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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Ethnobotanical study of indigenous knowledge of plant-material culture in Masha and Yeki Districts, Southwest Ethiopia

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Plants have been used throughout human history as a source of food, medicine and material culture. Several studies on plant material culture from Ethiopia, were limited to a certain geographical areas and ethnic groups. The purpose of this study was, therefore, to investigate the plants and associated indigenous knowledge of three ethnic groups (Shekacho, Sheko and Mejengir), residing in the Masha and Yeki Districts of Sheka Zone, southwest Ethiopia. A total of 80 informants between the ages of 20 and 80 were selected by the help of local administrators and knowledgeable elders. Ethnobotanical data were collected through semi-structured interview, guided-field-walk and field observation. Simple statistical methods such as percentage, ranking and comparison were applied for data analysis. A total of 113 plant species distributed in 91 genera from 48 botanical families were reported by informants of three ethnic groups used in making plant-based material culture. Of the 19 use categories recorded, the highest (69; 49.3%) were reported by Shekacho followed by Mejengir (38; 27.1%), and Sheko (33; 23.6%) ethnic groups. Preference ranking and direct matrix exercises on selected plants used for construction and as multipurpose indicated the highest preference of people for *Arundinaria alpina* and *Cordia africana* followed by *Ficus ovata, Baphia abyssinica* and *Cyathia manniana.* According to priority ranking, agricultural expansion was identified as the most destructive factor of forest plants, followed by illegal wood harvest and construction. The current study recommends the establishment of plant material culture centre. *Ex situ* and *in situ* conservation measures should be taken in the study area for sustainable use of plant resources and preservation of indigenous knowledge.

Key words: Ethnobotany, preference ranking, direct matrix ranking, paired comparison, Ethiopia.

INTRODUCTION

Plants have been used throughout human history as a source of food, medicine and material culture (Schlereth, 1982; Balick and Cox, 1996; Sophia, 2005). Plant material culture varies enormously and depends on the

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availability of plant species, specific environmental condition, and indigenous knowledge of a particular ethnic group (Cunningham, 2001; Terashima, 2001; Tika and Borthakur, 2008). In developing counties, the diversity of plant species remain vital in material culture for making homesteads, agricultural tools, fences, household furniture and/or utensils amongst the others (Parezo, 1996; Wiersum, 1997; Turner, 2000; Choudhary et al., 2008; Hattori, 2006). According to Cotton (1996), plant-based material culture of a given society refers to the total range of objects produced from the plants by that society including functional items such as tools, shelter and clothing, as well as more decorative arts and crafts. A range of plant extracts and exudates have provided pigments, dyes, resins and adhesives which have fulfilled a variety of functional and aesthetic requirements (Dunkelberg, 1992; Johnson, 1992; Miller and Tilley, 1996; Aumeeruddy and Shengji, 2003). For instance, Cotton (1996) mentioned that fibrous stem, roots and leaves have also provided materials for basketry, cordage and textiles; specific types of wood have been used for both construction and manufacturing of a wide range of tools, toys and small utensils; seed and flowers have been used in making necklaces and ceremonial garments.

Ethiopia is a land of topographical and climatic diversity suitable for the distribution of diverse plant taxa (Gebra-Egzabher, 1991). There are about 6,000 species of vascular plants in the country, out of which 10% are endemic (Gebra-Egzabher, 1991). Ethiopia has also diverse ethnic groups and varnishing cultures that possess a wealth of knowledge in the utilization of plants in material culture (Bahru et al., 2012). Although, there is a high utilization of in-county produce and imported synthetic industrial products in Ethiopia, much of the rural human population still depends on plant-based material culture due to a number of factors including the accessibility, economic affordability, and cultural acceptability (Hadera, 2000; Tamene et al., 2000; Jotte, 2007; Institute of biodiversity conservation (IBC), 2008; Abera, 2013).

The dependence of plant-based material culture by three ethnic groups (Shekacho, Sheko and Mejengir) could partly be attributed to underdeveloped infrastructures and expensive industrial products (Gemedo-Dalle, 2004; Anonymous, 2008). Unless the indigenous knowledge on plant-based material culture is documented and the potential plant species conserved both plant and knowledge sources, the knowledge could be lost forever especially in the current fast-growing and expansion of agriculture, urbanization and modern life style that leads to more acculturation. It is surprising that only two studies (Bahru et al., 2012; Abera, 2013) have been reported on plant-based material culture limited to a specific geographical location and ethnic groups in Ethiopia. Furthermore, due to the remoteness of the Masha and Yeki Districts and lack of well-established infrastructure, the indigenous knowledge of Shekacho, Sheko, Majengir ethnic groups remained unexplored.

Therefore, this study aimed to assess and document the plant species, and associated indigenous knowledge and practices related to plant material cultures among ethnic groups in the Sheka Zone, Southern Nations, Nationalities and Peoples Regional State (SNNPRS), southwest Ethiopia. The study was expected to play a role in prioritizing plants used in the manufacture of material culture in the districts for further evaluation and conservation.

Description of the study area and people

Sheka Zone is located in the Southern Nations, Nationalities and Peoples Regional State (SNNPRS), southwest Ethiopia. Sheka Zone covers about 2387.54 km^2 [(Sheka Zone Finance and Economy Development Department (SZFEDD, 2012)]. The administration center of Sheka Zone is located 676 km southwest of Addis Ababa. Geographically, the Zone lies between 7°24'-7°52' N latitude and 35°13'-35°35' E longitude and consists of three districts, namely the Masha, Andracha and Yeki (Figure 1). The Zone is bordered by Oromia Regional State to the North, Gambella Regional State to the West, Kaffa Zone to the East and Bench Maji Zone to the South. In total, there are 45 rural and 2 urban Kebeles (Kebele-The least administrative hierarchy in Ethiopia) in the two districts; Masha (town name, Masha) and Yeki (town name, Teppi) consist of 21 and 26 Kebeles, respectively.

According to Central Statistical Agency, CSA (2009), the total population of Sheka Zone was 226,090 residing in urban and rural areas with 114,661 males and 111,429 females. Of the total population of Sheka Zone (226,090), 27,406 (12.12%) live in Andracha District (14,000 males, 13,406 females), 122,469 (54.16%) in Yeki [(62,333 (50.89%)] were males and 60,136 (49.10%) were females and 37,983(16.79%) live in Masha District [(18,660 (49.12%)] were males and 19,323 (50.82%) were females. The rest 29,540 (13.06%) and 8,692 (3.84%) live in Tepi and Masha town administrations, respectively (CSA, 2009). Of these, the study was conducted in Mesha and yeki Districts.

There are different ethnic groups in the Sheka Zone including Shekacho, Sheko, Mejengir, Kafficho, Amhara, Oromo and Guraghe. The first three are indigenous peoples. According to Central Statistical Agency (CSA) (2009), the ethnic composition of Sheka Zone is 34.7% Shekacho, 20.5% Kafficho, 20.5% Amhara, 9.6% Oromo, 5.0% Sheko, 4.8% Bench and 2% Mejengir. These people do have their own culture, language, and life styles.

The Shekacho and Sheko were from Omotic and Mejengir from Nilotic language origins. This study focused on two selected districts (Masha and Yeki) of Sheka Zone, inhabited by three major ethnic groups

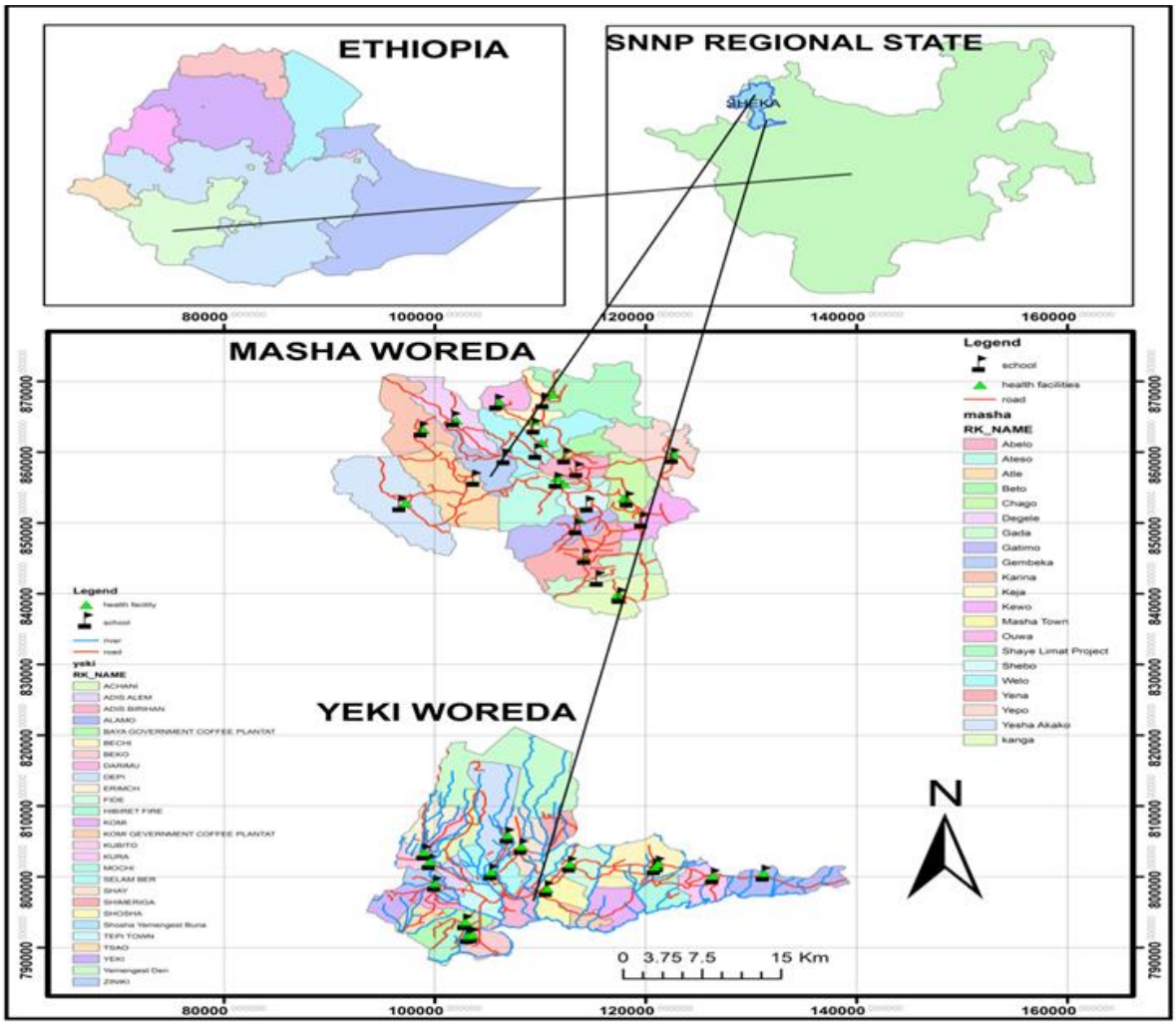

Figure 1. Map of the study districts.

namely Shekacho, Sheko, Mejengir [(Central Statistical Agency, (CSA, 2009)].

MATERIALS AND METHODS

Ethnobotanical inventory and informant selection

This study was conducted in eight Kebeles (Achani, Addis Alem, Bechi, Kubeto, Depi, Kura, Kubero and Yeki) of Yeki and seven kebeles (Abelo, Beto, Chago, Gatimo, Masha, Yena and Yego) of Masha districts inhabited by three indigenous ethnic groups (Shekicho, Shako and Mejengir) between December 2011 and March, 2012. Prior to data collection, an official letter was received from Jimma University Ethical Review Committee (ERC) while verbal informed consent was obtained from each informant who was participating during the study period. In addition, proposal write-up was guided under the supervision of advisor and co-advisors, evaluated and endorsed by two examiners and graduate committee of the College of Natural Science after public defense. The informants who participated in the current study were identified and selected with the help of Kebele leaders, Developmental Agents (DAs) and knowledgeable elders. A total of 80 informants (50 males and 30 females) between the ages of 20 and 80 were identified, of whom 50 were randomly selected from the community by asking every individual in the house and working fields (30 from Shekicho and 10 each from the rest). The rest 30 informants were key informants (20 from Shekacho ethnic group and 10 from the rest two ethnic groups based on the number of population). An interview was conducted in the presence of principal investigator (translating the questionnaire from English to Amharic) and then by three local knowledge translators. Data were collected based on a checklist of questions translated to the local languages of the three ethnic groups (Shakinono, Shekogna and Mejengiregna). Essential information about the plants such as local name, growth forms, and plant parts used were recorded during field survey whereas degree of management (wild/cultivated), and other related ethnobotanical data (plants which were employed in the manufacture of particular items for construction, as a source of handcrafts and arts) were documented by asking informants.

Specimens collection and identification

Voucher specimens were collected, preserved, pressed and dried

for identification. Preliminary identification was done in the field by using manuals and unidentified specimens were identified using herbarium materials, experts, and taxonomic keys in the various volumes of the flora of Ethiopia and Eritrea (Edwards et al., 1995, 1997, 2000; Hedberg and Edwards, 1995; Hedberg et al., 2006). The collected specimens with voucher numbers, family, species, vernacular names, dates and sites of collection were recorded and deposited at the Jimma Herbarium (Jimma University) and National Herbarium (Addis Ababa University).

Preference ranking

Martin (1995) noted preference ranking (PR) techniques as useful for gathering information on the different needs, feelings and priorities of different categories of individuals within a community and a numerical value was assigned to each item. Preference ranking was conducted for nine plant species out of the total plants selected based on their cultural value, strength, durability and their ability to protect themselves from termites. Of 30, 15 representative key informants were randomly selected to identify the best-preferred plant species for house construction and house hold articles. Each informant was provided with nine plants reported to be used for these purposes and asked to assign the highest value (9) for plant species best preferred, against these materials and the lowest value (1) for the least preferred plant and in accordance of their order for the remaining ones. These values were summed up and ranks were given to each plant species. In addition, PR was also applied to determine and rank the threat factors for the plant species in the study area following the same procedure stated above for nine plant species.

Direct matrix ranking

Direct matrix ranking technique was conducted for eight chosen multipurpose species out of 113 (Appendix 1) based on their cultural value, strength, durability and their ability to protect themselves from termites and seven use-categories from each ethnic group in order to evaluate their relative importance to the local people and the degree of the existing threats related to their use values (Martin, 1995). Thereafter, the plant species were listed for 15 randomly selected key informants to assign use values to each species. Each chosen key informants were asked to assign use values (5 = best, 4 = very good, 3 = good, 2 = less used, 1 = least used and 0 = not used). Using numerical scale in which the highest number is equal to the most preferred item whereas, the lowest to the least one. Then the informants were asked to rate their preferences. Finally, the values of each species were summed up and ranked. For the Mejengir and Shako ethnic groups, the ranking was done together because the most useful plants used in both of them were similar.

Paired comparison

Pair comparison was used for evaluating the degree of preference of 8, 5, 5 selected plants by Mejengir Shekacho and shako ethnic groups, respectively. Based on the information given by each ethnic group, availability of the plant species in the area, cultural value, strength, durability and their ability to protect themselves from termites for house construction. In this study, 15 selected informants of each ethnic group were asked to choose the best item from every pair according to personal perception. A list of pairs of selected items with all possible combinations was made and sequence of the pairs and the order within each pair was randomized and presented to selected informants following Martin (1995) and their responses were recorded and total scores were summed using the following formula:

$$\frac{n\,(n-1)}{2}$$

n = the number of medicinal plants being compared.

Data analysis

Ethnobotanical data were analyzed using both quantitative and qualitative methods as recommended by Martin (1995) and Cotton (1996). Direct matrix ranking, preference ranking, and paired comparison were used to analyze the data. All the data was compiled, organized and entered into SPSS version 16 for Windows and a descriptive statistics (percentage and frequency) were computed to describe the ethnobotanical information on the plants used for material cultures, associated knowledge and conservation. The qualitative data obtained from the focus-group discussion was subjected for thematic analysis. Finally, all the results were presented in tables and figures.

RESULTS

Plants diversity in material culture

In this study, a total of 113 plant species were recorded, being distributed in 91 genera and 48 botanical families. The family reported with the highest number of plant species was Rubiaceae (11 species, 9.7%), followed by Euphorbiaceae (8 species, 7.1%) and Astraceae (7 species (Appendix 1). Of 113 plants species used for material culture, 47(41.6%) were tree species, 28(24.8%) Trees/shrubs, 15 (13.3%) herbs, 13 (11.5%) climbers, 9 (7.9%) shrubs and 1 (0.9%) was a fern (Figure 2).

Source of plants used in material culture

Of the 113 useful plant species reported, 93 (82.3%) species were obtained from wild, 9 (7.9%) species were found in cultivated fields and home-gardens, and 11 (9.7%) species were obtained from both wild and cultivated fields (Figure 3).

Plant part(s) and mode of preparation

A total of 13 plant parts were reported to be used to produce various kinds of plant-based material objects in Masha and Yeki districts. The most widely used plant part for the preparations of materials was the stem, which accounts for (86; 62.8%) followed by leaves (20; 14.6%) and branch (6; 4.4%) (Table 1). However, the same plant parts (stem, leave and branch) were also reported to be used for a number of materials while various parts of a plant may be used for a single material. As a result, the total number of plant species increased from 113 to 137 (Table 1). Out of the preparation methods, a large

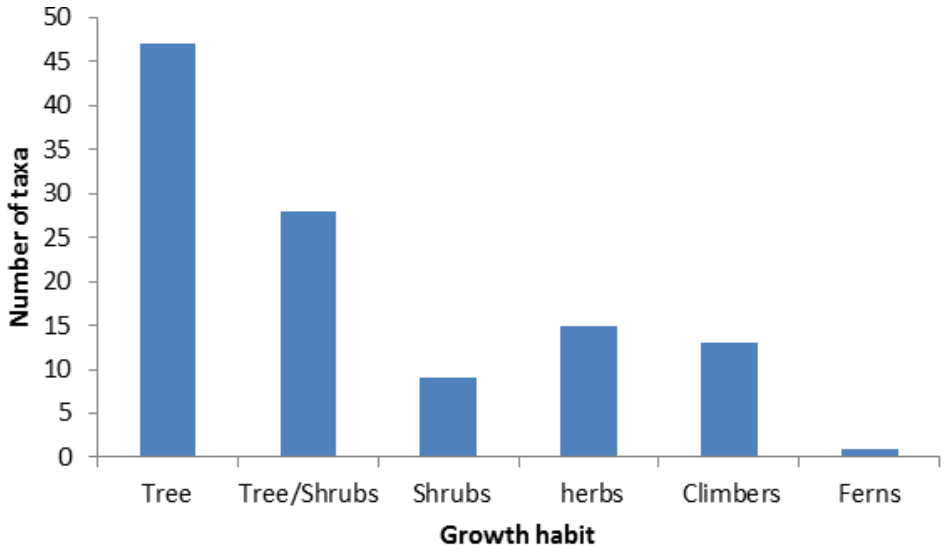

Figure 2. Growth habits of the plant species.

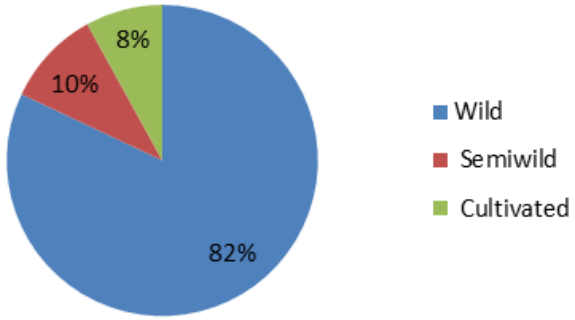

Figure 3. Source of plants used in plant-based material culture in the study area

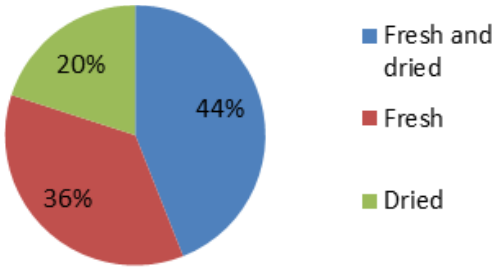

Figure 4. Plants condition used in the preparations of plant-based material culture.

number (59; 49.6%) of plants were reported to be prepared by carving followed by wrapping (15; 12.6%) and splitting (9; 7.6%) (Table 2). The use of similar preparation methods for a single plant increased the total number of plant species from 113 to 119 (Table 2).

Plants condition

A large number (44; 4%) of plants were identified to be used in dried and fresh forms in material preparations. Relatively few plants (41; 36.3%) were used in fresh form and the rest plants 23 (23; 20.4%) were reported to be used as dried forms (Figure 4). As informants indicated a substance like cold water, ash, clay and animal dung was reported to be used for wetting, washing and decoration during the preparation of materials.

Comparison of plant material culture among ethnic groups

The result of this study indicates that the three investigated ethnic groups (Shekacho, Sheko and Mejengir) are entirely dependent on plant-based material cultures for local kitchen utensils and/or household furniture, agricultural tools, fences, and musical instruments and others. Although there are some common plant species and plant material cultures reported by three ethnic groups (due to the similarity of the topography and cultural knowledge), this study also revealed that there is a significant difference ($P<0.05$) in the type of plant used and plant-based material culture between Shekacho and the rest but with no significant difference ($P>0.05$) between Sheko and Mejengir ethnic groups (Table 3). Of 19 uses recorded in this study, Shekacho ethnic group contributed a significantly higher (49.3%) as compared to 27 and 23.5% reported by Mejengir and Sheko ethnic groups, respectively. The difference is highly observed not only on the numbers but also on the kind of plant-based material cultures produced by these ethnic groups. For example, different

Table 1. Plant part used in preparation of plant material culture.

Plant part (s) used	Number of plant species	Percentage (%)
Stem	86	62.77
Leaf	20	14.6
Branch	6	4.4
Twig	5	3.6
Fruit	4	2.9
Root	4	2.9
Bark	4	2.9
Inflorescence	2	1.5
Culm	2	1.5
Latex	1	0.7
Pseudostem	1	0.7
Leaf stalk	1	0.7
Seed	1	0.7
Total	137	100

Table 2. Modes of preparation of plant material cultures.

Mode of preparation	Number of plants	Percentage (%)
Beating	1	0.8
Boiling	1	0.8
Burrowing	2	1.7
Carving	61	51.3
Latex collection	1	0.8
Mowing	4	3.4
Peeling	6	5.0
Pounding	2	1.7
Rubbing	7	5.9
Scooping and smoothing	1	0.8
Soaking	4	3.4
Splitting	9	7.6
Trampling	1	0.8
Weaving	4	3.4
Wrapping	15	12.6
Total	119	100

kinds of house construction (oval vs. round, one pole vs. two poles), musical instruments and household furniture (Appendix 1). In addition, in this study, there are several material cultures (such as ornaments, handcraft, Coffin) that were not reported by Sheko and Mejengir ethnic informants but only by Shekacho informants (Table 3).

Plant material culture categories versus number of plants reported by three ethnic groups

Of the 19 major use categories of material culture items reported by all ethnic groups about 38% of the plant species were used for house construction and to make utensils and house hold objects. However, the majority of the plant species (69%) were reported by Shekacho ethnic informants followed by 43 (48%) and 39 (43%) from Mejenger and Shako ethnic groups (Table 4). Moreover, this result showed the use of several plant species for the production of one or more plant-based material cultures mainly reported by Shekacho ethnic informants.

In addition, the difference was observed between Shekacho and the rest ethnic groups that there are plants that were reported by Shekacho informants but not by the rest for the construction of fence, ornaments, making of

Table 3. Number of plant material cultures used by Shekacho, Sheko, and Mejengir ethnic groups.

Ethnic groups	Number of material cultures used	Percentage (%)
Sheko	33	23.6[a*]
Mejengir	38	27.1[a*]
Shekacho	69	49.3[b*]
Total	140	100

*Means with standard deviations within the same column followed by same letters (a-e) are not significantly different (P < 0.05).

Table 4. Comparison of plants reported by Shekacho, Sheko and mejengir Ethnic groups used for the preparation of plant-based material objects.

Plant-based material culture Items	Number of plant species used by Ethnic groups			Total	Percentage (%)
	Shekacho	Shako	Mejengir		
House construction	34	10	6	50	19.2[a]
Fence	14	1	2	16	6.1[c]
Musical instruments	18	2	4	24	9.2[b]
Basketries or containers	9	1	4	14	5.4[c]
Ornaments	9	-	-	9	3.5[d]
Tooth brush	6	1	2	9	3.5[d]
Bathing brush	3	1	3	7	2.7[d]
Agricultural implements	14	5	3	22	8.4[b]
Utensils and house hold objects	32	10	8	50	19.2[a]
Weapon and Tool handles	7	1	1	9	3.5[d]
Decorations	2	-	3	5	1.9[e]
Traps and Nets	8	1	1	10	3.8[d]
Torches	3	2	1	6	2.3[d]
Traditional marks and tattoos	1	-	1	2	0.8[e]
Clothes	4	3	2	9	3.5[d]
Coffin	2	-	-	2	0.8[e]
Hand cart	1	-	-	1	0.4[e]
Beehives	11	1	1	13	4.9[c]
Foot bridge	1	1	1	3	1.2[e]
Total	179	39	43		
Percentage	69	43	48	261	100

*Means with standard deviations within the same column followed by same letters (a-e) are not significantly different (P < 0.05)

handcart and coffin. On the other hand, even though the number of plant species reported to make a specific item varies enormously, about 84% of 19 material cultures were reported by all ethnic groups.

Out of these, higher significance differences (P<0.05) was observed on the number of plant species used for house construction, and household furniture and/or utensils between Shekacho and the rest (Shako and Mejenger) ethnic groups (Table 5). For example, 34 and 32 plant species were reported to be used by Shekacho for house construction and household furniture and/or utensils making, respectively compared to 8 to 10 plant species reported by Shako and Mejenger ethnic groups for the same purposes.

Comparison of plant species for construction purposes

Arundinaria alpina (bamboo)*, Cyathea manniana* and *Syzygium guineense* were ranked, first, second and third, respectively by key informants of Shekacho ethnic group for construction (Table 5). However, *Cordia africana, Baphia abyssinica* and *Malotus oppositifolius* were ranked first, second and third, respectively for construction purposes as reported by key informants

Table 5. Preference ranking of nine plants used for construction in Shekacho ethnic group.

Species name	*R[1]	R[2]	R[3]	R[4]	R[5]	R[6]	R[7]	R[8]	R[9]	R[10]	R[11]	R[12]	R[13]	R[14]	R[15]	Total	Rank
Arundinaria alpina K. Schum. Poaceae	9	9	8	9	8	9	9	8	9	8	9	7	8	8	8	126	1st
Cordia africana Lam., Boraginaceae	4	6	4	9	9	8	9	8	9	8	6	7	4	5	5	101	4th
Cyathea manniana Lam. Cyatheaceae	8	9	9	9	9	8	9	8	9	8	6	7	8	7	8	122	2nd
Ekebergia capensis Sparrm. Meliaceae	6	6	6	5	3	5	3	2	3	4	3	2	1	1	2	52	8th
Galiniera saxifraga Hochst.) Bridson Rubiaceae	4	5	5	5	3	3	3	3	5	5	5	5	4	4	6	65	6th
*Hippocratea africana*_ Willd.) Loes., Celacteraceae	4	5	5	5	3	3	3	3	5	5	5	5	4	4	5	64	7th
Pouteria adolfi-friederici Engl.) Baehni, Sapotaceae	7	5	5	5	6	6	6	6	6	5	6	6	7	6	6	88	5th
Schefflera abyssinica(Engl.) Harms, Araliaceae	6	4	4	5	3	5	3	2	3	4	3	2	1	1	2	48	9th
Syzygium guineense subsp. *afromontanum* (Willd.) DC, Myrtacae	9	8	8	8	9	8	9	8	9	8	7	6	7	8	6	118	3rd

*Rs-Respondents

of Sheko and Mejengir (Tables 6 and 7) differing from Shekacho ethnic group.

