Abundance and Diversity of Arbuscular Mycorrhizal Fungi (AMF) in Soils under Different Rangeland Use Types in the Middle Awash Basin, Ethiopia

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Abstract

Background: Shrinking of rangelands/natural grasslands has led to various inter-communal conflicts and loss of livelihoods among pastoralists in Ethiopia. Restoring the rangelands is an important step to be taken to alleviate the problem. Various aggressively spreading invasive weed species are threatening the existence and sustainability of rangelands/natural grasslands. In addition, moisture stress is a major hindrance to any efforts made to restore the rangeland/natural grasslands. The role of Arbuscular mycorrhizal fungi (AMF) in establishing plant seedlings and regenerating natural grasslands through enhancing water and nutrient uptake is well documented.

Objective: The main objective of this study was to investigate the influence of land use and soil types on abundance and diversity of AMF in semi-arid rangeland areas of the Middle Awash Basin in the Ethiopian Rift Valley region.

Materials and Methods: Representative rhizosphere soil samples were taken from different land use types (cultivated, open grassland, shrubland, and prosopis-invaded land) and used to assess AMF diversity and abundance in relation to soil physical and chemical characteristics. The same soil samples were used to establish trap cultures for spore formation and colonization assessment.

Results: In the present study, morphological analysis from field soils and trap cultures revealed 16 distinct morphotypes belonging to 10 genera including *Glomus* (3), *Claroideoglomus* (3), *Funneliformis* (2), *Rhizophagus* (2) and one from each of *Acaulospora*, *Entrophospora*, *Gigaspora*, *Sclerocystis*, *Scutellospora* and *Septoglomus*. Spore abundance significantly varied, ranging from 265–481 and 319–488 (100-g^{-1}) in trap culture and field soil samples, respectively. Grassland soil samples displayed the highest spore abundance, followed by soil samples from shrubland and cultivated fields, with the lowest records from prosopis-invaded land use type. However, sporulation and level of colonization were higher in cultivated lands, which have lower spore abundance than open grass and shrublands.

Conclusion: The results of the study indicated that converting land use from traditionally managed rangelands/grazing system to cultivated lands leads to encroachment by *Prosopis* spp. on the lands and significantly reduces AMF spore abundance, diversity, and percentage root colonization. Thus, the ever-expanding encroachment of prosopis on the open grasslands and croplands around the Awash River necessitates implementing strict measures to decrease pressure on the soil biota underneath.

Keywords: Mycorrhizal inoculum potential; Open grassland; *Prosopis juliflora* invaded land; Shrub land; Trap cultures

1. Introduction

Arbuscular mycorrhizal fungi are one of the obligate mutualistic symbionts that form association with more than 80% of the plant species in the terrestrial ecosystem (Smith and Read, 2010). They are known to facilitate uptake of biologically essential plant nutrients such as phosphorus (P), nitrogen (N) and other micronutrients and improve water relations under stress conditions (van der Heijden *et al.,* 1999). AMF also induce plant resistance to drought stress, salt stress, give protection against pathogens and pests and reduce plant sensitivity to toxic substances in the soil (Siddiqui and Futai, 2008), enhance ecosystem sustainability through rapid response to degradation or any other stress (Smith and Read, 2010). A number of studies have documented that inoculation of AMF into degraded soil improve soil structure (and accelerate the establishment of native grasses (Brundrett *et al*., 1996; Smith and Read, 2010).

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ISSN 1993-8195 (Online), ISSN 1992-0407(Print)

The world is currently in a period of rapid, natural and human-induced driven environmental changes brought about by land cover changes, fragmentation, invasive species, and pollution (MEA, 2005). This fragmentation of natural systems leads to land degradation (LD) which is a global long-term loss of ecosystem function and productivity (Naseer and Pandey, 2018).

Land use changes in East Africa have transformed natural land covers, mainly rangelands to farmlands and human settlements and urban centers at the expense of natural vegetation and loss of biodiversity (Maitima, 2009). Many studies have shown that encroachment into woody plant species affect soil moisture, carbon storage, soil biochemistry and biological diversity (Richter and Stutz, 2002; Almaz Kebede*,* 2009).

The continuous land degradation can be reversed through "ecological restoration" which is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed, and to realize the environmental and socioeconomic benefits of natural resources (Hobbs & Cramer, 2008). It is established that the focus on indigenous plant species in natural vegetation restoration programs could return the previous species composition within a few years (Siddiqui and Futai, 2008; Ndoye *et al.,* 2012). However, reseeding alone is often not sufficient in restoration of degraded natural grassland areas.

One of the mechanisms that enable plants to survive in moisture-stressed environment is their ability to form a mutualistic association with soil microbes. Among the soil microbes, arbuscular mycorrhizal fungi (AMF) which occur in moisture-stressed environments form associations with plants and enhance water and nutrient uptake due to their rapid response to ecosystem changes (Siddiqui and Futai, 2008; Smith and Read, 2010). However, there is poor understanding of the impact of land use intensity and change on the abundance and diversity of AMF in tropical soils (Tachabi *et al*., 2008; Xiang *et al.,* 2014).

In Ethiopia, the arid and semi-arid rangelands covering 61-65% of the territory in the country are home to 12–15% of the human population and 26% of the total livestock population (Mohammed, 2009). However, the transformation of semi-arid grassland ecosystems into human settlement and farming has caused shrinkage of grazing land, imposed high stocking rate of animals and triggered continuous land degradation and bush encroachment in the area (Almaz Kebede*,* 2009; Mohammed, 2009).

For instance, the Middle Awash areas of central-eastern Ethiopia faced accelerated social and ecological change due to feed scarcity, intermittent drought, and land degradation (Solomon Beyene *et al.,* 2014). Over the past 30 years, there have been land use and land cover changes in the area. The change in land use was due to agricultural expansion, encroachment by aggressively spreading invasive alien plant species such as prosopis, and overgrazing (Almaz Kebede, 2009). Accordingly, assessing the composition and status of AMF species and diversity is important, as it would provide vital input for any management and restoration plans by the relevant stockholders.

