Phosphorus Requirement for Colonization by Arbuscular Mycorrhizal Fungi (AMF) and Effect of AMF Inoculants on Growth of Perennial Crops and Agroforestry Trees

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Abstract: In most tropical soils, phosphorus is deficient and high costs of phosphorus fertilizer made it difficult for smallholder farmers to use it when needed. Arbuscular mycorrhizal fungi is known to improve particularly P in P deficient soils. However, response of plant species to mycorrhizal fungi inoculation and application of different rates of P varies. Therefore, this study was conducted to investigate the effect of phosphorus (P) concentrations on arbuscular mycorrhizal fungi (AMF) colonization and growth of two perennial crops (*Catha edulis* and *Ensete ventricosum*) and four multipurpose agroforestry trees (*Cordia africana, Croton macrostachyus, Erythrina brucei* and *Millettia ferruginea*). The experiment was conducted in a glasshouse. The treatment consisted of 0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P/g substrate and three species of AMF. The experiment was laid out in CRD design in a factorial arrangement. The results showed that plant growth parameters (shoot length and dry weight) and P uptake increased significantly after inoculations with AMF, namely *Rhizophagus clarus,* and *Rhizophagus intraradices*, and the mixed AMF species. Results on effect of P application on total mycorrhizal dependency (MD) of the studied crops and agroforestry tree species showed that maximum (41.71%) MD value was recorded for *Rhizophagus clarus* in khat (*Catha edulis Forsk.)*, followed by 34.85 and 34.45% MD values for the same *Rhizophagus clarus* in Birbira (*Millettia ferruginea)* and Bisana (*Croton macrostachyus)*, respectively. The next MD values, ranging from 2.57% for *Catha edulis* to 30.67% in *Ensete ventricosum*, were recorded for inoculation with the mixed AMF species. The least MD values of 3.51, 16.46, 10.51, 7.71, 4.34, and 14.32 were recorded for treatments with *Rhizophagus intraradices* for all plant species (*Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea)* under the study respectively. Optimum P concentrations for maximum benefits from the AMF symbiosis in the aforementioned six plant species varied from 0.005 to 0.02 mg P g^{-1} substrate and the corresponding peaks of arbuscules, vesicles, percent colonization, and spore count per 50 cm^3 sand were noticed at similar P concentrations. Thus, the current research results revealed that the recorded plant growth peaks were attributed to AMF colonization of the perennial crops and agroforestry trees. Therefore, inoculating plant species with a suitable AMF inoculant could result in a benefit comparable to high P fertilizer input and lead to a significant cost saving from expenditure on inorganic P fertilizer. The information obtained on minimum P requirement for perennial crops and shade trees in Sidama agroforestry can form the basis for further pot/field experiments involving integration of chemical fertilizers with AMF

Keywords: Agroforestry; Crops; Inoculation; Phosphorus; Root colonization; Spore density; Trees.

1. Introduction

Agroforestry, a land use system/technology in which trees are deliberately planted on the same unit of land with agricultural crops, has been recognized as one of the most promising strategy for rehabilitating degraded areas and broadly practiced in Sidama Zone of Southern Nations, Nationalities and Peoples' Region (SNNPR), Southern Ethiopia. Some multipurpose shade trees play a vital role in the rural economy of the region. To meet the future demand for these trees and the perennial crops growing under these shade trees, their growth and productivity has to be hastened from the nursery stage onwards and their requirements for major fertilizers, like phosphorus, should be known. According to Tilman *et al.* (2002) and Foley *et al.* (2005), inappropriate and untimely application of fertilizers in agricultural fields generates several environmental pollution and soil problems.

Wrage *et al.* (2010) and de Carvalho *et al.* (2010) reported that side effects due to the practices of agro-ecosystem simplification, where the ecosystem services provided by the soil are increasingly bypassed. The perceived need for seeking alternatives to the current

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agricultural practices has resulted in an enhanced interest in agroforestry systems (Ingleby *et al*., 2007), which can conserve natural resources, improve environmental quality, rehabilitate degraded and deforested lands, and provide multiple outputs to meet the daily demands of the rural population (Pande and Tarafdar, 2004; Muleta *et al*., 2008). Under agroforestry, the needs for ecological sustainability can be reconciled with the needs for sustainable food production (Young, 1997).

Arbuscular mycorrhizal fungi (AMF) can rehabilitate degraded lands subjected to agroforestry systems (Mutuo *et al*., 2005; Cardoso and Kuyper, 2006). The common mycorrhizal network may further enhance the benefits of agroforestry through vertical niche expansion of AMF (Simard and Durall, 2004; Cavagnaro *et al*., 2005; Theuerl and Buscot, 2010). The low biomass production of agroforestry tree species in degraded areas can, therefore, be circumvented by the use of AMF (Shukla *et al*., 2009).

The key function of AM fungi is the exploration of the soil beyond the range of roots for better plant growth and nutrition (Oehl *et al*., 2002; van der Heijden *et al*., 2006). According to Jakobsen *et al.* (2005) and Ma and Rengel (2008), AMF have the potential to make crop cultivation successful in soils with low P level through effective exploitation of the P sources. The P level has been shown to significantly influence AMF colonization of crops and agroforestry trees (Koide, 1991; Covacevich *et al*., 2007).

To manage plant growth and productivity of agro-ecosystems, particularly agroforestry systems, knowledge of P requirement levels of the trees and crops practiced in a given area is mandatory. Therefore, the present study was conducted with the specific objective to investigate the effect of phosphorus (P) concentrations on arbuscular mycorrhizal fungi (AMF) colonization and growth of two perennial crops and four multipurpose agroforestry trees that grow in Sidama agroforestry practices.

2. Materials and Methods

2.1. Description of the Study Area

The study was carried out in the greenhouses of Hawassa University and Hawassa College of teacher education in the capital city of SNNPR located at about 275km form Addis Ababa.

2.2. Experimental Materials

In this study, seeds of selected plant species in Sidama agroforestry were used. Three native species of AM fungi isolated and purified were used as AMF inoculants. Dominant AMF were isolated from the rhizosphere soil of field grown trees, perennial and annual crops by wet sieving and decanting techniques of Gredman and Nicolson (1963).

2.3. Experimental Procedures

Trap culture was set using *Sorghum bicolor* as a host plant. After 5 months of growth, trap cultures were examined for efficiency of the isolates. Then the most vigorous species were selected for further culturing. To get the pure culture three successive inoculations on the same trap plant has been carried out.

Morphological taxonomic identification of spores (color, size, shape, cell wall layers, hyphal attachment, germination shield, etc) was checked to be matched with the description provided by the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM, 2006). Materials and inocula used in this study consisted of soil along with chopped root bits of *Sorghum bicolor*, spores, and extrametrical mycelia from trap culture pots.

2.3.1. Effect of P Concentrations on Plant Growth and P uptake due to AMF Inoculants

Experimental procedures 1: To study the effect of P concentrations on plant growth and P uptake after inoculation with AMF, separate experiments were carried out with two perennial crops and four most common multipurpose shade trees in the agroforestry. The trials consisted of six P concentrations (0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P g^{-1} substrate) and three mycorrhizal treatments and un-inoculated plants (control). Thus, a total of 24 treatment combinations were involved per plant species, and each experiment was replicated three times. Seeds of *Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea* were surface-sterilized with 2% sodium hypochlorite (NaOCl), washed five to six times with sterile distilled water and germinated at 30 ^{0}C in 20 cm top diameter plastic pots filled with 2 kg sterilized river sand.

At the time of sowing, 50 g of mycorrhizal inocula was applied to the hole in the pots where pre-germinated seedlings were individually transplanted. Phosphorus was applied to the pots at 0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg g^{-1} rates as KH_2PO_4. The potted plants were grown in greenhouse and were watered daily. One seedling was maintained per pot and half-strength Hoagland's solution in deionized water was applied at weekly interval. The composition of the Hoagland's solution was (0.51 g/L KNO_3, 0.246 g/L $Ca(NO_3)_2$, 0.245 g/L $MgSO_4.7H_2O$, 1.43 g/L H_3BO_3, 0.91 g/L $MnCl_2.7H_2O$, 0.11 g/L $ZnSO_4.5H_2O$, 0.04 g/L $CuSO_4.5H_2O$, and 0.04 g/L $H_2MoO_4.H_2O$). Pots were arranged in completely randomized design (CRD) and to reduce the risks of cross contamination, kept on separate benches, with a space of 40 cm between each treatment.

Data collection: Seedlings were harvested three months after transplanting and were analyzed for shoot length and dry weight by standard methods (Tanwar *et al*., 2013). Phosphorus uptake was recorded using molybdenum blue method according to Jackson (1973).

Mycorrhizal dependency (MD) was calculated according to Plenchette *et al.* (1983) as follows:

MD (%) = [(M-NM)/M] X 100

Where: M is the total dry biomass of mycorrhizal plant; NM is the total dry biomass of non-mycorrhizal plant.

2.3.2. Effect of P Application on AMF Colonization on Perennial Crops and Trees

Experimental procedure 2: To study the effect of P application on AMF colonization of the two perennial crops and the four component plants of the agro-forestry, 24 treatment combinations for each plant (1 plant X 6 p rates X 3 AMF species plus control) were replicated four times and six plants were maintained per replicate/pot (one *Ensete ventricosum* was grown per pot because of its broad canopy and large pseudostem).

Data collection: Two plants per pot (2 plants from 2 pots in the case of *Ensete ventricosum*) were harvested 1, 2, and 3 months after sowing to monitor formation of arbuscules and vesicles, and the colonization index was calculated and the spore concentration per 50 cm^3 sand was counted. Fine roots were cleared with 10% KOH and stained with acid fuchsin (0.01% in lactoglycerol) as reported by Phillips and Hayman (1970), and then colonization rates of arbuscules and vesicles were recorded. Colonization percentage was determined by gridline intersection method of McGonigle *et al.* (1990). Sporocarp and spores were isolated according to Gerdemann and Nicolson (1963), and were counted (mean of 40 counts for each subsample under field vision of the stereomicroscope was taken as number of spores/100g dry soil).

2.2. Data Analysis

All the data on plant growth were subjected to a one-way analysis of variance (ANOVA) for testing the effects of AMF inoculation and P application, and their interactions. The means were compared and ranked using Duncan's Multiple Range Test (DMRT) at 5% probability level. The means of the experiments were analyzed statistically using a general linear model for analysis of variance of completely randomized designs (CRD). Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 20.0).

3. Results

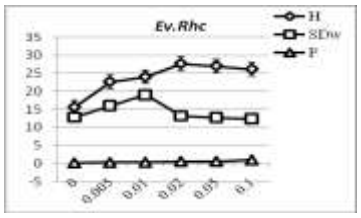
3.1. Plant Growth, Shoot Dry Biomass Yield, and P Uptake

The results on effect of AMF inoculation (*Rhizophagus clarus, Rhizophagus intraradices* and the mixed species) and P (0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P g^{-1} substrate) application on growth and P uptake by *Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei, and Millettia ferruginea* are presented (Figure 1). Most of the peaks of shoot lengths, and dry weights of these plant species occurred within the P concentrations ranges of 0.005 to 0.02 mg g^{-1}. For the un-inoculated plant species, such peaks increased with increase in P concentrations. For the two AM fungi separate and mixed inoculations studied, these peaks indicated that the optimum P concentrations for maximum benefits from the AMF symbiosis in plant species lied mostly within the ranges from 0.005 to 0.02 mg g^{-1} P concentrations (Figure 1).

For shoot length, the optimum P concentration for most effective AMF inoculants, *Rhizophagus clarus*, *Rhizophagus intraradices* and the two-mixed species in *Ensete ventricosum, Cordia africana, Erythrina brucei and Croton macrostachyus* was 0.02 mg P g^{-1} substrate. For *Catha edulis* inoculated with *Rhizophagus Clarus*, both plant height and shoot dry weight increased with increase in P concentration and in *Millettia ferruginea* there was a slight height increase in treatments inoculated with *Rhizophagus intraradices*; however, increase in shoot dry weight at 0.01 mg g^{-1} P concentration was consistent (Figure 1) with the other four plant species mentioned above.

Thus, except *Catha edulis,* which was inoculated with *Rhizophagus clarus* and that positively responded to increasing P concentrations, inoculating abovementioned perennial crops and agroforestry trees, with a suitable AMF inoculant (at lower P concentration) could be as effective as high inputs of recommended P fertilizer application. A similar benefit is expected in case of other tree seedlings, as the optimum P concentration for all selected agroforestry shade trees studied with different AM fungi for maximum benefit from the symbiosis was low (0.005–0.02 mg P g^{-1} substrate). Since different AM fungi can transport and transfer different amounts of P to plants, their effects on plant growth can also be different. Despite this fact, however, in the current study the two species from Glomeromycota and the mixture of the two-species produced similar results in the greenhouse as compared to the un-inoculated, which was given similar P concentrations with other treatments.

In all perennial crops and agroforestry trees studied, the AMF inoculants used, namely *Rhizophagus clarus* and *Rhizophagus intraradices* and the mixed species resulted in significantly ($p \leq 0.05$) increased shoot length, dry weight, and P uptake. Results obtained with *Rhizophagus clarus* were almost at par with the mixed species. Compared to *Rhizophagus clarus*, lower responses were recorded with *Rhizophagus intraradices* (Figure 1).



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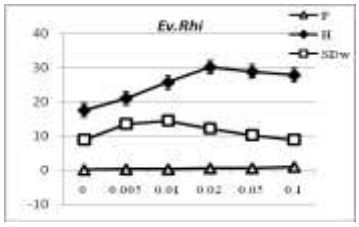

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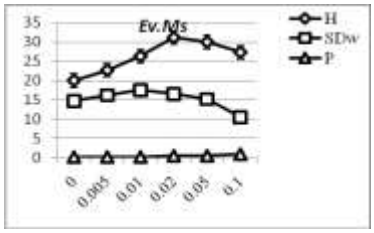

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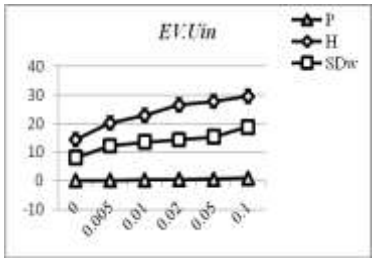

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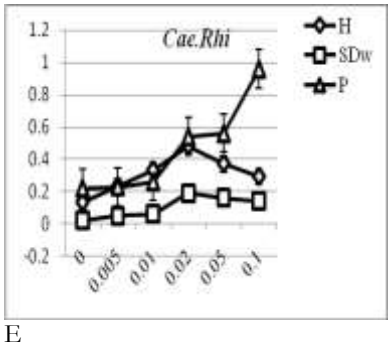

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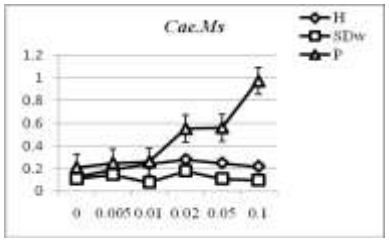

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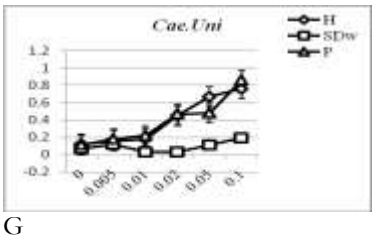

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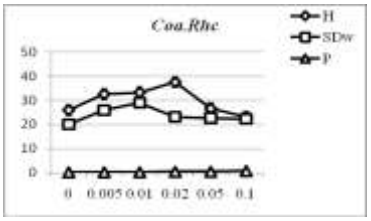

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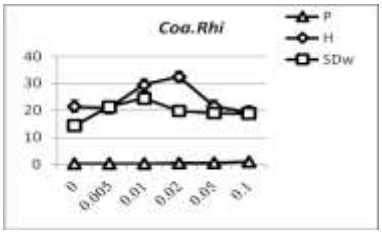

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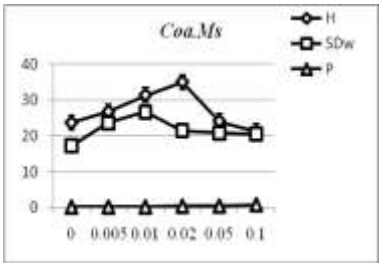

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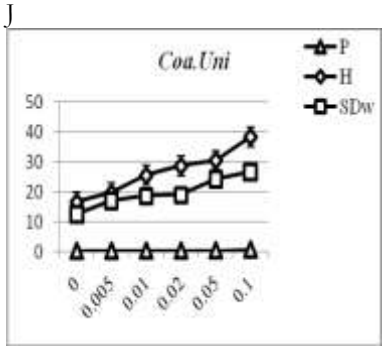

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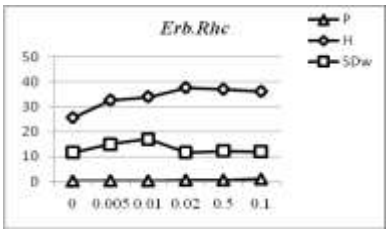

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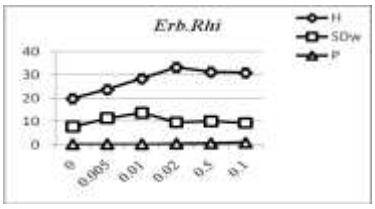

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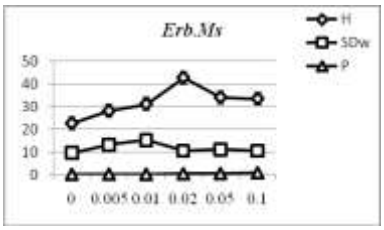

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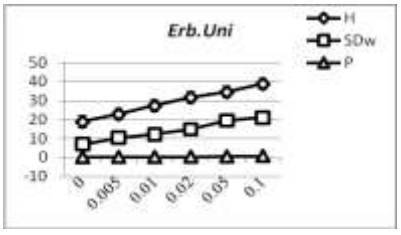

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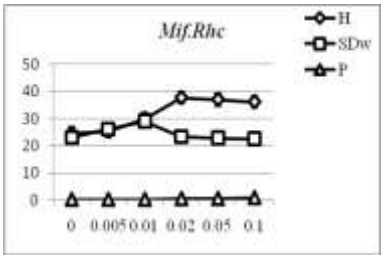

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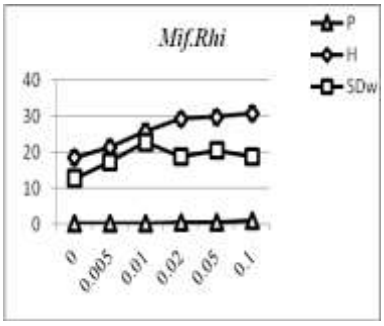

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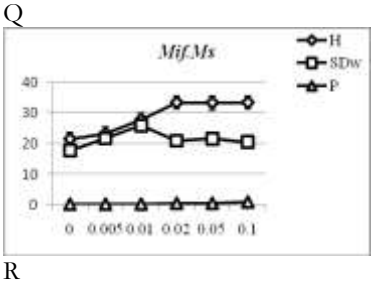

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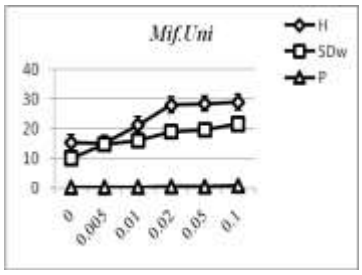

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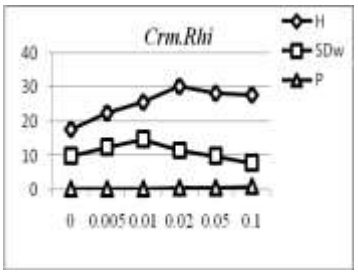

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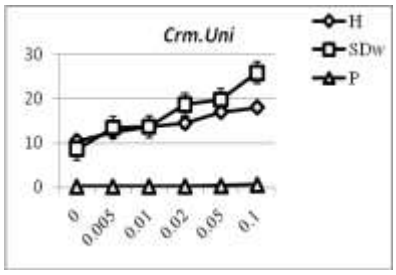

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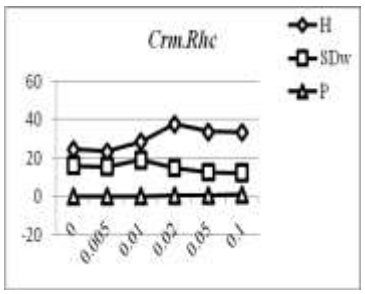

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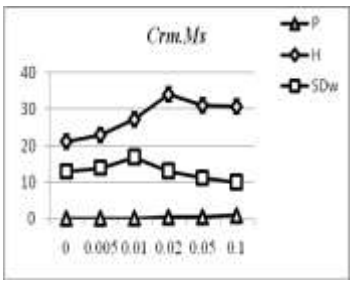

V

Figure 1. Plant height, shoot dry weight and P uptake at different P concentrations (mg/g) in AMF inoculated and un-inoculated treatments. Key: H, height; SDW, Shoot dry weight; P, Phosphorus; Ev, *Ensete ventricosum*; Cae, *Catha edulis*; Coa, *Cordia africana*; Erb, *Erythrina brucei*; Mif, *Millettia ferruginea*; Crm, *Croton macrostachyus*; Rhc, *Rhizophagus clarus*; Rhi, *Rhizophagus intraradices*; Ms, Mixed species; Uni, un-inoculated.

3.2. Mycorrhizal Dependence (MD) of Seedlings of the Selected Crop and Tree Species

Total results on mycorrhizal dependence (MD) of *Catha edulis, Cordia africana, Croton macrostchyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea* seedlings are presented (Table1). In perennial crops and the agroforestry shade trees, the three AMF inoculants, namely *Rhizophagus clarus, Rhizophagus intraradices* and the two-mixed species significantly ($p \leq 0.05$) increased shoot length. Except for *Catha edulis* inoculated with *Rhizophagus intraradices* and the two-mixed species, the total shoot dry biomass increased in all the treatments. Maximum (41.71%) MD value was recorded for inoculation with *Rhizophagus clarus* in *Catha edulis*, followed by MD value (34.85%) obtained from inoculation with the same *Rhizophagus clarus* in *Millettia ferruginea* and MD (34.45%) value with *Rhizophagus clarus* inoculant in *Croton macrostachyus*. For the two-mixed species, the next highest MD values ranged from 2.57% in *Catha edulis* to 30.67% in *Ensete ventricosum*. The least MD values were recorded in all the inoculation treatments with *Rhizophagus intraradices* in all plant species in the undertaken test (Table 1).

Table 1. Total shoot dry weight and mycorrhizal dependency (MD) of the perennial crops and agroforestry shade trees.

Plant species	SDW and MD%						Un-inoculated
	Rh. Clarus		*Rh. intraradices*		Mixed species		
	Total SDW	MD	Total SDW	MD	Total SDW	MD	SDW
Ensete ventricosum	86.03[c]	27.05a	62.76bc	7.71c	90.53d	30.67d	62.76bc
Catha edulis	0.97a	41.71d	0.57a	3.51a	0.76a	2.57a	0.55a
Cordia africana	143.37[e]	31.34b	117.83f	16.46f	130.6e	24.62c	98.44c
Erythrina brucei	79.7b	26.19a	61.5b	4.34ab	70.6b	16.67b	58.83b
Millettia ferruginea	146.03e	34.87c	111e	14.32e	128.52e	26.0cd	95.11c
Croton macrostachyus	90.53cd	34.45c	66.33d	10.55d	78.43c	24.35c	59.33b

Note: Rh, Rhizophugus; SDW, shoot dry weight; MD, mycorrhizal dependency. For each plant species means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan's multiple range test at P < 0.05 level.

3.3. Effect on AMF Structural Colonization and Spore Density

AM fungi structural colonization (arbuscules and vesicles) of the perennial crops and shade trees after inoculation with *Rhizophagus clarus, Rhizophagus intraracices* and the two-mixed species are presented in Table 2. This finding revealed that formation of arbuscules by the separate *Rhizophagus clarus and Rhizophagus intraradices* inoculations and inoculation with the two mixed species were more favored at lower P concentrations (between 0.05 to 0.02 mg P g^{-1} substrate) than either extremely lower or higher P concentrations. However, there were also some rates of colonization below and above 0.05 and 0.02 mg P g^{-1}

concentration in all inoculated trees and crop species (Table 2). The results also indicated that arbuscule formation occurred at the early stage during the 1st month of inoculation and that of formation of vesicles was intensive during the 2nd and 3rd months after the inoculation.

Table 2. Effects of different phosphorus concentrations (milligrams P per gram substrate) on AMF structural colonization (after 1st, 2nd and 3rd months of growth).

Plants	P mg/g	*Rhizophagus clarus*						*Rhizophagus intraradices*						Mixed					
		AC (%)			VC (%)			AC (%)			VC (%)			AC (%)			VC (%)		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	0	−	-	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
Ensete	0.005	+	+	++	−	+	+	+	+	+	_	+	+	+	+	++	-	+	+
ventricosum	0.01	+	+	++	+	+	+	+	+	++	−	+	++	+	+	+++	-	++	++
	0.02	+	++	++	−	++	++	−	+	+	-	++	++	+	+	++	-	+	++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	−	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	−	+	+	−	+	+	+	+	+	+	+	+	+	+	++	-	++	++
Catha edulis	0.01	+	+	++	−	+	++	+	+	++	−	+	++	+	+	++	+	++	++
	0.02	+	++	+++	+	+	++	+	+	++	−	++	+++	+	+	++	−	+	+++
	0.05	+	−	+	−	−	+	−	+	+	+	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
Cordia	0	−	−	+	−	−	+	-	+	+	−	+	+	−	+	+	−	+	+
africana	0.005	−	++	++	+	+	+	+	+	+	−	+	++	+	+	++	-	+	+
	0.01	+	+	+++	−	+	++	+	+	++	−	+	++	+	+	++	-	++	++
	0.02	+	++	+++	−	+	++	+	+	+	-	++	+++	+	+	++	-	++	+++
	0.05	+	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	-	+	−	−	+	-	+	+	−	+	+	−	+	+	−	+	+
	0.005	−	+	++	−	+	+	+	+	+	-	+	+	+	+	++	-	+	+
Erythrina	0.01	+	++	++	−	+	++	+	+	++	-	+	++	+	+	++	-	++	++
brucei	0.02	+	++	++	−	++	+++	+	+	+	-	++	++	+	+	++	-	++	+++
	0.05	−	−	++	+	+	+	−	+	+	−	+	+	−	+	+	−	++	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	-	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	+	++	++	−	+	++	+	+	+	+	+	++	+	+	++	-	++	++
Millettia	0.01	+	++	++	+	++	+++	+	+	++	−	+	++	+	+	++	-	++	++
ferruginea	0.02	+	+++	+++	−	++	++	+	+	+	+	++	+++	+	+	++	-	++	+++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	−	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	+	+	++	−	++	+	+	+	++	-	+	+	+	+	++	-	++	+++
Croton	0.01	+	++	++	+	++	+	+	+	++	−	+	+++	+	+	++	-	++	++
macrostachyus	0.02	+	++	+++	−	++	++	+	+	+	+	++	+++	+	+	++	+	++	++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+

Note: 1, 2, 3, number of months of plant growth*;* AC, arbuscular colonization; VC, vesicular colonization; absent (-), fair (+), moderate (++), high (+++ and above)

In conclusion, all the plants inoculated with AM fungi showed mycorrhizal colonization that was characterized by the presence of arbuscules and vesicles (Table 2). However, mycorrhizal colonization, arbuscule and vesicle formation decreased significantly with the increase in P concentrations. Also, similar trend was observed with mycorrhizal spore number (Table 3), and positive correlation was recorded between mycorrhizal spore number and percentage root colonization.

Table 3. Effects of different phosphorus concentrations (milligrams P per gram substrate) on root colonization and spore density (after three months of growth).

Plant species	P (mg/g)	*Rhizophagus clarus*		*Rhizophagus intraradices*		Mixed AMF species	
		RLC (%)	SD/50 cm^3 soil	RLC (%)	SD/50 cm^3 soil	RLC (%)	SD/50 cm^3 soil
	0	12.67bc	29.00b	13.87bc	31.67b	20.67c	26.67ab
Ensete	0.005	15.67c	35.00b	20.33cd	34.00bc	24.00c	37.67bc
Ventricosum	0.01	35.00d	58.00c	33.00e	54.67d	29.67d	62.33d
	0.02	17.33c	40.33b	22.33d	42.33c	23.00c	46.33cd
	0.05	9.33ab	7.33a	8.33ab	12.67a	15.33b	9.33a
	0.1	4.90a	5.67a	3.63a	6.67a	4.63a	10.00a
	0	9.33b	22.67b	12.50ab	32.67ab	15.67bc	22.67ab
	0.005	13.73c	28.67b	19.33b	36.67b	22.33bc	43.67c
	0.01	24.33d	58.33c	32.67c	65.33c	35.00d	65.33d
Catha edulis	0.02	16.00c	47.00c	20.00b	56.00c	26.33cd	35.67bc
	0.05	7.67b	6.67a	3.33a	11.67a	13.33ab	10.67a
	0.1	2.00a	2.33a	2.67a	6.67a	3.33a	7.00a
	0	13.83bc	29.00b	14.17b	32.67b	22.67c	28.67b
	0.005	16.83c	35.00b	21.67c	36.00bc	26.00c	39.67bc
	0.01	35.03d	58.00c	33.33d	56.67d	31.67d	56.33d
Cordia africana	0.02	17.87c	40.33b	23.33c	44.33c	25.00c	42.67c
	0.05	8.67ab	8.00a	9.33ab	14.67a	17.33b	11.33a
	0.1	4.93a	6.67a	4.33a	8.67a	6.67a	12.00a
	0	12.33ab	25.33b	16.33bc	32.67b	20.00bc	26.17b
	0.005	17.50bc	30.67b	22.67cd	35.67b	23.33c	36.67cd
	0.01	32.67d	57.33c	33.33e	56.67c	33.33d	39.33d
Erythrina	0.02	21.33c	49.67c	25.67de	42.33b	20.50bc	32.33c
brucei	0.05	10.33ab	6.67a	9.00ab	13.33a	14.67b	9.00a
	0.1	5.17a	3.33a	4.17a	6.67a	3.00a	7.50a
	0	13.33b	26.67b	16.50bc	36.67b	19.33bc	26.67ab
	0.005	17.73c	34.00b	23.33c	40.67b	26.33bc	47.67c
Millettia	0.01	28.67d	62.33c	36.33d	69.33c	39.00d	69.33d
ferruginea	0.02	20.00c	51.00c	24.00c	60.00c	30.33cd	39.67bc
	0.05	11.67b	10.67a	7.67ab	15.67a	17.33b	14.33a
	0.1	6.00a	6.00a	5.33a	10.67a	2.67a	11.00a
	0	11.07b	21.67b	12.00a	35.00c	17.33b	25.00b
Croton	0.005	18.33c	24.67bc	22.33b	34.67c	25.67c	39.00c
macrostachyus	0.01	27.33d	35.67d	28.67c	50.00e	25.43c	59.83d
	0.02	19.00c	33.00cd	20.67b	42.67d	24.00c	35.00bc
	0.05	9.33b	5.67a	9.00a	14.33b	14.33b	11.00a
	0.1	6.00a	3.33a	4.13a	7.00a	2.67a	7.00a

Note: RLC, root length colonization; SD, spore density. For each plant species means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan's Multiple Range Test at $P < 0.05$ level.

Maximum root colonization and spore count per 50 cm^3 sand was observed at P concentrations ranging from 0.005 to 0.02 mg g^{-1} in plants inoculated with AM fungi (Table 3). In this study, results showed that the optimum P concentration for maximum benefit from AMF symbiosis for inoculated agroforestry trees and perennial crops and tree seedlings was in between 0.005 and 0.02 mg g^{-1} and the plant growth decreased with increasing P concentration. Therefore, inoculating plants with a suitable AMF inoculants could result in a benefit comparable to high P input. However, extrapolation of the results to the real conditions of agroforestry systems should be done with precaution because of differences in the substrate used, i.e., sand in the present study. The information on P optimum can form the basis for further pot and/or field experiments involving integration of chemical fertilizers with AM fungi inoculants.

4. Discussion

Previous studies under field conditions have shown that agricultural management practices, such as tillage, fertilization and cropping systems, have a negative impact on the AMF associated with temperate and tropical crop plant species (Douds and Millner, 1999; Cardoso and Kuyper, 2006). Fertilization is an

important abiotic factor influencing growth, colonization, sporulation, composition and distribution of AMF (Wang *et al.*, 2009).

Other studies conducted in the greenhouse conditions have demonstrated that AM fungi usually have their maximum effect on host plant growth when the level of P in the growth medium is optimum (Habte and Manjunath, 1991). According to Habte and Manjunath (1991), when the soil solution P concentration is at or near 0.002 mg per liter, most plant species will respond dramatically to mycorrhizal colonization.

Results of the current study on perennial crops and agroforestry trees revealed the pick for maximum benefit at 0.02 mg P g^{-1} growth medium (sand) and that as P concentration is increased from 0.005 to 0.02 mg g^{-1}, the reliance of plants on AM fungi for P uptake increased and diminished progressively as P concentration increased from 0.05 to 0.1 mg g-1, after which only the very highly mycorrhizal-dependent species responded significantly to mycorrhizal colonization.

The current results also confirmed previous results (Vierheilig and Ocampo, 1991; Ravnskov and Jakobsen, 1995) on functional effectiveness of AMF. The mechanism underlying the reduction in plant growth just above the optimum P is probably due to both effects of P on root growth and direct effects on the fungi (Cardoso *et al.*, 2006). Increase in P supply may decrease the availability of organic substrates from roots to fungi. Azcon *et al.* (2003) reported that low P concentration in lettuce plants allowed the maximum colonization and occurrence of AM fungi. Koide (1991) showed that P levels influenced AMF colonization. Addition of P fertilizers above the optimum delayed and/or inhibited AMF colonization (de Miranda *et al.*, 1989; Baon *et al.*, 1992).

Several other authors have reported that mycorrhizal roots are able to absorb several times more phosphate than non-inoculated roots from soils and from solutions (Dela Cruz *et al.*, 1988). Increased efficiency of phosphorus uptake by mycorrhizal plants could have led to higher concentrations of P in the plant tissues.