In house construction, *Cyperus dichrostachyus, Cyprus* spp., *Eagrotis teff, Psychotria orophilia, Rhamnus prinodes, Ricimnus communis,* and *Pennisetum* sp., are used in roof thatching. The fibers obtained from the bark of *Clematis longicauda, Combretum paniculatum, Dombia torrida, Jasminum abyssinicum, Justica schempriana, Oncinotis tenuiloba, Landolphia buchanani* provide as ropes for tying the roofs, walls and fences during construction.

Comparison of multipurpose plant species (Direct matrix ranking)

The highest values was assigned by key informants of Sheko and Mejengir ethnic groups to *Cordia africana* as the most multipurpose plant species followed by *Ficus ovate* and *Pouteria adolfi-friederici* in the study area.

Diospyros abyssinica, Baphia abyssinica and *Manilkara butuji* were the other multipurpose species ranked, respectively. The least ranked species in multipurpose aspect varied between ethnic groups (Table 8, 9). This does not mean that the least ranked species are the less in multipurpose but either threatened and/or less preferred compared to other species. Thus, according to this rank, the survival of the most multipurpose species is under question due to the daily demand of the ethnic groups, which is not supplemented with follow-up and regular planting.

The highest value with the sum of 51 was given to *Arundinaria alpina* as one of the most multipurpose plant species in the area by key informants of shekacho ethnic group followed by *Cordia africana* and *Galiniera saxifraga* (49 and 40 in the 2nd and 3rd places respectively) *Ficus sur, Hallea rubrostipulosa* and *Hippocratea africana* are the other multipurpose species ranking 4th, 5th and 6th respectively (Table 10).

The least ranked species in multipurpose aspect from the selected species were *Croton macrostachyus* and *Maesa lanceolata.* According to the informants, the least ranked species are the less threatened and the dominantly distributed species in the area however, the survival of the species with the highest rank is under question, especially *Arundinaria alpina,* which is locally threatened as it is dying because of aging (Table 11).

Some studies made in Ethiopia (Tamene, 2000; Hadera, 2000; Hundei, 2001; Berhanu, 2002; Amenu, 2007) have used the method of pair wise ranking where informants made their choices on

Table 6. Preference ranking of nine plants used for construction in Mejengir ethnic groups.

Species name	R1*	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	Total	Rank
Albizia gummifera(J. F. Gmel.) C.A.Sm. Fabacae	4	2	5	6	6	7	3	6	6	5	6	4	5	6	2	73	4th
Diospyros abyssinica(Hiern) F. White, Ebenacae	7	6	6	7	7	8	8	7	5	7	8	5	7	7	6	101	3rd
Baphia abyssinica Brummitt, Fabacae	7	7	8	9	8	9	8	7	7	7	8	8	7	8	7	115	2nd
Mollotus oppositifolius (Geisel) Muell.Arg., Euphorbiacae	3	4	6	7	6	3	4	4	5	6	7	7	4	5	5	76	7th
Trilepsium madagascariense DC., Moraceae	4	6	4	5	3	5	3	1	3	4	3	2	1	1	2	47	9th
Erythrococa trichogyne(Muell. Arg) Prain., Euphorbiaceae	5	5	5	4	5	6	7	6	6	5	5	5	5	6	7	82	6th
Anthiaris toxicaria Lesch., Moraceae	8	8	7	8	7	8	8	7	6	7	8	5	7	7	6	107	5th
Celitis africana N.L.Burm.f., Ulmaceae	5	5	5	4	5	6	7	6	6	5	5	6	6	6	7	84	8th
Cordia africana Lam., Boraginaceae	8	8	8	9	8	9	8	7	9	7	9	9	7	8	7	121	1st

*Rs- Respondents.

Table 7. Preference ranking of nine plants used for construction in Sheko groups.

Species name	*R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	Total	Rank
Ficus ovate Vahl. Moraceae	4	2	5	6	6	7	3	6	6	5	6	4	5	6	2	73	8th
Diospyros abyssinica F. White, Ebenacae	7	6	6	7	7	8	8	7	5	7	8	5	7	7	6	101	4th
Baphia abyssinica Brummitt, Fabacae	7	7	8	9	8	9	8	7	7	7	8	8	7	8	7	115	2nd
Hippocratea pallens (Willd.) Loes., Celactrsae	3	4	6	7	6	3	4	4	5	6	7	7	4	5	5	76	7th
Lagenaria siceraria(Molina) Standl., Curcubitaceae	4	6	4	5	3	5	3	1	3	4	3	2	1	1	2	47	9th
Antiaris toxicaria Lesch., Moraceae	5	5	5	4	5	6	7	6	6	5	5	5	5	6	7	82	6th
Malotus oppositifolius Geisel) Muell.Arg., Euphorbiacae	8	8	7	8	7	8	8	7	6	7	8	5	7	7	6	107	3rd
Hippocratea africana Willd.) Loes., Celacteraceae	5	5	5	4	5	6	7	6	6	5	5	6	6	6	7	84	5th
Cordia africana Lam., Boraginaceae	8	8	8	9	8	9	8	7	9	7	9	9	7	8	7	121	1st

*Rs-Respondents

Table 8. Comparison of multipurpose plants in Mejengir ethnic group.

List of species	Use categories							Total	Rank
	Construction	Musical instruments	Farming implements	House hold articles	Weapon	Tool handles	Beehives		
Diospyros abyssinica F. White, Ebenacae	7	8	8	8	6	5	8	50	2nd
Albizia gummifera(J. F. Gmel.) C.A.Sm. Fabacae	8	6	8	4	4	8	3	41	4th
Baphia abyssinica Brummitt, Fabacae	8	2	8	8	3	8	1	38	5th
Mollotus oppositifolius Geisel) Muell.Arg., Euphorbiacae	8	2	0	8	1	3	5	27	7th
Trilepsium madagascariense DC., Moraceae	8	5	3	7	6	5	8	42	3rd
Erythrococa trichogyne(Muell. Arg) Prain., Euphorbiaceae	7	6	4	4	6	5	2	34	6th
Anthiaris toxicaria Lesch., Moraceae	8	1	5	5	8	5	0	32	8th
Cordia africana Lam., Boraginaceae	8	8	7	7	7	7	8	52	1st

Table 9. Comparison of multipurpose plants in Sheko ethnic groups.

List of species	Use categories							Total	Rank
	Construction	Musical instruments	Farming implements	Household articles	Weapon	Tool handles	Beehives		
Ficus ovate Vahl. Moraceae	7	8	8	8	6	5	8	50	5th
Diospyros abyssinica F. White, Ebenacae	8	6	8	4	4	8	3	41	2nd
Baphia abyssinica Brummitt, Fabacae	8	2	8	8	3	8	1	38	3rd
Hippocratea africana Willd.) Loes., Celacteraceae	8	2	0	8	1	3	5	27	7th
Pouteria adolfi-friederici(Engl.) Baehni, Sapotaceae	8	5	3	7	6	5	8	42	4th
Manilkara butuji Chiov. Sapotaceae	7	6	4	4	6	5	2	34	6th
Mallotus oppositifolius (Geisel) Muell.Arg., Euphorbiacae	8	1	5	5	8	5	0	32	8th
Cordia africana Lam., Boraginaceae	8	8	7	7	7	7	8	52	1st

Table 10. Comparison of multipurpose plants in Shekacho ethnic groups through direct matrix.

List of species	Use categories							Total	Rank
	Construction	Musical instruments	Farming implements	Household articles	Weapon	Tool handles	Beehives		
Cordia africana Lam., Boraginaceae	8	7	6	7	6	7	8	49	2nd
Maesa lanceolata Forssk. Myrcinaceae	6	1	4	3	3	4	1	22	8th
Hallea rubrostipulosa(K. Schum.) J. F. Leroy, Rubiaceae	7	5	3	7	5	3	8	38	5th
Galiniera saxifraga(Hochst.) Bridson, Rubiaceae	7	7	8	4	6	6	2	40	3rd
Ficus sur Forssk., Moraceae	7	2	5	7	8	2	8	39	4th
Croton macrostachyus Del., Euphorbiacae	7	4	5	5	2	5	6	34	7th
Arundinaria alpina K. Schum., Poaceae	8	8	6	8	7	6	8	51	1st
Hippocratea africana(Willd.) Loes., Celastraceae	8	4	4	8	1	4	6	35	6th

Table 11. Paired comparison of eight plants used for construction and house hold equipments in Shekacho ethnic group in reference to cultural value.

Species	R1-R15								Frequencies	Rank
	Arundinaria alpina	***Cordia africana***	***Cyathea manniana***	***Syzygium guineense***	***Galiniera saxifraga***	***Hallea rubrostipulosa***	***Macaranga capensis***	***Ficus sur***		
Arundinaria alpine K. Schum., Poaceae	-	Aa	Cm	Aa	Aa	Aa	Aa	Aa	6x	1st
Cordia africana Lam., Boraginaceae	-	-	Ca	Ca	Ca	Ca	Ca	Ca	6x	1st
Cyathea manniana Hook. Cyatheaceae	-	-	-	Sg	Cm	Cm	Cm	Cm	5x	2nd
Syzygium guineense subsp. *afromontanum* (Willd.) DC. Myrtaceae	-	-	-	-	Sg	Hr	Sg	Sg	4x	3rd
Galiniera saxifraga(Hochst.) Bridson Rubiacae	-	-	-	-	-	Hr	Mc	Fs	0x	6th
Hallea rubrostipulosa (K. Schum.) J. F. Leroy. Rubiaceae	-	-	-	-	-	-	Hr	Fs	3x	4th
Macaranga capensis Baill.) Benth. Eupphorbiaceae	-	-	-	-	-	-	-	Fs	1x	5th
Ficus sur Forssk. Moraceae	-	-	-	-	-	-	-	-	3x	4th

N (n-1)/2, 8(8-1)/2 =8x7/2= 56/2= 28

Table 12. Paired comparison of five plants used for construction and house hold equipments in Mejengir ethnic groups in reference to strength and cultural value.

Species	R1-R15					Frequencies	Rank
	Baphia abyssinica	***Cordia africana***	***Diospyros abyssinica***	***Ficus ovata***	***Hippocratea africana***		
Baphia abyssinica Brummitt, Fabacae	-	Ca	Ba	Ba	Ha	2x	3rd
Cordia africana Lam., Boraginaceae	-	-	Ca	Ca	Ca	4x	1st
Diospyros abyssinica F. White, Ebenacae	-	-	-	Da	Ha	1x	4th
Ficus ovate Vahl. Moraceae	-	-	-	-	Ha	0x	5th
Hippocratea africana Willd.) Loes., Celacteraceae	-	-	-	-	-	3x	2nd

Table 13. Paired comparison of five plants used for construction and house hold equipments in Sheko ethnic group in reference to strength and cultural value.

Species	R1-R15					Frequencies	Rank
	Baphia abyssinica	***Cordia africana***	***Diospyros abyssinica***	***Trichilia prieuriana***	***Hippocratea africana***		
Baphia abyssinica Brummitt, Fabacae	-	Ca	Ba	Ba	Ha	2x	3rd
Cordia africana Lam., Boraginaceae	-	-	Ca	Ca	Ca	4x	1st
Diospyros abyssinica F. White, Ebenacae	-	-	-	Da	Ha	1x	4th
Trichilia prieuriana Sond. Meliaceae	-	-	-	-	Ha	0x	5th
Hippocratea africana Willd.) Loes., Celacteraceae	-	-	-	-	-	3x	2nd

individual basis.

Paired comparison on plants used in material culture by the Mejengir ethnic group

In this study, 10 informants from Mejengir ethnic group were asked to indicate the efficacy and popularity of species used for construction and house hold equipments and they did paired comparison of five plants by using (N (n-1)/2, 5(5-1)/2 =5x4/2= 20/2= 10) formula (Table 12). *Cordia africana, Hippocratea africana, Baphia abyssinica, Diospyros abyssinica* and *Ficus ovata* were ranked 1st, 2nd, 3rd, 4th and 5th respectively.

Paired comparison on plants used for material culture by the Shako ethnic group

The same to the above ethnic groups, 10 informants from Mejengir ethnic group were asked to indicate the efficacy and popularity of species used for construction and house hold equipments and they did paired comparison of five plants (Table 13). *C. africana, Hippocratea africana, Baphia abyssinica, Diospyros abyssinica* and *Trichilia prieuriana* were ranked 1st, 2nd, 3rd, 4th and 5th respectively. Most of the results among the Sheko and Mejengir ethnic groups are similar.

Local threats of plants used for material culture and indigenous knowledge in the Study Areas

It was observed that plants species in the study area were being threatened. The major threat factor with the highest rank assigned by informants was agricultural expansion followed by illegal wood harvest and construction (Table 14).

The informants reported that there are beliefs, which have played great role for the preservation of plants. To cut a plant part is not allowed, even a climber. Hence,

Table 14. Ranking of threat factors to plant species in Masha and Yeki districts responded by 15 informants.

Threat factors	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}	R^{13}	R^{14}	R^{15}	Total	Rank
Agricultural expansion	9	8	7	8	9	9	9	8	9	6	8	9	8	9	8	124	1st
Illegal wood harvest	8	8	8	7	7	8	8	7	5	7	8	5	7	7	6	106	2nd
Construction	7	7	8	6	8	6	7	6	6	6	8	6	7	6	7	102	3rd
Fuel wood	3	4	6	7	6	3	4	4	5	6	7	7	4	5	5	76	6th
Deforestation	4	6	4	5	3	5	3	1	3	4	3	2	1	1	2	47	7th
Settlement	5	5	5	4	5	6	7	6	6	5	5	5	5	6	7	82	5th
Forest fire	5	8	7	5	7	5	8	7	6	7	8	5	7	7	6	98	4th

spiritual and religious beliefs of the three ethnic groups in Sheka, especially the Shekacho ethnic group have developed strong effect on its use and conservation. As reported by informants there are powerful beliefs, rituals and spirits and taboos for each ethnic group in Sheka zone. They believe that people who violate these taboos will die but there is high violation of these traditional rules by dwellers from other parts of Ethiopia, mainly in Yeki district.

DISCUSSION

Although the deterioration of forest species is increasing in the study area due to agricultural expansion and other threat factors, there is still rich indigenous knowledge of making plant material culture by Shekacho, Sheko and Mejengir ethnic groups in Masha and Yeki districts of Sheka Zone, Southwest Ethiopia. A total of 113 plant species that are in use in these districts serve as raw materials for various construction purposes, traditional arts and handcrafts. A study conducted in and around the Awash National Park in central Ethiopia reported 156 plant species (Bahru, 2012).

Another study conducted in Kersa district, Jimma Zone, Oromia Regional State, southwest Ethiopia revealed 46 plant species (Abera, 2013). Most of the plant species reported were also mentioned by authors in studies conducted elsewhere in Ethiopia; 26 plant species in Gemedo-Dalle (2004), 15 in Bahru et al. (2012), 19 in Rainer (2011). The most possible reasons for variations in number of plant species between the current and previous studies are due to difference in topography and traditional knowledge of the study sites.

The majority of plants used in material culture in the studied districts were obtained from the wild. The results concur with that of other studies conducted in Ethiopia (Bahru et al., 2012; Abera, 2013) and in India (Choudhay et al., 2008). Most of the species collected from the wild are highly exposed to various threat factors and as a result many of them including *C. Africana*, *F. ovate, G. saxifraga* and *Pouteria adolfi-friederici* are rarely encountered. Special attention should be given to those more preferred and multipurpose plants that were indicated by preference ranking exercise as the most threatened ones.

Tree was the most common growth habit used in the manufacture of plant material culture in the Masha and Yeki districts. This finding is in agreement with the reported study by Abera (2013) in the neighboring Ghimbi district of Oromia regional state, which may indicate their relatively better abundance in the forest area as compared to other growth forms. However, a study conducted in and around Awash National Park reported the dominance of shrubs used in making plant material culture (Bahru et al., 2012).

Stem was the most used plant part in the manufacture of plant material culture in the present study as compared to other plant parts. A study conducted in Kersa District, Jimma Zone of Oromia regional state in southwest Ethiopia also showed the dominance of woody stem in the manufacture of plant material culture (Abera, 2013). This implies the presence of hardwood plant species and the study area is largely covered by forest.

According to current study results, a large number of plants were identified to be used in dried and fresh forms in material preparations. There were also many plants from which, parts were claimed to either be used as dried or fresh materials. Of the preparation methods, a large number of plants were reported to be prepared by carving followed by wrapping and splitting. This might be due to the wide use of woody trees to make plant material culture in the study area. A substance like cold water, ash, and clay was

reported to be used for wetting,washing and decoration during the preparation of materials. Axes, blades, knives, sickle and other sharp materials are used for cutting, carving, peeling and mowing during the preparation of materials from the plants. Similarly, Abera (2013) reported the use of dried and fresh materials, and mainly curving method for preparation.

Of the plant material cultures reported by ethnic informants, Shekacho ethnic group contributed the highest proportion as compared to Mejengir and Sheko ethnic groups. This may be due to the availability of a diversity of plant species and large population of Shekacho ethnic group (CSA, 2009) with a wide knowledge in the study area. A study conducted in and around the Awash National Park of Central Ethiopia also showed that the highest number of plant species and plant material cultures were reported by Oromo informants as compared to Afar ones (Bahru et al., 2012). Local communities of the Shekacho, Sheko and Mejengir ethnic groups in Masha and Yeki districts of Sheka Zone are highly dependent on indigenous plant species for various construction, household furniture and/or utensils, dry fencing, roofs and/or walls thatching. Similarly, the study conducted in east Shoa Zone, Ethiopia (Bahru et al., 2012) indicated the similarity of the majority of the plant species for house construction reported by Oromo and Afar nationals but with the frequent use of *Acacia* species (as exotic species) due to the scarcity of indigenous plant species as a result of deforestation for the purpose of agriculture. In addition, the fiber obtained from the bark of several plant species provides ropes for tying the walls and roofs during house construction. Roofs, in turn, were thatched with a variety of grass species in the study area in agreement with the reported studies of Bahru (2012), Jacobs and Schloeder (1993). On the other hand, plants with thorns were reported to be used for fencing house, farmland and animal enclosures in the study area. However, almost all plant materials for house construction including corrugated iron sheets, stand metals and stone blockers are substituting the use of plant species for house construction through time mainly in urban kebeles due to acculturation.

On the other hand, the rest of the plants in the study area were reported to be sources of handcrafts and traditional arts while others are used for ritual values, tooth brush, tool handles, household utensils, fencing tool and musical instruments. Likewise, Gemedo-Dalle et al. (2004), Bahru (2012) and Munishi et al. (2006) reported many plant species in Borana and central Shoa of Ethiopia and Tanzania, respectively used as a source of handcrafts, traditional arts, and ritual.

The plant species of material culture in Masha and Yeki districts have also common cultural values for all ethnic groups of the study area due to cultural diffusion and sharing of resources. Similarly, a study reported in Bahru et al. (2012) confirmed such similar use of plant species between Oromo and Afar Nations in Ethiopia; it indicates the existence of common knowledge and cultural diffusion across a range of diverse cultures and geographical areas. In contrast, plants used in material culture also have certain cultural and ritual values within particular ethnic groups, which indicate that IK distribution can be influenced by socio-cultural factors of different ethnic groups.

Of the plant species used as material culture in the study area, some were found to have multipurpose values (use diversity). In this regard, the highest values assigned for the respective plant species by key informants were found to be as the most multipurpose plant species as compared to the least ranked species. However, according to the informants, this does not mean that the least ranked species are the less in multipurpose but either threatened and/or less preferred compared to other species. Thus, according to this rank, the survival of the most multipurpose species is under question due to the daily demand by the local community. A study reported from in and around the Awash National Park, central Ethiopia indicated that out of the total recorded plant species, which serve as a source of material culture, 16% of the species were found to have 4 and 5 distinct uses each, while 34% with 6 uses to the local people (Bahru et al ., 2012).

In this study, the major threat factor with the highest rank assigned by informants was agricultural expansion followed by illegal wood harvest and construction. These were probably due to the fertility of the land suitable for agriculture. Specifically, timber production by business people has severely accelerated the high rate of exploitation of plant species like *Cordia africana* as suggested from Yeki district informants. As a result, the number of material objects made from plants is decreasing at an alarming rate and is replaced by industrial products extending to the household of the rural communities. The habitat of most plants is increasingly becoming eroded due to over harvesting of trees for fuel, wood and urbanization throughout the region (Adal, 2004). The lack of ownership, diminished role of clan leaders in forest and expansion of private sector investment for temporary benefit have brought cultural changes that alter the importance of traditional resource management practices, exposing the community to various socio-economic problems (Woldemaiam and Fetene, 2007). In addition, as reported in Woldemariam and Fetene (2007) during the last decades several new threat factors, such as investment projects for new coffee and tea plantation development and fast population growth due to immigration related to settlement schemes and the agricultural development projects, have resulted in increasing pressures on the forests.

The informants reported that there were beliefs, which have played great role for the preservation of plants by the three ethnic groups. To cut a plant part is not allowed, even a climber. Hence, spiritual and religious beliefs of the three ethnic groups in Sheka, especially the Shekacho

ethnic group have developed strong effect on its use and conservation. In agreement with the present study, Adal (2004) has made similar conclusions in the study conducted in North Shoa Zone Ethiopia, about the roles of magical and religious beliefs and environmental perception on the use and management of plant species. As reported by Woldemaiam and Fetene (2007) there are powerful beliefs, rituals and spirits and taboos for each ethnic group in Sheka zone. They believe that people who violate these taboos will die but there is high violation of these traditional rules by dwellers from other parts of Ethiopia, mainly in Yeki district. However, these indigenous beliefs were dismantled as a result of non-indigenous settlement, agricultural expansion and state-led programme investments in new and fertile areas of the country.

Conclusion

A total of 113 plant species that have 19 major use categories in material culture were reported from three ethnic groups (Shekacho, Sheko, Mejengir) of Masha and Yeki Districts of Sheka Zone, SNNPRS, and southwest Ethiopia. Out of 19 major categories, 49.29, 23.57 and 27.14% were reported by Shekacho, Sheko and Mejengir ethnic groups, respectively. The most potential and multipurpose plant species exposed to a wide human devastation were also documented in this study for urgent conservation plan.

Conflict of interests

The authors have not declared any conflict of interest.

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Appendix 1. List of plant species used for material culture in Sheka Zone; Masha and Yeki district. (T = tree, Sh = shrub, T/Sh = tree/shrub, Sh/H= shrub/herb, H = herb, Cl = climber, F= fern: local names Sh1=Shakinono; Sh2= Shekogna and Mgr= Mejengiregna

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2237 •SY	*Acanthus eminens* C.B.Clarke	Acanthaceae	Peecho (Sh1)	Sh	Stem	Fresh	Splitting	"WUTO" (hole sealing) for roof NB: - The fresh is chosen for bending easily unless it is broken if dried.
2247	*Alangium Chinense* (Lour) Harms	Alangiaceae	Shotto (Sh1)	T	Stem	Dried and fresh	Carving and Sharpening	Beehive, beehive hanging, Torch or Match (twig), Construction NB: have light stem
2202	*Albizia gummifera* (J. F. Gmel.) C.A.Sm.	Fabaceae	C'ato (Sh1); Kasoy(Mgr)	T	Stem	Fresh and dried	Carving , peeling the bark	Beehive hanging, beehive(YEGILO), fence, house construction, bathing (in Shako ethnic groups) NB: They refer the dried one to handle easily
2187	*Allophylus abyssinicus* (Hochst.) Radlkofer	Sapindaceae	She'o (Sh1)	T	Stem and branch	Dried	Carving	Construction, farming implements
2210	*Antiaris toxicaria* Lesch.	Moraceae	Tengi (Mgr & Sh2)	T	Stem, bark and stem	Fresh and dried	Beating up and Peeling the bark, Soaking in the water and squeezing	Cloth (bark), Sac (to store grain), vessel cleaning sponge, Blanket and mat
2235	*Apodytes dimidiata* E. Mey. ex Am.	Icacinaceae	Wundabo (Sh1)	T	Stem	Fresh and dried	Carving	beehive hanging (because of suitable branches) and construction
2227	*Arundinaria alpina* K. Schum.	Poaceae	Ho'o(Sh1)	T/ Sh	Culm, culms sheath, branch and twigs,	Fresh	Splitting and Weaving	In basketries, irrigation pipe, brooms(leaf), cooking utensil, water bottles, processing Ensete (MAARO), construction (fence and house), beehive (both culms and Culm sheath are used), floor mat, chairs, DOLLO (water container), cups, GAMO (traditional tray), pipe used for smoking tobacco, bed, etc.
2255	*Arundo donax* L.	Poaceae	Shombok'o (Sh1, Sh2 and Mgr)	T/ Sh	Culm	Dried	Burrowing (making a hole)	Small musical instruments (Whistle and flute)
2256	*Baphia abyssinica* Brummitt	Fabaceae	Shifu(Sh2); Duwe(Mgr.)	T	Branch, stem	Fresh and dried	Carving	Tooth brush (twig), the most important plant for construction and farm implements in Shako and Mejengir ethnic group.

