg^{-1} for surface samples from non-cultivated and non-fertilized areas in Ethiopia. On the other hand, the results of the current study are comparable with the findings of Gichangi et al. (2008) which indicated that the amount of P required to maintain a soil solution concentration of 0.2 mg P L^{-1}(P 0.2) ranged from 2 to 28 mg P kg^{-1} for selected South African soil samples.

The amount of fertilizer applied on traditionally cultivated and the mechanized farm was 100 and 150 kg DAP per hectare, respectively, which are equivalent to 20.3 and 30.4 kg P ha^{-1} for traditionally cultivated and the mechanized farms, respectively. The reason for the low EPR value might possibly be due to early P saturation following repeated applications of P fertilizers. Soils that adsorb less than 150 mg P kg^{-1} soil to meet the SPR value of 0.2 mg L^{-1} solution are considered to be low sorbing soils and those adsorbing greater than this value are high P sorbing ones (Sanchez and Goro, 1980). Accordingly, all the land uses were low P fixers (Table 3) in the present study.

At the 0.2 mg P L^{-1} of equilibration, the EPR of different land uses were calculated based on the estimated value from the Langmuir equation. Accordingly, the EPR of 44 kg P ha^{-1}, 100.8 kg P_2O_5 ha^{-1}, and 219 kg DAP ha^{-1}) for traditionally cultivated farms, 36 kg P ha^{-1}, 82.5 kg P_2O_5 ha^{-1}, and 179 kg DAP ha^{-1} for mechanized farms, 26 kg P ha^{-1}, 59 kg P_2O_5 ha^{-1}, and 129.5 kg DAP ha-1for homestead land were estimated by Langmuir model. The EPR of traditionally cultivated land and mechanized farm were 22 and 18 mg P kg^{-1} respectively, which are equivalent to the application of 44 kg P ha^{-1} on traditionally cultivated and 36 kg P ha^{-1} on the mechanized farms and are greater than the amount of fertilizer application practiced on both land use systems by a factor of 2.2 for traditionally cultivated and 1.2 for the mechanized farms. Moreover, the rate of P being applied currently on the traditionally cultivated land is low compared to mechanize farm with respect to the EPR estimated from the Langmuir equation for each land use in this study. Thus, the application of P fertilizers based on the blanket recommendation in the study area might have resulted in a substantial yield deficit, i.e., which likely remained much below the maximum, owing to P deficiency.

Freundlich constants Kf and b, which represent the intercept and slope of the log-transformed sorption isotherm, may be taken as a measure of the extent of and energy of adsorption for the studied land use systems, respectively. The Kf could also be considered as capacity factor implying that a soil having a larger Kf value has superior adsorbing capacity than others and slope (b) show the effect of concentration on the adsorption capacity and represents adsorption intensity. Therefore, sorption capacity in Freundlich model determines whether the soil is high sorber or not relative to each other. The Freundlich coefficient Kf ranged from 44 to 70.5 mg P kg^{-1} with a mean value of 57.25 mg kg^{-1} (Table 3).

P sorption energy (b), which is also an indicator of the heterogeneity of the soil, ranged from 0.41 to 0.79 L kg^{-1}. The Kf values of the land use were in decreasing order of traditionally cultivated land >

mechanized > homestead > grassland > forest land systems.

Relationship between Soil and P Adsorption Parameters

The data revealed that P sorption maxima were positively correlated with clay content in both depths (r = 0.46) and (r =0.81) for 0-20 and 20-40 cm depth respectively (Tables 3 and 4). An increase in adsorption maxima values with an increasing clay content of the soil could be attributed to the availability of more surface for adsorption of added and native P as reported by (Bopari and Sharma, 2006).

Soil organic matter content was significantly ($p \leq 0.05$) and negatively correlated with Xm (r = -0.66) in 0-20 cm depth and (r = -0.75) in 20-40 cm depth. A significant ($p \leq 0.01$) negative correlation (r = -0.78) was observed between the soil available P and P adsorption maxima in 0-20 cm depth. Furthermore, sorption isotherm indices showed that Xm was negatively correlated with sorption energy of Langmuir (k) in both ranges of depth (r = -0.55) and (r = -0.65 for surface and sub-surface soils, respectively), showing that the soils might have high sorption sites but low sorption energy to hold P on the surface since it is a determinant factor for the soil to have high sorption capacity (Table 3).

Xm was positively correlated with SPR at both depth ranges indicating soils having high sorption maximum may also have high SPR. Soil pH was positively correlated with Xm in both ranges of depth, indicating that P sorption increases with increasing pH. Significant correlations were also observed between Freundlich adsorption indices and some soil physicochemical properties. There was a positive correlation between clay particle and Kf (r= 0.93 and r = 0.67) at 0-20 and 20- 40 cm depth respectively, illustrating that soil texture plays a major role in P adsorption of the soils types considered in the present study.

CONCLUSION

It is concluded that the selected soil physicochemical properties studied in the experiment varied widely in response to different land-use systems at Agarfa, southeastern Ethiopia, indicating that land-use systems have a profound impact on soil quality, fertility, and soil health in general. Soils in all land use systems are N and P deficient, which must be added to increase productivity.

The results of the P-adsorption isotherm study revealed that the P-adsorption data of soil samples taken from all land-use systems fitted well with both Langmuir and Freundlich equations suggesting that both equations can be equally employed to discriminate the soils of the study area with respect to P adsorption characteristics. The P-requirements of all experimental soils at P concentration of 0.2 mg L^{-1} were below 150 mg ha^{-1} indicating that all of them are low P-fixing soils. The blanket recommendation of P fertilizer in the study area may result in a substantial yield deficit, i.e., yield remaining much below the maximum, owing to P deficiency. Moreover, compared to the EPR estimated from the Langmuir equation for each land use in this study, the rate of P being applied currently on the traditionally cultivated land is low compared to mechanize counterparts. Therefore, the revision of fertilizer recommendation has to be considered for the different land-use types.

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Journal of Science and Development
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Discussion: Give interpretations and implications of the results obtained. Compare your findings with related previous studies. The results and discussion sections may be presented together or separately.

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

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Acknowledgements: (if required). These should be brief, *e.g.* five lines of text.

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Short communications should essentially follow the structure given for research articles.

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Books

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

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

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

Conference/workshop/seminar proceedings

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

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

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Thesis

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

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

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