The greater phosphate absorption by AMF has been suggested to arise due to superior efficiency of uptake from labile forms of soil phosphate, which is not attributable to a capacity to mobilize phosphate sources unavailable to non mycorrhizal roots (Pearson and Gianinaazzi, 1983). Mycorrhizal roots are known to have not only a considerably greater phosphate inflow rates, but also to possess a pathway of phosphate uptake with a much higher affinity for phosphate than non-mycorrhizal roots.

In this study maximum root colonization and spore count per 50 cm^3 river sand was observed at P concentrations ranging from 0.005 to 0.02 mg P g^{-1} in plants infected by AM fungi and effectiveness decreased with increasing P concentration. The current results are consistent with the findings of Kahiluoto *et al.* (2000), who observed and reported that with increasing P supply, there was a decrease in the colonization and the effectiveness of mycorrhizal colonization. The present results are also in agreement with many reports, which suggest that addition of phosphate fertilizers above optimum levels results in delay in colonization and reduction in chlamydospore production by AM fungi (Koide and Li, 1990; Koide, 1991; Thingstrup *et al.*, 1998).

In general, most of the perennial crops and agroforestry trees are fast-growing plants that require more nutrients during the initial stage of seedling establishment. During this period, the root system was not well developed and the AM fungal symbiosis might play a vital role by supplying the nutrients to the host plant (Muthukumar and Udaiyan, 2006). The results of the present study revealed that mycorrhizal inoculations increased the plant growth and P uptake in different treatments with a few exceptions. This can be due to increase in the sand volume explored for nutrient and water uptake by the mycorrhizal plants from the medium as compared to non-mycorrhizal plants. These results also support results of previous studies and the high rate of P fertilizer application, i.e. 0.05 and 0.1 mg g^{-1} lead to antagonistic inhibition of mycorrhizal colonization, whereas lower P doses with application of the vigorous AM fungus *Rhizophagus intraradices* were able to significantly increase the root colonization and spore density. However, increased P supply increased some growth parameters associated with plant height, shoot and root dry weight. Thus, soil amendment with AM fungi generally have the potential to possibly reduce the application of phosphorus fertilizer for crop improvement, growth, yield and nutritional value of the perennial crops and shade trees in Sidama agroforestry.

The current research results indicated that inoculating plants with a suitable AMF inoculant could result in a benefit comparable to high P input. However, extrapolation of the results to the real conditions of agroforestry systems should be done with precaution because of differences in the substrate used, i.e., sand in the present study. The information on optimum P concentration for better performance of AMF in the agroforestry trees can form the basis for further pot/field experiments involving integration of chemical fertilizers with AM fungi.

5. Conclusion

The present study demonstrated that the inoculation of perennial crops and multipurpose trees with *Rhizophagus intraradices, Rhizophagus clarus* and mixture of both inocula increased all plant growth parameters, but at the same time decreased percentage of mycorrhizal colonization and spore density as the concentration of P increased. Thus, soil amendments with AM fungi have the potential to possibly reduce the application of phosphorus fertilizer for crop and tree growth and improvement in agroforestry. However, to come up with more concrete, accurate and reliable information

on functional efficiency of the AMF species applied, further pot and/or field experiments should be carried out.

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Specific Gravity, Dry Matter Content, and Starch Content of Potato (*Solanum tuberosum* L.) Varieties Cultivated in Eastern Ethiopia

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Abstract: Several farmers' and improved potato varieties are cultivated by farmers in the eastern highlands of Ethiopia. However, sufficient information is not available on tuber quality characteristics of the potato varieties. Therefore, experiments were conducted at three locations in the region, namely, Haramaya, Hirna and Arbarakatte in eastern Ethiopia during the 2012 to 2014 main cropping season to elucidate the internal tuber quality characteristics of 17 potato varieties and prepare a specific gravity conversion chart. The treatments consisted of 17 potato varieties (Araarsaa, Badhasa, Belete, Batte, Bubu, Bulle, Chala, Chiro, Gabbisa, Gera, Gorebela, Gudanie, Guasa, Jalenie, Jarso, Mara Charre and Zemen). The experiment at location was laid out as a randomized complete block design and replicated three times per treatment. The results revealed that tuber specific gravity (SG), dry matter (DM) and starch contents (SC) were significantly influenced by variety, location and year, while the interaction of the three factors significantly influenced SG and SC. All improved cultivars produced tubers with >1.085, >21% and > 14% of SG, DM and SC, respectively, and were found to be suitable for making French fries, chips and flakes. However, tubers of the local varieties had <1.07 gcm^2, < 20% and < 11% SG, DM and SC, respectively, and were found to be suitable for making whole boiled potatoes. The dendrogram constructed using Unweighted Pair-group Method with Arithmetic means separated the varieties into three clusters of which Cluster I with distinct Sub-group I consisted of eight improved varieties (Ararsa, Bule, Marachere, Badhasa, Challa, Jalanine, Gabisa and Zemen) and Sub-group II six improved cultivars (Bubu, Gera, Gorbella, Gudenine, Gusa and Chiro) , and Clusters II and III consisted of one improved variety (Belete) and two farmers' varieties (Batte and Jarso), respectively. The varieties found to be suitable for making processed potato products (French fries, etc) were grouped in the Sub-group I as they produced tubers with SG, DM and SC contents of acceptable standard for making such products while cultivars in Sub-group II and Belete (Cluster II) produced tubers with high SG, DM and SC contents that may produce too hard, dry and brittle French fries and chips. The correlation of specific gravity with dry matter and starch contents being perfect or near to perfect (r=0.962 to 1) across locations and seasons with high coefficient of determination (R^2=0.924 to 0.999). It could, thus, be concluded that it is appropriate to use specific gravity and the conversion chart produced from this tuber quality trait to estimate the other two traits (dry matter and starch contents) and determine the quality of tubers for processing.

Keywords: Coefficient of determination; Conversion chart; Correlation; Internal tuber quality.

1. Introduction

Potato global production has exceeded 376 million tonnes from over 19.3 million hectares (FAOSTAT, 2013). There is some estimate that the crop yield will have to double by 2050 to meet the demand of global food security. Importantly, potatoes are affordable, putting them within reach of the economically disadvantaged groups of people. Potato contains high protein-calorie ratio (17g protein: 1000 kcal) and yields more edible energy, protein and dry matter per unit area and time compared to cereals (Anderson *et al.,* 2010). In Ethiopia, potato has been considered as a strategic crop to enhance food and nutrition security. In 2013/14, potato was produced on 179,159 hectares of land with the total 1,612,006 and average of 9.1 tonnes of yield in the country. In East Hararghe, potato is a co-staple food (ORARI, 2007) and export commodity. It is approximately grown by 52,710 farmers with a total area of 2,507.12 hectares and average yield of 19.3 t ha^{-1} (CSA, 2014).

In Ethiopia, research for potato variety development and other agronomic managements began in 1975. The first potato variety was released in 1987. Ever since, 33 potato varieties have been developed and released in the country for production under different recommendation domains by research centers, Haramaya University and private companies (Baye and Gebremedhin, 2013; MoA, 2013). The National potato research effort has been in developing high yielding and late blight resistant varieties mainly for different kinds of traditional foods, but less emphasis has been given to processing products such as French fries, chips and others. However, small scale potato chips processors are flourishing in cities and big towns (Elfnesh *et al*., 2011). Potato chips and French fries are commonly found in hotels, restaurants, supermarkets and small shops. In addition, the country has a potential in producing potatoes to supply large scale potato processing industries that might not be far from establishment. All varieties are not suitable for the production of processed products (Kabira and Berga, 2006). Therefore, it is necessary to evaluate the fitness of cultivars for the emerging economics of production until specific varieties are developed for specific end products.

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Potato tubers quality is often referred to as external and internal quality. The internal quality is determined by many traits of which the most important are dry matter content, type and amount of starch, sugar, and protein content (Van Eck, 2007). High tuber specific gravity, dry matter and starch content are important for processing by enhancing chip yield, crispness and reduces oil uptake in fried products (Johnson *et al.*, 2010; Freitas *et al.*, 2012). Potato cultivars are significantly different in tuber specific gravity, dry matter content, and starch content (Hassanpanah *et al.*, 2011; Kaur and Aggarwal, 2014; Ismail *et al.*, 2015). Significant influence of environment and genotypes on specific gravity and tuber dry matter content was also reported (Elfnesh *et al.*, 2011; Tefaye *et al.*, 2013; Ismail *et al.*, 2015). Therefore, it is necessary to evaluate potato cultivars for internal tuber quality traits across locations and over seasons.

There is a close relationship among specific gravity, total solids and starch content and relationship has been developed by several workers among these traits (Johanson *et al.*, 1967; Fitzpatrick *et al.*, 1969; Willson and Lindsay, 1969; Verma *et al.*, 1972; Vakis, 1978; AOAC, 1980; Kleinkopf *et al.*, 1987; Dale and Mackay, 1994). Specific gravity conversion tables are available in other countries to be used by the potato processing industry (Houghland, 1966; Lulai and Orr, 1980; DEPI, 1995; USDA, 1997; Ezekiel *et al.*, 2003; Dinesh *et al.*, 2005). But these tables cannot be used in other countries since the relationship vary with the variety, location, season and the year of cultivation (Verma *et al.*, 1972). Therefore, it is necessary to evaluate potato cultivars across locations and seasons to determine their fitness for varied processing products and to prepare a conversion chart for specific gravity. In Ethiopia, limited reports are available for some of the released varieties regarding to potato tuber internal quality traits and processing aspects (Elfnesh *et al.*, 2011; Tefaye *et al.*, 2013). However, these studies did not include most of the cultivars under cultivation and were conducted only for one cropping season. Moreover, conversion charts for specific gravity, dry matter and starch contents have not been developed for cultivars in the country at large and in eastern Ethiopia in particular. Therefore, this research was conducted with the objectives: i) to evaluate potato cultivars for internal tuber quality traits, ii) to study the effect of growing locations and seasons on internal tuber quality traits; and iii) to establish the relationship among internal tuber quality traits and prepare conversion chart for specific gravity, dry matter and starch contents in eastern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Sites

The field experiment was carried out at three locations namely; Haramaya, Hirna and Arberkete which represent mid and highland altitudes of potato growing areas of eastern Ethiopia. The experiment was conducted for two main cropping seasons (2012 and 2013) at all three locations. In addition, at Haramaya, potato cultivars were evaluated during the 2014 main cropping season. This made the total of seven environments considering one location and one cropping season as one environment.

Haramaya University research farm is located at 2020 meters above sea level, 9˚41"N latitude and 42˚03"E longitude. The area has a bimodal rainfall distribution with mean annual rainfall of 760 mm (Belay *et al.*, 1998). The long rainy season extends from June to October and accounts for about 45% of the total rainfall. The mean maximum temperature is 23.4^{0}C while the mean minimum annual temperature is 8.25^{0}C (Tekalign, 2011). The soil of the experimental site is a well-drained deep alluvial with a sub-soil stratified with loam and sandy loam. Hirna sub-station of Haramaya University is situated at a distance of about 134 km to the west of Haramaya. The site is located at 9 ^{o}12' North latitude, 41 ^{o}4'East longitude, and at an altitude of 1870 meters above sea level. The area receives mean annual rainfall ranging from 990 to 1010 mm. The average temperature of the area is 24^o C (Tekalign, 2011). The soil of Hirna is vertisol (HURC, 1996). Arbarakatte is located at a distance of about 171 km to the west of Haramaya. The site is located at 9 ^{o}14' North latitude, 41 ^{o}2'East longitude, and at an altitude of 2280 meters above sea level.

2.2. Experimental Materials

The experiment included 15 improved potato varieties which are under production and two farmers' varieties. These varieties were developed and released for different regions of Ethiopia by five Research Centers and Haramaya University (Table 1).

2.3. Experimental Design and Procedures

The experiment was laid out as a randomized complete block design (RCBD) with three replications in each location and season. Each potato variety was assigned to one plot in each replication and six rows with 12 plants. The gross plot size was 16.2 m^2 with 75 and 30 cm between rows and within plant spacing, respectively. The spacing between plots and replications was maintained at 1.5 m and 1 m, respectively. For measuring the specific gravity and dry matter content, tubers were harvested from plants in the four middle rows, leaving the plants growing in the two border rows as well as those growing at both ends of each row to avoid border effects.

The experimental fields were cultivated by a tractor (Haramaya and Hirna) to a depth of 25-30 cm and ridges were made by hand. Medium sized (39-75g) and well sprouted tubers were planted at the sides of ridges. Planting was at the end of June and first week of July during the main growing season after the rain commenced and when the soil was moist enough to support emergence. The planting depth was maintained at 10 cm. The whole recommended rate of phosphorus fertilizer (92 kg P_2O_5 ha^{-1}) was applied at planting in the form of Diammonium Phosphate. Nitrogen fertilizer was applied at the rate of 75 kg N ha^{-1} in the form of urea in two splits, half rate after full emergence (two weeks after planting) and half rate at the initiation of tubers.

2.4. Data Collection

Tuber dry matter content (%) was measured from five fresh tubers in each plot. The randomly taken tubers were weighed at harvest, sliced and dried in oven at 75°C until a constant weight was obtained and dry matter in percent was calculated according to Williams and Woodbury (1968) as follows.

$$\text{Dry matter (\%)} = \frac{Weight of\ sample after drying(g)}{Intial\ weight of\ sample(g)} \text{ x } 100 \quad (1)$$

Table 1. Studied potato varieties.

No.	Variety	Accession code	Year of release	Breeding center	Recommended altitude (m a.s.l.)
1	Araarsaa	CIP-90138.12	2006	Sinnana Research Center	2400-3350
2	Badhasa	AL-114	2001	Haramaya University	2400-3350
3	Belete	CIP-393371.58	2009	Holeta Research Center	1600-2800
4	Batte	Farmers' variety			
5	Bubu	CIP-384321-3	2011	Haramaya University	1700-2000
6	Bulle	CIP-387224-25	2005	Hwassa Research Center	1700-2700
7	Chala	CIP-387412-2	2005	Haramaya University	1700-2000
8	Chiro	AL-111	1998	Haramaya University	2700-3200
9	Gabbisa	CIP-3870-96-11	2005	Haramaya University	1700-2000
10	Gera	KP-90134.2	2003	Sheno Research Center	2700-3200
11	Gorebela	CIP-382173.12	2002	Sheno Research Center	1700-2400
12	Gudanie	CIP-386423.13	2006	Holeta Research Center	1600-2800
13	Guasa	CIP-384321.9	2002	Adet Research Center	2000-2800
14	Jalenie	CIP-37792-5	2002	Holeta Research Center	1600-2800
15	Jarso	Farmers' variety			
16	Mara Charre	CIP-389701-3	2005	Hwassa Research Center	1700-2700
17	Zemen	AL-105	2001	Haramaya University	1700-2000

Source: Plant Variety Release, Protection and Seed Quality Control Directorate, Crop Variety Register Issue No. 16, pp. 161-164 (MoA, 2013, June, Addis Abeba, Ethiopia); m a.s.l = meters above sea level

Specific gravity of tubers was measured using weight in air and weight in water method. Five kg tubers of all shapes and sizes were randomly taken from each plot and washed with water then weighed first in air and then in water. The specific gravity of tubers was calculated using the following formula (Kleinkopf *et al*, 1987).

$$\text{Specific gravity (gcm}^{-3}) = \frac{Weight in air}{Weight in air - Weight in water} \quad (2)$$

Total starch content (g/100g) was estimated from specific gravity. Starch (%) = 17.546 + 199.07 × (specific gravity-1.0988) (Talburt and Smith, 1959 as cited by Yildrim and Tokuşoğlu, 2005) where specific gravity was determined as indicated above by the weight in air and weight in water method.

In addition, dry matter and starch content in percent were calculated from the measured specific gravity and dry matter of tubers using different methods established by different researchers and institutions of other countries. The calculation was made by placing the measured tubers specific gravity or dry matter of each cultivar in the equation and the measured specific gravity also used to read and obtain the corresponding dry matter and starch contents in Canada (DEPI, 1995) and USA (USDA, 1997) specific gravity conversion chart. These methods are as follows:

i. Dry matter (%) = -214.9206 + 218.1852 (specific gravity) (Kleinkopf *et al*., 1987)
ii. Dry matter (%) = 3.33+211 (specific gravity-1) (Willson and Lindsay, 1969)
iii. Starch content (%)=17.565 + 199.07 (specific gravity – 1.0988) (Von Scheele equations cited by Hassel *et al*., 1997)
iv. Starch content (%) = 17.55 + 0.891 * (tuber dry weight% – 24.182) (AOAC, 1980).
v. Both dry matter and starch content (%) estimated from Canada specific gravity conversion table (DEPI, 1995)
vi. Both dry matter and starch content (%) estimated from USA specific gravity conversion chart (USDA, 1997)

2.5. Data Analysis

Data collected for specific gravity, dry matter and starch content were subjected to i) analysis of variance for each location and season to test the presence of significant differences among varieties in each location, ii) combined analysis of variance conducted for each location over cropping seasons/years, and iii) unbalanced general analysis of variance computed for seven environments considering the three seasons and locations. Homogeneity of error variances was tested using Bartlett's test for Haramaya site since the experiment was conducted for three cropping seasons while F-test was conducted for Hirna and Arbarakatte where varieties were evaluated for two cropping seasons. After the homogeneity of error variances was

observed in all locations across cropping seasons, combined analysis of variance was conducted for each location over cropping seasons. Similarly, Bartlett's test was conducted for seven environments and heterogeneity of the error variances was evident for specific gravity and starch content. Therefore, cultivars were compared for pooled means for each location over seasons and other analyses (regression and correlation) were made the same though the homogeneity of error variances was observed for dry matter content. Mean separation was employed following the significance of mean squares using Least Significant Differences (LSD) at 5% probability.

Linear regression analysis was used to establish the relationship among specific gravity, dry matter and starch content of which specific gravity was considered as independent variable and other two traits as dependent (response) variables. Linear regression analysis was conducted for each location and season as well as pooled mean values of each variety at each location over seasons to understand the differences of the relationships among each location and season and each location over seasons. However, specific gravity conversion table was prepared on the regression equation computed in each location over seasons using pooled mean values of each variety at each location over seasons. The specific gravity conversion for each location was presented in table and the computed regression was presented in graph for each location. Correlation analysis was conducted among the measured data and estimated values (using different methods and regression equation) for specific gravity, dry matter and starch content to test whether the recorded data were in agreement or in contrast to the established relationship among these traits.

The genetic distance of varieties was estimated using Euclidean distance (ED) calculated from, i) the measured mean values of each trait for each variety in seven environments, ii) estimated tuber dry matter and starch contents of each variety using regression equation computed for each location and season, and iii) estimated tuber dry matter and starch contents of each variety using regression equation computed for each location over seasons after standardization (subtracting the mean value and dividing it by the standard deviation) as established by Sneath and Sokal, (1973) as follows:

$$EDjk = \sqrt{\sum_{i=1}^{n} (Xij - Xik)^2}^{\frac{2}{2}} \quad (3)$$

Where: EDjk = distance between varieties j and k; xij and xik= tuber internal quality traits (specific gravity, dry matter and starch contents) mean values of the ith trait for varieties j and k, respectively; and n= number of traits used to calculate the distance.

The distance matrix from tuber internal quality traits was used to construct dendrograms based on the Unweighted Pair-group Method with Arithmetic means (UPGMA). The results of the cluster analysis were presented in the form of dendrogram.

3. Results

3.1. Analysis of Variance and Mean Performance of Varieties

Potato varieties showed significant differences for specific gravity, dry matter and starch content in all environments (at each location and growing season) (data not presented). The combined analysis of variance for each location over growing seasons revealed that tuber dry matter content was significantly influenced by variety and the interaction of variety x season at Haramaya while it was significantly influenced by variety and season at Hirna and Arbarakatte (Table 2). Specific gravity and starch content showed significant variations due to variety, growing season and interaction of variety x season at Haramaya but only due to variety and the interaction of variety x season at Hirna and variety and season at Arbarakatte. Mean squares from unbalanced combined analysis of variance over years and locations revealed that dry matter content was significantly influenced by variety, season, location, interactions of variety x location and location x season while starch content was significantly affected by all possible interactions(Table 3). Specific gravity was significantly influenced by variety, season and interaction effect of variety x season and variety x location x season.

Table 2. Mean squares from analysis of variance in three studied locations.

Location	Source of variation	Dry matter content (%)	Specific gravity	Starch content (g/100g)
	Replication (2)	6.058	0.0000116	0.5149
	Variety (16)	40.191**	0.000780**	30.716**
Haramaya	Season	4.245	0.0001867**	7.871**
	Variety x Season (32)	2.269**	0.0000538**	2.1184**
	Error (100)	2.178	0.0000146	0.5744
	CV (%)	6	0.4	5.1
	Replication (2)	0.498	0.00000683	1.175
	Variety (16)	26.503**	0.00032602**	12.025*
Hirna	Season (1)	63.961**	0.00005775	5.13
	Variety x Season (16)	2.778	0.00008344*	4.847**
	Error (66)	1.696	0.00003203	1.481
	CV (%)	5.60	0.50	8.3
	Replication (2)	2.821	0.0000823	1.745
	Variety (16)	21.139**	0.00052976**	19.579**
Arbarakatte	Season (1)	3.491*	0.00028872*	4.743*
	Variety x Season (16)	1.78	0.0000662	2.108
	Error (66)	0.749	0.0000302	1.441
	CV (%)	3.5	0.50	10.3

*Note: * and **, significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis represented degree of freedom.*

Table 3. Mean squares from unbalanced combined analysis of variance in three studied locations.

Source of variation	df	Dry matter content (%)	Specific gravity	Starch content (g/100g)
Replication	2	0.456	0.00005058	1.085
Variety	16	80.765**	0.00132426**	55.031**
Location	2	51.237**	0.00003343	4.260*
Season	2	8.196*	0.00013145*	4.964*
Variety x Location	32	2.788*	0.00005739	2.555*
Variety x Season	32	2.211	0.00008097*	2.669*
Location x Season	2	35.758**	0.00008907	7.062*
Variety x Location x Season	32	2.229	0.00006724*	2.815**
Error	236	1.782	0.00003885	1.291
CV (%)		5.5	0.57	7.65

*Note: * and **, significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis represented degree of freedom.*

Belete followed by Gera, Bubu and Gorebela had highest specific gravity, dry matter and starch content across locations and years though the order of varieties varied according to the rank differences across locations (Table 4). Belete produced tubers with >27% dry matter content at Hirna and Arbarakatte while Gera and Gorebela at Haramaya and Bubu at Arbarakatte produced tubers >26% dry matter content. Chirro and Chala also produced tubers with >25% dry matter content in all locations. None of the improved varieties produced tubers with <1.08 specific gravity and <14% starch content except Badhasa had <14% starch content at Haramaya and Hirna. On the other hand, the two farmers' varieties produced tubers with <1.07 specific gravity except Jarso at Hirna. The highest tubers dry matter content, specific gravity and starch content were observed at Haramaya (2014) and Arbarakatte (2012 and 2013), respectively.

Table 4. Mean specific gravity, dry matter and starch contents of 17 potato varieties in three locations.

Location	Haramaya			Hirna			Arbarakatte		
Variety	DM	SG	Starch	DM	SG	Starch	DM	SG	Starch
Ararsa	24.4^{efg}	1.085^{efg}	14.79^{efg}	21.81^{f}	1.082^{efg}	14.19^{efg}	23.26^{g}	1.083^{ef}	15.27^{cd}
Badhasa	24.23^{fg}	1.081^{h}	13.9^{h}	22.63^{ef}	1.076^{gh}	13.28^{gh}	23.58^{fg}	1.082^{ef}	14.76^{d}
Belete	26.63^{a}	1.095^{a}	16.72^{a}	27.18^{a}	1.096^{a}	16.97^{a}	27.14^{a}	1.099^{a}	17.58^{a}
Batte	19.88^{h}	1.064^{i}	10.59^{i}	19.12^{g}	1.068^{i}	11.79^{i}	20.99^{h}	1.067^{g}	11.46^{e}
Bubu	25.79^{a-d}	1.09^{bc}	15.86^{bc}	25.35^{b}	1.089^{bcd}	15.57^{a-e}	26.82^{ab}	1.095^{ab}	17.23^{a}b
Bulle	24.84^{d-g}	1.087^{def}	15.11^{def}	22.33^{ef}	1.084^{def}	14.53^{d-g}	23.86^{efg}	1.085^{def}	15.21^{cd}
Chala	25.7^{a-e}	1.084^{fgh}	14.52^{fgh}	24.26^{bcd}	1.085^{c-f}	14.78^{c-f}	25.53^{cd}	1.089^{bcd}	15.52^{bcd}
Chirro	25.93^{a-d}	1.092^{ab}	16.23^{ab}	24.49^{bcd}	1.088^{b-e}	13.55^{fgh}	25.16^{cd}	1.089^{bcd}	15.62^{bcd}
Gabbisa	23.5^{g}	1.088^{cde}	15.3^{cde}	23.83^{cde}	1.087^{b-f}	15.15^{b-e}	24.57^{def}	1.081^{f}	14.79^{d}
Gera	26.27^{abc}	1.09^{bc}	15.87^{bc}	25.55^{b}	1.091^{abc}	15.94^{abc}	25.84^{bc}	1.091^{bc}	15.64^{bcd}
Gorebela	26.56^{ab}	1.092^{ab}	16.21^{ab}	25.13^{bc}	1.092^{ab}	16.27^{ab}	25.52^{cd}	1.091^{bcd}	15.95^{a-d}
Gudanie	25.25^{a-f}	1.089^{bcd}	15.56^{bcd}	24.75^{bc}	1.088^{b-e}	15.45^{b-e}	25.81^{c}	1.088^{cde}	16.65^{abc}
Guasa	25.59^{a-f}	1.092^{ab}	16.07^{ab}	23.77^{cde}	1.091^{abc}	15.97^{abc}	25.96^{bc}	1.088^{cde}	15.78^{bcd}
Jalenie	24.87^{d-g}	1.083^{gh}	14.37^{gh}	23.05^{def}	1.081^{fg}	14.21^{efg}	25.41^{cd}	1.09^{bcd}	15.33^{cd}
Jarso	19.08^{h}	1.061^{i}	10.00^{i}	19.27^{g}	1.072^{hi}	12.19^{hi}	20.11^{h}	1.061^{g}	10.32^{e}
Mara Charre	25.21^{b-f}	1.084^{efg}	14.69^{efg}	22.34^{ef}	1.084^{def}	14.59^{c-g}	23.65^{efg}	1.081^{f}	16.05^{a-d}
Zemen	25.17^{c-f}	1.087^{cde}	15.29^{cde}	23.66^{cde}	1.088^{b-e}	15.77^{a-d}	24.58^{de}	1.086^{c-f}	15.01^{cd}
Mean	24.64	1.085	14.77	23.44	1.085	14.72	24.576	1.085	15.19
LSD (5%)	2.39	0.0062	1.228	2.123	0.0092	1.984	1.4202	0.009	2.54
Year									
2012	24.36	1.083^{a}	14.32^{b}	22.65^{b}	1.08421	14.49	24.76^{a}	1.084^{b}	15.4^{a}
2013	24.62	1.086^{b}	14.96^{a}	24.23^{a}	1.08572	14.94	24.39^{b}	1.087^{a}	14.97^{b}
2014	24.94	1.086^{b}	15.03^{a}	--------	--------	-------	--------	--------	-------
LSD (5%)	NS	0.0015	0.298	0.515	NS	NS	0.344	0.0022	0.41

Note: Means in each column with similar letter(s) are not significantly different each other. DM = dry matter content in percent, SG gcm² = specific gravity, Starch = starch content g/ 100g of fresh tuber weight and LSD (5%) = least significant difference at 5% probability.

The dendrogram constructed using Unweighted Pair-group Method with Arithmetic means (UPGMA) clearly divided the varieties in to three clusters of which the first Cluster consisted of 14 released varieties which was divided in to two sub-groups (Figure 1). The first Sub-group consisted of eight varieties released between 2001 to 2006. The mean tuber specific gravity, dry matter and starch content of these varieties were either equal or less than the mean values of varieties but most of these varieties performed higher than the mean of varieties for dry matter and starch content at Haramaya and Arberkete, respectively. These varieties relatively perform better at these two locations for specific gravity. The second Sub-group consisted of six varieties which included the very old variety Chiro released in 1998 to recently (in 2011) released variety Bubu. This group had mean values higher than the overall mean values of varieties for all traits in all locations, but they had much higher tuber dry matter content (≥25%) and specific gravity (≥1.09) at Haramaya. Belete was formed a solitary Cluster II with highest specific gravity (≥1.096), dry matter (>27%) and starch content (>16.72%) except specific gravity of 1.095 and dry matter content of 26.63% at Haramaya. The third Cluster consisted of the two farmers' varieties (Batte and Jarso) which had <1.07, <20%, and <11% specific gravity, dry matter and starch content, respectively, in all locations, but these varieties had 1.07 and 11% of specific gravity and starch content, respectively, at Hirna and 20% dry matter content at Arberkete for different seasons.

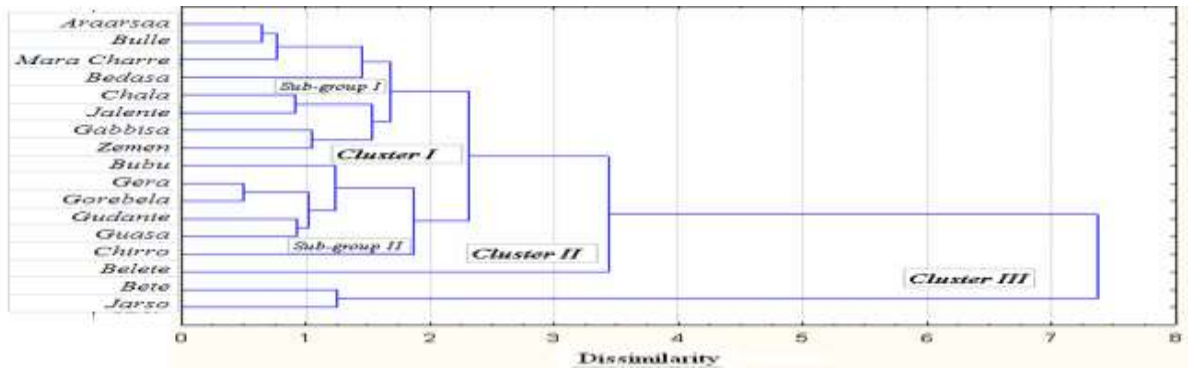


Figure 1. Dendrogram generated based on UPGMA clustering method depicting relationship among 17 potato varieties based on tuber specific gravity, dry matter and starch contents over years at three locations.

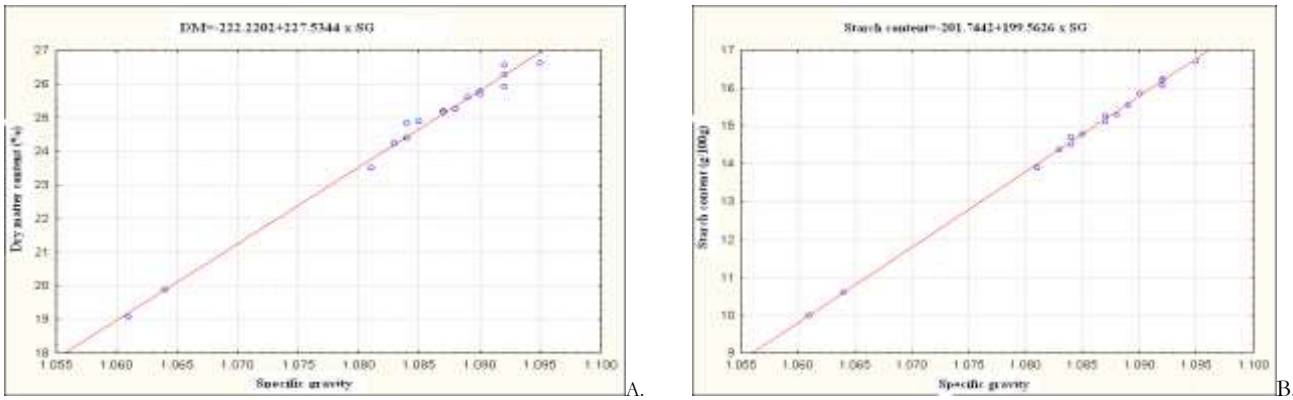
3.2. Relationship among Internal Tuber Quality Traits and Conversion Chart

The regression equations for each location and cropping season and pooled means over years for each location are presented in Table 5. The highest coefficient of determination ($R^2 \geq 0.924$) and correlation ($r \geq 0.962$) were computed for the regression of specific gravity, starch and dry matter contents for all locations in each year. However, regression computed on the basis of pooled means over years for each location showed that both coefficient of determination and correlation values were ≥ 0.99, ≥0.96 and ≥0.97 for Haramaya, Hirna and Arbarakatte, respectively. The graphic presentation of regression computed on the basis of pooled means over years for Haramaya, Hirna and Arbarakatte are presented in Figure 2, 3 and 4, respectively.

Table 5. Regression equation, coefficient of determination (R^2) and correlation of separate years and pooled mean.