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and notes
2152	*Bersama abyssinica* Fresen.	Melianthaceae	Booko (Sh1)	T	Stem	Fresh and dried	Burrowing and carving	Beehive hanging, wood to make mortar, fence, Pestle, ploughs. Wood is referred for musical instruments and for house construction because it is not eaten by ants and beetle. For carving a pillow NB: The smoke and the plant smell are bad. Can be used as insect repellent
2172	*Brucea antidysentrica J. F. Mill.*	Simaroubaceae	Nuqaasho (Sh1)	T	Stem	Fresh and dried	Carving	
2192	*Canthium oligocarpum* Hiern	Rubiaceae	C'oche–afo (Sh1)	Sh	Stem	Dried	Sharpening	Construction
2151	*Cassipourea malosana* (Baker) Alston.	Rhizophoraceae	Waraallo (Sh1)	T	Stem and branch	Dried	Sharpening	Beehive hanging, farm implements, pestle, Weapon, "MATO", trap, ornaments(branch)
2223	*Celtis africana* N.L.Burm.f.	Ulmaceae	Gonji (Mgr)	T	Stem	Dried	Carving	Tools; wood used to make ploughs
2228	*Chionanthus mildbraedii* (Gig & Schellenb.) Stearn	Oleaceae	Shigawo (Sh1)	T	Stem	Dried	Carving	Farm implement, tool handle
2195	*Clausena anisata* (Willd.) Benth.	Rutaceae	Ermicho(Sh1); Ermasoy (Mgr);Enshu (Sh 2)	T/ Sh	young stems and branch	Fresh and dried	Peeling or carving the tip	Tooth brush
2160	*Clematis longicauda* Steud. ex A. Rich.	Ranunculaceae	Shego(Sh1)	Cl	Stem	Fresh	Wrapping	Rope for construction of fence
2220	*Coffee arabica* L.	Rubiaceae	Moye (mgr)/buno (Sh1)	T/ Sh	Stem	Dried	Carving	Construction It is forbidden to cut it unless it fallen down or out of use for drinking b/c they believe it has so many uses rather than material culture.
2253	*Colocasia esculenta* (L.) Schott.	Araceae	Baaka or Goddarree (sh2 &Mgr)/Qiiddo (Sh1)	H	Leaf	Fresh	Wrapping	To wrap market foods, plate, use for drinking water as cup.
2232	*Combretum paniculatum* Vent.	Comberetaceae	Bergo (Sh1)	Cl	Stem	Fresh	Wrapping	Rope for construction
2199	*Cordia africana Lam.*	Boraginaceae	Di'o (Sh1); Danpe (Mgr); Danpa(Sh2)	T	Stem , bark, leaf	Dried and fresh	Carving	Construction (timber), house hold equipments, the bark peeled and use as a rope, the buttress roots use for musical instrument (drum), plate(leaf)
2168	*Croton macrostachyus Del.*	Euphorbiaceae	Shomo(Sh1)	T	Stem and root	Dried	Carving	Tool handle, beehive, house construction (roof), root for tool handle(knife handle)

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and notes
2159	*Cyathea manniana* Hook.	Cyatheaceae	Sesino(Sh1)	T	Stem	Dried	Carving and removing the spines and leaves	house wall construction and fencing
2254	*Cympopogon citratus* (DC.) Stapf.	Poaceae	Toocho (Sh1)	H	Leaf	Fresh	Rubbing	Vessel cleaning specially for milk bottles
2240	*Cyperus dichroostachyus* Hochst.	Cyperaceae	Tono (Sh1)	H	Leaf	Fresh and dried	Mowing	Roof thatching in the wild, Weave floor mats.
2257	*Cyprus sp.*	Cyperaceae	Disho(Sh1)	H	Grass leaf	Dried	Mowing	Roof thatching ; the grass is sawn when it starts drying
2179	*Deinbollia kilimandscharica* Taub.	Sapindaceae	Qaaso(Sh1)	T/ Sh	Stem and leaf	Fresh and dried	Carving	Wedge, leaf bedding in forest (camp)
2213	*Diospyros abyssinica* (Hiern) F. White	Ebenaceae	Kuri (Mgr)	T	Stem	Dried	Sharpening	Strong wood for construction , farm implements, pestle, cloth (for males in Shako ethnic groups), tool handle NB: wood used for the pestle is always hard
2205	*Dombeya torrida (J.F. Gmel.) P. Bamps*	Sterculiaceae	Bo'aro(Sh1)	T	Bark or Peel	Fresh	Peeling and tearing	Fiber, bark peeled to make a rope and used in construction and for trapping.
2251	*Dracaena afromontana Mildbr.*	Dracaenaceae	Fishino (Sh1)	T	stem	Dried	Splitting	Fence
2198	*Dracaena fragans (L.) Ker Gawl.*	Dracaenaceae	Emo(Sh1)/ serte / Tesit(Mgr)	T	stem	Dried	Splitting	Cloth, fence
2252	*Dracaena steudneri Engl.*	Dracaenaceae	yudo(Sh1)	T	Stem, leaf	Dried	Splitting	Fence
2173	*Drynaria volkensii Hieron.*	Polypodiaceae	Oko(Sh1)	F	Leaf	Dried	Wrapping for sealing	Seal beehive
2214	*Ehretia cymosa Thonn.*	Boraginaceae	Jigemoy (Mgr) ; Yoogaamo (Sh1)	T/ Sh	stem	Dried	Carving	Farm implement
2167	*Ekebergia capensis Sparrm.*	Meliaceae	Ororo(Sh1)	T	Stem	Dried	Carving	Beehive hanging, door, window,
2244	*Ensete ventricosum (Welw.) Cheesman*	Musaceae	Qocho /qaawo(Sh1) Oudu(sh2)	H	Pseudo stem, leaf and leaf stalk	Dried and fresh	Chopping the pseudostem to extract the fiber and weaving	Fiber cloth, bedding, vessel and hand cleaning, bathing, shade, umbrella, pipe (leaf stalk or "oqqo"). Leaves used to wrap the dough or "Kocho" before putting it in the fire wood stove so that it does not burn.

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2241	*Eragrostis tef* (Zucc.) Trotter	Poaceae	Gasho (Sh1)	H	Culm	Dried	Mowing with a sickle and trampling (walk over), then tying	Roof thatching , pillow and mattress (by trampling the straw/TUJJO (in Shakinono) inside the pillow case made from *Phoenix reclinata* leaf), and broom
2200	*Erythrina abyssinica* Lam.	Fabaceae	Bero (Sh1)	T	Stem	fresh	Carving	Construction
2174	*Erythrina brucei* Schweinf.	Fabaceae	Kocho(Sh1)	T	Stem	Fresh and dried	Carving	Fence, hanging beehive, furniture, Tools; wood used to make the pestle for large mortars.
2194	*Erythrococca trichogyne* (Muell. Arg) Prain.	Euphorbiaceae	Bichirekucho (Sh1); Gidigr(Mgr)	T/ Sh	Stem	Fresh and dried	Sharpening and carving	Construction
2239	*Euphorbia ampliphylla* E.	Euphorbiaceae	Qakaro (Sh1)	T	Stem	Fresh	Carving	Beehive, fence, sliver for granaries
2218	*Ficus exasperata* Vahl	Moraceae	Balantay (Mgr)	T	Leaf	Fresh and dried	Rubbing	For cleaning kitchen utensils, sliver for granaries
2209	*Ficus ovata*Vahl	Moraceae	Acha(Sh2); dokey(Mgr)	T	Stem, latex	Fresh and dried	Carving , latex collection	Torch(milky latex)
2178	*Ficus sur.* Forssk.	Moraceae	Et'o(Sh1)	T	Stem	Fresh and dried	Carving	Timber for furniture, Beehives (wood), Coffin
2157	*Galiniera saxifraga* (Hochst.) Bridson	Rubiaceae	Diido(Sh1)	T/ Sh	Stem and fruit	Fresh and dried	Carving and burrowing	small mortar, tool handle Weapon, mortar, house roofing, "OKO", farm implement, (Yoke and plough), children use the fruit as weapon , sliver for granaries
2204	*Girardinia bullossa* (Steudel) Wedd.	Urticaceae	Shekirato(Sh1)	H	Leaf	Fresh	Rubbing or pounding	Fiber for hunting net, sack, Cloth
2190	*Hallea rubrostipulosa* (K. Schum.) J. F. Leroy	Rubiaceae	Oppo(Sh1)	T	Stem and leaf	Dried and fresh	Carving	Beehive, mortar, beehive hanging, timber for door, plate (leaf)
2185	*Hibiscus macranthus Hochst. ex A.Rich.*	Malvaceae	Gaahijo(Sh1)	H	Stem	Dried and fresh	Peeling	Fiber /Rope; bark peeled for rope used in construction and stem for trap
2231	*Hippocratea africana (Willd.) Loes.*	Celastracae	P'ijo (Sh1), Pizoy(Mgr)	Cl	Stem	Fresh	Wrapping , splitting and Soaking in water if dried	Used to make strong cordage for rope, containers and construction, house hold equipments
2184	*Hippocratea goetzei Loes.*	Celastracae	T'ero(Sh1)	Cl	Stem	Fresh	Wrapping	Construction (fence) , sieve

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and notes
2258	*Hippocratea pallens* Planchon.ex Oliver	Celastraceae	Gelenchi (Mgr)	Cl	Stem	Fresh	Splitting	Basketries, construction
2163	*Ilex mitis* (L.) Radlk.	Aquifoliaceae	Qeto(Sh1)	T	Stem	Fresh and dried	Carving	beehive, fence,
2219	*Jatropha curcas* L.	Euphorbiaceae	Gebuy (Mgr)	T/ Sh	Stem	Dried and fresh	Sharpening	Fence
2182	*Jasminum abyssinicum* Hochst. ex DC.	Oleaceae	Hawuto(Sh1)	Cl	Bark	Fresh	Wrapping	Rope; used in house construction
2193	*Justicia schimperiana* (Hochst. ex Nees) T. Anders.	Acanthaceae	Shesharo(Sh1)	Sh	Stem	Fresh and dried	Peeling	rope and small stems for construction(roof) and (MAGER) because it is not eaten by termites easily,
2259	*Lagenaria siceraria* (Molina) Standl.	Cucurbitaceae	Boto (sh1); Gini (Mgr)	Cl	Fruit	Dried	Scooping, Scrubbing and smoothing the inside of the gourd	Kitchen utensils, and household objects (cups, containers, vessels, bowls and ladles.
2233	*Landolphia buchananii (Hall. F.) Stapf*	Apocynaceae	Yeebo (Sh1), Yakat (Mgr)	Cl	Stem	Fresh	Splitting and wrapping	Rope , basketries for chicken house and other household objects
2249	*Lepidium sativum L.*	Brassicaceae	Shiipo(Sh1)	H	Twig	Dried	Wrapping in a bunch	Broom, Shed for livestock
2183	*Lepidotrichilia volkensii (Gurke) Leroy*	Meliaceae	Shaayo (Sh1)	T	Stem	Fresh and dried	Carving	Tools; wood to make ploughs.
2236	*Leucas deflexa Hook. f.*	Lamiaceae	Tinbe –gube (Sh1)	H	inflorescence	Fresh	Rubbing	Vessel cleaning
2243	*Lippia adoensis Hochst. ex Walp.*	Verbenaceae	Shoobbo (Sh1)	T/ Sh	Leaf	Fresh	Rubbing	milk utensils cleaning (Fresh or dried
2188	*Lobelia giberroa Hemsl.*	Campanulaceae	Gedrano (Sh1)	T/ Sh	Inflorescence	Dried	sharpening the stalk of the infllorecense after removing the flower	Musical instruments for children
2158	*Macaranga capensis (Baill.) Benth.*	Euphorbiaceae	Werango (Sh1)	T	Stem	Fresh	Sharpening	Construction (mager)
2154	*Maesa lanceolata Forssk.*	Myrsinaceae	Chego (Sh1)	T/ Sh	Stem	Dried and fresh	Carving	Construction, house hold equipments , sliver for granaries

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2211	*Mallotus oppositifolius* (Geisel) Muell. Arg.	Euphorbiaceae	Birekechi or Dasoy (Mgr)	T/ Sh	Stem	Dried and fresh	Sharpening	Construction (wall), farming implements
2206	*Malva verticillata* L.	Malvaceae	T'oso (Sh1)	T/ Sh	Leaf and bark	Fresh	peeling and soaking in the water	Bathing brush
2212	*Manihot esculenta* L.	Euphorbiaceae	Anchute (Mgr)	T/ Sh	Stem	Fresh and dried	Splitting the stem	Fence
2262	*Manilkara butugi* Chiov.	Sapotaceae	Gawo (Sh 1) Goji (Mgr)	T	Stem	Fresh and dried	Carving	Musical instruments, construction
2176	*Maytenus gracilipes (Welw. ex Oliv.) Exell subsp. arguta (Loes.) Sebsebe*	Celastraceae	Atato(Sh1)	Sh	Stem and spine	Fresh and dried	Carving and Sharpening	Spines use for making tattoos (traditional marks) and stems for making ornaments
2153	*Maytenus undata (Thunb.) Blakelock.*	Celastraceae	Getto (Sh1), p'ey(Mgr)	Sh	Stem	Fresh and dried	Carving	Ornaments ,comb
2263	*Microglossa pyrifolia (Lam.) 0. Kuntze*	Astraceae	Nibasho (Sh1)	Sh	Stem	Fresh	Splitting	House hold articles like sieve ("chimo")
2175	*Millettia ferruginea subsp. darassana (Hochst.) Bak.*	Fabaceae	Yaago(Sh1)	T	Stem	Fresh and dried	Carving	Construction (timber)
2217	*Momordica foetida Schum. & Thonn.*	Cucurbitaceae	Munji (Mgr)	Cl	Leaf	Fresh	Wrapping on the head	Cloth, ornamental uses for dancing on celebrations
2250	*Ocimum basilicum L.*	Lamiaceae	Kefo (Sh1)	H	Leaf	Fresh	Rubbing	Vessel cleaning
2201	*Olea welwitschii (Knobl.) Gilg & Schellenb.*	Oleaceae	Yeho(Sh1)	T	Stem	Fresh and dried	Carving	Bee hive, farm implements, tool handle
2161	*Oncinotis tenuiloba Stapf*	Apocynaceae	C'eno(Sh1)	Cl	Stem	Fresh	Wrapping	Rope for a fence ,
2234	*Oncoba spinosa Forssk.*	Flacourtiaceae	Shurato(Sh1)	T	Fruit and seed	Dried	Burrowing	House hold article (the dried fruit burrowed to put butter and salt), ornaments(seed)
2208	*Oxyanthus speciosus DC.*	Rubiaceae	A'emato(Sh1)	T/ Sh	Stem	Fresh	Carving and sharpening	House construction, fence, weapon like spear

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2196	*Pavetta abyssinica* Fresen.	Rubiaceae	Qorbandaro(Sh1)	T/ Sh	Stem	Fresh and dried	Carving	Construction
2216	*Pennisetum* sp.	Poaceae	Peta/elti or solakak(Mgr)	H	Leaf	Fresh and dried	Mowing	Roof thatching
2226	*Phoenix reclinata Jacq.*	Arecaceae	Zenbaba (yeebo) (Sh1)	T	Leaf, stem, petiole, midrib and sheath	Fresh and dried	Weaving and sewing	Leaves and midribs are woven and sewn as handcraft, containers, floor mat, broom, basketries and hat; sheathes are used for Sieve, leaves for ornamental and the thick midribs use for tooth brush.
2156	*Phytolacca dedocandra L'Herit.*	Phytolaceae	Yingo(sh1); Shorshu(Sh2)	Sh	Root and leaf	Fresh	Pounding & rubbing	Bathing brush (sponge) during itching problem (leaf); root for cloth washing
2181	*Polyscias fulva (Hiern) Harms*	Araliaceae	Karasho(Sh1)	T	Stem	Fresh and dried	Carving	Beehive making, fence and construction
2238	*Pouteria adolfi-friederichi (Engl.) Baehni*	Sapotaceae	Sha'o (Sh1)	T	Stem	Fresh and dried	Carving	Beehive, house hold articles, timber, saucer
2222	*Pouteria alnifolia (Bak.) Roberty*	Sapotaceae	Fana (Sh2)	T	Stem and branch	Fresh and dried	Carving	Ornamental (comb)
2166	*Prunus africana (Hook. F.) Kalkm*	Rosaceae	Omo(Sh1)	T	Stem	Fresh and dried	Carving	Mortar (large one), beehive hanging, tool handle
2260	*Psychotria orophila Petit*	Rubiaceae	Neche Aimato(Sh1)	Sh	Stem	Fresh	Carving	Construction (roof)
2197	*Rhamnus prinoides L'Herit.*	Rhamnaceae	Geesho(Sh1)	T/ Sh	Stem	Fresh	Carving	Construction (roof)
2180	*Ricinus communis L.*	Euphorbiaceae	Eho(Sh1)	Sh	Stem	Fresh	Carving	Construction (roof), except for clan leaders home. In Shekacho ethnic group it is forbidden for traditional house of gepitatos.
2261	*Rothmannia urcelliformis (Hiern) Robyns*	Rubiaceae	Diibo (Sh1)	T/ Sh	Stem, fruit and leaf	Fresh	Carving and Boiling	Coloring handcrafts, construction (fence).
2189	*Rubus steudneri Schweinf.*	Rosaceae	Garoo(Sh1)	Cl	Stem	Fresh	Carving and bending	house construction, fence
2245	*Ruta chalepensis L.*	Rutaceae	Chediramo (Sh1)	H	Twigs	Fresh	Carving the tip of the twigs.	Tooth brush (twigs).
2246	*Rytigynia neglecta (Hiern) Robyns*	Rubiaceae	Nachato(Sh1)	T	Stem	Fresh	Carving	Side wall construction (mager)

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2162	*Schefflera abyssinica* (Hochst. ex A. Rich.) Harms	Araliaceae	Manjo(Sh1)	T	Stem	Fresh & dried	Carving	Beehive hanging, beehive, timber, house hold equipments (furniture), farming implements (yoke)
2171	*Schefflera volkensi* (Engl.) Harms	Araliaceae	Qero(Sh1)	T	Stem	Fresh & dried	Carving	Construction (door support)
2242	*Sida rhombifolia* L.	Malvaceae	Sheto (Sh1)	H	Root, twig	Fresh & dried	Wrapping, tying in a bunch	Broom, vessel cleaning, tooth brush(root)
2203	*Solanecio gigas* (Vatke) C. Jeffrey	Asteraceae	Donbirako (Sh1)	T	Stem	Fresh	Carving	Construction
2177	*Solanecio mannii* (Hook. f.) C. Jeffrey	Asteraceae	Eqibelo(Sh1)	T/ Sh	Stem	Fresh	Weaving	chickens basket for laying and hatching egg
2264	*Sorghum bicolor* (L.) Moench.	Poaceae	Donqe(Mgr)	H	Twig	Dried	Wrapping in a bunch	Broom, granaries and Vessel cleaning
2169	*Syzygium guineense* subsp. *afromontanum* (Willd.) DC.	Myrtaceae	Yino(Sh1)	T	Stem	Fresh & dried	Carving	pole for house construction, wall
2229	*Teclea nobilis Del.*	Rutaceae	Molawo (Sh1)	T / Sh	Stem	Fresh & dried	Carving	Tools; wood to make ploughs.
2230	*Tiliachora troupinii Cufod.*	Menispermaceae	Acho (Sh1)	Cl	Stem (liana)	Fresh	Wrapping and soaking if dried	house construction, cordage
2224	*Trichilia dregeana Sond.*	Meliaceae	Luya /desha (Sh1)	T	Stem	Fresh & dried	Carving	Construction
2225	*Trichilia prieuriana A. Juss.*	Meliaceae	Deka (Sh2)	T	Stem	Fresh & dried	Carving and sharpening	Construction
2215	*Trilepsium madagascariense DC.*	Moraceae	Gebo /gemuy(Mgr)	T	Stem	Fresh & dried	Carving	Construction
2170	*Urera hypselodendron (A. Rich.) Wedd.*	Uritacaceae	Imano(Sh1)	Cl	Stem (liana)	Fresh	Wrapping	Cordage used for construction
2207	*Vangueria madagascariensis Gmel.*	Rubiaceae	Gujimato(Sh1)	T/ Sh	Stem	Fresh	Carving	Spear handle
2248	*Vangueria volkensii K. Schum.*	Rubiaceae	Ambilato (Sh1)	T/ Sh	Stem	Fresh & dried	Carving	Construction and simple farm implements
2155	*Vepris dainelli (Pichi-Serm.) Kokwaro*	Rutaceae	Mergeto(Sh1)	T/ Sh	Stem	Fresh & dried	Carving	Tool handles; wood to make ploughs handle (“Irf- in Amharic language”)
2164	*Vernonia amygdalina Del.*	Asteraceae	Girawo(Sh1) ; Gesi (Mgr)	T/ Sh	Stem , branch and leaf	Fresh	Carving	Construction, Fence, Vessel cleaning , tooth brush and sliver for granaries

Appendix 1. Contd.

Collection number	Scientific name	Family	Local Name (Mejengir, Shako and Shakinono)	Habit	Parts used	Form used	Methods of preparation	Uses for material culture and Notes
2165	*Vernonia auriculifera* Hiern	Asteraceae	Dengrato(Sh1)	T/ Sh	Stem	Fresh	Carving	Fence and granaries
2191	*Vernonia hochstetteri* Sch. Bip. ex Walp.	Asteraceae	Kusino(Sh1)	T/ Sh	Stem	Fresh	Carving	House construction
2221	*Vernonia lasiopus* Hoffm.	Asteraceae	Soyoma or Degorgnan (Mgr)	T/ Sh	Stem	Fresh	Carving	YEY (movable bed) in mgr ethnic groups, cloth (skirt in Shako ethnic groups), torch

Collected by •SY = Saeda Yassin

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Full Length Research Paper

Hairy roots production in *Phyllanthus odontadenius* Müll. Arg. by seedlings transformed with *Agrobacterium rhizogenes* A4RS/pHKN29

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Phyllanthus odontadenius **Müll. Arg is one of the most important medicinal plants in the genus *Phyllanthus* (Phyllanthaceae). It is used in different regions in the world for the treatment of various diseases for example malaria caused by *Plasmodium falciparum* causing millions deaths in tropical and subtropical regions. In this work, we experimented *P. odontadenius* seeds germination and seedlings infected by *Agrobacterium rhizogenes* A4RS/pHKN9. We obtained 36% of seeds that germinated for 84.6% of seedlings transformed which showed positive *gfp* activities. The number of hairy roots formed in infectious sites for seedlings infected with *A. rhizogenes* A4RS/pHKN29 is valued at 6.3±2.71 against 0 for control seedlings. Diagrams showed that mean length for the new-formed roots were 1.92±0.55 cm and 1.59±0.49 cm for hairy root.**

Key words: *Phyllanthus odontadenius*, hairy roots, *Agrobacterium rhizogenes*, malaria, secondary metabolites.

INTRODUCTION

In many tropical and subtropical regions, malaria remains one of the main global health problems of our time, causing more than 1 million deaths per year, with about 90% of deaths and 60% cases occurring in South Africa in the Sahara. It is caused by the protozoan parasite *Plasmodium falciparum* and transmitted by female Anopheles mosquitoes, which bite mainly between sunset and sunrise (WHO, 2007; Orhan et al., 2006; Mitaine-Offer et al., 2002).

The widespread resistance of *P. falciparum* against classical antimalarial drugs through the tropics (Olliaro and Yuthavong, 1999) has led to a research of new drugs with new modes of action (Tona et al., 2004). It is well known that plant species used in traditional or folk

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medicine synthesize and accumulate various secondary metabolites (Cimanga et al., 2004). *P. odontadenius* is one of most important medicinal plants in the genus *Phyllanthus* (Phyllanthaceae) used in different regions in the world for the treatment of various diseases (Unander et al., 1991; Bajaj, 1999; Luyindula et al., 2004).

In recent years, rapid procedures for obtaining transgenic roots have been developed using *Agrobacterium rhizogenes*, a soil pathogen which elicits adventitious, genetically (Ri-T-DNA) transformed roots (Chabaud et al., 2006). A. rhizogenes mediated transformation has also been used to produce transgenic hairy root culture and plantlets have been regenerated (Anand, 2010). Several studies on *A. rhizogenes* (Tepfer, 1983, 1984, 1990; Chabaud et al., 2006) have shown that although many dicotyledonous plants are susceptible to infection by these bacteria and can be transformed and produce hairy roots, some of these species have no ability to regenerate plantlets through their hairy roots.

Although *Phyllanthus niruri* hairy roots have been previously reported (Ishimaru et al., 1992; Bajaj, 1999) and showed the main constituents contained in these hairy roots. The objective of this work was to compare firstly the standard methods and modifications to those methods for *P. odontadenius* seeds germination from Kinshasa in DRC (Democratic Republic of the Congo) and secondarily to investigate *P. odontadenius* hairy roots production using *A. rhizogenes* A4RS/pHKN29 for later identification or isolation of secondary metabolites against *P. falciparum* causing malaria disease.

MATERIALS AND METHODS

Plant material and culture methods

Seeds of *P. odontadenius* from CGEA/ CREN-K in Kinshasa (DRC) were subjected to six essays of germination (A, B, C, D, E and F). Firstly, seeds were surface scarified with sulfuric acid 96% and disinfected firstly with 70% (v/v) ethanol for 1 min, and then in 0.1% (p/v) aqueous mercuric chloride ($HgCl_2$) for 3 min (Zhao et al., 2006). Secondarily, seeds were dipped in 20% Domestos for 10 min followed by four rinses with sterile water. Then seeds were dipped in 200 ppm gibberellic acid (GA_3) solution during 4 h (Jimenez et al., 2007). Thirdly, H medium (Hoagland and Arnon, 1938) was prepared then adjusted to pH 5.6 and solidified with 8 g/l agar before autoclaving at 121°C for 15 min. Seeds were placed aseptically on hormone-free H medium and incubated at 27°C for 16 h photoperiod with 50 µmol/m^2/s. Leaved seedlings were used for inducing hairy roots.

Bacterial strain culture

A. rhizogenes, strain A4RS harboring pHKN29 plasmid, containing GFP only, was used as a binary vector for the generation of control hairy roots (Kumagai and Kouchi, 2003; Nakatsukasa-Akume et al., 2005), activated by culturing on Luria and Bertani (LB) medium (LB medium: 1 L containing 10 g Bacto-tryptone, 5 g of yeast extract, 10 g of sodium chloride salt and 15 g of agar with pH adjusted to 7.2 before autoclaving). 200 mg/l of kanamycin and 300 mg/l of streptomycin were prepared for incorporation. Previously, bacteria suspensions were prepared using single colonies of bacteria in LB medium supplemented with related antibiotics and cultured overnight at 28°C shaken at 120 rpm (Zhao et al., 2006) .The cultured A4RS/ pHKN29 was transferred on LB agar for 1 or 2 days infection later on hypocotyl *Phyllanthus* seedlings.

Maintenance of hairy root cultures

Axenic *P. odontadenius* seedlings were infected directly with *A. rhizogenes* strain A4RS/ pHKN29 using a needle G26. Each of the infected seedlings was cultured on solid MS/2 medium (Murashige and Skoog, 1962; Saitou et al., 1999; Bhattacharyya and Bhattacharya, 2004) and incubated in darkness. After the first appearance of hairy roots, seedlings were transferred to hormone-free solid MS/2 medium gelled with 0.8% agar containing 3% sucrose and 500mg/l cefotaxime. About three weeks after infection, several hairy roots appeared at the wounded sites. These were cut off and cultured in hormone-free MS/2 semi-solid medium containing 500 mg/l cefotaxime for one week. This process was repeated three times to eliminate completely bacteria from the transformed roots (Ridgway et al., 2004). For investigation, number of hairy roots was counted seedling by seedling. They were cut and transferred on M/2 medium in square plate for growth.

Localization of hairy root gfp activity and calli produced at hypocotyl plants

Seedlings observation was made directly on seedling cultivated in Petri dish (plates) on solid MS/2 medium incubating after 2 weeks with bi-ocular lens MZFIII (Leica). The filters used were the *GFP1* (excitation 480/40 nm; stopped: 510 nm).

Number and length measurement

Number and length for transformed roots or untransformed roots (10) were measured with OPTIMAS 6.1 programs. Means were determined for each measurement.

RESULTS AND DISCUSSION

Seeds germination of *P. odontadenius*

Table 1 shows 36 ± 16.59% as total number of *P. odontadenius* seeds that germinated and seeds not dipped in GA_3 that did not germinate. 36±16.59% divided as well as 76.4% for Jimenez *et al.,* 2007 protocol (F) and 23.6% for *Casuarina* Protocols (E). 41.87% of seedlings from Jimenez et al. (2007) protocols were infected with *A. rhizogenes* A4RSpHKN29 with 5.4% only for seedlings from *Casuarina* protocols. On the other hand, 4.13% of seedlings obtained were immature for their infection with *A. rhizogenes* A4RSpHKN29. Others protocols used have not produce germination or seedlings because sulphuric acid showed a fatal effect on *P. odontadenius* seeds germination and on the other hand, gibberellic acid (GA_3) has positive or stimulate effect in raising of

Table 1. Percentage of *P. odontadenius* seeds germination.

Protocol types	Percentage of germination (%)	Percentage of seedlings infected with A4RSpHKN29 (%)	Percentage of seedlings not infected with A4RSpHKN29 (%)	Percentage of control seedlings (%)	Percentage of immature seedlings (%)
F	76.4	41.87	16.50	13.91	4.13%
E	23.6	5.4	7.3	0	10.9%
Total	36±16.59% of seeds germinated in the total				

F: Protocol F: Protocol of Jimenez et al. (2007); E: Protocol E: Protocol of *Casuarina (*Rhizogenesis Laboratory, IRD)*; Gfp*: Green fluorescent proteins; A4RSpHKN29: *Agrobacterium rizogenes s*train A4RS harboring pHKN29 plasmid.

Table 2. Distinctive morphological features for seedlings infected with A4RSpHKN29.

Parameter	Wounded seedlings	Presence of calli	Absence of calli	Seedlings with *gfp*	Seedlings without *gfp*
Seedlings wounded with A4RS/pHKN29	100%	73.10%	26.90%	84.60%	15.40%
Seedlings not wounded with A4RS/pHKN29	100%	0	100%	0	100%

GFP: Green fluorescent protein.

