Journal of Science and Development

Volume 4 No. 1, 2016

HAWASSA UNIVERSITY

Journal of Science and Development

Volume 4, No.1 2016

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

Contents

Agricultural and Veterinary Sciences Vol. 4, No. 1 (2016)

Previously Journal of Science and Development, JSD

Effects of Lime and Phosphorus Applications on Soil Chemical Composition, Growth and Nutrient Uptake of Maize (*Zea mays* L.) in an Acid Soil of Gununo, Southern Ethiopia

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Abstract

Soil acidity and phosphorus deficiency are some of the constraints affecting crop production in highlands of Ethiopia. A pot experiment was conducted with maize (*Zea mays* L. ACV 6) to determine the effects of lime ($CaCO_3$) and phosphorus (P) application on highly acidic and P-deficient clayey soil from Gununo area in southern Ethiopia. The experiment consisted application of lime at three rates (0, 3, and 6 g $CaCO_3$ kg^{-1} soil) and P at four rates (0, 50, 100 and 150 mg P kg^{-1} soil), each quadruplicated in a completely randomized design (CRD). Plants harvested at 60 days after plating were partitioned into roots and shoots to record dry matter yields. Changes in soil properties and macronutrient concentrations in roots and shoots were determined. Application of the lime at the highest tested rate (6 g $CaCO_3$ kg^{-1} soil) increased the soil pH by 1.73 units over control, and increased the concentrations of Ca and Mg in the soil and plants. Liming, when applied with P, also improved dry matter production and P concentration in shoots. However, liming alone did not influence available P in the experimental soil, and increasing rates of lime resulted in a slight decline in plant growth and biomass production. On the other hand, P application significantly enhanced available P and exchangeable Ca in the soil; plant heights, root and shoot dry weights, and uptake and concentration of nutrients in shoots and roots. Interestingly, application of 50 g P kg^{-1} soil improved root and shoot dry weights by 1332 and 4184%, respectively, as compared to controls (P omitted pots) demonstrating that P was a more important limiting nutrient for maize growth in the study soils. The results demonstrated that 3 g $CaCO_3$ kg^{-1} together with 50 g P kg^{-1} soil could be recommended for maize growth on the acidic soils of the Gununo area, Boloso Sore district of southern Ethiopia.

Key words: acid soil; dry weight; nutrient concentration; plant height; soil characteristics
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INTRODUCTION

Soil acidity and fertility depletion are among the major constraints limiting agricultural production in high rainfall areas of sub-Saharan Africa (Sanchez et al., 1997; Kisinyo et al., 2014). Soil acidification is one of the major environmental factors emerging as an important land degradation issue. Acid soils constitute about 40% of the cultivated land in Ethiopia and the problem of soil acidity in the country is apparently increasing both in area and severity (Mesfin, 2007). It has become a serious threat to crop production in highlands of the country in general and in the southern region in particular (Desta, 1987; Abdena et al., 2007).

In acid soils, the availability of certain nutrients like aluminum, iron and manganese increases due to high dissolution rates (Upjohn et al., 2005; Sarker et al., 2014). This has been one of the main factors limiting agricultural productivity. Consequently, some barley and wheat growing farmers in southern highland areas have shifted to producing oats, a crop more tolerant to soil acidity. In addition, 70 to 75% of the agricultural soils of the highland regions of Ethiopia are P deficient (Desta 1982; Tekalign and Haque 1991). Phosphorus is therefore considered to be the most limiting nutrient for food production in the soils of Ethiopian highlands (Tekalign and Haque, 1991; Sanchez et al., 1997; Solomon et al., 2002).

Soil acidity adversely affects crop yields, seedling emergence and survival, legume nodulation and root growth, as well as microbial growth, especially if soil pH ($CaCl_2$) is less than 4.5. In strongly acidic soil (pH < 5) aluminum and manganese become more soluble and toxic in most soils, and deficiencies of essential plant nutrients such as P, Ca, K, Mg, and Mo arise (Wang et al., 2006). Correcting soil acidity and nutrient deficiencies, especially P deficiency, by lime and P fertilizer applications, respectively, are the general practices (Fageria et al., 1995; Jibrin et al., 2002). Liming improves the physical, chemical and biological properties of soils and increases crop production on acid soils by raising the pH, Ca and Mg concentrations, and P availability by improving nutrient uptake by plants (Haynes and Ludecke, 1981; Naidu et al., 1990; Oguntoyinbo et al., 1996; Oluwantoyinbo et al., 2005; Loncaric et al., 2007; Uzoho et al., 2010). Liming is more effective in increasing dry matter yields and changing soil properties when combined with phosphorus fertilization (Oluwantoyinbo et al., 2005; Uzoho et al., 2010). Lime materials applied as calcium hydroxide [$Ca(OH)_2$], calcium oxide (CaO) or calcium carbonate ($CaCO_3$) have been found to effectively counteract soil acidity by raising the pH of acidic soils, providing Ca^{2+} and decreasing Al-toxicity, hence stimulating crop

growth (Kamprath, 1984; Kanyanjua et al., 2002; Omenyo et al., 2010). Increases in the available P in soil and resultant high maize production have been reported in acid soils of western Kenya due to P fertilizer application (Kisinyo et al., 2014).

At Gununo, Wolaita Zone of southern Ethiopia, agricultural soils are P deficient due to continuous mining, poor external nutrient return, and soil acidity. This suggests that P availability is the most commonly limiting factor for crop production in the area (Gifole et al., 2011; Sheleme, 2011; Wondwosen and Sheleme, 2011). Further works have also confirmed that most soils in the region are poor in available P and addition of fertilizer is a must for optimum crop production (Ashenafi et al., 2010; Mulugeta and Sheleme, 2010). Application of lime is also one of the important practices, to enhance P availability in acidic soils. However, detailed studies are scanty regarding pH levels, liming requirements and P fertilizer application rates for profitable crop production. In view of this, a lath house experiment was designed to evaluate the effects of lime and P fertilizer application on soil properties, growth, dry matter production and nutrient uptake of maize on acidic soil from Gununo, Wolaita Zone, Southern Ethiopia.

MATERIALS AND METHODS

Site description and soil sampling

Twelve random surface soil samples (0-20 cm) were collected from a farmer's field with known acidity in Gununo (06º 56.316' N, 37º 39.503' E and altitude 1920 meters above sea level) Boloso Sore district, Wolaita Zone in southern Ethiopia. The area is characterized by undulating topography with well-drained Alfisols formed from basaltic parent material. The main crops grown in the area include cereals such as maize (*Zea mays*), wheat (*Triticum aestivum*), pulses such as haricot bean (*Phaseolus vulgaris*), root and tuber crops such as sweet potato (*Ipomea batatas*) and enset (*Ensete ventricosum*).

Soil sample preparation and experimental setting

Soils were homogenized and each pot (10 L capacity) was filled with 5 kg soil and set in a Lath house. Pot experiment was conducted in 2013 with treatment combinations of three lime (0, 3 and 6 g lime kg^{-1} soil) and four phosphorus (0, 50, 100 and 150 mg P kg^{-1} soil) levels, each quadruplicated in a completely randomized design. A recommended rate of N was uniformly applied at 200 mg N kg^{-1} soil using urea (46-0-0) as a fertilizer source, while P (as per the treatment) was applied as triple superphosphate (0-46-0). Four seeds of maize (*Zea mays* L. cv ACV 6) per pot were sown and thinned to two plants per pot 10 days after germination.

Collection of plant data and soil samples from the pot experiment

The pots were kept in a Lath house that was protected from incoming dust and watered regularly using deionized water to maintain the moisture level at about field capacity. Under each pot, a saucer was placed to prevent drainage loss of nutrients. Plant heights were measured at harvest (60 days after planting). Roots and shoots were carefully harvested and their dry weights recorded after oven drying the samples at 80^0C to constant weights (until the same weights were obtained in three consecutive measurements). Soil samples were also collected from each pot at the time of harvest.

Soil and plant tissue analyses

Soil samples (pre-sowing and post-harvest) were analyzed following standard procedures for soil analysis (Sparks, 1996). Accordingly, determinations of pH (0.01 M $CaCl_2$) in 1:2.5 soil: liquid, available P (Bray and Kurtz method), exchangeable Ca, Mg and K (using 1M NH_4OAc extract) and organic carbon content (following the method of Walkley and Black, 1934) were made. In addition, particle size (in accordance with the pipette method of Gee and Bauder, 1986), total nitrogen (Kjeldahal method), NH_4-N and NO_3-N (using ion-selective electrodes), total P, dithionite extractable Fe and Al, pyrophosphate extractable Fe and Al, and oxalate extractable Fe, Al and Si of the test soil were also determined. Plant samples were analyzed for N by a modified Kjeldahal procedure (Nelson and Sommers, 1973), and P, K, Ca and Mg were measured following dry ashing method (Wolf, 1982).

Data analyses

Data from post-harvest soil analysis, plant height, root and shoot dry weights, and nutrient uptakes and concentrations in the plants were subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS, 2007). Mean comparisons were made using Least Significant Difference (LSD), and a PROC CORR option of ANOVA was used to determine the relationships between nutrient availability, growth parameters and nutrient uptake.

RESULTS

Soil characteristics before lime and P application

The experimental soil was clayey in texture, highly acidic (pH 4.01) and very low in available phosphorus (Table 1). It also demonstrated low levels of organic carbon and total nitrogen concentrations. The C:N ratio (13:1) however suggests a dominance of mineralization in the soil even with the acidic conditions of the soil. Nevertheless, this does not guarantee sufficient N availability for plants, as soil microbes often out-compete plants. Out of 1300 mg kg^{-1} total N (0.13 per cent), only

0.32% (4.10 mg kg^{-1}) was NH_4-N and 0.80% (10.4 mg kg^{-1}) NO_3-N, while the remaining 98.9% of the total nitrogen was present in soil as organic N (NH_2-N). The low level of total N coupled with the acidic soil reaction are causes of deficiencies of N in these soils. Furthermore, the very low mineral forms of N show that N availability is extremely low, which might be due to poor microbial activity in the acidic conditions. Additionally, the available P content of the soil (1.07 mg kg^{-1} soil) was also very low (Landon, 1991) indicating that an external supply of N and P would be required for optimum plant growth.

Table 1. Some selected physical and chemical properties of the experimental soil

Sand (%)	8.94	**Dithionite-citrate-bicarbonate (DCB) extractable**	
Clay (%)	65.03	Fe (%)	0.35
pH (0.01M $CaCl_2$)	4.01	Al (%)	0.37
Organic C (%)	1.70	**Sodium pyrophosphate extractable**	
Total N (%)	0.13	Fe (%)	0.24
Total P (mg kg^{-1})	381	Al (%)	0.09
Avail. P (mg kg^{-1})	1.07	**Ammonium oxalate extractable**	
Exch. K (mg kg^{-1})	300	Fe (%)	7.3
Exch. Ca (mg kg^{-1})	456	Al (%)	0.65
Exch. Mg (mg kg^{-1})	58	Si (%)	0.70
		Mineral Nitrogen	
NH_4-N (mg kg^{-1})	4.10	NO_3-N (mg kg^{-1})	10.44

The soil, being acidic, demonstrated appreciable amount of exchangeable Ca, followed by K and, less yet, by Mg. With such inherent properties, the effects of liming as well as P application on some selected soil properties and growth parameters of maize appeared to be specific and selective. The concentrations of Ca (2.28 cmol kg^{-1}), Mg (0.48 cmol kg^{-1}) and K (0.77 cmol kg^{-1}) in the experimental soil could be considered low, medium and high, respectively, in accordance with the ratings of Landon (1991). These results indicate that the Ca:Mg ratio (4.75:1) was in an adequate range, whereas the K:Mg ratio (1.6:1) was high in the experimental soil. Although the absolute Mg concentration (0.48 cmol kg^{-1}) is about optimum threshold for clay soils, deficiencies of Mg could result from its imbalance with K (Landon, 1991). A potassium-to-magnesium ratio of 0.7:1 was suggested as optimum (Loide, 2004) and values higher than 1:1 may affect Mg uptake in clayey soils.

The amounts of Fe and Al extracted from the soils increased in the order following: pyrophosphate-, dithionite-citrate-bicarbonate (DCB-) and oxalate-extractable Fe and Al. Higher values of oxalate-extractable Fe than DCB-extractable Fe might be due to the dissolution of minerals such as magnetite by acid oxalate (Evans and Wilson, 1985; Loepprt and Inskeep, 1996). Furthermore, Fe-oxalate/Fe-DCB ratios (about 21) suggested a high amount of extractable Fe in the soils in the form of active iron oxides. The ratio of Al:Si [(Al-oxalate – Al-pyrophosphate)/Si-oxalate] of about 0.8 indicated dominance of 1:1 clay minerals and the experimental soils being at advanced stages of weathering.

Selected properties of the soils after liming and P application

Soil pH ($CaCl_2$) was raised significantly ($P \leq 0.05$) with increasing doses of lime indicating the usefulness of adding liming material for amelioration of acidity in soils, and corroborating previous findings (Ernani et al., 2006; Torkashvand et al., 2010; Uzoho et al., 2010; Kisinyo et al., 2014; Muindi et al., 2015). Addition of P with lime has also resulted in significant ($P \leq 0.05$) pH increments, although much variation was not recorded among the different levels of applied P (Table 2). However, it did significantly correlate ($P \leq 0.001$) with exchangeable Ca and Mg (Table 6) showing that the release of these basic cations from liming material altered soil reactions. Increases in soil pH after P application without lime might be attributed to contribution of Ca from TSP [$Ca(H_2PO_4)_2$].

Table 2. Soil pH, available P, and exchangeable Ca, Mg and K as influenced by increasing levels of lime and P application

Treatment		pH ($CaCl_2$)	Avail. P	Exch. Ca	Exch. Mg	Exch. K
Lime (g kg^{-1} soil)	P (mg kg^{-1} soil)		(mg kg^{-1} soil)			
0	0	4.43d	1.10f	486.75c	73.50d	279.25a
	50	4.72d	3.54f	503.00c	71.25d	158.75bc
	100	4.64d	12.05cd	523.25c	74.25d	134.50c
	150	4.79cd	17.67a	549.50c	99.00d	174.00bc
3	0	4.87cd	1.03f	1028.75b	209.50bc	292.25a
	50	5.37bc	3.29f	1046.25b	213.25bc	154.00bc
	100	5.82ab	9.45de	1065.75b	206.25c	163.00bc
	150	5.59ab	15.88ab	1086.50b	234.00bc	171.25bc
6	0	5.56ab	1.22f	1427.00a	328.75a	297.00a
	50	6.16a	3.77f	1431.75a	280.00ab	180.50bc
	100	5.87ab	8.38e	1448.00a	278.50ab	166.75bc
	150	5.93ab	13.87bc	1446.00a	278.75ab	193.25b
LSD *(0.05)*		0.63	3.26	199.69	70.72	51.49

Means followed by the same letter(s) within a column are not significantly different at P≤0.05.

Tremendous increases in available P in soils were observed with increasing doses of P, particularly at high P rates (Table 2), whereas increasing levels of lime resulted in a trend of decreasing available P in soil, perhaps due to P fixation as calcium phosphate. Previous studies revealed that a reducing effect on available P was pronounced with high rates of lime application owing to complexation of P (Oluwatoyinbo et al., 2005; Torkashvand et al., 2010; Kisinyo et al., 2014). Additionally, insignificant changes in the levels of available P might also be attributed to the low level of total P and precipitation of phosphate with Fe as structural parts of clay minerals that would not be easily dissociated in acid soil (Mengel and Kirkby, 2001).

Exchangeable Ca significantly ($P \leq 0.05$) increased with increasing doses of lime, whereas its increments with increasing P application rates were not significant (Table 2). The increase in exchangeable Ca was due to its release from the liming material and its slight increase with P rates could be attributed to the release of Ca from the TSP [Ca $(H_2PO_4)_2$] fertilizer. Liming has also significantly enhanced the amount of Mg^{2+} in the soil, whereas the influence of added P on exchangeable Mg was slightly suppressed when combined with the highest lime level (Table 2). The results are in line with previous findings (Torkashvand et al., 2010; Uzoho et al., 2010), and confirmed increments in soil pH, Ca and Mg contents with combined application of P fertilizer and lime. On the other hand, liming had no significant influence on the exchangeable K while P application reduced its content in the soil, which might be attributed to improved K uptake by plants due to synergistic effect of P. Exchangeable K was also negatively correlated ($P \leq 0.001$) with all growth parameters and nutrients' uptake (Table 6) indicating its removal by plants was the cause for the decline in the post-harvest soil.

Plant height and dry matter production

Plant height increased significantly by application of P alone or in combination with lime, although lime alone had no effect (Table 3). Maize plants were vigorously grown with successive increase in P rates when combined with lime. Application of P alone increased root dry weight up to 100 mg P kg^{-1} soil, and root dry weight significantly decreased at the highest P rate indicating declining response of root growth to high P level. Liming combined with P enhanced root dry weights and the highest value was obtained at 3 g kg^{-1} lime and 50 mg P kg^{-1} soil, but further increase in P levels reduced root yields (Table 3). On the other hand, no significant effect of lime alone was observed on root dry weights.

Similarly, shoot dry weight was significantly ($P \leq 0.05$) increased by application of P alone and lime in combination with P application. The highest shoot dry weight (53.5 g pot^{-1}) was recorded at application of 3 g and 150 mg kg^{-1} soil lime and P, respectively (Table 3). This was followed by the treatment combination having the highest rate of lime (6 g kg^{-1} soil) and 100 mg P kg^{-1} soil, whereas the lowest dry weight of shoots was obtained from the pots that received the highest rate of lime without P. There was a slight, reduction in shoot dry weight when increasing levels of lime applied alone indicating that increased rates of lime without P

negatively affects plant growth. In contrast, the application of P alone tremendously increased the shoot dry weight. The shoot dry weight was also reduced by combining the highest rates of both lime and P. The yield obtained from this treatment was significantly lower compared to that of the highest level of P and 3 g lime kg^{-1} soil (Table 3).

Generally, the results show that P rates higher than 50 mg kg^{-1} soil did not significantly increase plant height, and dry weights of roots and shoots when applied in combination with lime. Thus, low level of lime combined with low level of P enhanced maize yield showing that P fertilizer utilization efficiency was enhanced at low level of liming. This is consonant with previous reports showing the presence of low amount of lime reduces the amount of fertilizer P required for optimum crop performance (Oluwatoyinbo et al., 2005; Ernani et al., 2006). Sarker et al. (2014) also reported that shoot and root dry weights were boosted by combining lime with P application. Control plants were remarkably shorter and weighed less than plants treated with lime and/or phosphorus fertilizer. The increments in plant height and dry weights could be attributed to enhanced nutrient uptake, which in turn were influenced by P availability following application of the fertilizer. This was also confirmed by the correlation between the nutrient uptakes and available P in the soil (Tables 2 & 6), showing the essentiality of P, and its deficiency limiting plant growth (Schactman et al., 1998). Previous studies revealed that P deficiency highly limits good crop growth and development in the soils of the study area, and as a result 70, 56 and 76% reductions in plant height, root and shoot dry weights, respectively, were recorded due to P-omission as compared to the optimum treatment with adequate P supply (Wondwosen and Sheleme, 2011).