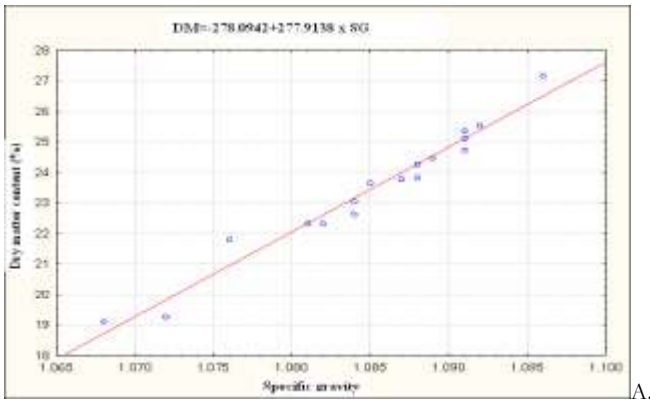
Location	Year	Regression equation	R^2	Correlation (r)
		DM=-171.2307+180.6728 x SG	0.937	0.968
	2012	Starch=-199.7252+197.7151 x SG	0.999	0.999
		DM=-242.9437+246.4027 x SG	0.976	0.988
Haramaya	2013	Starch=-202.1383+199.9253 x SG	0.999	0.999
		DM=-260.2067+262.5102 x SG	0.986	0.993
	2014	Starch=-203.0845+200.8024 x SG	0.999	0.999
Pooled means over three years		DM=-222.2202+227.5344 x SG	0.992	0.996
		Starch=-201.7442+199.5626 x SG	0.999	0.999
		DM=-289.8848+288.2386 x SG	0.95	0.975
	2012	Starch=-208.3541+205.5233 x SG	0.984	0.992
Hirna		DM=-229.6209+233.7771 x SG	0.932	0.965
	2013	Starch=-202.199+199.9677 x SG	0.999	1.00
Pooled means over two years		DM=-278.0942+277.9138 x SG	0.964	0.982
		Starch=-189.9193+188.6054 x SG	0.978	0.989
		DM=-154.9683+165.8926 x SG	0.974	0.987
	2012	Starch=-176.3201+176.960 x SG	0.924	0.962
Arbarakatte		DM=-220.4252+225.2465 x SG	0.968	0.984
	2013	Starch=-188.4728+187.126 x SG	0.959	0.979
Pooled means over two years		DM=-192.1592+199.745 x SG	0.982	0.991
		Starch=-192.189+191.5153 x SG	0.976	0.988

Note: Correlation (r) = correlation coefficient, R^2 = coefficient of determination, DM = dry matter content, SG = specific gravity and Starch = starch content.

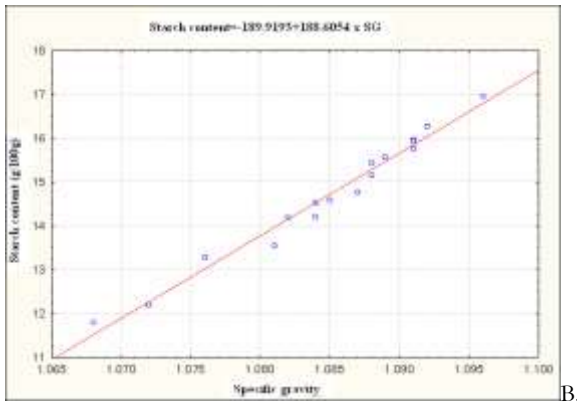


A. B.

Figure 2. Linear regression of tuber specific gravity on A). Dry matter (DM) and B). Starch contents of 17 potato varieties with equation of best-fit line on the basis three years mean values at Haramaya.

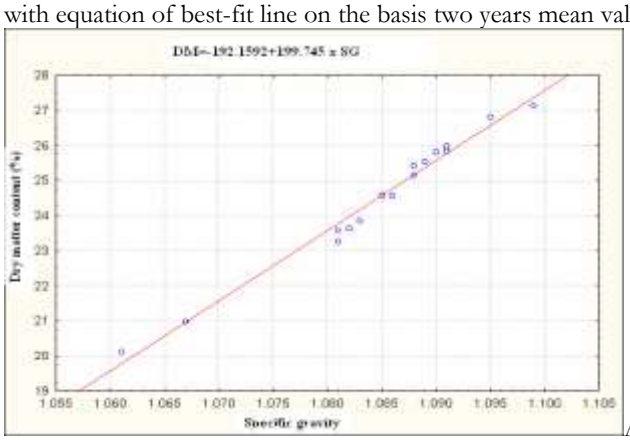


A.

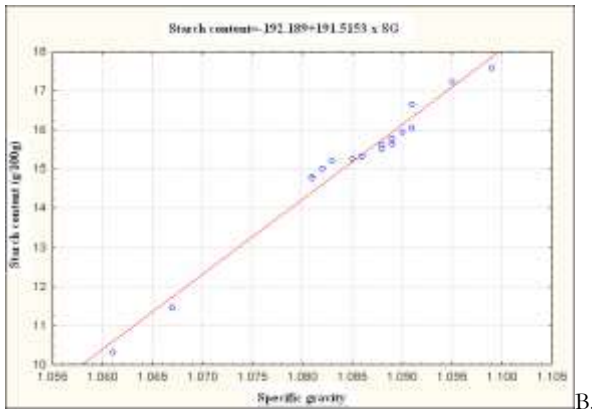


B.

Figure 3. Linear regression of tuber specific gravity on A). Dry matter (DM) and B). Starch contents of 17 potato varieties with equation of best-fit line on the basis two years mean values at Hirna.



A.



B.

Figure 4. Linear regression of tuber specific gravity on A). Dry matter (DM) and B). Starch contents of 17 potato varieties with equation of best-fit line on the basis two years mean values at Arberkete

A specific gravity conversion chart is presented in Table 6. The dry matter and starch contents were 18.97 and 9.79%, respectively, for the lowest specific gravity of 1.06 at Haramaya, while the values were 16.49% (dry matter content) and 10% (starch content) at Hirna and 19.57 (dry matter content) and 10.82% (starch content) at Arberkete. Similarly, 28.09% (dry matter) and 17.79% (starch content) were computed for the highest specific gravity of 1.001 at Haramaya, while it was computed 27.64 (dry matter content) and 17.57% (starch content) at Hirna and 27.58 (dry matter content) and 18.50% (starch content) at Arberkete.

3.3. Correlation among the Observed and Estimated Internal Tuber Quality Traits

The correlation was highly significant among the observed (measured) and estimated (using different methods) tubers specific gravity, dry matter and starch contents. In most cases the correlation was perfect (r = 1.00) or near to perfect (r = 0.97 to 0.99) (Table 7). The measured tubers specific gravity showed perfect or near to perfect correlations with all calculated and estimated dry matter and starch contents except the correlations with measured dry matter content and estimated from regression equation and estimated starch content using AOAC (1980) method (r = 0.91 to 0.96). On the other hand, the observed dry matter content showed perfect or near to perfect correlations (r = 1.00 & r = 0.99) only with estimated dry matter and starch content using regression equation and AOAC (1980) methods, respectively. The measured dry matter content had correlation coefficient of r = 0.94 & r = 0.95 with estimated dry matter and starch contents from all other methods. As compared to measured dry matter content, the observed starch content had higher correlation coefficients ($r \geq 0.97$) with observed and estimated specific gravity, dry matter and starch contents except for the correlation with the estimated dry matter and starch content from regression equation and AOAC (1980), respectively

Table 6. Conversion of specific gravity to dry matter and starch content for three locations calculated from regression equation on the basis of pooled means.

Location	Haramaya		Hirna		Arbarakatte	
SG (gcm^2)	DM (%)	Starch (%)	DM (%)	Starch (%)	DM (%)	Starch (%)
1.060	18.97	9.79	16.49	10.00	19.57	10.82
1.061	19.19	9.99	16.77	10.19	19.77	11.01
1.062	19.42	10.19	17.05	10.38	19.97	11.20
1.063	19.65	10.39	17.33	10.57	20.17	11.39
1.064	19.88	10.59	17.61	10.76	20.37	11.58
1.065	20.10	10.79	17.88	10.95	20.57	11.77
1.066	20.33	10.99	18.16	11.13	20.77	11.97
1.067	20.56	11.19	18.44	11.32	20.97	12.16
1.068	20.79	11.39	18.72	11.51	21.17	12.35
1.069	21.01	11.59	19.00	11.70	21.37	12.54
1.070	21.24	11.79	19.27	11.89	21.57	12.73
1.071	21.47	11.99	19.55	12.08	21.77	12.92
1.072	21.70	12.19	19.83	12.27	21.97	13.12
1.073	21.92	12.39	20.11	12.45	22.17	13.31
1.074	22.15	12.59	20.39	12.64	22.37	13.50
1.075	22.38	12.79	20.66	12.83	22.57	13.69
1.076	22.61	12.99	20.94	13.02	22.77	13.88
1.077	22.83	13.18	21.22	13.21	22.97	14.07
1.078	23.06	13.38	21.50	13.40	23.17	14.26
1.079	23.29	13.58	21.77	13.59	23.37	14.46
1.080	23.52	13.78	22.05	13.77	23.57	14.65
1.081	23.74	13.98	22.33	13.96	23.77	14.84
1.082	23.97	14.18	22.61	14.15	23.96	15.03
1.083	24.20	14.38	22.89	14.34	24.16	15.22
1.084	24.43	14.58	23.16	14.53	24.36	15.41
1.085	24.65	14.78	23.44	14.72	24.56	15.61
1.086	24.88	14.98	23.72	14.91	24.76	15.80
1.087	25.11	15.18	24.00	15.09	24.96	15.99
1.088	25.34	15.38	24.28	15.28	25.16	16.18
1.089	25.56	15.58	24.55	15.47	25.36	16.37
1.090	25.79	15.78	24.83	15.66	25.56	16.56
1.091	26.02	15.98	25.11	15.85	25.76	16.75
1.092	26.25	16.18	25.39	16.04	25.96	16.95
1.093	26.47	16.38	25.67	16.23	26.16	17.14
1.094	26.70	16.58	25.94	16.42	26.36	17.33
1.095	26.93	16.78	26.22	16.60	26.56	17.52
1.096	27.16	16.98	26.50	16.79	26.76	17.71
1.097	27.39	17.18	26.78	16.98	26.96	17.90
1.098	27.61	17.38	27.06	17.17	27.16	18.09
1.099	27.84	17.58	27.33	17.36	27.36	18.29
1.100	28.07	17.77	27.61	17.55	27.56	18.48
1.1001	28.09	17.79	27.64	17.57	27.58	18.50

Note: SG (gcm^2) = specific gravity, DM (%) = dry matter content and Starch (%) =starch content.

Table 7. Correlation coefficient among the measured and estimated specific gravity, dry matter and starch contents computed for each location and cropping season (above diagonal) and pooled means of three locations over years (below diagonal) (2012-2014)

	OSG	ODM	OSTAR	CDM	CSTAR	WDM FSG	KlDM FSG	DEPI DMFSG	DEPI STAR	VSSTAR	USADDM	AOACST
OSG		0.91**	0.97**	0.96**	0.98**	1.00**	1.00**	1.00**	1.00**	1.00**	0.99**	0.91**
ODM	0.94**		0.93**	0.95**	0.91**	0.91**	0.91**	0.91**	0.91**	0.91**	0.92**	1.00**
OSTAR	0.98**	0.95**		0.95**	0.97**	0.97**	0.97**	0.97**	0.97**	0.97**	0.96**	0.93**
CDM	0.95**	0.99**	0.96**		0.96**	0.96**	0.96**	0.96**	0.96**	0.96**	0.96**	0.95**
CSTAR	0.97**	0.95**	0.99**	0.96**		0.98**	0.98**	0.98**	0.98**	0.98**	0.97**	0.91**
WDMFSG	1.00**	0.94**	0.98**	0.95**	0.97**		1.00**	1.00**	1.00**	1.00**	0.99**	0.91**
KlDMFSG	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**		1.00**	1.00**	1.00**	0.99**	0.91**
DEPI DMFSG	1.00**	0.95**	0.99**	0.95**	0.97**	1.00**	1.00**		1.00**	1.00**	0.99**	0.91**
DEPI STAR	1.00**	0.94**	0.99**	0.95**	0.97**	1.00**	1.00**	1.00**		1.00**	0.99**	0.91**
VSSTAR	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**	1.00**	1.00**	1.00**		0.99**	0.91**
USADDM	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**	1.00**	1.00**	1.00**	1.00**		0.92**
AOACST	0.94**	1.00**	0.95**	0.99**	0.95**	0.94**	0.94**	0.95**	0.94**	0.94**	0.94**	

*Note: **, significant at P < 0.01 probability. OSG = observed specific gravity, ODM = observed dry matter content, OSTAR = observed starch matter content, CDM = calculated dry matter content on the basis of regression equation, CSTAR = calculated starch content on the basis of regression equation, WDMFSG = estimated dry matter content from specific gravity using Willson and Lindsay (1969) method, KlDMFSG = estimated dry matter content from specific gravity using Kleinkopf et al. (1987) method, DEPI DMFSG = estimated dry matter content from specific gravity using specific conversion table of Department of Environment and Primary Industries of Canada (1995), DEPI STAR = estimated starch content from specific gravity using specific conversion table of Department of Environment and Primary Industries of Canada (1995), VSSTAR = estimated starch content from observed specific gravity using Von Scheele equations (cited by Hassel et al., 1997), USADDM = estimated dry matter content from observed specific gravity using specific gravity conversion chart of United States Agriculture Standard (USDA, 1997), AOACST = estimated starch content from observed dry matter content using official methods of analysis, Association of Official Analytical (AOAC, 1980).*

4. Discussion

The presence of wide variations among varieties for tuber specific gravity, dry matter and starch contents indicated the genetic factor was important to influence the tuber internal quality traits. The observed differences are a good opportunity for the producers to select the varieties for production that fit the market demand. Many other researchers also reported the presence of significant differences among potato cultivars for these tuber quality traits (Elfnesh *et al*., 2011; Hassanpanah *et al*., 2011; Tesfaye *et al*., 2013; Kaur and Aggarwal, 2014; Ismail *et al*., 2015). These traits were also significantly influenced by growing season and location. The influence of growing location on starch content in dry matter was reported (Dorota *et al*., 2011; Hassanpanah *et al*., 2011; Kaur and Aggarwal, 2014). Specific gravity and tuber dry matter content are influenced by both the environment and cultivars (Elfnesh *et al*., 2011; Ismail *et al*., 2015). However, the interaction of variety x location x season was significantly influenced specific gravity and starch content indicating the unstable expression of these traits in different varieties across locations and seasons. These quality traits are genetically controlled and also influenced with growing locations and seasons (Dorota *et al*., 2011; Hassanpanah *et al*., 2011; Kaur and Aggarwal, 2014). The result suggested the importance of testing potato varieties across locations and seasons to identify wide adaptable varieties that could produce tubers with uniform specific gravity and starch content in all environments since it benefit producers, processors and consumers.

All improved varieties produced tubers $\geq$1.08 and $\geq$23% specific gravity and dry matter content, respectively, in all locations and growing seasons except two varieties at two locations. On the other hand, the farmers' varieties had tubers with low values of <1.07 and <20% specific gravity and dry matter content, respectively. Tesfaye *et al.* (2013) reported dry matter content ranged from 17.05 to 29.88% for 25 potato genotypes studied at three locations of northwestern Ethiopia. Elfnesh *et al.* (2011) also reported 20.33 to 27.33% and 1.078 to 1.110 gcm^{-3} dry matter content and specific gravity, respectively, for five improved potato varieties tested at three locations in eastern Ethiopia. Specific gravity values considered as low (<1.077) intermediate (between 1.077 and 1.086) and high (>1.086) (Fitzpatrick *et al*., 1969). Potato cultivars with a dry matter content of 20% or higher are the most preferred for processed products (Kirkman, 2007). Kabira and Berga, (2006) suggested a dry matter content of 20 to 24% are ideal for making French fries while those with up to 24% for preparing crisps. They suggested also, potato tubers should have a specific gravity value of more than 1.080 and those with less than 1.070 are generally unacceptable for processing. This suggested the evaluated potato varieties were suitable for processing but farmers' varieties were not suitable for processing to French fries and chips.

The eight varieties (Ararsa, Bule, Marachere, Badhasa, Chala, Jalenine, Gabisa and Zemen) included in the first Sub-group of Cluster I had with mean tuber starch content of 14.56 to 15.24% whereas the six varieties (Bubu, Gera, Gorbella, Gudenine, Gusa and Chiro) in the second Sub-group had a mean starch content $\geq$15.46%. Belete formed solitary Cluster II had highest mean values up 17.58% of tuber starch content while farmers' varieties in Cluster III had lowest mean values of <12%. Tesfaye *et al.* (2013) reported starch content ranged between 10.44 and 18.51% for 25 potato genotypes that Betete had the highest tuber starch content. Starch is of special importance for the nutritional value ranges between 15-20% (Schafer-Pregl *et al*., 1998), important role in the cooking quality (Binner et al., 2000), starch production in starch processing industries (Liu *et al.,* 2003) and healthy food processing and consumption in relation to moderating blood glucose levels. Esendal (1990) suggested three groups: the highest starch content (>19.0%, mashing), high starch content (between 16.0 and 19.0%, roasting), intermediate starch content (between 13.0 and 15.9%, cooking or roasting), and low starch content (up to 12.0%, boiling). In general, potato varieties with a starch content of 13% and above are the most preferred for processed products (Kirkman, 2007). All improved varieties could be grouped under intermediate starch content fit for processed products either for cooking or roasting while Belete with high starch content for roasting and farmers' varieties with low starch content for boiling.

Starch concentration represents the dry matter content of potatoes (Hogy and Fangmeier, 2009). Since starch content has direct influence on technological quality, especially with regard to the texture of the processed products. High dry matter content increases chip yield, crispy consistency, and reduces oil absorption during cooking (Rommens *et al*., 2010). However, tubers with very high dry matter content produces too hard and dry French fries and the crisps will be too brittle. Potatoes with a dry matter content of 20 to 24% are preferred for French fries 22 to 24 for chips and >21% are preferred for flakes production (NIVAA, 2002). In this regard, the eight potato varieties under Cluster I, Sub-group I might serve for all purpose (French fries, chips and flakes) while the six varieties in same cluster in Sub-group II might be used for flakes production. Varieties in Sub-group II and more likely Belete may not serve for both French fries and chips because the products may have a higher chance to be too hard, dry and brittle due to tubers high dry matter content. The high tuber starch content of these varieties may result cell separation, reduced cohesiveness and softening during cooking (Binner *et al*., 2000) and may not preferred by diabetic patients. In this regard the two farmers' varieties may be preferred as producing healthy food due to their low starch content. Potato due to its high starch content mainly carbohydrate thought to contribute to some health problems such as diabetes

and weight gain (Cordain, 2005; Mozaffarian *et al*., 2011). Studies showed variability among potato genotypes for glycemic index values (Henry *et al*., 2005; Parada and Aguilera, 2009). The waxy potatoes are with high moisture and low starch content and had medium glycemic index and the floury potatoes are high in starch and had high glycemic index (Henry *et al*., 2005). Glycemic index is a measure of foods ability to affect human blood sugar levels. Foods with low glycemic index values are considered healthy food choices since they have the innate property of moderating blood glucose levels, while foods with a high value are considered to be the opposite (Jenkins *et al*., 1981).

The relationship of specific gravity with tuber dry matter and starch contents was linear with high coefficient of determination and high positive correlation. The relationship was differing from location to location and season to season in the same location. However, the measured specific gravity showed perfect or near to perfect correlations with the estimated tuber dry matter and starch contents from regression equation. The relationship between specific gravity dry matter and starch contents of potatoes has been developed by several workers and associations (Johanson *et al*., 1967; Fitzpatrick *et al*., 1969; Willson and Lindsay, 1969; Verma *et al*., 1972; Vakis, 1978; AOAC, 1980; Kleinkopf *et al*., 1987; Dale and Mackay, 1994; Hassel *et al*., 1997). The relationship among internal tuber quality traits has been found to vary with the variety, location, season and the year of cultivation (Verma *et al*., 1972). On the other hand, the correlation of measured and estimated dry matter content with specific gravity and tuber starch content had lower coefficient values. This suggested measuring specific gravity and estimating starch content is preferred than estimating starch content from measured dry matter content. Tekalign and Hammaes (2005) reported the positive and significant correlation of tuber dry matter content and specific gravity and suggested specific gravity as a true indicator of the amount of tuber dry mater.

Specific gravity of potatoes is commonly used by the potato processing industry as a tool for quick estimation of dry matter content. The preparation specific conversion charts need to test genetically different potato genotypes at different locations and seasons. Johanson *et al.* (1967) suggested the importance of testing varieties for a few years under local conditions and to select wide adaptable varieties with the same specific gravity when grown across environments. Many authors reported the significant influence of growing season and location other than genotype on specific gravity and the two traits to be converted (Dorota *et al*., 2011; Elfnesh *et al*., 2011; Hassanpanah *et al*., 2011; Kaur and Aggarwal, 2014; Ismail *et al*., 2015). The prepared conversion chart for specific gravity was: i) the result of testing considerable number of potato varieties at representative potato growing areas of eastern Ethiopia for a couple of years, ii) it was observed positive and highly significant correlations of the measured specific gravity with the measured dry matter and starch contents, iii) most importantly, it was observed perfect or near to perfect correlations of the measured specific gravity with the estimated dry matter and starch contents using regression equation, and iv) it was also observed perfect or near to perfect correlations of the measured specific gravity with the estimated dry matter and starch contents with several methods. These could allow recommending the importance of measuring specific gravity and using the prepared specific gravity conversion chart as reliable indicator of tuber quality traits of the tested varieties and other potato genotypes in eastern Ethiopia.

5. Conclusion

The research results suggested the importance of evaluating varieties for internal tuber quality traits (specific gravity, dry matter and starch content) across representative locations of growing region over seasons. Because the observed significant influence of variety x location x season interaction on these traits make difficult to predict the tuber quality of potato varieties by testing them in one location over seasons. This also suggested the important of identifying wide adaptable (stable) varieties that produce tubers with uniform specific gravity and starch content throughout the production areas of the region that benefit producers, processors and end consumers. Though, the varieties were developed for high tuber yield, all the improved varieties produced tubers above the minimum requirements to fit different processing products (French fries, chips flakes etc.). However, some varieties produced tubers with high dry matter and starch content that might not be preferred for French fries and chips, because the products may have a higher chance to be too hard, dry and brittle. The farmers' potato varieties might be preferred to be used for making whole boiling tubers but not for making French fries and chips due to the low starch and high moisture contents of the tubers.

The research also suggested that measuring specific gravity of tubers is most appropriate to determine the quality of tubers. The prepared specific gravity conversion chart can be used as indicator of tuber dry matter and starch contents of potato genotypes and thereby to determine the internal quality of tubers for processing in eastern Ethiopia. From the research results it is possible to make conclusion and recommendation such as: i) it is necessary to develop wide adaptable varieties in the country that produce tubers with the same specific gravity through evaluation of varieties across major potato growing regions of the country, ii) use of specific gravity than dry matter content as good indicator of internal quality of tubers for processing, iii) it is necessary to prepare specific gravity conversion chart in the country at least for major potato growing regions of representative locations to be used by processors, other consumers and researchers, and iv) it is necessary to evaluate the varieties further for other physical tuber quality and

quality of processed products to identify which variety(ies) fit to which processing to produce healthy food. These could not be accomplished with separate efforts of researchers at different research centers rather it will be successful with the coordinated joint efforts of potato researchers in the country.

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Grain Yield and Economic Benefit of Intercropping Barley and Faba Bean in the Highlands of Southern Ethiopia

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Abstract: Farmers in the highland areas of southern Ethiopia own less cultivable land. Barley and faba bean are important crops in the southern highlands of Ethiopia. However, rapid population growth in the region, which has led to scarcity of cultivable land, is threatening cultivation of these crops. Therefore, farmers often resort to alternative ways of maximizing crop yields from the small plots of land they own through intercropping. However, little empirical information is available on the agronomic and economic benefits obtained from intercropping barley and faba bean as well as on the influence of pattern of intercropping the two crops on productivity. Thus, a study was conducted during 2011 and 2012 years to evaluate the effect of barley (Ba)-faba bean (Fb) intercrop on yield, yield related traits and economic benefit in the highlands of southern Ethiopia. The treatments consisted of planting patterns of one (1Fb), two (2Fb) and three (3Fb) rows of faba bean combined with one (1Ba), two (2Ba) and three (3Ba) rows of barley. The experiment was laid out as a randomized complete block design in a factorial arrangement with three replications per treatment. Data were collected on a number of plant parameters on both crops. The results indicated that there were significant main effects of year and planting pattern on grain, straw and total biomass yields, harvest index and net income of barley. The number of barley seeds per spike was significantly influenced by the main effect of year, and was 12% less in 2011 than in 2012. Grain yield of barley in 2011 was 67% more than in 2012 while straw and total biomass yields were 45 and 23% less, respectively. Intercropping of 1Faba bean: 1Barley yielded 2176 kg ha^{-1} grain, HI of 96%, LER of 1.56, system productivity index of 3013, better monetary benefit of 9056 Ethiopian birr, and additional land benefit of 36% over the control treatment. Intercropping in this pattern also produced 91% more energy and significantly more income (167%) compared to sole crop barley. Intercropping of 1Faba bean: 1Barley, 1Faba bean: 2Barley and 1Faba bean: 3Barley yielded 52 to 79% less grain of faba bean than sole faba bean. The productivity of barley-faba bean intercrop was more (LER>1) and varied between 32 and 56%. In conclusion, this study indicated that farmers with subsistence and low-input farming can benefit more from intercropping of one row of faba bean combined with one, two and three rows of barley in terms of productivity and economic benefit.

Keywords: Land equivalent ratio; Land benefit; Monetary benefit; Planting pattern; System productivity index; Row ratio.

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal crops grown in the highlands of southern Ethiopia. According to CSA (2014), barley covered 76,763.7 ha of land. The mean grain yield of barley in southern Ethiopia is low (1724 kg ha^{-1}) (CSA, 2014) while the potential yield varies between 2000 and 4900 kg ha^{-1} under research and 1500 to 4300 kg ha^{-1} under on-farm condition (MoARD, 2007). Faba bean in southern region covers 71763 ha of land. The average grain yield of faba bean at the regional level is 1461 kg ha^{-1} (CSA, 2014). The low productivity of barley is attributed to low soil fertility, mainly soil nitrogen and phosphorus deficiency (IFPRI, 2010), and weed and insect pests (CSA, 2014) and cultivation of low-yielding local cultivars for production and limited use of improved barley varieties (about 1% of the total barley production area). Application of urea, Diammonium Phosphate (DAP) and farmyard manure to the crop is at 0.91, 28.3 and 15.42% of the total production area, respectively (CSA, 2014). Currently, the highland areas of southern Ethiopia are densely populated and led to a decline in farm size (Headey *et al*., 2013). Farmers, thus, practice intercropping of cereals with legumes and horticultural crops due to less cultivable land ($\leq$ 0.5 ha) (CSA, 2014) per household. In addition, farmers expect increased crop yield, better soil fertility and economic return (Tenaw *et al*., 2006). The finding of Andersen *et al*. (2007) indicates that barley benefited more from pea-barley intercrop. An increase in barley grain N concentration when intercropped with faba bean was also reported by Knudsen *et al*. (2004). According to Strydhorst *et al*. (2008), faba bean–barley, lupin–barley, and pea–barley intercrops produced 64, 27 and 55% more protein yields, respectively, compared to sole crop barley. Getachew *et al*. (2006) reported that total yield, land equivalent ratio (LER) and system productivity index (SPI) of barley-faba bean mixtures exceeded those of sole crop barley.

The component crops in cereal-legume intercropping efficiently utilize the different sources of nitrogen (Willey, 1979) that may be considered as monetary benefit for farmers in the high land areas of southern

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Ethiopia (Dordas *et al.*, 2012). Crop combination of 50:50% seed ratio of barley-field pea yielded 20% less grain than the respective sole crops, and LER of 23 and 68% more yields of field pea and faba bean, respectively, compared to crops grown separately (Pristeri *et al.*, 2012), Eslami-Khalili *et al* (2011) reported that intercropping of 25% faba bean and 75% barley was better in LER compared with 50:50% faba bean-barley combination; however, more yield was obtained from sole crops of barley and faba bean. Various studies (Sadeghpour *et al.*, 2014; 2013; Takim, 2012; Yilmaz *et al*, 2008) have shown that cereals are more competitive in intercropping but legumes can fix atmospheric nitrogen symbiotically if effective strains of rhizobium are present in the soil. The complementary use of growth resources by the component crops is particularly important in low input subsistence farming such as those in the highlands of southern Ethiopia. However, limited research has been done to elucidate the effect of intercropping faba bean and food barley on the productivity of the crops. Therefore, this research was conducted with the objective of evaluating the effect of barley-faba bean intercrop on growth, grain yield of the component crops, and economic benefit.

2. Materials and Methods

2.1. Experimental Site Description

Field experiment was conducted during the 2011 and 2012 main cropping season at Bulle testing site located in the southern region of Ethiopia. Bulle is a highland area with an altitude of 2700 meters above sea level. It is located between 6.11 and 6.37^{o}E latitude and 38.29-38.44^{o}N longitude. It has an annual rainfall varying between 1401 and 1800 mm with a mean annual temperature of 12.6 to 20^{o}C. The soil of Bulle area is classified as Haplic luvisol and Leptic phaeozems (FAO/UNESCO, 1988) in which the latter is the dominant soil type. The soil texture of the study site is clay loam with a bulk density of 1.04 g cm^{-3}, while pH of the soil varies between 5.8 and 6.1 (medium). The soil has EC 0.02 to 0.6 dS/m, total nitrogen (TN) content ranging between 0.393 to 0.510% (medium), organic carbon (OC) content of 3.8 to 5.6% (medium), and available phosphorus content of P 32.2 to 82 ppm (high). The exchangeable Na, K, Ca, and Mg contents of the soil varies between 0.35-0.52, 0.60-0.98, 12.03-17.86, and 1.48-2.06 cmol (+) kg^{-1}, respectively, while its CEC varies between 30.0 and 41.6 cmol (+) kg^{-1} (high) (Hawassa Centre report, 2011, unpublished report) and the ratings were indicated according to Landon (1984).

2.2 Treatments and Experimental Design

The treatments consisted of planting patterns of one (1Fb), two (2Fb) and three (3Fb) rows of faba bean combined with one (1Ba), two (2Ba) and three (3Ba) rows of barley. The experiment was laid out as a randomized complete block design in a factorial arrangement and replicated three times per treatment. Sole crops of barley and faba bean were planted as control treatments, and the net harvestable area during the two growing years was 8.4 m^2. Plant and row spacing of sole faba bean were 0.1m and 0.4 m, respectively, while the row spacing of sole barley was 0.20 m.

Improved cultivars of six-rowed food barley (var HB-42) and faba bean (var Messay) were used for this study. The seeding rates of barley and faba bean were 85 and 150 kg ha^{-1}, respectively. Only Diammonium phosphate (DAP) fertilizer was applied to both sole and intercropped barley and faba bean at the rate of 100 kg ha^{-1} (18 N and 46 kg P_2O_5 ha^{-1}). The crops were planted simultaneously on 27 August 2011 and 25 August 2012 cropping years/seasons.

2.3. Data Collection

Data were collected on plant height, spike length, seeds spike^{-1}, 1000 seed weight, grain and total biomass yields, and harvest index of barley. Similarly, plant height, pods plant^{-1}, seeds pod^{-1}, grain and total biomass yields of faba bean were measured. Moisture contents of barley and faba bean seeds were adjusted to 12.5% and 10%, respectively, using a moisture tester. All data of growth and yield components of both crops were collected from 10 randomly selected plants from the central rows while data on total biomass yields were collected from a net plot size of 8.4 m^2 for intercrop barley and 9.6 m^2 for sole barley while harvest index was derived from grain and total biomass yields.

Partial and total land equivalent ratios of the companion crops (LER_P and LER_T), energy yield (EY), system productivity index (SPI), monetary advantage index (MAI), economic and land benefits of the system were calculated as follows.

$$LER_T = (Y_{BaFb}/Y_{Ba}) + (Y_{FbBa}/Y_{Fa}) \quad (1)$$

Where: Y_{BaFb} and Y_{FbBa} were intercrop grain yields of barley and faba bean, respectively; Y_{Ba} and Y_{Fa} were yields of sole barley and faba bean, respectively (Yilmaz et al., 2008). Land equivalent ratio (LER) values >1 indicates resources are used more efficiently by the intercrop than sole crop. Energy yield (EY) using caloric measurement was used to evaluate how much energy is produced from intercropping system relative to sole crops, and coefficient values of 17.8 and 16.8 KJ g^{-1} were used to convert crop yield into energy yield for barley and bean, respectively (Tsubo et al., 2004).

Energy Yield (EY):

$$EY_{Ba}=EY_{Ba}*YS_{Ba} \text{ (barley)} \quad (2)$$
$$EY_{Fb}=EY_{Fb}*YS_{Fb} \text{ (faba bean)} \quad (3)$$

Where: EY_{Ba} and EY_{Fb} are energy yields of barley and faba bean while YSba and YSfb are yields of sole barley and faba bean, respectively.

Total energy yield (EY_T) from intercropping was calculated as:

$$EY_T = EY_{Ba}*Yi_{Ba} + EY_{Fb}*Yi_{Fb-} \quad (4)$$

Where: Yi_{Ba} and Yi_{Fb} are yields of barley and faba bean in intercrop, respectively.

System productivity index (SPI), which standardizes the grain yield of the secondary crop faba bean in terms of the primary crop barley, was computed to assess the efficiency of intercropping (Getachew *et al*. 2006) using the model:

$$SPI = (S_{Ba}/S_{Fb}*Y_{Fbi}) + Y_{Bai} \quad (5)$$

Where: S_{Ba} and S_{Fb} are mean grain yields of barley and faba bean, respectively, in sole crop and Y_{Fbi} and Y_{Bai} are mean yields of faba bean and barley in intercrop, respectively.

Monetary advantage index (MAI) was calculated as described by Esmaeili *et al* (2011), where MAI= (value of combined intercrops) was calculated as (LER−1)/LER.

Economic benefit of barley-faba bean intercrop was assessed using grain yield and the mean market prices of faba bean and barley as 15 and 10 Ethiopian birr kg^{-1}, respectively. Variable costs of improved seeds (costs of barley and faba bean), and labor (planting and harvesting) were determined for each treatment. The costs of improved seeds of barley and faba bean were 8.53 and 14.50 ETB kg^{-1}, respectively (ESE, 2012), where 1USD was 17.60 ETB. Other costs were also estimated from the market price during the experimental period. Net income (NI) was determined as the difference of gross income and variable cost (Babatunde, 2003).

2.4. Data Analysis

Analysis of variance (ANOVA) was carried out using SAS version 9 (2000) on the variables measured (SAS, 2000). Combined analysis across years/seasons was conducted after test of homogeneity with pooled error variance (Gomez and Gomez, 1984). The comparison of means was made for the variables that exhibited significant differences due to the applied treatment in ANOVA using Tukey's HSD (honest significant difference) test of significance at 5% probability level.