Despite their importance, few studies to date have assessed the diversity of AMF in Ethiopia. Most of the studies have assessed AMF diversity and abundance in relation to dry woodland trees (Emiru Birhane *et al.,* 2010; Zerihun Belay *et al.,* 2013), coffee and shade trees (Tadesse Chane and Fasil Assefa, 2013), agroforestry and crop land ecosystems (Beyene Dobo *et al.,* 2016) and high land grazing areas with terraces (Emiru Birhane *et al*., 2017). However, there is dearth of information on the diversity and abundance of AMF in semiarid rangeland/grassland areas of central-eastern Ethiopia, and the impact of land use change on their pattern and composition. Understanding of the AMF status and studying the connection between land use legacies, thus, becomes the first step to design better management and restoration strategy (Soka*et al*., 2015; Muchane *et al*., 2012; Emiru Birhane *et al*., 2017). Therefore, we conducted research to address the following questions. Does land use type affect AMF abundance and diversity in semi-arid rangelands? How do soil physical and chemical properties influence AMF diversity? By answering these questions, we intended to provide a novel insight into AMF diversity in semi-arid rangelands of Ethiopia and how it could contribute to sustainability of rangelands/natural grassland. The information obtained can serve as a basis for designing appropriate rangeland resource management and conservation practices.

Thus, the aims of this study were to (1) examine the influence of land use type/cover on the diversity and abundance of AMF in the middle awash basin of the Ethiopian Rift Valley; (2) examine AMF diversity, soil physical and chemical properties and their relations to different land use types in semi-arid rangeland areas of Ethiopia; and (3) to examine the mycorrhizal inoculum potential (MIP) of the soils from different land use types.

2. Materials and Methods

2.1. Description of Study Area

The study was conducted in four land use types in the middle awash basin of the Ethiopian Rift Valley (Figure 1). The basin is located at 90^{o} 16' N and 400^{o} 9' E, and categorized as semiarid rangeland with altitude ranging from 500–752 meters above sea level (Chekol and Mnaluk, 2012). The rainfall is bimodal with mean annual rainfall of 277–653 mm with the main rainy season extending from July to September, and short rainy season from February to April. The mean daily minimum and maximum temperatures are 18 and 34 ^{O}C, respectively (Tessema Zewdu *et al.,* 2012).

The mean annual total evaporation ranges from 1810 to 2348 mm. The soils of Middle Awash basin are derived from an alluvial (deposition of streams) and

colluvial process (weathering of bedrock materials) associated with fluvial process at one time or another (Wondimagegne Chekol and Abere Minalu, 2012)

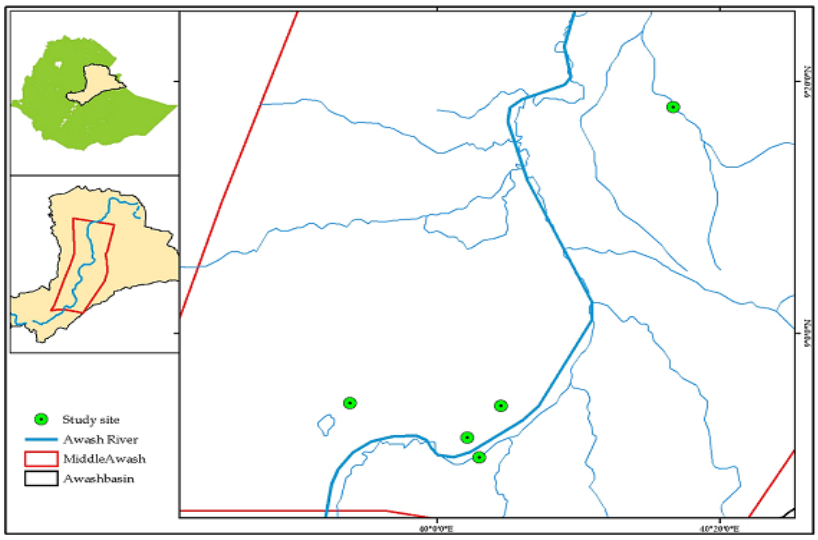

Figure 1. Map of the study area and sampling locations.

There are three land uses, namely, protected area (Awash National Park), large-scale irrigation farming and traditional use by the indigenous pastoral and Agro-pastoral communities. In addition, vast areas of grazing land have been seriously encroached by the invasive plant *Prosopis juliflora* (Almaz Kebede, 2009).

Table 1. Geographical positioning and description of study sites.

Land use [a]	Location	Altitude	Dominant plant species
OGL	08°54'15.2" N 040°04'32'.1" E	1040 m.a.s.l.	*Cenchrus ciliaris, Cenchrus setigerus, Chrysopogon plumulosus, Ischaemum afrum Ocimum canum, Panicum coloratum, Sporoblus ioclades,*
PIL	09°17'57.2"N 040°16'42.5" E	960 m.a.s.l.	*Acacia senegal, Prosopis juliflora, Sporob ioclades,*
CL	08°54'26.7"N 039°53'52.5" E	1022 m.a.s.l.	Sorghum, maize and onion
SL	08°51'42.7"N 040°02'09.1" E	808 m.a.s.l.	*Acacia melifera, Acacia senegal, Chrysopogon plumulosus, Dobera glabra, Grewia bicolar,*

Note: [a] OGL = Open grassland; PIL = Prosopis-invaded land; CL = Cultivated land; and SL = Shrubland; m.a.s.l = meters above sea level.

2.2. Experimental Design

Fieldwork was conducted in October 2018 after the end of the long rainy season in the Middle Awash Basin of the Ethiopian Rift Valley system. Soil samples were collected from four land use types, namely; Open grass, Shrub, Prosopis invaded and Cultivated lands. A total of 24 samples (4 land use type X six replicates of soil samples from each land use) were collected at a depth of 0–20 cm.

2.3. Soil Sample Collection

Six replicates of sampling points (25m apart) were established for each land use type and soil samples were collected at the depth of 0–20 cm. The six soil samples from a land use were mixed and a composite soil sample was drawn. The samples were sealed in sterile polyethylene bags, transported and stored at 4°C in a refrigerator for further studies. Composite soil sample from each land use was partitioned into three sets of sub samples. The first set of soil sample was used to establish bioassays; the second set of soil sample was used for AMF spore analysis; the third set of soil sample was used to analyze soil physicochemical properties.