Nutrient concentrations and uptake in maize plant parts

Applying lime alone had significantly increased Ca and Mg concentrations in the plant material, whereas the concentrations of the other macronutrients in roots and shoots were not affected (Table 4). The highest concentrations of Ca and Mg in the shoot were recorded at the highest lime rate without P, while addition of P significantly suppressed the Ca concentration in shoots at the highest lime rate. On the other hand, increasing P levels significantly improved root Ca concentration. Highest rates of both lime and P together showed the highest concentrations of Ca and Mg in the roots, indicating a synergistic effect of lime with applied P. In contrast, the least concentration of leaf Ca was obtained from the highest rates of P and $CaCO_3$, owing to low soil reaction that might have reduced its uptake by plants (Oluwatoyinbo et al., 2005).

Table 3. Influence of lime and P levels on maize plant height, and root and shoot dry weights

Treatments		Plant height (cm)	Root dry weight (g pot^{-1})	Shoot dry weight (g pot^{-1})
Lime (g kg^{-1} soil)	P (mg kg^{-1} soil)			
0	0	14.9c	1.08d	1.30d
	50	66.8b	11.43bc	34.68c
	100	75.1ab	12.75ab	42.53bc
	150	74.0ab	8.15c	33.58c
3	0	13.1c	0.83d	0.93d
	50	73.3ab	15.30a	48.38ab
	100	75.8ab	13.40ab	45.43ab
	150	79.8a	14.23ab	53.53a
6	0	13.8c	0.95d	0.83d
	50	77.1ab	14.08ab	48.13ab
	100	80.5a	13.78ab	50.10ab
	150	80.5a	13.88ab	41.83bc
LSD (0.05)		10.51	3.57	9.72

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

Table 4. Nutrient concentrations in maize roots and shoots as influenced by increasing levels of lime and P applications on acidic soils of Gununo area, southern Ethiopia

Treatment		Concentration in Roots (%)					Concentration in Shoots (%)				
Lime ($g\ kg^{-1}$)	P ($mg\ kg^{-1}$)	N	P	K	Ca	Mg	N	P	K	Ca	Mg
0	0	2.58a-c	0.13f	0.62	0.14d	0.06c	3.58a-d	0.13f	2.18a	0.34de	0.15d
	50	2.63a-c	0.19d-f	0.57	0.16d	0.07c	3.54a-d	0.27de	1.69bc	0.30e	0.10e
	100	2.35bc	0.23c-e	0.52	0.18cd	0.08bc	3.62a-d	0.37bc	1.72bc	0.37c-e	0.11de
	150	3.23a	0.38a	0.67	0.21bc	0.09bc	4.03a	0.45a	1.70bc	0.39b-d	0.12de
3	0	2.16bc	0.14f	0.52	0.21bc	0.09bc	3.24b-d	0.13f	2.06ab	0.45bc	0.23bc
	50	2.18bc	0.16ef	0.58	0.22bc	0.11ab	3.24b-d	0.24e	1.68bc	0.39b-d	0.20c
	100	2.46bc	0.26b-d	0.65	0.24b	0.12a	3.45a-d	0.35bc	1.37c	0.40b-d	0.22bc
	150	2.84ab	0.34ab	0.61	0.25ab	0.12a	3.82ab	0.40ab	1.33c	0.42b-d	0.24a-c
6	0	2.08c	0.15f	0.76	0.25ab	0.12a	3.08d	0.13f	2.25a	0.59a	0.28a
	50	2.45bc	0.16ef	0.57	0.23bc	0.12a	3.16dc	0.22e	1.44c	0.46b	0.26ab
	100	2.57a-c	0.26b-d	0.62	0.24b	0.13a	3.58a-d	0.33cd	1.44c	0.42b-d	0.28a
	150	2.80a-c	0.30bc	0.63	0.29a	0.13a	3.76a-c	0.35bc	1.31c	0.46b	0.25ab
LSD (0.05)		0.748	0.082	NS*	0.048	0.029	0.616	0.059	0.425	0.093	0.041

Means followed by the same letter(s) within a column are not significantly different at P≤0.05. *NS= Non-significant

The concentrations of P in roots and shoots increased significantly and linearly with increasing levels of applied P; though increasing lime rates suppressed its influence (Table 4). Despite the pronounced increments in concentrations of P, the dry weights of the plant parts did not significantly increase after the first level of applied P, indicating luxury consumption of the nutrient at higher P levels. In contrast to many previous findings (Ivoilov et al., 1990; Oluwatoyinbo et al., 2005; Torkashvand et al., 2010), liming neither increased the concentration of P nor its uptake by plants in the present study, which may be due to the absence of a solubilizing effect of lime on soil P. Generally, N concentrations in both shoots and roots were not significantly affected by liming or applied P; however, the highest P dose indicated appreciable increases in concentrations of N in roots and shoots (Table 4). The K concentration of shoots declined with added P due to the dilution effect.

Table 5. Nutrient uptake by maize root and shoots as influenced by increasing levels of lime and P applications on acidic soils of Gununo area, southern Ethiopia

Treatment		Uptake by Roots ($mg\ pot^{-1}$)					Uptake by Shoots ($mg\ pot^{-1}$)				
Lime ($g\ kg^{-1}$)	P ($mg\ kg^{-1}$)	N	P	K	Ca	Mg	N	P	K	Ca	Mg
0	0	27.6c	1.4f	6.7c	1.5d	0.7c	47.0d	1.7e	28.5d	4.4e	2.0e
	50	294.6ab	21.0e	64.2ab	17.6c	8.4b	1236.3c	92.7d	584.3bc	102.8d	33.3d
	100	288.5b	29.3c-e	65.4ab	22.3bc	10.5b	1577.5a-c	154.0bc	710.1a-c	153.4bc	47.0d
	150	264.6b	30.7dc	54.2b	17.0c	7.0b	1356.3bc	149.3bc	561.7bc	132.6cd	41.8d
3	0	17.9c	1.1f	4.3c	1.8d	0.7c	29.6d	1.2e	19.6d	4.2e	2.2e
	50	333.7ab	23.5de	85.0a	33.0a	16.0a	1551.2bc	117.2b-d	819.1a	187.4ab	96.7c
	100	328.9ab	34.4bc	87.6a	31.6ab	15.7a	1574.0a-c	158.8b	613.2bc	179.9a-c	99.3c
	150	386.2a	46.0a	83.6a	36.5a	17.1a	2025.4a	218.2a	736.4ab	228.7a	129.4ab
6	0	20.8c	1.5f	6.5c	2.4d	1.1c	25.4d	1.0e	16.8d	4.6e	2.3e
	50	348.2ab	22.0e	81.4ab	31.6ab	16.4a	1532.8bc	108.5cd	685.0a-c	223.6a	124.6a-c
	100	343.9ab	34.2bc	83.7a	33.9a	17.2a	1802.8ab	164.6b	722.4a-c	211.8a	139.3a
	150	383.8a	39.9ab	86.4a	39.9a	17.9a	1577.8a-c	147.6bc	546.1c	189.5ab	105.1bc
LSD *(0.05)*		94.6	8.4	27.6	10.6	4.5	468.2	48.4	186.6	49.0	28.4

Means followed by the same letter(s) within a column are not significantly different at P≤0.05.

Increasing levels of lime had no significant influence on the uptake of the macronutrients (N, K, Ca and Mg) by roots and shoots of maize (Table 5), whereas the uptakes of these nutrients were increased in both plant parts by applying P alone; but with no significant difference between the rates of applied P. Combined use of lime and

P had a pronounced effect on the uptake of the nutrients, being maximum at 3 g lime and 150 mg P kg^{-1} soil (Table 5). However, the results of plant growth and yields indicated that application of lime and P above 3 and 50 mg kg^{-1} soil, respectively, was not necessary (Table 3). Increased nutrient uptake with application of P fertilizer was due to high plant growth as was evident by strong correlations between these parameters and corroborates with previous findings (Torkashvand et al., 2010). In addition, very high correlations between nutrient uptakes (Table 6) indicated that their supply should be balanced by adequate availability of other nutrients for optimum growth and development (Wondwosen and Sheleme, 2011).

Table 6. Correlations between soil pH, available P, exchangeable cations, nutrients' uptake and growth parameters

	SDW	RDW	PH	N-UP	P-UP	K-UP	Ca-UP	Mg-UP	AvP	Ex.Ca	Ex.Mg	Ex.K
RDW	0.89***											
PH	0.92***	0.87***										
N-UP	0.97***	0.85***	0.92***									
P-UP	0.91***	0.79***	0.84***	0.91***								
K-UP	0.96***	0.90***	0.89***	0.90***	0.89***							
Ca-UP	0.96***	0.89***	0.87***	0.91***	0.90***	0.93***						
Mg-UP	0.89***	0.80***	0.78***	0.85***	0.81***	0.82***	0.94***					
AvP	0.50***	0.44**	0.64***	0.57***	0.70***	0.47***	0.50***	0.39**				
Ex.Ca	0.45**	0.44**	0.42**	0.43**	0.47***	0.39**	0.56***	0.64***	0.39**			
Ex.Mg	0.11	0.13	0.02	0.09	0.08	0.05	0.25	0.40**	-0.09	0.84***		
Ex.K	0.81***	-0.70***	0.79***	0.79***	0.74***	0.82***	0.71***	0.60***	0.49***	-0.16	0.21	
S-pH	0.40**	0.40**	0.37*	0.41**	0.26	0.25	0.46***	0.60***	0.11	0.70***	0.69***	-0.01

*, **, *** = significant at $P \leq 0.05, 0.01, 0.001$, respectively

SDW=Shoot dry weight; RDW=Root dry weight; PH=Plant height; N-UP=N uptake; P-UP=P uptake; K-UP=K uptake; Ca-UP=Ca uptake; Mg-UP=Mg uptake; AvP=Available P; Ex.Ca=Exchangeable Ca; Ex.Mg=Exchangeable Mg; Ex.K=Exchangeable K; S-pH=Soil pH

CONCLUSION

The study showed that increasing rates of lime increased soil pH and concentrations of nutrients in soil and plants. Liming also had positive effects on dry weights of maize roots and shoots and P concentrations in shoots, when applied with P. However, liming alone did not influence available P in soil, and an increasing rate of lime application without P resulted in a slight decrease in growth and biomass production of the plants. On the other hand, P application significantly enhanced available P and exchangeable Ca in the soil; plant height, root and shoot dry weights and; concentration and uptake of the nutrients in maize shoots and roots. The results demonstrate that P is the most important limiting nutrient; more important than liming for maize growth on the acid soil of the study area. Furthermore, the findings indicate that liming should be used with appropriate level of P to improve plant growth and production. However, the experiment should be repeated in the field to draw a sound conclusion.

Acknowledgements

The study was conducted with the financial support of the government of Canada, International Development Research Center and Global Affairs Canada, through the Canadian International Food Security Research Fund. The support provided by the Research Assistants and Laboratory Staff of the College of Agriculture at Hawassa University was highly appreciated.

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Phytochemical Analysis of Roots of *Aloe gilbertii* and *Millettia ferruginea*

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Abstract

There are about 6500 species of higher plants in Ethiopia making the country one of the most diverse floristic regions in the world. Many medicinal plants are found in southern and south western parts of the country. Nevertheless, there are limited ethnobotanical information and knowledge on the chemical constituents of these medicinal plants. As part of the ongoing project to identify the chemical constituents of medicinal plants of Southern Ethiopia, a comprehensive phytochemical analysis was conducted on the roots of *Aloe gilbertii* and *Millettia ferruginea*. Phytochemical screening tests of the crude root extracts were done accompanied by complete isolation and spectroscopic characterization of compounds isolated from the extracts. Phytochemical screening tests revealed the presence of alkaloids, anthraquinnes, and flavonoids in the roots of both plants whereas terpenoids were absent. Two flavonoids (**1** and **2**) and one known anthraquinone, 8-methoxychrysopanol (**3**), were identified from the roots of *M. ferruginea* and *A.gillbertii*, respectively. The structures of these compounds were determined using spectroscopic techniques (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHSQC and gHMBC) and comparison with literature. The presence of flavonoid and anthraquinone derivatives, known in literature for their various pharmacological activities, in the roots may be attributed to the wide traditional use of the plants.

Key words: anthraquinone, ethnobotany, flavonoids, medicinal plants

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INTRODUCTION

There are about 6500 species of higher plants in Ethiopia making the country one of the most diverse floristic regions in the world. The greater concentrations of these medicinal plants are found in southern and south western parts of the country. Nevertheless, there are limited ethnobotanical information and knowledge on the chemical constituents of these medicinal plants. As part of the ongoing project to identify the chemical constituents of medicinal plants of Southern Ethiopia, a comprehensive phytochemical analysis was conducted on the roots of *Aloe gilbertii* and *Millettia ferruginea* Phytochemical screening tests of the crude root extracts were done accompanied by complete isolation and spectroscopic characterization of compounds isolated from the extracts. Phytochemical screening tests revealed the presence of alkaloids, anthraquinnes, and flavonoids in the roots of both plants whereas terpenoids were absent in the roots of both plants. Two flavonoids (**1** and **2**) and one known anthraquinone, 8-methoxychrysopanol (**3**), were identified from the roots of *M. ferruginea* and *A.gillbertii*, respectively. The structures of these compounds were determined using spectroscopic techniques (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHSQC and gHMBC) and comparison with literature. The presence of flavonoid and anthraquinone derivatives, known in literature for their various pharmacological activities, in the roots may be attributed to the wide traditional use of the plants.

Throughout history, mankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. In Ethiopia, peoples have been using plants in various forms of formulations to treat various diseases (Abebe 1986). For instance, *M. ferruginea* (Figure 1) fruits powder mixed with butter is topically applied to treat skin infection (Mesfin et al., 2009) and fruits powder mixed with honey is taken orally for amoeba and treating 'mujele' (chigger) with fruit paste mixed with butter (Teklehaymanot andand Giday, 2007). The fresh leaf and roots of *A. gilbertii* (Figure 2) is traditionally used to treat liver related disease in Ethiopia (Lulekal et al., 2008). In an

ongoing project to identify the chemical constituents of medicinal plants of Southern Ethiopia, so far we have done a comprehensive phytochemical analysis of roots of *Zanthoxylum chalybeum* (Anza et al., 2014), *Senna didymobotrya* (Alemayhu et al., 2015), *Crotalaria incana* (Anza et al., 2015), *Clerodendrum myricoides* (Esatu et al., 2015), *Lantana camara* (Taye et al., 2015), and *Vernonia auriculifera* (Albejo et al., 2016). We hereby report a comprehensive phytochemical analysis of the roots of *M. ferruginea* and *A. gilbertii*. The genus *Aloe* is known for its bioactive anthraquinones and phenolic components including C-glucosyl derivatives such as barbaloin, aloin A and B, aloenin, aloe-emodin (Fanali et al., 2010). The genus *Millettia* is known for its bioactive flavonoids components including leptobotryanone, maximaisoflavone B, robustigenin, medicarpin, maackiain, genistein, biochanin A, prunetin, chrysoeriol, kaempferol and desmoxyphyllin A (Feng et al., 2007; Sritularak et al., 2006; Rayanil et al., 2011; Pancharoen et al., 2008).

Figure 1. *Millettia ferruginea* (picture taken in Dec., 2014)

Figure 2. *Aloe gilbetii* (picture taken in Dec., 2014)

MATERIALS AND METHODS

General procedures and equipment

UV-Vis spectrum was measured with GENESY's spectrometer (200-400nm) in methanol at room temperature. Infrared (KBr pellet) spectrum was recorded on Perk-Elmer BX infrared spectrometer in the range 400-4000cm^{-1}. Nuclear Magnetic Resonance (NMR) analysis was recorded on Bruker Avance 400MHz spectrometer with tetramethylsilane (TMS) as internal standard. Structural assignments were performed on the basis of 1D NMR (^{1}H NMR, ^{13}C NMR, DEPT-135) and 2D NMR (COSY, gHMQC, gHMBC) spectra. Thin Layer Chromatography (TLC) was conducted using silica gel 60 F_{254}. Column chromatography was performed on silica gel 60 (60-100 mesh).

Plant materials

The roots of *M. ferruginea* were collected in January 2013 from Alamura hill (7° 00' N; 38° 30' E ; 1800 m a. s. l.), in Sidama Zone of SNNPR, Ethiopia whereas roots of *A. gilbertii* were collected from Jello Kebele, Shashemene Woreda, Oromia region that is found 250 km south of Addis Ababa, Ethiopia. All plant materials collected were identified and authenticated by a botanist from Department of Biology, Hawassa College of Teachers Education. Specimen of each plant species was deposited at the herbarium of Hawassa College of Teachers Education, Hawassa, Ethiopia.

Extraction and isolation

The collected plant materials were dried and grounded into fine powder with the help of mortar and pestle. The grounded roots (each 500 g) were extracted by cold percolation with CH_2Cl_2/CH_3OH (1:1) three times for 24 hrs while shaking at speed of 230 r/min and temperature controlled at 28.0 ^{O}C. The marc left was further extracted with methanol (100%) as above. The extracts were concentrated using rotary evaporator (40 ^{o}C) and gave yield of 59 g (11.8%) for *M. ferruginea*, and 49.2 g (9.84 %) for *A. gilbertii*. The crude extract obtained was screened for the presence of various classes of secondary metabolites following the standard protocols (Pradeep et al., 2014; Saleem et al., 2014). Isolation of compounds from the crude extracts was carried out using silica gel column chromatography and solvent mixture of n-hexane and ethyl acetate with gradual increase in polarity.

Phytochemical screening tests for secondary metabolites

Phytochemical screening test was carried out on the crude extract of CH_2Cl_2:CH_3OH (1:1) using standard procedures reported in literature (Pradeep et al., 2014; Saleem et al., 2014) in order to identify the type of secondary metabolites present in the crude extracts.

Alkaloid was screened by adding 1mL of 1% HCl to 3mL of the test extract, dissolved in methanol, in a test tube. The mixture was heated for 20min, cooled and filtered. Then 1mL of the filtrate was tested with 0.5mL Wagner's, Hager's and Mayer's reagents. Formation of reddish brown precipitate for Dragendorff's and Wagner's reagents, yellow precipitate for Hager's reagent and cream precipitate for Mayer's reagent indicated the presence of alkaloids (Pradeep et al., 2014; Saleem et al., 2014).

Flavonoids were determined by Mg-HCl reduction test. A piece of magnesium ribbon (powder) and 3 drops of conc. hydrochloric acid were added to 3mL of the test extract dissolved in methanol. A red coloration indicated the presence of flavonoids. Five milliliters of dilute ammonia solution was added to 5mL of the aqueous filtrate of extract followed by the addition of 1mL concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids. The yellow color disappeared on standing for 10 min (Pradeep et al., 2014; Saleem et al., 2014).

Terpenoids were isolated by Salkowski test i.e. about 5mL of the extract, dissolved in methanol, was mixed with 2mL of chloroform and 3mL of concentrated H_2SO_4 was added. A reddish brown coloration at the interface confirmed the presence of terpenes (Pradeep et al., 2014; Saleem et al., 2014).