3. Results

3.1 Growth and Yield Components

Plant height and spike length were not significantly affected by year and planting pattern despite an increase of plant height and spike length by 7.3 and 14.3%, respectively, compared with sole crop barely. On the other hand, the straw yield of barley was significantly affected by variation in year and intercrop (Table 1) and there was 80% increase in 2012 (Table 3). The straw from all intercrops was significantly low ranging from 20.6 to 71% compared to the straw of sole barley. On the other hand, the straw from 1Fb:2Ba and 1Fb:3Ba combinations was significantly 53.3 and 75% more, respectively, compared with single alternate row intercrop (Table 4).

Table 1. Growing year and intercrop combination mean squares from analysis of variance (ANOVA) of barley under faba bean-barely intercropping during 2011 and 2012 in the highland area of Bulle, southern Ethiopia.

	Mean squares of variables for barley			
Variables	Error (40)	Year /Y/ (1)	Intercrop combination/IC/ (9)	Y*IC (9)
Seeds $spike^{-1}$	15.51	156.49**	13.59	31.21
Grain yield, kg	0.26	12.51**	1.14**	0.13
Stover yield, kg	1.67	92.75**	16.55**	1.99
Total biomass yield, kg	2.27	37.13**	23.47**	1.60
Harvest index	0.005	0.97**	0.03**	0.02
Land equivalent ratio/barley/	0.13	0.03	0.27*	0.03

*Note: * and ** significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis indicate degrees of freedom.*

Table 2. Mean squares of growing year and intercrop combination combined ANOVA across years/2011 and 2012/for pods $plant^{-1}$, grain and total biomass yields, and harvest index in the highland area of Bulle, southern Ethiopia.

	Mean squares of variables for faba bean			
Variables	Error (40)	Year /Y/ (1)	Intercrop combination/IC/ (9)	Y*IC (9)
Plant height (cm)	77.83	2870.4**	103.09	73.7
Pods $plant^{-1}$	7.35	141.07**	13.19	12.25
1000 seed weight, (g)	1799.77	883112.5**	1450.1	1545.3
Grain yield (kg)	0.46	27.47**	4.76**	0.63
Total biomass yield (kg)	0.88	11.79**	18.06**	2.08**
Harvest index	0.02	0.63**	0.03	0.04

*Note: * and ** significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis indicate degrees of freedom.*

The results showed that variation in year significantly affected seeds spike^{-1} (Table 1) but no interaction effect of year by intercrop was observed. Seeds spike^{-1} was significantly 14.6% more in 2012 (Table 3). Neither year nor intercrop or their interaction significantly affected thousand seed weight of barley. Variation in year significantly affected total biomass of barley in which a 29.3% increase was observed in 2012 (Tables 1, 3). All intercrops produced significantly less biomass of barley relative to that of sole barley while combinations of 1Fb: 2Ba and 1Fb: 3Ba produced more biomass (30.3 and 43% more, respectively) compared to single alternate row intercrop. Planting pattern and year significantly affected harvest index of barley (Tables 1). There was more dry matter partitioning (125%) in 2011 (Table 3). Single alternate and 3Fb:1Ba intercrops had significantly more dry matter partitioning (95.8 and 83.3%, respectively) compared to sole barley (Table 4).

The biomass of faba bean was significantly affected by variation in year (Table 2) and it was 21.3% more in 2011 compared to 2012. All planting patterns had significantly less biomass compared to sole crop faba bean in which the reduction varied between 25 and 71% (Table 5). Statistically, variation in year affected the dry matter partitioning of faba bean (Table 2) and in 2011 harvest index was 51.3% more while all intercrop combinations except 1Fb:3Ba were at par with sole bean harvest index.

Table 3. Effect of growing year on growth, yield and yield components and harvest index of barley under faba bean- barely intercropping.

	Season	
Parameter	2011	2012
Seeds spike^{-1} (number)	22 b	25a
Grain yield (kg ha^{-1})	2104.9a	1259.2b
Straw yield (kg ha^{-1})	2873.4b	5175.8a
TBMY (kg ha^{-1})	4978.3b	6435.0a
Harvest index	0.45a	0.20b

Note: TBMY = total biomass yield; Means followed by the same letter(s) in a row indicate non-significant difference at 5% probability level.

3.2 Yield of Component Crops

Variation in year and intercrops significantly affected grain yield of barley (Table 1). The yield in 2011 was 67.2% more compared with 2012 (Table 3) while single alternate row intercrop significantly increased grain yield by 6% but it was at par with the yield of sole barley and 1Fb:2Ba and 1Fb:3Ba (Table 4). On the other hand, lower grain yield of barley that varied between 21.8 and 52.6% was observed from two and three rows of faba bean combined with the different rows of barley.

Table 4. Effect of planting pattern on grain, straw and total biomass yields and harvest index of barley in barley-faba bean intercrop from combined ANOVA over years/2011 and 2012/Bulle, southern Ethiopia.

	Barley yield (kg ha^{-1})			
Year/season	Grain	Straw	Total biomass	Harvest index
Sole barley	2052.4ab	7206.6a	9259.0a	0.24 c
Planting pattern				
1Fb: 1Ba	2175.9a	3271.5de	5447.4c	0.47a
1Fb: 2Ba	2083.3ab	5015.3bc	7098.6b	0.30bc
1Fb: 3Ba	2067.9ab	5725.1b	7793.0b	0.28bc
2Fb: 1Ba	1589.5bc	3657.3d	5246.8c	0.34b
2Fb: 2Ba	1604.9bc	3950.5cd	5555.4c	0.29bc
2Fb: 3Ba	1574.0bc	3518.5d	5092.5c	0.34b
3Fb: 1Ba	1466.0cd	2083.3e	3549.3e	0.44a
3Fb: 2Ba	972.2d	2731.4de	3703.6e	0.28bc
3Fb: 3Ba	1234.5cd	3086.4de	4320.9cde	0.31bc

Note: The same alphabets in a column show no significant variation at 5% probability level. 1, 2 and 3 are row ratio/ planting patterns/ of either faba bean or barley in faba bean/ barley intercrop.

Variation in grain yield of faba bean was observed between years and among treatments and interaction effect (Table 2). There was 83.4% more grain in 2011 than in 2012 (Table 5). The finding showed that intercrops of 2Fb:1Ba, 3Fb:1Ba, 3Fb:2Ba and 3Fb:3Ba produced yields not statistically different from the yield of sole faba bean. Faba bean yielded 52.2, 54.7 and 79.3% less from combinations of 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba relative to the yield of sole faba bean (Table 5).

Table 5 Faba bean grain and total biomass yields and harvest index (HI) of faba bean in barley-faba bean intercropping.

Year/ Season	Faba bean yield (kg/ha)		Harvest Index
	Grain	TBM*	
2011	3485. 1a	5750.6a	0.59a
2012	1899.8b	4742.2b	0.39b
Planting pattern			
Sole faba bean	4027.9a	8594.0a	0.47ab
1Fb:1Ba	1924.6c	3968.4de	0.52ab
1Fb:2Ba	1825.4c	3095.3ef	0.58a
1Fb:3Ba	833.3d	2480.2f	0.33b
2FB:1Ba	3154.8ab	6051.6bc	0.53ab
2Fb:2Ba	2638.9bc	4861.1cd	0.54a
2Fb:3BA	2480.2bc	4861.1cd	0.50ab
3Fb:1Ba	3254.0ab	6150.8b	0.49ab
3Fb:2Ba	3670.6a	6448.4b	0.46ab
3Fb:3Ba	3115.1ab	5952.4bc	0.51ab

Note: TBM = Total biomass; The same alphabets in a column show no significant variation at 5% probability level. Fb-faba bean; Ba-barley*

3.3 Productivity and Profitability of Intercropping

Productivity of barley in 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba planting patterns showed partial LER >1 with the respective productivity of 12, 12 and 15% higher than sole crop barley. The contribution of barley to the total productivity from 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba planting patterns were 71.8, 74.7 and 86.5%, respectively (Table 6). In all these treatments, the total productivity of barley-faba bean intercrop (LER_T) was greater than one which varied between 32 and 56%. System productivity index (SPI) of barley-faba bean intercrop did not exhibit statistically significant differences due to treatments but better SPI was achieved from planting patterns of 1Ba:1Fb, 1Fb:2Ba and 2Fb:1Ba. At a constant row of faba bean, there was a decrease in SPI as the number of rows of barley increased from one to three.

The energy yield was significantly influenced by the treatments and growing years. Single alternate and 1Fb:2Ba intercrops produced significantly 153.9 and 141.8% more energy, respectively, compared to the energy from sole-cropped barley (Table 6). On the other hand, the energy yield from one and two rows of faba bean intercropped with one, two and three rows of barley was at par with the energy from single alternate row. Combinations of one row of faba bean with one and two rows of barley had relatively higher monetary advantage compared to the other row arrangements.

Planting patterns of 1Fb:1Ba, 1Fb:2Ba and 2Fb:1Ba had 35.9, 33.3 and 34.2% saved more land, respectively, compared with other row arrangements. The net income from barley-faba bean intercrop was significantly higher than the income of sole crop barley. Except 1Fb:2Ba and 1Fb:3Ba planting patterns, other intercrops showed no significant variation in net benefit (Table 6). On the other hand, the net income in 2011 was 86.7% more than the income in 2012. Overall, the benefit from barley faba bean intercrop varied between 71.4 and 248.6% more over the income from sole crop barley.

Table 6. Effect of cropping system on LER, SPI, EY, MAI and land benefit (LB) of barley in barley faba bean intercropping.

Cropping system	Partial LER		Total LER	SPI	EY	MAI	LB (%)	Net income (Etb/ha)
	LER_{Fb}	LER_{Ba}						
Sole crop		-	-		39.5b		-	17979c
Planting pattern								
1Fb:1Ba	0.44cd	1.12a	1.56	3012.9	102.9a	9056	35.9	48083ab
1Fb:2Ba	0.38de	1.12a	1.50	2903.0	95.5a	7860	33.3	45908b
1Fb:3Ba	0.18e	1.15a	1.33	2430.8	89.6ab	4677	24.8	30813c
2Fb:1Ba	0.67abc	0.85ab	1.52	2979.6	72.8ab	6940	34.2	61157ab
2Fb:2Ba	0.57a-d	0.82ab	1.39	2793.8	75.9ab	5404	28.1	53087ab
2Fb:3Ba	0.51b-d	0.85ab	1.36	2653.2	72.2ab	4682	26.5	50688ab
3Fb:1Ba	0.70ab	0.71ab	1.41	2787.0	70.7ab	3689	29.1	61471ab
3Fb:2Ba	0.79a	0.54b	1.33	2661.9	43.0b	3006	24.8	62672a
3Fb:3Ba	0.66a-c	0.66ab	1.32	2647.7	56.3ab	3832	24.2	56527ab

Note: LER_{Fb}-partial land equivalent ratio of faba bean; LER_{Ba}- partial land equivalent ratio of barley; SPI-system productivity index; EY-energy yield; MAI-monetary advantage index; LB--Land benefit from intercrop; Etb-Ethiopian birr/currency. The same alphabets in a column shows no significant variation at 5% probability level.

4. Discussion

The dominancy of barley (RY>1) in 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba intercrops resulted in higher mean grain yield of the crop. However, grain yield of faba bean was reduced due to competition from the intercropped barley and lower bean population. The above intercrops might have more canopy cover due to higher tillering capacity that might have intercepted more light in which barley became more competitive resulting in higher plant height, spike length and more seeds spike^{-1}, leading to better resource use. In addition, more dry matter partitioning from 1Fb:1Ba intercrop might show more resource flow to the grain and thus better crop performance. Various studies have shown that cereals are more competitive in intercropping (Sadeghpour *et al.*, 2014; 2013; Takim, 2012; Yilmaz *et al*, 2008). Similarly, Esmaeili *et al* (2011) reported more grain yield of barley from more cropping ratio of barley in barley-annual medic (*Medicago scutellata* CV Robinson) intercrop.

Reduction in barley grain yield from intercrops of two and three rows of faba bean combined with one, two and three rows of barley varied between 22 and 53%, which was attributable to low plant density of barley but more population of faba bean. Consistent with the results of this study, Getachew *et al.* (2006) reported lower grain yield of barley in a barley-faba bean mixed intercropping due to more plant density of faba bean. The work of Mariotti *et al.* (2006) indicates that the yields of cereals and vetch was reduced by about 40 and 20%, respectively, compared to sole crop yields.

Increased population of faba bean caused a competitive effect towards barley that reduced the relative yields of both crops (RY<1), which could be competition within and between crops and larger seed size of faba bean (Benincasa *et al.*, 2012). Reduction in grain yield of faba bean from various planting patterns of one row of faba bean with one, two and three rows of barley that varied between 52 and 79% was because barley was more competitive and aggressive in most planting patterns, which is also supported by the finding of Esmaeili *et al.* (2011).

Barley in 1Fb:1Ba intercrop might have also benefitted from faba bean due to high N uptake from the transfer of nitrogen from the associated faba bean crop, which is supported by the work of Fujita *et al.* (1990) who reported the transfer of N from the legume crop to the associated maize crop. Increase in barley grain yield from 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba combinations might also be due to the contribution of N and P from faba bean to the growth and yield of barley. In addition, more growth and grain yield of intercropped barley with faba bean might be less use of water by faba bean than barley as indicated by the finding of Papastylianou *et al.* (1981). The report of Eskandari *et al.* (2009) shows supply of N from legumes in grass –forage legume intercrop thus more forage yield than sole crop grass grown alone. Their report shows more protein yield of barley (64, 27, and 55%) in faba bean-barley, lupin-barley and pea-barley intercrops compared to sole crop barley, respectively.

Intercropping of 1FB:1Ba, 1Fb:2Ba and 1Fb:3Ba was more efficient in using resources than sole crop barley (RYT>1). Single alternate row intercrop had a 12 % increase in grain yield, higher yield advantage, system productivity index, and land benefit. Partial land equivalent ratios indicated more contribution of barley to the total productivity of the system and was higher (72 to 87%) in 1Fb:1Ba, 1Fb:2Ba and 1Fb:3Ba intercrops as compared to faba bean, and it showed intercropping of barley was more efficient (partial LER >0.5) in land use. But the current research result was in contrast to Benincasa *et al.* (2012) finding that resource use efficiency in wheat-faba bean intercrop compared with one species crop was lower.

Higher grain yield of barley in some planting patterns was reported by Dordas *et al.* (2012) and Workayehu and Wortmann (2011). It was also reported higher total land equivalent ratio (Workayehu and Wortmann, 2011), system productivity index (Dordas *et al.,* 2012), monetary advantage index (Dordas *et al.*, 2012), land benefit, energy yield (Workayehu and Wortmann 2011), and net income (Dordas *et al.,* 2012) relative to sole crop barley.

Energy yield obtained from intercrop (91%) compared with the energy obtained from sole crop barley would help the low input farmers in developing countries to supplement dietary balance with protein and carbohydrate nutrition.

The net income in 2011 (83% more) might be due to rainfall variation in which better amount and distribution of rainfall contributed to better growth and grain yield of the crops and less weed infestation, and this resulted in higher monetary benefit from intercropping and this agrees with the finding of Esmaeili *et al.* (2011) because of shade effect and more competition from both intercropped barley and faba bean crops. Increased growth and productivity in barley intercropping might be due to better N uptake obtained from faba bean which fixes atmospheric nitrogen (Kopke and Nemecek, 2010).

In mixed farming systems, faba bean offers the potential of enhancing the productivity and sustainability of intercropping, which is in agreement with the work of Reynolds *et al.* (1994) in that faba bean add N to the soil making additional soil N available. This achievement becomes an example of ecological intensification of cereal systems of the farmers who live in the highland areas at the same time intercropping exploiting the resources of the environment.

5. Conclusions

The finding showed that intercropping of one, two and three rows of barley combined with one row of faba bean produced higher partial and total LER of barley, energy yield, system productivity index, monetary

advantage, land benefit and net income. On the other hand, more grain yield and harvest index was obtained from single alternate row of barley and faba bean intercrop. The overall result suggests that intercropping of cereals (barley) with legume (faba bean) can be of a benefit to the low-input farming system in particular in the highland areas of the country due to better productivity, economic benefit and energy yield while the system could be an eco-friendly approach. Barley-faba bean intercrop would provide not only carbohydrate but also improved protein supply for balanced diet of farmers' family members. This finding showed that barley-faba bean intercrop is beneficial for farmers in the study area who cannot afford to buy inorganic chemical fertilizers.

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Efficacy of Pepper Tree (*Schinus molle*) Extracts to Suppress Growth of *Botrytis fabae* and Manage Chocolate Spot Severity on Faba Bean (*Vicia faba*) at Sinana, Bale Zone, Southeastern Ethiopia

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Abstract: Fungal phytopathogens cause considerable crop yield reduction if not managed well. Use of synthetic chemicals is a conventional method of managing plant pathogens. However, the negative environmental impact of using synthetic chemicals prompted the search for plant-based compounds, which are relatively safer to the environment than synthetic agro-chemicals. In this study, crude extracts of pepper tree (*Schinus molle*) were evaluated through *in vitro* and *in vivo* tests to suppress growth of *Botrytis fabae* and to manage chocolate spot of faba bean (*Vicia faba* L.), respectively. Growth inhibitory effects were evaluated by applying different concentrations of aqueous, methanol and ethanol leaf extracts of *S. molle* or extraction solvents (control) using agar diffusion method in the laboratory. The laboratory experiment was done in a completely randomized design with three replications. The efficacies of leaf extracts of *S. molle* were evaluated through *in vivo* test after spraying the extracts and scoring the disease incidence and severity on *V. faba* (variety Shallo) grown in the field right after the detection of disease symptoms at 61 day after planting (DAP) up to 89 DAP on a weekly basis. The design for the field experiment was randomized complete block design (RCBD) with three replications. Mancozeb 80WP was used as a check or control treatment. Results of the *in vitro* test showed that all extract types had antifungal properties and significantly reduced mycelial growth in a concentration-dependent manner. Growth inhibitory effects also varied with extraction solvent. Results of the *in vivo* experiment showed that disease severity was significantly reduced by all types of *S. molle* extracts and particularly that of methanolic extract was on a par with the synthetic fungicide, Mancozeb 80WP. Corresponding to reduced disease severity, extract application increased grain yield by ca. 38-95% when compared with the negative control. Therefore, it could be concluded that, *S. molle* extracts can be considered as one of the alternative means of suppression of the negative effects of this fungus. However, screening of the active principle(s) of *S. molle* leaf extracts is subject to further research for effective utilization of this plant in crop protection.

Keywords: Disease incidence/severity, *In vitro*, *In vivo*, Plant extract, *Schinus molle*, Shallo, *Vicia faba*

1. Introduction

Ethiopia is considered to be the secondary center of diversity for faba bean (*Vicia faba* L.) (Asfaw *et al.,* 1994; Yohannes, 2000; Torres *et al*., 2006). The country is also among the major faba bean producing nations in the world ranking second next to China (FAO, 2006; Torres *et al.,* 2006). Faba bean is a valuable source of cheap protein for the poor that cannot afford to buy animal protein. Moreover, the crop naturally improves soil fertility through biological nitrogen fixation (Samuel *et al.,* 2008).

In spite of the above-mentioned advantages, faba bean yield is substantially lower in Ethiopia than the world's average, mainly due to negative impacts of various diseases. Among faba bean diseases, chocolate spot, caused by the fungus *Botrytis fabae* Sard, is the most widespread and highly destructive one causing a yield loss ranging roughly from 34 to 61% on susceptible cultivars in Ethiopia (ICARDA, 2006). The disease is found to be severely affecting yield wherever it occurs provided that a susceptible host and conducive environmental factors prevail (ICARDA 2006; Samuel *et al*., 2008).

So far, several disease management options have been devised for the prevention and management of diseases among which the use of synthetic fungicides is one. Even though synthetic protection chemicals appear to be effective, resistance development by the pathogens against them is a prevailing problem (Maggie *et al.,* 2006). Additionally, synthetic chemicals are hazardous to human health and non-target beneficial organisms, and can result in environmental pollution because of poor biodegradability (Isman, 2006).

Plant products (botanicals) are sought as alternative to synthetic chemicals as they are cheap and environmentally friendly. Plant-based chemicals are rich sources of secondary metabolites that have antimicrobial properties (Srivastava *et al*., 1996). They are easily biodegradable and hence considered safe to the environment and human health compared to the synthetic ones (Isman, 2006; Koul, 2008; Nerio *et al.,* 2010).

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Plant pathogens including *B. fabae* have been managed by the use of plant extracts. For example, complete inhibition of mycelial growth of *B. fabae* was recently reported by Hoda et al. (2016). Roman (2010) also had demonstrated growth inhibitory effects of crude extracts of 7 plant species (*Croton macrostachyus, Solanum incanum, Datura stramonium, Solanum marginatum, Calpurnia aurea, Clematis simensis,* and *C. hirsute*) on *B. fabae* under laboratory and greenhouse conditions. However, compared to the rich plant resources of Ethiopia, researches done so far on natural products that can effectively suppress fungal plant diseases like chocolate spot is limited. The objective of this study was, therefore, to investigate the efficacy of crude extracts of pepper tree (*Schinus molle*, Anacardiaceae) against *B. fabae*, a causative agent of chocolate spot of faba bean (*V. faba*, Fabaceae) both under laboratory and field conditions.

2.Materials and Methods

2.1. Test Plant Material and Pathogen

Fresh mature leaves of pepper tree (*Schinus molle*) were collected from around Sinana Agricultural Research Center (SARC) located at 07° N and 40° 10' E in Bale Zone, south-eastern Ethiopia. *Botrytis fabae* isolate was obtained from infected leaves of faba bean grown at SARC. Small portions of the infected faba bean leaves were cut from the advancing margin and dipped into 0.5% sodium hypochlorite for 2-3 min (Haggag *et al.*, 2006). Thereafter, the leaves were washed three times using sterilized distilled water. Then, the specimens were placed on the Faba bean dextrose agar (FDA) prepared as indicated in section 2.3 below and incubated at the temperature of 20±2 °C for seven days and then sub-cultured until a pure isolate was obtained. The isolate was identified by comparing with previous pure isolates from SARC and further cross checked with the reference from Barnett and Hunter (1982) for confirmation.

2.2. Preparation of Plant Extracts

Fresh leaf samples of *Schinus molle* were washed, air-dried in the laboratory and powdered using sterilized blender. The powder was then extracted using distilled water, methanol and ethanol separately. Stock extract was prepared by soaking leaf powder in distilled water, 99.8% methanol or 99.8% ethanol in 1:4 (w:v) ratio of leaf powder to the respective solvents left in the laboratory for 24 h with intermittent stirring using a sterile glass rod to ensure uniform soaking (Simeon *et al.,* 2008; Egigu *et al.*, 2010). The extract was then filtered first by using 4-fold cheese cloth (Wokocha and Okereke, 2005) and then using sterilized Whatman No. 1 filter paper. After centrifuging (at 6000 rpm for 15 min) the filtrate was serially diluted in the respective solvents to get 5, 10, 20 and 40% concentrations (Shovan *et al.*, 2008; Yeni, 2011). The extracts were then stored in air-tight bottles at 4 °C in a refrigerator until use in bioassay (Naduagu *et al.,* 2008; Prince and Prabakaran, 2011).

2.3. Preparation of Growth Medium

Faba bean dextrose agar (FDA) was prepared and used as a growth medium (Hanounik and Maliha, 1986; Haggag *et al.*, 2006). For this, coarsely chopped fresh faba bean leaf (400 g) was mixed with 1 L of tap water in a 1.5 L conical flask and autoclaved at 121 °C for 20 minutes. The sterilized leaf-water mixture was then filtered and the filtrate was mixed with agar medium (18 g) and dextrose (20 g). Thereafter, the mixture was heated along with uniform mixing and the volume was made up to 1 litre with tap water. This mixture was again autoclaved at 121 °C for 20 minutes, cooled down to about 40 °C and poured into Petri dishes (with 9 cm diameter) to serve as growth medium.

2.4. *In Vitro* Growth Test

Prior to pouring the growth medium into Petri dishes, two perpendicular lines that divided the Petri dishes into four equal sections were drawn at their bottom (Amadioha and Obi, 1999). The point of intersection of the lines represented the centre of the Petri dishes. Thereafter, the growth medium was poured into the Petri dishes with immediate addition of 2 ml of the different concentrations of the extract (Joseph *et al.,* 2008; Shovan *et al.,* 2008). Petri dishes that received distilled water or organic solvents represented negative control. Subsequently, a 4 mm diameter disk of mycelial pure culture was cut out using sterile cork borer and placed into the hole cut out from the solidified medium-extract mixture just at the point of intersection of the two lines drawn at the bottom of the plate (Yeni, 2011). The medium was modified by adding 0.2% (v/v) lactic acid to prevent bacterial contamination. The culture was incubated at 20±2°C for about 7 days and mycelial radial growth measurement was done when the growth in the control plates reached maximum. Fungi-toxicity of test extracts was then calculated in percentage mycelial growth inhibition using the formula indicated in Sundar *et al.* (1995) and Ahmed *et al.* (2002). The experiment was arranged in a completely randomized design (CRD) with three replications.

$$\%\text{Growth inhibition} = \frac{DC - DT}{DC} X100 \quad (1)$$

Where: DC = the average growth diameter from control (distilled water) plates, DT = the average growth diameter of fungal mycelia from extract treated plates

2.5. *In Vivo* Experiment

A faba bean variety, Shallo, which is released by Sinana Agricultural Research Center (Oromia Agricultural Research Institute, OARI) in 1999/00 was used for the field experiment. The trial was arranged in a factorial randomized complete block design (RCBD) with three replications. Plots of 2 m length and 1.2 m width were

used to cultivate faba bean. The plots had three rows of 2 m long spaced by about 0.4 m from one another. Adjacent plots were spaced by 1 m within a block, whereas adjacent blocks were spaced by 2 m. Plants were subjected to natural infection and disease development (Ogbebor and Adekunle, 2008; El-Sayed *et al.,* 2011). Thereafter, extracts were applied to the treatment plots soon after disease infection was noticed. Plots that received extracts were sprayed with 100% concentration of crude extracts (i.e., 1:4 w/v of plant material to solvent ratio) of individual solvents to sufficient wetting of the leaves. The extract concentration was not diluted as in the case of *in vitro* experiment deliberately to compensate for the probable loss of active principles under field conditions.

A standard synthetic fungicide, Mancozeb 80WP, was applied at the recommended rate (2.5 kg ha^{-1}) and distilled water and organic solvents were sprayed on control plots. Extract and fungicide application was done right after the disease symptom was seen in either one of the plots on the 61 days after planting and subsequent applications were made in a seven days interval up to 89 DAP. Prior to extract application, potential phytotoxic effects of the extracts and solvents were also evaluated by applying onto sample leaves of the crop to inspect the development of leaf injury symptoms for a week time.

For collection of agronomic data including number of aborted flowers $plant^{-1}$, number of tillers m^{-2}, number of pods $plant^{-1}$, number of seeds pod^{-1}, hundred seed weight (HSW) in gm and grain yield (kg ha^{-1}), five plants from middle row were randomly taken and marked with colored threads for identification. Disease incidence was assessed by counting plants showing symptoms of the disease and expressed in percent (%) infection. Disease severity was assessed as the proportion of plant structure affected by the disease from the five randomly tagged plants in the middle row. Disease severity was recorded using 1-9 scale: where, 1= no disease symptoms or very small specks; 3= few small discrete lesions; 5= some coalesced lesions with some defoliation; 7= large coalesced sporulating lesions, 50% defoliation and some dead plant; and 9= extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (Bernier *et al.,* 1993). Disease severity data was then converted to Percent Severity Index (PSI) using the following formula developed by Wheeler (1969) and presented as:

$$PSI = \frac{Snr}{NprXMss} X100 \quad (2)$$

Where: PSI = Percent severity index, Snr=Sum of numerical ratings, Npr = Number of plants scored and Mss = the maximum scale of the disease

2.6. Data Analysis

Statistical data analyses were conducted with the statistical packages of the computer software SPSS for Windows 16.0 (SPSS; Chicago, IL, USA). Data were first checked for normality of distribution and logarithmically transformed as necessary. Analysis of variance (ANOVA) was used to analyze data and the treatments means were separated by the least significant difference (LSD) and differences between means were considered to be statistically significant at $p \leq 0.05$ (Gomez and Gomez, 1984). Regression analysis was done to reveal the efficacy of extract with increasing concentration.

3. Results

3.1. Efficacy of *S. molle* Extracts on *in Vitro* Growth of *Botrytis fabae*

Both organic (methanol and ethanol) and aqueous extracts of *S. molle* at all concentration levels, including 10, 20 and 40% (i.e. with the exception of ethanolic extract at 5% level), highly and significantly ($p \leq 0.01$) reduced mycelial growth of *B. fabae* when compared to the control (distilled water: data for the control is not presented as it is zero). Growth inhibitory effects of extracts increased with extract concentration though no significant difference was observed between 5 and 10% concentrations of aqueous extracts (Figure 1A, B and C). Regression analysis showed that each unit increase in methanol, ethanol and aqueous extracts concentrations increased their respective efficacies by 1.6, 1.3 and 1.4%, respectively, where the coefficients of determination (R^2) were explained by over 80%.

There was significant ($p \leq 0.05$) difference among the different extracts due to the solvents in inhibiting growth of *B. fabae* at each concentration level (Figure 2). At the two lower concentrations (i.e. 5 and 10%), aqueous extracts resulted in maximum growth inhibition followed by methanolic and ethanolic extracts. However, at the two upper concentration levels, methanolic extracts showed the highest efficacy, followed by aqueous and ethanolic extracts.

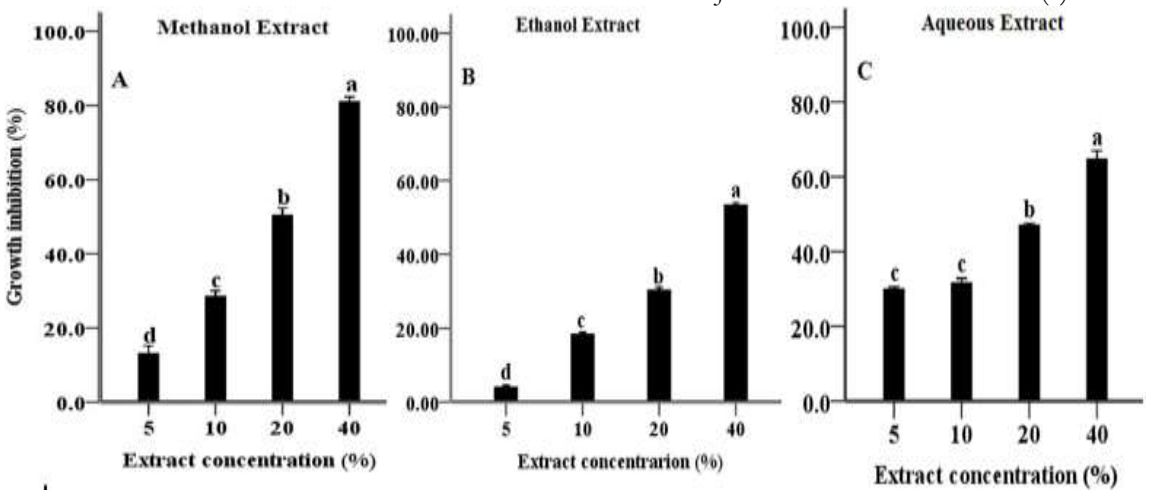

Figure 1. *In vitro* growth inhibitory effect of the three solvents' extracts of *S. molle* on *B. fabae*. Values are Mean ± S.E., n = 3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P<0.05$.

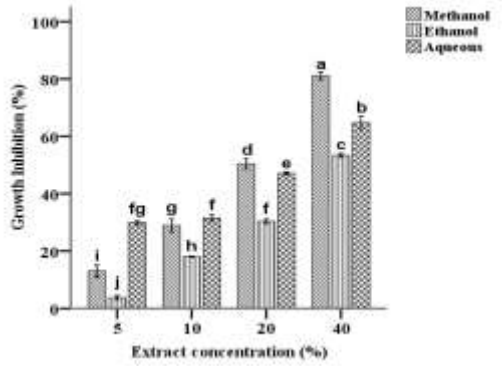

Figure 2. Comparison of the three solvents' extracts of *S. molle* on *B. fabae* mycelial *in vitro* growth. Values are Mean ± S.E., n =3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P < 0.05$.

3.2. Effects of *S. molle* Extracts on the Disease Incidence and Severity Caused by *B. fabae* Under Field Conditions

Treatment application with extracts was done on the 61st day after planting (DAP) immediately on the onset of infection and continued on a weekly basis up to 89 DAP at which final percent incidence and disease severity were assessed. On the 89 DAP, disease incidence was 100% in plots sprayed with distilled water (control). This value was significantly decreased in plots sprayed with synthetic fungicide and *S. molle* extracts. Disease incidence reduction was highest in methanol extract and synthetic fungicide sprayed plots, followed by ethanol and aqueous extracts sprayed plots (Figure 4).

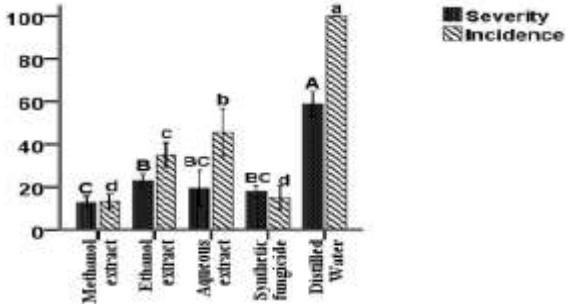

Figure- 3. Percent disease severity (black bar) and percent disease incidence (striped bar) caused by *B. fabae* on *V. faba* treated with *S. molle* extracts under field conditions. Values are Mean ± S.E., n=3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P < 0.05$. Capital letters compare disease severity between treatments, whereas small letters compare disease incidence between treatments.

Disease severity was significantly reduced by all types of *S. molle* extracts and synthetic fungicide compared to the control. The synthetic fungicide and all types of *S. molle* extracts showed a similar efficacy in disease severity reduction (Figure 3). Disease severity kept advancing throughout the experimental period in control plot, but halted at about constant level (<25%) in extract and synthetic fungicide-treated plots (Figure 4).