2.4. Analysis of Soil Physical and Chemical Properties

Soil physical and chemical properties were analyzed following standard procedures. Soil texture, pH, Total N, available phosphorus (P), and soil organic matter (OM). Soil texture was determined using Bouyoucos' hydrometer method (Day, 1965). Soil pH was measured

by glass electrode in a 1:2.5 soil: water suspension (Ziadin, 2007). Total N was determined by Kjeldahl method following the procedure described by Hinds and Lowe (1980). Available phosphorus (P) was extracted with a solution of 0.5 M $NaHCO_3$ at pH 8.5 (Olsen and Sommers, 1982). Soil organic matter (OM) was analyzed using the dichromate oxidation method (Walkley and Black, 1934).

2.5. Establishment of Trap Culture

Trap cultures were established from fresh field soils collected from the four land use types to induce sporulation of fungi present in the soil. Composite soil samples containing root fragments from each land use type were mixed with autoclaved sand (1:1; v/v) and accommodated in 15cm plastic pots to serve as a substrate. Five surface-sterilized (using 0.5% sodium hypochlorite solution for 15 minutes) sorghum seeds (*Sorghum bicolor*) were sown in each pot (three pots per land use type) and thinned down to three after establishment. They were placed in a greenhouse in completely randomized design under natural light condition; mean morning/midday/night temperatures of 15/30/21^{o}C and watered daily as needed. Watering was reduced during the final weeks to maximize spore production. At the end of 4 months, the plants were cut near the base, and the cultures were air dried and checked for the presence of spores.

2.6. Determining Mycorrhizal Inoculum Potential (MIP) of the Soil

Bioassay was performed to determine mycorrhizal inoculum potential of soil samples from different land use types according to Brundrett *et al.* (1996). Thus, 400g of soil samples with root fragments were put into plastic pots. Sorghum (*Sorghum bicolor*) seeds were surface sterilized and sown as before on five pots per land use type and they were then thinned down to one per pot after emergence and placed in a greenhouse in completely randomized design under natural light condition for 45 days. Roots were harvested and preserved in 50 % ethanol and stored at 4^{0}C for further assessment of root colonization.

2.7. Root Staining and Quantification of AMF Root Colonization

Roots were washed carefully with tap water and cut into segments of about 1 cm long and put in a test tube (15 ml) containing 10% (w/v) KOH and heated at 90°C in a water bath for 1 hour to be bleached. Thereafter, the roots were washed to remove the KOH and treated with 10% HCl (v/v) for 15 min at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90°C for 30 min in a water bath (Brundrett *et al.*, 1996). Fungal colonization was quantified using the magnified intersection method (McGonigle *et al.*, 1990) under a compound-light microscope at a magnification of x 200. Thus, 100 root segments were mounted on microscope slides to examine the presence and percentage colonization of AMF structures; hyphae, arbuscule, and vesicle.

2.8. AMF Spore Extraction and Identification

AMF spores from field soil and trap cultures were isolated using the wet sieving and decanting method (Gerdemann and Nicolson, 1963), followed by sucrose gradient technique (Brundett *et al.*, 1996). Hundred grams of dry soil sample was soaked in 1000 ml water and left for 5 minutes to settle soil particles and was decanted through 500 μm, 250 μm, 100 μm and 45 μm sieve layers. The contents left in 250–45μm sieves were collected in a test tube and suspended in water and centrifuged at 2000 RPM for five minutes, and the supernatant was decanted.

The soil materials in the test tubes were re-suspended in a 50% sucrose solution and centrifuged at 2000 RPM for one minute. Then, the supernatant containing the spores was poured over a 45-μm size sieve and washed with tap water thoroughly to remove the sucrose and transfer the spores to a Petri Dish. The AMF spores were counted under a dissecting microscope (ISO 1006) at x4 magnification. Enumeration of spore numbers per gram of dry soil was undertaken according to INVAM, *http://invam.caf.wvu.edu*. Thereafter, healthy looking spores with similar morphology were picked and mounted on slides in polyvinyl-lactic acid-glycerol (PVLG) or in PVLG mixed with Melzer's reagent (1:1 v/v), and examined under the compound microscope [NOVEX Holland] at x400 – 1000 magnification (Brundett *et al.*, 1996). Species identification and matching of morphotypes were based on original descriptions and identification manuals of Schenck and Perez (1990), online references of species descriptions provided by INVAM (2004) (*http://invam.caf.wvu.edu*), West Virginia University, USA, University of Agriculture in Szczecin, Poland (http://www.zor.zut.edu.pl/Glomermycota), Schüßler and Walker (2010) and the Schüßler AM fungi phylogeny website (http://www.lrz.de/~schuessler/amphylo/).

2.9. AMF Diversity and Density

Spore abundance of Arbuscular mycorrhizal fungi was expressed as the number in 100 g soil. Isolation frequency (IF): number of samples in which a given species was observed as the percentage of total soil samples for each land use type, relative abundance of spores (RA) and the importance value (IV) were calculated to determine the dominance or rarity of AMF species. Diversity of AM fungi among the land use types was determined using the Shannon Wiener diversity index (H') calculated by the formula:

$$H' = \sum_{i=1}^{K} (P_i \ln P_i)$$

Where, P_i= the proportion of individuals in the i^{th} species and ni = the number of species with i individuals. Pielous' evenness index was also used to measure species evenness (J) using the formula J = H/log(S): where, H = observed Shannon index; S= total number of species in the habitat. The Simpson dominance index (D) was calculated using the formula:

$$D = 1/\sum_{i=1}^{S}(n_i / N)^2:$$

Where, n_i = the total number of individuals in a species i; N is total number of species in the sample and S is the number of species.

2.10. Data Analysis

Pearson correlation coefficient was performed to figure out the relationship between AMF root colonization, spore density (SD), richness (R) and soil parameters. Significant differences among land use types in SD, R and soil parameters were examined using one-way ANOVA while mean separation was done using Tukey's HSD multiple comparison test at *P* values < 0.05. All statistical analyses were performed using R 3.6 software.

3. Results

3.1. Land Use and Soil Physicochemical Properties

The soil physical and chemical analyses showed that all land use types have a loamy soil textural class. The soils are alkaline ranging from 7.67 for shrub land (SL) to 8.84 for OGL land use type). Soil organic carbon and total nitrogen content were significantly higher in grazing areas (OGL & SL) than in CL and PIL land use types (Table 2). The data also showed that cultivated land (CL) contained significantly higher available P (25.6 ± 0.35) and K (117.37 ± 0.55) content compared to the others.