For Tannin identification, about 0.2g of the dried powdered samples was boiled in 10mL of distilled water in a test tube and then filtered. Addition of 0.1% $FeCl_3$ solution resulted in a characteristic blue, blue-black, green or blue-green color which confirmed the presence of tannins (Pradeep et al., 2014; Saleem et al., 2014).
In order to screen saponins about 0.2g of powdered sample extract was boiled in 2mL of distilled water on a water bath and filtered. A fraction of aqueous filtrate about 1mL was mixed with 2mL of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously. Formation of an emulsion confirms the presence of saponins (Pradeep et al., 2014; Saleem et al., 2014).

RESULTS AND DISCUSSION

Phytochemical screening tests of the crude CH_2Cl_2/CH_3OH (1:1) extracts of the roots of the plant materials used in the experiment revealed the presence of alkaloids, anthraquinnes, and flavaonoids in the roots of both plants whereas terpenoids were absent in both plants (Table 1). Moreover, the screening test indicated the presence of tannins and saponins in the roots of *M. ferruginea* only (Table 1). The presence of flavonoid and anthraquinone derivatives, known in literature for their various pharmacological activities, in the roots may be attributed to the wide traditional use of the plants.

The crude extracts obtained from the roots of the two plant species were subjected to isolation using column chromatography. The extracts in the column were eluted using a mixture of n-hexane and ethyl acetate with gradual increase in polarity. This led to isolation of flavonoids (1, 2) and 8-methoxy chrysopanol (3) from roots of *M. ferruginea* and *A.gillbertii*, respectively. The structures of these compounds were identified by exhaustive spectroscopic analysis (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHSQC and gHMBC) and comparing with literature spectral data.

Table 1: Phytochemical screening test results of the crude dichloromethane: methanol (1:1) extract

Plant Constituent	**Reagent used**	***A. gilbertii***	***M. ferruginea***
Alkaloids	Dragendroff's reagent	+	+
Tannins	$FeCl_3$	-	+
Anthraquinones	$HCl + CHCl_3 + NH_3$	+	+
Saponins	Warming in water bath	-	+
Terpenoides	Chloroform and conc. Sulphuric acid	-	-
Flavonoides	Dilute ammonia solution + dil. HCl	+	+

+ indicates presence, - indicates absence

Compound 1 was obtained as white crystalline solid (7mg) from the dichloromethane/methanol (1:1). The UV-Vis spectrum in H_2O showed maximum absorption band at $\lambda_{max;}$ 263nm indicating the presence of π-π^* transition in conjugated system of C=C double bond. The IR spectrum exhibited strong absorption band of conjugated carbonyl at 1641cm^{-1}, and medium absorption around 1600cm^{-1} and 1470cm^{-1} suggesting the presence of aromatic system. The ^{1}H NMR spectrum suggested the presence of two methyls at δ 1.56 (6H, *s*), two methoxy at δ 3.96 (3H, *s*) and δ 3.92 (3H, *s*), methine proton singlet at δ 7.81,H-2 (1H, s), three doublet protons at δ7.1 H-2'(1H, *d*), δ 6.83, H-5' (1H, *d*) and δ 6.76, H4" (1H, *d*) and a double doublets at δ 6.96, H-6' (1H, *dd*) in aromatic region and one doublet at δ 5.70, H-3" (1H, *d*) in sp^2 system and one methylene dioxy (-OCH_2O-) protons at δ 6.00, H-2"' (2H, *s*) (Table 2).

The ^{13}C NMR spectrum coupled with DEPT-135 showed twenty two carbons attributed to one carbonyl carbon at δ 175.4, six oxygenated quaternary aromatic carbons at δ 140.1, 147.6, 147.6, 149.2, 151.2 and 153.1, one olefinic quaternary carbon at δ 125.4, three quaternary aromatic carbons at δ 125.7, 113.2 and 106.2; three methine

carbons in aromatic region at δ 122.6, 110.1, 108.3,one oxygenated olefinic methine carbons at δ 150.4, two olefinic methine carbons at δ 129.4 and 115.1, one oxygenated quaternary aliphatic carbon at δ 78.2, two methoxy carbons at δ 62.3 and 61.5, one methylene dioxy (OCH_2O) at δ 101.1 and two methyl carbons at δ 28.1 (Table 2). The peaks at δ 62.25 and 61.49 suggest the existence of methoxy group attached to aromatic sp^2 system. The peak at δ 101.1 suggests the existence of oxymethylene group.

The UV-Vis (λ_{max} = 263nm) spectrum coupled with the ^1H NMR chemical shifts at δ 7.81, (1H, *s*, H-2) and ^{13}C NMR chemical shifts at δ 150.4 (C-2), δ 125.4 (C-3) and δ 175.4 (C-4) suggest isoflavone skeleton for this compound. Moreover, the HMQC spectrum which showed correlation between methine protons at δ 7.81, 7.1 6.96, 6.83, 6.76, 6.00 and 5.70 correlate with the carbons at δ 150.4, 110.1, 108.3, 122.6, 129.2, 101.1, and 115.1, respectively(Table 2). From the ^1H-^1H COSY spectrum evidence, the doublet protons at δ 6.76 (H-4", *d*, J= 8.0) and the doublet protons at δ 5.70 (H-3", *d*, J= 8.0) couple each other suggesting that the two protons are on the adjucent sp^2 carbon atoms. HMBC spectrum (Table 2) revealed that protons H-2 (δ 7.81, *s*) and H-2' (δ 7.1, d, J= 2.4) correlate with the same quaternary carbon at δ 125.7 (C-1') in support of isoflavone skeleton. Additional correlations observed between the protons H-2' (δ 7.1, d, J= 2.4), H-6' (δ 6.96, *dd*, J= 2.4, 7.8), H-5' (δ 6.83, *d*, J= 7.8) and H-6'' (δ 6.00, *s*, 2H) with the same oxygenated quaternary aromatic carbon C-3' (δ 147.6) coupled with the correlation of protons H-6' (δ 6.96, *d*, , J= 2.4, 7.8), H-5' (δ 6.83, *d*, , J= 7.8) and methylene dioxy protons (H-6'', δ 6.00, *s*, 2H) suggest that the oxymethylene is connected at C-3' and C-4' and ring B have an ABX spin pattern. HMBC correlations between proton H-3" (δ 5.70, *d*, J= 8.0) and H-4" (δ 6.76, *d*, J= 8.0) with the oxygenated aliphatic quaternary carbon C-2'' (δ 78.2) and coupled with correlation of prpton H-4'' (δ 6.76, *d*, J= 8.0) with that of quaternary carbon C-8a at δ 153.0 suggest that the pyran ring is connected to ring A. Finally, from the HMBC spectrum correlation observed between H-4" (δ 6.76, *d*, J= 8.0) with that of oxygenated quaternary carbons C-8a (δ 153.1) and C-7 (δ 149.2) support unequivocal placement of the two methoxy groups at C-5 (δ 151.17) and C-6 (δ 140.06) positions of ring A. Thus, all spectral data were in good concurrence with those reported in the literature (Dagne *et al*. 1989) and this compound was previously reported from the ethanolic extract of the stem bark of the same species and the trivial name was given as 5-methoxy durmillone (1, Figure 3) (Dagne *et.al*, 1989).

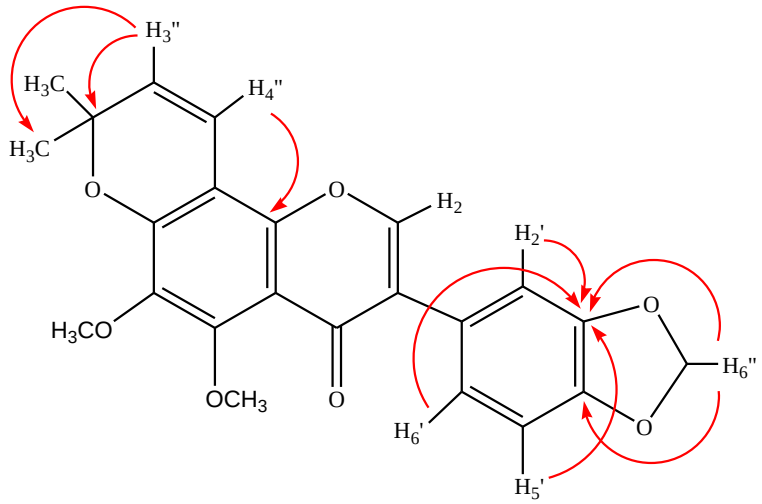


Figure 3: Important HMBC correlations of 5-methoxy durmillone (**1**)

Table 2. Complete NMR data of 5-methoxydurmillone (**1**) (chemical shift (δ) is given in ppm) (400MHz, $CDCl_3$)

Position	^{13}CNMR data of compound 1 (ppm)	^{1}HNMR data of compound 1 (ppm)	^{1}H-^{1}H correlation	HMBC		Reported ^{13}CNMR*	Reported ^{1}HMR data*
				^{2}J	^{3}J		
C-2	150.4	7.81 (H-2, s)			C-1',C-4, C-5	150.2	7.81
C-3	125.4					125.3	
C-4	175.0					175.0	
C-4a	106.7					106.5	
C_5	151.2					151.0	
C_6	140.1					140.0	
C_7	149.2					149.1	
C_8	113.2					113.1	
C_{8a}	153.0					153.0	
$C_{1'}$	125.7					125.6	
$C_{2'}$	110.1	7.10 (H-2', d, J= 2.4)			C-3"	110.0	7.08
$C_{3'}$	147.6					147.5	
$C_{4'}$	147.6					147.5	
$C_{5'}$	108.3	6.83 (H-5', d, J= 7.8)		C-3'	C-1'	108.2	6.85
$C_{6'}$	122.6	6.96 (H-6', dd, J= 2.4, 7,8)		C-1'	C-6, C-2'	122.5	6.93
$C_{2"}$	78.2					68.1	
$C_{3"}$	115.1	5.70 (H-3", d, J= 8.0)	H-7↔H-5		C-2", C-3, C-7	114.9	5.68
$C_{4"}$	129.2	6.76 (H-4", d, J= 8.0)	H-5↔H-7	C-2",	C-8, 2"-CH_3	129.0	6.76
2"-CH_3	28.1	1.56 (H-5", s)				28.0	1.55
O-CH_2-O	101.1	6.00 (H-6", s)				100.0	5.98
5-OCH_3	62.2	3.96 (H-7", s)				62.1	3.96
6-OCH_3	61.5	3.92(H-8", s)				61.3	3.90

*Dagne et al., 1989

Compound **2** have a very comparable NMR data with 5-methoxy durmillone (**1**) except the ^{13}C NMR spectrum. It showed eleven additional signals that can be attributed to two sugar units, two glucopyranosyl moiety of which two signals at δ 66.2 and δ 66.4 are due to methylene carbons of the sugar moeity. The existence of sugar moiety was also supported by signals at δ 4.98 (^{1}H NMR spectrum) and anomeric carbon peaks (^{13}C NMR spectrum) at δ 100.0 (C-1"') and δ 100.9 (C-1""). The downfield chemical shift of C-2"' (δ 87.9), 2""' (δ 87.8) and C-6"' (66.2), C-6"" (66.3) suggested an interglycosidic linkage between the two glucopyranosyl (1""→6"'). In addition, the chemical shift values of oxygenated quaternary aromatic carbons at δ 167.4 C-7 and δ 106, C-7 and C-8, respectively, reveal the existence of -OH at C-7 and prenyl group at C-8. In agreement with this, the absence of downfield singlet which is a characteristic of the chelated OH group suggest that the position of glucose moiety to be at C-7 (δ 100.0, C-1"', δ_H 5.05). Thus, based on the above spectral data, the structure of compound **2** was proposed to be a close derivative of 5-methoxy durmillone (**1,** Figure 3).

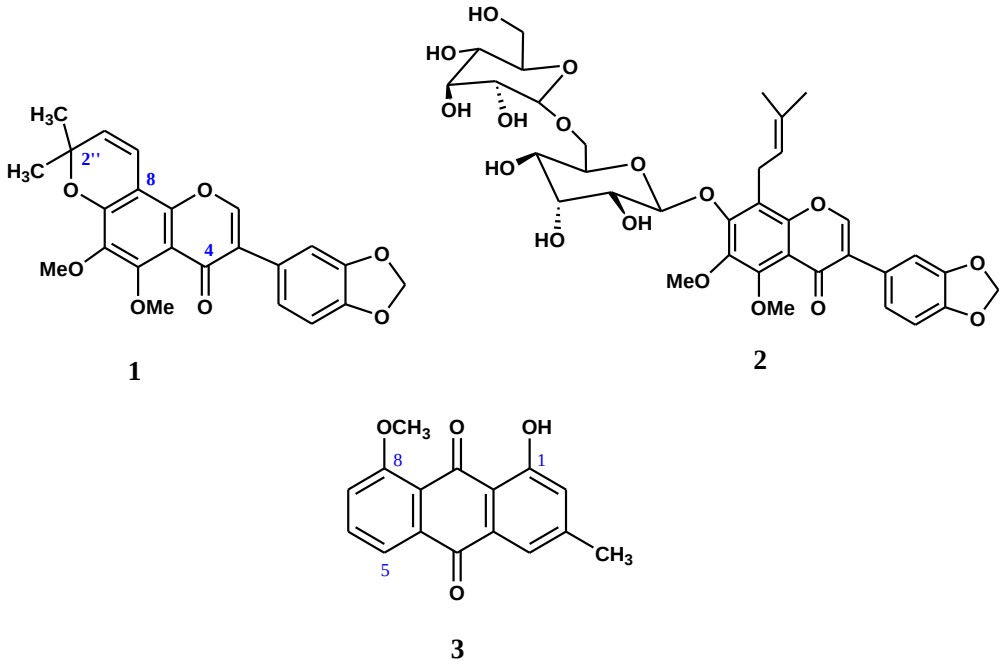
Compound **3** is isolated as yellow powder with R_f value of 0.57 in *n*-hexane/ethylacetae (7:3) solvent system. The IR spectrum showed broad and weak absorption approximately at 3400 cm^{-1} attributed to hydroxyl group. The strong absorption band at approximately 2920 and 2850cm^{-1} revealed the presence of aliphatic C-H stretching vibration. The strong stretching vibration at 1615 cm^{-1} showed the presence of aromatic functionality in the molecule. The absorption bands at 1715 cm^{-1} and 1665 cm^{-1} indicate the absorption of the un-chelated and chelated carbonyl carbon, respectively. The ^{1}H NMR spectrums revealed a singlet signal at δ 12.90 integrated to one proton indicate the presence of chelated hydroxyl group at C-1 *peri* to carbonyl carbon C-9. A triplet peak at δ 7.64 coupled with a doublet proton at δ 7.64 indicates the presence of an ABX aromatic pattern on one ring of anthraquinone skeleton. Two protons showed a weak coupling not well resolved multiplicity (close to broad singlet) at δ7.32 and 7.28 indicates the aromatic protons on C-4 and C-2 of the other ring of the anthraquinone skeleton. A singlet peak at δ 4.08 and 2.99 ppm integrated to three protons indicates the presence of methoxy proton with *peri* effect at C-8 position of ring C

of anthraquinone and a methyl directly connected to an sp^2 carbon (C-3 of ring A) of anthraquinone skeleton. Based on close comparison with literature (Hui et al., 2014), the structure of the compound was found to be a derivative of chrysopanol except substituent on C_8 (hydorxyl replaced by methoxy group). The chemical shift of the methoxy group was desheilded to 4.1 due to the *peri* effect with the carbonyl carbon C-9. Thus, based on the above data, compound **3** was found to be 1-hydroxy-8-methoxy-3-methylanthracene-9,10-dione, known as 8-methoxychrysophanol.

Table 3. Comparison of the observed ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic dataof 8-methoxy chrysopnaol (**3)** with the reported value of Chrysophanol

Position	^{1}HNMR data of compound 3 (ppm)	Reported ^{1}HNMR data of Chrysophanol**
1-OH	12.9 s	12.03 *s*
2-H	7.28 *brs*	7.11 *br s*
3-CH_3	2.90 *s*	2.47 s
4-H	7.32 *brs*	7.30, *dd*, *J*=1.2
5-H	7.78 *dd* (*J*=7.5, 1.2)	7.83 Hz
6-H	7.66 *dd*	7.66, *t*
7-H	7.78 *dd* (*J*=8.0, 1.2)	7.81 *dd*, *J*=1.1, 7.5 Hz
8-OH	-	12.13 *s*
8-OCH_3	4.10 *s*	

**Hui et al., 2014



1 **2**

3

Figure 4. Structures of compounds 1-3.

CONCLUSION

In order to promote Ethiopian herbal drugs and traditional use of medicinal plants, there is an urgent need to evaluate the therapeutic potentials of the drugs as per the WHO guidelines. Bioactive extracts should be validated and standardized on the basis of phytochemical constituents. Despite the rich biodiversity of Ethiopian flora, there are limited information about the type of secondary metabolites present in most of these plants and their biological activity. This paper is a summary of the phytochemical analysis works we have carried out on two plants; *A. gilbertii* and *M ferruginea*. From the two plants, three secondary metabolites (**1-3**) are fully characterized. The study is one of the few attempts to phytochemically analyze the polar extracts of these

plants and further work is recommended on polar extracts of the various parts of the plants so as to identify more novel and bioactive secondary metabolites in support of their traditional use.

Acknowledgements
We are grateful to botanist Reta Regassa, Department of Biology, Hawassa Teacher's College for plant identification. School of Graduate Studies, Hawassa University is duly acknowledged for offering the graduate studies opportunity to Berhanu Eribo. We are thankful to Department of Chemistry, Addis Ababa University for access to NMR, UV-Vis and IR instruments.

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Genetic Variability and Association among Grain Yield and Yield Related Traits in Tef [*Eragrostis tef* (Zucc.) Trotter] Germplasm Collections from Different Parts of Ethiopia

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Abstract

Genetic variability is a key for successful selection of better crop varieties. The present study was, therefore, conducted to determine the magnitude of genetic variation, the broad sense heritability and expected genetic advance and the association among grain yield and yield related traits of recent tef germplasm collections from different parts of Ethiopia. Seventy tef genotypes (68 germplasm collections and 2 released varieties) were evaluated in 7-by-10 alpha lattice design with two replications at Holetta and Debre Zeit Agricultural Research Centers during the main cropping season of 2015. Data were collected on 18 quantitative traits. Analysis of variance showed highly significant ($P<0.01$) genotypic differences for all quantitative traits except for thousand seed weigh and the Genotype x Environment interaction was significant for 14 of the traits. This indicates that breeding for specifically adaptable varieties would be important. The genotypic coefficients of variability (GCV) ranged from 0 to 14.87 % while phenotypic coefficient of variability (PCV) ranged from 7.88 to 31.04 %. The lowest and the highest heritability estimates were observed for grain filling period (0%) and thousand seed weight (0%), and first basal culm internode diameter (46.07%), respectively. The estimates of genetic advance as percent of mean (GAM) ranged from 0% for grain filling period and thousand seed weight to 17% for number of spikelets per panicle and first basal culm internode diameter. Diameters of the first and second basal culm internodes, and panicle length showed relatively high heritability combined with high GAM; these traits can successfully be improved through selection. Moreover, the character of correlation showed selecting longer plant, longer panicle height and high spikelet number increases grain yield, and that higher number of first and second culm internode diameters minimize lodging index.