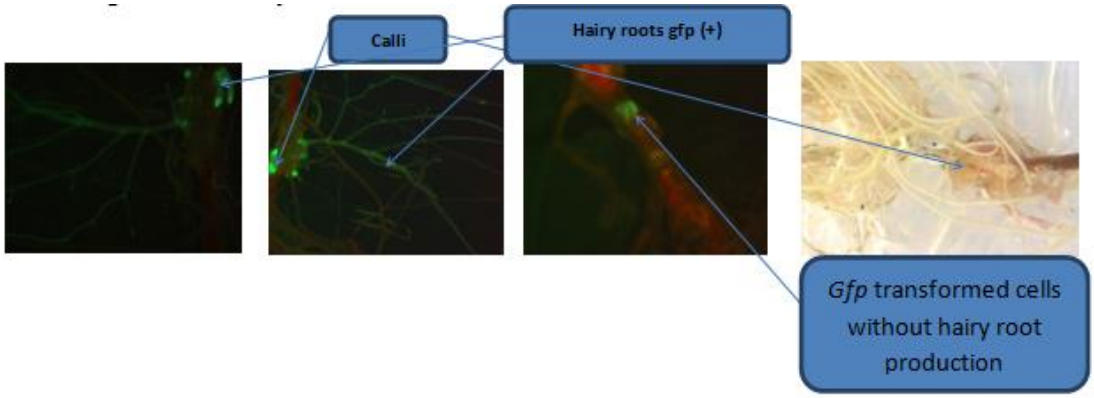

Figure 1. Enzymatic evidence and callus transformation.

dormancy seeds (Tourte, 2002).

Localization of hairy root gfp activity and calli produced on hypocotyl plantlets

Observations made under LEICA microscopic in order to certify gene reporter expression of gfp are illustrated in Table 2 and Figure 1.

Table 2 shows that 73.1% plantlets wounded with *A. rhizogenes* A4RSpHKN29 have produced calli on infected sites against 26.9 and 84.6% seedlings presenting positive gfp on the infected sites against 15.4% with negative *gfp* on the infected sites with *A. rizogenes*A4RS/pHKN29. All seedlings wounded without *A. rizogenes*A4RS/pHKN29 did not present calli or *gfp* on the infected sites. Figure 1 show enzymatic evidence and callus transformation.

Hairy roots counting and length measurement

Hairy roots number by seedlings in the infected sites and their length are presented in Figures 2 and 3. Figure 2a shows that the infected plantlets produced most new-formed roots with 6.17±3.37 in the wounded sites of *P. odontadenius* seedlings against 4.83±1.83. These two values did not present difference at P=5% (F-cal. = 0.73

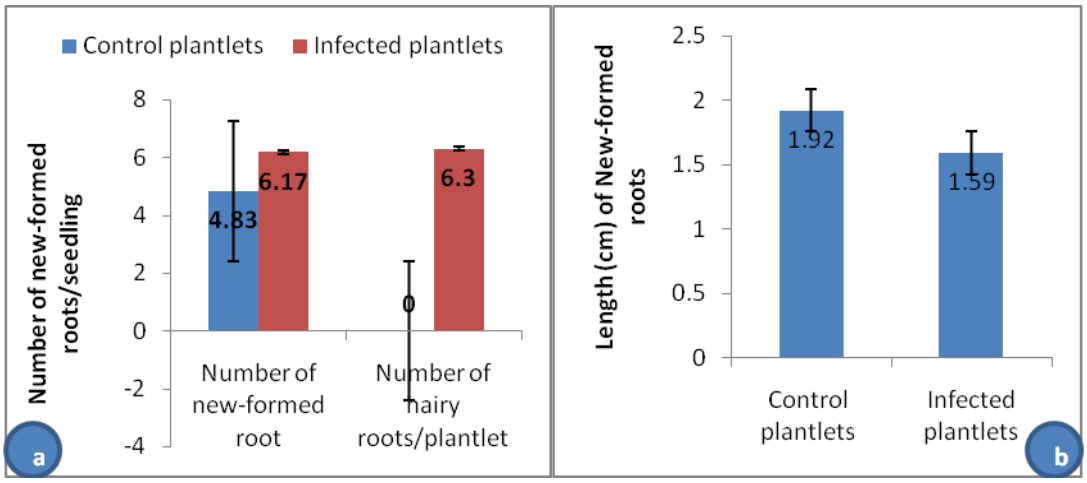

Figure 2. Number of newly-formed roots on plantlets wounded sites and length of newly-formed root on the wounded sites of *P. odontadenius.*

Figure 3. Autonomous growth of hairy roots on MS/2 in square disk.

< F-Tab. = 5.05). Hairy roots obtained with infected plantlets by *A. rhizogenes* A4RSpHKN29 were 6.3±2.71 against zero for control seedlings.

Figure 2b shows that control seedlings produced newly-formed roots in the wounded sites which measured 1.92±0.55 cm against 1.59±0.49 cm. These two values do not differ at P=5% (F-cal. = 0.97 < F-Tab. = 5.05).

Proof of autonomous hairy roots growth

Hairy roots obtained by infection of *P. odontadenius* seedlings were cut and transferred into the square disk for their autonomous growth in on MS medium with cefotaxime 300 mg/ml. The growth of hairy roots is illustrated in the Figure 3.

Figure 3 shows that all hairy roots (1, 2, 3 and 4) marked respectively (a-a'), (b-b'), (c-c') and (d-d') placed on MS/2 in square plate were increasing in length. The means length was 0.88±0.62 cm as presented in Figure 4.

DISCUSSION

The main objective of this investigation was to establish protocol of germination and hairy roots production for *P. odontadenius*, an important herbal medicinal plant. In this work, we have been showed on one hand, the role of gibberellic acid in seeds germination or seeds lifting dormancy and embryo lifting dormancy (Tourte, 2002). On the other hand, the sulphuric acid toxicity when it is used in *P. odontadenius* seeds scarification which did not germinate after their dipping in gibberellic acid solution 200 ppm. Contrary to the results obtained by Jimenez et al. (2007) with the higher percentage (60%) of *P. niruri* seeds germination *in vitro*, we obtained 36% of germinated seeds. These results are better than the various results (3.8 - 30.20%) obtained by Unander et al. (1995) after the experiments on factors affecting germination. Authors reported that the hours after harvest, the temperature, hours of exposure to light and the environment of seeds derived are the important factors in *P. amarus* seeds germination.

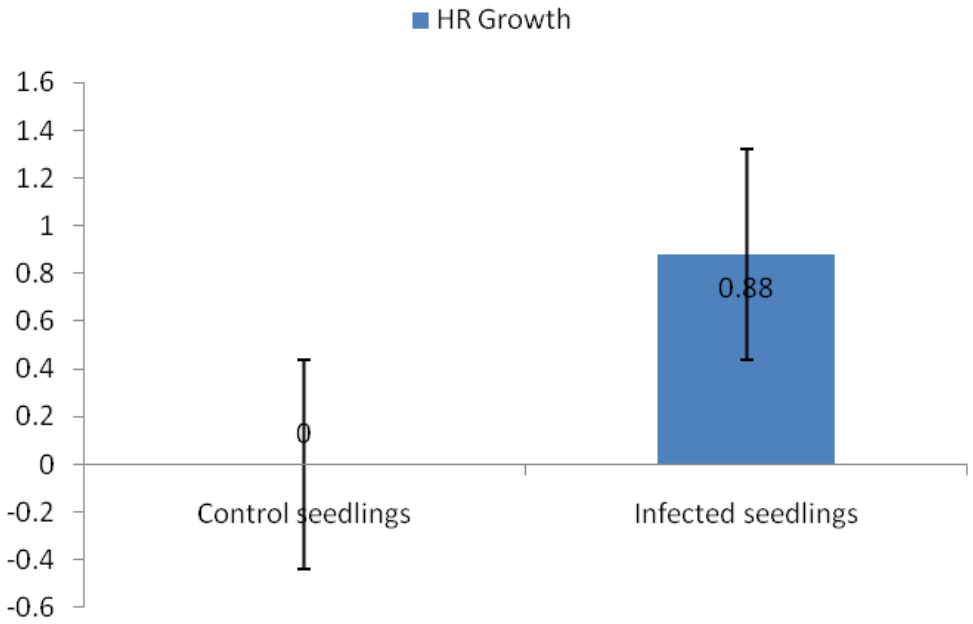

Figure 4. Length of increasing hairy roots on MS/2 in square disk.

The variation in our results shows the explanation in Unander et al. (1995) and by the Kinshasa environment issue (DRC) and the cultured media (MS and H). Another fact, we have obtained new-formed roots with all seedlings, wounded with needle and infected or uninfected with *A. rhizogenes*. It can be caused by the stress due to wounds by needle and by gibberellic acid used in seeds germination. These factors will be active on auxin hormone tenor which is increasing frequently whether by synthesis stimulations or by auxin - oxydase inhibitions for the uninfected seedlings (Augé et al., 1984). Then basic peroxydases have been particularly involved in the metabolism of auxinique catabolism, the application of gibberelic acid has been known in the increase of endogenous auxins (Zrÿd, 1988).

A. rhizogenes A4 strain, resistant to rifampicine and spectinomycine, have been shown their efficiency in the hairy roots production in Coffee (*Coffea arabica*) (Alpizar et al., 2008). Pirian et al. (2012) reported that the A4 strain was less efficient than AR15834, 9534 and C318 in the hairy root induction of *Portulaca oleracea* for adrenalin production. All strains used by these authors were rifampicin resistant.

Further, the infected or uninfected seedlings have been distinguished by the presence of callus, principle characteristic of *A. rhizogenes* strain A4RS/ pHKN29 and the green fluorescent protein (*gfp*) activities produced by the reporter gene coding *gfp* (Kumagai and Kouchi, 2003; Nakatsukasa-Akume et al., 2005) in the seedlings infected by A4RS/pHKN29. Another characteristic was the growth of hairy roots on MS/2 in the square plate than the new-formed root of control seedlings.

However, this experiment did not show difference between number and length of hairy roots (Figure 2) obtained and new-formed roots from seedlings of *P. odontadenius* uninfected with A4RS/pHKN29. This experiment has also noticed the difference in the length of the roots produced by seedlings of *P. odontadenius* and the non-multiplication of roots from the non-tainted seedlings when they were put in cultures on MS/2 without phyto-hormones whereas this growth is manifested for the roots from tainted seedlings with A4RS/pHKN29.

Conclusion

This survey revealed that *A. rhizogenes* A4RSpHKN29 encouraged the transgenic root production when the seedlings of *P. odontadenius* from germination of seeds (36%) were soaked beforehand in the gibberellic acid (200 ppm) for four hours. The proof of effectiveness of transformation has first been shown by the presence of the calluses instead of infection and then by the positive *gfp* activity when those roots are observed under the luminescent microscope. The growth of hairy roots on MS/2 in square disk was also the characteristic which confirm transgenic root production in *P. odontadenius.*

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Morphometric study of accessions of *Sesamum indicum* L. collected from Nigeria

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Sesamum (*Sesamum indicum* L.), is a very beneficial plant with manifold implementation in human life hence the identification of different species on the basis of morphological basis is indeed a need especially in less developed parts of the world. Morphometric studies of 12 accessions were carried out in order to find out characters which may be used for the delimitation of the accessions. Both quantitative and qualitative characteristics of the leaves, stems, flowers, stipule, node, fruit pods and growth type were studied and coded. Numerical analysis of these character data using cluster analysis – average linkage, single linkage, complete linkage and centroid method delimit the 12 accessions into five cluster groups which conform to the results of the morphological analysis. The results of these morphological variability analyses may set the foundation to detect promising accessions for mapping out future breeding schemes and also will be helpful for other researchers working on other aspects such as medicinal or nutritive consumption of different accessions of this multi-purpose crop.

Key words: Morphology, *Sesamum indicum*, accessions, taxonomy.

INTRODUCTION

Sesamum (*Sesamum indicum* L.), a member of the order Tubiflorae, family Pedaliaceae is perhaps the oldest oilseed known and used by humans (Weiss, 1971). It is an important annual oilseed crop in the tropics and warm subtropics, where it is usually grown in small plots (Bedigian and Harlan, 1986). Renowned for its high oil content with seeds that can contain up to 60% oil, the oil has a composition that provides good health benefits including high levels of unsaturated fatty acids (80%) and antioxidants. Possibly for this reason, sesame oil is widely considered to prevent diseases of different kinds (Pham, 2011). Farri (2012) reported that it is used in culinary and traditional medicines for its nutritive, preventive and curative properties. Beside food, sesame also finds its uses in application areas such as pharmaceutics, industrial and as biofuel. Sesame is a very drought-tolerant plant, often called a survivor crop because of its ability to survive in regions where most crops fail (Pham, 2011).

It can survive in both tropical and temperate conditions

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Table 1. List of accessions studied.

S/N	Accessions of *S. indicum*
1	Adaw-ting
2	Adaw-ting (improved)
3	Adaw-wula
4	Ex-Gombe 1
5	Ex-Gombe 2
6	Ex-Gombe 3
7	Ex-Gombe 4
8	Ex-Gombe 5
9	Ex-Gombe 6
10	Ex-Sudan
11	Kenana 4
12	Lale-duk

and grows well on stored soil moisture with minimal irrigation or rainfall. Sesame is a short-day plant and is normally self-pollinated, although cross pollination ranging from 5 to over 50% occurs (Rheenen, 1980; Pathirana, 1994). It is an erect herbaceous annual plant that has two growth characteristics, indeterminate and determinate, with the plants reaching heights of up to two meters. Most varieties show an indeterminate growth habit, which is shown as a continuous production of new leaves, flowers and capsules as long as the environment remains suitable for growth (Carlsson et al., 2008). The growth period range from 70 to 150 days depending on the variety and the conditions of cultivation (Ashri, 1998).

India and China are the world's largest producers of sesame, followed by Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan and Paraguay (FAOSTAT, 2008). Nigeria is a major exporter of sesame, which is rated second to cocoa in export volume. Nigeria has the potential to become the largest sesame seed producer in Africa and the world because FAO (2012) reported that Nigeria exported 140,800 tonnes of sesame seed worth $139 million in 2010.

Alege et al. (2013) reported that the wide diversity in a sizeable number of sesame characteristics poses a problem to proper identification of the plant's taxon and addressed this identification problem by indicating the vegetative and pod characteristics that are species specific for the purpose of their easy and quick identification. According to Abubakar et al. (2011), a prerequisite of any genetic improvement programme is a focus on morphological, anatomical and genetic variability in the local germplasm so that breeding strategies can be mapped out. Morphological and anatomical characters of plants have been used by many authors in plant identification (Sapir et al., 2002; Agbagwa and Ndukwu, 2004; Noraini and Cutler, 2009; Soladoye et al., 2010; Sharma et al., 2010). Taxonomic identification has been the basis on which plant breeding effort are founded such that diagnostic characters are assigned to specific or varietal parentage. Thus, progress in crop improve-ment depends to a great extent on the ability of the breeder to select high yielding varieties with good plant characteristics.

In view of the above facts, the present study was undertaken to develop a system of varietal identification in *S. indicum* that can be used for further improvement through morphological characters. This research will also be helpful for other researchers working on other aspects such as medicinal or nutritive consumption of different species of this plant.

MATERIALS AND METHODS

Collection of materials

Twelve (12) sesame accessions were used in this study, which consisted of the list of the accessions in Table 1. The field experiment was carried out at the Research Farm, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

Data collection

At maturity (at about 12 weeks after planting), quantitative morphological characters which include plant height, leaf length, leaf breadth, petiole length, pod length, pod breadth and pod size were measured using standardized centimetre rule and electronic digital calliper. The mean and the corresponding standard errors of ten measurements taken for each character as suggested by Lawal et al. (2007) were recorded. The average number of primary branches per plant, number of leaves per plant and number of pod per plant were also recorded. Qualitative morphological features which include growth type, leaf shape, leaf type, leaf margin, leaf arrangement, leaf surface, form of stipule, colour of dot at stipule, leaf orientation, leaf colour, leaf apex, leaf base, stem type, stem texture, stem colour, calyx colour, corolla colour, type of cymose inflorescence, flower aestivation, flower shape, flower colour, fixation of anther, fruit type, fruit shape, fruit size, fruit colour (immature, mature and dry colour), fruit end shape, fruit lobes and fruit surface were recorded based on visual assessment. Terminologies used throughout were those of Pandey and Misra (2009).

Numerical taxonomy

Selection of operation taxonomic units (OTUs)

Altogether, data matrix comprising forty-two characters by twelve accessions were used for the cluster analysis which was based on Adansonian principles (Sokaland Sneath, 1973; Pandey and Misra, 2009) which suggested that many characters should be used for classif ication purposes. These included both qualitative and quantitative morphological characters.

Data analysis

The data matrix was analyzed using hierarchical cluster analysis. The agglomeration schedule employed were the average linkage between groups, complete linkage, single linkage and the centroid method, Squared Euclidean distance as recommended by Lawal et al. (2007) was used to measure the distance between OTUs. The ranges of variations of these characters among the OTUs were observed and noted. For the qualitative characters, the character

Table 2. Qualitative morphological characters of accessions of *S. indicum* studied.

Acc.	Leaf type	Leaf shape	Leaf margin	Leaf base	Leaf apex	Leaf surface	leaf arrangement	Leaf colour	Stem texture	Stem colour	Stem type	Spot at stipule
1	Unipinnate	Lanceolate	Serrated	Acute	Acute	Glabrous	Dou Opposite	Green	Glabrous	L Green	Postrate	Brown
2	Unipinnate	Lanceolate	Entire	Acute	Acute	Pubescent	Alt Spiral	Green	Glabrous	L Green	Postrate	Yellow
3	Unipinnate	Lanceolate	Entire	Acute	Acute	Pubescent	Opp Spiral	Green	Glabrous	L Green	Postrate	Brown
4	Unipinnate	Lanceolate	Entire	Acute	Acute	Glabrous	Opp Spiral	Green	Pubescent	L Green	Postrate	Brown
5	Unipinnate	Lanceolate	Entire	Acute	Acute	Glabrous	Opposite	Green	Glabrous	Green	Postrate	Brown
6	Bipinnate	Cordate	Biserrated	Cordate	Acute	Pubescent	Opp Spiral	Green	Pubescent	Green	Postrate	Brown
7	Unipinnate	Lanceolate	Entire	Obtuse	Acute	Glabrous	Alternate	Green	Glabrous	L Green	Postrate	Yellow
8	Unipinnate	Ovate	Serrated	Obtuse	Acute	Pubescent	Alt Spiral	Green	Pubescent	L Green	Postrate	Brown
9	Unipinnate	Ovate	Serrated	Acute	Obtuse	Pubescent	Opp Spiral	Green	Pubescent	L Green	Postrate	Brown
10	Unipinnate	Lanceolate	Entire	Acute	Acute	Pubescent	Opposite	Green	Pubescent	L Green	Postrate	Yellow
11	Unipinnate	Lanceolate	Entire	Acute	Acute	Pubescent	Opp Spiral	Green	Pubescent	L Green	Postrate	Brown
12	Unipinnate	Lanceolate	Entire	Acute	Acute	Glabrous	Opp Spiral	Green	Glabrous	L Green	Postrate	Yellow

opp = Opposite, alt = alternate, L = light, dou = double.

states were written out against the respective characters in a table. For the quantitative characters, the following formulae were used to determine the number of states K = 1.0 + 3.332 log n, where K = number of states and n = number of OTU's. The character values in the number of states were divided based on the range of variations (Hill, 1980). The programmes were run on a personal computer using the IBM Statistical Package for Social Sciences (SPSS) version 20.

RESULTS

Tables 2 to 6 and Figures 1 to 11 give the summaries of the results of this study. The qualitative morphological studies showed a very close relationship between the twelve accessions. All the accessions had the same unipinnate leaf type, green leaf colour, indeterminate growth type, erect stem type, lateral stipule form, green calyx colour, campanulate form of calyx, valvate flower aestivation, bell flower shape (Figure 8), basifixed anther, capsule fruit type, oblong fruit shape, green immature fruit colours, yellow mature fruit and brown dry fruit colour, hairy fruit surface and tetralobed fruit lobes (Tables 2 to 4). The diagnostic morphological features used in the delimitation of accessions are the leaf shapes which were observed to be ovate, lanceolate and cordate (Figures 3 to 5); leaf margins- entire, serrate or double serrate; leaf bases- acute, cordate or obtuse; leaf apices- acute or obtuse (Figures 3 to 5 and Table 2); leaf arrangement- opposite (Figure 7), alternate (Figure 6), double opposite, alternate spiral or opposite spiral; leaf surfaces- pubescent or glabrous; stem texture- pubescent or glabrous; stem colour- green or light green; dot at stipule- black or yellow; corolla colour- cream or purplish white; cymose inflorescence- monochasial cyme, dichasial cyme or polychasial cyme; fruit end form-dentate, cirrhose or cordate (Figure 10) and fruit sizes- small, medium or large (Table 3 to 4 and Figure 9).

Morphometric features in accessions of *S. indicum* studied are shown in Table 5. The average plant heights range from 67.86 to 115.08 cm in all the accessions with the lowest mean recorded in accession lale-duk (67.86 cm) and the highest mean in Adaw-wula (115.08 cm). The number of branches per plant showed a wide variation in the accessions; it ranges from 1.6 (Ex-Gombe 1) to 89.2 cm (Lale-duk).

The lowest leaf length mean (3.32 cm) and leaf breadth (2.26 cm) were recorded by Lale-duk, while the highest leaf length (12.29 cm) and leaf breadth (4.52 cm) by Ex-Gombe 2 and Ex-Gombe 6, respectively. The accession showing the highest average number of pods per plant in all the samples studied was Lale-duk (120.20) and the lowest recorded by Ex-Gombe 6 (17.00). The length ranges between0.82 – 4.34 cm. Fruits of adaw-wula are the largest among the accessions with the average size of 232.81 mm while Lale-duk recorded the smallest fruit size (75.32 mm).

Table 3. Floral morphological characters in accessions of *S. indicum* studied.

Accessions	Corolla colour	Cymose inflorescence
Adaw-ting	Dark pink	Dichasial cyme
Adaw-ting (improved)	Dark pink	Polychasial cyme
Adaw-wula	Dark pink	Polychasial cyme
Ex-Gombe 1	Dark pink	Polychasial cyme
Ex-Gombe 2	Light pink	Polychasial cyme
Ex-Gombe 3	Light pink	Monochasial cyme
Ex-Gombe 4	Dark pink	Dichasial cyme
Ex-Gombe 5	Light pink	Dichasial cyme
Ex-Gombe 6	Dark pink	Dichasial cyme
Ex-Sudan	Dark pink	Polychasial cyme
Kenana 4	Dark pink	Monochasial cyme
Lale-duk	Dark pink	Dichasial cyme

Table 4. Fruit pod morphological features in accessions of *S. indicum.*

Accessions	Fruit size	Fruit end form
Adaw-ting	Medium	Dentate
Adaw-ting (improved)	Medium	Dentate
Adaw-wula	Large	Cirrhose
Ex-Gombe 1	Small	Dentate
Ex-Gombe 2	Medium	Cirrhose
Ex-Gombe 3	Medium	Cirrhose
Ex-Gombe 4	Medium	Cirrhose
Ex-Gombe 5	Large	Cirrhose
Ex-Gombe 6	medium	Dentate
Ex-Sudan	Medium	Cirrhose
Kenana 4	Large	Cirrhose
Lale-duk	Small	Cordate

The results of hierarchical clustering procedures are presented in the form of phenograms, which are diagrams of relationship. The abscissa shows the spacing out of the accessions employed in the study while the ordinate on the other hand represents degree of similarity between and among the accessions. Figures 1 and 2 are phenograms of 42 x 12 data matrix obtained from average linkage cluster analysis and complete linkage cluster analysis using both qualitative and quantitative morphological characters.

The analysis of the values indicated five cluster groupings (Figures 1 to 2 and Table 6). Complete linkage (CL) clustering method showed that cluster 1 is comprised of Ex-Gombe 6, Adaw-ting (improved) and Adaw-ting; cluster 2 constitute accession Kenana 4, Adaw-wula, Ex-Gombe 3 and Ex-Gombe 2; cluster 3 has only accessions lale-duk while two accessions each make up cluster 4 (Ex-Gombe 5 And Ex-Gombe 4) and cluster 5 (Ex-Sudan and Ex-Gombe 1). Although branching occurred at low phenon levels which showed broad similarities among the accessions, there are some accessions that stand as outliers and do not correspond to any group.

DISCUSSION

The varietal description for identification of crop accessions has assumed critical importance in national and international seed programmes and there is a considerable need for the development of reliable methods and identifiable characters for the purpose. The characters for which a variety is distinct from other could be morphological, chemical and biochemical in nature which aids in varietal identification (Suhasini, 2006).

The use of plant diagnostic characteristics to identify a

Table 5. Quantative morphological characters in accessions of *S. indicum.*

Acc.	Plant height (cm)	No. of branches per plant	No. of leaves per plant	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	No. of pods per plant	Fruit length (mm)	Fruit breadth (mm)	Fruit size
1	90.58±3.00	2.00±0.63	43.60±1.33	7.44±0.33	4.20±0.15	2.42±0.11	45.20±6.43	26.93±1.78^{bc}	8.90±0.27	185.88±19.74
2	96.38±5.81	3.20±0.49	45.60±3.83	9.18±0.59	3.48±0.34	3.20±0.39	25.80±1.62	24.87±0.67^{bc}	7.78±0.17	154.35±6.56^{b}
3	100.22±10.01	2.40±0.40	38.00±2.43	12.29±1.11	4.42±o.25	4.34±0.29	45.00±6.77	27.16±1.51^{bc}	8.84±0.16	190.33±13.36
4	102.60±1.43	24.80±5.12	166.40±2.23	7.90±0.67	3.92±0.27	1.88±0.18	89.20±18.76	26.94±1.72^{bc}	7.57±0.21	162.01±13.98
5	90.66±2.34	1.6±0.40	46.20±1.62	10.92±0.62	4.18±0.58	3.14±0.32	17.00±2.53	24.48±0.52^{bc}	7.15±0.17	139.34±5.11^{b}
6	67.86±4.66	89.2±26.98	227.10±10.43	3.32±0.13	2.26±0.14	1.36±0.04	120.20±9.05	13.63±0.38^{a}	7.00±0.13	75.32±3.16
7	101.70±12.89	3.6±0.75	56.00±3.75	9.66±0.57	2.32±0.45	0.82±0.04	48.60±3.46	27.35±2.18^{bc}	9.52±0.20	206.16±17.86
8	85.70±4.53	22.40±4.44	63.60±6.85	8.28±0.40	4.52±0.29	0.98±0.15	65.00±7.81	23.94±0.77^{bc}	7.60±0.22	144.18±8.30
9	89.08±6.71	16.80±3.67	167.20±2.06	8.08±0.44	3.56±0.23	2.74±0.32	45.20±6.95	25.73±1.18^{bc}	7.39±0.17	151.39±8.11^{b}
10	97.04±7.14	2.80±0.49	16.8±1.24	11.04±0.4	2.72±0.27	1.24±0.19	70.00±5.36	30.75±1.98^{b}	8.71±0.37	212.81±19.58
11	115.08±6.84	3.80±0.20	47.00±3.87	11.62±0.86	3.88±0.32	3.86±0.17	46.20±2.15	31.65±2.28^{b}	9.24±0.18	232.18±20.93
12	93.52±8.33	2.80±0.49	43.20±4.60	10.2±0.48	2.66±0.27	2.40±0.08	39.00±4.44	26.68±3.31^{bc}	8.52±0.49	183.12±30.52

Table 6. Groups of accessions according to the method of analysis.