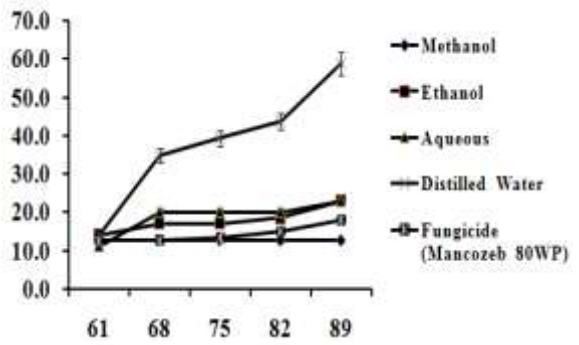

Figure 4. Disease severity pattern as assessed in a weekly basis of extract application.

3.3. Effect of Crude Extracts of *S. molle* on the Yield and Yield Components of Faba Bean

No significant difference was observed among the treatments and the negative control with regard to the number of pods per plant, number of seeds per pod and hundred seed weight (HSW). Except that of ethanol, all types of extracts of *S. molle* significantly reduced flower abortion when compared with that of the negative control. Moreover, the number of tillers counted from plots and pods per plant in plots treated with all types of extracts of *S. molle* were significantly higher than that of the control plot. Grain yield obtained from plots treated with extracts and synthetic fungicide significantly increased when compared with that of the negative control (Table 1). Percent infection of faba bean by *B. fabae* and chocolate spot disease severity were significantly ($p < 0.01$) lower in plots sprayed by *S. molle* leaf extracts than the negative control plots (Figure 3). Assessment of disease severity on a weekly basis revealed that symptoms were consistently advancing in negative control plot than in extract and fungicide sprayed plots (Figure 4).

Table 1. Effect of crude extracts of *S. molle* on yield and yield components of faba bean.

Treatments	No. of aborted flowers per plant	No. of tillers/m²	No. of pods/plant	No. of seeds/pod	HSW (g)	Grain yield (kg/ha)
Methanol extract	12.6±1.3c	22.0±0.2ab	20.2±1.6a	3.2±0.0a	56.5±1.3a	3739.3±43.3a
Ethanol extract	21.0±3.5ab	17.6±1.8b	22.0±1.2a	3.3±0.1a	54.9±0.5a	4236.9±15.2a
Aqueous extract	14.0±3.5bc	13.0±0.9c	16.1±0.2a	3.1±0.1a	58.2±0.6a	2995.7±12.1a
Fungicide (Mancozeb 80 WP)	11.8±3.6bc	17.3±0.8b	18.1±2.7a	3.3±0.1a	60.8±1.5a	3404.0±22.7a
Distilled water	23.9±3.2 a	8.3±0.9d	11.0±5.3b	3.2±0.1a	60.6±3.3a	2167.1±39.4b

Note: Means with different letters within a column are significantly different, whereas means with the same letter within a column are not significantly different from each other at P < 0.05. Values are Mean ± S.E., n=3; HSW= hundred seed weight.

4. Discussion

4.1. Growth Inhibition of *B. fabae* due to *S. molle* Extracts

Results of the *in vitro* experiment showed that all *S. molle* leaf extracts were considerably efficacious in inhibiting the growth of *B. fabae* in a concentration-dependent manner. Some organic compounds used as extraction solvents may have growth inhibitory effect on microbes. In this experiment, both methanol and ethanol that were used as solvents of extraction were used as negative control and compared with that of distilled water. The result showed that the two organic solvents had no negative effect on *B. fabae* growth, suggesting the observed growth inhibitory effects of the extracts were entirely attributed to the extract constituents.

Similar to the results of this study, Ibrahim and Al-Naser (2014) reported the growth inhibitory effect of fruit extracts of *S. molle* on *Botrytis cinerea* and revealed the presence of terpenoids such as α-pinene, β-pinene, α--phellandrene β-phellandrene and limonene in the extracts. Recently, Muhd *et al.* (2015) had also reported that *S. molle* leaf extracts possess secondary compounds such as terpenoids (monoterpenes and sesquiterpenes), which are reported to have antimicrobial properties. The antifungal effects of *S. molle* leaf essential oil had also been reported on other fungal species, such as *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Fusarium culmorum* and *Alternaria alternata* (Gundidza, 1993). Though all solvents' extracts were effective to suppress growth at all concentration levels, that of ethanol performed less compared to methanol and aqueous extracts. Especially, its performance at 5% concentration did not vary from the control. Moreover, aqueous extracts were superior to both methanol and ethanol at 5 and 10% while methanol extract was superior to aqueous and ethanol at 20 and 40% extract concentration. This shows that solvents of different polarity have different potential of extracting and yielding compounds of varying bioactivity (Egigu *et al.*, 2010).

4.2. *S. molle* Extracts Suppress Chocolate Spot Disease of Faba Bean

Under the field condition, infection of faba bean by *B. fabae* and the accompanying disease severity were greatly reduced in plots sprayed with *S. molle* extracts and the positive control synthetic fungicide, Mancozeb 80WP. Last assessment made on the 89 DAP showed that disease incidence and severity were at ca. 100 and 60%, respectively. Methanolic extract was on a par with synthetic fungicide in disease incidence reduction,

followed by ethanolic and aqueous extracts. Assessments made at a seven days' interval showed that disease severity advanced in control plots, but progress was effectively halted and kept below 25% by *S. molle* extracts. This shows that leaf extracts from *S. molle* could also suppress chocolate spot severity under field conditions as they suppress the growth of *B. fabae* under laboratory conditions. Similar to its efficacy under laboratory conditions, methanolic extracts appeared to perform better than ethanolic and aqueous extracts under field conditions, suggesting the stability of its extract constituents under field conditions. Some compounds are easily degradable under field conditions due to ultraviolet light and/or extreme temperatures (Schmutterer, 1990).

The rate of disease severity showed a sharp increase in control plots when one week after the onset of chocolate spot measured. Severity kept on increasing afterwards between 68 and 82 DAP, but the rate of increase was slower. This shows that naturally faba bean produces defense chemicals to fight back pathogens once attacked. Plants produce novel defense chemicals and/or increase the production of constitutive defense chemicals upon attack by pathogens and herbivores (Turlings *et al.*, 1990; Loughrin *et al.*, 1994; McCall *et al.*, 1994; Dudareva *et al.*, 2006). The fact that disease severity regained its momentum between 82 and 89 DAP in the control plots shows that production of defense chemicals may be age dependent in which plants invest less resources to defense chemicals than other purposes, reproduction, for example. Time series measurement is worth taking in the future to elucidate the pattern of defense compounds (secondary compounds) production specific to faba bean.

Corresponding to disease severity reduction, seed yield, number of tillers plot^{-1}, number of pods plant^{-1} and flower retention were positively affected by extract application. Interestingly, for example, estimate of grain yield obtained from extract sprayed plots increased in an order of 38 to 95% when compared to the negative control plots. This result is in agreement with the report of ICARDA (2006) which mentions that *Botrytis fabae* can cause a yield loss ranging roughly from 34 to 61% on susceptible faba bean cultivars in Ethiopia.

5. Conclusion

The results from the *in vitro* and *in vivo* experiments have demonstrated that *S. molle* extracts have negative impacts on *B. fabae*. Compared to the control, mycelial growth was significantly reduced by extracts under lab conditions. Results of field experiment showed significant reduction in disease severity that helped to increase grain yield in plots sprayed with extracts when compared with that of negative control. This indicates that *S. molle* extracts possess bioactive compounds against *B. fabae,* which needs safety evaluation of crude extracts not to damage non-target organisms and screening of active principle(s) for use by farmers.

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Microbiological Quality of Raw Cow Milk across the Milk Supply Chain in Eastern Ethiopia

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Abstract: The risk of milk contamination with spoilage and pathogenic microorganisms is high for milk produced in developing countries like Ethiopia especially in lowland region as their milk production practices is traditional type which lack appropriate hygienic control. To protect the raw cow milk from spoilage loss and consumers from milk born public health risk, the availability of documented information on the microbiological quality of raw milk across the milk supply chain is critically important as such information may be important for different organization to undertake relevant development intervention on hygienic practices essential for safe milk production and handling**.** This study was, therefore, conducted to determine the microbiological quality of informally marketed raw cow milk across the milk supply chain in eastern Ethiopia. A total of 360 pooled raw cow milk samples (each with a volume of 450 mL) were collected from udders and milk handling equipment of producers in rural areas of Babile district; from the equipment of collectors/transporters in Harar and Dire Dawa towns as well as from the equipment of vendors and consumer at Babile, Harar and Dire Dawa towns during February 2014 to January 2015. The milk samples were subjected to laboratory analyses to evaluate total aerobic mesophilic bacteria count (TAMBC), total coliform count (TCC), yeast count (YC) and mold count (MC) in the laboratory to determine the microbiological quality of the milk. Mean TAMBC, TCC, YC and MC for raw cow milk samples collected directly from the udders were 6.02±0.14, 4.23±0.12, 2.57±0.10 and 2.67±0.10 $\log_{10}$ cfu mL^{-1}, respectively. The values for the samples collected from the equipment of producers upon arrival at their selling points were 7.17±0.14, 5.86±0.12, 3.46±0.10 and 3.70±0.10 $\log_{10}$ cfu mL^{-1} for TAMBC, TCC, YC and MC, respectively. Mean TAMBC, TCC, YC and MC for samples collected from the equipment of collectors/transporters were 7.96±0.10, 6.49±0.07, 3.99±0.07 and 4.37±0.07 $\log_{10}$ cfu mL^{-1}, respectively. The microbial counts for samples collected from the equipment of vendors were 8.78±0.08, 7.32±0.07, 4.98±0.06 and 5.04±0.07 $\log_{10}$ cfu mL^{-1} for TAMBC, TCC, YC and MC, respectively. The values for samples collected from equipment of consumers were 8.82±0.08, 7.37±0.07, 5.10±0.06 and 5.11±0.07 $\log_{10}$ cfu mL^{-1} for TAMBC, TCC, YC and MC, respectively. It could be concluded that raw cow milk samples collected from all towns and milk source were severely contaminated with aerobic mesophilic and coliform bacteria, yeast and molds, with loads exceeding the respective acceptable limits.

Keywords: Dairy production system; Herd size; Microbiological quality; Milk supply chain, Raw cow milk

1. Introduction

Milk is universally recognized as a complete diet due to its composition of essential nutrients (Pandey and Voskuil, 2011; Melese and Tesfaye, 2015). Cow milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of different products in the world (Ali, 2010). It is also an economically important farm commodity and investment option for smallholder farmers in developing countries such as Ethiopia (Haile *et al.,* 2012).

However, milk serves as an excellent growth medium for a wide range of microorganisms because of its high water content, nearly neutral pH, and a variety of available essential nutrients (Ruegg, 2003). Although fresh milk, which is aseptically drawn from clean and healthy cow normally contains low (less than 1000 cfu mL^{-1} of milk) microbial count, it picks many microbes from the time it leaves the teat of the cow until it is used for consumption (Torkar and Teger, 2008). The load of microbes in milk is an indicator of the manner of milk handling from time of milking to consumption (Torkar and Teger, 2008). The microbial load and types found in the milk are influenced by factors such as health and hygiene of milking animal as well as its environment, cleanness of storage and transport

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equipment, milk holding temperature during storage and transport, cleanness of water used for hygienic practices, health and personal hygiene of milkers and milk handlers (Bytyqi *et al.,* 2011).

Mishandling and disregard of hygienic measures by milk handling personnel may enable undesirable microbes to come into contact with milk and in some cases to survive and multiply in sufficient numbers and make the milk unsafe for both direct consumption and further processing (Chatterjee *et al.,* 2006). A high microbial count in milk is an indication of poor hygiene, and reduces the nutritional quality of milk, causes unpleasant effect on the taste and also affect the physical quality of milk. Moreover, it reduces the market value of milk causing income losses to producers and traders. Furthermore, high microbial count in milk threatens the health of consumers due to toxic metabolites produced by different organisms growing in it (Karmen and Slavica, 2008).

The potential risk of milk contamination by spoilage and pathogenic microbes is high for milk produced in traditional system and marketed through the informal channels (Coorevits *et al*., 2008). This is because, in such systems it is a common practice to handle, transport and vend milk in inappropriate equipments and temperature as there is little or no quality control measures. Such practices are very common in developing countries such as Ethiopia and pose a threat to public health as chances of consuming unsafe milk is very high (Kurwijila *et al.,* 2006; Yilma and Faye, 2006).

In Ethiopia, the demand for cow milk is rapidly increasing because of population growth, increase in per capita income and urbanization. Therefore, provision of milk and milk products of good microbiological quality is desirable from consumers' health point of view (Zelalem, 2012). However, there is no well documented information available on the microbiological quality of raw cow milk at different milk source (cow's udder, producers, collectors/tranpsorters, vendors and consumers) across the milk supply chain in the study area. On the other hand, almost all milk produced from pastoral and agro-pastoral areas is supplied to urban centers through informal milk marketing channels. It is, therefore, important to document the microbiological quality of raw milk across the milk supply chain to ensure safety and suitability of raw milk for intended use. The information will be relevant to dairy value chain actors and service providers for introduction of pertinent interventions through the participation of the community. The objective of this study was, therefore, to determine the microbiological quality of informally marketed raw cow milk across the milk supply chain in eastern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in eastern Ethiopia, specifically in Babile district, and Harar & Dire Dawa towns. Babile district is the site of milk production whereas Harar and Dire Dawa towns are the sites of milk distribution (by vendors via informal marketing channel) and consumption.

Babile district is located at $9^{0}08'$ N latitude and $42^{0}21'$E longitude at the distance of about 557 km east of Addis Ababa. The altitude of the district ranges from 950 to 2000 meter above sea level. It has mean annual minimum and maximum temperatures of 18 and 28 ^{o}C, respectively. The mean annual rainfall and humidity of the area ranges from 700 to 900 mm and 33 to 38%, respectively (CSA, 2008). The two prevailing agricultural production systems in the district are pastoral and agro-pastoral production system (CSA 2008). Cattle are the most dominant in population size (56,355 heads) followed by goat (23,020), sheep (12,216) and camel (9,704) (BDLDHA, 2015). The district produced about 12,000 and 6,745 liters of raw cow milk during the wet and dry seasons, respectively (BDLDHA, 2015). Of the total cow milk produced daily in the district, about 50% was used for sale (Bedilu *et al.,* 2015). The total human population of the district is estimated at 115,229, out of which about 21.5% live in Babile town (CSA, 2013).

Harar town is located between 9.11^{0}-9.24^{0} N latitude and 42.03-42.16^{0} E longitude at the distance of about 526 km east of Addis Ababa and 31 km west of Babile district (Abdulwasi, 2009). The altitude of the town is 1850 above sea level and its mean annual rainfall and humidity is 596 mm and 60.3%, respectively (Dinkineh *et al.,* 2014). The town has mean annual maximum and minimum temperatures of 25 and 10^{o}C, respectively (Abdulwasi, 2009). The total human population of the town was estimated at 125,000 with a growth rate of 2.6% (CSA, 2013).

Dire Dawa town is located in the eastern part of Ethiopia at 9°36' N latitude and 41°52' E longitude (Belachew and Zeleke, 2015). The town is situated at the distance of 515 km east of Addis Ababa and 86 km west of Babile district. The altitude of the town is about 1200 meters above sea level. The mean annual rainfall and humidity are 594 mm and 41.82%, respectively. The town has mean annual maximum and minimum temperatures of 31.4 and 18.41 ^{0}C, respectively (Arabali and Amare, 2015). The total human population of the town was estimated at 288,000 with a growth rate of 2.5% (CSA, 2013).

According to Diro *et al.* (2009) and Shimelis *et al.* (2015), the study area has three seasons based on the distribution of rainfall. These are the long rainy season extending from June to September, the short rainy season from February to May, and the dry season that

extends from October to January (NMSA, 1996; Cheung *et al.,* 2008).

2.2. Study Design

A longitudinal study was conducted from February 2014 to January 2015 to determine the microbiological quality of informally marketed raw cow milk across the milk supply chain in the study area. Pooled raw cow milk samples were taken repeatedly from each milk source (from udder, milk handling equipment of producers, collectors/transporters, vendors and consumers) at every month during the study period. Thus, milk samples were collected throughout the year in order to assess the effect of season. The laboratory analysis was done in dairy technology laboratory, Haramaya University, Ethiopia.

2.3. Sampling Targets Across the Supply Chain

Babile district was stratified into pastoral and agro-pastoral production systems. Each production system was further stratified into peasant associations (PAs; the lowest administration unit in Ethiopia). Thus, a total of ten PAs (5 from pastoral and 5 from agro-pastoral systems) with high cow milk production potentail were purposively selected for the study. Each PA was then further stratified into small (1-3 cows) and medium (4-10 cows) herd size groups based on the number of milking cows they possessed (Dayanandan, 2011). Large herd size groups with more than 10 cows were not encountered in the study area. Milk producers households were selected from each herd size group randomly.

Unlike the milk producers in the Babile district, milk traders (collectors/transporters, and vendors), and consumers in Harar and Dire Dawa towns, were selected following a snowball sampling technique. Moreover, the same technique was used to select milk vendors and consumers in Babile town.

2.4. Milk Sampling Across the Supply Chain

A total of 360 raw cow milk (each with 450 mL) samples were collected from the udder, milk handling equipment of producers, collectors/transporters, vendors, and consumers following the sampling stratification described above. The numbers of pooled raw milk samples taken from the cow's udder directly and from the equipment of producers at their selling points (nearby the road side) were 36 each. Similarly, 72 pooled raw milk samples were collected from milk handling equipment of collectors/transporters in Harar (n = 36) and Dire Dawa (n = 36) towns. Moreover, 108 pooled raw milk samples were collected from milk handling equipment of vendors in Babile (n = 36), Harar (n = 36) and Dire Dawa (n = 36) towns. The total number of pooled raw milk samples taken from milk handling equipment of consumers in Babile (n = 36), Harar (n = 36), and Dire Dawa (n = 36) towns was 108. Raw milk samples were collected aseptically using sterile screw-capped sampling bottles. The bottles were then securely capped, labeled with markers and kept in an ice box filled with ice packs and brought to Haramaya University dairy technology laboratory within 3-4 hours of collection. The analysis was carried out within a period of 24 hours after collection. The samples were collected once every month over a period of 12 months (February 2014 to January 2015). In each month, three raw milk samples were collected from the equipment of milk producers, collectors/transporters, vendors, consumers, and from the udders of lactating cows.

2.5. Media Preparation for Microbial Quality Analysis

The total aerobic mesophilic bacterial counts (TAMBC) and total coliform count (TCC) were determined using sterile standard plate count agar and violet red bile agar (VRBA), respectively. Yeast count (YC) and mold count (MC) were also done using sterile Potato Dextrose Agar (PDA) whose pH was adjusted to 3.5 by adding 10 mL of sterile 10% lactic acid to a 1 L volume of the medium. All media except VRBA were sterilized by autoclaving at 121ºC for 15 minutes, while VRBA was sterilized by boiling for two minutes. After sterilization, all media were cooled to 45-47ºC in a water bath before use. The preparation of media was generally done according to the instructions given by the respective manufacturers. Peptone water that was autoclaved at 121ºC for 15 minutes and cooled to 30ºC was used for serial dilution of the milk samples to determine TAMBC, TCC, YC and MC. Each analysis was made in a duplicate.

2.6. Determination Total Aerobic Mesophilic Bacterial Count (TAMBC)

TAMBC was determined using standard plate count agar. One mL of raw milk sample was added into a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-11} and duplicate samples from the appropriate dilution (1 mL) was pour-plated using a 15-20 mL of cooled but still molten standard plate count agar (Oxoid, UK) solution and mixed thoroughly. The resulting plates were allowed to solidify and then incubated at 32 ºC for 48 hours (Murphy, 1996). The plates with colonies ranging from 30-300 colony forming units (cfu) mL^{-1} were selected for determination of TAMBC (Kiiyukia, 2003). TAMBC was determined as the total number of cfu per milliliter of milk sample which was calculated using the formula provided by IDF (2004).

$$N = \frac{\sum C}{[(1xn1) + (0.1xn2)]d} \quad (1)$$

Where: N is the number of cfu per milliliter of milk sample; ΣC is the sum of colonies on all plates counted; n_1 is the number of plates in the first dilution counted; n_2 is the number of plates in

the second dilution counted; and d is the dilution from which the first counts were obtained.

2.7. Determination Total Coliform Count (TCC)

TCC was determined using sterile violet red bile agar (VRBA). One mL of raw milk sample was added into a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-9} and duplicate samples (1mL) were pour-plated using a sterile 15-20 mL VRBA (Oxoid, UK). After thoroughly mixing, the resulting plates were allowed to solidify and then incubated at 32 ºC for 24 hours (Murphy, 1996). After incubation, typical dark red or purplish-red colonies appearing on the plates were counted as coliforms. For confirmatory test, five to ten typical colonies from each plate were transferred into tubes containing 2% Brilliant Green Lactose Bile Broth (Oxoid, UK) and incubated at 37 °C for 48 hours. Growth and gas production within incubation period was considered as sufficient evidence for the presence of coliforms (Richardson, 1985). Plates with 15 to 150 cfu mL^{-1} were used (Kiiyukia, 2003) for determining total coliform counts using the formula provided by IDF (2004).

2.8. Determination Yeast and Mold Counts

Yeast count (YC) and mold count (MC) were determined using sterile Potato Dextrose Agar (PDA). One mL of raw milk sample was added into a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-7} and duplicate samples of 0.1 mL were spread-plated on pre-dried surfaces of media containing PDA (Oxoid, UK). The plates were then incubated at 25°C for 5 days (Andrews, 1992; Roberts and Greenwood, 2003). Creamy to white/gray colonies were counted as yeasts whereas, filamentous (fuzzy) colonies of various colors (yellow, green, light brown) were counted as molds (Yousef and Carlstrom, 2003). When difficulties were faced to differentiate some colonies whether they are yeast or mold, a microscopic examination using the oil immersion objective was carried out to identify whether the cells in the colonies were unicellular or multi-cellular. Plates with 10 - 150 colonies were used for determining yeast and mold counts (IDF, 2004) using the formula provided by IDF (2004).

2.9. Data Analysis

Data on microbial counts, which were expresed as colony forming unit (cfu) per mL were transformed into logarithmic scales (log_{10}) and analyzed using the General Linear Model (GLM) procedure of SAS (SAS, 2008). Mean comparison was made using Tukey's adjustment. The following models were used for the analysis:

Model 1: Effect of production system, herd size group and season on microbial counts of raw cow milk collected directly from the udder of cow at Babile district

$$Y_{ijkl}= \mu+P_i+H_j+S_k+H_jxS_k+E_{ijkl} \quad (2)$$

Where: Y_{ijkl}=Total aerobic mesophilic bacterial count, total coliform count, yeast count and mold count; μ=Population mean (Overall mean); P_i= the effect of i^{th} production system (i=1, 2); H_j= the effect of j^{th} herd size group (j = 1, 2); S_k = the effect of k^{th} seasons (k = 1…3); H_jxS_k = interaction of herd size group with seasons; E_{ijkl} = random error

Since interaction between production system and season (P_i x S_k), production system and herd size group (P_ixH), and production system with herd size group and season (P_i x H_j x S) had no significant effect on microbial load of raw milk samples collected directly from the udder of milking cow, they were excluded from the model.

Model 2: Effect of production system, herd size group and season on microbial counts of raw cow milk collected from milk handling equipment of producers at Babile district

$$Y_{ijkl}= \mu+P_i+H_j+S_k + P_ixS_k+H_jxS_k+ E_{ijkl} \quad (3)$$

Where: Y_{ijkl} = Total aerobic mesophilic bacterial count, total coliform count, yeast count and mold count; μ = Population mean (Overall mean); P_i = the effect of i^{th} production system (i=1, 2); H_j= the effect of j^{th} herd size group (j = 1, 2); S_k = the effect of k^{th} seasons (k=1…3); H_jxS_k = interaction between herd size group and seasons; P_ixS_k= interaction between production system and seasons; E_{ijkl} = random error

Since the interaction between production system and herd size group (P_ixH_j) as well as interaction among production system, herd size group and season (P_i x H_j x S_k) had no significant effect on microbial load of raw milk samples collected from handling equipment of producers, they were excluded from the model.

Model 3: Effect of sources of milk and seasons on microbial count of raw cow milk samples collected in the study area

$$Y_{ijk}= \mu+M_i+S_j+M_ixS_j+ E_{ijk} \quad (4)$$

Where: Y_{ijk} =Total aerobic mesophilic bacterial count, total coliform count, yeast count and mold count; μ=Population mean (Overall mean); M_i=the effect of i^{th} milk source (I = 1…5); S_j = the effect of j^{th} season (j = 1…3); M_ixS_j = interaction between milk source and season; E_{ijk} = Random error

Model 4: Effect of locations and seasons on microbial count of raw cow milk collected from the equipment of

traders (collectors/transporters, and vendors) and consumers in the study areas

$$Y_{ijk} = \mu + L_i + S_j + L_i x S_j + E_{ijk} \quad (5)$$

Where: Y_{ijk} *= Total aerobic mesophilic bacterial count, total coliform count, yeast count and mold count;* μ *= Population mean (Overall mean);* L_i *= the effect of* i^{th} *location (I = 1…3);* S_j *= the effect of* j^{th} *season (j = 1…3);* $L_i x S_j$ *= interaction between location with season;* E_{ijk} *= Random error.*

3. Results and Discussion

3.1. Microbial Count of Raw Cow Milk Samples Collected from the Udder

Mean total aerobic mesophilic bacterial count (TAMBC) and total coliform count (TCC) of raw milk samples were influenced ($P < 0.05$) by herd size group and season differently (Table 1). Thus, milk samples collected from medium size herd during the short rainy season had significantly higher ($P < 0.05$) mean TAMBC than samples collected from small size herd during the dry season. Moreover, among medium size herds, milk samples collected during the short and long rainy seasons had significantly higher TCC than samples collected during the dry season (Table 1). Such differences might be due to the variation in health and hygiene of milking cows between herd size groups as well as between rainy and dry seasons.

Raw cow milk samples collected from medium-sized herds had significantly ($P < 0.05$) higher mean TAMBC and TCC than milk samples collected from small-sized herds (Table 1). This might be due to higher accumulation of effluents in night enclosure areas for cows from medium-sized herds than for small-sized herds. The mean TAMBC, TCC, yeast count (YC) and mold count (MC) of raw cow milk were not influenced ($P > 0.05$) by production system (Table 1).

The mean value of TAMBC and TCC for raw milk samples collected during the short and long rainy seasons were significantly ($P < 0.01$) higher than that for the dry season (Table 1). This might be due to over contamination of teats and udders of milking cows during the rainy seasons compared to the dry season, which might result in milk contamination with bacteria during milking. Moreover, it might be due to higher prevalence of mastitis during the rainy seasons than the dry season (Fox *et al.,* 1995) as the level of udder and teat contamination with mud while lying in night enclosure area is high during the former than the latter season. Moreover, the warm temperature and high humidity during the rainy season favor the growth of organisms, which might result in increased prevalence of mastitis.

The overall mean TAMBC for raw milk samples collected from the udder of milking cows in Babile district was 6.02 ± 0.14 $\log_{10}$ cfu mL^{-1} (Table 1). This was higher than 4.57 ± 0.21 $\log_{10}$ cfu mL^{-1} reported for raw milk samples collected from the udder of milking cows in Hawassa city in Ethiopia (Haile *et al.,* 2012). However, it is lower than 7.18 ± 0.1 $\log_{10}$ cfu mL^{-1} reported for raw milk samples collected from the udder of milking cows in Borana pastoral community in Ethiopia (Tollossa *et al.,* 2012). This difference might be due to the variation in health/hygiene of the milking cows and their environments as well as health care management practices performed by milk producers.

The overall mean TCC of raw milk samples collected from the udder of milking cows in the district was 4.23 ± 0.12 $\log_{10}$ cfu mL^{-1} (Table 1). The finding is in agreement with 4.00 $\log_{10}$ cfu mL^{-1} reported for milk samples collected from the udder of milking cows in Debre Zeit area in Ethiopia (Alehegne, 2004). However, it was lower than 6.88 ± 0.04 $\log_{10}$ cfu mL^{-1} reported for raw milk samples collected directly from the udder of milking cows in Borana pastoral community, Ethiopia (Tollossa *et al.,* 2012). The difference could be attributed to variation in cleanliness of night enclosure area and hygiene of milking cows (e.g. level of soiling of teats, udders, flanks and tails of the milking cows while lying in night enclosure area).

For raw milk samples collected from the udder of milking cows, the overall mean MC was 2.67 ± 0.10 $\log_{10}$ cfu mL^{-1} (Table 1), which was relatively comparable to 3.03 ± 0.26 $\log_{10}$ cfu mL^{-1} reported for raw cow milk samples collected from the udder of milking cows in Hawassa city in Ethiopia (Haile *et al.,* 2012). The overall mean yeast count (YC) for raw milk samples collected from the udder of milking cows was 2.57 ± 0.10 $\log_{10}$ cfu mL^{-1} (Table 1), which was relatively comparable with 2.87 $\log_{10}$ cfu mL^{-1} reported for udder milk samples in the Republic of Benin (Souaibou *et al.,* 2012).

Table 1. Least square mean (± S.E.) microbial counts (log_{10} cfu mL^{-1}) and temperature of raw cow milk samples collected directly from the udder across the different production systems, herd size groups and seasons in Babile district.

	Microbial count				Milk temperature
Variables	TAMBC	TCC	YC	MC	Temperature (°C)
Production system (n=36)	ns	ns	Ns	ns	ns
Pastoral	5.95(0.14)	4.26(0.12)	2.57(0.11)	2.72(0.12)	33.42(0.28)
Agro-pastoral	6.09(0.14)	4.21(0.12)	2.55(0.11)	2.62(0.12)	33.00(0.28)
Herd size group (n=36)	**	*	Ns	ns	ns
Small	5.73(0.14)^{b}	3.99(0.13)b	2.43 (0.11)	2.57(0.21)	33.194(0.28)
Medium	6.31(0.14)^{a}	4.47(0.13)a	2.70 (0.11)	2.78(0.21)	33.22(0.28)
Season (n=36)	**	**	ns	ns	ns
Short rainy season	6.33(0.16)^{a}	4.37 (0.14)^{a}	2.75(0.14)	2.70(0.15)	33.58(0.35)
Long rainy season	6.12(0.16)^{a}	4.57 (0.14)^{a}	2.59(0.14)	2.78(0.15)	33.08(0.35)
Dry season	5.60(0.16)^{b}	3.76(0.14)^{b}	2.36(0.14)	2.54(0.15)	32.96(0.35)
Herd size group X Season	*	*	ns	ns	ns
Medium X Short rainy season	6.66(0.22)^{a}	4.73(0.22)^{a}	2.92(0.20)	2.66(0.21)	33.33(0.49)
Medium X Long rainy season	6.33(0.22)^{ab}	4.84(0.22)^{a}	2.70(0.20)	2.98(0.21)	33.42(0.49)
Medium X Dry season	5.93(0.22)^{ab}	3.85(0.22)^{b}	2.48(0.20)	2.68(0.21)	32.92(0.49)
Small X Short rainy season	6.00(0.22)^{ab}	4.01 (0.22)^{ab}	2.57(0.20)	2.74(0.21)	33.83(0.49)
Small X Long rainy season	5.79(0.22)^{ab}	4.30 (0.22)^{ab}	2.49(0.20)	2.59(0.21)	33.00(0.49)
Small X Dry season	5.39(0.22)^{b}	3.67(0.22)^{b}	2.23(0.20)	2.40(0.21)	32.75(0.49)
Overall mean	6.02(0.14)	4.23(0.12)	2.57(0.10)	2.67(0.10)	33.21(0.24)

*Note: TAMBC = Total aerobic mesophilic bacterial count; TCC = Total coliform count; YC = Yeast count; MC = Mold count; Significance: *=p < 0.05, **= p < 0.01, ns = not significant; S.E. = Standard error; Column mean values with different superscript letters (a, b, ab) are significantly different.*

According to Marshall (1992) and Heeschen (1997), the acceptable limit of TAMBC and TCC for raw milk is 5.30-5.60 and 2.18 log_{10} cfu mL^{-1}, respectively, which was lower than the present findings. Moreover, the mean values of YC and MC for udder milk samples exceeded the upper acceptable limit (2.1 and 1.7 log_{10} cfu mL^{-1} for YC and MC, respectively) (Torkar and Vengust, 2008). This might be due to poor herd/farm hygiene and health care management practices performed by smallholder milk producers.

3.2. Microbial Count of Raw Milk Samples Collected from the Equipments of Producers

The microbial count of raw milk samples collected from the equipment of producers in the Babile district was influenced by the interaction between the production system and season ($P < 0.05$), and herd size group and season ($P < 0.05$) (Table 2). In most cases, milk samples collected during the short rainy season had significantly higher mean TAMBC, TCC, YC and MC than milk samples collected during the dry season within a given production system and herd size group.

Raw milk samples collected from pastoral production system had significantly ($P < 0.05$) higher mean TAMBC, TCC, YC and MC than raw milk samples collected from agro-pastoral production system (Table 2). This might be due to higher milk temperature for the former than the latter production system. Moreover, the quality of water used for hygienic practices in agro-pastoral production system is better than the quality of water used in pastoral production system (Tadele *et al.,* 2016).

The overall mean TAMBC for raw milk samples collected from the equipment of producers upon arrival at their selling points (nearby road side) in Babile district was 7.17±0.14 log_{10} cfu mL^{-1} (Table 2). The finding is in agreement with that of Alehegne (2004), who reported 7.20±0.13 log_{10} cfu mL^{-1} for raw cow milk collected from the equipment of producers in Debre Zeit, Ethiopia. However, it was lower than 7.96±0.07 log_{10} cfu mL^{-1} as reported for Yabello district of Ethiopia by Gurmessa (2015). On the contrary, it is higher than 6.36 log_{10} cfu mL^{-1} as reported by Asrat (2010) for raw cow milk samples collected from the equipment of producers in Wolayta zone in southern Ethiopia. The difference could be attributed to differences in the levels of hygiene of milking equipment, animal, milker wash water and the environment. Moreover, it might be due to the differences in milk holding time and temperature during storage and transportation.

Table 2. Least square mean (± S.E.) microbial load of raw cow milk samples (log_{10} cfu mL^{-1}) collected from milk handling equipment of producers across the different production systems, herd size groups, and seasons in Babile district.