Key words: Genotypic correlation, GCV, genetic advance, heritability, PCV, Tef

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INTRODUCTION

Tef (*Eragrostis tef* (Zucc.) Trotter) is a C4, self-pollinated, chasmogamous annual cereal crop (Seyfu Ketema, 1997). Tef owes its center of origin and diversity in Ethiopia (Vavilov, 1951). The unleavened bread made of tef flour, "*injera*", is the mainstay of the Ethiopian diet and the straw, "*chid*", is an important feed for livestock (Hailu Tefera and Peat, 1997; Hailu Tefera and Seyfu Ketema, 2001). Mekonnen Melaku *et al*. (2014) noted that the nutrient composition of tef grain has high potential to be used in foods and beverages worldwide. In addition it serves the farmer as a cash crop because both its grains and straw fetch higher market prices than that of the other cereals (Kebebew Assefa *et al.,* 1999; Kenea Yadeta *et al.,* 2001). It has long shelf life and minimal post-harvest damage since the grains are resistant to attack by storage pests (Seyfu Ketema, 1997; Kebebew Assefa *et al*., 1999). It performs better than other cereals including maize and sorghum under moisture stress conditions and it also performs better than maize, wheat or sorghum under excess moisture (waterlogged) conditions (Hailu Tefera and Seyfu Ketema, 2001). In spite of the enormous food, feed, adaptive, nutritive, health, agronomic and economic qualities, the productivity of tef is relatively low (1.56 t/ha) (CSA, 2015) compared to other cereals. One of the major yield limiting factors is lack of cultivars tolerant to lodging which causes yield losses of up to 25%. Lodging remains the major constraint in tef production because it decreases straw yield and deteriorates the quality of both grain and straw produced. It also imposes restrictions to the use of high rates of nitrogen fertilizers. Furthermore, drought and pests play prominent role in reducing tef yield (Kebebew Assefa *et al*., 1999; 2011; 2013).

The wide range of agro-climatic conditions in Ethiopia, generally, favors the existence of large amount of genetic diversity for characters that impart adaptation to specific environments and contribute to yield improvement of the crop. On the other hand, successful selection to develop better varieties with high grain yield is dependent on the

existence of genetic variability. Therefore, estimating the existing genetic variation among landraces will enable us to determine their potential for further breeding activities (Kebebew Assefa *et al*., 2015; Tiruneh Kefyalew *et al*., 2000). In addition to genetic variability, high estimate of heritability with relatively high genetic advance value can be used as an indicator for the efficiency of the phenotype-based selection (Kebebew Assefa *et al*., 2001b). Moreover, as yield is a complex trait and its inheritance is influenced by many genes which are linked with it, assessment of its correlations with important traits facilitates selection of desired traits directly or in directly affecting it. The objectives of the current study were therefore,: to determine i) the magnitude of genetic variation, ii) the broad sense heritability and expected genetic advance and iii) the association among grain yield and yield related traits of tef germplasm recently collected from different parts of Ethiopia.

MATERIALS AND METHODS

The experiment was conducted at Holetta and Debre Zeit Agricultural Research Centers (HARC and DZARC) during the 2015/16 main cropping season. DZARC is located at 8°44'N and 38°58' E, and HARC is located at 9°03'N and 38°30'E at an elevation of 1860 m.a.s.l and 2390 m.a.s.l., respectively. The soil of the experimental site at Holetta and Debre Zeit Research Center is Nitosol and Vertisol, respectively.

A total of 70 tef genotypes recently collected (2012-2014) by the National Tef Research Program from six zones and two released varieties ('Quncho' and 'Tsedey') were included in the present study (Table 1). These materials originated from panicles, which were sown in separate rows for purification at DZARC during the 2013 and 2014 main-cropping seasons and the 2015 off-season. The check variety 'Quncho' was released mainly for high potential areas and 'Tsedey' for low moisture areas. A 7x10 alpha lattice design with two replications and 10 blocks per replication was used at both locations. Each plot with an area of 1mx1m consisted five rows with spacing of 0.2m. All other pre- and post-planting management practices were made as per the recommendations for tef husbandry in the respective test locations.

Data collection

Data were collected on 18 quantitative traits. Out of these Days to heading; Days to maturity; Days to grain filling period; Lodging Index [following the method of Caldicott and Nuttall, (1979), who calculate lodging index as the sum of product of each scale (0-5) of lodging on 0 being erect and 5 completely lodged plant and their respective percentage divided by five]; Total biomass (g); Grain yield (g); Straw yield (g); Thousand seed weight (g); and Harvest Index (HI) were determined on whole plot basis. On the other hand, Plant height (cm); Panicle length (cm); Culm length (cm); Number of total tillers per plant; Number of fertile tillers per plant; Number of spikelets per panicle; Number of primary branches per main panicle; First basal culm internode diameter (mm) and Second basal culm internode diameter (mm) were determined on individual plant basis using five plants randomly sampled from the central parts of the middle rows of each plot.

Statistical analyses

Data of all quantitative variables were subjected to combined analysis of variance using SAS (SAS Institute, 2002). Homogeneity of error variance was tested using the F-max method of (Hartley, 1950).

To estimate the variation among the germplasm, all quantitatively measured variables were subjected to analysis of variance using SAS (SAS Institute, 2002). In the analysis, both genotype and location were considered as fixed effects. The total phenotypic variance of each of the traits were partitioned into contributions by genetic and non-genetic factors using the analysis of variance components method suggested by Singh and Chaudhury (1996). The variance components were determined from mean square values of the ANOVA for each trait according to Prasad et al (1981) as follows:

$\sigma^2G = [(MSG) - (MSE)] / r$

$\sigma^2P = [\sigma^2G + (\sigma^2E/r)]$,

where: σ^2G = Genotypic variance; σ^2P = Phenotypic variance; σ^2E = environmental variance (error mean square from the analysis of variance); MSG = mean square of genotypes; MSE = error mean square; r = number of replications.

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) of each trait were calculated following the method suggested by Burton and Devane (1953).

PCV= $(\sqrt{\sigma^2_p}/\bar{X})$ *100

GCV= $(\sqrt{\sigma^2_g}/\bar{X})$* 100

Where: $\sigma^2_{p=}$ phenotypic variance, $\sigma^2_{g=}$ genotypic variance and $\bar{X}$= grand mean of the trait.Heritability in broad sense, genetic advance (GA) and GA as % of mean were computed as suggested by Allard (1960).

a) Heritability (H^2) = (σ^2_g/σ^2_p)*100

b) GA= K $(\sqrt{\sigma^2}p)$ (H)

c) GA (as % of mean) = (GA/grand mean)*100

Where: GA= Genetic advance; K= A constant which at selection intensity of 5% is 2.06.

Genotypic and phenotypic correlation coefficients and their tests of significance were obtained from CANDISC procedure of SAS (SAS Institute, 2002).

Table 1. List and area of collection of the tef genotypes used in the study

No.	Name	Collection Zones	No.	Name	Collection Zones
1	Amh-ACC#1-L50	North Wello	36	Oro-ACC#16-L38	Jima
2	Amh-ACC#1-L51	North Wello	37	Oro-ACC#16-L48	Jima
3	Amh-ACC#1-L56	North Wello	38	Oro-ACC#16-L51	Jima
4	Amh-ACC#1-L59	North Wello	39	Oro-ACC#16-L52	Jima
5	Amh-ACC#5-L4	North Wello	40	Oro-ACC#7-L1	Horo Gudru
6	Amh-ACC#5-L63	North Wello	41	Oro-ACC#7-L15	Horo Gudru
7	Amh-ACC#6-L5	North Wello	42	Oro-ACC#7-L19	Horo Gudru
8	Amh-ACC#6-L11	North Wello	43	Oro-ACC#9-L2	Horo Gudru
9	Amh-ACC#6-L41	North Wello	44	Oro-ACC#9L5	Horo Gudru
10	Amh-ACC#8-L13	North Wello	45	Oro-ACC#9-L26	Horo Gudru
11	Amh-ACC#8-L20	North Wello	46	Oro-ACC#9-L28	Horo Gudru
12	Amh-ACC#8-L51	North Wello	47	Oro-ACC#9-L38	Horo Gudru
13	Amh-ACC#8-L61	North Wello	48	Oro-ACC#1-L1	South West Shewa
14	Amh-ACC#9-L4	North Wello	49	Oro-ACC#1-L21	South West Shewa
15	Amh-ACC#9-L45	North Wello	50	Oro-ACC#1-L37	SouthWest Shewa
16	Amh-ACC#11-L13	North Wello	51	Oro-ACC#4-L18	South West Shewa
17	Amh-ACC#11-L44	North Wello	52	Oro-ACC#4-L25	South West Shewa
18	Amh-ACC#11-L22	North Shewa	53	Oro-ACC#4-L47	South West Shewa
19	Amh-ACC#11-L36	North Shewa	54	Oro-ACC#8-L10	South West Shewa
20	Amh-ACC#12-L2	North Shewa	55	Oro-ACC#8-L17	South West Shewa
21	Amh-ACC#12-L4	North Shewa	56	Oro-ACC#8-L25	South West Shewa
22	Amh-ACC#12-L29	North Shewa	57	Oro-ACC#15-L8	South WestShewa
23	Amh-ACC#14-L21	North Shewa	58	Oro-ACC#15-L12	South West Shewa
24	Amh-ACC#14-L23	North Shewa	59	Oro-ACC#15-L30	South West Shewa
25	Amh-ACC#14-L24	North Shewa	60	Oro-ACC#16-L42	West Shewa
26	Oro-ACC#8-L13	Jima	61	Oro-ACC#16-L49	West Shewa
27	Oro-ACC#8-L30	Jima	62	Oro-ACC#19-L32	West Shewa
28	Oro-ACC#8-L32	Jima	63	Oro-ACC#19-L36	West Shewa
29	Oro-ACC#8-L5	Jima	64	Oro-ACC#27-L3	West Shewa
30	Oro-ACC#9-L34	Jima	65	Oro-ACC#27-L17	West Shewa
31	Oro-ACC#9-L37	Jima	66	Oro-ACC#30-L7	West Shewa
32	Oro-ACC#9-L45	Jima	67	Oro-ACC#30-L14	West Shewa
33	Oro-ACC#11-L15	Jima	68	Oro-ACC#30-L29	West Shewa
34	Oro-ACC#11-L26	Jima	69	Quncho (DZ-Cr-387)	Released variety(2006*)
35	Oro-ACC#11-L36	Jima	70	Tsedey (DZ-Cr-37)	Released variety(1984*)

*Year of release

RESULTS AND DISCUSSION

Descriptive statistics of qualitative traits

Phenological traits varied widely ranging from 38.8- 52.8 days for heading, 91.3-123.0 days for maturity and 50.0-73.3 days for grain filling (Table 2). The days to heading values of all tef genotypes in this experiment fall within Kebebew Assefa *et al*. (1999; 2000; 2001b) ranges but the maximum values of days to maturity were lower than these reports. Plant height, panicle length and culm length ranged from 74.7-115.5 cm, 26.5-44.9 cm and 47.7-72.7cm, respectively. The panicle length values in the present experiment fall within the ranges of other stated studies, except that reported by Kebebew Assefa *et al*. (2001a). Furthermore, the mean values for other traits varied from 234.9-536.5 for number of spikletes per main panicle, 17.4-32.6 for number of primary branches per main panicle, 1.3-2.9 mm for first basal culm internode diameter and 1.5 and 2.9 mm for second basal culm internode diameter. In line with the current results, Kebebew Assefa *et al*. (2001a) also reported similar mean values for number of primary branches of the main panicle. Regarding the diameters of the first and second basal culm internodes, Kebebew Assefa *et al*. (1999; 2001a&b) reported slightly lower and Habte Jifar *et al*. (2015a) found lower mean values than those recorded in the current experiment. Besides, wide ranges of lodging index values of 48.5-81.5 were noted with the low end corresponding to accessions Oro-ACC#16-L38 and Oro-ACC#9L-5 while the upper end was recorded for accession Oro-ACC#4-L18. On the other hand, studies with released tef varieties by Habte Jifar *et al*. (2015a) showed lower lodging index (10.5) as compared to the results in the current study. Moreover, the range between the lowest and the highest total biomass yielder genotypes was 9375 Kg/ha. Also 3155 Kg/ha difference in grain yield was noted between the lowest yielder Amh-ACC#11-L44 and the highest yielder genotype Oro-ACC#30-L29. Likewise, 6890Kg/ha differences in straw yield were found between the locally collected gemplasm lines Amh-ACC#1-L56 and Oro-ACC#15-L30. Lastly, among the tested tef germplasm lines, Oro-ACC#4-L25 had the lowest (22.9) and Amh-ACC#1-L51the highest (37.0) harvest index values. In comparison, the present results for tiller numbers (total and fertile) were relatively lower than those previously reported by Kebebew Assefa *et al*. (1999; 2000, 2001a&b) and harvest index were relatively higher than those formerly reported by Habte Jifar *et al*. (2015a).

Table 2.Minimum, maximum, mean and standard error (SE) for 18 traits of 70tef germplasms tested at DZARC and HARC in 2105.

Traits	Min.	Genotype	Max.	Genotype	Mean	SE (±)
DH	38.75	Amh-ACC#1-L50	52.75	Oro-ACC#7-L19, Oro-ACC#9-L38 Oro-ACC#4-L18	48.09	0.736
DM	91.25	Tsedey (DZ-Cr-37)	123.00	Amh-ACC#14-L23	109.76	1.233
GFP	50.00	Tsedey (DZ-Cr-37)	73.25	Amh-ACC#14-L23	61.67	0.659
PH (cm)	74.73	Amh-ACC#1-L56	115.46	Oro-ACC#15-L12	99.03	0.702
PL (cm)	26.49	Amh-ACC#1-L51	44.90	Oro-ACC#15-L12	36.91	0.317
CL (cm)	47.68	Amh-ACC#1-L56	72.65	Amh-ACC#8-L61	62.12	0.528
NSPP	234.90	Amh-ACC#1-L59	536.49	Oro-ACC#15-L8	395.33	6.870
NPB	17.44	Oro-ACC#4-L25	32.63	Oro-ACC#9-L37	25.52	0.313
FBCD (mm)	1.25	Oro-ACC#8-L30	2.90	Oro-ACC#11-L26	2.12	0.024
SBCD (mm)	1.53	Oro-ACC#11-L36	2.90	Oro-ACC#11-L26	2.24	0.024
LI (%)	48.50	Oro-ACC#16-L38 Oro-ACC#9L-5	81.50	Oro-ACC#4-L18	67.05	0.797
NTT	2.55	Oro-ACC#1-L37	6.75	Tsedey (DZ-Cr-37)	4.04	0.080
NFT	2.40	Oro-ACC#1-L37	6.50	Tsedey (DZ-Cr-37)	3.59	0.070
TSW (g)	0.24	Amh-ACC#8-L51 Oro-ACC#9-L45	0.36	Amh-ACC#11-L22, Amh-ACC#14-L21 Oro-ACC#4-L25	0.30	0.003
TBM (Kg/ha)	6875	Amh-ACC#11-L44	16250	Oro-ACC#15-L30	12659	1.841
GY (Kg/ha)	1921	Amh-ACC#11-L44	5076	Oro-ACC#30-L29	3690	0.586
SY (Kg/ha)	4842	Amh-ACC#1-L56	11732	Oro-ACC#15-L30	8968	1.366
HI (%)	22.88	Oro-ACC#4-L25	37.03	Amh-ACC#1-L51	29.22	0.246

DH=days to heading, DM=days to maturity, GFP=grain filling period, PH=plant height, PL=panicle length, CL=culm length, NSPP=number of spikletes per main panicle, NPB=number of primary branches per main panicle, FBCD= first basal culm internode diameter, SBCD=second basal culm internode diameter, LI=lodging index, NTT=number of total tillers, NFT=number of fertile tillers, TSW= thousand seed weight, BM= Total biomass, GY=grain yield, SY=Straw yield and HI=harvest index.

Analysis of Variance

The combined analysis of variance, across two locations revealed highly significant (P<0.01) variation among the tef genotypes tested for all of quantitative traits considered, except for thousand seed weight which did not exhibit significant genotype effects (Table 3). Similarly except lodging index and thousand seed weight at Holetta and thousand seed weight at Debre Zeit the individual location, the analysis of variance of all traits showed significant genotype differences (Table 4). Consequently, the presence of highly significant genetic variability among the 70 germplasm accessions for all traits indicated the possibility to exploit the variability existing in tef germplasm in future breeding programs. Supportive results to the present findings were also reported by Kebebew Assefa *et al*. (1999; 2000; 2001a&b; 2002), Ayalneh Tilahun*et al*. (2012), and Habte Jifar *et al.* (2015a&b), who also found significant genotype difference in important yield related traits.

On the other hand, 14 of the grain yield and yield related traits evaluated were significantly affected by genotype x environment interactions (Table 3). This indicated that for these traits the performance of genotypes was not consistent across the two test locations. The highly significant genotype × location interactions noted for the various traits were in agreement with the previous reports (Kebebew Assefa *et al*., 1999, 2000, 2001b, 2002; Wondewosen Shiferaw *et al*., 2012; Habte Jifar *et al.*, 2015a&b). Presence of such interaction for most of the traits is in line with the current focal strategic direction of the tef breeding program (shift from wide to specific adaptation) (Kebebw Assefa *et al*., 2011). Moreover, except grain yield all traits measured in this experiment significantly (p<0.01) varied between locations.

Phenotypic and Genotypic Coefficients of Variation

The coefficients of variation measure the magnitude of variability present in the population (Jalal and Ahmad, 2012). In this experiment, the variances of the genotype × location interaction of 11 traits were higher than the genotypic variances. But genotypic variance of the remaining traits (plant height, panicle length, number of spikletes and primary branches per main panicle, lodging index and the diameters of the two basal culm internodes) showed either comparable or higher genotypic variation than the genotype × location interaction variances (Table 5). These were also reflected in the relatively wider gaps between the corresponding estimates the phenotypic and genotypic coefficients of variation for these traits.

The GCV values ranged from (0%) for grain filling period and thousand seed weight (where variance component due to genotypes was negative) to (14.87%) for number of fertile tillers per plant (Table 5). Similarly, low GCV values were obtained for days to maturity (2.23%) and days to heading (2.87%). This indicates that improvement of those traits through selection may not be effective in this population due to non-genetic sources of variation (Solomon Chanyalew *et al*., 2009). On the other hand, relatively high values of GCV were detected for number of spikelets per panicle (14.72 %), total number of tillers (14.59 %), first basal culm internode diameter (12.34%) and straw yield (11.10%). The lowestPCV values (7.88%) were estimated for days to heading and the highest (31.04 %) for number of fertile tillers. Apart from these, the other phenological and height related traits scored low PCV values. On the other hand, traits like number of total tillers (30.10%), grain yield (26.25%), number of spikelets per main panicle (26.08%), straw yield (24.76%), and total biomass (23.85 %) exhibited high PCV values.