Method of analysis	Cluster membership				
	Group 1	Group 2	Group 3	Group 4	Group 5
Average Linkage	Ex-Gombe 6	Kenana 4	Lale-duk	Ex-Gombe 5	Ex-Sudan, Adaw-wula, Adaw-ting, Adaw-ting (improved), Ex-Gombe 4, Ex-Gombe 2, Ex-Gombe 3
Complete Linkage	Ex-Gombe 6, Adaw-ting (improved), Adaw-ting	kenana 4, Adaw-wula, Ex-Gombe 3, Ex-Gombe 2	lale-duk	Ex-Gombe 5, Ex-Gombe 4	Ex-Sudan, Ex-Gombe 1
Single Linkage	Ex-gombe 6	Kenana 4	Lale-duk	Ex-Gombe 5	Ex-Sudan, Adaw-wula, Adaw-ting, Adaw-ting (improved), Ex-Gombe 4, Ex-Gombe 2, Ex-Gombe 3
Centroid Linkage	Ex-Gombe 6	Kenana 4	Lale-duk	Ex-Gombe 6	Ex-Sudan, Adaw-wula, Adaw-ting, Adaw-ting (improved), Ex-Gombe 4, Ex-Gombe 2, Ex-Gombe 3

variety has been classical taxonomic approach for both varietal purity and varietal identification (Suhasini, 2006; Pandey and Misra, 2009). The general appearance of the leaves of *S. indicum* in all of the accessions are unipinnate with heterogeneous leaf shapes, lanceolate (Figure 3), ovate (Figure 4) and cordate (Figure 5).

Though, Suhasini (2006) observed only one type of leaf shape (lanceolate), Kashiram (1930)and Weiss (1971) observed varied leaf shapes such as lobed, lanceolate and linear in sesamum genotypes. All the accessions have acute or

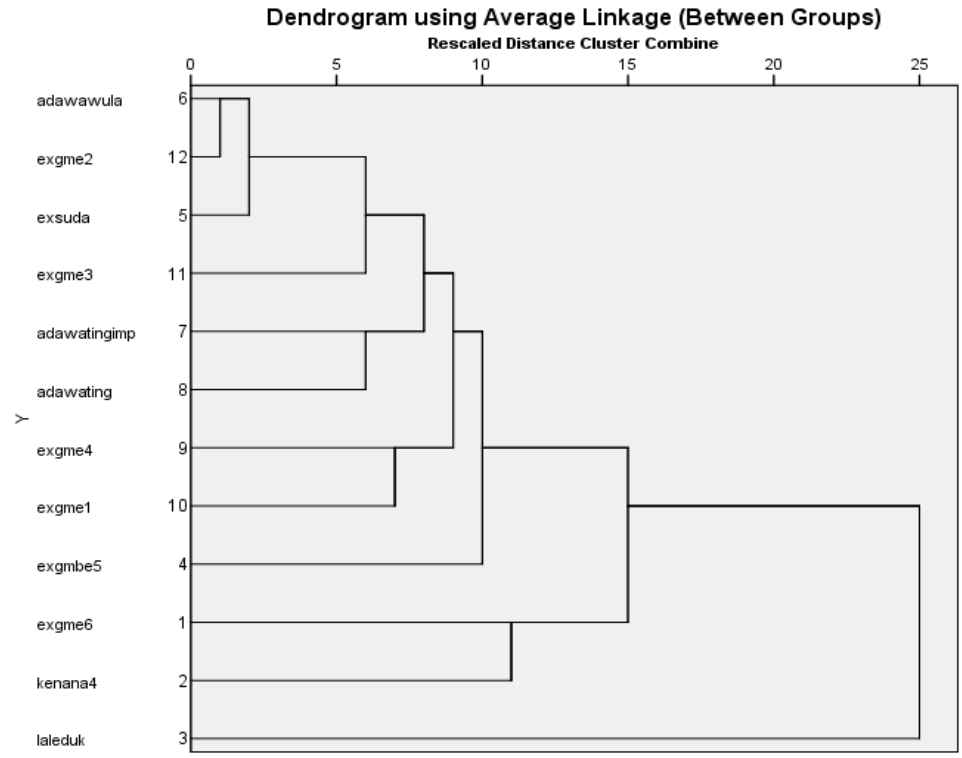

Figure 1. Dendrogram using nearest neighbour squared Euclidean distance in accessions of *S. indicum.*

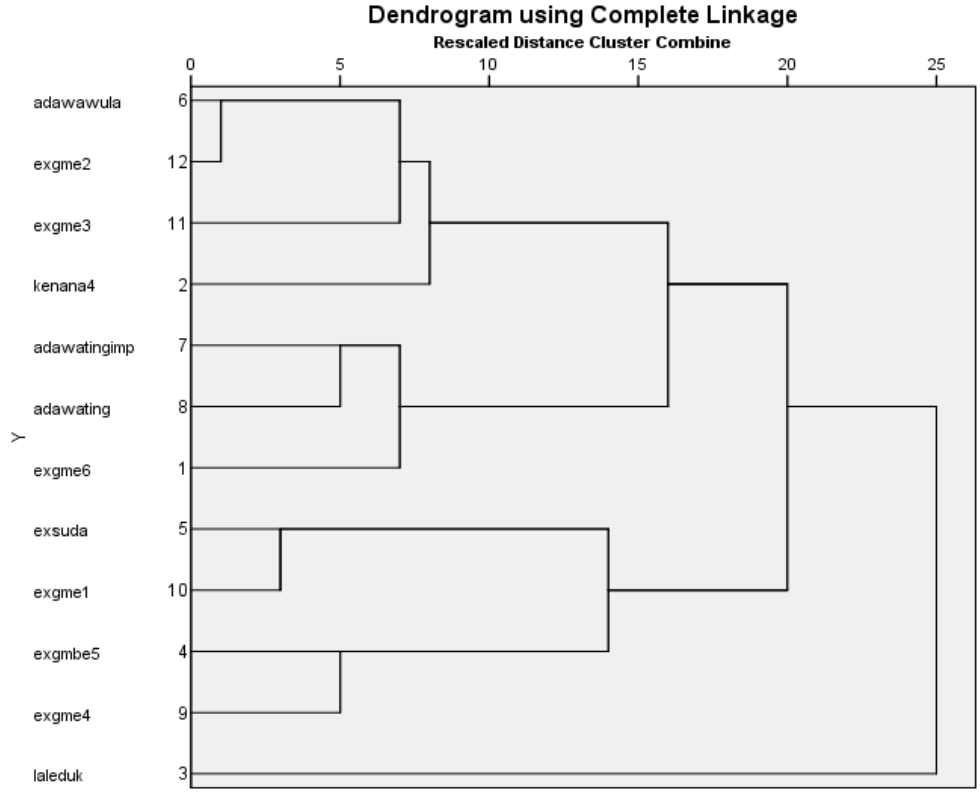

Figure 2. Dendrogram using furthest neighbour squared Euclidean distance in accessions of *S. indicum.*

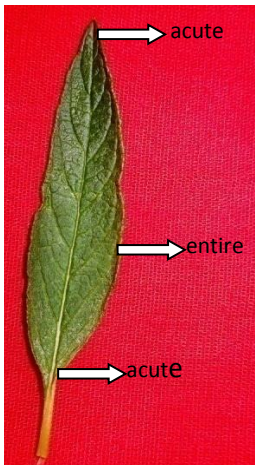

Figure 3. Surface view of lanceolate leaf shape with entire margin, acute leaf base and apex.

Figure 4. Surface view of ovate leaf shape with biserrate margin.

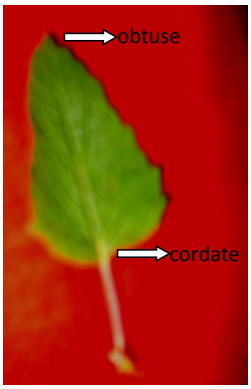

Figure 5. Surface view of cordate leaf shape with obtuse apex.

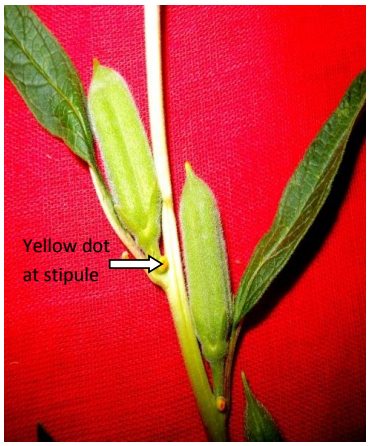

Figure 6. Surface view of node with yellow spot at stipule.

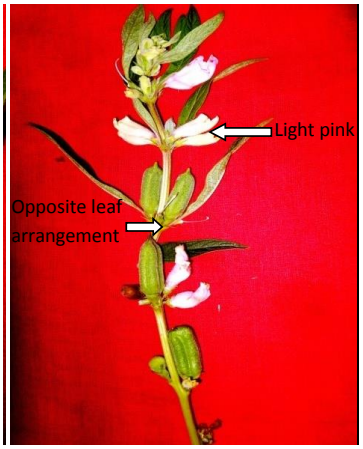

Figure 7. Surface view of Ex-Gombe 3 showing opposite leaf arrangement and light pink flower.

Figure 8. Surface view of Adawting showing dark pink corolla.

Figure 9. Side view of *S. indicum.*

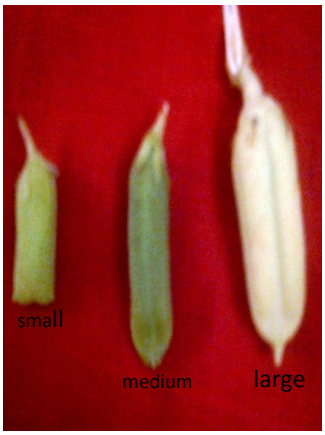

Figure 10. Surface view of the three types of pod sizes.

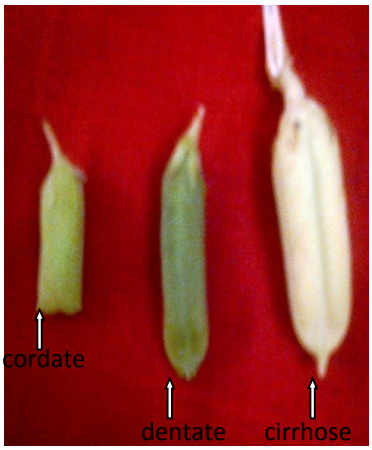

Figure 11. Surface view of three types of pod beaks.

obtuse leaf apices, entire, serrate or biserrate leaf margins; acute, cordate or obtuse leaf bases; opposite, alternate, double opposite, alternate spiral or opposite spiral leaf arrangement and pubescent or glabrous leaf surfaces which suggest these features to be inherent characters. The leaf colour is one of the important characteristics used for grouping of the accessions. The leaf colour depends upon the intensity of chlorophyll pigmentation which in turn varies with genotypes according to their genetic constitution. The genotypic variation in colour of the leaf is also governed to some extent by the varied response to environmental conditions such as light intensity and nutrition (Suhasini, 2006). Thus, in the present investigation, leaf colour of the sesamum accessions varied from light green to green. Changes in plant morphological features in terms of variation have been attributed to small-scale evolutionary processes, a view supported by the works of Serebrayanaya and Shipunov (2009) in different species of plants. In addition to this, leaf size variation in taxonomy forms a basis for evolutionary changes in plants; also, it can modify the distribution of leaf biomass between support and functional tissues (Xu et al., 2008). With the stem textures being pubescent or glabrous; stem colour- green or light green; dot at stipule– black or yellow (Figure 6).

The floral morphology showed the presence of four to five, green, gamosepalous calyx and four to five light pink observed in 3 accessions (Figure 7) or dark pink observed in 9 accessions (Figure 8) gamopetalous corolla. The petal colour of the flower is one of the important characters for characterization. Similar results were observed by Kashiram (1930) in sesamum, Tarasatyavathi et al. (2004) and Kumar et al. (2005) studied petal colour in jute accessions and reported that the actions of the genes were responsible for variations in the petal colour of the genotypes. The genes determine the colour of the petal by developing or blocking of anthocyanin pigmentation

The pod characteristics influence the yielding ability of the plant. Variations were observed for various characteristics such as number of pods per plant, pod length, pod breadth, pod size and pod shape, pod beak and number of locules per pod which help in classifying the accessions into different group (Tables 4 and 5). There was no variation in fruit shape as all the accessions were observed to have oblong fruit shape with four lobes. The variation in pod number may be due to pod bearing ability of the accession itself. The fruit end forms have great diagnostic features among the accessions. The accessions were observed to have cirrhose, dentate or cordate fruit end form (Table 4). Suhasini (2006) reported that the inheritance of capsule length was found to be conditioned by two to five pairs of factors with a heritability value of 50 to 70%, indicating the genetic influence in determining the pod length. The number of locules per pod did not vary with the accession;

all the accessions were observed to have four locules. Similar results by Suhasini (2006) in chickpea sesame accessions showed that the genotypic expression of four and six loculed pods is mainly controlled by the gene actions as four locules expression is governed by dominant gene and six loculed by recessive gene in *Sesamum*.

In hierarchical cluster analysis, accessions were grouped based on the coded data matrix of both quantitative and qualitative traits. Harch et al. (1997) and Escribano et al. (1998) used quantitative and qualitative traits to determine genetic diversity among the world groundnut collection and common bean, respectively. The dendrograms constructed from the pooled data (Figure 1) clearly showed, five major clusters namely cluster 1, 2, 3, 4 and 5. Only one accession (lale-duk) was found on cluster 3 in the four methods of analysis. Figures 1 to 2 and Table 6 show that Ex-gombe 6, kenana 4 and ex-gombe 5 were found in cluster 1, 2 and 4, respectively, using the average linkage, single linkage and centroid linkage methods (Table 6). These indicated the morphological diversities between and within these accessions, hence, the need to delimit the taxa.

In conclusion, the present analysis using morphological features in accessions of *S. indicum* indicated the existence of variation among the samples which can form a basis for developing a system of varietal identification and the possibility of improving the crop. Specifically, the accession lale-duk showed the highest representation of distinct morphological characters in all the groups. However, additional effort using other taxonomic markers to support the present findings with a view to enhance the delimitation of the accessions of *S. indicum* for mapping out breeding schemes for improved yield is hereby recommended.

Conflict of interest

The authors have not declared any conflict of interest.

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In-situ morphological characterization of coconut in the Coastal Lowlands of Kenya

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Characterization of *in situ* coconut (*Cocos nucifera* L.) genotypes using morphological descriptors is of primary importance for genetic resources. Variations in five traits were recorded on 48 selected coconut palms located at 0 to 177 m above sea level in the Coastal Lowlands of Kenya. The objective of our study was to characterize coconut palms in the coastal Kenya using morphological markers. Data was recorded on palms that included tall, dwarf and hybrid varieties. Data was analyzed using Genstat Ver 14.2 software. Phylogenetic tree construction, using the neighbour joining method, revealed that the germplasm fell into three major clusters comprising of 2, 25 and 21 genotypes, respectively. Principal component analysis (PCA) showed that the first component (CP1) accounted for 65.54% of the total variation and was associated with the number of green leaves. The second component (PC2) explained 19.71% of the total variation and was associated with stem height. The results from this study indicate that morphological variation for coconut germplasm at the Kenyan coast exists for both qualitative and quantitative characters. Coconut palms did not cluster on the basis of their origin based on the counties grown.

Key words: Morphological characterization, *Cocos nucifera* L., variation, genetic resources, genetic diversity, cluster analysis.

INTRODUCTION

Coconut (*Cocos nucifera* L.), is a monocotyledonous plant of the family Arecaceae (Palmaceae), subfamily Cocoideae and the monospecific genus *Cocos.* It is an important crop at the Kenyan coast, providing its growers with a source of livelihood. It also plays a significant role in the economic, cultural and social life of over 80

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tropical countries in the world. Coconut is cultivated mainly as an oil seed crop. The oil is rich in lauric acid and besides being cooking oil it has a variety of other uses (Harries, 1995). Almost every part of the coconut tree can be used in either making commercial products or meeting the food requirements of rural communities (Teulat et al., 2000). Perera et al. (2000) considered Southeast Asia as the centre of origin of coconut, whilst Melanesia is thought to be the most likely region for coconut domestication along the coasts and islands between Southeast Asia and the Western Pacific (Harries, 1995). Coconut has been found distributed in many parts of the world including Central and South America, East and West Africa, Southeast Asia, East Asia and the Pacific islands. According to Ohler (1984) dissemination was achieved by seeds floating in sea currents and subsequent germination on the shore, followed by further human dispersal. Geographic isolation, introgressive hybridization, mutation, and selection are the most likely causes of population differentiation of coconut. The mode of dispersal of coconut is likely to have resulted in founder effects influencing population differentiation (N'Cho et al., 1993). Tall and dwarf types are the two main stature and breeding habit categories used as the primary classification of coconut (Menon and Pandalai, 1958). Tall coconuts (talls) grow to a height of about 20-30 m and are allogamous, late flowering, and their nuts are medium to large in size. They are hardy and thrive in a wide range of environmental conditions. Dwarf coconuts (dwarfs) grow to a height of about 10-15 m and are autogamous, early flowering, and generally produce a large number of small nuts with distinctive colour forms (Menon and Pendalai, 1958). Introgression of talls and dwarfs and further selection and dissemination by man produced the wide range of varieties and pan-tropical distribution of coconut seen today (Harries, 1978).

The improvement of crop genetic resources depends on continuous infusion of desired traits of wild relatives, traditional varieties and use of improved breeding methods. These require an assessment of diversity to select superior varieties (Mondini et al., 2009). Diversity analyses in coconut palm to date have been done by morphological traits, biochemical and molecular markers (Dasanayaka et al., 2009). Using morphological and biochemical markers have disadvantages due to the long juvenile phase of the plants, high cost, long-term of field evaluation, potential environment factors' influence on the phenotype and limited number of available phenotypic markers (Manimekalai et al., 2006). However, morphological markers are easy to study. Assessment of the genetic diversity present within a species is a prerequisite for future sustainable breeding efforts and germplasm management. Molecular markers provide an important technology for evaluating levels and patterns of genetic diversity and have been utilised in a variety of plant species (Rafalski et al., 1996). To date, there are over 300 recorded ecotypes of coconut (Coconut Genetic Resources Network Database v. 2.2 COGENT/IPGRI), with evaluation and characterization being mainly carried out on morphological and reproductive traits (Fernando et al., 1995).

Currently, coconut production in Kenya is low despite its potential and adequate farmer-to-farmer dissemination of market access (Muhammed et al., 2012). The present level of productivity is low, which is probably due to pest and disease attacks, old and unproductive orchards and inadequate production of quality planting material for replanting and new planting (Muhammed et al., 2013). Decline of soil fertility, lack of a reliable and fast means of generating clean planting materials, general lack of value additions and poor agronomic packages contribute to low production (Muhammed et al., 2013).

The tall and dwarf coconut types as well as their intermediates, which are thought to be their hybrids, have traditionally existed at the Kenyan coast. However, no genetic or morphological diversity studies on coconut at the Kenyan coast have been conducted and documented. Therefore, an *in situ* morphological characterization of the coconut grown at the coastal lowlands of Kenya was conducted with a view to understand and document the morphological diversity among coconut populations at the Kenyan coast. Knowledge of the degree of morphological relationships is of importance for crop improvement and may help in establishing core collection for future research work.

MATERIALS AND METHODS

The study was conducted in the coastal lowlands of Kenya in April 2013. A total of 48 samples were included in the study. There were 45 tall palms designated East African Talls (EAT), including the suspected hybrids and three East African Dwarfs (EAD) that broadly represented all varieties of coconut from different coconut-growing regions at the Kenyan coast. For the character assessment, eight varieties were included that researchers and farmers considered as hybrids. Of these, 15 palms were sampled from gardens/house yards, 16 from small farm fields while 17 were sampled from large farm fields. Samples were specifically taken from areas where the palms grown were morphologically different and there was a marked change in altitude or cropping systems, where a formidable barrier such as a mountain or a river existed or where local people were ethnically different (in terms of dialect) from previous collection sites. This strategy aimed at reducing chances of sampling duplicates. Selected areas in coastal regions were sampled to cover different agro ecological zones, designated as Coastal Lowland (CL), Sugarcane zone (CL2), Coconut - cassava Zone (CL3), Cashew-cassava Zone (CL4) and Millet-livestock zone (CL5) (Table 1 and Figure 1). The range of altitudes covered was 0 m above sea level at Faza in Lamu County to 177 m above sea level at Rabai, Kilifi County. Palms with similar features growing in ecologically distinct sites, were assumed to be of different eco-strains and were both sampled and characterized. For every palm tree sampled, Global Positioning System (GPS) data was recorded and the tree photographed while notes were taken on the general structure and appearance of the population, origin of the population and the collection sources. Adjacent palm status was also recorded.

Table 1. Names and sources of the Kenyan coast coconut germplasm used in the study.

Characterized germplasm code	Local Name	County	District	Division	Location	Village	Dialect	Type
KLF/MAG/MAG/GOG/NGA/03	Nazi	Kilifi	Magarini	Magarini	Gogoni	Ngarite	Giriama	Tall
KLF/MAG/MAG/GOG/NGA/01	Nazi	Kilifi	Magarini	Magarini	Gogoni	Ngarite	Giriama	Tall
KLF/MAG/MAG/GOG/NGA/04	Nazi	Kilifi	Malindi	Magarini	Gogoni	Ngarite	Giriama	Tall
KLF/MAG/MAG/GOG/NGA/02	Nazi	Kilifi	Malindi	Magarini	Gogoni	Ngarite	Giriama	Tall
KLF/MAL/MAL/GED/CHA/03	Nazi wa panda	Kilifi	Malindi	Malindi	Gede	Chafisi	Giriama	Tall
KLF/MAL/MAL/WAT/JIB/01	Mnazi	Kilifi	Malindi	Malindi	Watamu	Jimba	Giriama	Tall
KLF/MAL/MAL/WAT/JIB/02	mnazi	Kilifi	Malindi	Malindi	Watamu	Jimba	Giriama	Tall
KLF/RAB/KAM/KAM/MBU/09	Mtsamuri	Kilifi	Rabai	Kambe/Ribe	Kambe	M'bungoni	Kambe	Tall
KLF/RAB/KAM/PAN/MAE/10	-	Kilifi	Rabai	Kambe/Ribe	Pangani/Maereni	Maereni	Kambe	Tall
KLF/RAB/MWA/MIK/MAW/08	Mtsamuli	Kilifi	Rabai	Mwawesa	Mikahani	Mawe Mabomu	Rabai	Dwarf
KLF/RAB/RAB/RAB/BUN/02	-	Kilifi	Rabai	Rabai	Rabai	Buni	Rabai	Tall
KLF/RAB/RAB/RAB/BUN/03	Mnazi msemwa	Kilifi	Rabai	Rabai	Rabai	Buni	Rabai	Tall
KLF/RAB/RAB/RAB/BUN/04	Mnazi Mtune	Kilifi	Rabai	Rabai	Rabai	Buni	Rabai	Tall
KLF/RAB/RAB/RUR/JIB/05	Mnazi wa Kawaida	Kilifi	Rabai	Rabai	Ruruma	Jimba B	Rabai	Hybrid
KLF/RAB/RAB/RUR/JIB/06	Mnazi wa Kawaida	Kilifi	Rabai	Rabai	Ruruma	Jimba B	Rabai	Hybrid
KLF/RAB/RAB/RUR/JIB/07	Mnazi wa Kawaida	Kilifi	Rabai	Rabai	Ruruma	Jimba B	Rabai	Hybrid
KWL/KWA/MAT/KIK/KIG/02	Mnazi	Kwale	Kwale	Matuga	Kikoneni	Kigato	Digo	Tall
KWL/KWA/MAT/KIK/KIG/01	Mnazi	Kwale	Kwale	Matuga	Waa	Kigato	Digo	Tall
KWL/KWL/MAT/WAA/KOM/05	Gao	Kwale	Kwale	Matuga	Waa	Kombani	Digo	Tall
KWL/KWL/MAT/WAA/KOM/04	Gao	Kwale	Kwale	Matuga	Waa	Kombani	Digo	Tall
KWL/KWL/MAT/WAA/MWA/03	mnazi	Kwale	Kwale	Matuga	Waa	Mwatate	Digo	Tall
KWL/KWL/MAT/WAA/TSU/06	Kisamili/Kipemba	Kwale	Kwale	Matuga	Waa	Tsunguni	Digo	Dwarf
KWL/MSA/LUN/KIK/KAM/03	Kipini	Kwale	Msambweni	Lungalunga	Kikoneni	Kambe	Digo	Tall
KWL/MSA/LUN/KIK/KAM/05	Tonga	Kwale	Msambweni	Lungalunga	Kikoneni	Kambe	Digo	Tall
KWL/MSA/LUN/KIK/KAM/02	Nazi yaKinyamwezi	Kwale	Msambweni	Lungalunga	Kikoneni	Kambe	Digo	Tall
KWL/MSA/LUN/KIK/KAM/01	Kipemba	Kwale	Msambweni	Lungalunga	Kikoneni	Kambe	Digo	Tall
KWL/MSA/LUN/KIK/KAM/04	Kisamli	Kwale	Msambweni	Lungalunga	Kikoneni	Kambe	Digo	Tall
KWL/MSA/MSA/MIL/MAB/01	-	Kwale	Msambweni	Msambweni	Milalani	Mabatani	Digo	Hybrid
LMU/LMUE/FAZ/FAZ/FAZ/38	Mnazi waKawaida	Lamu	LamuEast	Faza	Faza	Faza	Arab	Tall

Table 1. Contd.

Characterized germplasm code	Local Name	County	District	Division	Location	Village	Dialect	Type
LMU/LMUE/FAZ/FAZ/FAZ/41	Mnazi Mfupi	Lamu	LamuEast	Faza	Faza	Faza	Bajuni	Hybrid
LMU/LMUE/FAZ/FAZ/FAZ/40	Mnazi Mfupi	Lamu	LamuEast	Faza	Faza	Faza	Bajuni	Hybrid
LMU/LMUE/FAZ/FAZ/FAZ/36	Mnazi wa Kawaida	Lamu	LamuEast	Faza	Faza	Faza	Arab	Tall
LMU/LMUE/FAZ/FAZ/FAZ/42	Kongoo	Lamu	LamuEast	Faza	Faza	Faza	Bajuni	Hybrid
LMU/LMUE/FAZ/FAZ/FAZ/43	Kongoo	Lamu	LamuEast	Faza	Faza	Faza	Bajuni	Tall
LMU/LMUE/FAZ/FAZ/FAZ/37	Mnazi wa Kawaida	Lamu	LamuEast	Faza	Faza	Faza	Arab	Tall
LMU/LMUE/FAZ/FAZ/FAZ/39	Mnazi wa Yellow	Lamu	LamuEast	Faza	Faza	Faza	Arab	Tall
LMU/LMUW/AMU/AMU/POW/50	Jongo	Lamu	LamuWest	Amu	Amu	Power	Bajuni	Tall
LMU/LMUW/AMU/MKO/MAK/49	Mnazi Mfupi	Lamu	LamuWest	Amu	Mkomani	Makafuni	Bajuni	Hybrid
LMU/LMUW/AMU/MKO/MAK/48	Mnazi wa Pombe	Lamu	LamuWest	Amu	Mkomani	Makafuni	Bajuni	Tall
LMU/LMUW/AMU/MKO/MAK/47	Zipue	Lamu	LamuWest	Amu	Mkomani	Makafuni	Bajuni	Tall
LMU/LMUW/AMU/MKO/MSU/46	Kongoo	Lamu	LamuWest	Amu	Mkomani	Msumarini	Bajuni	Tall
LMU/LMUW/AMU/MKO/MSU/45	Mnazi wa Kipemba	Lamu	LamuWest	Amu	Mkomani	Msumarini	Bajuni	Dwarf
LMU/LMUW/AMU/MKO/MSU/44	Kongoo	Lamu	LamuWest	Amu	Mkomani	Msumarini	Bajuni	Tall
TR/TD/KIP/KIP/BAH/35	-	Tana River	Tana Delta	kipini	kipini	Bahongo	Arab	Tall
TR/TD/KIP/KIP/WAK/31	Mnazi Kongoo	Tana River	Tana Delta	kipini	kipini	Wakehoya	Meru	Tall
TR/TD/KIP/KIP/WAK/32	-	Tana River	Tana Delta	kipini	kipini	Wakehoya	Meru	Tall
TR/TD/KIP/KIP/WAK/33	Mnazi Kongoo	Tana River	Tana Delta	kipini	kipini	Wakehoya	Arab	Tall
TR/TD/KIP/KIP/WAK/34	Kithamli	Tana River	Tana Delta	kipini	kipini	Wakehoya	Arab	Tall

A total of five traits were characterized, which included stem, crown, leaf and fruit morphology as well as stress severity of the crop assessment using the descriptors listed for coconut by Bioversity International (Batugal et al., 2005). The characters assessed for the 47 selected palms are listed in Table 2. In addition, vegetative, reproductive parts, apple and nut traits were assessed based on the protocol of the coconut descriptor list of the International Plant Genetic Resources Institute (IPGRI, 1995).