Variables	Microbial count				Milk temperature
	TAMBC	TCC	YC	MC	temperature °(C)
Production system (n=36)	*	**	*	*	*
Pastoral	7.38(0.14)^{a}	6.14(0.12)^{a}	3.66(0.10)^{a}	3.89(0.11)^{a}	27.39(0.20)^{a}
Agro-pastoral	6.95(0.14)^{b}	5.57(0.12)^{b}	3.25(0.10)^{b}	3.51(0.11)^{b}	26.75(0.20)^{b}
Herd size group (n=36)	**	**	ns	ns	ns
Small	6.86(0.14)^{b}	5.58(0.12)^{b}	3.52(0.10)^{a}	3.81(0.11)^{a}	26.92(0.20)a
Medium	7.48(0.14)^{a}	6.13(0.12)^{a}	3.38(0.10)^{a}	3.58(0.11)^{a}	27.19(0.20)a
Season (n=36)	**	***	***	***	**
Short rainy season	7.54(0.17)^{a}	6.09(0.14)^{a}	3.94(0.12)^{a}	4.17(0.13)^{a}	27.96(0.25)^{a}
Long rainy season	7.48(0.17)^{a}	6.31(0.14)^{a}	3.50(0.12)^{a}	3.74(0.13)^{a}	27.26(0.25)^{a}
Dry season	6.48(0.17)^{b}	5.18(0.14)^{b}	2.94(0.12)^{b}	3.18(0.13)^{b}	26.00(0.25)^{b}
Production system X Season	*	*	*	*	**
Pastoral X Short rainy season	7.73(0.23)^{a}	6.37(0.20)^{a}	4.25(0.17)^{a}	4.41(0.19)^{a}	28.42(0.35)^{a}
Pastoral X long rainy season	7.67(0.23)^{a}	6.53(0.20)^{a}	3.62(0.17)^{ab}	3.84(0.19)^{ab}	27.32(0.35)^{ab}
Pastoral X dry season	6.74(0.23)^{ab}	5.53(0.20)^{b}	3.10(0.17)^{bc}	3.40(0.19)^{bc}	26.42(0.35)^{bc}
Agro-pastoral X short rainy season	7.36(0.23)^{a}	5.80(0.20)^{ab}	3.62(0.17)^{ab}	3.85(0.19)^{ab}	27.50(0.35)^{ab}
Agro-pastoral X long rainy season	7.28(0.23)^{ab}	6.10(0.20)^{ab}	3.48(0.17)^{b}	3.71(0.19)^{b}	27.10(0.35)^{b}
Agro-pastoral X dry season	6.22(0.23)^{b}	4.82(0.20)^{c}	2.68(0.17)^{c}	2.96(0.19)^{c}	25.58(0.35)^{c}
Herd size group X Season	**	*	*	*	*
Medium X short rainy season	8.03(0.21)^{a}	6.32(0.20)^{a}	3.90(0.17)^{a}	4.15(0.19)^{a}	27.83(0.35)^{a}
Medium X long rainy season	7.69(0.21)^{ab}	6.54(0.20)^{a}	3.30(0.17)^{ab}	3.77(0.19)^{a}	27.68(0.35)^{a}
Medium X dry season	6.71(0.21)^{bc}	5.54(0.20)^{b}	2.95(0.17)^{b}	3.52(0.19)^{ab}	26.07(0.35)^{b}
Small X short rainy season	7.05(0.21)^{b}	5.85(0.20)^{ab}	3.96(0.17)^{a}	4.20(0.19)^{a}	28.08(0.35)^{a}
Small X long rainy season	7.26(0.21)^{b}	6.09(0.20)^{ab}	3.70(0.17)^{a}	3.70(0.19)^{a}	26.83(0.35)^{ab}
Small X dry season	6.26(0.21)^{c}	4.81(0.20)^{c}	2.92(0.17)^{b}	2.84(0.19)^{b}	25.83(0.35)^{b}
Overall mean	7.17(0.14)	5.86(0.12)	3.46(0.10)	3.70(0.10)	27.06(0.21)

*Note: TAMBC=Total aerobic mesophilic bacterial count; TCC = Total coliform count; YC = Yeast count; MC = Mold count; Significance: *p < 0.05, ** p < 0.01,*** p < 0.001, ns = not significant; S.E.= Standard error*

The overall mean TCC for raw milk samples collected from the equipment of producers was 5.86 ± 0.12 log_{10} cfu mL^{-1} (Table 2), which is relatively comparable with 6.19 ± 0.03 log_{10} cfu mL^{-1} as reported by Gurmessa (2015) for raw cow milk samples collected from the equipment of producers in Yabello district in Ethiopia. However, it is higher than 4.84 log_{10} cfu mL^{-1} as reported by Derese (2008) for raw cow milk samples collected from a milk shed in Bahir Dar in Ethiopia and 4.03±0.09 log_{10} cfu mL^{-1} as reported by Abebe *et al.* (2012) for equipment samples in Ezha district of the Gurage zone in Southern Ethiopia.

The overall mean YC and MC of the present study were 3.46±0.10 and 3.70±0.10 log_{10} cfu mL^{-1}, respectively (Table 2). The result is lower than 4.9±0.6 and 4.61±0.5 log_{10} cfu mL^{-1} for yeast and mold, respectively as reported by Teshome and Ketema (2014) for raw cow milk sample collected from the equipment of producers in Kersa district in Jimma Zone of southwestern Ethiopia. This might be due to differences in hygienic practices during production and postharvest handling as well as due to the variations in milk holding temperatures and time during storage and transportation.

The mean TAMBC and TCC for raw cow milk samples collected from the equipment of producers at Babile district were much higher than the upper acceptable limit given by Marshall (1992) and Heeschen (1997). Moreover, the mean YC and MC of raw milk samples collected from the equipment of producers exceeded the upper acceptable limit as reported earlier (Torkar and Vengust, 2008). This indicates that milk is produced and handled under unhygienic condition at producer's level. Moreover, delayed milk transportation and lack of cooling facilities during milk storage and transportation, which are the common practice in the area might also be another important factor contributing to high microbial loads in the milk. Omore *et al.* (2005) also provided similar suggestion.

3.3. Microbial Count of Raw Milk Samples Collected across the Milk Sources and Seasons

The mean TAMBC, TCC, YC and MC of raw milk samples were significantly ($P < 0.05$) influenced by milk source and season interaction (Table 3). In most cases, raw milk samples collected during the short and long rainy seasons had significantly higher mean TAMBC, TCC, YC and MC than samples collected during the dry season within a given milk source. The mean TAMBC, TCC, YC and MC for raw milk samples collected from the udder were significantly ($P < 0.001$) lower than samples collected from the equipment of producers, which were significantly ($P < 0.001$) lower than samples collected from the equipment of collectors/transporters (Table 3). The mean TAMBC, TCC, YC and MC for raw milk samples collected from the equipment of vendors and consumers were significantly ($P < 0.001$) higher than milk samples collected from udder as well as from the equipment of producers and collectors/transporters (Table 3). This might be due to further contamination of the milk during storage and transportation. The possible sources of contamination might be the use of poorly cleaned equipment, lack of proper protection of milk equipment from risk factors after cleaning, the use of untreated water for hygienic practices, lack of cooling system, poor personal hygiene of milk handlers etc. Moreover, the longer storage time (about 4 hours) elapsed during milk vending by vendors might also contribute to such differences. However, there is there is no difference ($P>0.05$) in mean TAMBC, TCC, YC and MC between milk samples collected from the equipment of vendors and consumers (Table 3).

The mean TAMBC for raw cow milk samples collected from the equipment of collectors/ transporters upon arrival at selling points i.e., 7.96±0.10 log_{10} cfu mL^{-1} (Table 3) is in agreement with 8.0l log_{10} cfu mL^{-1} for raw cow milk samples collected from most dairy cooperatives operating in Ethiopia (Francesconi, 2006), but slightly lower than 8.26±0.31 log_{10} cfu mL^{-1} as reported by Mustefa (2012) for raw cow milk samples collected from the equipment of collectors in Sululta and Welmera districts, Ethiopia. The mean TAMBC for raw milk samples collected from the equipment of vendors in the study areas (8.78±0.08 log_{10} cfu mL^{-1}) (Table 3) was lower than 10.28±0.28 log_{10} cfu mL^{-1} as reported by Haile *et al.* (2012) for raw cow milk samples marketed in Hawassa city, Ethiopia but higher than 7.35±0.18 log_{10} cfu mL^{-1} as reported by Shunda *et al.* (2013) for raw cow milk collected from the equipment of vendors in Mekelle town, Ethiopia. The differences might be attributed to variation in the level of hygiene of cleaning water, milk handling equipment and milk marketing places used by milk vendors.

The mean value of TCC (6.49±0.07 log 10 cfu mL^{-1}) of raw cow milk samples collected from the equipment of collectors/ transporters upon arrival at their selling points (Table 3) is in agreement with 6.46±0.03 log_{10} cfu mL^{-1} reported for raw cow milk samples collected from market in Yabello district, Ethiopia (Gurmessa, 2015), but much higher than 4.11±0.01 log_{10} cfu mL^{-1} reported for raw cow milk samples collected from market at Khartoum in Sudan (Ali and Abdelgadir, 2011). The mean TCC for samples collected from the equipment of vendors was 7.32±0.07 log_{10} cfu mL^{-1} (Table 3). The finding was comparable with Alehegne (2004) who reported mean TCC of 7.32 log_{10} cfu mL^{-1} for raw cow milk samples collected from market upon arrival at processing plant in Addis Ababa, Ethiopia.

Table 3. Least square mean (± S.E.) microbial count (log_{10} cfu mL^{-1}) of raw cow milk samples collected across the milk sources and seasons in the study area.

Variables	Microbial count				Milk temperature
	TAMBC	TCC	YC	MC	temperature(°C)
Source of milk (n=360)	***	***	***	***	**
Udder	6.02(0.14)^{d}	4.23(0.12)^{d}	2.57(0.10)^{d}	2.67(0.10)^{d}	33.21(0.25)^{a}
Producers equipment	7.17(0.14)^{c}	5.86(0.12)^{c}	3.46(0.10)^{c}	3.70(0.10)^{c}	27.06(0.25)^{c}
Collectors equipment	7.96(0.10)^{b}	6.49(0.07)^{b}	3.99(0.07)^{b}	4.37(0.07)^{b}	28.12(0.18)^{b}
Vendors equipment	8.78(0.08)^{a}	7.32(0.07)^{a}	4.98(0.06)^{a}	5.04(0.07)^{a}	28.49(0.18)^{b}
Consumers equipment	8.82(0.08)^{a}	7.37(0.07)^{a}	5.10(0.06)^{a}	5.11(0.07)^{a}	28.37(0.18)^{b}
Season (n=360)	***	***	***	***	**
Short rainy season	8.18(0.09)^{a}	6.47(0.07)^{a}	4.31(0.07)^{a}	4.40(0.07)^{a}	29.85(0.17)^{a}
Long rainy season	8.03(0.09)^{a}	6.64(0.07)^{a}	4.12(0.07)^{a}	4.31(0.07)^{a}	29.09(0.17)^{b}
Dry season	7.04(0.09)^{b}	5.66(0.07)^{b}	3.63(0.07)^{b}	3.82(0.07)^{b}	28.21(0.17)^{c}
Milk Sources X season	*	*	*	*	*
Udder X short rainy season	6.33(0.17)^{d}	4.37(0.16)^{de}	2.75(0.15)^{de}	2.70(0.15)^{de}	33.58(0.41)^{a}
Udder X long rainy season	6.13(0.17)^{de}	4.57(0.16)^{d}	2.59(0.15)^{de}	2.78(0.15)^{de}	33.08(0.41)^{a}
Udder X dry season	5.59(0.17)^{e}	3.76(0.16)^{e}	2.36(0.15)^{e}	2.54(0.15)^{e}	32.96(0.41)^{a}
Producers equipment X short rainy season	7.54(0.24)^{c}	6.09(0.15)^{c}	3.94(0.14)^{bc}	4.17(0.14)^{bc}	27.96(0.41)^{cd}
Producers equipment X long rainy season	7.48(0.24)^{c}	6.31(0.15)^{bc}	3.50(0.14)^{c}	3.74(0.14)^{c}	27.26(0.41)^{d}
Producers equipment X dry season	6.48(0.17)^{d}	5.18(0.15)^{d}	2.94(0.14)^{d}	3.18(0.14)^{d}	26.00(0.41)^{e}
Collectors equipment X short rainy season	8.39(0.17)^{b}	6.68(0.14)^{bc}	4.20(0.13)^{b}	4.54(0.13)^{b}	28.90(0.35)^{bc}
Collectors equipment X long rainy season	8.32(0.17)^{b}	6.81(0.14)^{b}	4.10(0.13)^{bc}	4.51(0.13)^{b}	28.26(0.35)^{c}
Collectors equipment X dry season	7.18(0.17)^{c}	6.00(0.14)^{c}	3.68(0.13)^{c}	4.06(0.13)^{bc}	27.21(0.35)^{d}
Vendors equipment X short rainy season	9.31(0.14)^{a}	7.56(0.11)^{a}	5.25(0.11)^{a}	5.27(0.11)^{a}	29.04(0.28)^{b}
Vendors equipment X long rainy season	9.10(0.14)^{a}	7.76(0.11)^{a}	5.13(0.11)^{a}	5.26(0.11)^{a}	28.68(0.28)^{bc}
Vendors equipment X dry season	7.93(0.14)^{bc}	6.67(0.11)^{bc}	4.55(0.11)^{b}	4.63(0.11)^{b}	27.75(0.28)^{cd}
Consumers equipment X short rainy season	9.33(0.14)^{a}	7.61(0.11)^{a}	5.41(0.11)^{a}	5.33(0.11)^{a}	29.44(0.28)^{b}
Consumers equipment X long rainy season	9.19(0.14)^{a}	7.80(0.11)^{a}	5.26(0.11)^{a}	5.30(0.11)^{a}	28.56(0.28)^{bc}
Consumers equipment X dry season	7.95(0.14)^{bc}	6.71(0.11)^{bc}	4.62(0.11)^{b}	4.70(0.11)^{b}	27.13(0.28)^{d}

*Note: TAMBC = Total aerobic mesophilic bacterial count; TCC = Total coliform count; YC = Yeast count; MC = Mold count; Significance: *p < 0.05, ** p < 0.01, *** p < 0.001, ns = not significant; S.E.= Standard error*

The mean values of TAMBC and TCC for raw milk samples collected from the equipment of traders (collectors/transporter and vendors) and consumers in the study area far exceeded the upper acceptable limit as reported earlier (Marshall, 1992; Heeschen, 1997). Moreover, they were higher than the upper acceptable limit (6.30 and 4.70 $\log_{10}$ cfu mL^{-1} for TAMBC and TCC, respectively) given by East African Community Standard (EACS, 2007). This implies that the sanitary conditions in which milk is being handled in the study area is substandard and leads to high degree of microbial contamination and multiplication. Moreover, unavailability of cooling facilities during milk storage and transportation in the study area could also be another important factor contributing to high TAMBC and TCC in the milk (Omore *et al.,* 2005).

A high TAMBC in the milk may reduce shelf life stability and the nutritional quality of milk (Yousef and Carlstrom, 2003), and also threatens the health of consumers due to toxic metabolites produced by of different organisms growing in it (Karmen and Slavica, 2008). Moreover, the high number of coliform bacteria in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more enteric pathogens being present. Apart from safety and public health concerns, occurrence of coliforms in raw milk in high numbers could result in spoilage that makes the milk unsafe for processing (Gamal *et al.,* 2015).

The mean of YC for raw milk samples collected from the equipment of collectors/transporters upon arrival at selling points were 3.99±0.07 $\log_{10}$ cfu mL^{-1} (Table 3), which is relatively similar to 4.15 $\log_{10}$ cfu mL^{-1} reported for raw cow milk marketed in Cairo town, Egypt (Gamal *et al*., 2015). The mean values of YC of raw milk samples collected from the equipment of vendors in the study area was 4.98±0.06 $\log_{10}$ cfu mL^{-1} (Table 3). The finding is far lower than 7.13±0.33 $\log_{10}$ cfu mL^{-1} reported for raw cow milk samples collected from distribution equipment upon arrival at selling points in Hawassa city, Ethiopia (Haile *et al.,* 2012). However, it is higher than 4.11±0.02 $\log_{10}$ cfu mL^{-1} as reported by Gemechu *et al.* (2014) for raw cow milk samples collected from small milk vending shops in Shashemene town, Ethiopia. Such variations might be due to the differences in hygienic handling practices performed by milk vendors and previous actors (like collectors/transporters and producers). Variations in milk holding temperature and time during storage and transportation at each milk source might also contribute to such differences.

The mean MC for raw cow milk samples collected from the equipment of collectors/transporters upon arrival at their selling points was 4.37±0.07 $\log_{10}$ cfu mL^{-1} (Table 3), which was comparable with 4.46±0.04 $\log_{10}$ cfu mL^{-1} as reported by Gurmessa (2015) for raw cow milk samples collected from market in Yabello district in Ethiopia). The mean value of MC (5.04±0.07 $\log_{10}$ cfu mL^{-1}) for raw milk samples collected from the equipment of vendors in the study areas was lower than 5.63±0.24 $\log_{10}$ cfu mL^{-1} reported for raw milk samples collected from El-Beida city in Egypt (El-Diasty and El-Kaseh, 2009). This variation might be due to differences in initial contamination during production, further contamination during post harvest handling as well as due to the differences in milk holding temperatures and time during storage and transportation

The mean YC and MC counts for samples collected from the equipment of traders and consumers in the study area were also much higher than the upper acceptable limit indicated above (Torkar and Vengust, 2008). The presence of high numbers of yeast and mold in milk indicates that the milk has been contaminated with soil, dusts, air and other contaminants due to poor hygienic practices during milk production and postharvest handling. In the study area, delivery of raw milk to the next actors in the study area is carried out at roadsides on the grounds, which are dusty and not protected from wind and road traffic (Tadele *et al.,* 2016). This might be a possible reason for the high yeast and mold counts observed in the present study. Moreover, the absence of milk cooling system at all critical points in the study area might contribute higher YC and MC than the upper acceptable limit indicted above.

High yeast and mold counts in foods including milk cause spoilage (Gamal *et al.,* 2015). Moreover, some molds, however, are public health concerns due to their ability to produce toxic substances (mycotoxins), which may not be easily destroyed during food processing or cooking (Wouters *et al.,* 2002). Therefore, training and guidance should be given to traders and consumers on general hygienic practices to be followed during milk postharvest handling to avoid/minimize the risk of milk contamination with yeasts and molds.

3.4. Microbial Count of Raw Milk Samples Collected from the Equipment of Traders and Consumers across Locations and Seasons

Mean TAMBC, TCC, YC and MC of raw milk samples were significantly ($P < 0.05$) influenced by location and season interaction (Table 4). In most cases, within a season, there is no difference ($P>0.05$) in mean TAMBC, TCC, YC and MC among milk samples collected from Babile, Harar and Dire Dawa towns. However, there is difference ($P<0.05$) in mean TAMBC, TCC, YC and MC among milk samples collected during the short rainy season, the long rainy season and the dry season within a location, except for MC in Harara and YC in Dire Dawa, in that, milk samples collected during short and long rainy seasons had significantly higher microbial load than that for dry season. This might be due to higher milk holding temperature and initial contamination during short and long rainy seasons than during dry season.

The milk samples collected in Dire Dawa and Babile towns had higher ($P<0.01$) mean TAMBC and TCC than milk samples collected in Harar town (Table 4). This might be mainly due to higher milk holding

temperature for Dire Dawa and Babile towns than for Harar town. Although milk holding temperature for Dire Dawa town is significantly higher (P<0.001) than that for Babile town, mean TAMBC and TCC were not significantly (P>0.05) different between milk samples collected in Dire Dawa and Babile towns (Table 4). This might be due to the higher level of contamination of milk with microorganisms for Babile town than for Dire Dawa town.

The mean YC and MC for milk samples collected from Babile town were higher (P<0.05) than for milk samples collected from Harar town (Table 4). This might be mainly due to the higher milk holding temperature of Babile town than Harar town. Moreover, the variation in the level of milk contamination with microorganisms during production, storage and transportation might contribute such differences.

Table 4. Least square mean (± S.E.) microbial count (log_{10} cfu mL^{-1}) of raw cow milk samples collected from the equipment of traders and consumers across location and season.

	Microbial count				Milk temperature
Variables	TAMBC	TCC	YC	MC	temperature(°C)
Location (n=288)	**	**	**	*	***
Babile	8.70(0.09)^{a}	7.26(0.09)^{a}	5.03(0.09)^{a}	5.03(0.08)^{a}	28.67(0.08)^{b}
Harar	8.35(0.11)^{b}	6.95(0.08)^{b}	4.52(0.08)^{b}	4.75(0.07)^{b}	26.40(0.08)^{c}
Dire Dawa	8.76(0.09)^{a}	7.23(0.08)^{a}	4.78(0.08)^{ab}	4.95(0.07)^{ab}	30.10(0.08)^{a}
Season(n=288)	***	***	***	***	***
Short rainy season	9.10(0.10)^{a}	7.37(0.08)^{a}	5.08(0.08)^{a}	5.13(0.07)^{a}	29.31(0.09)^{a}
Long rainy season	8.93(0.10)^{a}	7.54(0.08)^{a}	4.96(0.08)^{a}	5.09(0.07)^{a}	28.45(0.09)^{b}
Dry season	7.79(0.10)^{b}	6.54(0.08)^{b}	4.29(0.08)^{b}	4.51(0.07)^{b}	27.42(0.09)^{c}
Location X season	*	*	**	*	***
Babile X short rainy season	9.15(0.16)^{a}	7.49(0.16)^{a}	5.37(0.16)^{a}	5.32(0.15)^{a}	29.52(0.17)^{bc}
Babile X long rainy season	8.88(0.16)^{a}	7.59(0.16)^{a}	5.22(0.16)^{ab}	5.28(0.15)^{a}	28.81(0.17)^{c}
Babile X dry season	8.08(0.16)^{b}	6.72(0.16)^{b}	4.50(0.16)^{bc}	4.48(0.15)^{b}	27.69(0.17)^{d}
Harar X short rainy season	8.88(0.16)^{a}	7.18(0.13)^{ab}	4.85(0.13)^{ab}	4.96(0.12)^{ab}	27.01(0.14)^{de}
Harar X long rainy season	8.74(0.16)^{ab}	7.41(0.13)^{a}	4.70(0.13)^{b}	4.79(0.12)^{ab}	26.61(0.14)^{e}
Harar X dry season	7.41(0.16)^{c}	6.28(0.13)^{c}	4.00(0.13)^{c}	4.51(0.12)^{b}	25.57(0.14)^{f}
Dire Dawa X short rainy season	9.26(0.16)^{a}	7.45(0.13)^{a}	5.03(0.13)^{ab}	5.13(0.12)^{a}	31.39(0.14)^{a}
Dire Dawa X long rainy season	9.18(0.16)^{a}	7.63(0.13)^{a}	4.94(0.13)^{ab}	5.20(0.12)^{a}	29.92(0.14)^{b}
Dire Dawa X dry season	7.85(0.16)^{bc}	6.61(0.13)^{bc}	4.37(0.13)^{bc}	4.54(0.12)^{b}	28.99(0.14)^{c}

*Note: TAMBC = Total aerobic mesophilic bacterial count; TCC = Total coliform count; YC = Yeast count; MC = Mold count; Significance: *p < 0.05, ** p < 0.01,*** p < 0.001; S.E. = Standard error*

4. Conclusions

The results of the present study revealed that the raw milk samples collected from the equipment of producers in pastoral production system and medium size herds had significantly higher microbial counts than samples collected from the equipment of producers in agro-pastoral production systems and small-sized herds, respectively. Moreover, there were significant microbial quality differences among milk samples collected during the short rainy season, the long rainy season, and the dry seasons at all milk sources except for milk samples collected from the udders (for which the effect of season was not significant for yeast and mold counts). In most cases, milk samples obtained during the short and long rainy seasons had greater microbial loads than those obtained during the dry season. There were significant microbial quality differences among milk samples collected from udder, producers, collectors/transporters, vendors and consumers. Samples collected from the equipment of consumers and vendors had significantly higher microbial loads than samples collected from the equipment of collectors/transporters, which had greater microbial loads than those obtained from the equipment of producers; and also samples collected from the equipment of producers had significantly higher microbial counts than samples collected directly from the udder of milking cows. The mean values of TAMBC, TCC, YC and MC for raw milk samples collected from all milk sources (from udder, producers, collectors/transporters, vendors and consumers) in the study area exceeded the upper acceptable limit. This indicates that the sanitary conditions in which the milk is being produced and handled are substandard. In general, it could be concluded that, the microbial quality of raw cow milk produced and marketed in the study area was poor and a threat to human health. Therefore, improved milk hygienic practices across the milk supply chain is recommended to protect the milk from being unsafe for consumption as well as from being spoiled. Thus, awareness creation and capacity development of producers, collectors/transporters, vendors, consumers and other people involved in the milk supply chain should be done on hygienic practices of producing and handling raw milk. Moreover, introduction of pertinent interventions such as milk cooling facilities, organized and efficient milk storage and transportation systems at all across the supply

chain are highly important. Further investigation is recommended to identify pathogenic strains of mold (like aflatoxins) and coliform (like *Escherichia coli* O155:H7) that cause a potential health risk.

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Radiation Levels in Buildings on the Main Campus of Haramaya University and at the Towns of Harar and Dire Dawa, Eastern Ethiopia

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Abstract: Indoor radiation is a concern for people living in buildings constructed from materials with high emission of radionuclides. In this study, radiation rate measurements of 39 rooms in nine buildings of three different age groups at three locations were made using Electronic Personal Dosimeter (EPD). The measurements included both interiors and exteriors of the rooms. Interior measurements were made in two perpendicular directions from two adjacent walls at distance interval of 0.5 m. The EPD measurement revealed a decrease in the magnitude of the radiation as the days of measurement progressed, and that necessitated the need of correction factors, which were evaluated using background radiation rates of each location separately. All measured radiation rates were then corrected using the respective correction factors. The results obtained are summarized as follows. Background radiation doses at HU campus and Harar and Dire Dawa towns, averaged over the measurement days is, 4.1, 2.8 and 2.4 mSv/y, respectively. These values reflect effective external doses of 0.82, 0.55 and 0.47 mSv/y, respectively, for the three locations. Dire Dawa old building differed from all the other buildings of the three locations and it exhibited the highest interior radiation of average dose of 0.027±0.011 mSv/y above the background radiation. There were no significant differences between the new and the intermediate buildings of the three locations. When averaged out, irrespective of building ages of each location, HU buildings showed average dose of 0.004±0.004 mSv/y, Harar, -0.008±0.006 mSv/y and Dire Dawa, 0.009±0.008 mSv/y. No difference in radiation rates were observed between the two directions but radiation rates slightly increased from walls to the centers of rooms up to a certain point. Radiation rates of the interior and exterior of each room did not show a significant difference. Though differences were observed among buildings of the three different ages, the differences were not uniform at the three locations. The doses from all the rooms were within the limit set by IAEA for indoor radiation.

Keywords: Background radiation variability; Indoor radiation; Electronic Personal Dosimeter; Radiation rate; Building age

1. Introduction

Ionizing radiation is one of the potential risks human beings have been experiencing ever since its existence. It occurs naturally and from man-made sources. Natural (background radiation), which has worldwide average of 2.4 mSv/y per person, at sea level (IAEA, 2010; Thabayneh and Jazzar, 2012) comes from two sources. The first source is cosmic, which is due to interaction of cosmic rays with atomic nuclei in the atmosphere, and it accounts for about 10% of the total external natural radiation. Primordial terrestrial radiation is formed by nucleosynthesis and makes up 25% of the external and about two third of the internal exposures (inhalation and ingestion) (UNSCEAR, 2010). Overall, background radiation accounts for about 80% of the total radiation (natural and manmade dose of 2.8 mSv a person is exposed to in a year) (Taskin *et al*., 2008).

Soils and rocks are the main sources of terrestrial radiation since volcanic geographic structures as well as rocks that are rich in phosphate, granite and salt contain natural radionuclides like uranium-238 (^{238}U), thorium-232 (^{232}Th) and potassium-40 (^{40}K) (EC, 1999; STUK, 2010). The three elements are the main sources of gamma radiation (Lust and Realo, 2012). Sometimes, ^{226}Ra, which accounts for 98% of ^{238}U decay subseries, is considered instead of ^{238}U (Kinsara *et al.,* 2014). Radon (^{222}Rn is the daughter of Ra) and ^{232}Th are responsible for internal radiation since they can get into the air as gases (IAEA, 2010).

Knowledge of concentrations of radionuclides in building materials is important in the assessment of population exposure as most individuals spend approximately 80% of their time indoors (Steger and Grün, 1999). The presence of the naturally occurring radionuclides in building materials is a source of indoor radioactive pollution, since building materials are obtained from soil and rocks and contain ^{226}Ra, ^{232}Th and ^{40}K. Therefore, trace amounts of these radionuclides are found in all buildings (EC, 1999). But only buildings in which there are higher concentrations of these radionuclides that increase the probability of health problems (EC, 1999; Aamidalddin *et al*., 2015).

Radiation exposure due to building materials can be divided into external and internal. External exposure is caused by direct gamma radiation, whereas internal

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exposure is caused by inhalation or ingestion of radon and its short-lived decay products. Buildings are generally constructed using different materials, among which the predominant ones are cement, metal frames and other materials such as stones, bricks, aggregates, sand, etc. In addition, wood and any other material which at one time was living contains carbon-14 (Othman & Mahrouka 1994).

Radiation risk from buildings depends on a number of factors. These include the nature of the material and the quantity of the material used in the construction of the building, age and condition of the building, the floor level of the room in the building, the rate of ventilation and how long the inhabitants spend indoors (EC, 1999; Markkanen, 1999: Salih *et al.,* 2014).

The nature of material (type of material and where it is from) can determine the amount of radionuclides in the material since natural building materials reflect their geologic formation and origin (Lust and Realo, 2012). Generally, wood has lower amounts of the three radionuclides except trace amounts of ^{14}C (Othman & Mahrouka, 1994) and therefore, countries such as Newzeland, Iceland and USA who mostly use wood for construction of residential houses experience less than half (28 nSv/h) radiation rate than those countries with stone constructions (UNSCEAR, 2000). The worldwide average indoor effective dose due to gamma rays from building materials is estimated to be about 0.4 mSv per year (Jwanbot *et al.*, 2014).

From among the construction materials the ones containing granite or igneous rock of granite composition, are enriched with ^{238}U (average 5 ppm) and ^{232}Th (average 15 ppm) compared with Earth's crust average of 1.8 and 7.2 ppm, respectively (Alharbi *et al.,* 2011). For instance, Aamidalddin *et al.* (2015) in their study of building materials used in Saudi Arabia found highest value of effective dose of 1.17 mSv/y in granite materials and this value is in excess of the limit set for public (1 mSv/y) over background radiation (UNSCEAR, 2000; STUK, 2010; USNRC, 2015). Dose *et al.* (2014) also found high level of activity in granitoid aggregates compared with other aggregates. According to Alharbi *et al.* (2011), Kinsara *et al.* (2014) and Dose *et al.* (2014), granitoides contain higher percentages of ^{232}Th compared to other rocks.

Recycled industrial by-products containing Technologically Enhanced Naturally Occurring Radioactive (TENOR) materials may also be used in the construction industry. Industrial byproducts such as coal fly-ash, ballast furnace slag incorporated in cement and byproduct gypsum (phosphogypsum) can increase radiation from buildings and consequently internal and external absorbed doses to residents (Othman & Mahrouka 1994; Aamidalddin *et al.*, 2015). These industrial byproducts have especially high activity concentrations of ^{226}Ra compared to other building materials such as concrete, bricks, building stone and natural gypsum (EC, 1999).

In addition to the nature of material, the quantity of a specific building material used in building construction matters. The radiation limit set for materials used in bulk such as aggregates, sand, cement, stone, bricks, etc. is generally lower than materials used in small quantities such as marbles and tiles (Markkanen, 1999). Buildings with massive walls and floors can partially shield against gamma radiation from undisturbed Earth's crust (EC, 1999), but it has also a proportionally higher emission of ^{222}Rn from the massive walls and floors (Markkanen, 1999; UNSCEAR, 2000; Tzortis *et al.,* 2003).

The rate of emission, however, and hence, dose rates may decline over time due to radioisotope decay (Othman and Mahrouka 1994). Therefore, for buildings with all conditions the same, dose rates are assumed to be lower for older buildings than newer buildings (Markkanen, 1999; Othman and Mahrouka, 1994). However, buildings generally deteriorate (show up cracks in walls and floors) with age, which serve as passageways for ^{222}Rn from inside the walls by the process of diffusion and convection and from the soil underneath. In such buildings, there is a possibility of elevated radiation especially if the building materials and the soil below contain elevated concentrations of radon (EC, 1999). Such exhalation causes buildup of radon especially if the building is not well ventilated (Salih *et al.*, 2014). For building levels close to the ground such as basements, the amount of radon in the rooms would be higher (Tubosun *et al.*, 2013).

Even though several studies have been done on different types of building materials as mentioned earlier, not much has been done regarding radiations in buildings. Concerns such as variability of radiation within a room, dependence of radiation on the type of building materials and ages of buildings have not been sufficiently addressed. In Ethiopia, no studies have been conducted to elucidate radiation levels and there is also little public awareness on radiation levels from buildings. In this work, total gamma emission from buildings of different ages was studied using electronic personal dosimeter at three different locations of Eastern Hararghe zone, Ethiopia. The objectives were to look at several factors such as radiation variability within a room, differences in radiation between the interior and exterior of a room and whether building age differences show significant differences in the amount of radiation both in the interior and the exterior of rooms in buildings.

2. Materials and Methods

2.1. Study Areas

This study was conducted at three locations, namely, Haramaya University's (HU) campus, Harar and Dire Dawa towns. HU campus is located at the distance of about 505 km from Addis Ababa, to the east. Geographically this area lies between 9°15'N latitude and 42°0'E longitude and has an average altitude of 2006 meters above sea level. The area has a temperature ranging from 12.6 to 28.5°C with average relative humidity of 65%. It receives an average annual rainfall of 790 mm with bimodal distribution of the

seasonal pattern peaking in mid-April and mid-August of the year.