Table 2. Physical and chemical properties of experimental soils in four different land use types of the Middle Awash Basin, Ethiopia.

Soil Parameter	Land use type					
	Open grassland	Prosopis invaded	Cultivated land	Shrub land	F value	P value
pH	8.84 ^{a} ±0.05	8.52^{ab} ±0.23	7.87 ^{bc}±0.05	7.67 ^{c} ±0.05	55	0.000
EC dsm^{-1}	0.51^{b} ± 0.01	1.21 ^{a} ± 0.02	0.33 ^{C} ± 0.02	0.31 ^{C} ± 0.01	2816	0.000
OC %	2.7^{a} ± 0.04	1.91^{b} ± 0.02	2.10^{b} ± 0.12	2.35^{ab}± 0.31	14.77	0.001
TN (%)	0.18^{ab} ± 0.04	0.13^{c} ± 0.01	0.17 ^{b} ± 0.00	0.20 ^{a} ± 0.00	50.65	0.000
C/N	15.1^{a} ± 0.36	15.3^{a} ± 1.88	12.62^{ab} ± 0.52	11.97^{b} ± 1.5	5.628	0.022
P (mg/kg)	6.27 ^{b} ± 0.03	5.85 ^{b} ± 0.79	25.62^{a} ± 0.35	1.44 ^{c} ± 0.01	1888	0.000
Ca	22.10^{a} ± 0.63	12.04^{b} ±1.49	9.57^{c} ± 0.02	11.36^{b} ±1.03	382.4	0.000
Mg	4.41^{b} ± 1.11	4.86^{a} ± 1.03	3.82^{c} ± 0.83	4.8^{ab} ± 0.62	26.16	0.0001
Na	1.46^{a} ± 1.93	0.37^{c} ± 0.52	0.52^{b} ± 0.47	0.42^{c} ± 0.01	1325	0.000
K (mg/kg)	82.97^{c} ± 0.48	95.14^{b} ±0.15	117.37^{a} ±0.55	62.73^{d} ± .61	6733	0.000
CEC	25.12^{a} ± 0.83	18.57^{b} ±0.40	15.62^{c} ± 0.37	16.99^{bc}±0.04	214.8	0.000
Sand (%)	54.3 ^{d} ± .58	58.2 ^{c} ± .06	68 ^{a} ± 1.00	62.1 ^{b} ± .08	304.3	0.000
Clay (%)	31^{a} ± 0.00	19.2^{b} ± 0.61	13.3^{d} ± 1.53	17.7^{c} ± 1.15	169.4	0.000
Silt (%)	15.33^{d} ± 0.58	23.61^{a} ±0.51	19.2b^{c} ± 0.26	21.17 ^{b}± .21	289.8	0.000

Note: [a]Ca, Mg, Na in cmolc/kg; C/NC = ratio; CEC in meq/100g; Means in the same row followed by same letter do not differ significantly at P < 0.05 probability level.

3.2. AMF Root Colonization

The percentage hyphal, arbuscular, and vesicular colonization on the sorghum plant established on soils from the four land use types ranged from 18.6% - 34%, 1.6%–5.0% and 0.6%-3.7%, respectively (Table 3). The land use types also showed different patterns of AMF structural distribution. Cultivated land displayed the highest percentage of hyphal (34%) and arbuscular (5.0%) colonization while shrub land showed the highest vesicular colonization (3.7%) on the roots of sorghum plants. There were significant differences in levels of hyphal and vesicular colonization. However, post hoc Tukey's HSD test did not show significant difference (*P* = 0.25) in hyphal colonization level between OGL and SL.

Table 3. Mean percentage colonization of mycorrhizal structures in samples of roots from sorghum trap plant (MIP across land use types).

	Percentage of colonization		
Land use type	Arbuscules	Vesicles	Hyphae
Open Grass Land (OGL)	3.0 ± 2.6	1.7 ^{b} ± 0.6	25.6 ^{bc} ± 2.3
Prosopis Invaded Land (PIL)	2.3 ± 1.0	0.6 ^{b} ± 1.2	18.6 ^{c} ± 4.2
Cultivated Land (CL)	5.0 ± 2.64	2.7 ^{ab} ± 0.5	34 ^{a} ± 3.1
Shrub Land (SL)	1.6± 2.1	3.7 ^{a} ± 2.1	29 ^{b} ± 4.0
	F = 2.02; P = 0.19	F = 8.57; P = 0.007	F = 2.02; P > 0.05

Note: Means followed by different letters in each column denote significant difference at P < 0.05.

3.3. AMF Spore Density in Field Soils and Trap Cultures

The data showed OGL displayed the highest mean spore count (488 ± 8.5) while, CL land contained the lowest (319 ± 5.1) mean spore count per 100g of field soil (Figure 2). The impact of land use types on AMF spore density was significant (F = 15.6, P<0.05). Sporulation in AMF was variable amongst the land use types where OGL and PIL induced a smaller number of spores with trap cultures and CL and SL contained more spores than their respective from field soils. In fact, no significant (F = 0.001, P = 0.97) difference was observed in the overall spore density between trap culture and field soil from different land use types. The number of spores recovered from trap culture from SL and CL were 14.3% and 25.8% higher than their respective field soil, respectively (Figure 2). However, trap cultures established from OGL and PIL soils were not efficient and number of AMF spores recovered were 25% and 28.1% less than the field soil, respectively.

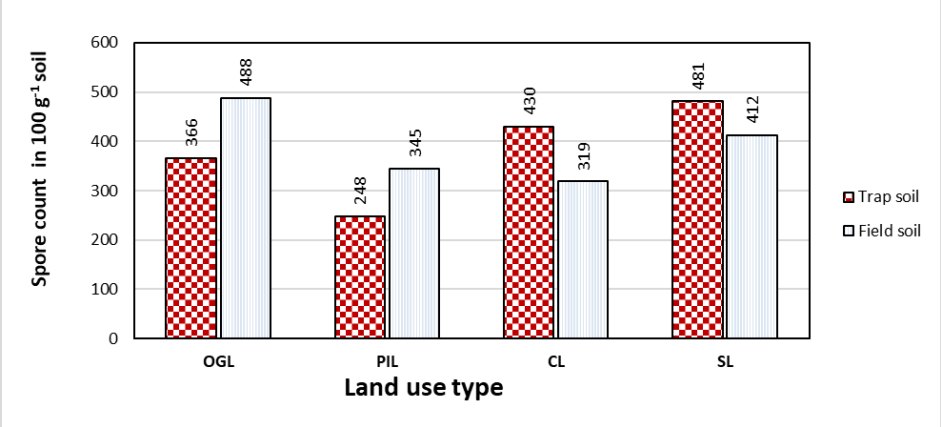

Figure 2. AMF spore density in field and trap culture.