In contrast to our findings, Kebebew Assefa *et al.* (2000) reported high variability in most quantitative traits considered in the experiment but, in line with the present findings, the diameters of the two basal cum internode diameters scored lower variability (7.50% and 6.02% GCV;10.60% and 9.52% PCV). Likewise, the results of Kebebew Assefa *et al*. (2001b) for GCV estimates were comparable to those found in the present study for most of the traits except for the two diameters of basal culm internodes that were lower than those found in the current experiment. Moreover, Ayalneh Tilahun *et al*. (2012) reported similar estimates of PCV and GCV for plant height, while their results were also comparable with respect to PCV values of days to heading and grain yield, and with regard to GCV values of days to maturity and culm length. However, the remaining characters showed low variability coefficients than the current study. Moreover, most of the traits considered in the current experiment showed higher GCV values than those reported by Hailu Tefera *et al*. (2003) in recombinant inbreed lines of tef. In other aspects, Solomon Chanyalew *et al*. (2009) obtained comparable PCV and GCV estimates to those reported here for traits like panicle length and lodging index, plant height and harvest index.

Table 3. Mean squares from the combined analysis of Variance for 18 traits of 70 tef genotypes

Traits	Location (DF=1)	Rep. (DF=1)	Block /Rep. (DF=18)	Genotype (DF=69)	Genotype × Loc. (DF=69)	Error (DF=121)	SEM (±)	CV (%)
DH	38282.41**	6.30ns	7.41ns	25.16**	20.29**	4.58	0.736	4.45
DM	92456.23**	284.01**	33.79ns	136.58**	135.55**	40.82	1.233	5.82
GFP	11752.13**	205.71*	42.84ns	98.28**	125.22**	41.14	0.659	10.40
PH	749.84**	35.03ns	36.19ns	276.25**	127.40**	45.54	0.702	6.81
PL	823.61**	2.17ns	6.26ns	55.97**	18.02**	9.89	0.317	8.52
CL	3145.18**	54.62ns	28.21ns	121.92**	75.46**	24.50	0.528	7.97
NSPP	559070.12**	81.48ns	6557.11ns	19989.08**	9816.62**	4827.88	6.870	17.58
NPB	1837.21**	21.14ns	18.28*	35.23**	16.26**	9.82	0.313	12.28
FBCD	2.40**	1.22**	0.053ns	0.284**	0.083ns	0.08	0.024	13.37
SBCD	7.75**	0.85**	0.091ns	0.263**	0.072ns	0.072	0.024	12.00
LI (%)	3514.51**	1064.70**	176.46ns	220.51**	138.43ns	135.57	0.797	17.37
NTT	81.01**	12.73**	0.56ns	2.93**	1.75**	0.51	0.080	17.71
NFT	26.56**	8.41 **	0.56ns	2.33**	1.46**	0.45	0.070	18.76
TSW	0.04**	0.0003ns	0.002ns	0.002ns	0.0025ns	0.002	0.003	13.99
TBM	5177.20**	432.51ns	556.01ns	1519.92**	1179.69**	382.03	1.841	15.44
GY	8.04ns	67.40ns	52.29ns	151.86**	145.93**	32.31	0.586	15.40
SY	5580.09**	157.67ns	292.90ns	850.64**	568.25**	222.29	1.366	16.63
HI (%)	300.85**	2.62ns	3.8094ns	29.62**	19.40**	5.078	0.246	7.71

DF=degree of freedom, **, * significant at 5% and 1% probability level, ns=non-significant.

Heritability and Expected Genetic advance

Heritability values ranged from 0% for grain filling period and thousand seed weight to 46.11% for first basal culm internode diameter (Table 5). According to Robinson (1966) cited in Dabholkar, (1992), Heritability estimates are classified as low (5-10%), medium (10-30%) and high (30-60%). Therefore, traits such as panicle length (46.07%), second basal culm internode diameter, panicle length (45.38 %) and plant height (37.01%) showed relatively high broad sense heritability estimates, while the values for days to maturity (6.39%) and grain yield (6.13%) were relatively low. Due mainly to the high genotype x location interactions values in the current study, most of traits exhibited low broad sense heritability estimates than those reported by Kebebew Assefa *et al*. (1999; 2000; 2001b). However, diameters of both basal culm internodes showed relatively low and similar heritability values to those previously reported by Kebebew Assefa *et al*. (2000; 2011b). In addition, compared to the other traits, Kebebew Assefa *et al*. (1999) obtained relatively low heritability for the first basal culm internode diameter. Similarly, except plant height and culm length which scored relatively low heritability estimates, most traits studied by Ayalneh Tilahun *et al*. (2012) demonstrated high heritability than those found in the present experiment. Moreover, relatively high heritability values were also reported for different comparable traits of brown-seeded tef genotypes (Habte Jifar *et al*., 2015b), released tef varieties (Habte Jifar *et al*., 2015a) and gynogenically derived tef lines (Habte Jifar and Likyelesh Gugssa, 2013). On the other hand, the heritability value obtained for harvest index in the present study was in agreement with the findings of Solomon Chanyalew *et al*. (2009). Nevertheless, a similar experiment with recombinant inbred lines of tef showed relatively lower heritability values for plant height, panicle length, lodging index and shoot biomass (Hailu Tefera *et al*., 2003).

The estimates of GA as percent of mean ranged from 0% for grain filling period and thousand seed weight to (17%) for number of spikiests per main panicle and first basal culm internode diameter (Table 5). Since, Johnson *et al.* (1955), categorize genetic advance as percent of mean (GAM) as high (>20%), moderate (10-20%) and low (0-10), second basal culm internode diameter (15.13%), number of total tillers (14.56%) and number of fertile tillers (14.68%) exhibited moderate GA estimates as percent of mean, while days to maturity (1.16), days to heading (2.16%) and grain yield (3.31 %) revealed low GA values expressed as per cent of the mean.

Table 4. Mean squares from the individual location analysis of Variance for 18 traits of 70 tef genotypes tested at Holetta and Debre zeit during the 2015 main cropping season

	Holetta							Debre zeit						
Traits	**Rep. (DF=1)**	**Block(Rep.) (DF=18)**	**Genotype (DF=69)**	**Error (DF=51)**	**Mean**	**SE(±)**	**CV**	**Rep. (DF=1)**	**Block(Rep.) (DF=18)**	**Genotype (DF=69)**	**Error (DF=51)**	**Mean**	**SE(±)**	**CV**
DH	0.71ns	9.98ns	29.25**	6.85	59.79	0.38	4.38	7.31ns	2.00ns	13.34**	2.37	36.40	0.26	4.23
DM	5.21ns	53.17ns	86.81**	36.94	127.94	0.69	4.75	682.01**	26.08ns	167.58**	35.96	91.59	0.94	6.55
GFP	9.78ns	57.62ns	65.74*	37.93	68.15	0.63	9.04	548.06**	29.36ns	146.04**	37.20	55.19	0.86	11.05
PH	3.15ns	11.51ns	186.35**	25.25	100.67	0.92	4.99	102.91ns	63.41ns	198.85**	67.74	97.40	1.04	8.45
PL	8.55ns	7.14ns	36.05**	6.20	35.20	0.42	7.07	0.71ns	12.08ns	36.54**	12.55	38.63	0.43	9.17
CL	1.32ns	8.51ns	81.07**	16.11	65.48	0.62	6.13	86.54ns	35.02ns	102.94**	35.97	58.77	0.76	10.20
NSPP	19426.63**	3372.23ns	12850.81**	2403.01	350.65	8.10	13.98	23148.00*	11427.78*	15821.12**	5308.93	440.02	9.75	16.56
NPB	1.15ns	5.16ns	32.88**	8.61	28.08	0.40	10.45	29.49ns	27.33**	17.29*	9.48	22.96	0.37	13.41
FBCD	0.35**	0.04ns	0.13**	0.02	2.03	0.03	7.79	4.63**	0.05ns	0.23**	0.08	2.21	0.04	12.88
SBCD	0.12*	0.03ns	0.15**	0.03	2.07	0.03	8.25	2.70**	0.14*	0.18**	0.07	2.40	0.03	11.44
LI	820.86**	237.20**	106.72ns	80.18	70.59	0.94	12.68	306.06 ns	319.98**	205.45**	105.88	63.51	1.22	16.20
NTT	9.00**	0.43ns	1.28**	0.32	3.50	0.08	16.10	4.18**	1.12*	3.09**	0.54	4.58	0.12	16.00
NFT	7.71**	0.49ns	1.16**	0.33	3.28	0.08	17.58	1.75ns	0.61ns	2.27**	0.53	3.89	0.11	18.71
TSW	0.001ns	0.001ns	0.001ns	0.001	0.29	0.003	11.90	0.000ns	0.003ns	0.003ns	0.002	0.32	0.004	15.22
TBM	1946.31**	743.16**	673.84**	295.94	130.89	2.33	13.14	5406.43**	486.01*	1576.46**	237.16	122.29	2.81	12.59
GY	158.83**	67.06**	56.09**	19.46	36.73	0.67	12.01	586.30**	48.29*	189.40**	21.64	37.07	0.96	12.55
SY	995.86*	388.34*	407.97**	185.60	94.14	1.76	14.47	2431.94**	255.55ns	802.75**	153.80	85.21	2.02	14.55
HI	0.04ns	4.01ns	12.36**	4.27	28.19	0.26	7.33	6.19ns	3.86ns	33.10**	6.27	30.26	0.40	8.28

DF=degree of freedom, **, * significant at 5% and 1% probability level, ns=non-significant.

Table 5. Components of variance, coefficient of variation, heritability, genetic advance (GA) and GA as per cent of the mean for 18 characters in 70 tef genotypes evaluated at Holetta and Debre Zeit during the 2015 main cropping season

Traits	σ^2 l	σ^2 g*l	σ^2 e	σ^2 p	σ^2 g	PCV	GCV	H %	GA	GA (as % of mean)
DH	273.30	7.85	4.59	14.35	1.91	7.88	2.87	13.29	1.04	2.16
DM	659.43	47.90	39.75	93.63	5.98	8.82	2.23	6.39	1.27	1.16
GFP	83.11	38.12	40.59	78.70	0.00	14.39	0.00	0.00	0.00	0.00
PH	4.45	41.57	44.26	136.27	50.44	11.79	7.17	37.01	8.90	8.99
PL	5.75	4.33	9.37	25.39	11.70	13.65	9.27	46.07	4.78	12.96
CL	21.93	25.41	24.65	66.77	16.71	13.15	6.58	25.03	4.21	6.78
NSPP	3923.20	2572.70	4671.10	10629.10	3385.30	26.08	14.72	31.85	67.64	17.11
NPB	13.01	3.37	9.53	18.81	5.91	16.99	9.53	31.43	2.81	11.00
FBCD	0.02	0.003	0.078	0.15	0.07	18.17	12.34	46.11	0.37	17.26
SBCD	0.06	0.01	0.07	0.13	0.06	16.18	10.90	45.38	0.34	15.13
LI (%)	24.12	0.34	137.76	159.97	21.88	18.86	6.98	13.68	3.56	5.31
NTT	0.57	0.62	0.51	1.48	0.35	30.10	14.59	23.49	0.59	14.56
NFT	0.18	0.50	0.45	1.24	0.29	31.04	14.87	22.96	0.53	14.68
TSW	0.0003	0.0004	0.0017	0.0021	0.00	15.13	0.00	0.00	0.00	0.00
TBM	28.55	399.94	379.81	911.37	131.62	23.85	9.06	14.442	8.98	7.10
GY	0.00	55.90	32.15	93.80	5.75	26.25	6.50	6.127	1.22	3.31
SY	35.80	174.24	219.77	493.09	99.08	24.76	11.10	20.094	9.19	10.25
HI (%)	2.01	7.29	4.83	15.79	3.68	13.60	6.56	23.288	1.91	6.52

σ^2 l= location variance, σ^2 g*l=genotype by location interaction variance, σ^2 e=error variance, σ^2 p=phenotypic variance, σ^2 g=genotypic variance, PCV=phenotypic coefficient of variability, GCV=genotypic coefficient of variability, H=heritability in broad sense , GA=genetic advance, DH=days to heading, DM=days to maturity, GFP=grain filling period, PH=plant height, PL=panicle length, CL=culm length, NSPP=number of spikletes per main panicle, NPB=number of primary branches per main panicle, FBCD first basal culm internode diameter, SBCD=second basal culm internode diameter, LI=lodging index, NTT=number of total tillers, NFT=number of fertile tillers, TSW= thousand seed weight, TBM= total biomass, GY=grain yield, SY=Straw yield and HI=harvest index.

On the other hand, the estimates of genetic advance (% of the mean) for days to heading and maturity found in this experiment were similar with what was reported in Hailu Tefera *et al*. (2003). In addition, the GAM values obtained for the first and second basal culm internode diameters in present investigation are in agreement with those found by Kebebew Assefa *et al*. (1999), but lower than those reported by Kebebew Assefa *et al*. (2000; 2001b). In addition, compared to the present results, Ayalneh Tilahun *et al*. (2012) obtained genetic advance (% of the mean) estimates that were similar for plant height and that were higher for panicle length. But the GAM values for both plant height and panicle length in the present study were comparable to those reported by Habte Jifar *et al*. (2015b). Accordingly, the estimated genetic advance values of number of primary branches, number of spiklets per panicle and shoot biomass in current experiment are in line with those found by Ayalneh Tilahun *et al*. (2012); Habte Jifar *et al*. (2015b) and Solomon Chanyalew *et al*. (2009). Moreover, GAM estimate for harvest index in the current study was greater than what was reported by Solomon Chanyalew *et al*. (2009) and Ayalneh Tilahun *et al*. (2012), while it was comparable with that found by Kebebew Assefa *et al*. (2001b). Moreover, compared to the findings in this study, gynogenically derived tef lines of Habte Jifar and Likyelesh (2013) showed similar genetic advance (% of the mean) estimates for days to maturity, culm length and lodging index.

Nevertheless, both heritability and genetic advance (% of mean) values of traits must show high values for effective phenotypic selection (Johnson *et al*., 1955; Ahsan*et al*., 2015). Consequently, in this study first basal culm internode diameter, second basal culm internode diameter, panicle length, number of spikletes per main panicle and number of primary branches showed high heritability combined with moderate GA values. In comparison, days to heading, days to maturity and grain filling period, thousand seed weight, grain yield, lodging index and total biomass revealed low heritability and genetic advance as percent of mean values, while most of the remaining traits depicted relatively medium heritability with moderate GAM values. Therefore, since the combinations of high heritability and GAM indicated the existence of additive gene action and lodging is the major constraint in tef (Kebebew Assefa *et al*., 2011), phenotype based selection of lodging related traits such as first and second basal culm internode diameters would appear effective to bring about improvements in the development of lodging resistant tef varieties.

Phenotypic and Genotypic Correlations

Selection criteria takes into account the information on interrelationships among agronomic characters, their relationship with grain yield and their direct influence on grain yield (Dewey and Lu, 1959). Therefore, in the present study, phenotypically**,** lodging index was correlated positively and significantly with all phenological traits i.e. total biomass, grain yield, straw yield and harvest index and culm length. On top of this, grain yield, harvest index, total and fertile number of tillers had positive and significant genotypic correlation with lodging index, indicating their role in aggravating lodging incidence. In contrary, first and second basal culm internode diameters had negative and significant correlation at both phenotypic and genotypic level with lodging index which showed their role in reducing lodging (Table 6). Similarly, number of primary branches per main panicle revealed significant and negative genotypic correlation with lodging index. On the other hand quantitative traits like total biomass, straw yield, harvest index and lodging index showed positive and highly significant ($P<0.01$) phenotypic and genotypic correlation with grain yield (Table 6). In the same way, positive and highly significant phenotypic correlations were observed between grain yield and other height related traits. However, apart from number of spikletes per main panicle which showed significant ($P<0.01$) correlation, all the remaining traits considered in this experiment did not show significant phenotypic correlation with grain yield. Likewise, plant height, panicle length, number of spikletes per main panicle also revealed significant ($P<0.05$) positive genotypic correlation with grain yield and those traits could serve as pointer of high yielding ability.

On other hand, excluding days to heading and grain filling period, similar phenotypic correlation of grain yield with different yield related traits was observed by Habte Jifar *et al*. (2013). But genotypically, comparable results regarding associations with grain yield were reported only for traits like days to maturity, culm length and total biomass. Moreover, among the different traits measured by Habte Jifar *et al*. (2015a), number of primary panicle branches, thousand seed weight, second basal culm internode diameter, total biomass and the above stated phenological traits showed similar phenotypic correlation with grain yield. Besides those traits, culm length and harvest index showed similar genotypic association with grain yield. Likewise, the strong and positive correlations of grain yield with yield related traits agree with the previous findings of Solomon Chanyalew *et al*. (2009) and Wondewosen Shiferaw *et al*. (2012). However, compared to the present results, the correlations of grain yield reported by Habtamu Ayalew *et al*. (2011) were not in agreement for height related and number of fertile tillers, but it was similar with total biomass and harvest index.