Harar town is found at the distance of 517 km to the east of Addis Ababa. The town is located at 42°04' - 42°22'E longitude and 9°15' – 9°27'N latitude. It has an average altitude of 1780 meters above sea level and average temperature of 22.65°C. The annual rainfall, on average is 700 mm.

Dire Dawa town is located at the distance of 527 km to the east of Addis Ababa. The area is located between 9°27' N and 9°49' E latitudes and 41°38' and 42°19'E longitude. The rainfall pattern of the area is characterized by small rainy season from February to May and big rainy season from July to September. The average annual rainfall in the study area varies from 550 mm in the lowland northern part to above 850 mm in the southern mountains. The monthly average maximum and minimum temperature ranges from 34.6°C to 14.5°C, respectively. The altitude where the study was conducted is about 950 meters above sea level.

The three locations were selected for their proximity and also because they have old and new buildings made from different materials. They were also assumed to have three different background radiations because of their altitudinal differences. Figure 1 shows the location map of the three areas, namely, HU campus, Harar and Dire Dawa towns.

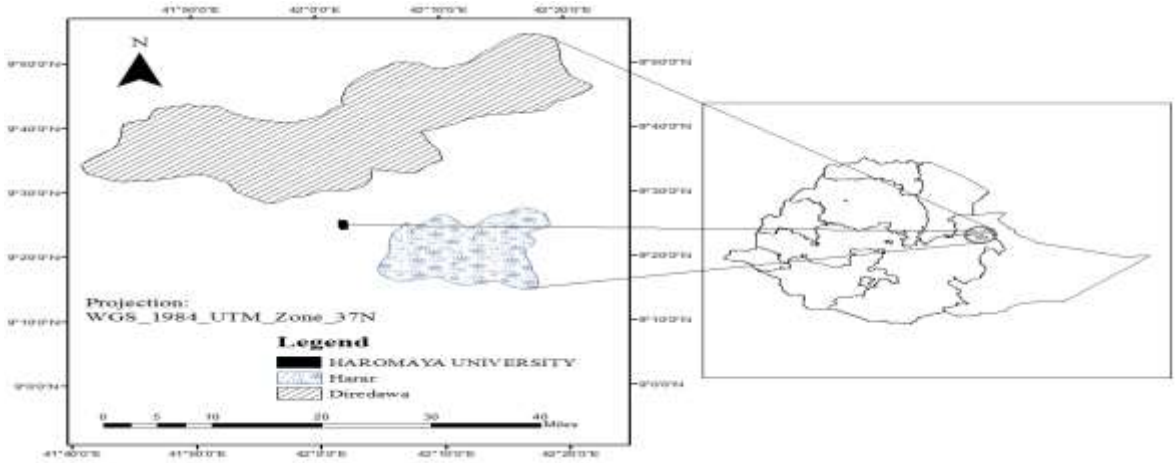


Figure. 1. Location map of HU campus, Harar and Dire Dawa towns.

2.2. Instrument Used for Data Collection

Measurements of background and building radiations were made using Electronic Personal Dosimeter (EPD model type MINI-6100), which evaluated ionizing radiation exposure by measuring the amount of visible light emitted from a crystal in the detector. The instrument measures dose, dose rate and run time. It has dose range of 0 - 9,999 mSv and dose rate range of 0 - 99.9 mSv/h.

2.3. Data Collection

A total of three locations [Haramaya University (HU) campus, Harar and Dire Dawa towns] were selected for this study. At each location, three buildings of different age (recent, those with intermediate age, and relatively old) were identified for the study. Approximate age of each building was obtained from people who know the building and the materials, from a visual assessment of predominant materials used to construct the buildings. This anecdotal method of gauging the age of the buildings was used because of lack of documentation on the history of the buildings. Selection of buildings containing classrooms was purposefully made for the study because such buildings house many people at a time and contain no household materials such as furniture and utensils other than chairs, which may bias the data and prevent easy access to rooms.

On HU campus the buildings selected were categorized as relatively recent, intermediate and old buildings. The selected buildings included two new classroom buildings, which are about 15 years old; one building of intermediate age, which belongs to the College of Natural and Computational Sciences (a little over 40 years of age), and one old building belonging to the College of Agriculture and Environmental Sciences (over 60 years of age). A total of six rooms were selected from the recent buildings, and five and four rooms from the intermediate and the old buildings, respectively.

The three buildings selected in Harar town included one of the new classroom buildings of the College of Medical Science (about 10 years old) and one building of an intermediate age and another one of an old age both on the campus of Harar Teachers' Education and Business College. Four rooms were selected from each building at this location.

In Dire Dawa, the new building used for this test was on Dire Dawa University campus. The old building was selected from Dire Dawa Alliance France School whereas Mariam Sefer Junior Secondary school was selected as a building of intermediate age. The number of rooms selected here were similar to those of Harar town.

Even though it is generally recommended to take background radiation at 1 m height (Markkanen 1999),

prior to each day study background radiations were always measured outside, far from any building at five different heights, i.e., zero or ground level, 0.5, 1.0, 1.5 and at 2.0 meters. The purpose was to verify by how much the 1 m height differed from the values obtained at other heights. This test was necessary since we conducted all other measurements at ground level. Since rooms of different buildings at different locations were numbered differently (sometimes with identical room numbers), the rooms were re-numbered sequentially (for ease of reference) as shown in Table 1.

Table 1. Sequential numbers given to each room of the three locations and buildings of three age groups.

Building type	Recent		Intermediate		0ld	
Location	Actual Room No.	Given Rm. code	Actual Room No.	Given Rm. code	Actual Room No.	Given Rm. code
HU	XXI-4	1	R-201	7	R-007	12
	XXI-3	2	R-202	8	R-206	13
	XXI-7	3	R-203	9	R-207	14
	XII-12	4	LTH-III	10	R-208	15
	XI-3	5	R-12	11		
	XII-10	6				
Harar	LTH-1	16	R-10	20	R-5	24
	LTH-3	17	R-11	21	R-6	25
	R-B	18	R-7	22	R-7	26
	R-C	19	R-8	23	R-8	27
Dire Dawa	R-5	28	R-1	32	R-1	36
	R-6	29	R-2	33	R-2	37
	R-3	30	R-3	34	R-3	38
	R-4	31	R-4	35	R-4	39

2.4. Data Analysis

In this work six points were considered. In order to test whether the background radiation measured at 1 m height differed from the background radiation values measured at different heights, the 1 m values were compared with the values of other heights. The daily measured background radiations for the same location showed a declining trend that also reflected on hourly values. Since we took the background radiation measurement only once per day (during morning hours), it was imperative to find the mathematical pattern the background radiation followed so as to make corrections on the hourly values. For this purpose, curve fittings were made for all the three locations and the values obtained were used as correction factors for the respective locations.

Variation in radiation from walls was also considered first by making time corrections and comparing the measured values. In addition, since the rooms did not have equal width and length, comparisons were made to check whether radiation rates measured in the two directions depended on the directions of measurement from the wall. Comparisons were also made to see differences between the interior and the exterior (not the background) radiations. Finally, radiation rate dependence on the age of each building was considered by comparing radiation values obtained for the recent, intermediate and old buildings, at the three locations.

2.5. Mathematical Formulations Used for Data Analysis

Understanding the concept of dose and dose rate helps to control the dose an individual can receive while staying around any radiation source. Dose is the total amount of radiation absorbed over time. Dose rate is the rate at which the radiation is absorbed. Dose *(D)* and dose rate *(D_r)* are related as (RSSC, 2013).

$$D = D_r t \quad (1)$$

Where: t = time. The radiation dose a person receives is equal to the time the person spends in the area multiplied by the dose rate of the area. Generally, the unit of dose rate is Sievert per hour (Sv/h) such that the dose calculated over a year ((24 h/day × *365 days/y* $=8.76 \times 10^3$ h/y) *is:*

$$D = \left(D_r \times 8.76 \times 10^{-3}\right)\ m\text{Sv/y} \quad (2a)$$

D can also be given in milli-Sievert per year (mSv/y) or micro-Sievert per year (μSv/y)such that:

$$D = \left(D_r \times 8.76 \times 10^{-3}\right)\ \text{mSv/y} = \left(D_r \times 8.76 \times 10^{-6}\right)\ \mu\text{Sv/y}. \quad (2b)$$

When outdoor and indoor radiation rates are different, the indoor radiation *(D_{in})* is obtained by multiplying the indoor rate, D_{rin} by a factor of 0.8 (taking into consideration the 80% time a person spends indoors).
Hence,

$$D_{in} = \left(D_{rin} \times 7.008 \times 10^{-3}\right)\ m\text{Sv/y}. \quad (3a)$$

If the value of dose is very small, sometimes using a unit of micro-Sievert ($\mu Sv/y$) is preferable as shown next.

$$D_{in} = \left(D_{rin} \times 7.008 \times 10^{-6}\right) \mu Sv/y. \quad (3b)$$

Similarly, for outdoor *(D_o)* a factor of 0.2 of the outdoor rate, D_{ro} is used (i.e., assuming a person spends 20% outdoors) and

$$D_o = \left(D_{ro} \times 1.752 \times 10^{-3}\right) mSv/y. \quad (4)$$

Since the magnitude of the radiation dose rate is in the order of Nano-scale (10^{-9} Sv/h), the radiation annual dose is in the order of 10^{-6} ~ 10^{-4} Sv/y, 10^{-3} ~ 10^{-1} mSv/y or 1 ~ 10^{2} μSv/y.

The total dose a person receives per year is then given as the sum of the indoor and the outdoor radiation;

$$D = D_{in} + D_o. \quad (5)$$

After calculating the dose, the value is compared with the international limits to know whether the dose is within the acceptable limit or not. In the case of net radiation, it can independently be compared with the limit (1 mSv/y) above the background radiation (USNRC, 2015). All calculations were performed and graphs were drawn using Microsoft Office Excel.

3. Results and Discussion

3.1. Dependence of background radiation on height of measurement

Background radiation is dependent on cosmic and terrestrial radiations. Out of the two, only cosmic radiation is dependent on altitude (height from ground surface). Because of this, when background radiation is measured it is important to choose the height at which to measure it. Though the height of 1 m is recommended (Markkanen, 1999) it is important to know how much error is committed from the recommended value by variation in small heights. For this reason, measurements were made at five different heights to see the influences of small height differences. Table 2 shows the result of percent difference (P_d) calculated as

$$P_d = \frac{D_{rh} - D_{rr}}{D_{rr}} (100\%) \quad (6a)$$

Where: D_{rh} = dose rate at height h and D_{rr} = dose rate at reference height, which in this case is the dose rate at 1 m height as shown in Table 2.

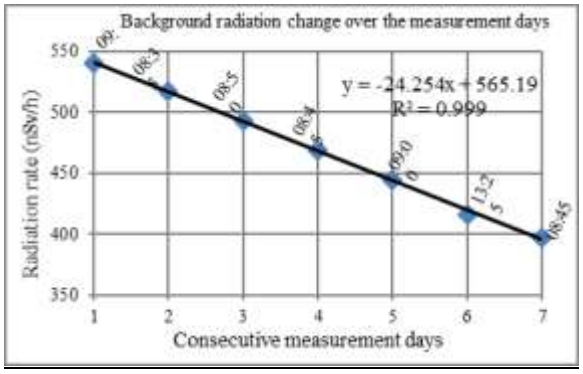
Table 2. Percent differences between background radiation rates measured at different heights and the values measured at 1 m height.

		Percent differences from values measured at 1 m height					
		Day					
Location	Height (m)	1	2	3	4	5	6
HU campus	0.0	0.02	0.03	0.01	0.10	0.01	0.01
	0.5	0.01	0.01	-0.01	0.10	-0.01	-0.01
	1.0	0.00	0.00	0.00	0.00	0.00	0.00
	1.5	0.01	0.01	-0.01	0.01	-0.02	-0.02
	2.0	0.01	-0.01	-0.01	-0.01	-0.01	-0.01
Harar	0.0	0.01	**0.14**	0.01			
	0.5	-0.01	-0.01	-0.01			
	1.0	0.00	0.00	0.00			
	1.5	-0.02	-0.02	**-0.15**			
	2.0	-0.01	-0.01	**-0.14**			
Dire Dawa	0.0	0.01	-0.03	0.01			
	0.5	0.02	-0.01	0.03			
	1.0	0.00	0.00	0.00			
	1.5	0.01	0.01	0.01			
	2.0	0.02	-0.01	-0.01			

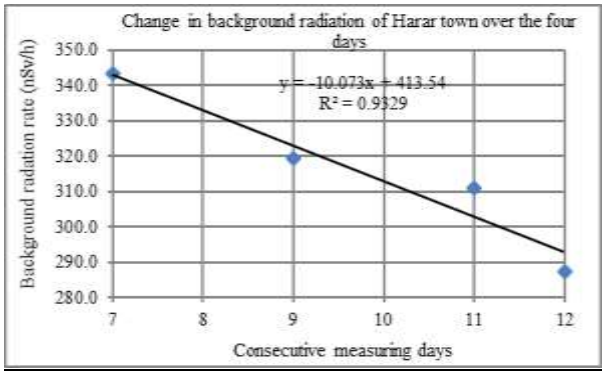
As observed in Table 2, only in three instances (shown in bold face in the Table) did the percent difference come to a little over one thousandth of the value measured at 1 m height. It is not surprising to find such closeness in the values since for the same location the only difference with height is in cosmic radiation, which is not high at such small differences in height. Hence, there were no significant differences in dose rates measured at other heights compared with the 1 m values.

3.2. Variation in Background Radiation over Time

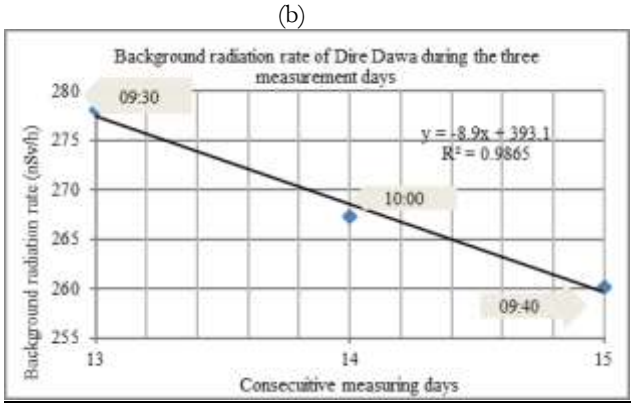
Over the entire days of measurement, background radiation showed time dependence. To know whether the time dependence followed a certain pattern or not, data of the three locations were taken independently and plotted against time. For illustration, the plots of background radiations against time of the three locations are shown in Fig. 2.



(a)



(b)



(c)

Figure. 2. Background radiation rates measured on (a) HU campus over seven consecutive days (8/5/2015 – 8/11/2015), (b) Harar town and (c) Dire Dawa town. The numbers written adjacent to the data points are the ltimes during which measurements were conducted on the respective day.

As observed in Figure 2, background radiation rate on HU campus showed a decline from one day to the next and the amount of decline was 24.25 nSv/h per day (slope of the linear fit). The curve showed a very good linear fit (R^2 = 0.999), which is indicative of a decreasing linear trend for the particular location. Hourly variability was obtained from interpolation of the daily variability, i.e., about 1 nSv/h (= 24.25/24) every hour.

Similar linear fits were observed for Harar and Dire Dawa towns with slopes and correlation coefficient values of 10.07 and 0.933, and 8.9 and 0.987, respectively. What they translate into is declines of about 0.42 nSv/h and 0.37 nSv/h every hour, for Harar and Dire Dawa towns, respectively.

Plot of background radiations of the three locations together indicates lower reduction at lower altitudes. The combined background radiation data of the three locations showed better quadratic fit than linear fit as shown in Fig. 3. The quadratic relationship takes care of both altitude and daily decline in background radiation, which was perhaps due to power reduction in the EPD in sensing gamma radiation. When power reduces the sensing ability of EPD also decreases (Kinsara *et al.*, 2014). According to Döse *et al.* (2014) there is partial loss of radon when measuring in an open space. Variability in temperature and speed and direction of wind (Aamidalddin *et al.,* 2015), humidity, cosmic radiation and terrestrial background affect radiation rate measurement in the field using portable hand-held radiation sensors (Döse *et al.*, 2014). In our case all these did not matter since we followed the method suggested by Markkanen (1999) and Hameed *et al.* (2014), and considered the net radiation (difference between the background radiation and radiations measured both indoors and on the exteriors of the rooms).

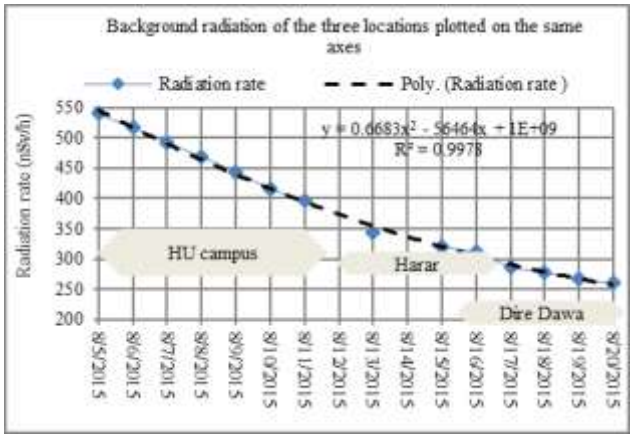


Figure. 3. Plot of background radiation against days of measurement at the three locations.

3.3. Dependence of Radiation rate on Distance from Wall

For this particular test, large rooms with measured radiation rates up to distance of 4 m were considered. Only five rooms from HU campus' recent building satisfied this condition and considered. The patterns of the plotted lines are as shown in Fig. 4.

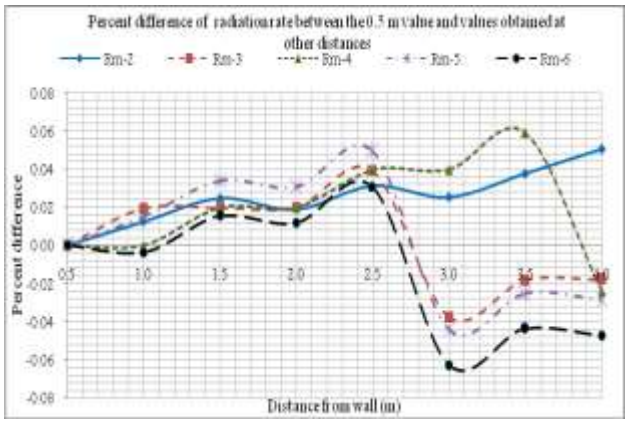


Figure. 4. Percent differences between the actual indoor radiation rates measured at 0.5 m distance and the other distances. Room numbers are as given in Table 1.

The percent difference (P_d) was calculated as,

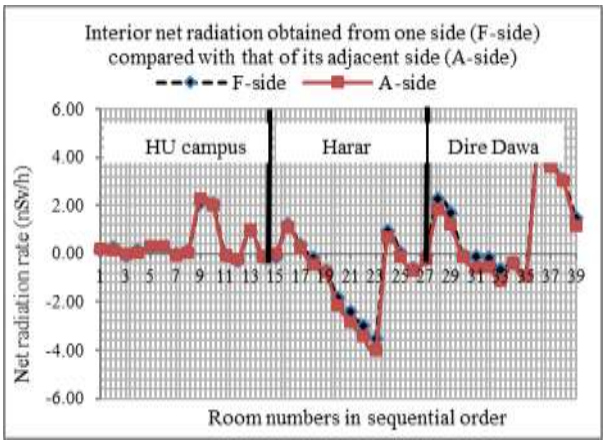
$$P_d = \frac{D_{r(0.5)} - D_{r(x)}}{D_{r(0.5)}}(100\%) \quad (6b)$$

Where: $D_{r(0.5)}$ is the dose rate at 0.5 m and $D_{r(x)}$ is the dose rate at any other distance x where x represents any one of the distances from 1.0 to 4.0 m.

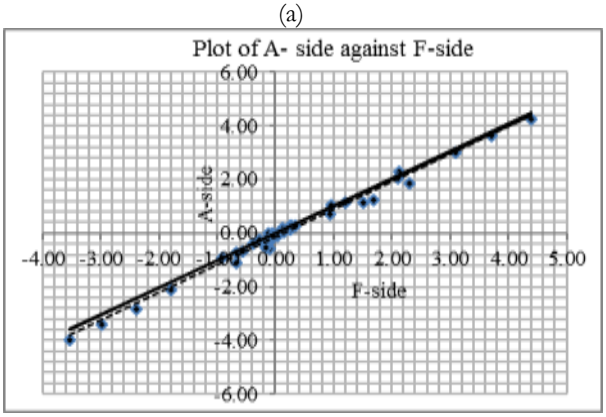
Hence, the points above the 0.00 percent difference line indicate that the measured value at 0.5 m was higher than that of the other distance while points below the 0.00 line indicate the opposite. As observed in Fig. 4, the curves for all the rooms, though slightly oscillating, showed an increasing trend in percent difference up to 2.5 m from the wall and showed mixed patterns thereafter. Such positive percent difference is interpreted as decreasing tendency (value at 0.5 m exceeding the value at the other point) of actual radiation a little distance before reaching the center of the room, for the three rooms (3, 5 and 6). For these rooms there were shifts to the negative percent difference at around 2.75 m. Since the lengths of the rooms were around 7 m on average, the shift of P_d from the positive to the negative shows high net radiation at the center of the rooms. This is perhaps due to the contribution of radionuclides emitted from the other walls as one moves toward the center. Lust and Realo (2012) mentioned about the dimensions of rooms having relatively small effect on dose rate in a room but for the rooms they considered they calculated the dose rate in the middle of the rooms. For room 4 the decreasing trend continued up to 3.5 m while for room 2 it did not stop even at 4 m. Since all the rooms were roughly identical in terms of ventilation and room sizes (except small differences in their lengths), we could not find adequate explanations for the differences between the two rooms.

3.4. Comparison of Radiation Rates obtained from Two Directions (from Walls) in a Room

A room wall which extends to the exterior and a partition wall are assumed to be different in the amount of radiations they emit since they are made from different materials. Radiation rate is assumed to be high on the side of the wall which has higher emission of radionuclides. Fig. 5 is plotted to check if there is indeed a difference in net radiation rates emitted when measured from two perpendicular directions in the room.



(a)



(b)

Figure. 5. Plot of interior net radiation rates obtained from measurements made from two adjacent walls. The first direction is named as F-side while, the second adjacent direction is named as A-side. (a) Plot of net radiation against room number and (b) Linear fit between A-side against F-side. The room numbers are as given in Table 1. The plots are shown for the three locations together.

In Fig. 5, plots of net radiations obtained from two perpendicular directions were compared by plotting them together for all the three locations. As observed in Fig.5a, for almost all of the rooms, the two curves (except their irregularities) are almost overlapping at all the three locations. The linear fits shown in Fig. 5b with the solid line drawn as 1:1 line and the dotted line as the linear fit line (of slope 0.0106 and $R^2 = 0.98$) are also almost overlapping. This means, the direction of measurement did not make significant difference in the amounts of radiation rates measured. This happened regardless of the differences in the widths and lengths of the rooms. It also did not matter if one of the walls had windows and the other, a solid wall. Generally,

such directional differences could be observed if the amount of radiation emitted from one wall differed from that of the other possibly because of differences in materials from which the wall was constructed. In the rooms selected, the interior walls were of similar nature and that must have contributed to their identical results. The fact that there were adequate ventilations in the rooms could also have affected the result since ventilation immediately circulates radon emitted from the walls such that the room shows identical results in the two directions.

3.5. Comparison of Radiation Rates of Buildings of Different Ages

Radionuclides decay overtime and given all other conditions to be the same, one may expect lower emissions from older buildings compared with the recently constructed buildings (Othman and Mahrouka, 1994). But the science of building construction has evolved over the years and one can see differences in the types and quantities of materials used in old and recent buildings. Such differences can also reflect in the amount of radionuclides emitted from buildings of different ages. Fig. 6 shows two things at the same time. First it shows net radiation differences among buildings of different age groups. Along the x-axis, the three locations are separated by long and solid vertical lines and within each location; differences among buildings of three different ages are separated using shorter solid lines. The figure also shows differences between interior and exterior radiations of all the rooms of all the buildings at the three locations together. In the figure, instead of taking the raw data, background radiations were subtracted from the measured interior and exterior radiations to get net radiation as suggested by Markkanen (1999) and Hameed *et al.* (2014).

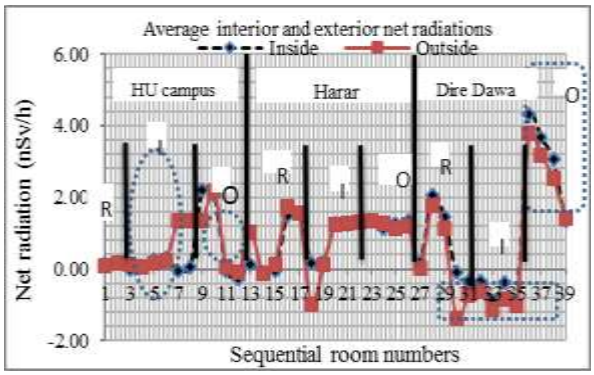


Figure. 6. Interior and exterior net radiation rates of rooms of buildings of three age groups at the three locations shown together. The letters R, I and O shown in white backgrounds represent recent, intermediate and old, respectively, to indicate the relative ages of the buildings.

As far as age differences are concerned, the figure reflects three different scenarios. The first is the case where the data points are overlapping with the zero net radiation rate line. For example, the recently erected buildings and two rooms from the old building on HU campus reflected this case. This case indicates that the rooms have radiation rates identical to the background. It does not, however, imply that the rooms lack indoor radiation. What it actually reflects is that, what is emitted within the room is balanced with what the walls of the room prevent from getting into the room from outside. The explanation is consistent with the proposition that building materials act as sources of radiation and also as shields against outdoor radiation (EC, 1999; Tzortzis *et al.,* 2003). A person living in such a room is experiencing the same radiation effect as outdoor.

The second case is where the net radiations are positive. Rooms with such values reflect higher indoor radiation rates compared with the background. The building of intermediate age (shown within dotted ellipse) and one room from old building of HU campus (shown within a dotted circle), all buildings of Harar town (except room 16) and the recent and old buildings (shown in dotted rectangle) of Dire Dawa town fall under this category. What it reflects is indoor radiation exceeding the background radiation. Manifesting positive net radiations is not a concern unless the values exceed the limit of 1 mSv/y in dose (EC, 1999; STUK, 2010). In this particular case none of the rooms exceeded this limit and details are given in Table 3 (section 3.7).

The fact that in some cases new buildings and in others old buildings showed slightly higher net radiations deserves explanation. HU intermediate building was constructed from massive concrete and red clay bricks on the exterior and the rooms are separated by unplastered hollow blocks on the inside. Rooms 7, 8 and 9 showed slightly elevated radiations perhaps because of the clay bricks and massive concrete materials both of which inherently have higher emissions next to stones (EC, 1999; Kinsara *et al.,* 2014; Aamidalddin *et al.,* 2015). Besides, the rooms do not have adequate ventilation. For instance, room 7 is a lecture theater with exposed clay bricks on two of the walls on the inside. Such bricks have radionuclide concentrations slightly less than masonry stones but higher than concrete (EC, 1999). Rooms 8 and 9 are small office rooms with additional items such as computers, printers and other materials and those must have slightly elevated the net radiation. Rooms of HU old building all showed radiations comparable to those of the new building (except for Room no 13). The reason why old buildings show lower rates of radiation may be because of the gradual decay of radioactive elements in the building materials. The difference between the recent and the intermediate buildings is attributable to the materials from which the buildings were constructed. Concrete and bricks have slightly higher emissions than hollow cement blocks (Salih *et*

al., 2014) because of differences in the materials and their bulk densities.

The recent building in Harar town showed slightly elevated radiation (especially rooms 16 and 17) both on the inside and on the outside. These rooms are lecture theaters with fixed chairs anchored to metal frames and massive floors made of concrete. The two could be the reason for such elevated rates of radiation. Whatever small emissions there were from the two, they were active on account of their young age (not yet decayed enough). The rate of radiation in the new buildings is higher because of its age, i.e., the radionuclides in the materials of the building might have not decayed sufficiently. Buildings of intermediate and old ages of Harar also showed slightly elevated net radiations. On the other hand, radiation rates from old buildings were higher because of the large quantities of materials used for construction and lack of adequate ventilation (at least these were observed in rooms of Dire Dawa old building). Radon diffusion from the walls or the floors (Masok *et al.,* 2015) can only be minimized with adequate ventilation. Some old buildings which are over sixty years were generally made from stones without or with fewer iron reinforcements. In order to make the structures safe, walls were generally made thicker than what is observed in the recent buildings. The bulkiness of the structure and the nature of the materials are assumed to have effect on how much radiation is emitted.

In Dire Dawa recent and old buildings showed slightly more elevated radiations than the building of intermediate age. Elevated net radiations from recent buildings are due to the materials from which they are constructed and due to their recent age. The old buildings generally show higher emissions because of their massive structures or possibly due to radon diffusions from the walls or floors.

The last case is where the net radiation remained in the negative territory. Rooms of Dire Dawa intermediate age building fall under this group. Such rooms generally play significant roles in shielding background radiation while the amount they emit is slightly lower than the amount they shield. The rooms are found in low cost school building and the low emission may be due the use of low quantities of materials.

3.6. Comparison of Radiation Rates of the Interior and Exterior of Rooms

As shown in Fig. 6, differences between net radiation rates of the interiors and exteriors of rooms were observed in few instances. Only three rooms (7, 8 and 9) of HU building of intermediate age, room number 13 of HU building, room 18 from Harar and all except room 39 of Dire Dawa showed slightly different values between the interior and the exterior. In most cases, however, the two were identical. Radiation on the inside equals that of the outside when the rooms have identical materials on both sides and when the inside has adequate ventilation (Salih *et al.*, 2014). The presence of multiple windows, which allows the radon that is possibly emitted from the walls to disperse to the outside, has one impact on the similarity of the two sides. The other reason is possibly very low emission from the materials from which the building was constructed. This can be attributed to the plastering and painting of the inside walls which has lower gamma emission compared to granite and concrete walls. For instance, Aamidalddin *et al.* (2015), while they found effective dose of 1.17 mSv/y for granite, they only obtained 0.03 mSv/y in paints. Since building materials can shield against gamma radiation emitted from the soil (UNSCEAR, 1993; Tzortis *et al.,* 2003), it is possible to also assume that what is exhaled from the building material of the room is balanced with what is kept out.

Table 3. Annual doses of net indoor radiations calculated for all the rooms.

HU			Harar			Dire Dawa		
Room No.	NIR rate (nSv/h)	Annual Dose (μSv)	Room No.	NIR Rate (nSv/h)	Annual Dose (μSv)	Room No.	NIR rate (nSv/h)	Annual Dose (μSv)
1	0.17	1.16	16	1.17	8.17	28	2.07	14.53
2	0.20	1.37	17	0.31	2.15	29	1.45	10.16
3	-0.03	-0.20	18	-0.30	-2.11	30	-0.09	-0.61
4	0.10	0.67	19	-0.70	-4.92	31	-0.35	-2.48
5	0.28	1.93	20	-1.96	-13.72	32	-0.35	-2.45
6	0.29	2.03	21	-2.62	-18.33	33	-0.89	-6.25
7	-0.04	-0.25	22	-3.20	-22.41	34	-0.38	-2.64
8	0.07	0.50	23	-3.77	-26.41	35	-0.92	-6.44
9	2.21	15.47	24	0.81	5.70	36	4.33	30.38
10	2.07	14.47	25	-0.04	-0.31	37	3.68	25.79
11	-0.06	-0.43	26	-0.63	-4.38	38	3.05	21.39
12	-0.26	-1.79	27	-0.13	-0.90	39	1.34	9.37
13	0.49	3.44						
14	-0.10	-0.67						
15	-0.06	-0.45						

Note: NIR rate = net indoor radiation rate (radiation rate above the background); Room numbers are as given in Table 1.

3.7. Dose Comparisons with the Radiation Limit

Annual dose is the amount of radiation a person absorbs while staying indoors. Values in Table 3 were calculated using Eq. (3b) based on the assumption that inhabitants spend 80% of their time indoors.

Any dose above background to which a public is exposed is limited to 1 mSv/y (USNRC, 2015). Since the dose values given in Table 3 are in micro-Sievert (μSv), even the extreme values are lower by more than an order of magnitude. Because almost all of the rooms tested were classrooms, the percentage that the students use these classrooms is even lower than 80%, which means the risk is even lower than what is indicated in the table.

What was observed in the table was less than what was estimated for indoor radiations. Worldwide average effective indoor dose is 0.42 mSv/y (Thabayneh and Jazzar, 2012). EC (1999) estimated effective dose from apartment blocks to be about 0.25 mSv/y in excess of the background radiation. Aamidalddin *et al.* (2015) estimated dose of indoor radiation of 0.39 mSv/y for masonry buildings. Tzortzis *et al.* (2003) in their work on commercially-used natural tiling rocks found indoor radiation doses between 0.02 -2.97 mSv/y. Hameed *et al.* (2014) found mean indoor annual effective dose of 0.58 mSv from igneous rock but one order of magnitude less (0.056 mSv) for sedimentary rocks. None of the net radiations in our study came close to any one of the values mentioned.

4. Conclusion

This study has demonstrated that, all the rooms of buildings of the three locations exhibited radiation doses below the IAEA recommended limit of 1.0 mSv/y, and hence pose no radiation threat to the occupants. From among buildings of the three age groups the Dire Dawa old building was different and had the highest dose of 0.027±0.011 mSv/y. Even though the highest indoor radiation dose was observed at Dire Dawa (0.03 mSv/y over the background), the value is still below the world average by an order of magnitude. The corresponding highest values at Harar and on HU campus are 0.008 mSv/y and 0.015mSv/y, respectively.

In all the rooms studied, direction of radiation rate measurement from walls did not affect the outcome of the radiation rate. However, when it comes to distance from walls, net radiation rate slightly increased close to the center of the room. For all the buildings, radiation rates in the interior and exterior of the rooms did not show distinct differences. Except for rooms with materials of different emission rates on the inside and the outside, in most cases the inside and outside emissions were mostly identical. Even though we observed radiation differences between the recent, intermediate and old buildings, we did not observe similarity at the three locations. It seems rather than age, the material from which the buildings were constructed (in terms of quantity and its rate of radionuclide emission) and the amount of ventilation in the building seem to have a profound influence on the amount of net radiation. Higher net radiation in area of low background radiation indicates the relative risk (with respect to background radiation) rather than the total risk.