The data did not show significant correlation between spore abundance and the occurrence of mycorrhizal structures [hyphal (r = 0.19, P = 0.53), arbuscular (r = –0.14, P = 0.66) and vesicular (r = 0.33, P = 0.28)] colonization percentages. The Pearson correlation coefficient showed that AMF spore density from field soil was correlated to TN (r = 0.8, P< 0.05), CEC (r = 0.63, P< 0.05), Ca (r = 0.71, P< 0.05), Na (r = 0.72, P< 0.05) and OC (r = 0.86, and P< 0.05) of the soil characteristics.

However, spore density did not show any correlation with and soil P, pH and Mg content (Table 4). Spore density in trap culture was not correlated to most of the parameters except soil total nitrogen content (r = 0.88, P< 0.05).

Table 4. Correlation between AMF spore density [100 g^{-1}] and some soil parameters in different rangeland use types in the Middle Awash basin, Ethiopia.

Soil parameter	Spore density (SD)			
	Field soil		Trap culture	
	R	*P* value	r	*P* value
Ca	0.71	0.00	-0.19	0.55
CEC	0.63	0.02	-0.30	0.33
Mg	0.87	0.05	-0.33	0.28
Na	0.72	<0.05	-0.01	0.95
N/C	-0.08	0.79	-0.75	<0.05
P	-0.39	0.6	0.16	0.83
pH	0.13	0.68	-0.71	<0.05
TN	0.78	<0.05	0.88	<0.05
K	-0.65	0.02	-0.27	0.37
OC	0.86	<0.05	0.34	0.26
EC	-0.58	0.04	-0.95	<0.05
Sand	-0.45	0.13	-0.52	0.08
Clay	0.68	0.01	-0.265	0.404
Silt	-0.72	<0.05	-0.263	0.408

Other soil physical properties such as K, pH, EC, N/C, and silt also indicated negative relation with spore density, while soil clay content had significant positive correlation (r = 0.68, and $P< 0.01$) with spore density in field soil, but sand content was negatively correlated to AMF spore density in trap culture.

3.4. AMF Species Composition in Different Land Use Types

In the present study, morphological analysis from field soil and trap culture revealed the presence of 16 distinct morphotypes, belonging to 10 genera (*Acaulospora, Claroideoglomus, Entrophospora, Funneliformis, Gigaspora, Glomus, Rhizophagus, Sclerocystis, Scutellospora and Septoglomus*) (Table 5). Four of the morphotypes (*Acaulospora*, *Glomus species* 1& 2 and *Entrophospora*) could not be identified to species level. The genus *Glomus* represented the most abundant group accounting for 33.6% of the total genera, followed by *Claroideoglomus* (23.4%), and *Funneliformis* (20.5%). The remaining 6 genera account for 22.5%, of the morphotypes and two morphotypes: belonging to *Gigaspora* and *Entrophospora* constituted to less than 1% of the total genera.

With regard to species diversity, the dominant genus *Glomus* included three species; but only one morphotype (*Glomus hoi*) was identified at the species level. Similarly, the genus *Claroideoglomus* contained three species including *Claroideoglomus claroideum, Claroideoglomus etunicatum* and *Claroideoglomus lutum*. Two genera *Funneliformis* and *Rhizophagus* contained two species (*Funneliformis mosseae*, *Funneliformis geosporum* and *Rhizophagus aggregatus*, *Rhizophagus fasciculatus*, respectively).

Table 5. Relative abundance of AMF genera in field soil and trap culture of the different rangeland use types in Middle Awash basin, Ethiopia.

AMF genera	Trap culture		Field soil		Total	
	SD	RA	SD	RA	SD	RA
Acaulospora	24	5.43	23	4.90	47	5.16
Claroideoglomus	109	24.66	104	22.17	213	23.38
Funneliformis	96	21.72	91	19.40	187	20.53
Gigaspora	4	0.90	-	0.00	4	0.44
Glomus	138	31.22	168	35.8	317	33.6
Entrophospora	-	0.00	3	0.64	3	0.33
Rhizophagus	-	-	11	2.35	-	1.21
Sclerocystis	28	6.33	31	6.61	59	6.48
Scutellospora	20	4.52	19	4.05	39	4.28
Septoglomus	23	5.20	19	4.05	42	4.61

Note: SD = spore density per 100 g^{-1} soil and %RA = relative abundance.

The data also showed that the most frequently isolated AMF species in field soil and trap culture samples of all land use types (100%) were *Calarodium caloradum*, *Funuliform mossae* and *Glomus* sp. 2. The other frequently isolated from three of the four land use types (75%) AMF were, *Acaulospora* sp., *Glomus* sp.1, and *Sclerocystis sinuosa*. The other morphotypes such as *Septoglomus constrictum, Scutellospora pellucida,* and *Claroideoglomus lutum* were isolated from two of the four land use types. The isolation frequency of the three top AMF species: *Claroideoglomus claroideum, Funueliformis mosseae* and *Glomus* sp. 2 from trap culture were 31%, 30.6%, 30.0%, respectively, whereas *Claroideoglomus claroideum* (30%), *Funueliformis mosseae* (33.3%) and *Glomus* sp.1 (29.3%) were the most frequently isolated species from field soil samples (Table 6).