Table 6. Phenotypic (above diagonal) and Genotypic (below diagonal) correlation coefficient for 18 quantitative traits of 68 tef populations and two improved varieties

Variable	DH	DM	GFP	PH	PL	CL	NSPP	NPB	FBCD	SBCD	LI	NTT	NFT	TSW	TBM	GY	SY	HI
DH	1.00	0.90**	0.56**	0.20**	-0.24**	0.41**	-0.30**	0.51**	-0.15**	-0.31**	0.22**	-0.40**	-0.28**	-0.22**	0.19**	0.01ns	0.25**	-0.29**
DM	0.58**	1.00	0.87**	0.20**	-0.20**	0.40**	-0.29**	0.46**	-0.12*	-0.28**	0.20**	-0.40**	-0.29**	-0.14*	0.16**	-0.05ns	0.24**	-0.38**
GFP	0.19ns	0.91**	1.00	0.16**	-0.12*	0.29**	-0.21**	0.30**	-0.05ns	-0.17**	0.12*	-0.31**	-0.22**	-0.01ns	0.09ns	-0.09ns	0.17**	-0.38**
PH	0.44**	0.33**	0.18ns	1.00	0.71**	0.91**	0.33**	0.41**	0.36**	0.32**	0.10ns	-0.24**	-0.25**	0.07ns	0.43**	0.27**	0.46**	-0.22**
PL	0.42**	0.31**	0.16ns	0.86**	1.00	0.34**	0.56**	0.13*	0.41**	0.41**	-0.04ns	-0.07ns	-0.15**	0.12*	0.27**	0.24**	0.26**	-0.02ns
CL	0.38**	0.30**	0.16ns	0.94**	0.63**	1.00	0.11ns	0.46**	0.23**	0.18**	0.16**	-0.29**	-0.24**	0.02ns	0.41**	0.22**	0.46**	-0.28**
NSPP	0.40**	0.30**	0.15ns	0.66**	0.72**	0.52**	1.00	0.23**	0.51**	0.56**	-0.08ns	0.10ns	0.02ns	0.13*	0.24**	0.20**	0.23**	-0.04ns
NPB	0.34**	0.33**	0.22ns	0.55**	0.51**	0.48**	0.68**	1.00	0.31**	0.20**	0.07ns	-0.31**	-0.23**	-0.04ns	0.21**	0.03ns	0.26**	-0.28**
FBCD	0.40**	0.48**	0.38**	0.65**	0.55**	0.61**	0.58**	0.68**	1.00	0.81**	-0.18**	-0.13*	-0.17**	0.20**	0.06ns	-0.02ns	0.09ns	-0.21**
SBCD	0.43**	0.49**	0.38**	0.70**	0.55**	0.69**	0.62**	0.73**	0.90**	1.00	-0.22**	0.04ns	-0.02ns	0.20**	0.05ns	0.02ns	0.07ns	-0.09ns
LI	-0.12ns	-0.10ns	-0.06ns	-0.16ns	-0.06ns	-0.20ns	-0.18ns	-0.28*	-0.39**	-0.33**	1.00	-0.04ns	0.03ns	-0.11ns	0.37**	0.42**	0.32**	0.22**
NTT	-0.22ns	-0.30**	-0.25*	-0.38**	-0.36**	-0.34**	-0.22ns	-0.30**	-0.43**	-0.33**	0.24*	1.00	0.91**	0.07ns	-0.06ns	0.05ns	-0.11ns	0.26**
NFT	-0.26*	-0.34**	-0.28*	-0.40**	-0.41**	-0.32**	-0.22ns	-0.26*	-0.43**	-0.33**	0.24*	0.94**	1.00	0.03ns	-0.10ns	-0.01ns	-0.14*	0.23**
TSW	0.06ns	0.24*	0.25*	0.30**	0.29**	0.26*	0.15ns	0.12ns	0.33**	0.26*	0.06ns	-0.05ns	-0.08ns	1.00	-0.03ns	-0.04ns	-0.02ns	-0.05ns
TBM	0.33**	0.21ns	0.09ns	0.48**	0.45**	0.42**	0.50**	0.19ns	0.17ns	0.24*	0.20ns	-0.09ns	-0.12ns	0.15ns	1.00	0.86**	0.98**	-0.07ns
GY	0.09ns	-0.03ns	-0.08ns	0.23*	0.27*	0.17ns	0.28*	0.04ns	-0.05ns	0.01ns	0.39**	0.01ns	-0.03ns	0.08ns	0.85**	1.00	0.74**	0.42**
SY	0.40**	0.29**	0.15ns	0.54**	0.49**	0.49**	0.55**	0.23*	0.25*	0.31**	0.10ns	-0.12ns	-0.15ns	0.17ns	0.98**	0.72**	1.00	-0.28**
HI	-0.42**	-0.48**	-0.37**	-0.43**	-0.30**	-0.45**	-0.35**	-0.28*	-0.46**	-0.43**	0.40**	0.28*	0.28*	-0.17ns	-0.15ns	0.38**	-0.36**	1.00

*, ** **significant** at 5% and 1 % probability level, respectively. DH=days to heading, DM=days to maturity, GFP=grain filling period, PH=plant height, PL=panicle length, CL=culm length, NSPP=number of spikletes per main panicle, NPB=number of primary branches per main panicle, FBCD first basal culm diameter, SBCD=second basal culm diameter, LI=lodging index, NTT=number of total tillers, NFT=number of fertile tillers, TSW= thousand seed weight, SBM= shoot biomass, GY=grain yield, SY=Straw yield and HI=harvest index.

CONCLUSION

The present study revealed substantial variability in some yield related traits of the locally collected tef germplasm lines which can be exploited in the tef breeding program. Specifically these materials could serve as a source of lodging resistant tef varieties. Besides, the high genotype x location interactions on many of the agronomically important traits evaluated in the present study indicates that breeding for specifically adaptable varieties would be important while still exploring for widely adapted varieties. Complementary uses of phenotypic evaluations along with modern genomic tools would be important to avoid confounding effects of environment and genotype x environment interactions in genetic diversity evaluation and further studies involving diverse tef genotypes and evaluation techniques would be worth for more conclusive and comprehensive recommendations.

Acknowledgments

The authors thank the Ethiopian Institute of Agricultural Research (EIAR) for covering the costs of this research. Our appreciation also goes to DZARC and HARC for providing the tef accessions included in the study as well as the necessary man-power for this work. HARC and DZARC Tef program technical assistants are duly acknowledged for their role in the execution of the experiments.

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Gastrointestinal Helminthes of Dogs in Yirgalem Town, Southern Ethiopia

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Abstract

The study was conducted from November 2011 to March 2012 to estimate the prevalence and major gastrointestinal parasites burden of dogs (n=427) in Yirgalem town. The overall prevalence of parasites was 72.8% and the prevalence of *Ancylostoma caninum*, *Dipylidium caninum* and *Toxocara canis* were 53.4%, 43.1% and 54.3%, respectively. Significant difference was observed between the prevalence of the *A. caninum* and *D. caninum* (x^2 =9.1, p = 0.003), and between *T. canis* and *D.* caninum (x^2 =10.7, p= 0.001). The highest relative proportion of solitary infection was recorded for *A. caninum* (12%) followed by *D. caninum* (8%) and *T. canis* (7%). On the other hand, mean egg count of *T. canis* were the highest followed by *A. caninum* and *D. caninum* with the significant mean difference between *A. caninum* and *D. caninum* (x^2 =67, p = 0.0481), and between *T. canis* and *D. caninum* (x^2 =79, p = 0.0149). There were significant differences in the mean egg count between sexes (F = 4.14, p = 0.0429) and purpose of keeping the dog (F = 9.22, p=0.001). Male dogs harbor higher number of eggs than female. A significantly greater mean egg count (p = 0.000) was observed in dogs kept as guard and for both joy and guard compared to dogs used only for joy. In conclusion, this study revealed the importance of some gastro-intestinal tract parasites of dog in the study area that need attention for subsequent control measures.

Key words: *Ancylostoma caninu, Dipylidium caninum*, dog parasites, prevalence, *Toxocara canis*
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INTRODUCTION

The most common gastrointestinal parasites of dogs are roundworm, especially the *Toxocara* species, and hook worm, primarily *Ancylostomas* species (Lee et al., 2010). *Ancylostoma caninum* occurs in the small intestines of dogs and more commonly in puppies before they are born and transmitted via the dam's milk. As dogs live in close proximity with humans there is transmission of zoonotic parasites of dogs to human resulting in serious consequences. Children are the most vulnerable to these parasites as they are usually in close contact with these animals especially in the developing countries where there is no practice of regular deworming of dogs. It is recommended that all dogs should get regular fecal examination, treatments and prevention of internal parasites (Lee et al., 2010). Intestinal parasites are among the most common pathogenic agents causing serious intestinal pathology in dogs. Among canine intestinal parasites, *T. canis, Ancylostoma* spp. and *D. caninum* have received great attention due to their zoonotic potential (Blagburn et al., 1996). One of the most common cosmopolitan parasites of dogs is the canine roundworm, *T. canis*. Apart from its veterinary importance, this species is responsible for the most widely recognized form of visceral larva migrans (VLM) in human (Taylor et al., 2007).

The significance of dog parasites with regard to both animal health and zoonotic importance has been recorded in Ethiopia. Hence, several works were carried out so far to identify dog parasites in a broader sense by different researchers including Zewdu et al. (2010) in Ambo town, Degefu et al. (2011) in Jimma Town and Abere et al. (2013) in Bahir Dar which are located at Central, South-western and North-western Ethiopia, respectively. However, information regarding the status of dog helminths in the Southern part of Ethiopia is scant. Hence, this work estimated the prevalence and intensity of dog parasite and identified the associated risk factors.

MATERIALS AND METHODS

Study area

The study was conducted at Yirgalem town, which is located in the Southern Nation Nationalities and People Region (SNNPR) and 320km far from Addis Ababa. Geographically the town lies between 6^0 44 and 6^0 84 N latitude and 37^0 92 and 38^0 06 E longitudes.

Study population

The study animals were dogs of all age groups, breeds (local and exotic) and both sexes. Dogs aged up to one year were classified as puppies, from one to three years as young and those more than three years as adult. They were randomly selected from confined (indoor), semi-confined (mixed) and loses (outdoor) housing system.

Sample size determination
The sample size required for the study was calculated according to the formula given by Thrusfield (2005). The sample size calculated was 384 dogs by using the expected prevalence of 50% but with the intention of maximizing the precision, it was raised to 427.

Study methodology
Coprological examinations were performed on 427 randomly selected dogs. The freshly collected feces were put in tightly closed universal bottles and processed to examine for internal parasitic eggs. The presence of eggs was determined by the floatation techniques as described by Kassai (1999). Eggs were counted using McMaster counting technique conducted according to Chauhan and Agarwal (2006) and Foreyt (2001) and the result was considered as positive when at least one parasite egg was present (Lorenzinl et al., 2007). Maximum effort was made to count, characterize and classify the different eggs observed under 10 x magnifications (Hendrix, 2003; Soulsby, 1982). Furthermore, gravid parasites were collected and transported to Yirgalem Veterinary Clinic laboratory for examination.

Data management and analysis
Preliminary analysis was done in Microsoft Excel and descriptive statistics was used to summarize the prevalence and relative percentage of each parasite. The association between the risk factors and the occurrence of the helminth parasites was analyzed with Logistic regression and confidence interval and *p*-value were employed to investigate the presence of association. Additionally, Odds Ratio was used to assess the strength and direction of this association using STATA statistical soft ware version 9. Moreover, the average mean egg counts were subjected to Analysis of Variance (ANOVA). The variation within each category was further evaluatedusing multiple comparisons of Bonferroni test.

RESULTS

Prevalence
Out of the 427 dogs examined, 311 (72.8%) were positive for one or more types of parasitic eggs. The prevalence of *A. caninum*, *D. caninum* and *T. canis* were 53.4%, 43.1% and 54.3%, respectively (Table 1). There was significant difference between the prevalence of A. **c**aninum and *D. caninium* (x^2 =9.1, p = 0.003), and between *T. canis* and *D. caninium* (x^2 =10.7, p= 0.001), while no significant diffrence was observed between the occurence of *A. caninium* and *T. canis*. The highest relative proportion of solitary infection was for *A. caninum* (12%) followed by *D. caninum* (8%) and T. canis (7%). The record on mixed infection of these parasites was highest for all the three parasites mixture (34%) followed by double infection with *A. caninum* and *T. canis* (21%), and *D. caninum* and *T. canis* (12%). The least mixed infection recorded was in case of *A. caninum* and *D. caninum* (6.4%) (Table 1).

There was a significant difference between the mean egg count of *A. caninum* and *D. caninum* (p = 0.0481) and between *T. canis* and *D. caninum* (p = 0.0149) (Table 2). The mean the highest mean egg count was for *T. canis* followed by *A. caninum* and *D. caninum.*

Table 1. Prevalence and relative proportion of GIT parasites in dog in the study area

Species	Prevalence (%) (95%CI)	Relative proportion (%)
A. caninum	53.4 (228/427)	11.9 (37/ 311)
T. canis	54.3 (232/427)	8.0 (25/311)
D. caninum	43.1 (184/427)	7.1 (22/311)
A.C+ T. canis	15.2 (65/427)	20.9 (65/311)
A. C +D. C	4.7 (20/427)	6.4 (20/311)
T. C + D. C	7.7 (33/427)	11.6 (36/311)
A. C + T. C + D. C	24.4 (104/427)	34.1 (106/311)

Table 2. Comparison of the mean egg count of each parasites (n= 427)

Species of parasites	Mean (95%CI)	Range	SE	SD	Mean difference
A. caninium	274 (224, 323)	50-6000	25.08	518.26	1 and 2 (p = 0.725)
T. canis	286 (241, 332)	50-4500	23.21	479.70	1 and 3 (p = 0.0481)
D. caninum	207 (163, 252)	10-7500	22.52	465.34	2 and 3 (p = 0.0149)

Analysis of mean egg count of the parasites with different risk factors

The analysis of variance revealed the existence of significant association between the mean Egg Per Gram of feces (EPG) with sex (F = 4.14, p=0.0429) and purpose of keeping the dog (F = 9.22, p = 0.001) (Table 3). Male dogs harbor higher number of eggs than female. Within purpose comparisons indicated that the mean egg count was significantly greater (p = <0.001) in the dogs kept for guard and for both joy and guard than the dogs used only for joy. On the other hand, there were no difference between age groups, breeds, and kept indoor outdoor or mixed (Table 3).

The study showed that ticks infestation was prevalent in 66.5% of the animals with a higher prevalence observed during minor wet season (82.3%) compared to dry season (58.4%) (Table 2). Contrary to the observed mange mite prevalence (Table 1), higher tick prevalence was observed in Borana camel herds, and female animals. Tick prevalence also increased with age of the animals and was more prevalent in camels with poor body conditions.

Analysis of the prevalence of dog parasites with risk factors

With the logistic regression analysis, no significant association (p>0.05) was observed between parasitism and the potential risk factors in this study (Table 4).

Table 3. Analysis of the mean egg count with various risk factors

Risk factors		Mean	SD	F (p-value)	Bonferroni result	
					Category	*p* value
Sex	Female	860	431			
	Male	1106	957	4.14 (0.0429)		
Age	<1	979	1115			
	[1,3]	1001	883			
	>3	1159	743	1.24 (0.2903)		
Breed	Local	1033	822			
	exotic	1232	1290	1.37 (0.2430)		
Purpose	Guard	3600	818		Guard and Joy	1.000
	Joy	775	317	9.22 (0.001)	Guard and both	0.000
	*Both	1042	4738		Joy and Both	0.000
keeping	Indoor	1043	918			
	Outdoor	1044	743	0.24 (0.7830)		
	mixed	1165	545			

*Both means dogs kept for both guard and Joy purposes

Table 4: Univariate and multivariate logistic regression analysis of potential risk factors in association with occurrence of gastrointestinal helminthes in the study area

Risk factors		No. Tested	No. (%) positive	Univariate analysis		Multivariate analysis	
				Crude Odd Ratio (95% Cl)	P-value	Adjusted Odd Ratio (95% Cl)	*P*-value
Sex	Female	83	66 (80. 0)	1		1	
	Male	344	245 (71.0)	1.6 (0.9, 2.8)	0.13	2.0 (1.2, 4.3)	0.06
Age	≤ 1	68	47 (69.0)	1		1	
	(1,3)	218	154 (70.6)	1.1(0.6,1.9)	0.81	1.4 (0.65,3.0)	0.401
	>3	141	110 (78.0)	1.6 (0.82,3.0)	0.17	1.3(0.6,3.03)	0.503
Breed	Local	383	281 (73.3)	1		1	
	Exotic	44	30 (68.1)	1.3(0.7,2.5)	0.5	1.2(0.5,3.03)	0.709
Purpose	Guard Joy	413	303 (73.3)	1		1	
	Mixed	11	6 (54.5)	2.3(0.7,7.7)	0.18	1.4 (0.22, 8.0)	0.829
		3	2 (66.7)	1.4(0.12,15.3)	0.8	3.0(0.13,53.3)	0.517
How kept	Indoor	355	259 (72.9)	1		1	
	Outdoor	32	24 (75.0)	1.1(0.48,2.6)	0.8	1.7 (0.6, 5.0)	0.340
	Mixed	40	28 (70.0)	1.2 (0.6, 2.4)	0.7	1.3 (0.5, 3.6)	0.606

DISCUSSION

The current prevalence of dog helminthes (72.8%) is in agreement with some of the previous authors (Martinez-Moreno et al., 2006; Minnaar et al., 2002) who reported a prevalence of 71.3% in Spain and 76% in South Africa. On the other hand, Zewdu et al. (2010), Benito et al. (2003), Fontanarros et al. (2006) and Blagburn et al. (1996) reported relatively lower prevalence of 52.9% in Ethiopia, 53.6% in Spain, 52.4% in Argentina and 35.9% in USA, respectively. The reported prevalence of 53.4% for *A. caninum*, 43.1% for *D. caninum* and 54.3% for T. canis were higher than the previous reports of Zewdu et al. (2010) who recorded the prevalence of 35.7% for *A. caninum*, 25.6% for *D. caninum* and 17.1% for *T. canis*. Additionally, in the present study, infection with more than one helminth parasites (polyparasitism) was common. It was noted that, out of the 311 positive dogs, concurrent infection with three different species of helminthes was more common (34.0%) than infection with single or two types of parasites. Similarly, Zewdu et al. (2010) reported higher prevalence of mixed infections with three species of helminthes (35.6%) than single infection (24.4%). Higher incidences of concurrent infections with more than one species of helminthes (75.6%) were also reported by other studies in Ethiopia (Reshid, 1988; Shihun, 1994). In contrast, the dominance of single parasite infection was reported in studies conducted abroad (Anene et al., 1995; Bugg et al., 1999; Papazahariodous et al., 2007). This difference might be attributed to the level of awareness about dog parasites, regular deworming, housing and other management activities practiced in these areas.

The most prevalent adult helminthes observed in the present study was *T. canis* (54.3%). This prevalence was considerably higher than the result reported by Zewdu et al. (2010), Papazahariodous et al. (2007), Himonas (1968) and Haralabidis et al. (1988) who reported 17.14% in Ambo zone central Ethiopia, 12.8%, 25.4% and 22.4% in Greece.

This variation could be attributed to the difference in management system, health care and degree of environmental contamination with infective stage and exposure to naturally infection more than owned dogs.

The second prevalent parasite in this study was A. caninum (53.4%) and this is in agreement with those studies conducted in Wolaita (Reshid, 1988), Dire Dawa and Eastern Hararge (Temesgen, 1990), and Debre Zeit (Shihun, 1994). However, Zewdu et al. (2010) reported lower prevalence of 35.7%.

The prevalence of *D. caninum* in the present study (43.1%) was also found to be comparable to the reports from South Africa (Minnaar et al., 2002) and Debre Zeit (Shihun, 1994) with prevalence of 44% and 47.54%, respectively. However, higher prevalence was reported by Dada et al. (1979) in Zaria, Nigeria (97.8%) and Temesgen (1990) in Dire Dawa and eastern Hararge (83%). On the other hand, Reshid (1988) and Zewdu et al. (2010) reported lower prevalence of 32.4% and 25.6% from Wolaita and Ambo Zones, respectively. Very low prevalence of *D. caninum* occurred in European countries such as Greece (Papazahariodous et al., 2007), Spain (Martinez-Moreno et al., 2006), and Australia (Bugg et al., 1999). The routine use of antihelminthics, particularly in puppies is the most likely cause for the reduced prevalence of gastrointestinal helminthes in those countries (Robertson et al., 1991).

Concerning the average egg count of the three species of parasites, the mean egg count was the highest in case of *T. canis* followed by *A. caninum* and *D. caninum* and was higher than the mean count reported by Degefu et al. (2011). Likewise, this variation could be attributed to the differences in health care and degree of environmental contamination with infective stage, frequent mixing of pets with stray dogs which might have the infections, lack of awareness of dog parasites and their control strategies. On the other hand, the dogs that used for joy were found with relatively lower mean egg count (317) and this could be due to the better care (management) given to the joy type of dogs.

In conclusion, the coprological examination in the current study revealed the existence of three different types of dog helminthes namely, *T. canis*, *A. caninum* and *D. caninum*. The prevalence of mixed infections with three species of helminthes was more common than single or double infections. In general, the prevalence observed in the current study is high. As common principles of parasitic disease control scheme, the life cycle of these helminthes parasites should be broken by regular deworming and restriction from other sources that cause contamination with intermediate stages of dog parasites. Control of stray dog population should be carried out to reduce environmental contaminations.