The quantitative and qualitative morphological traits of coconut characterized were classified using dissimilarity coefficients and the neighbour joining from the usual Euclidean distance of complete linkage clustering method.

RESULTS AND DISCUSSION

In this study, seven trees had yellow stalks and immature nuts; two had red yellow; one had red; 12 exhibited green; 19 had green yellow; four showed red yellow-green (brown) while two trees had orange stalks as well as immature nuts. The fruit morphology from five trees were oblong; 13 ovoid; 26 angled and four round. Seventeen trees sampled had no boles while 18 and 13 exhibited low and high bole categories, respectively. Crown morphology was categorized as spherical (14 trees), hemispherical (seven trees), V-shaped (seven trees) and X-shaped (19 trees). Twenty one trees were observed to have almost round fruit shape while 15, nine and eight trees had ovoid, oblate and pointed fruit (without husk) respectively. Tree height ranged from 12.1 m (KLF/RAB/KAM/PAN/MAE/10) to 1.4 m (TR/TD/KIP/KIP/WAK/34). Width of scar at 1.5 m height varied from 1.2 m (KLF/RAB/KAM/PAN/MAE/10) to 0.4 m

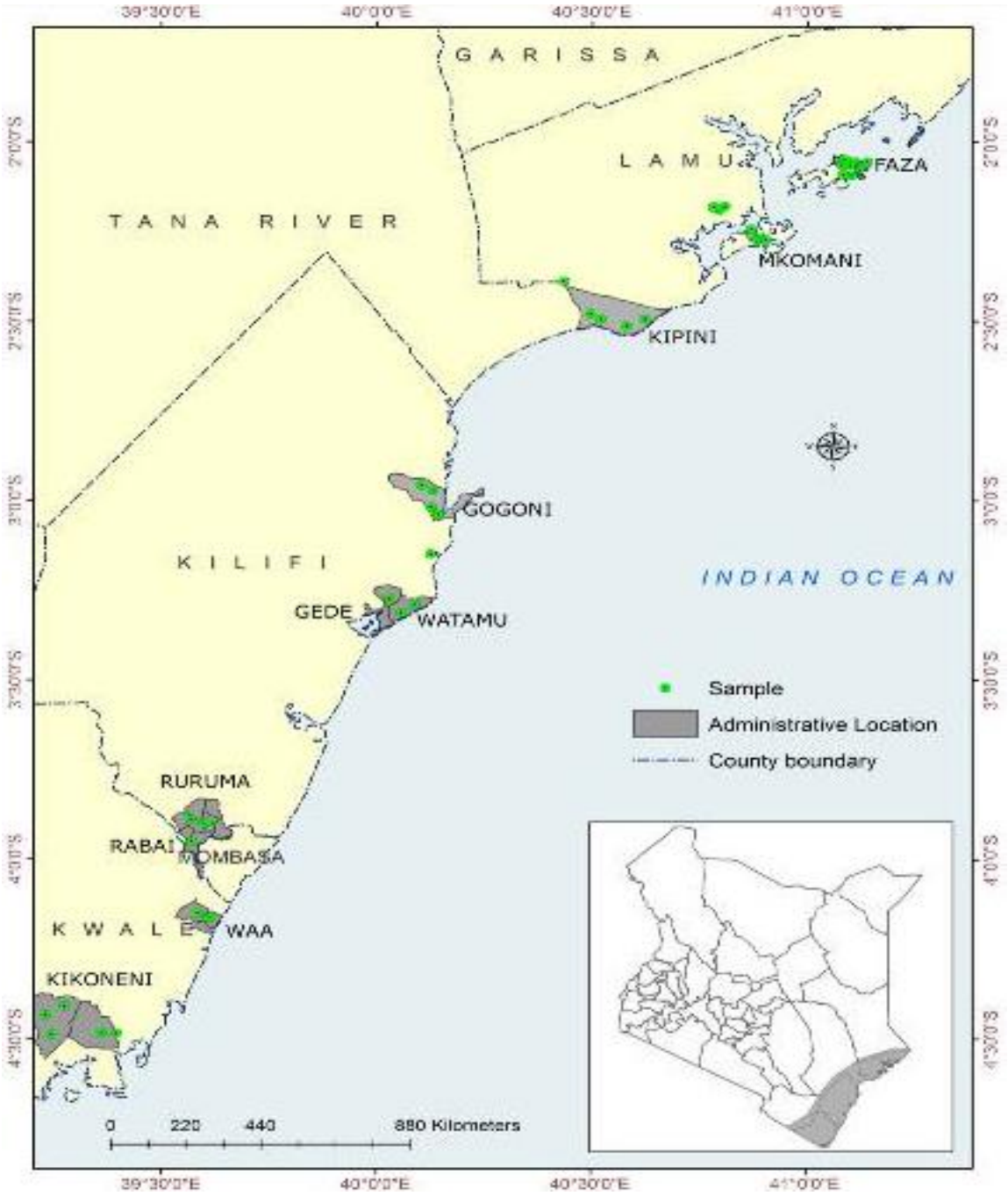

Figure 1. Map of the Coastal Lowlands of Kenya indicating the sites where *in situ* characterization of coconut (*Cocos nucifera*) was conducted in this study.

(KLF/RAB/BUN/03). On vertical descriptors, 31 trees were characterized as erect, one angled, one bowed while 15 had curved stems. The number of green leaves showed variation ranging from 51 (LMU/MUW/AMU/AMU/POW/50) to 9 (TR/TD/KIP/KIP/BAH/35). Longest petiole length recorded was 3.5 m for KWL/MSA/MSA/MIL/MAM/01 and the shortest was 0.6 m, recorded for LMU/MUW/AMU/MKO/MSU/44 and TR/TD/KIP/KIP/BAH/35.

The Counties and their respective divisions where the study was conducted are given in Table 1. In addition, global positioning system (GPS) data is shown in Figure 1. Generally, genotype clustering did not follow the geographical origins from which the genotypes were sampled, suggesting an exchange of germplasm among

Table 2. Descriptors for coconut characters as listed by IPGRI .

Trait type	Character assessed
Stem Morphology	Height from the ground to the oldest green leaf (m) Height of 10 leaf scars starting from 15 m from ground surface (m) Width of leaf scar at 1.5 m height (m) Height of 10 leaf scars starting from 15 m from ground surface (m) Vertical descriptors of the stem Bole category Stem type - dwarf, tall or hybrid
Crown morphology	Number of green leaves Overall appearance /Shape of crown
Leaf Morphology	Length of leaf 14 (m) Petiole length (m)
Fruit morphology	Fruit appearance Fruit (without husk) appearance/shape Color of immature nuts
Stress severity assessment	General biotic stress susceptibility

Table 3. Principle component loadings (latent vectors) of 11 traits in 48 genotypes of coconut at the coastal lowlands of Kenya.

Trait	PC1	PC2	PC3	PC4
Plant height (m)	-0.004	-0.115	0.036	0.025
Width of leaf scar at 1. 5 m height	-0.003	0.019	0.032	0.008
Bole Category	0.016	0.146	0.922	-0.203
Crown morphology	-0.006	0.007	-0.014	-0.234
Fruit appearance (with husk)	-0.004	0.026	0.063	-0.079
Fruit appearance (without husk)	0.014	-0.062	-0.329	-0.718
Length of 14^{th} leaf (m)	0.009	-0.016	0.049	0.098
No of green leaves	0.999	-0.021	-0.010	0.035
Petiole length (m)	0.002	-0.009	0.022	0.041
Stem height (m)	0.019	0.980	-0.156	-0.002
Vertical descriptor of the stem	0.040	0.007	0.090	-0.608
Percent variation	65.54	19.71	8.14	2.74
Latent roots	2649.2	796.4	328.9	110.9
Cumulative percent of total variation	65.54	85.25	93.39	96.13

the coastal population probably due to the fact that farmers could have acquired planting materials from fellow farmers across the region. It is evident from our results that many genotypes from different origins over-lapped; an indication of morphological redundancy in the characterized germplasm.

Coconut can cross-pollinate and it is possible that crop at the coast has several hybrids which could not be discriminated by morphological analysis probably due to environmental influence on the traits and not necessarily that coconut at the coast share a common ancestry. Principal Component Analysis (PCA) showed that the first four components explained 96.13% of the total variation (Table 3). Principal component 1 (PC1) accounted for 65.54% and was associated with the number of green leaves. PC2 explained 19.71% of the variation and was associated with stem height. PC3 accounted for 8.14% of the total variation and was mainly associated with bole category and fruit husk appearance, while PC4 explained 2.74% of the total variation and was associated with

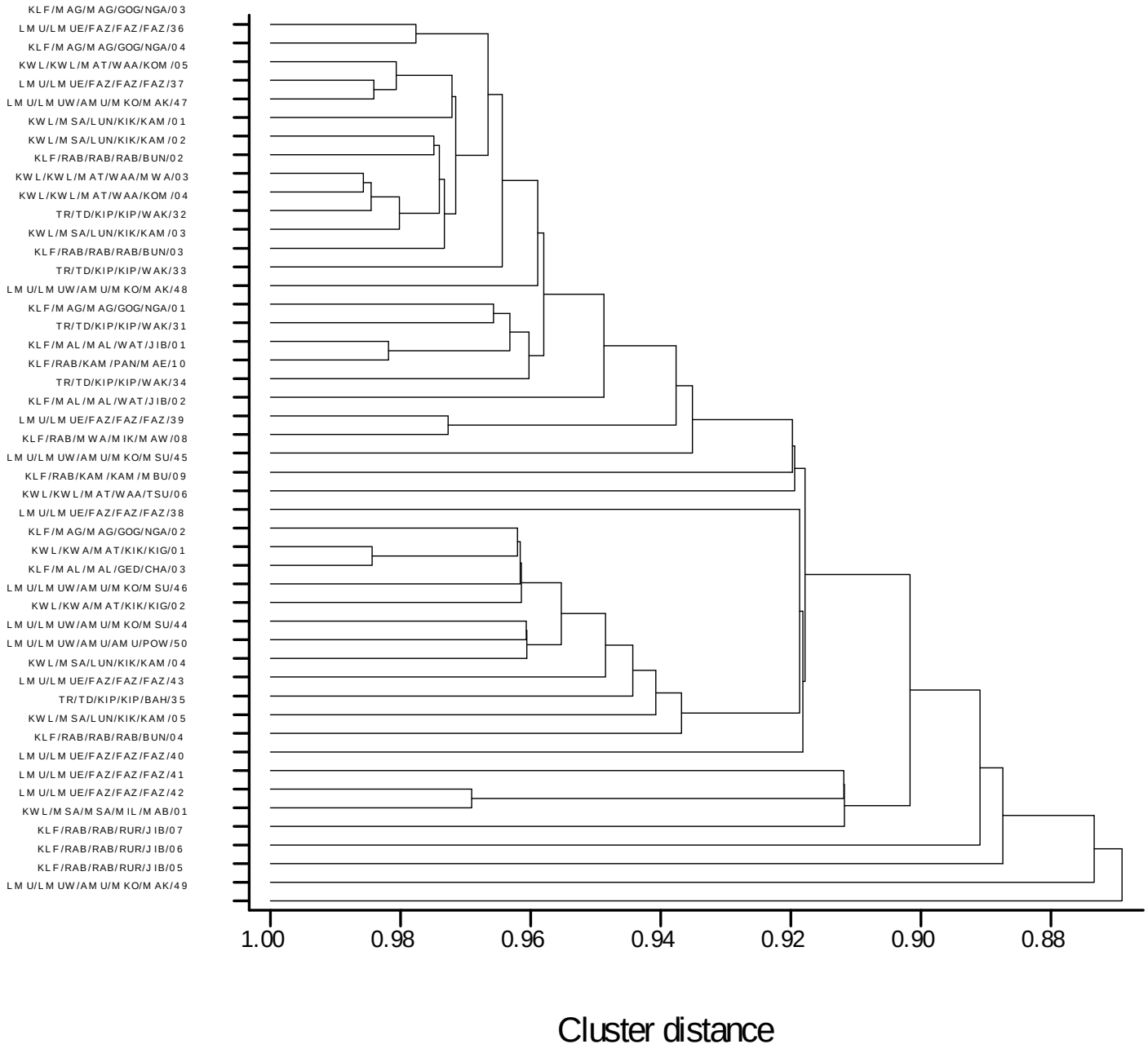

Figure 2. Dendrogram of the similarities among 48 coconut genotypes using the nearest neighbour method of cluster analysis.

vertical stem descriptors.

Dissimilarity coefficients and the neighbour joining from the usual Euclidean distance of complete linkage clustering method divided the 48 coconut genotypes into two major clusters (Figure 2). Genotype LMU/LMUW/AMU/MKO/MAK/49 was grouped in a cluster of its own while other genotypes were grouped together. LMU/LMUW/AMU/MKO/MAK/49 was the shortest nut at on 2.28 m. Genotypes KLF/RAB/RAB/RUR/JIB/05,KLF/RAB/RAB/RUR/JIB/06 and KLF/RAB/RAB/RUR/JIB/07 formed a minor cluster mainly due to their tall palm types, stalk color and height of 10 leaf scar starting from 1.5m from the ground; as did genotypes KLF/MAG/MAG/GOG/NGA/03, KLF/MAG/MAG/GOG/NGA/04, LMU/LMUE/FAZ/FAZ/FAZ/36, KWL/KWL/MAT/WAA/KOM/05 and LMU/LMUE/FAZ/FAZ/FAZ/37. They were dwarf, had the same crown morphology, vertical stem type as well as length of 14th leaf (m). Other genotypes that belonged to the same cluster were LMU/LMUW/AMU/MKO/MAK/48, KLF/MAG/MAG/GOG/NGA/01, TR/TD/KIP/KIP/WAK/31 and KLF/MAL/MAL/WAT/JIB/01. They were all tall palm types with brown stalk, erect vertical stem as well as high

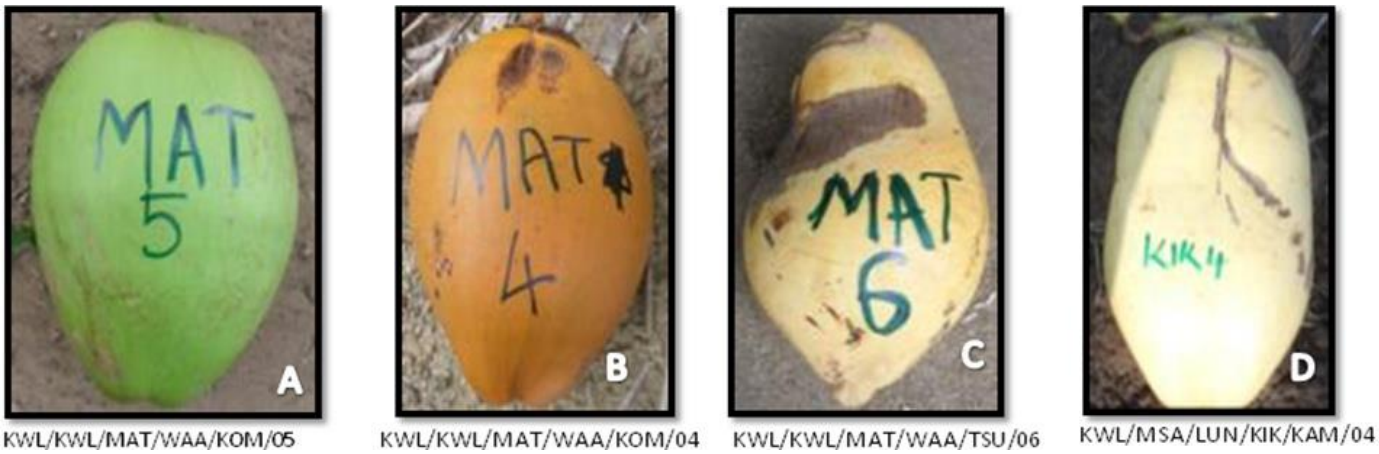

Plate 1. Appearance and colour of the palm fruits of some of the nuts at the coasts of Kenya. A, ovoid and green; B-angled and yellow red; C, angled and yellow; D, yellow.

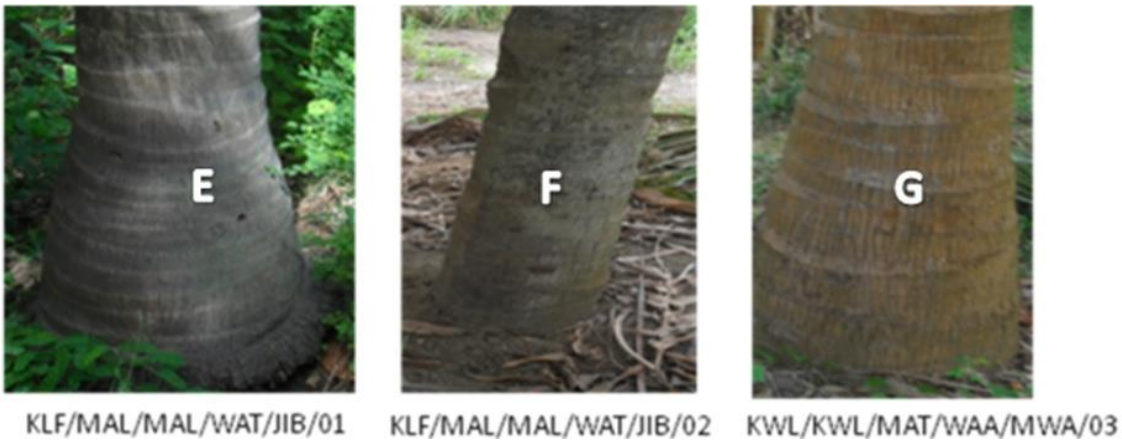

Plate 2. The bole categories of palm tress at the coastal lowlands of Kenya. E, Low bole; F, no bole; G, high bole.

bole type and same length of the 14th leaf. Similarly, close proximity was observed for LMU/LMUE/FAZ/FAZ/FAZ/38, KLF/MAG/MAG/GOG/NGA/02, KWL/KWA/MAT/KIK/KIG/01, KLF/MAL/MAL/GED/CHA/03 and LMU/LMUW/AMU/MKO/MSU/46.

It is clear from the dendrogram, PCA and as shown by the Plates 1 to 4 that coconut germplasm at the Kenyan coast is diverse in various morphological categories. It is our view that in Kenya, just as reported in Sri Lanka (Fernando et al., 1995), coconut can be found growing in a range of environmental conditions other than the optimal conditions and maintain their productivity despite the stress and management conditions which included palm trees not of uniform age, unweeded fields, progeny generations mixed in a population, no pure stands and lack of fertilization. In almost all instances, even the age of the palms was not known. In addition, we noted that coconut plantations in Kenya, especially of EATs have undergone preferential selections for yield, nut size, nut shape, nut colour, kernel thickness and tolerance to drought, pest and disease as argued by Perera et al. (1996).

In some cases it was evident there could have existed hybrid varieties as some EATs bearing green yellow or red green fruits could be seen suggesting hybridity between the EATs, which usually bear green fruits and the EADs that commonly bear yellow fruits. There also existed tall palms with yellow nuts, and intermediates with the dwarfs suggesting under-dominance of yellow nuts over green ones. Selecting for variations explained by the PCA and cluster patterns obtained from different genotypes and probably environments could be useful for selection of genetically diverse genotypes for improvement programs in coconut. However, owing to the fact that morphological characters are under heavy control of the crop growing environment, diversity studies using DNA markers is recommended for thecharacterized

Plate 3. Fruit appearances/shapes of some of the nuts at the coastal lowlands of Kenya. H, almost round; I, almost round; J, almost round; K, pointed.

Plate 4. Crown morphology of some of the palm trees at the coasts of Kenya. L-X, shaped; M, spherical.

germplasm in this study.

Conflict of interests

The authors have not declared any conflict of interest.

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Coffee leaf damaging insects' occurrence in the forest coffee ecosystem of southwestern Ethiopia

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Insects are diverse and abundant in forest ecosystems, but poorly documented in afromontane rainforests in Southwestern Ethiopia where *Coffea arabica* L. originated and is distributed worldwide. Therefore, the present study was initiated to study the occurrence of coffee leaf damaging insect pests in the afromontane rainforest of southwestern Ethiopia. Accordingly, surveys on insect pest of coffee were conducted to investigate pest status and; their damaging intensity in three (Yayu, Berhane-kontir and Bonga) forest coffee ecosystems. Based on general uniformity of forest coffee population, each forest coffee was stratified into three forest sites and 16 trees were systematically selected for leaf insect damage assessment. Survey and damage assessment results reveal that, coffee leaf damaging insect pests in afromontane rainforests of Southwestern Ethiopia include 12 insect families from five insect orders. Mean incidence data showed, significant different (P< 0.05) during different survey seasons within and between three forest coffee populations. Among the frequently occurred and damage causing insect pests were *Leucoplema doherthyi*, *Cryphiomystis aletreuta*, coffee giant looper and *Leucoptera* species and had 72.20 ± 1.42, 14.41 ± 5.15, 10.73 ± 4.30 and 2.63 ± 1.24 proportion damage across forest coffee populations, respectively. There is also high variation among major coffee leaf damaging insect during different seasons (rainy, rainy to dry transition, dry and dry to rainy season transition season). Generally, there were differences in insect pest incidence between and within forest coffee populations with regard to coffee leaf damaging insects and relatively low incidence observed compared to other coffee production systems. The present findings complement other research results meant for the conservation of remnant forest and its biodiversity especially to explore the natural enemies in these forest ecosystems.

Key words: *Coffea arabica* L., forest coffee, southwestern Ethiopia, insect pests, season.

INTRODUCTION

Coffee is an evergreen perennial crop grown in ecosystems, which favors insect pest to survive from year to year (Wrigley, 1988). According to Le Pelley (1968), one particular consequence for this reason is; it is not

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possible without coffee to employ the useful method of pest control applied for as annual crops particularly cultural control like “closed season” during which the crop is completely removed from the field. In general, succulent green shoots, leaves, flowers, and young fruits of coffee plant parts are attacked by different chewing and sucking insects (Wrigley, 1988; Mugo, 1994).

Despite the existence of suitable natural conditions for coffee production, the average national yield is very low in Ethiopia. Insect pests are among a number of factors that contribute to low yield (Mesfin, 1989; Million, 2000). Over 49 species of insect pests were recorded on coffee in Ethiopia, which were categorized as major, potential and minor pests (Million, 1987; Esayasl et al., 2006). Antestia bugs, *Antestiopsis intricata* (Ghesquiere and Carayon) and *Antestiopsis facetiodes* (Greathead) and coffee leaf miner, *Leucoptera caffeina* (Washbour) are considered as major insect pests of coffee particularly in large scale farms. Coffee berry borer, *Hypothenemus hampei* (Ferriere) is a potentially important insect pest of coffee in Ethiopia. Coffee insect pest status and category have been studied in plantation, garden and to some extent in semi-forest coffee production systems, but there is no single study so far conducted in forest coffee ecosystem, where coffee is grown as wild and used as organic product.

Furthermore, the diversity and the economic value of the Ethiopian coffee gene pool and its forest habitat as well as the institutional framework of forest users have been studied. The *in-situ* conservation of wild coffee offers an interesting approach in biodiversity conservation including both flora and fauna (Chemeda et al., 2011a, b; COCE, 2007). However, still the status of insect pest has not been assessed and described; and no reliable information exists in such genetically diversified ecosystems. In order to supplement *in-situ* conservation of the genetically diversified forest coffee plant and associated fauna for sustainable use, it is crucial to study the status of insect pests in different afromontane rainforest coffee populations and examine the extent of damage they inflict. Hence, the current study was initiated to obtain base line information on the status of forest coffee leaf damaging insect pests to contribute significantly to the overall conservation of forest coffee which is considered as source of organic coffee to the world market. Therefore, the study was initiated with the objective to investigate occurrence and incidence of coffee leaf damaging insect pests in afromontane rainforests of southwestern Ethiopia.

MATERIALS AND METHODS

Description of the study sites

The study sites were selected based on previous studies of joint project between the Center for Development Research (ZEF), Bonn University, Germany and the Ethiopian Institute of Agricultural Research (EIAR), Ethiopia, which focuses on “conservation and use of the wild populations of *C. arabica* in the montane rainforests of Ethiopia”. It was undertaken in Yayu (Gaba-dogi), Berhane-kontir and Bonga forest coffee populations, which are located 580, 620 and 420 km away from Addis Ababa to southwestern part of the country, respectively. Yayu forest coffee population is found in western part of Oromia Regional State, while Berhane-kontir and Bonga coffee populations are found in the Southern Nation Nationalities Peoples Regional state" (SNNPRs). The forest coffee populations are representatives of the different agro-ecologies for forest coffee production areas with elevations ranging from 1050 to 1900 m.a.s.l. and all exhibit tropical humid climate. From each forest coffee population, three representative sites of approximately one hectare was selected for the study. Plots were ecologically described including the slope, aspect (direction) and altitude within different gradient. Clinometer and compass were used to measure the slope and the aspect, respectively. Co-ordinates for the study sites were recorded using Geographic Positioning System (GPS) (Table 1).

In order to characterize shade structure (canopy coverage), forest inventories was made at each site. A 20 by 20 m plot replicated three times was used to describe the shade status for each site. Shade canopy coverage was visually estimated and classified into three classes: Low, medium and dense shade tree coverage which coincides with < 40, 40 - 60% and > 60% canopy coverage, respectively.

Experimental tree selection, insect specimen collection and damage assessment

A reconnaissance survey was carried to find out general information about each forest coffee populations, to select and tag experimental trees in each forest site. Based on general uniformity of coffee trees, shade status and elevation of forest coffee population, each population was classified into three forest sites (Table 1). Forest coffee population refers to three forest localities, while sites are strata in each population. Latter on from each site 16 trees were systematically selected employing the zigzag sampling method and tagged for leaf damage assessment. Furthermore, each tree was stratified into three-canopy layer and a pair of branch from each layer was selected for the assessment of leaf damaging insects.

Insect samples were collected during different coffee fruit phenological stages four times in all selected forest populations. Specimens were collected using different methods including hand collection, aspirator and sweep net. Collected insect samples were labeled with information including co-ordinates, altitudes, slopes, date of collection and developmental stage of the pest. All insect specimens were identified at the Biosystematics Department of the International Center for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

Damage assessment was made by counting total and damaged leaves from lower, middle and upper canopy branch leaves in July, October, January and April 2007/08 cropping season, representing rainy, autumn (rainy to dry season transition), winter (dry season) and spring (dry to rainy season transition) of the year, respectively. The percentages of damaged leaves were computed from the cumulative number of damaged leaves to total number of leaves for each canopy branches.

Data processing and analysis

Forest coffee populations, sites, seasons (referring to data collected month) and percentage leaf damage were considered for statistical analysis. All measurements of count data of damaged leaves were tested for normality using SAS software Proc Univariate. Data violate the assumption of ANOVA or normality was square root

Table 1. Descriptions of forest coffee study sites.