On the basis of importance value (IV), three AMF species, *Funneliformis mosseae* (57.6), *Claroideoglomus claroideum* (56.8), and *Glomus* sp.1 (50.4) were dominant in the field soil samples. Similarly, three species *Claroideoglomus claroideum* (60.6), *Funneliformis mosseae* (59.2) and Glomus sp. 2 (57.6) were dominant in trap culture. Five species and three species were categorized as common species in field soil and trap culture, respectively. Of the fourteen species isolated from field soil samples, four species were categorized as rare of which *Rhizophagus fasciculatus*. *Rhizophagus aggregatus*, *Glomus hoi,* and *Entrophospora* sp. failed to appear in trap culture. Interestingly, *Glomus* spp.1 which was dominant in field soil became common in trap culture, while, *Glomus* spp. 2 which was common in field soil become dominant in trap culture.

3.5. AMF Richness and Diversity

AMF species richness in field soil was in the range of 5 –11. The highest mean AM fungi species richness per field soil sample was recorded for OGL, followed by SL, FL and PIL (Table 7). However, in the case of trap culture, the highest mean AM fungi species richness per field soil sample was recorded in SL (10), followed by CL, OGL and PIL. The lowest number of morphotypes (5) was recorded from PIL use type. The number of morphotypes in field soils of OGL and PIL use types decreased in number compared to their trap culture counterpart. For instance, four AMF species (*Rhizophagus fasciculatus, Glomus hoi* and *Entrophospora* from OGL and *Rhizophagus aggregatus* from CL field soils) failed to appear in their respective trap cultures. However, two new morphotypes, *Gigaspora gigantica* and *Funuliform geosporum*, were recovered from trap cultures of CL and SL land use types, respectively. The AMF *Claroideoglomus etunicatum* was isolated only from SL field soil and trap culture samples.

Table 6. Isolation frequency, relative abundance and important value of AMF in field soil samples.

AMF species	Trap culture					Field soil				
	IF	RA	IV	Status	Occur	IF	RA	IV	Status	Occur
Acaulospora sp.	8.00	19.3	13.7	Com	CL, OGL	7.67	18.9	13.9	Com	CL,OGL,SL
Claroideoglomus claroideum (Schenck and Smith,2010)	31.0	88.6	60.6	Dom	All	30.00	81.1	56.8	Dom	All
Claroideoglomus etunicatum (Becker and Gerdemann; Walker and Schussler, 2010)	3.00	6.5	4.7	Rare	SL	3.00	7.1	5.2	Rare	SL
Claroideoglomus lutum (Stutz and Morton; Walker and Schüßler, 2010)	2.67	8.9	5.8	Rare	OGL, PI	1.67	3.2	2.5	Rare	OGL, PI
Funneliformis geosporum (Nicolson and Gerdemann; Walker and Schüßler, 2010)	1.67	3.6	2.6	Rare	SL	-	-	-	-	-
Funneliformis mosseae (Nicolson and Gerdemann; Walker and Schüßler, 2010)	30.66	86.1	59.2	Dom	All	30.33	82.1	57.6	Dom	All
Gigaspora gigantea (Nicolson and Gerdemann; Gerdemann;and Trappe, 1974)	1.33	3.3	2.3	Rare	PI	-	-	-		
Rhizophagus aggregatus (Schenck andSmith.1982)	-	-	-	-	-	2.00	5.7	4.1	Rare	CL
Rhizophagus fasciculatus (Gerdemann andTrappe,1974)	-	-	-	-	-	1.67	3.2	2.5	Rare	OGL
Glomus hoi (Berch andTrappe, 1985)	-	-	-	-	-	3.00	5.8	4.5	Rare	PI
Glomus sp.2	30.00	79.6	57.6	Dom	OGL,CL,PI	24.00	62.3	43.7	Com	All
Glomus sp.1	17.33	44.1	29.7	Com	OGL,CL,PI	29.33	68.1	50.4	Dom	CL,OGL,SL
Entrophospora sp.	-	-	-			1.00	1.9	1.5	Rare	OGL
Sclerocystis sinuosa (Gerdemann and Bakshi, 1976)	9.33	28.6	19.0	Com	OGL,CL,SL	10.33	26.8	18.9	Com	OGL,PI,SL
Scutellospora pellucida (Nicolson and Schenck; Walker and Sanders, 1986)	6.67	23.2	14.9	Com	CL,SL	6.33	20.1	13.6	Com	PIL,CL
Septoglomus constrictum (Silva and Oehl, 2011)	7.67	18.5	13.1	Com	OGL, SL	6.33	13.5	10.2	Rare	OGL, SL

Note: CL = cultivated land, OGL = open grassland, PI = prosopis invaded land, SL = Shrubland, IF = isolation frequency, RA = relative abundance, IV = importance value, Occur = occurrence, Dom = dominant, and Com = common.

However, two new morphotypes, *Gigaspora gigantica* and *Funuliform geosporum*, were recovered from trap cultures of CL and SL land use types, respectively. The AMF *Claroideoglomus etunicatum* was isolated only from SL field soil and trap culture samples.

Figure 3. Some AMF species identified form the samples: A) *Claroideoglomus claroideum* B) En*trophospora nevadensis* C) *Septoglomus constrictum* D) *Gigaspora gigantea* E) *Rhizophagus aggregatus*; F) *Glomus species (Brown)* G) *Glomus hoi* H) *Glomus* spp. (Red brown; thick wall) I) *Calarodium ethonicatum*.

Furthermore, ecological measures of diversity such as species richness, Simpson dominance, Shannon and Pielou indices showed variation of AMF species among land use types (Table 7). The data showed that the highest AMF species richness was recorded from OGL with Shannon Weiner diversity index of H'= 2.1, and the lowest species richness for PIL (H'= 1.51) from field soil.

Table 7. Species richness, Shannon and Pielous evenness indices of AMF in different rangeland use types of the Middle Awash basin, Ethiopia.

Land use	Richness		Shannon H'		Simpson D		Evenness J	
	Field	Trap	Field	Trap	Field	Trap	Field	Trap
Open grass land (OGL)	11	7	2.16	1.83	0.87	0.82	0.90	0.94
Prosopis invaded land (PIL)	5	5	1.51	1.54	0.75	0.77	0.94	0.96
Cultivated land (CL)	7	8	1.87	1.89	0.84	0.83	0.96	0.91
Shrub land (SL)	8	10	1.92	2.08	0.84	0.85	0.93	0.91

Nevertheless, in the case of trap cultures the result was vice versa; soil samples from SL land use type showed the highest AMF species richness with Shannon Weiner diversity of H'= 2.08, followed by OGL (H'= 1.83).