The general recommendation that can be given based on this study is first, to use less quantity of materials in the construction of new buildings. Secondly, it is advisable to regularly maintain and paint buildings to minimize cracks and pores. Besides, interior radiations have to also be studied in relation to ventilation, especially for old buildings.

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Crop Productivity as Influenced by Commercial Orientation of Smallholder Farmers in the Highlands of Eastern Ethiopia

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Abstract: Smallholder farmers in Ethiopia are characterized by low crop production and productivity. As a result, production is primarily for self-consumption with a possibility of supplying only a small part of total output to the local markets. Despite their undisputed importance, most studies in Ethiopia focused on smallholder farmers' commercial orientation and analyzed the determinants of the proportion of output sold in crop markets and failed to analyze the relationship between crop productivity and commercial orientation. Therefore, this research was conducted to elucidate synergies existing between commercial orientation and total factor productivity (TFP) among smallholder farm households in the highlands of Eastern Ethiopia. The study was conducted in four districts: two districts, namely, Gurawa and Haramaya were selected from eastern highlands of the region, and two districts, namely, Tullo and Habro were selected from eastern Hararghe highlands). A total of 385 sample household heads were selected randomly and interviewed using a semi-structured questionnaire to elicit data pertaining to crop production input and output market during the year 2015. A two-stage least squares (2SLS) regression model was applied for the analysis. Results of the 2SLS regression indicated that total factor productivity was strongly and positively influenced by the endogenous commercial orientation index. In addition, the number of oxen owned, market distance, extension visits, amount of manure used, quantity of labor used, and location dummy influenced TFP.

Keywords: Commercial orientation; Total factor productivity; Two-stage least square

1. Introduction

The agriculture sector is the most important segment in the Ethiopian economy. This is because the share of the sector to the national gross domestic product (GDP) is 38.5%. Out of this, crop production accounts for 27.4% (NPC, 2016), and provides employment for 72.7% of the total population (UNDP, 2015). Moreover, Ethiopian agriculture is dominated by smallholder farming which accounts for 96% of the total area cultivated and 97% of agricultural output produced (MoARD, 2010). This shows that smallholder farming takes a major share in the overall efforts being exerted to realize the agricultural growth and development plan of the country.

Today, increasing the productivity of agriculture through commercialization is an inevitable reality throughout the world. As a result, Ethiopia has espoused a policy of commercializing smallholder agriculture as a strategy towards attaining economic transformation (MoFED, 2010; NPC, 2016). Empirical studies elsewhere indicate that increasing the rates of market participation or productivity could have bidirectional synergies, and increasing both could boost living standards of farmers (von Braun, 1995; IFAD, 2001, 2003; Barrett, 2008). Thus, an understanding of the effects of commercial orientation on crop productivity would provide policy makers with information on how to design programs or develop strategies that can contribute to increasing production potential among smallholder farmers.

Despite efforts made to commercialize and transform the Ethiopian agriculture from production of staple crops to that of high value crops, performance has been considerably below expectations (NPC, 2016). Many other studies reveal very low smallholder farmers' crop commercialization scale with differentiated factors determining commercial orientation decisions (Moti and Gardebroek, 2008; Adam, 2009; Adane, 2009; Bedaso *et al*., 2012). Most importantly, it is of critical importance to generate up-to-date information on the relationship between smallholder farmers' commercial orientation and productivity. Therefore, this research was done to elicit data on commercial orientation of smallholder farmers and measure synergistic relationships existing between the commercial orientation and total factor productivity in the highlands of eastern and western Hararghe Zones in the Oromia Regional State, Ethiopia.

2. Methodology

2.1. Description of the study area

The study area, Hararghe highlands are situated in the Eastern part of Ethiopia, circumscribed by East and West Hararghe zones, Oromia Regional State and covers about 10% of the total population of highland farming systems in Ethiopia. Oromia is the largest region in terms of population and area coverage. According to the 2012 intercensal population survey projection, it has a total population of more than 31.9 million (CSA, 2012).

Farming system in the East and West Hararghe zones constitute complex production units involving a diversity of interdependent mixed cropping and livestock activities. The known cash crops predominantly produced are *khat* (Catha edulis), coffee, and other crops such as potatoes, onions/shallots and other vegetables. The major

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annual crops grown in the two zones are sorghum, maize, groundnuts, potato, wheat, haricot beans, barley, and so on (CSA, 2008). Cereal production in both zones is mostly for home consumption; only about 5.2% of the produce in East Hararghe, and 4.6% of the produce in West Hararghe were sold in 2008 (CSA, 2009).

The agro-climatic range includes lowlands (locally called *kola* or *gammoji*) with rainfall distribution of less than 700 mm and constituting about 30 to 40%; midlands (*weyna-dega* or *badda-daree*) with rainfall distribution ranging from 700 mm to 1200 mm and constitutes 35 to 45%; and highland (*dega or baddaa*) with rainfall distribution of more than 1200 mm and constitutes 15 to 20% of the whole areas in these zones. There are two rainy seasons in these zones, the short (*belg or badheessa*) rainy season extending from March to May and the main (*meher or ganna*) rainy season extending from June to September (CSA, 2009).

2.2. Data Sources and Sampling Frame

The study was conducted based on data obtained from primary and secondary sources. Secondary data regarding the priority of most important crops, livelihood strategy, population, type of credit and technology available were collected. The primary data were elicited through face-to-face personal interviews using semi-structure questionnaire. Thus, a two-stage sampling procedure was employed to draw sample households for an interview. In the first stage, a random sampling procedure was employed to draw the sample highland districts. Accordingly, two districts from eastern Hararghe Zone and two districts from western Hararghe Zone were randomly selected. In the second stage, a total of eight *kebeles* were randomly selected from the four districts. To determine the sample size, the formula given by Kothari (2004) was used as follows:

$$n = \frac{Z^2 pqN}{e^2(N-1) + Z^2 pq} = \frac{(1.96)^2(0.5)(0.5)(126382)}{(0.05)^2(126382) + (1.96)^2(0.5)(0.5)} \approx 383 \quad (1)$$

Where, n is the sample size; Z is the standard cumulative distribution that corresponds to the level of confidence with the value of 1.96; e is desired level of precision; p is the estimated proportion of an attribute present in the population with the value of 0.5 as suggested by Israel (1992) to get the desired minimum sample size of households at 95% confidence level and ± 5% precision; q=1-p; and N is the size of the total population from which the sample is drawn.

Finally, samples of 385 farm household heads were selected from eight *kebeles* using a random sampling procedure with probability proportional to size as shown in Table 1.

Table 1. Respondent sample households based on districts and *Kebele* administrations.

Sample District			Sample *Kebele*		
Districts	Total households	Sample households	*Kebeles*	Total households	Sample households
Gurawa	38545	117	Raasaa Jannata	803	43
			Leenca	1402	74
Haramaya	34732	106	Daamota	1483	62
			Finqilee	1041	44
Tullo	28832	88	Ifaa Handodee	635	43
			Kufa Kaas	676	45
Habro	24273	74	Haro-Chercher	876	34
			Bareda	1027	40
Total	126382	385		7,943.00	385

Source: Eastern Hararghe and western Hararghe Zones Bureaus of Agriculture and Rural Development, 2015.

2.3. Methods of Analyses

Data analyses were made following three steps indicated below:

(1) Measurement of crop productivity: Index of TFP involving elements of outputs and inputs were defined over gross values of crops output, labor and traction power, rental value of cultivated land and value of purchased inputs (fertilizer, chemicals and seeds) and then estimated by TFP Index Program version 1.0 which is a DOS computer program developed by Coelli and Battese (1998) and a widely used Tornqvist TFP index.

The general equation in its logarithmic form is:

$$lnTFP = ln\frac{O}{I} = lnO\text{-}lnI \quad (2)$$

[

Where, TFP = total factor productivity, O = output index, I = input index.

$$\ln TFP_{io} = \underbrace{\left[\frac{1}{2}\sum_{i=1}^{n}(\omega_i + \omega_{io})(\ln y_{io} - \ln y_i)\right]}_{\text{Output}} - \underbrace{\left[\frac{1}{2}\sum_{i=1}^{n}(v_i + v_{jo})(\ln x_{jo} - \ln x_j)\right]}_{\text{Input}} \quad (3)$$

Where; ω = value share of outputs; v= value share of input; y= output (s) in physical quantities; x= input (s) in physical quantities; i = i^{th} output (n selected crops); j = j^{th} input (human labor, animal traction, land, seed, fertilizer, chemicals); o = observations (sample farm households).

(2) Measurement of crop commercial orientation: Commercial orientation of smallholder farmers is defined in a scale neutral measure adapted from von Braun *et al.* (1994) and Strasberg *et al.* (1999). Based on the proportion of total amount sold to total production, a crop specific marketability

index (α_k) was computed for each crop produced at household level as follows:

$$\alpha_k = \sum_{i=1}^{N} \frac{S_{ki}}{Q_{ki}} \quad ; \; Q_{ki} \geq S_{ki} \text{ and } 0 \leq \alpha_k \leq 1 \qquad (4)$$

Where: a_k is the proportion of crop k sold (S_{ki}) to the total amount produced (Q_{ki}) aggregated over the total sample households in a farming system.

Then, household's market orientation index in land allocation is derived from equation (3) as:

$$MOI_i = \sum_{k=1}^{k=k} \alpha k \frac{L_{ik}}{tL_i} \quad ; tL_i > 0 \text{ and } 0 < moicr_i \leq 1 \qquad (5)$$

Where: MOI_i is market orientation index of the household i, L_{ki} is amount of land allocated to crop k, and $t_{1,i}$ is the total crop land cultivated by the household i.

(3) Establishing the mathematical relationship between commercial orientation and crop productivity: As a strategy, it is worthy to start with commercial orientation index is supposed to be endogenous with TFP. Hence, the mathematical relationship between commercial orientation and TFP is established using two-stage least squares (2SLS) procedure in equations (5) and (6) as follows:

$$Y_i = \alpha_0 + \alpha_1 \hat{C}_i + \alpha_2 X_{1t} + \varepsilon_{1t} \qquad (6)$$

$$C_i = \beta_0 + \beta_1 X_{1t} + \beta_2 X_{2t} + u_{1t} \qquad (7)$$

Where Y_i is productivity (measured as TFP) for agricultural crop production for household i, a_0, a_1, a_2, β_0, β_1 and β_2 are unknown parameters of interest, x_{1t} is a vector of common exogenous variables hypothesized to affect both TFP and market orientation, $\hat{C}_i$ is the predicted value of market orientation index, C_i is market orientation index itself, x_{2t} is a vector instruments for market orientation, ε_{1t} and μ_{1t} are error terms such that $E(\varepsilon_{1t})=0$ and $cov(\varepsilon_{1t}, \mu_{1t})=0$.

Variables description and expected sign of the hypothesized determinants are presented in Annex Table 1.

3. Results and Discussion

3.1. Endogeneity and Instrumental Variable (IV) Estimation Tests

Before the decision to use IV regression to evaluate the effects of market orientation on TFP, the necessary tests for endogenity and instrumental variables (such as tests of endogeneity, under-identification and weak-instruments and over-identifying restrictions) were made. These tests were applied to make sure whether households' market orientation index is simultaneously determined by TFP that usually geared towards markets.

Test results obtained from 2SLS confirmed that the use of IV estimation was assured because the Durbin χ^2 value of 24.61 enables us to reject the null hypothesis that commercial orientation index is exogenous at conventional significance level (p=0.000). Similarly, the robust regression-based test of Wu-Hausman F-statistic of 25.06 does reject the null hypothesis of exogeneity at 1% significant level. Thus, the significant χ^2 and F-statistic results confirmed the assumption that commercial orientation indices and TFP of crops are endogenous.

The under-identification is checked using the Lagrange multiplier (LM) test of whether the equation is identified or not, i.e., the excluded instruments are relevant, meaning correlated with the endogenous regressors. The test is essentially a test of the rank of a matrix. Anderson (1951) canonical correlation test (=52.95) indicated the rejection of the null (P=0.000) and confirmed the matrix is full column rank, i.e., the model is identified.

Sargan score test and Basmann tests of over-identifying restrictions were performed and resulted values of 3.46 (p=0.18) and 3.32 (p= 0.19), respectively predicated the errors being independently distributed. Moreover, Wooldridge's robust score test of over-identifying restrictions was also made and resulted a value of 4.31 (p=0.12) which is insignificant and hence no over-identification is confirmed.

Stock and Yogo (2005) test result of weak-instruments indicated that the value of test statistic (=19.46) exceeds all the critical values of 2SLS relative bias. Thus, we can tolerate a relative bias of 5%, 10%, 15%, 20%, or 25% and conclude the instruments used are not weak. Furthermore, from the result of Stock and Yogo's (2005) second characterization of weak instruments, we can reject the null hypothesis of weak instruments since the value of test statistic (=19.46) exceeds the rejection rate of 10% (=6.46). This assures the instruments are not weak.

In addition to the above tests, diagnostic test for multicollinearity that seriously affects the parameter estimates was conducted among explanatory variables. The results confirmed that multicollinearity is not a problem in the estimated model since the largest VIF test result in the participation model is 2.81 and the Mean VIF is 1.66 (see Annex Table 2).

3.2. Results of the Synergies between Crop Commercial Orientations and TFP

The 2SLS estimation results (as shown in Table 2) assured that farm households' market orientation index, when instrumented by road distance, annual crop income and land allocated to *khat*, strongly and positively influenced TFP. This indicates that households who are more commercial oriented are found to be higher in crop productivity. The reasons behind were commercial orientation may provide a source of cash that allows households to overcome key agricultural production constraints such as purchase of inputs. Further, farm households' participation in increased crop sales would allow them to acquire resources for reinvestment to improve agricultural productivity and obtain income. The result is consistent with Strasberg *et al.* (1999), Govereh and Jayne (2003), and Adam *et al.* (2010).

It is known that the effect of instruments on TFP is expressed through market orientation. Results of first-stage regression (Annex Table 3) indicated that increasing market orientation behavior of farm households through income from sales of food crops leads to improvements in crop productivity. In contrast, allocating more land to *khat* crop and farm distance from residence to the main road did not favor enhanced productivity since it negatively influenced households' market orientation index. Moreover, additional six variables were found to influence total factor productivity of crops beside their contribution to commercial orientation. These factors included the number of oxen owned, market distance from residence, extension visits, amount of manure used, labor used, and location dummy.

Oxen availability, being the main sources of draught power, plays a crucial role in crop production at smallholder level in Ethiopia (Melaku, 2011). Although a pair of oxen is normally required to carry out the normal task of ploughing, oxen ownership patterns were not evenly distributed in the study area. Farm households who did not own oxen might have other ways of getting draft oxen power, such as sharing and/or hiring arrangements so as to cope with the unequal oxen distribution. However, this type of getting draft power might have negative impact on planting time and cultivation operations. Consequently, the results confirmed that farm households who owned higher numbers of oxen had higher crop productivity.

The role of extension services has been to support and facilitate people engaged in agricultural production to obtain information, skills, and technologies to solve problems and to improve the livelihoods and well-beings of farmers (Lerman, 2004; Berhanu *et al.*, 2006). Frequent extension visits in giving technical advice on productivity enhancing inputs encourage farmers to think of acquiring the particular inputs (Adam *et al.*, 2010). The results of this study assured that the coefficient of number of extension contacts was positive and statistically significant, implying that those sample farm households who got large number of extension contacts also experienced improved crop productivity.

Market distance affected crop TFP negatively and significantly. Sample farm households that were located relatively far away from market places are expected to be less productive probably due to their relative inaccessibility to inputs and outputs (Adam, 2009). Concurrent with this postulation, in this study, distance from market was found to be a transaction cost that worked against productivity. Thus farmers that were located relatively far away from the nearest markets were less productive than those that were located nearby. The other important factor that affected crop productivity was labor (in man-days). Labor available for agricultural production affects TFP negatively probably due to the unemployment caused by capacity limitation in access to physical capital (Adam, 2009; Adam *et al.,* 2010). The coefficient of labor used is negative and the result of the current study assured the previous results.

Manure, locally called *dike*, is widely used as means to improve soil fertility and is considered by farmers as one of the major practices that enhance crop productivity in the study areas. The finding confirmed that crop TFP increased with increased use of manure. Furthermore, the agro-ecological variable expressed in the location dummy had a positive and significant influence on the TFP. This implies that farmers in eastern Hararghe highlands are less fortuitous in crop productivity than their counterparts, i.e. farmers in western Hararghe highlands.

Table 2. 2SLS estimation results of factors influencing TFP.

Factors influencing productivity (TFP)	2SLS Estimation	
	Coefficients	Std. Err.
Commercial orientation index	2.756***	0.54
Sex of household head	0.029	0.069
Active members to land ratio	0.011	0.012
Farming experience	0.003	0.003
Non-oxen livestock owned	-0.018	0.016
Number of oxen owned	0.064*	0.035
Education status	-0.014	0.026
Credit use (log)	-0.006	0.008
Off/ non-farm income (log)	-0.004	0.006
Extension contacts	0.041***	0.007
Distance to nearest market	-0.029*	0.016
Amount of manure used	0.004*	0.002
Amount of fertilizer used	-0.001	0.00
Quantity of labor used	-0.299***	0.078
Annual livestock income (log)	0.005	0.006
Location dummy	0.344***	0.056
Constant	1.567***	0.276
Waldχ^2 (16)	162.280	
Prob > χ^2	0.000	
R^2	0.087	
Root MSE	0.396	

*Note: *, ** and *** represent statistical significance of factors at 10%, 5% and 1% levels, respectively.*
Source: Authors computation from sample survey data (2015).

4. Conclusion

The findings of this study indicated that commercial orientation of farm households described by their respective indices of increased volume of crop sales is a requirement for increased crop productivity. This is an indication for households who are more commercial oriented are found to be higher in crop productivity because commercial orientation provides cash that allows households to purchase productivity enhancing inputs. The findings of this study also demonstrated that strategies aimed at improving crop productivity of smallholder farmers in the study area should fully address other determining factors (such as oxen ownership, market distance, extension visits, amount of manure used, and labor used) in addition to commercial orientation. Further, the current study could not

verify the reverse causality of productivity on commercial orientation behavior of farm households instead it suggests this concern for future research outlooks.

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7. ANNEXES

Annex 1. Variables description and expected sign of the hypothesized determinants.

Variable Description	Measurement	Expected sign
Sex of household head	Binary (0- female, 1- male)	+/-
Educational status	Binary (1-literate, 0 otherwise)	+
Farming experience	Continuous (years)	+/-
Commercial orientation index	Continuous (%)	+/-
Active members to land ratio	Continuous (%)	+/-
Off /non-farm income	Continuous (ETB)	+
Income from livestock	Continuous (ETB)	_
Non-oxen livestock owned	Continuous (TLU)	+
Number of oxen owned	Continuous (TLU)	+
Amount of fertilizer used	Continuous (qt/ha)	+
Amount of credit used	Continuous (ETB)	+
Number of extension visits	Discrete (count)	_
Distance to nearest market	Continuous (km)	+
Amount of manure used	Continuous (qt)	+
Amount of labor used	Continuous (Man- days)	+
Annual livestock income	Continuous (ETB)	+/-
Location dummy	Binary (0- East Hararghe, 1- otherwise)	+/-

Note: TLU-Tropical Livestock Unit; qt-quintal; ETB-Ethiopian Birr; km-kilometer; ha-hectare

Annex 2. Diagnostic test for multicollinearity using VIF.

Variable	VIF	1/VIF
Annual crop income (log)	2.81	0.36
Amount of manure used	2.7	0.37
Number of oxen owned	2.29	0.44
Non-oxen livestock owned	2.24	0.45
Quantity of labor used	1.81	0.55
Amount of fertilizer used	1.78	0.56
Location dummy	1.6	0.62
Annual livestock income (log)	1.59	0.63
Farming experience	1.49	0.67
Active members to land ratio	1.43	0.7
Education status	1.4	0.71
Land allocated to khat	1.35	0.74
Distance to nearest market	1.34	0.75
Distance to nearest road	1.34	0.75
Credit use (log)	1.2	0.83
Number of extension visits	1.18	0.85
Sex of household head	1.15	0.87
Off/ non-farm income (log)	1.14	0.88
Mean VIF	1.66	

Annex 3. First-stage regressions result expressing the effect of instruments on market orientation.

Market orientation index	RC	Std. Err.	P>t
Sex of household head	-0.009	0.018	0.607
Active members to land ratio	-0.009**	0.003	0.008
Farming experience	-0.0003	0.001	0.646
Non-oxen livestock owned	0.0002	0.004	0.959
Number of oxen owned	0.009	0.01	0.389
Education status	0.003	0.007	0.669
Credit use (log)	0.0003	0.002	0.858
Off/ non-farm income (log)	0.003**	0.001	0.029
Distance to nearest market	0.002	0.004	0.524
Extension contacts	-0.052***	0.002	0.979
Location dummy		0.013	0.000
Amount of manure used	-0.001*	0.001	0.08
Amount of fertilizer used	0.0001**	0.0002	0.005
Quantity of labor used	0.067***	0.016	0.000
Annual livestock income (log)	0.001	0.001	0.400
Distance to nearest road*	-0.011***	0.003	0.001
Annual crop income (log)*	0.082***	0.023	0.000
Land allocated to khat*	-0.343***	0.066	0.000
Constant	0.860***	0.204	0.000

*Note: RC = Robust Coefficients * indicates instruments; Number of observation = 385; F (18,366) = 21.21; Probability >F = 0.000; R^2 = 0.411; Adjusted-R^2=0.382; Root MSE=0.096*

Short Communication

Effect of Physical Exercise on Physiological Changes and Performances of First Year Students at Haramaya University

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Abstract Physical exercise is important for maintaining physical fitness and contributes positively to maintaining a healthy weight, promoting physiological well-being, and strengthening the immune system. It is a fact that many life threatening conditions can be prevented by regular exercise. This research attempted to investigate the effects of intensified physical training on physiological changes and performance efficiencies on Haramaya University first year sport science students. An informal design (i.e. before and after without control) was applied. Twenty participants were selected from first year sport science department. Ten male and ten female students participated in different physical training programs of varying intensities for 3 consecutive months, i.e. 3 days per week and 60 minutes duration per day. Pre and post training performances and laboratory tests were conducted and analyzed for performance efficiency levels and major physiological changes. Findings of this study revealed a significant effect of physical exercise on cardio vascular endurance, muscular endurance, muscular strength, flexibility and body composition as well as some physiological changes. Based on the findings, it was concluded that intensified physical training had a positive effect on performance and physiological changes of the subjects.

Key words*:* Intensified physical training; Performance efficiency; Physiological changes

1. Introduction

Physical education programs are designed and intended to promote general health and overall fitness. The exact regime of education may vary among programs, but physical education remains critical in achieving an overall healthy society. The main purpose of physical education is the process of becoming physically active for the rest of our lives (Watson, 1983).

Physical exercise is important for maintaining physical fitness and can contribute positively to maintaining a healthy weight, building and maintaining healthy bone density, muscle strength, and joint mobility, promoting physiological well-being, reducing surgical risks, and strengthening the immune system. Exercise reduces levels of cholesterol, which causes many health problems, both physical and mental (Cornil *et al*., 1965). Frequent and regular aerobic exercise has been shown to help prevent or treat serious and life-threatening chronic conditions such as high blood pressure, obesity, heart disease, Type 2 diabetes, insomnia, and depression (Menoutis, 2008).

The beneficial effect of exercise on the cardiovascular system is well documented. There is a direct relation between physical inactivity and cardio vascular endurance, and physical inactivity is an independent risk factor for the development of coronary artery disease. Most beneficial effects of physical activity on cardiovascular disease mortality can be attained through moderate- intensity activity (40% to 60% of maximal oxygen uptake, depending on age) (Stampfer *et al*., 2000).

Intensified physical training on the other hand is a person's ability to perform a specific activity by making more intense, stronger or more marked and by increasing in extent (Brandon, 2009). Exercises are performed with a high level of effort, or intensity, where it is thought that it will stimulate the body to produce an increase in muscular strength and size (Philbin, 2004). It seems clear that physical training is not designed for achieving muscle failure and it should be done in proper sets with repetitions. This training exercise involves a combination of weight and repetitions, which helps in the maximum development of the muscles.

The reason for selecting first year students of sport science department was that: They are considered as beginners. Participating in this research will be a base for them in their future activities in the department. They are going to do a lot of activities related to physical exercise in their ongoing under graduate program in the department. This research lays the foundation of their performance and will help identify their initial level.

The effect of exercise on physiological changes and performance is more visible and noticeable on beginners. This study was designed to examine the effects of twelve week program of strength, endurance

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and flexibility exercises using 3 days per week and 60 minute sessions per day.

The end result of this study may have possible effects on physiological changes and performance efficiency of beginners, the exercise trainers, fitness center users, instructors, participants of the study, and physical education institutions in the country. It may also have great significance in improving student's participation in physical activity and achievement in high performance and quality of lives. The objective of this study is to investigate the effects of intensified physical training on physiological changes and performance efficiency on Haramaya University first year sport science students.

2. Materials and Methods

2.1. Experimental Design

Experimental Design which is a kind of informal design (i.e. before- and- after without control) was used to conduct this research. The participants of this study were the selected students of first year sport science department.

2.2. Data Collection Instruments

The data collection was more quantitative, including a questionnaire/check list, laboratory and performance test results. The use of these principal data collection instruments was intended to explore a range of quantitative information. The Physical activity Readiness questionnaire (from now onwards PAR-Q) was prepared based on reviewing the available literatures on similar studies, journals and other sources. The main purpose of the questionnaire was to select the appropriate subjects who would provide authentic, valid and reliable data to answer the general and specific objectives of this research.

2.3. Procedures of Data Collection

Based on the objectives of the research, the physical activity readiness questionnaire (PARQ) was distributed for 39 volunteer students in the class. But, the researcher selected 20 students (10 male and 10 female students) from the total population (first year sport science students) by considering the PARQ as an inclusion and exclusion criteria. Purposive sampling method was used specifically in the selection process. All selected subjects were at the age of 18 – 25 and they were active participants in different performance and health related exercise training programs. This resulted in physiological change and performance efficiency for three months (12 weeks) of total training, 3 days per week and 60 minutes per session (including warm up, cool down and stretching exercises). The intensity was progressively increased as the subjects adapted themselves to the training.

2.4. Methods of Data Analysis

The data that were collected through field and laboratory tests, before and after intervention, were analyzed and interpreted. The analyses were carried out by the Descriptive Statistical Analysis Code and by using SPSS version 16.0 software to summarize fitness and performance status as well as physiological changes. Calculating measures of central tendency like mean and calculating measures of dispersion like standard deviation were also carried out.

2.5. Ethical Consideration

The study protocol was approved by the Department Graduate Committee, School of Graduate Studies and then by Ethical Review Committee of Haramaya University College of Health Sciences. Information on the study was given to the participants, including purposes and procedures, potential risks and benefits. It was explained that participation was voluntarily and private information was protected. Verbal informed consent form for focus group was obtained.

3. Results and Discussion

An improvement was seen in the participants' health that was related to their physical fitness components (cardio vascular endurance, muscular strength and endurance, flexibility rate and body composition). Because of the physical appearance and conditions of the majority of the participants, the selected exercise types that were designed for the program of intensified physical training were more related with weight gain and strength and relatively endurance activities like aerobic exercises.

Table 1 shows that the mean score of selected subjects step test performance before intensified training is higher than that of after intensified training. This result implies that physical training has a positive effect on the improvement of cardio vascular endurance. The above table indicates that the mean score of push up test of the subjects before training (9.75) is lower than after training (36.05). Slowly increasing the amount of weight and number of repetitions performed gives even more benefits, irrespective of age (Campos *et al.*, 2002).

Table 1 also indicates that the mean score value for sit up test before training (5.35) is much lower than the value after training (37.35). This test measures the strength and endurance of the abdominals and hip-flexor muscles. As the test was repeatedly carried out the muscles became stronger. The effectiveness of an abdominal exercise is dependent upon how well it recruits your stomach muscles. Sit ups on a decline bench and on an exercise ball target your abdominal muscles; however, because of the slight differences in technique, one is more effective than the other (Mikesh, 2012).

Table 1. The Mean change of performance tests before and after intensified physical training (*For N=20).*

Pair	Variables	Values
Pair 1	Step test before training	144.60 ± 17.45
	Step test after training	107.05 ± 12.25
Pair 2	Push up test before training	9.75 ± 6.22
	Push up test after training	36.05 ± 12.79
Pair 3	Sit up test before training	5.35 ± 6.06
	Sit up test after training	37.35 ± 9.12
Pair 4	Sit and reach test before training	6.05 ± 3.83
	Sit and reach test after training	17.75 ± 4.22
Pair 5	Shoulder flexion test before training	4.64 ± 2.33
	Shoulder flexion test after training	9.91 ± 3.83
Pair 6	Body mass index before training	19.89 ± 2.33
	Body mass index after training	20.55 ± 2.36
Pair 7	Waist to hip ratio before training	0.77 ± 0.06
	Waist to hip ratio after training	0.79 ± 0.06

Similarly the mean value for sit and reach flexibility test is higher in the test after (17.75) intensified training than before (6.05). This test only measures the flexibility of the lower back and hamstrings. Rate of this test is highly increased with regular physical training. As table 1, the mean value of shoulder flexion shoulder stretch test before intensified training (4.64) is much lower than the mean value of the test after training (9.91). The improvement of the rate of this test as shown on the data is one indicator of the improvement of the participants' range of motion in joints (flexibility).

Mean value of body mass index also increases after the training (20.55) than before (19.89). One of the major benefits of physical training is that it reduces the risk of obesity. As it is shown in the above table, body mass index is highly affected by physical training. But it depends on the type, duration and intensity of exercise. In case of this research, the designed exercises were more of weight gain which was done by free weight materials. That is why the level of body mass index shows an improvement.

The mean value of white blood cell (WBC) increased in the after intensified training (7.25) than before training (5.73). When there is regular exercise, these cells increase their numbers and circulate more quickly through the whole body. If exercise becomes too much or too heavy, increased activity by the white blood cells can improve the ability to fight off viral and bacterial infections (Mikesh, 2012).

The mean value of red blood cell (RBC) count before training was also lower than the mean value after training. The above table also shows that the mean value of hemoglobin (HGB) count in the test before the training (14.42) is lower than the count which was held in the test after the training (16.14). Exercise training can increase total HGB and red cell mass, which enhances oxygen-carrying capacity. The possible underlying mechanisms are proposed to come mainly from bone marrow, including stimulated erythropoiesis with hyperplasia of the hematopoietic bone marrow, improvement of the hematopoietic micro environment induced by exercise training, and hormone- and cytokine-accelerated erythropoiesis (Hu and Lin, 2012).

The bellow table 2 shows that the mean value of hematocrit before training (45.91) is lower than the value of it after the intensified training (48.17). The increase of the number of hematocrit is because of the increasing of red blood cells.

The mean value of platelet before the training is also lower than the mean value after the training. In single sessions of resistance exercise, such as weight-lifting, increase circulating platelet levels immediately after exercise. However, such changes are not lasting, with a 21-week resistance training program having no effect on baseline platelet levels (Bobeuf *et al*., 2009).

Table 2. Paired Samples Statistics for Laboratory Test Results *(For N=20).*

Pair	Tests	Value
Pair 1	Pre training WBC (mg/dl)	5.73 ± 1.51
	Post training WBC (mg/dl)	7.25 ± 2.23
Pair 2	Pre training RBC(mg/dl)	4.80 ± 0.41
	Post training RBC (mg/dl)	5.55 ± 1.09
Pair 3	Pre training Hgb test (g/dl)	14.42 ± 1.72
	Post training Hgb test (g/dl)	16.14 ± 3.16
Pair 4	Pre training Hct test (mg/dl)	45.91 ± 4.44
	Post training Hct test (mg/dl)	48.17 ± 7.92
Pair 5	Pre training PLT (mg/dl)	315.95 ± 99.50
	Post training PLT (mg/dl)	357.65 ± 66.22
Pair 6	Pre training creatinine test	0.80 ± 0.30
	Post training creatinine test	1.22 ± 0.19
Pair 7	Pre training albumin test	47.96 ± 7.20
	Post training albumin test	46.43 ± 6.80
Pair 8	Pre training triglyceride test	80.59 ± 20.91
	Post training triglyceride test	77.59 ± 20.92
Pair 9	Pre training uric acid test	5.61 ± 1.60
	Post training uric acid test	3.51 ± 1.14

As table 2 indicates, the mean value of creatinine before training is slightly decreased in the test after training. The mean value of albumin is increased in the test after the training than the test before the training. These results indicate that increased albumin synthesis after intense upright exercise contributes to the maintenance of greater plasma albumin content. Moreover, the impact of exercise on the control of albumin synthesis is modulated by posture (Haskell *et al*., 1997).

In case of triglyceride, increase in mean value also shows in the test after training than the test before. Triglycerides are fats in the blood. It can reduce the levels by physical exercise because burning fat stored in the body cuts the fat levels in the blood. But in case of this research, the reason behind the increase of the mean value of triglyceride in the test after training is that the exercises that were designed were more of weight gain activities for training (Banach *et al*., 2005).

The above table also shows that the mean value of uric acid before the intensified training is higher than the value after training. It shows that the proper nutrition and exercise will lower uric acid levels to help prevent gout.

4. Conclusions and Recommendations

Based on the major findings of the study, intensified training has a significant effect on the improvement of health related physical fitness components. Most of the increase in muscle size form training is the result of an increase in the size of the muscle fibers, and not an increase in their number. The changes of amount of RBC, WBC, HGB, HCT, PLT, creatinine, albumin, triglyceride and uric acid is an indicator for the physiological changes as a result of intensified physical training.

Considering the major findings, it was recommended that, the exercise training program needs to be long term and the nutritional status of participants should be emphasized for more beneficial from intensified training in all dimensions (physiological, psychological and sociological). In addition, long term effect of intensified physical training for health related and performance skills, more physiological system change needs to be adopted.

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