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Ectoparasitic Burden of Camels under Pastoral Management in Southern Ethiopia

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Abstract
Camels are suffering from wide range of diseases of which external parasites are the most common ones. Three seasonal surveys were carried out in Borana areas to investigate the external parasitic burden among 1227 camels. Results showed that mange mite (35.4%) and ticks (66.4%) were widely prevalent among the study camels. Sarcoptes mange identified from skin scrapings was found to be responsible for camel skin infestation. A total of 1164 ticks were collected and six tick species were identified. *Rhipicephalus pulchellus* was the most prevalent species (77.5%) followed by *Amblyoma gemma* (12.6%), *Hyaloma dromedarii* (4.3%), *R. evertsi* (3.6%), *A. variegatum* (1.5%) and *A. lepidum* (0.4%). Mange mite was more prevalent during the dry season, in young and male camels, camels owned by Gabra, and large herds compared to their counter parts. There was also a significant association between poor body condition and mange mite occurrence. Tick infestations were higher during minor wet season (82.3%) compared to dry season (58.4%). Higher prevalence of tick infestations were recorded for Borana herds, older and female camels compared to Gabra and young camels. Animals in poor body condition were more infested with ticks than those in good body conditions. High prevalence of mange mite and tick infestation among the study camels indicates inadequate ecto-parasite control in the area. Hence, mange mite and ticks are destructive parasites that hinder productivity of camels and require effective control measures.

Key words: Camels, Mange-mites, Ticks, Risk factors, Borana, Ethiopia
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INTRODUCTION

In Ethiopia, camels represent a subset of large livestock resources and are kept by pastoralists under diverse constraints in arid and semi-arid marginal areas of eastern and southeastern lowlands. Camels are generally versatile animals that play key role in ensuring food security and fulfilling the livelihood priorities of households inhabiting the arid environments (Schwartz and Dioli, 1992). They provide households with cash income, food supply, transportation services and other benefits including ceremonial uses, insurance and risk buffering options. Camels have both economic and ecological advantages in multiple herding as they represent a minimal competition with other ruminants, and enhance wise use of the rangeland resources with minimum pressure on the environment (Megersa et al., 2014).

The Borana people who have been based on cattle pastoralism are increasingly engaged in camel production in response to changing climate and rangeland ecosystem i.e. increased frequency of droughts and bush encroachment which reduced grazing land for cattle (Megersa et al., 2014). Thus, camels have been an indispensable component of pastoralism to cope up with the looming climatic changes and rangeland degradations, and are reliable source of milk under such environmental constraints. Thus, camels of the study area are managed by pastoralists of different herding experience. For instance, camel pastoralism among the Gabra people constitutes an age-old tradition and the Gabra herders have also played an instrumental role in the introduction of camels to the Borana areas (Coppock, 1994). Gabras are endowed with rich indigenous knowledge in camel husbandry and health management. But the Boranas who are late adopters of camel pastoralism may have less experience and inadequate knowledge of camel production and health care (Megersa et al., 2014). Thus, such difference in the level of indigenous knowledge of camel husbandry and health management between the two clans may be anticipated to result in variations in disease occurrences (Megersa et al., 2008). Despite its ecological and economic importance of keeping camels with multiple livestock species, challenge of infectious diseases and parasitic burden on camels is not clear and require further investigations.

Infectious and parasitic diseases are among the major constraints that hinder the production and reproduction performances of pastoral camels in Ethiopia and elsewhere in East Africa. Research findings on camel diseases showed that camels are either carriers or suffering from a wide range of infectious and parasitic diseases (Richard, 1987; Agab and Abbas, 1999; Wernery and Kaaden, 2002; Abbas and Omer, 2005). Trypanosomiasis, camel pox, contagious skin necrosis, pneumonia, mange mite infestations and internal parasites are among the major camel health problems previously reported from Borana areas (Richard, 1979; Megersa, 2010). Various authors who provided extensive accounts of camel diseases have indicated that mange

mite infestation to be of greater economic importance (Wernery and Kaaden, 2002; Abbas and Omar, 2005). Camel mange is a highly contagious disease which can spread to animals associated with infected animals. *Sarcoptes scabie var cameli* is the most commonly identified from skin scrapings, and was regarded as the most destructive parasitic disease hampering production and productivity of camels in Ethiopia (Dinka et al., 2010; Megersa et al., 2012; Feyera et al., 2015).

Ticks are blood sucking parasites and are of high economic importance in the tropical environments. In addition to blood sucking, they cause tick paralysis and responsible for tick born diseases (Musa and Osman, 1990). The most important tick species reported to infest camels in Ethiopia include *Amblyomma gemma, A. variegatum, Boophilus decoloratus, Hyalomma dromedarii, Rhipicephalus pulchellus* (Zeleke and Bekele, 2004; Dinka et al., 2010; Megersa et al., 2012; Taddese and Mustefa, 2013). The present study is therefore to investigate the ecto-parasites burden of camels, associated risk factors and major tick and mange species affecting camels of the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Yabelo district of Borana zone in southern Ethiopia. The area has semi-arid climate with bimodal rainfall distribution during the major wet season, from mid-March to May, and the minor wet season from September to November. A cool dry period occurs from June to August while warm dry season extends from December to February (Coppock, 1994). The area is characterized by extensive pastoral production system. Cattle dominate the livestock species biomass followed by small ruminants and camels. Involvement in camel production among the previously cattle herders is on rise due to increased climate variability and rangeland ecosystem changes (Megersa et al., 2014).

Study design and sampling methods

Repeated cross-sectional surveys were carried out during three seasonal herd investigations. A total of 12 villages were randomly selected from six pastoral associations (Kebeles), namely Surupa, Jijido, Dadim, Dida Yabello, Dida Hara and Dartu with some restrictions depending on accessibility to villages by vehicle and presence of camel population. Subsequently, a total of 70 camel herds (i.e. sampling all herds found in the study villages) were selected and seasonally investigated during the dry period, and major and minor wet seasons. This study is part of a broader study on "Epidemiological investigations of major camel diseases in Borana" (Megersa, 2010). An assumption was made that at least six camels exist per herd, so that 420 animals were anticipated to be examined over the three seasons (i.e. making an overall sample size of 1260 animals). However, the dynamic nature of pastoral herds, moving out and in of animals or herds in the study area, indeed made revisiting of the same animal or herd difficult. Since newly introduced animals or herds were used to replace those moved out of the selected villages, the study can be considered as repeated cross-section. Accordingly, a total of 442, 423 and 362 animals were clinically examined during dry, major wet and minor wet seasons, respectively.

Clinical examinations were performed on individually identified animals during each visits for presence of ectoparasite (ticks) and mange mite lesions. Participatory discussions on camel diseases and health care practices were also carried out on 12 groups (in each village), each having six to eight key informants. Since finding a tick free camels is unlikely, an animal having about ten or more ticks (rough count) was considered to be infested with ticks (tick burden). Ticks were collected carefully by hand and were preserved in universal bottles containing 70% ethyl alcohol for species identifications. A total of 1164 ticks were collected for laboratory analysis. The specimens were transported to Yabelo Regional Veterinary Laboratory for primary identifications using stereomicroscope following key identification procedures described by Walker et al. (2003). Further species identifications were performed at Veterinary Parasitology laboratory of Hawassa University. Skin scrapings were also taken from 125 suspected cases for mange mite identification during the first visit and preserved in 10% formalin. In the laboratory 10% KOH was added to the samples to allow the release of mites from scabs and crusts, and examined under stereomicroscope. Identification of mange mites at genera level was performed according to morphological features described by Urquhart et al. (1996) and Taylor et al. (2007).

Statistical analysis

In addition to descriptive summary of the data, further statistical analyses were performed using Stata version 11 (StataCorp 2009, College Station, TX 77845, USA). Prevalence of ecto-parasites was presented as proportion of positive cases to number of examined. Potential risk factors associated with parasitic prevalence were analyzed for herd and animal level variables using logistic regression analysis. Potential risk factors such as ethnic group, seasons, herd size, body condition score, sex and age of animals were analyzed using logistic regression analysis.

RESULTS

Camel health care and parasite treatments were mainly practiced by herders and traditional healers. Participatory discussions showed that higher proportion of health care, especially parasitic treatments (90%) was provided by herders themselves, while veterinary services only contributed about 10%. The clinical examination showed that study camels were highly infested with mange mite (35.4%) and tick (66.5%). The study animals were also concurrently affected by other health problems of the integument system, such as contagious ecthyma (12.9%), camel pox (4.8%) and contagious skin necrosis (8.8%), traumatic wounds (2.1%) and abscesses (9.5).

Out of a total of 1227 animals examined for external parasites, 35.4% the animals were infected with mange mite (Table 1). Examination of skin scrapings showed that camels in the study area were affected by *Sarcoptes* mange. Mange mite prevalence was significantly higher during the dry season, in Gabra camels, large herds and young animals compared to their counterparts. There was also a significant association between poor body conditions and mange mite prevalence. Mange mite prevalence was found to be higher in male than female camels.

Table 1. Prevalence and associated risk factors of mange mites in camels

Factors	Number	Prevalence	Odds Ratio	P-value
Season				
Dry	442	42.1		
Major wet	423	31.2	0.58	0.000
Minor wet	362	32	0.46	0.000
Ethnic group				
Borana	581	28.2		
Gabra	646	41.8	1.82	0.000
Herd size				
< 10 animals	107	27.1		
10-20 animals	718	33.1	1.24	0.371
> 20 animals	402	41.5	1.7	0.031
Sex of camels				
Male	239	41		
Female	988	34	0.74	0.043
Age of camels				
< 4 years	396	42.4		
4-15 years	711	32.6	0.7	0.015
> 15 years	120	28.3	0.51	0.005
Body conditions				
Poor	158	40.8		
Good	767	33.5	0.43	0.000
Medium	302	37.3	0.43	0.003
Total	1227	35.4		

The study showed that ticks infestation was prevalent in 66.5% of the animals with a higher prevalence observed during minor wet season (82.3%) compared to dry season (58.4%) (Table 2). Contrary to the observed mange mite prevalence (Table 1), higher tick prevalence was observed in Borana camel herds, and female animals. Tick prevalence also increased with age of the animals and was more prevalent in camels with poor body conditions.

Table 2. Prevalence and associated risk factors of ticks in camels

Factors	Number	Prevalence	Odds Ratio	P-value
Season				
Dry	442	58.4		
Major wet	423	61.7	1.3	0.084
Minor wet	362	82.3	4.7	0.000
Ethnics				
Borana	581	69.7		
Gabra	646	63.8	0.77	0.028
Herd size				
< 10 animals	107	71		
10-20 animals	718	64.6	0.75	0.195
> 20 animals	402	68.9	0.9	0.672
Sex of camels				
Male	239	51.5		
Female	988	70.2	2.23	0.000
Age of camels				
< 4 years	396	43.4		
4-15 years	711	77.5	5.31	0.000
> 15 years	120	78.3	5.04	0.000
Body conditions				
Poor	158	79.6		
Good	767	67.3	0.53	0.003
Medium	302	58.1	0.35	0.000
Total	1227	66.5		

A total of 1164 ticks were collected and identified to belong to three genera, namely *Rhipicephalus* (81.2%), *Amblyoma* (14.6%) and *Hyaloma* (4.3%). *Rhipicephalus pulchellus* was the most dominant tick species (77.5%) followed by *A. gemma* (12.6%), *H. dromedarii* (4.3%), *R. evertsi* (3.6%), *A. variegatum*,(1.5%) and the least abundant species was *A. lepidum* (0.4%) in which case only male ticks were encountered (Table 3).

Table 3. Genera and species of ticks collected from camels

Tick Genera	Species	Male	Female	Total
		No (%)	No (%)	No (%)
Ambyloma	*A. gemma*	80 (12.9)	67 (12-3)	147 (12.6)
	A. variegatum	12 (1.9)	6 (1.1)	18 (1.5)
	A. lepidum	5 (0.8)	0	5 (0.4)
Hyloma	*H. dromedarii*	30 (4.8)	20 (3.7)	50 (4.3)
Rhipicephalus	*R. pulchellus*	468 (75.)	434 (79.8)	902 (77.5)
	R .evertsi	25 (4.0)	17 (3.1)	42 (3.6)
Total		620	544	1164

Discussion

In addition to external parasites such as ticks and mange mites, study camels were found to suffer from other diseases affecting cutaneous system. The observed high prevalence of Sarcoptic mange (35%) and ticks (66.5%) is comparable to previous reports from Borana area (Megersa et al., 2012; Regassa et al., 2015). In particular, mange mite is more damaging and can be regarded as the major health problem in the study areas similar to reports elsewhere (Richard 1987; Abbas and Omar, 2005; Megersa et al., 2012; Zahid et al., 2015; Awol et al., 2014; Feyera et al., 2015). Sarcoptic mange is a burrowing mite that penetrates deep into skin and lead to pruritus, irritation, development of papules, hairless areas and scab formation (Driot et al., 2011; Megersa *et al.*, 2012). As a result of intense pruritus and irritations, affected camels stop feeding and rub against objects, bite or scratch the affected areas. This indeed affects the feed intake of infested animals in addition to facilitating spreading of infestation to other body parts and to healthy ones (Schwartz and Dioli, 1992).

The prevalence and severity of mange was higher during the dry period perhaps due to aggregation of camels at water points and prevailing feed shortage which might have reduced the resistance of the animals. Mange mite infestation is a highly contagious disease which spreads from affected to susceptible animals especially during aggregation at water point and night resting. The mite may spread directly by contact or indirectly through objects such as harnessing materials, bedding and tree trunk (Schwartz and Dioli, 1992). Thus, close contacts of camels particularly at water points coupled with high feed shortage could account for the observed higher mange mite prevalence during the dry period. Contrary to the present finding, other studies suggested that the mite actually tends to spread more quickly during cold weather when animal coats usually grow long and the animals huddle together more often (Richard, 1987; Zahid et al. 2015; Kotb and Abdel-Rady, 2015). In general, several factors have an effect on occurrences of mange mite infestation including poor hygienic condition, climate (temperature and humidity), overcrowding, feed shortage (including mineral deficiency) and poor animal health services (Wernery and Kaaden, 2002; Driot et al., 2011; Kotb and Abdel-Rady 2015). Since, animal health service is too limited to access such a highly mobile pastoral herds; animals continuously suffer from such a high disease burden. Once a herd is infested with Sarcoptic mite, the disease circulates within herd and severely affects susceptible animals such as young animals. Thus, observed higher prevalence with severe clinical manifestation in young animals also suggests their vulnerability to mange infestations similar to other reports (Hussain et al., 2012; Megersa et al., 2012; Awol et al., 2014; Zahid et al., 2015). Severely affected animals were also in poor body conditions and often had concurrent infections and other skin problems.

Mange mite infestation was more prevalent in Gabra camels which were large sized herds and affected by feed shortage due to limited mobility. Large herd size and overcrowding of animals coupled with nutrition deficiency favors mite transmission and infections. Feyera et al. (2015) also observed increased prevalence of mange mite with large herds which augment close contact between animals during herding, housing and watering. Mange mite prevalence was found to be higher in male than female animals, and this is in agreement with a report from Morocco (Driot et al., 2011) in which male camels (35.1%) had higher prevalence than females (19%). Our result could be explained by increased proportion of young animals in male groups compared to females (most of which were breeding animals). Conversely, Zahid et al. (2015) reported a higher prevalence of mange mites in females than the males, and ascribed their findings to physiological stresses and hormonal effects. In general, improving herd management and treatment of affected animals particularly during feed scarcity, young and those in poor body conditions may reduce the disease burden and further halt the spread of the disease within herds (Richard, 1987).

Similar to the present finding, high level of tick occurrences among camels have been reported from different parts of Ethiopian (Dinka et al., 2010; Megersa et al., 2012; Taddese and Mustefa, 2013; Kiros et al., 2014; Regassa et al., 2015). Tick prevalence progressively increased during the subsequent wet seasons and peaked in the minor wet season, which is in line with the findings of other studies (Zeleke and Bekele, 2004; ElGhali and Hassan, 2009; El Tigani et al., 2010; Megersa et al., 2012). In wet season, climatic factors (high humidity and lower temperature) and ecological factors such as increased vegetation cover of bush and shrubs may create a conducive conditions that favor the growth and survival of tick at all developmental stages.

The effects of animal factors such as age and sex on tick prevalence were observed in this study. Older animals and female camels harbored more ticks than their counterparts similar to the results of El Tigani et al. (2010) and Taddese and Mustefa (2013). The effect of sex may also be coupled with age of animals in which adults and old females carry more ticks than males (which were relatively younger). Other studies found no significant difference in tick infestations between the two

sexes and age groups (Dinka et al., 2010; Megersa et al., 2012).

Similar to the findings of this study, earlier studies also reported that *A. gemma, H. dromedarii,* and *R. pulchellus*, are the major tick species infesting camels in Ethiopia (Zeleke and Bekele, 2004; Dinka et al., 2010; Megersa et al., 2012; Taddese and Mustefa, 2012; Kiros et al., 2014). Unlike the present results, other studies have reported low proportion of *Boophilus decoloratus* such as 1.2% by Tadesse & Mustefa (2012), 4.2% by Megersa et al. (2012) and 1.8% by Kiros et al. (2014). *B. decoloratus* is generally widely prevalent in the highland areas, but lowest in arid environments. Among the tick species identified, *Amblyoma* and *Hyaloma* species are long mouthed ticks that are more important in inflicting udder and skin damages and being a risk factor for opportunistic infections. Likewise, *Hyalomma* and *Rhipicephalus* species are responsible for causing tick paralysis among camels. For instance, Musa and Osman (1990) reported an outbreak of tick paralysis in Sudanese camel herd, and incriminated *Hyalomma* and *Rhipicephalus* ticks to be responsible. Additionally, tick infestation causes tick worry and reduces feed intake, leading to decreased production performances (Schwartz and Dioli, 1992).

In conclusion, this study revealed high prevalence of mange mite and tick infestations of camels and existence of associated risks in the southern pastoralist areas as shown in Garba and Borana herds. Owning to their destructive effects, mange mite and ticks deserves implementation of effective ecto-parasite control and strategic treatment interventions.

Acknowledgements

The study was supported by the Drylands Coordination Group (DCG) of Norway. The authors duly acknowledge the contribution of camel owners in Gabra and Borana for sharing their knowledge on camel husbandry and the district veterinary assistants for effective support during the field works.

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Prevalence of Hepatitis B virus Infection and Associated Risk Factors Among Pregnant Women Attending Antenatal Care Clinics in Kofele Town, Oromia Region, Ethiopia

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Abstract

Hepatitis B virus (HBV) infection is one of the major public health problems affecting many people worldwide. This cross-sectional study was conducted to assess the prevalence and associated factors of HBsAg carriage among pregnant women attending antenatal care clinics in Kofele Town, Oromia Region, Ethiopia from January to April, 2016. A total of 270 pregnant women were recruited using systematic random sampling method. Structured questionnaires were used to collect data on socio-demography and risk factors for HBsAg carriage. Moreover, venous blood samples were collected from all study participants and sera were analyzed for HBsAg marker using SD BIOLINEHBsAg strip test. The prevalence of HBsAg among pregnant women was 5.9%. The age of the women varied from 17–41 years (mean age 24.5 years) and 40.0% were in the age category of 20–24 years. Participants with no formal education (AOR= 6.2; 95% CI= 1.35-28.74, P= 0.019) and had a history of abortion were at higher odds of being HBsAg carrier (AOR= 6.23; 95% CI= 1.74-22.5, P= 0.005). The study showed an intimidate prevalence of HBV infection on the basis of the World Health Organization HBV endemic definition (5-7% HBsAg prevalence). Therefore, screening pregnant women for HBV infection and making the vaccine available to exposed babies need to be emphasized.