Forest coffee Population	Forest site[1]	Shade status[2]	Elevation (m)	Co-ordinates		Slope (%)	Aspect or Direction
	PIVSI	Low	1493	N-08° 24' 11"	E-035° 47' 44"	10	East
Yayu	PIVSII	Medium	1491	N-08° 23' 98"	E-035° 47' 40"	16	West
	PIVSIII	Dense	1496	N-08° 23' 10"	E-035° 47' 62"	20	West
	PIIISI	Medium	1051	N-07° 07'43"	E-035° 26' 16"	15	North
Berhane-Koniter	PIIISII	Dense	1084	N-07° 07' 16"	E-035° 26' 29"	13	N-East
	PIIISIII	Low	1134	N-07° 06' 52"	E-035° 26' 33"	20	East
	PIISI	Medium	I744	N-07° 20' 01"	E-035° 13' 39"	5	East
Bonga	PIISII	Dense	1739	N-07° 20' 31"	E-035° 13' 32"	10	East
	PIISIII	Medium	1894	N-07° 19' 04"	'E-035° 03' 31"	8	N-West

[1]PII, PIII and PIV = Codes given to Bonga, Berhane-Kontir and Yayu forest populatins, while SI, SII and SIII are Site one, Site two and three, respectively. [2]Low = shade coverage < 40%, Medium= shade coverage 40% - 60%, and Dense = shade coverage > 60%.

Table 2. Coffee leaves damaging insects in the forest coffee ecosystem of southwestern Ethiopia.

Common name	Order	Family/species	Occurrence / status	Collected FCP*
Black thread scale	Homoptera	Diaspididae	Rare	2 and 3
Brown totrix	Lepidoptera	Tortricidae	Rare	2 and 3
Coffee aphid	Hemiptera	Aphididae	Rare	2 and 3
Coffee leaf miner	Lepidoptera	Lyonetiidae *Leucoptera spp.*	Frequent	1, 2 and 3
Coffee cushion scale	Homoptera	Stictococcidae	Rare	2 and 3
Coffee hawk moth	Lepidoptera	Sphingidae	Rare	2 and 3
Coffee thrips	Thysanoptera	Thripidae	Rare	1 and 2
Coffee leaf skeletonizer	Lepidoptera	Epiplemidae *Leucoplema doherthyi* Warren)	Frequent	1, 2 and 3
Dust brown beetle	Coleoptera	Tenebrionidae	Rare	1 and 3
Coffee giant looper	Lepidoptera	Geometridae	Frequent	1, 2 and 3
Helmet scale	Homoptera	Coccidae	Rare	2and3
Mussel scale	Homoptera	Diaspididae	Rare	2 and 3
Serpentine leaf minor	Lepidoptera	Gracillariidae *Cryphiomystis aletreuta (*Meyrick)	Frequent	1, 2 and 3
Stinging caterpillar	Lepidoptera	Limacodidae	Rare	2 and 3
Systates weevil	Coleoptera	Curculionidae	Rare	2 and 3

*[1]Yayu, [2]Berhane-Kontir and [3]Bonga forest coffee populations (FCP).

transformed to normalize the data and to meet assumption of ANOVA before executing statistical analysis. Transformed percentage damaged leaves were compared using the SAS package for windows-v8 (SAS Institute Inc. Cary NC, USA). One-way ANOVA with nested design was performed to analyze coffee plant-insect relations.

Nested design of SAS proc mixed was used to analysis variation in infestation across locations and data collection months (seasons). Wherever significant difference ANOVAs (F-ratios) was found for means at the 5% probably level, means were separated using Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Level of damage by coffee leaf insect pests

Surveys result of coffee insect pests in afromontane rainforests of southwestern Ethiopia recorded 12 insect families from five insect orders (Table 2). Not all insect pests collected frequently occurred in all forest coffee ecosystems. Based on number of recorded site and

Table 3. Mean (± SE) damaged coffee leaves during different survey seasons and forest coffee populations in afromontane rainforests of southwestern Ethiopia.

Season during data collected	Forest coffee population			P- value
	Yayu	Berhane- Kontir	Bonga	
Rainy	35.73 ± 2.127bA	46.05 ± 1.611aA	17.40 ±1.053cA	<.0001
Transition (rainy to dry)	8.47 ± 0.579 C	8.82 ± 0.552D	9.16 ± 0.664C	0.7150
Dry	11.01± 0.903b B	13.40 ± 0.875abC	14.18 ± 1.241a B	0.0325
Transition (dry to rainy season)	10.04 ± 0.580cB	21.10 ± 0.741a B	16.83 ± 0.814b A	<.0001
P-value	<.0001	<.0001	<.0001	-

Means followed by the same letter(s) within a raw (lower case) and means followed by the same letter within a column (upper case) are not stastically significant at 5%, Tukey´s Honestly Significant Difference (HSD) test.

frequency of occurrence, insects in forest ecosystems of southwestern Ethiopia were categorized into two: rarely and frequently occurring insect pests of coffee. Generally rarely occurred insects were recorded once or twice in one and/two forest populations, however frequently occurring insect pests cause visible damage symptoms and recorded for more than two times in all forest coffee populations. Of the insect pests of coffee leaves, *L. doherthyi*, *C. aletreuta*, *Leucoptera* spp and Coffee giant looper, Geometridae were very common and causing visible damage on coffee leaves in all forest coffee forest surveyed in southwestern Ethiopia.

The mean incidence of coffee leaf damage varied between 10 and 56% with an average of 33% across the study coffee populations during rainy season. Of the total sites, 62% of them showed leaf damage incidence between 20 and 50%, 12% had more than 50% incidence and only 25% had less than 20% leaf damage with the lowest mean incidence of 10% recorded at Bonga forest coffee population. Analysis of Variance result showed a significant difference ($P < 0.05$) among forest coffee populations in level of coffee leaf damage insect pest with the highest mean recorded at Berhane-Kontir (46.05%) followed by Yayu (35.73%) and Bonga coffee population (17.40%) (Table 3). However, there is no significant difference ($P > 0.05$) among forest coffee populations in the level of leaf damage during rainy to dry season transaction survey period (Table 3). However, the mean incidence vary from 4-15% with the mean of 9% at Bonga, 8% at Berhane-Kontir and 8% at Yayu forest coffee populations. Similarly, dry season survey result revealed, there was significant difference ($P<0.05$) between Yayu and Bonga forest coffee populations. However, there was no significant difference between Berhane-Kontir and Yayu, and Berhane-Kontir and Bonga forest coffee population. But, variations in mean damages were observed across forest coffee populations (Table 3).

Data collected during dry to rainy season revealed that, 79% of the forest coffee populations showed more than 10% leaf infestation. However, there was a significant difference ($P < 0.05$) among forest coffee populations (Table 3).

Major coffee leaf damaging insects

Coffee leaf skeletonizer, Leucoplema doherthyi

It can be noticed that all the coffee tree under observation showed damage caused by insects. However, damage proportion caused varies among the major coffee leaf damaging insects at different seasons and forest coffee populations. Coffee leaf skeletonizer was among the most commonly occurring insect pests causing coffee leaf damage and showing the highest proportion of damage incidence (59%) out of 33% leaf damage incidence caused by the four most frequently occurring coffee leaf insect pest during rainy season data collected. Also, data collected three month later revealed, the pest showed the highest proportional damage incidence (70%) across forest coffee populations with and as high as 84 and 78% for data collected during dry and dry to rainy season transition, respectively (Table 4). Considering Yayu, Berhane-konter and Bonga forest coffee populations, coffee leaf skeletonizer shared about 71, 72 and 74% proportional damage, respectively to other three major coffee leaf damaging insects (Figure 1).

Serpentine leaf miner, Cryphiomystis aletreuta

Among all commonly occurring insect pests of coffee, coffee leaf skeletonizer ranked first followed by serpentine leaf miner with 72 and 14% proportion incidence throughout the study seasons and forest coffee populations, respectively. Coffee serpentine leaf miner showed 20, 14, 15 and 8% proportion incidence in rainy, rainy to dry, dry and dry to rainy seasons data collected time, respectively, compared to other coffee leaf

Table 4. Mean (± SE) proportion damage of coffee leaf by major insect pests coffee during different seasons in afromontane rainforest southwestern of Ethiopia.

Season	Serpentine leaf miner	Coffee leaf miner	Coffee leaf skeletonizer	Coffee gaint looper
Rainy	20.56 ± 1.09	9.12 ± 6.69	59.37 ± 8.90	10.95 ± 9.76
Transition (rainy to dry)	14.14 ± 14.10	1.09 ± 1.11	66.91 ± 11.04	17.93 ± 14.51
Dry	15.11 ± 12.89	0.08 ± 0.13	84.20 ± 12.31	0.62 ± 0.74
Transition (dry to rainy)	7.84 ± 5.64	0.25 ± 0.24	78.32 ± 8.09	5.25± 3.19

± = Mean plus or minus standard error of mean

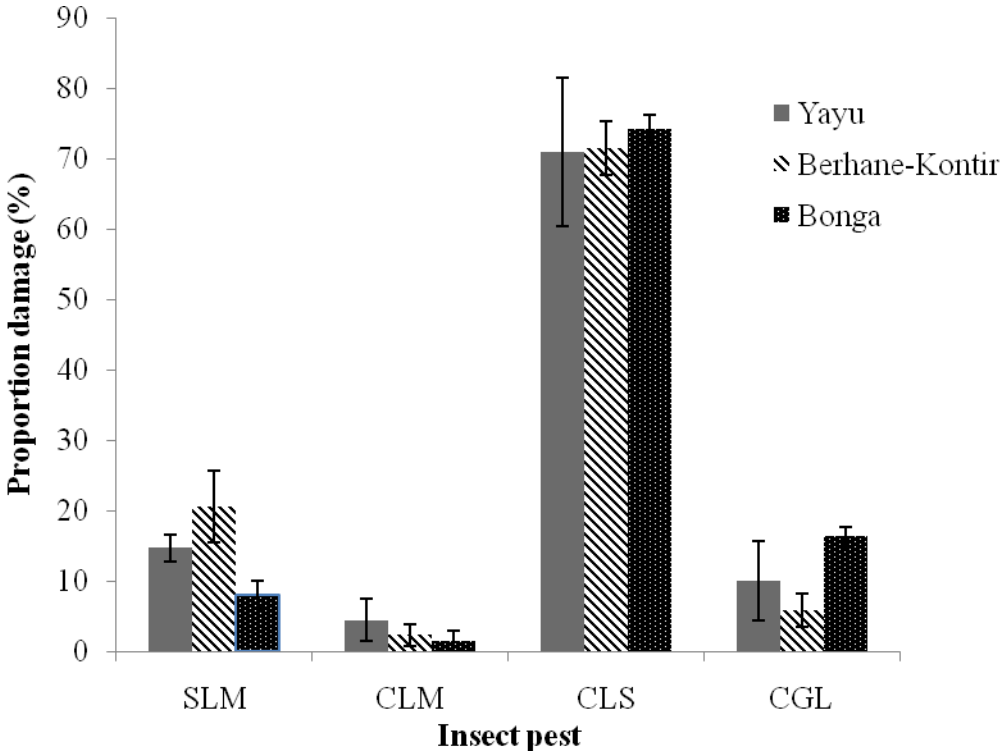

Figure 1. Mean (± SE) damage proportion by coffee leaf insect pests in three forest coffee ecosystems of southwestern Ethiopia. SLM= Serpentine Leaf Miner; CLM= Coffee Leaf Miner; CLS = Coffee Leaf Skeletonizer; CGL= Coffee Gaint Looper. Error bar (±) = Mean plus or minus standard error of mean

damaging insect pests (Table 4). However, 15% (Yayu), 21% (Berhane-konter) and 8% proportional damage were observed at Bonga forest coffee population compared to other leaf damaging insects (Figure 1)

Coffee gaint looper

Coffee gaint looper ranked third in terms of proportional incidence as it showed incidence of 10% (rainy), 18% (rainy to dry season transition), 0.20% (dry) and 13% (dry to rainy season transition) (Table 4). Also, 10%, 6% and 16% proportional damage were recorded at Yayu, Berhane-konter and Bonga forest coffee populations, respectively (Figure 1).

Coffee leaf miner, Leucoptera spp.

Among all commonly occurring insects pests of coffee leaf, coffee leaf miner ranked at last with the mean proportional incidence of 3% across seasons and forest coffee populations. Coffee leaf miner showed variation in terms of proportional incidence of 9% in rainy season and 0.08% in dry season compared to the other coffee leaf damaging insects (Table 4). The target pest showed about 5% damage as maximum at Yayu and 2% as lowest proportional damage at Bonga forest coffee popu-

lation compared to other leaf damaging insects (Figure 1).

DISCUSSION

Twelve (12) insect families from five insect orders of coffee leaf damaging insect were recorded in afromontane rainforests of southwestern Ethiopia. Afromontane rainforests in southwestern part of the country are characterized by complex ecosystems, in which many components including insect pests interact towards a prevailing equilibrium with natural enemies towards a steady state. As a result, occurrence and distribution of coffee insect pests was relatively low, probably due to the efficient role of natural enemies in stable ecosystems as there is minimum human intervention in these protected ecosystems. Different authors reported vegetation diversity play key role in insect pest management and their population response (Altier and Nicholls, 1999; Andow, 1991; Gibson and Jones, 1977).

Generally, data collected during different seasons highlights that there is variation within and between forest coffee populations in seasonal abundance and effects of coffee leaf insects pests. This result is in line with the hypothesis that, for tropical rainforests the rainy season is the most favorable period for the activities of leaf eating insects (Gombauld and Rankin-de-Merona, 1998), which totally agree with current study of coffee leaf damaging insects with highest incidence of 33.06%, when the amount of rain fall was very high compared to the other seasons. Furthermore, difference in genetic diversity of wild coffee populations as well as the intensity of cultural practices from one forest populations to another and within the same forest coffee ecosystem could be resulted in variations in the extent of insect incidence across forest coffee populations. Similarly, damage intensity by major coffee leaf damaging insects showed a great variation in their intensity across forest coffee populations.

Coffee leaf skeletonizer was observed in all forest coffee populations during all seasons varying and more abundantly compared to other leaf damaging insects. Million and Bayisa (1986) reported coffee leaf skeletonizer as potentially occurring insect pests of coffee in plantation and other production system; however the intensity was lower than coffee leaf miner. It reveals that different coffee production systems resulted with different level of occurrences/and damage level with the same insect pest in the country. Similarly, report from Kenya by Waikwa (1981) indicated that coffee leaf skeletonizer is a common pest of coffee in the lower and medium altitudes of coffee growing areas. However, Crowe (2004) reported that coffee leaf skeletonizer considered is a minor pest of coffee Arabica and Robusta in Kenya, Uganda and Democratic Republic of Congo without mentioning incidence can be inflected by the target pest.

Coffee serpentine miner comes after coffee leaf skeletonizer in importance in forest coffee populations of the study areas. However, there was no report on the incidence of coffee serpentine leaf miner from Ethiopia, but Million and Bayisa (1986) stated that the pest was very common in most coffee growing areas of Ethiopia though their population was highly suppressed by the natural enemies. The authors also reported, coffee serpentine miner can easily build its population and reach damaging level if its parasitoids are killed by pesticides as witnessed by the application of dieldrin to control ants in Mizan Teferi.

Coffee gaint looper ranked third in level of incidence in forest coffee populations with about 11% infestation among all major coffee leaf damaging insects. Waikwa (1981) also reported that Coffee gaint looper is most common and densely populated in areas of hot weather coffee growing areas in Kenya. Similarly, Crowe (2004) stated this insect pest became prominent in Kenya in 1961 following the spraying of parathion. However, coffee leaf miner, *Leucoptera* species showed relatively the lowest proportional incidence among major leaf damaging insects across forest coffee populations. However, study by Million and Bayisa (1986) and Million (1987, 2000) reported that coffee leaf miner comes next to Antestia bug in importance causing heavy defoliation when there is sever infestation in other coffee production systems other than forest. Studies conducted at Agaro Sub-Center, southwestern Ethiopia indicated that percentage leaf damage due to this insect pest ranged from 2.2 - 55% with an average of 13% infestation (IAR, 1986).

The result of this study indicated that there was no coffee tree free from insect pest attack. At least one insect pest species is associated with it. However, damage was mostly negligible and very low. And, generally incidence of forest coffee insect pests observed was relatively low compared to the other coffee production systems in the country and other coffee producing countries.

As the result, present research findings suggest that, these coffee stands may not urgently recommend additional control for leaf damaging insects, but it supplements other research findings to conserve remnant forest and its biodiversity in southwestern part of the country. Especially, it can serve as areas for exploration of different natural enemies for key and chronic pests of coffee production worldwide.

Conflict of interest

The authors have not declared any conflict of interest.

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Integrated management of *Cercospora* leaf spots of groundnut (*Arachis hypogaea* L.) through host resistance and fungicides in Eastern Ethiopia

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Cercospora **leaf spots caused by** ***Cercospora arachidicola*** **and** ***Cercosporidium personatum*** **are the most widespread diseases of groundnut that result in severe yield losses in Ethiopia. Field trials were conducted at Babile and Dire Dawa to evaluate the effect of integrated use of host resistance and fungicides on the temporal epidemics of leaf spots and yield of groundnut. The experiments were conducted during the 2010 main cropping season using three groundnut varieties and six fungicide treatments. The experiment was laid out as RCBD in a factorial arrangement with three replications. Severity, disease progress rate, area under disease progress curve (AUDPC) and yield of groundnut were used to evaluate the effects of treatments. High levels of disease control were achieved by weekly application of chlorothalonil at both locations. Up to 25 and 65% severity levels were recorded on sprayed and unsprayed plots of the varieties, respectively. Fungicide applications also significantly reduced disease progress rate and AUDPC value on the susceptible variety. Seed yield harvested from fungicide sprayed plots was consistently greater than the yield harvested from unsprayed plots.**

Key words: *Arachis hypogaea* L., *Cercospora* leaf spots, disease progress rate, disease severity and integrated disease management.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an important leguminous oilseed crop belonging to the family Fabaceae (Mali and Bodhankar, 2009). It is one of the most popular and universal crops cultivated in over 100 countries in six continents but mainly Asia, Africa and America with a world production of 37.1 million metric tons from an area of 23.1 million hectares (FAO, 2007). Its cultivation is mostly confined to countries ranging from 40° N to 40° S. Major groundnut producing countries are China, India, Nigeria, U.S.A and Indonesia, respectively. Groundnut is very important cash crop for small scale farmers of developing countries. In Ethiopia, it is grown over an area of 41,761 ha, with an annual production of 46,887 t (MoARD, 2009).

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Groundnut is one of the three economically important oilseed crops consisting of nigerseed/noug and sesame in Ethiopia. It serves as a source of cash income for many small-scale farmers and the country also obtains foreign currency by exporting the crop (Geleta et al., 2007). However, eastern Ethiopia holds primary position in producing and supplying for both domestic and export markets as compared to other parts of the country. Groundnut is commonly cultivated by farmers under rain-fed conditions. Particularly in East Hararghe Zone, the crop ranked third next to maize and sorghum in area coverage and seed production.

Despite its importance, the average national yield of groundnut in Ethiopia is very low. This might be due to a number of biotic and abiotic constraints. Among the biotic constraints, fungal diseases are one of the major factors affecting the production and productivity as well as the quality of the crop. Amongst the fungal diseases, *Cercospora* leaf spots, caused by *Cercospora arachidicola* (early leaf spot) and *Cercosporidium personatum* (syn. *Phaeoisariopsis personata*) (late leaf spot) are the major destructive diseases of groundnuts worldwide including Ethiopia (Backman and Crawford, 1984; Smith et al., 1992). The damages done by these diseases generally ranged from defoliation to reduction in pod, seed and haulm yield (Brenneman and Culbreath, 2000). Yield losses due to *Cercospora* leaf spots are as high as 50% in the USA (Shokes and Culbreath, 1997; Hagan et al., 2006).

Cercospora leaf spots are widespread and economically important diseases of groundnut in Ethiopia, and they can cause about 65% yield losses in high diseases pressure areas of the country (Teklemariam et al., 1985). The study on leaf spot and rust resistance, which was conducted at Babile and Bisidimo, showed that most of the genotypes tested suffered a high disease severity and yield was not obtained from most plots due to heavy defoliation of leaves in different years depending on weather conditions (Abraham, 2009). Although, the diseases are highly destructive in the areas, farmers do not apply any control measures like fungicides applications and selection of improved varieties. Therefore, it is necessary to develop suitable disease management practices for groundnut crop in Ethiopia.

As indicated by different studies in different countries, effective control of *Cercospora* leaf spot can be achieved by applying recommended fungicides. Significant yield improvement (up to 75%) was observed with fungicide applications (Naab et al., 2005). Synthetic fungicides that combat phytopathogenic fungi can increase crop yields and provide stability of crop production and market quality (Kishore et al., 2007). Chemical method still plays a vital role in the management of plant diseases. However, repeated application of fungicides could lead to reduced efficacy, greater production costs and environmental pollution. It can also kill or negatively affect beneficial microorganisms used as bioagents and nitrogen fixer in the soil. In addition, farmers in the arid and semi-arid areas of Africa in general including Ethiopia are resource poor and so most of them cannot afford the cost of chemical control measure as the sole method of disease management. Plant disease can be effectively managed by a combination of fungicides and host plant resistance (Pande et al., 2001).

The use of resistant varieties to a particular disease is one of the main methods of disease management. Therefore, the planting of moderately resistant cultivars will reduce the use of fungicides and associated expenses and increase economic gain. Thus farmers will benefit economically from planting resistant varieties (Johnson and Beute, 1986). Therefore, development of integrated disease management programs could be effective in decreasing the production costs and improving productivity and quality as well as reducing the detrimental effects of chemicals on the ecosystem. So, this study was conducted to determine the effect of integrated use of host resistance and fungicides spray intervals on the epidemics of *Cercospora* leaf spots and yield of groundnut.

MATERIALS AND METHODS

Experimental sites

Field experiments were conducted at Babile and Dire Dawa research stations of the Haramaya University during the main cropping season in 2010. The sites are located in a semi-arid and arid agroclimatic zone in the country and they differ mainly in their altitude, temperature, and annual total rainfall. Babile is situated in Oromia Regional State at 30 km East of Harar at 9° 08' 40" N latitude, 42° 21' 30" E longitudes and at altitude of 1650 m.a.s.l. The district is characterized by weather conditions conducive for leaf spots epidemic development. It receives annual rainfall of over 600 mm (Mitiku, 1989; Tadele and Tana, 2002). Mean annual maximum and minimum temperatures are 28.05 and 15.52°C, respectively. The type of the soil at Babile research field is a well drained sandy loam with pH of 7.0 (Mitiku, 1989). While Dire Dawa is located at 40 km North of Haramaya University at 9° 31' N latitude, 41° 51' E longitudes and at altitude of 1160 m.a.s.l. It receives annual rainfall of 520 mm, and has mean maximum and minimum temperatures ranging from 28.1-34.6 and 14.5-21.6°C, respectively. The dominant soil type is well-drained loamy sand with a pH of 8 (Tana et al., 2002).

Experimental materials and treatments

Three groundnut varieties currently under production and differing in their resistance level to leaf spots were used. The varieties were: Oldhale (local variety), Betisedi (ICG-273) and Werer-962 (ICGV-86928). The varieties Betisedi and Werer-962 were released in Ethiopia in 1993 and 2004 by Werer Agricultural Research Center, respectively. Werer-962 is a late maturing variety and resistant to leaf spots. Different severity levels of leaf spots were created by varying intervals of application of chlorothalonil (Odeon 825 WDG) and carbendazim (Bavistin 50% DF) at the rate of 1.4 and 0.5 kg ha^{-1},

respectively. Chlorothalonil was sprayed at intervals of 7, 14 and 21-days on each variety while carbendazim was sprayed at intervals of 14 and 28-days. Foliar spray with the fungicides was started after the appearance of the symptoms of the disease using a manual Knapsack sprayer. Unsprayed plots were left as a control to allow maximum leaf spots development on each variety. At Babile, eight sprays were made on the most frequently sprayed plots (7-days interval), three sprays were made on the plots that received chlorothalonil every 21-days and carbendazim every 28 days and five sprays were made on the plots that received chlorothalonil and carbendazim every 14-days.

Experimental design and management

A randomized complete block design in a factorial arrangement was used with three replications at both locations. The total treatment combination of 18 (three varieties × six fungicide treatments) were used. 10 and 60 cm spacing between plants and rows were used, respectively. Spacing between blocks and adjacent plots were 1 m. Each plot had a size of 2.4 × 4 m and consisted of four rows (with two harvestable central rows) of the groundnut plants. Planting of the crop was done on 30 April at Babile and 8 May in 2010 at Dire Dawa. Seeding was done at the rate of two seeds per hill and seedlings were thinned to one plant per hill two weeks after emergence. Leaf spot was allowed to develop naturally on each cultivar without any artificial inoculation at Babile. Specifically, the experimental field at Dire Dawa is not known to be planted with groundnut in previous years. Due to this reason, equal amount of infected groundnut residues were applied on each plot at the time of planting to enhance the development of *Cercospora* leaf spot. All agronomic practices, including N and P fertilizers, were done for all treatments as required at both locations.

Disease assessment

The disease severity was assessed at weekly intervals by estimating the percentage of leaf area affected using a 1-to-9 scale of Subrahmanyam et al. (1995) on all leaves of 10 randomly selected and tagged plants in each plot beginning from the disease appearance, where 1 = no disease, all leaves healthy and 9 = almost all leaves defoliated; leaving bare stems; some leaflets may remain; but show severe leaf spots; 81-100% leaf area damaged by the disease. Disease scoring was done for both *Cercospora* leaf spots (early and late) together and separate data for the two leaf spots was not presented because the chemicals control both diseases equally. In all assessments, disease severity was averaged for the 10 plants in each plot. The severity grades were converted into percentage severity index (PSI) for analysis using the formula of Weeler (1969).

The area under the disease progress curve (AUDPC) from PSI was calculated for each plot using the formula of Shaner and Finnery (1977):

$$\mathbf{AUDPC} = \sum_{i=1}^{n-1} \mathbf{0.5[(x_i + x_{i+1})(t_{i+1} - t_i)]}$$

Where, n = total number assessment times, t_i = time of the i^{th} assessment in days from the first assessment date, x_i = percentage of disease severity at i^{th} assessment. AUDPC was expressed in percent-days because severity(x) will be expressed in percent and time (t) in days. AUDPC values were used in the analysis of variance to compare amount of disease among plots with different treatments. Disease progression from each treatment was calculated by transforming the percent disease severity values to the logistic model as ln(y/1-y), where y is leaf spot severity in proportion (Van der Plank, 1963). The transformed data were then regressed over time (as DAP) so as to get the disease progress rate, which is the coefficient of the regression line.

Assessment of seed yield

Seed yield data were recorded from each plot at harvest. Yield data were recorded from the central two rows of each plot by leaving the border two rows to avoid border effects. The yields were presented on per ha basis.