4. Discussion

4.1. AMF Spore Abundance in Field Soils and Trap Cultures

The average AMF spore density from field soil and soil culture showed significant variation across land use types differing in vegetation cover and intensity of use. The difference in the average AMF spore density was in the range of 319 in PIL and 488 in the OGL per 100g of soil (Figure 2). Spore densities were higher in less disturbed natural ecosystems than in disturbed ones (cultivated and invaded). This is similar to the report that indicated the conversion of semi-arid rangeland to other land use type decreases AMF spore abundance because of the sensitivity of AMF to soil biochemical changes (Guo *et al*., 2008).

Alien species encroachment or change in vegetation (from OGL to PIL or SL) alters AMF spore density and infectivity potential. The AMF spore count recorded in this study was lower than the mean spore count of 994 per 100g soil according to Zerihun Belay *et al*. (2013) who reported their study results on a similar environment in central Ethiopia. In fact, 4790 spores per 100g of soil was reported from different land use types in the semi-arid area of Senegal near to Serengeti (Soka *et al*., 2015). However, 35–130 spores per 100g of soil were reported from different land use types of the semi-arid Taita-Tavveta district in Kenya (Jefwa *et al*., 2012); from semiarid wooded grassland in western Ethiopia (Yonas Yoannes and Fasil Assefa, 2007) and from soils of grazing areas of Northern Ethiopia (Emiru Birhane *et al*., 2017) also reported 58 and 176 spores per 100g soil; mean spore count 13.6 per 100g soil was also reported from different land use types in Kenya (Muchane *et al*., 2012)

In this study, spore yield was variable, and there was no correlation (r = 0.19, P = 0.53) between field soil spore density and AMF sporulation in the culture. Trap cultures established from CL and SL were better and produced more spores compared to those from field soils of the same land use types. However, sporulation of AM fungi is soils form OGL and PIL inv were poor and produced fewer spore than those from field soils of the same land use types. Interestingly, a soil from CL showed the lowest spore abundance next to PIL, but rate sporulation and level of colonization was far better than OGL and SL areas (Figure 2). The high sporulation rate in trap cultures from CL may be attributed to adaptability of AMF in CL which has been accustomed to different farming disturbances or implies irrigation water used for cultivation was stressful to AMF sporulation than the tap water used in the greenhouse; whereas lower sporulation rate in PIL may also be due to the loss of host plants or host plant specificity (Smith and Read, 2008; Muchane *et al.,* 2012).

4.2. AMF Root Colonization

In this study Arbuscular mycorrhizal infectivity potential varied across land sue types (Table 3). The percentage of hyphal root colonization in the present study was between 18.6 and 29% (Table 3). The lowest colonization value was recorded from soil samples of PIL and the highest colonization was from CL. The percentage root colonization was similar to the ones reported from various land use types in Ethiopia (19.4% – 53.7%) (Zerihun Belay *et al*., 2015) and in Sorghum trap culture in Kenya (18.8% – 45%) (Muchane *et al.,* 2012) Although, soil from cultivated land (CL) displayed the highest level of mycorrhizal infectiveness in *sorghum* bioassay, it actually harbored lower number of spores than OGL and SL land use types.

These findings are consistent with other reports in which infectivity of soil from CL showed the highest mycorrhizal colonization levels (Zerihun Belay *et al*., 2015), particularly in low input monocropping farming systems (Muchane *et al.,* 2012). However, there were contrasting reports by Beyene Dobo *et al*. (2016), and Jefwa *et al*. (2012). Pearson correlation analysis revealed that no significant correlation existed between AMF spore density and percentage of hyphal and vesicular colonization, which is consistent with other reports (Beyene Dobo *et al*., 2016; Zerihun Belay *et al*., 2015).

4.3. Relationship between AMF Spore Abundance and Soil Physicochemical Properties

This study showed a relation between land use types and the soil physicochemical properties (Table 2). Pearson correlation coefficient showed that AMF spore abundance showed significant positive correlation with soil OC, Ca, Na and TN (Table 4) but not with P, N/C and EC. Thus, the observed variation in this study might be due to other edaphic or environmental factors (Jefwa *et al*., 2012; Soka *et al*., 2015).

AMF spore density showed no significant correlation with sand and clay contents of field soil. Root colonization showed significant negative correlation with silt content in field soils (Table 4). Hyphal colonization showed significant positive correlation with vesicular colonization (Table 8). In contrast, arbuscular colonization had no significant relationship with vesicular (r = 0.2, *P* = 0.53) and hyphal colonization (r = 0.33; *P* = 0.28). This result is in line with Zerihun Belay *et al*. (2015). No significant correlation was observed between spore abundance and percentage colonization by mycorrhizal structures [hyphal (r = 0.19, *P* = 0.53), arbuscular (r = -0.14, *P* =

0.66) and vesicular (r = 0.33, *P* = 0.28)] in field soils (Table 8). In contrast, in trap culture, the spore abundance was positively correlated with vesicular (r = 0.84, P<0.05) and hyphal (r = 0.81, 0.001) colonization.

Table 8. Pearson correlation coefficient between AMF spore abundance and colonization (%).

Parameters	Arbuscules		Vesicles		Hyphae	
	r	*P*	r	*P*	R	*P*
AMF spore abundance in field soil	-0.14	0.660	33	0.28	.19	0.53
AMF spore abundance in trap culture	0.13	0.665	0.84	<0.05	0.81	0.001
Arbuscules	--	--	0.20	0.52	0.33	0.28
Vesicles	--	--	--	--	0.64	0.02

4.4. AMF Composition

Simpson dominance index values similarity analysis showed difference in AMF species composition, where the highest index of dominance for AMF in SL as compared to the other land use types indicates lower number of shared dominances of AMF species. However, the lowest Simson index values [(0.75) in field soil and (0.77)] in trap culture from PIL land use type showed higher number of shared dominance (uniformity in the abundance of species) than the other land use types. The highest J values 0.96 and 0.94 in filed soil samples of CL and PIL show uniform distribution of species, which indicated how cultivation and encroachment by invasive plants negatively affect rare AMF spp. composition. Other studies also showed that land use cover and management have a strong impact on AM fungi morpho types by modifying their composition and pattern (Ndoye *et al*., 2012; Xiang *et al.,* 2014).