Key words: Hepatitis B virus, HBsAg, pregnant women

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INTRODUCTION

Hepatitis B virus (HBV) infection is a challenging global health problem (Liaw and Chu, 2009). There have been more than 2 billion HBV infected people globally; 240 million of which are chronically infected. In the absence of expanded and accelerated response, the number of people living with HBV is projected to remain high for the next 40–50 years, with a cumulative 20 million deaths occurring until 2030 (WHO, 2016). An estimated 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer result from HBV infection (Perz et al., 2006). Chronic viral hepatitis also results in loss of productivity (Su et al., 2010).

The prevalence of chronic HBV infection varies widely according to geographical area, and predominant routes of transmission (Maddrey, 2000). The global prevalence of chronic HBV infection varies widely, from high (≥8 %, e.g. Africa and Asia) to intermediate (2-7%) e.g. Southern and Eastern Europe) and low (<2%, e.g. Western Europe, North America and Australia) (World Health Organization, 1990). A medium to high endemicity of HBV was reported in Ethiopia (Tsega, 2000).

Perinatal transmission is the major route of HBV transmission in many parts of the world, and an important factor in maintaining the reservoir of infection (Franco et al., 2012). In the absence of prophylaxis, a large proportion of viraemic mothers, especially those who are seropositive for HBeAg, transmit the infection to their infants at the time of, or shortly after birth (Franco et al., 2012). The risk of perinatal infection increased if the mother has acute hepatitis B in the second or third trimester of pregnancy or within two months of delivery. The risk of developing chronic infection is about 90% following perinatal infection (up to 6 months of age) but decreases to 20–60% between the ages of 6 months and 5 years(Ranger-Rogezand Denis, 2004).

HBV infection in pregnancy can cause coagulation defects, postpartum hemorrhage, organ failure, high maternal mortality and poor outcomes of their newborns such as stillbirths, neonatal deaths, acute and chronic liver disease, hepatocellular carcinoma and increased premature delivery (Elsheikhet et al., 2007). Globally, perinatal HBV transmission accounts for an estimated 21% of HBV related deaths in neonates. It ranges from 13% in the Eastern Mediterranean region to 26% in the Western Pacific region (Sookoian , 2006).

In Ethiopia, HBV screening is not recognized as an essential component of a quality antenatal care service package and the infection is often left undiagnosed among pregnant women. Thus, infected mothers may pass the infection on to their babies. In the absence of a large country data on HBV prevalence among pregnant women, small scale studies in various localities may generate valuable information to plan interventions. This study aimed to determine the prevalence and risk factors of HBsAg carriage among pregnant women in Ethiopian

settings where HBV screening is not a part of antenatal care (ANC) services.

MATERIALS AND METHODS

A facility based cross- sectional study was conducted in two health centers (Kofele and Roba health centers) in Kofele District of the West Arsi Zone, Oromia Region from January to April, 2016. The majority of the population in the area belongs to Oromo ethnic group (97%) and Muslim religion (93.7%). The health centers provide antenatal care services including HIV and syphilis screening.

The study population consisted of all pregnant women who attended the antenatal care (ANC) clinics of the health centers during the study period. However, those women who were mentally and physically incapable of being interviewed or refused to consent were excluded. The sample size was estimated using a single population proportion formula, with the assumption of HBV prevalence among pregnant women to be 6.1 % (Ramos et al., 2011) and level of confidence 95%. A precision of 3 % was taken considering the suggestion that one half of the estimated prevalence would be appropriate incases prevalence is lower than 10% or higher than 90% (Nainget al., 2006). Thus, the sample size was calculated to be 270. ANC attendees were recruited into the study using a systematic random sampling method. On the basis of the health center's plan and a prior three-month performance document review, a total of 562 pregnant women were estimated to visit the ANC clinics of the health centers. This estimate was divided by the sample size to determine sample interval (k value), which would be two. The 1stserved pregnant woman and every 2nd woman thereafter were invited to participate in the study until the required sample size was obtained.

A pre-tested and structured questionnaire was used to collect information on socio-demography, risky sexual behavior, history of hospital admission, history of abortion and contact with HBV infected individuals. Nurses or health officers working in the ANC clinics of the health centers interviewed the study participants. Trained laboratory personnel drawn 5 ml of venous blood from each study participant, and sera were tested for HBsAg marker using SD BIOLINE Strip Test (Standard Diagnostic Inc, Korea) according to the manufacturer's instruction. The sensitivity and specificity of the kit was >99.9%.

Data were summarized and analyzed using SPSS version 23 software. Multivariable logistic regression analysis was performed for those socio-demographic and risk behavior factors found to be significantly associated with HBsAgsero status in bivariate logistic regression analysis. Odds ratio (OR) and its corresponding 95% confidence interval (CI) were calculated to measure the strength of association. In all cases p-value less than 0.05 was considered statistically significant.

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Ethical approval was obtained from the Institutional Review Board of College of Medicine and Health Sciences, Hawassa University. Permission to conduct the study was also obtained from Oromia Health Bureau, West Arsi Zone Health Department and respective health centers administrations. All participants were given adequate information regarding the purpose, risk, benefit, and confidentiality of the study. Participation was fully voluntary based on informed consent. Code numbers were used in place of identifiers to maintain the confidentiality of participant's information. The study incurred no cost to the study participants and HBsAg testing was performed free of charge. Participants who tested HBsAg positive were managed by responsible health care workers.

RESULTS

In the study period, a total of 270 women who attended the antenatal clinics, 170 in Kofele and 100 in Roba Health Centers, were included. Thus, 62.6% of the participants were from Kofele Health Center and 37.4% from Roba Health Center. The mean age was 24.5 years (standard deviation of 4.59; range 17–41years), and 40.0% were in the age category of 20–24 years. Urban residents accounted for 50.4%, and most participants (95.2%) were married. The majority (85.2%) of the respondents was housewives, and 40.0% had completed a secondary school level education (Table 1).

Table 1: Socio-demographic characteristics of pregnant women attending antenatal clinics at Kofele town, Oromia Region, Ethiopia, 2016

Variable	Category	Kofele Health Center (N=170) (%)	Roba Health Center (N=100) (%)	Total (N=270) (%)
Age (in years)	15-24	54.7	43.0	50.4
	25-34	43.5	53.0	47.0
	35 and above	1.8	4.0	2.6
Residence	Urban	79.4	1.0	50.4
	Rural	20.6	99.0	49.6
Marital status	Single	2.9	1.0	2.2
	Married	95.3	95.0	95.2
	divorced	1.8	3.0	2.2
	Widowed	0.0	1.0	0.8
Educational status	No formal education	18.2	28.0	21.9
	Primary education	30.6	51.0	38.1
	Secondary and above	51.2	21.0	40.0
Occupation	Employed	8.2	0.0	5.2
	Housewife	78.2	97.0	85.2
	Merchant	10.0	0.0	6.3
	Others	3.5	3.0	3.3

Out of the 270 pregnant women tested for HBsAg, 16 were found to be positive, making the sero-prevalence 5.9% (Table 2). The highest sero prevalence of HBsAg was observed among pregnant women in the age range of 35 years and above (28.6 %), followed by those aged 25-34 years (7.1%). Concerning site of residence, 5.9 % of urban dwellers were sero-positive for HBsAg compared to 6%in rural dwellers. The sero-positivity rate was 16.7% among women who never married. It seems that HBsAgsero-positivity was higher among merchants (11.8%) and in those pregnant women with no formal education (16.9%), although difference was not found to be statistically significant (P > 0.05). Moreover, there was no statistically significant association between HBsAg and residence, marital status, or occupational status.

In bivariate analysis, the prevalence of HBsAg positivity increased with age, with the highest risk at 35 years of age and above compared with those in the age range 15-24 years (COR 10.48; 95% CI 1.62-67.8, P = 0.014). Similarly, the educational status was significantly associated with HBsAg (COR 7.14; 95% CI 1.88-27.11).

Further analysis, after adjustment for those significantly associated variables, using multivariable logistic regression showed that educational status of the study participant influenced the rate of HBV infection and those with no formal education had 6.2 times higher risk of acquiring HBV infection (AOR= 6.2; 95% CI= 1.35-28.74, P= 0.019) compared to women who had completed secondary school and above level. However, the association between HBsAg and age did not remain statistically significant in the multivariable analysis.

Table 2: Distribution of HBsAg by socio-demographic characteristics of pregnant women attending the antenatal care clinics of KofeleTown, Oromia Region, Ethiopia, 2016

Socio-demographic variables	Categories	Total N (%)	Positive for HBsAg N (%)	Crude OR (95% CI)	Adjusted OR(95%CI)	P-value
Age(in years)	15-24	136(50.4)	5(3.7)	1	1	
	25-34	127(47.0)	9(7.1)	1.99(0.65-6.13)	1.55(0.39-6.08)	0.527
	35 and above	7(2.6)	2(28.6)	10.48(1.62-67.8)	6.72(0.36-124.2)	0.200
Residence	Urban	136(50.4)	8(5.9)	0.98(0.35-2.7)		
	Rural	134(49.6)	8(6.0)	1		
Marital Status	Single	6(2.2)	1(16.7)	3.47(0.38-31.76)		
	Married	257(95.2)	14(5.4)	1		
	Divorced	6(2.2)	1(16.7)	3.47(0.38-31.76)		
	Widowed	1(0.4)	0(0.0)			
Educational Status	No formal education	59(21.9)	10(16.9)	7.14(1.88-27.11)	6.2(1.35-28.74)	0.019
	Primary education	103(38.1)	3(2.9)	1.05(0.20-5.32)	1.36(0.25-7.37)	0.723
	Secondary and above	108(40.0)	3(2.8)	1	1	
Occupation	Employed	14(5.2)	1(7.1)	1.39(0.16-11.59)		
	Housewife	230(85.2)	12(5.2)	1		
	Merchant	17(6.3)	2(11.8)	2.42(0.49-11.83)		
	Others	9(3.3)	1(11.1)	2.27(0.26-19.66)		

NB: *Candidate variable for multivariate analysis at P<0.25 *variable significant at P<0.05,1: reference

HBsAg sero positivity was not significantly higher in pregnant women who had ear piercing (6.3%) than who did not have this practice (3.3%) (Table 3). Among pregnant women that reported a history of abortion, 22.6% were positive for HBsAg compared to those with no history of abortion (3.8%). The bivariate logistic regression analysis showed that a history of abortion was significantly associated with HBsAg (COR 7.45; 95% CI 2.54-21.80; P <0.001). The association of HBsAg sero-positivity with previous contact with patients having history of liver disease was marginally non-significant (COR 4.39; 95% CI 0.85-22.65; P = 0.077). In multivariable logistic regression analysis, a history of abortion was also found to be a significant predictor of HBV infection (AOR 6.23; 95% CI 1.74-22.5, P= 0.005). However, there was no statistically significant association between HBV infection and body tattooing, history of surgical procedures, gestational age, gravidity, previous place of birth, hospital admission, history of multiple sexual partners, and history of blood transfusion.

Table 3.Bivariate and multivariate analyses of factors associated with HBV infection of pregnant women attending the antenatal care clinics of Kofele town, Oromia Region, Ethiopia, 2016

Socio-demographic variables	Categories	Total N (%)	positive for HBsAg N (%)	Crude OR (95% CI)	AOR (95%CI)	P-value
Ear piercing	Yes	240(88.9)	15(6.3)	1.9(0.24-15.18)		
	No	30(11.1)	1(3.3)	1		
History of multiple sexual practice	Yes	16(5.9)	1(6.3)	1.06(0.13-8.59)		
	No	254(94.1)	15(5.9)	1		
History of surgical Procedure	Yes	7(2.6)	1(14.3)	2.75(0.31-24.38)		
	No	263(97.4)	15(5.7)	1		
History of blood transfusion	Yes	2(0.7)	0(0)			
	No	268(99.3)	16(6)			
Gravidity	Primigravida	83(30.7)	4(4.8)	1		
	Multi gravida	187(69.3)	12(6.4)	1.35(0.42-4.33)		
History of abortion	Yes	31(11.5)	7(22.6)	7.45(2.54-21.80)	6.23(1.74-22.5)	0.005*
	No	239(88.5)	9(3.8)	1	1	
Previous place of delivery	No birth	83(30.7)	4(4.8)	1	1	
	Home	93(34.4)	10(10.8)	2.38(0.71-7.89)	0.85(0.18-3.90)	0.832
	Health institution	94(34.8)	2(2.1)	0.43(0.07-2.40)	0.15(0.02-1.16)	0.69
Gestational age	1st trimester	19(7)	1(5.3)	0.95(0.11-8.05)		
	2nd trimester	145(53.7)	8(5.5)	1		
	3rd trimester	106(39.3)	7(6.6)	1.21(0.42-3.45)		
Pervious contact with liver disease	Yes	10(3.7)	2(20)	4.39(0.85-22.65)	2.23(0.22-22.0)	0.494
	No	260(96.3)	14(5.4)	1	1	
Hospital admission	Yes	20(7.4)	3(15)	3.22(0.83-12.39)	2.99(0.53-6.89)	0.214
	No	250(92.6)	13(5.2)	1	1	
Tattooing	Yes	86(31.9)	8(9.3)	2.26(0.81-6.23)	0.8(0.22-2.87)	0.733
	No	184(68.1)	8(4.3)	1	1	

NB: *Candidate variable for multivariate analysis at P<0.25 *variable significant at P<0.05, 1: reference

DISCUSSION

In this study, the overall sero-prevalence of HBsAg among pregnant women was (5.9 %) which is an intermediate endemicity of HBV infection according to the classification criteria of (World Health Organization, 1990). The prevalence we reported is similar with results in various localities in Ethiopia: 6.1% in south Ethiopia (Ramos et al., 2011),3% in Addis Ababa (central Ethiopia) (Tegegne et al., 2014),4.9 % in Dessie (northeast Ethiopia) (Seid et al., 2014)and 3.8%in Bahir Dar (Zenebe et al., 2014).This highlights that pregnant women in Ethiopia may have similar risk of exposure to and/or rate of HBsAg clearance.

The current finding is also similar to the reported rates of HBsAg among pregnant women in different African countries: 8% in Mali (MacLean et al., 2012), 4.2% in Nigeria (Ezechi et al., 2014), and 6.3% in Tanzania (Hasegawa et al., 2006). However, contrasting the result of this study, higher prevalence rates were also reported from high endemic African countries: 12.5% in Benin (Lai and Yuen, 2007) and 16% in Ghana (Andernach et al., 2009). The difference in the rate of HBsAg may be due to diverse risk factors involved in various geographical regions and different diagnostic methods employed. There are studies that used more sensitive laboratory methods (ELISA and PCR) compared to the rapid diagnostic test employed in the present study.

Regarding socio-demographic status of the study participants, educational status had significant association with HBV infection where pregnant women with no formal education had 6.2 times higher risk of having HBsAg compared to those who completed a secondary school level education and above. This finding was also supported by other reports from Dessie in Ethiopia (Seid et al., 2014), Cameroon (Ezegbudo et al., 2004) and Nigeria (Scott et al., 1997).The possible cause for this higher prevalence of HBV infection among pregnant women with no formal education may be related to a low level of awareness about the transmission and methods of prevention.

This study showed a significant association between HBsAg positivity and history of abortion. Those who had history of abortion were 6.23 times more likely to have HBsAg marker than their counter parts. A similar study, which was conducted in Dessie (Seid et al., 2014) identified this variable as significant associated factor for HBsAg prevalence. Abortion may be related to unprotected sexual intercourse resulting in unplanned pregnancies. Thus, HBV infection might be contracted as a result of unprotected sexual contact or due to abortion with inadequately sterilized instruments since most induced abortion in Ethiopia remains predominantly unsafe and clandestine (Sundaram et al., 2010).

Although the difference was not significant, a higher rate of HBsAg among pregnant women in the age range of 35 years and above in the current study agrees with a similar study done in Nigeria (Alegbeleye et al., 2013). An increasing HBsAg carriage rate with this age group may be explained by the cumulative effect phenomena in which an exposure to HBV infection increase with time (Mansour et al., 2012).

The current study showed a history of blood transfusion was not associated with rate of HBsAg carriage, which is also in line with the result in Jimma (Awole and Gebre-Selassie, 2005). However, in contrast to this finding, blood transfusion was significantly associated with sero-prevalence of HBV in other studies in Ethiopia (Walle et al., 2008; Zenebe et al., 2014). In fact, the small number of pregnant women with a history of blood transfusion in the current study may result in weaker statistical power to be able to assess the rate difference. Similar to a finding reported from Sana'a, Yemen (Murad et al., 2013), we also observed no difference in seropositivity of HBsAg between urban and rural pregnant mothers. However, a study from Eastern Sudan has shown a significantly higher prevalence of HBsAg among pregnant mothers from urban areas than the rural counterparts (Abdallah and Mohamed, 2013). The observed similar distribution in seropositivity in our context may highlight no difference in rate of HBV transmission and/ or HBsAg clearance rate by residence site.

Our study has some limitations in light of which results need to be interpreted. We used rapid diagnostic tests, which are less sensitive than ELISA or PCR tests and may underestimate the prevalence. We investigated HBV prevalence based on HBsAg marker and did not use other markers such as HBeAg, anti-HBe antibodies. Furthermore, the smaller sample size used resulted in a widened confidence interval for some of the variables. Despite these shortcomings, this study generated valuable information on HBV infection among pregnant women in a setting of very limited epidemiological data

CONCLUSION

In conclusion, the prevalence of HBsAg among pregnant mothers attending ANC clinics in Kofele town was intermediate. Pregnant women with no formal education and those with a history of abortion were at higher odds of being HBsAg carrier. Therefore, screening pregnant women for HBV infection and making the vaccine available to exposed babies has to be emphasized. In addition, Oromia Regional Health Bureau and health facilities in the region need to work on preventive measures against unwanted pregnancies and unsafe abortion to child bearing age women. Moreover, Kofele and RobaHealth Centers should give health information on transmission and prevention of HBV to child bearing age women.

Acknowledgments

We would like to thank nurses and health officers at Kofele and Roba Health Centers for assisting in data collection. We appreciate the valuable inputs in the paper by Mr. DemolishWachamo. Financial supporting for the study was given by the Department of Medical Laboratory, College of Medicine and Health Sciences, Hawassa University. We acknowledge the Oromia Region Health Bureau, West ArsiZone Health Bureau for providing us all the support needed during data collection

Conflict of interest

The authors have no conflict of interest to declare for this study.

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Journal of Science and Development
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Conference/workshop/seminar proceedings
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ISSN (Online): 2789-2123; (Print): 2222-5722