The diversity study showed that the AMF were diversified into nine genera and sixteen species (Table 5, 6). Some of the species identified in the field were not recovered in the trap culture. *Funneliformis, Claroideoglomus*, and *Glomus* were the dominant genera that were also reported by other researchers from Ethiopia (Beyene Dobo *et al*., 2016; Zerihun Belay *et al*., 2015) and Kenya (Muchane *et al*., 2012 and Tachabi *et al*., 2008). Of the 14 spp. identified in the field soil, *Funneliformis mosse, Claroideoglomus claroideum*, *Glomus* spp 2 were distributed in all land use systems and categorized as generalists, whereas four species *Rhizophagus fasciculatus* (4.5) *Rhizophagus aggregatus* (4.0%), *Glomus hoi* (2.5%), and *Entrophospora* (1.5%) were described as rare species. However, the three species: *Claroideoglomus claroideum*, *Funneliform mosse* and *Glomus* sp. 2, showed high isolation frequency (59.3, 58 and 54.0%, respectively) in trap culture. This phenomenon shows their capability to sustain in the prevailing biotic and abiotic change in the region, as well as their potential to adapt to new environmental conditions. Thus, these species could be selected as candidates for inoculum development initiative.

4.5. AM Richness

In this study, the highest AMF richness was observed in OGL followed by SL; while relatively lower and lowest values were recorded for cultivated land and PIL, respectively. Two new species were recovered in the trap culture soil but not in field soils; and some species, which were recorded in field soils failed to appear in trap culture. For instance, four species failed to appear in trap culturewhereas two new species were identified in this study. The result suggested that different environmental and management conditions in the field and greenhouse might be the cause for the absence and presence of AM spores between field soil and trap culture samples as reported earlier by Xiang *et al.* (2014) and Leal *et al.* (2018). In fact, many studies have indicated higher proportion of AMF sporulating under field conditions were unable to sporulate under greenhouse conditions (Antoniolli *et al*., 2002; Muchena *et al*., 2012). This phenomenon emphasizes the need for trap culturing and subsequent isolation of spores to reinforce AMF species survey obtained by direct isolation from field soils (Brundrett *et al*., 1996; Siddiqui and Futai, 2008).

AMF species richness recorded in this study was lower than that reported by other studies conducted in semi-arid areas worldwide; 43 recorded in Brazil (Sturmer and Siquueira, 2011), 42 in different land use types in Ethiopia (Zerihun Belay *et al.* 2015) and 29 under different plant and soil properties in Southern Ethiopia (Beyene Dobo *et al.* 2016). Nonetheless, the species richness was comparable to other studies from tropical regions elsewhere in the world. Soka *et al.* (2015) recorded 9 species in the Serengeti ecosystem, Tanzania; Jefwa *et al.* (2012) reported species richness of 12 from seven land use types in Kenya; Antoniolli *et al.* (2002) reported 12 from different land use types in southern Australia. The differences may be attributed to sampling intensity (Brundett *et al*., 1996), isolation technique (Leal *et al*, 2018) and identification methods (Smith and Read, 2010). Moreover, the large variation in spore density and species richness of AMF in different rhizosphere can be attributed to other factors such as soil disturbance (Tachabi *et al*., 2008), ecological degradation (Muchena *et al*., 2012) complex

underground structure (van der Heijden *et al.,* 1999), and host-preference (Antoniollil *et al*., 2002).

4.6. AMF Species Diversity

The variations in Shannon Wiener diversity index observed in this study reflect the substantial impacts of land use type on AMF diversity. Open grassland harboring highest number of AMF species as compared to the other land use types had higher H' value. Actually, traditionally managed ecosystems (OGL and SL) showed higher species diversity in field soil samples, respectively; whereas in trap cultures the higher H' values were recorded in SL and CL, respectively (Table 7). Leal *et al.* (2018) showed that land use type and condition have enormous impact on AMF diversity in field soil as well as in trap culture. AMF diversity in areas where there is less human and animal interference shows higher richness and diversity. The relatively higher AMF species richness and Shannon Weiner diversity (H'= 2.16 and 1.96, respectively) observed in OGL and SL than in CL and PIL could be taken as an evidence to support the argument. Soil disturbance caused by cultivation and overgrazing followed by invasion negatively affected AMF diversity. Lower Shannon Weiner diversity indices were recorded in PIL and CL lands. The high available P and tillage practice (Smith and Reads, 2010; Xiang *et al*., 2014) might be the cause for the lower AMF diversity in CL land use type; whereas in PIL, overgrazing coupled with degradation followed by prosopis invasion or land use change (from open grass land to thick shrubland) could be the cause for the lower AM fungi diversity (Siddiqui and Futai, 2008; Xiang et al., 2014).

5. Conclusion

This study has demonstrated that the Arbuscular Micorrhizea (AMF) community structure of the four land use types in semi-arid soils of Middle Awash basin is variable and mainly characterized by low abundance and diversity. However, the results revealed that open grassland and shrub land, which have been managed by pastoralists for centuries, harbor more species with higher spore number than the newly cultivated and prosopis-encroached areas, but sporulation and trap plant colonization is higher in soils from cultivated lands. The use of brackish water for irrigation has increased available soil P and K and this will inevitably alter physical and chemical properties of the soil, and thereby AMF abundance and diversity. This effect could have a significant impact on the productivity of mechanized farms in the study area as well as in the lower Awash basin. Two AMF species, namely, *Claroideoglomus claroideum* and *Funneliformis mosse* were found to be the most ubiquitous in all land use types and the most sporulating ones in *Sorghum* trap cultures. The two AMF species are recommended as potential candidates for their bio-inoculum production. Furthermore, the ever-expanding prosopis encroachment on the open grassland and cultivated land around Awash River necessitates implementing strict measures to decrease pressure on the soil biota underneath and enhance sustainable use of rangeland resources.

6. Acknowledgments

We thank Haramaya University and the Ethiopian Ministry of Science and Higher Education for funding the research. We also thank Haramaya University, Adama Science and Technology University, and Addis Ababa University for allowing us to use their greenhouse and laboratory facilities. We are very much indebted to Dr. Tesfaye Araya for fetching laboratory chemicals from abroad for the research.

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