Statistical analysis

Data of disease severity from each assessment date, AUDPC values, disease progress rates, and seed yield were subjected to analysis of variance (ANOVA) to determine the effects of treatments. The two locations were considered as different environment because of the significant variation in weather conditions during the experiments. Due to this reason, data were not combined for analysis. Analyses of variances (ANOVA) were performed using SAS computer software (SAS System 9). Comparison of treatment means was made using least significance difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Disease severity

Varieties by spray interval interactions showed very highly significant ($P < 0.001$) difference at final date of assessment at Babile and Dire Dawa. This might indicate a differential cultivar effect on *Cercospora* leaf spot development at different spraying intervals. Almost complete control was achieved by application of chlorothalonil at 7-day intervals on all varieties at both locations. At Babile, the highest leaf spot severity (65.3%) was observed on unsprayed plots of the susceptible variety "Betisedi" while the lowest (24.6%) was observed on plots sprayed chlorothalonil at 7-days interval of the moderately resistant variety "Werer-962" at assessment of 106 DAP (Figure 1B and C). Carbendazim spray at 28-days interval reduced the disease severity significantly as compared to unsprayed plots of each cultivar. Disease severities of plots sprayed fungicide at 14- and 21-days

$$\mathbf{PSI} = \frac{\mathbf{Sum\ of\ numerical\ ratings}}{\mathbf{No.\ of\ plants\ scored\ x\ maximum\ disease\ score\ on\ scale}} \mathbf{x\ 100}$$

intervals were not significantly different from each other on the moderately resistant variety "Werer-962". This study is in agreement with that of Culbreath et al. (1992) who reported that the application of chlorothalonil, or alternating applications of chlorothalonil and tebuconazole or azoxystrobin at 21-days or longer intervals, can provide good control of early and late leaf spot when

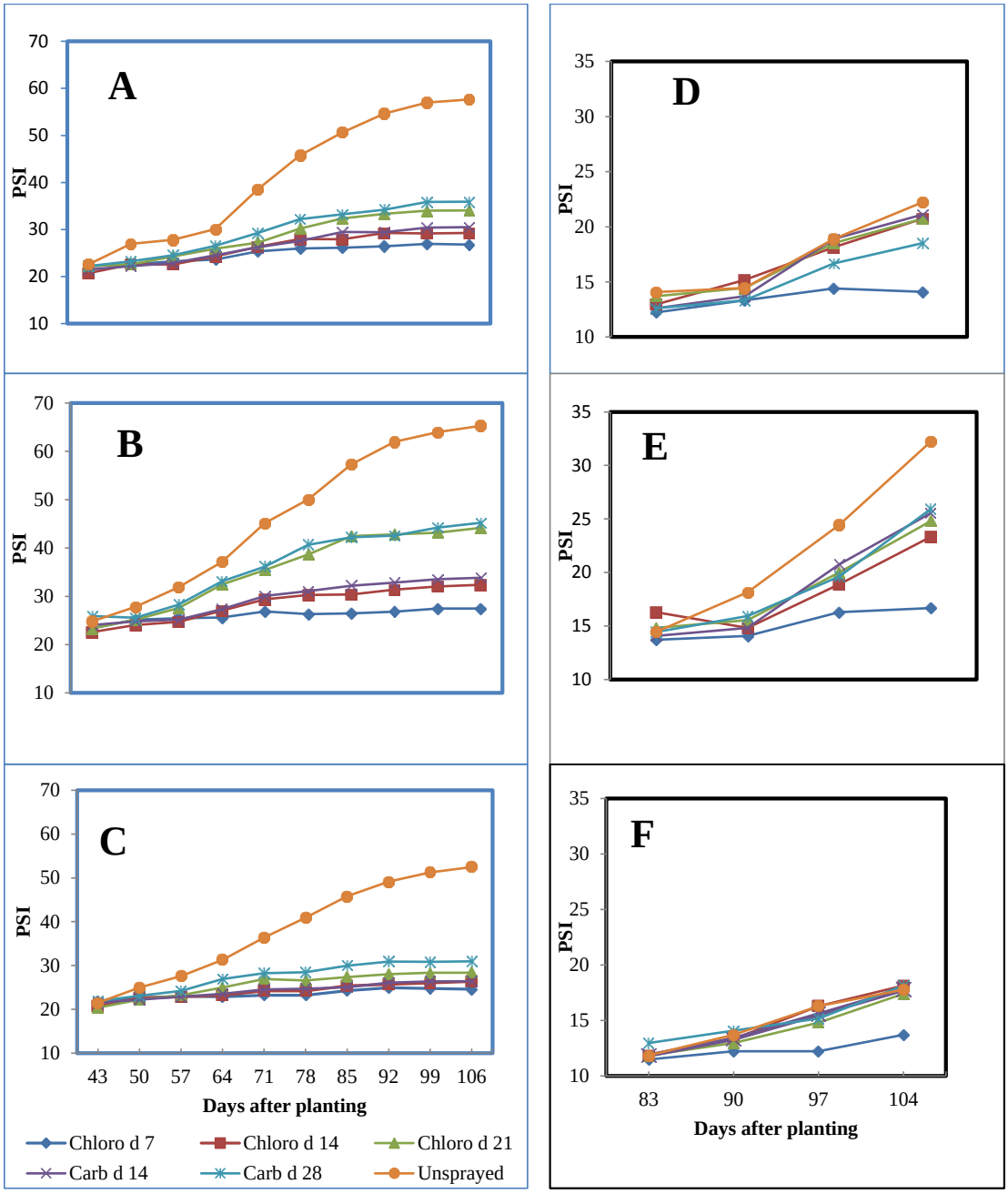

Figure 1. *Cercospora* leaf spot progress curves under different fungicides spray intervals on three groundnut varieties (A) Oldhale, (B) Betisedi and (C) Werer-962 at Babile and (D) Oldhale, (E) Betisedi and (F) Werer-962 at Dire Dawa in 2010 main cropping season. Disease severity was assessed every 7-days starting from 43 DAP at Babile and starting from 83 DAP at Dire Dawa.

combined with a cultivar possessing moderate levels of resistance to those pathogens. The ultimate value of partial resistance to control of groundnut leaf spot was a means to reduce fungicide use (Gorbet et al., 1982).

At Dire Dawa, the highest PSI value (32.2%) was also observed on unsprayed plots of the variety "Betisedi" while the lowest (13.7%) on plots sprayed chlorothalonil at 7-days interval of the variety "Werer-962" at 104 DAP (Figure 1E and F). In plots sprayed with chlorothalonil at 7-days interval (a total of two sprays), mean leaf spot severity ranged from 13-16% on all varieties. Except chlorothalonil at 7-days intervals, disease severities of all fungicides treatments were not significantly different from the unsprayed plots on the moderately resistant variety.

Table 1. AUDPC of *Cercospora* leaf spot on three groundnut varieties sprayed with chlorothalonil and carbendazim at Babile and Dire Dawa in 2010 main cropping season.

Cultivars	Fungicides Treatments (Spray Intervals)	AUDPC (%-days)	
		Babile	Dire Dawa
Oldhale	Chl 7-days	188.0	99.7
	Chl 14-days	204.6	136.1
	Chl 21-days	238.2	137.4
	Carb 14-days	213.1	140.0
	Carb 28-days	251.2	123.1
	No spray	401.3	143.9
	Mean	249.4	130.0
Betisedi	Chl 7-days	192.3	115.4
	Chl 14-days	225.4	147.8
	Chl 21-days	305.5	156.9
	Carb 14-days	235.8	162.0
	Carb 28-days	313.1	159.4
	No spray	452.4	198.3
	Mean	287.4	156.6
Werer-962	Chl 7-days	172.7	90.7
	Chl 14-days	183.4	120.6
	Chl 21-days	198.5	112.8
	Carb 14-days	184.7	116.7
	Carb 28-days	216.2	116.7
	No spray	363.1	119.3
	Mean	219.8	112.8
Site Mean		252.20	133.15
LSD (0.05)		22.96	8.53
CV (%)		5.49	3.86

Chl = chlorothalonil and Carb = carbendazim.

In general, *Cercospora* leaf spot was less severe on the varieties at Dire Dawa. This might be due to the prevailing weather conditions of the area. Dire Dawa is characterized by severe drought (arid) conditions and during the experiment, the mean minimum and maximum temperatures were higher as compared to the previous year and RH of the area was also low. On the other hand, for *Cercospora* leaf spot, prolonged periods of humidity and leaf wetness, as well as a late rainy season favor sporulation which results in greater disease (Smith et al., 1992; Butler et al., 1994; Nutter and Shokes, 1995).

Area under the disease progress curve (AUDPC)

The AUDPC is a very convenient summary of plant disease epidemics that incorporates initial intensity, the rate parameter and the duration of the epidemic which determines final disease intensity (Madden et al., 2008). Varieties by fungicides spray interval interactions showed very highly significant ($P < 0.001$) difference in terms of AUDPC values both at Babile and Dire Dawa. At Babile, the highest AUDPC value (452.4%-days) was calculated from unsprayed plots of the variety "Betisedi" while the lowest AUDPC value (172.7%-days) was from plots sprayed chlorothalonil at 7-days interval of the variety "Werer-962" (Table 1). AUDPC values of 401.3%-days and 452.4%-days were calculated from unsprayed plots of the varieties Oldhale and Betisedi, respectively, which had the highest disease severity at Babile. The AUDPC value of unsprayed plots of the variety Werer-962 was lower by 38 and 89%-days as compared to unsprayed plots of the varieties Oldhale and Betisedi, respectively. At Dire Dawa, the lowest AUDPC value (90.7%-days) was also calculated from plots sprayed chlorothalonil at 7-days interval of the variety "Werer-962" while the highest AUDPC value (198.3%-days) was from unsprayed plots of the variety "Betisedi" (Table 1). Our finding is in agreement with that of Mukankusi et al. (1999) who stated that weekly applications of fungicides resulted in the lowest *Cercospora* AUDPC and the highest yields. Shokes et al. (1982) also reported that earlier initiation of fungicide applications on a calendar schedule reduced severity and defoliation, and resulted in higher yield.

Disease progress rate

Disease progress rate showed significant difference among the varieties and fungicide treatments both at Babile and Dire Dawa. The varieties by spray intervals interactions effect were not significant at the 5% probability level. Therefore, the main effects (varieties and spray intervals) were used for comparison of the rates (Table 2). Leaf spot developed at different rates on the varieties. At Babile, the fastest leaf spot progress (0.042 units per day) occurred on the variety Betisedi while the slowest progress was from the variety Werer-962 (0.033 units per day). The varieties Betisedi and Oldhale were not significantly different from each other in terms of disease development rate. At Dire Dawa, the rate of disease development was relatively slower. Leaf spot increased by 0.030, 0.029 and 0.017 units each day on the cultivar Betisedi, Oldhale and Werer-962, respectively. The moderately resistant variety Werer-962 reduced the rate of disease development consistently over the locations. Johnson and Beute (1986) reported that reduced infection rates may delay the onset of defoliation beyond some critical point in host development and yield accumulation. The ultimate value of partial resistance to control of groundnut leaf spot was as a means to reduce fungicide use (Gorbet et al., 1982).

The fungicides spray intervals also created very significantly variable ($P<0.001$) rates of leaf spot development. The fastest disease progress was observed on unsprayed plots than on sprayed plots as shown by the results at

Table 2. Disease progress rate of *Cercospora* leaf spot on three groundnut varieties sprayed with chlorothalonil and carbendazim at Babile and Dire Dawa in 2010 main cropping season.

Treatment	Disease progress rate (units/day)	
Cultivars	**Babile**	**Dire Dawa**
Oldhale	0.040	0.029
Betisedi	0.042	0.030
Werer-962	0.033	0.017
Mean	0.038	0.026
LSD (0.05)	0.005	0.007
Spray Interval		
Chl 7-days	0.015	0.010
Chl 14-days	0.031	0.028
Chl 21-days	0.043	0.027
Carb 14-days	0.028	0.035
Carb 28-days	0.041	0.022
No spray	0.072	0.031
Mean	0.038	0.026
LSD (0.05)	0.007	0.010

Chl = chlorothalonil and Carb = carbendazim

both locations. At Babile, leaf spot epidemics developed at 0.072 units per day on unsprayed plots (Table 2). This rate is about five times faster than the rate on plots sprayed chlorothalonil every 7-days interval (0.015 units per day). This indicated that leaf spot development was at much higher rate on unsprayed check plots as compared to other spray intervals. At Dire Dawa also, the lowest disease progress rate was obtained from plots sprayed fungicides and from the variety Werer- 962 (Table 2). The finding of this study is in agreement with that of Beard et al. (2004) who reported that application of fungicide can retard the rate of disease progress. Application of fungicides consistently resulted in greater groundnut yields and biomass as compared to untreated control (Nutsugah et al., 2007).

Seed yield

Interactions between effects of varieties and foliar spray intervals showed very highly significant ($P < 0.001$) differences in terms of seed yield at Babile but not at Dire Dawa. At Babile, the highest yield (1750.8 kg/ha) was obtained from plots sprayed chlorothalonil at 7-days interval of the variety “Werer-962” while the lowest yield (687.9 kg/ha) was from unsprayed plots of the variety “Betisedi” (Table 3). Fungicides spray at different intervals also significantly varied seed yield harvested from the variety “Betisedi”. This result indicates the yield of susceptible variety “Betisedi” decreased as the intervals between sprays increased. However, the yields obtained from different spray treatments of the moderately resistance variety Werer-962 were not significantly different from each other except yield of unsprayed plots. This means all fungicides intervals increased yields of this variety almost in the same amounts. Our finding also agree with that of Gorbet et al. (1990) who reported that moderate resistance cultivars gave little or no additional yield response when the fungicide schedule was intensified from 20 to 10-days. Gorbet et al. (1982) also stated that there is no difference in yields between applications of chlorothalonil at 14 and 20-days intervals. The ability to use pyraclostrobin at 21-days intervals and maintain control of early leaf spot similar to that of application of currently available fungicides at 14-day intervals represents potential savings in time, fuel, labor and equipment costs (Culbreath et al., 2002).

At Dire Dawa, however, *Cercospora* leaf spot appeared at the end of the season (about 75 DAP) on all disease resistant and susceptible varieties and it did not cause serious leaf defoliation up to the time of harvest. Due to these reasons, significant differences were not detected in yield among fungicide treatments. However, the varieties significantly differed in their seed yield and, this might be due to their differences in genetic makeup level. The highest seed yield (1108.8 kg/ha) was obtained from the variety “Werer-962” while the lowest seed yield (895.4 kg/ha) was obtained from Betisedi. The yield obtained from Werer-962 was not significantly different from the yield of Oldhale. The yield losses caused by leaf spot are mainly because of decrease in photosynthetic leaf area caused by necrotic spots and defoliation (Boote et al., 1980; Bourgeois and Boote, 1992; Naab et al., 2005). The time of leaf spot diseases occurrence depends on the weather conditions and the field cropping history (Virginie, 1999).

In general, seed yield harvested from fungicide sprayed plots was consistently greater than the yield harvested from unsprayed plots. The highest yield was obtained from fungicide sprayed plots of moderately resistant variety when compared with the yield of susceptible varieties. Therefore, integrated use of host resistance and foliar applications of fungicides are recommended to reduce the epidemic level of *Cercospora* leaf spots and to achieve optimum yield of groundnut.

Conflict of interest

The authors did not declare any conflict of interest.

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Table 3. The effect of integrated use of host resistance and fungicide treatments on the yield of groundnut at Babile and Dire Dawa in 2010 main cropping season.

Treatment		Seed yield (kg/ha)	Treatments	Seed Yield (kg/ha)
Cultivars	Spray Intervals	Babile	Cultivars	Dire Dawa
Oldhale	Chl 7-days	1658.3	Oldhale	1049.9
	Chl 14-days	1590.1	Betisedi	895.4
	Chl 21-days	1475.1	Werer-962	1108.8
	Carb 14-days	1430.9	Mean	1018.0
	Carb 28-days	1413.4	LSD (0.05)	130.2
	No spray	974.1	Spray Intervals	
Betisedi	Chl 7-days	1504.0	Chl 7-days	1030.6
	Chl 14-days	1476.1	Chl 14-days	1019.0
	Chl 21-days	993.7	Chl 21-days	1017.9
	Carb 14-days	1324.1	Carb 14-days	1014.0
	Carb 28-days	1054.2	Carb 28-days	1020.6
	No spray	687.9	No spray	1006.2
Werer-962	Chl 7-days	1750.8	Mean	1018.0
	Chl 14-days	1700.4	LSD (0.05)	184.18
	Chl 21-days	1685.4	CV (%)	18.9
	Carb 14-days	1681.7		
	Carb 28-days	1631.4		
	No spray	1167.9		
Site Mean		1400.0		
LSD (0.05)		131.54		
CV (%)		5.7		

Chl = Chlorothalonil and Carb = Carbendazim.

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Efficacy of selected plant extracts against *Tribolium castaneum* Herbst in stored groundnut (*Arachis hypogaea* L.)

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The efficacy of powders of plant parts from *Azadirachta indica, Lawsonia inermis, Annona senegalensis* and *Hyptis suaveolens* at 10, 15 and 20 g/250 g seeds was tested using Complete Randomized Design (CRD) against the storage pest *Tribolium castaneum* (Herbst) in groundnut in the laboratory. At 28 days after application, mean number of seeds damaged was 0.33±0.33 for *A. indica* and 2.33±0.33 for *L. inermis* at 20 g concentration when compared to untreated seeds (10.00±1.15). Aluminium phosphide gave complete control (0.00±0.00). Twenty grammes (20 g) at 28 days of *A. indica* was efficacious as aluminium phosphide in protecting seeds against damage by *T. castaneum*. All powders provided some control of *T. castaneum*. The percentage mortality of adult pest in 20 g of *A. indica* (53.33%), *L. inermis* (33.33%) is high compared to *H. suaveolens* (16.66%) and *A. senegalensis* (20.00%) as compared to untreated (0% mortality). At the end of 6 weeks of storage, the weight loss from original weight of 250 g was 40.89 g (16.36%) for untreated seeds, 1.88 g (0.75%) for *A. indica* and 5.05 g (2.02%) for seed treated with *L. inermis* at 20 g concentration each. Aluminium phosphide-treated seeds suffered minimal weight loss (0.16 g, 0.06%) at 20 g concentration. *A. indica* and *L. inermis* are recommended for post-harvest control of *T. castaneum* in stored groundnut for planting.

Key words: Plant extracts, biocidal activity, *Tribolium castaneum,* groundnut.

INTRODUCTION

Groundnut is an important oil and protein source to a large portion of the population in Asia, Africa and the Americas. It is predominantly used for oil extraction in many Asian and African countries. In China, the average pod yields have hovered around 1.8 t/ha and total production has reached 7.0 mt annually (Ryan, 1992). However, potential yields reported are much higher between 7,500 kg and 11,000 kg pods/ha in experimental farms situated at Shandong province in China. In Southern Africa, the average groundnut yields are lower at 700 kg/ha, in comparism to potential yields of 4,000 kg pods/ha. Obviously, soil fertility, abiotic and biotic stress, factors limit groundnut crop

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growth and yield in many ways. However, in response to it a measurable progress in incorporating disease and pest resistance, and tolerance to abiotic stress factors have been achieved, during the previous two decades. Breeding options to enhance yield, its quality and/or to impart tolerance to biotic and abiotic stress factors have been carefully prioritized on a regional basis by the international and national groundnut agencies to suit consumer preferences (Ryan, 1992).

Groundnut, also known as peanuts, is considered a very healthy snack. Groundnut is the member of the legume family and is native to regions like South America, Mexico and Central America. However, it is successfully grown in other parts of the World as well. The name of the plant combines the morpheme 'pea' and 'nut'. In the culinary sense, it is regarded as a nut, but in the botanical sense, the fruit of the plant is a woody legume. Since that is the case, the groundnut is actually a kind of pea. The groundnut is also known by different names like earthnuts, manila nuts and monkey nuts. Throughout the World, they are known for their nutrition and health benefits.

In storage, groundnut pods/seeds are attacked by several stored products pests including the groundnut borer *Caryedon serratus* (Oliver), the merchant grain beetle *Oryzaephilus mercator* (Fauvel), the khapra beetle *Trogoderma granarium,* the black fungus beetle *Alphitobius spp*., the flat grain beetle *Cryptolestes ferrugineus,* the tropical warehouse moth *Ephestia cautella* (Walker), the Indianmeal moth *Plodia interpunctella* (Hubner), the rice moth *Corcyra cephalonica* (Stainton). It is known that more than 100 species of insects are capable of infesting stored groundnuts (Redlinger and Davis, 1982; Ofuya and Lale, 2001). Of these, insect pests of groundnut, *T. castaneum* are the first to colonize a new stock. It consumes the endosperm and causes caking, musty smell and loss of grain weight (Davey et al., 1959). It is also known to cause up to an economic damage rate of 30 and 40%, respectively, of stored millet and wheat flour (Ajayi and Rahman, 2006).

Over the years, synthetic chemical pesticides have provided an effective means for pest control. The short-comings of the use of chemicals which include; resistance by insects, adverse effect of non-target species, pollution of the environment including; soil, water, air and hazard of residue necessitated the evolution of natural insect-cides of plant origin (Deedat, 1994). Plants are composed of chemical directly beneficial for the growth and development of the plant. Rather they are part of the plant's defense against plant feeding insects and other herbivores (Rosenthal and Janzen, 1979). Recently, a number of plant materials have been explored as sustainable alternatives for controlling short-lived insect pests during storage of grains and found to be quite effective. Some of these botanical pesticides that have been reported to be efficacious against pests wchich include powders from *Piper guineensis* (Ivbijaro and Agbaje, 1986), *Piper nigrum* (Rajapakse, 1990), *Zanthoxylum xanthoxyloides* (Ogunwolu and Odunlami,1996), root bark of *Annona senegalensis* (Aku et al.,1998), *Capsicum frutescens* (Echezona, 2006; Ofuya, 1986), plant mixtures (Arannilewa et al., 2006), essential oil derived from *Artemisia sieberi* (Negahban et al., 2007), powders from the seed of neem (*Azadirachta indica* A. Juss) (Ivbijaro, 1983) and neem leaf extracts (Epidi et al., 2005), leaf powders of the Dragon tree (*Dracaena arborea*) and *Vitex grandifolia* (Epidi et al., 2008) and methanol leaf extracts of *Vitex negundo, V. trifolia, V. peduncularis* and *V. altissima* (Kannathansan et al., 2007). Others are vegetable oils from groundnut, palm kernel, coconut (Hall and Herman, 1991; Lale, 1995), rhizomes of ginger (Vijayalakshmi et al., 1997), leaf extracts of *Teprosia vogeli* (Mallaya, 1985), oil from fresh garlic (Ho et al., 1997), leaves and seeds of *Ocimum basilium* (Grainge and Ahmed, 1988) and leaves of *Dracaena arborea* (Boeke et al., 2004). The Derived Savanna is known to have a wide array of plant species with great insecticidal potentials yet to be discovered. Furthermore, different insects react in varying ways to different plant products. In this study, powders from four plant species viz *Lawsonia inermis, Hyptis suaveolens, Annona senegalensis* and *Azadirachta indica* were evaluated for their efficacy against *T. castaneum.* In several studies *H. suaveolens* essential oil has shown useful insecticidal properties against many foodstuff pests (Peerzada, 1997; Othira et al., 2009). *H. suaveolens* essential oil shown a toxic activity against *Plutella xylostella* (L.) (Lepidoptera Plutellidae) larvae and Callosobruchus maculatus (F.) (Coleoptera Bruchidae) adults (Kéïta et al., 2006; Tripathi and Upadhyay, 2009). In recent studies, it was reported that *H. suaveolens* essential oil had a marked toxic and repellent activity against adults of both *S. granarius* and *S. zeamais* (Motschulsky) (Coleoptera Dryophthoridae) (Conti et al., 2010; 2011).

L. inermis (Lythraceae) commonly called henna produces a burgundy dye, lawsone. This molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, fingernails, leather, silk and wool. The dye molecule, lawsone is primarily concentrated in the leaves, and is in the highest levels in the petioles of the leaf. Women use Henna (*L. inermis*) to dye or decorate their feet, hand, and skin and are not easily affected by fungal infection and bacterial infection. Henna has been found to exhibit antibacterial, antifungal and dermatological properties (Wren, 1988). *A. senegalensis* (Annonaceae) generally known as 'African custard-apple' is a potent medicinal plant generally used traditionally in the treatment of many diseases. Larvicidal effect of plant extracts belonging to the family of Annonaceae including *Annona muricata*, *A. cherimolia*, *A. squamosa*, etc against Anopheles sp., *A. aegypti* and *Culex quinquefasciatus* was reported (Saxena et al., 1993, Isman, 2006; Bobadilla-Alvarez et al., 2002). *A. senegalensis* has shown his insecticidal effect on different

development stages of *Caryedon serratus* (Coleoptera: Chrysomelidae) (Gueye et al., 2011). In northern part of Cameroon, both the leaves of *A. senegalensis* and *B. dalzielii* were used locally to protect maize, millet and sorghum against weevils' attacks (Ngamo et al., 2007). Neem tree (*A. indica*) is a tree in the mahogany family Meliaceae; is evergreen tree found in most tropical countries. Traditional and Ayurvedic uses of neem include the treatment of fever, leprosy, malaria and tuberculosis. Various folk remedies are used as an anthelmintic, antifeedant, antiseptic, diuretic, emmenagogue, contraceptive, febrifuge, parasiticide, pediculicide and insecticide (Makeri et al., 2007; Subramanian et al., 1996; NRC, 1992; Mukherjee et al., 1955; Ernst, 2007). In view of the importance of these plants for their insecticidal properties, the insecticidal efficacies are to be tested on *T. castaneum* (Herbst).

The objectives of the study included to find the effect of different concentration(s) of powders of *L. inermis, H. suaveolens, A. senegalensis, A. indica* on *T. castaneum* and to evaluate the effects of these plants on the quality of groundnut seeds vis-a-viz weight and deterioration.

MATERIALS AND METHODS

Study area

This study was carried out in the Biological Science Laboratory of Kogi State University, Anyigba, Kogi State, Nigeria.

Sources and preparation of plant extracts

Fresh leaves of Neem (*A. indica*), Horehound (*H. suaveolens* L.), Wild custard apple (*A. senegalensis* Pers.) and Henna (*L. inermis* L.) were collected from Kogi State University environs. The plants were authenticated at the department of Botany, Kogi State University. The leaves were air dried for 2 weeks. When fully dried they were separately ground into fine powder. The powders were kept in air tight jars prior to use.

Preparation of groundnuts

Groundnut kernel (*A. hypogaea* L.) was purchased from Anyigba main market, Kogi State. Healthy groundnut seeds were carefully selected into a container and were preserved.

Source of pest (*Tribolium castaneum* Herbst)

The red flour beetle (*Tribolium castaneum* Herbst) were naturally obtained from infested stored groundnuts in Anyigba main market and kept in a jar containing groundnut to feed on. The adult beetles of uniform size were used for the experiments.

Experiment 1: The effects of the plant powders on damage of groundnut seeds by *Tribolium castaneum*

The four different powders were evaluated for their ability to protect seeds of groundnut against damage by *T. castaneum.* Three doses (10, 15 and 2g) of each plant powders were thoroughly mixed with250 g of groundnut in separate 2 L capacity jars. Thirty adult beetles were introduced into each of the jars. Aluminium phosphide (10, 15 and 20 g) was used as a standard and a control jar was included. The open end of each jar was covered with a lid lined with mosquito net to prevent escape of the insects. For 14, 21 and 28 days after introduction of the adult, the number of damaged seeds (seeds with holes) in each sample were determined by counting. The experiment was a completely randomized design (CRD) replicated thrice.

Experiment 2: Effect of the different plant powders on recovery of adult beetle of *T. castaneum* two weeks after treatment of groundnut

In a similar but separate experiment observations were taken on the number of surviving adults two weeks after treatment. Adults were considered dead if they did not move when touched or tilted.

Experiment 3: Effect of the plant powders on weight of groundnut seeds 6 weeks after introduction of plant powders

This experiment was also similar to experiment 1 except that the set up was left for 6 weeks. At the end of 6 weeks, the content of each jar was poured into a 5 mm sieve and mechanically shaken to separate the groundnut seeds from both the insects and the plant powder. The seeds were thereafter weighed. The effect of the plant powders on the weight of groundnut was determined by subtracting the final weight from the original weight of 250 g:

$W_0 = W_1 - W_2$

Where W_0= weight difference; W_1 = original weight (before infestation); W_2 = final weight (after infestation).

Data analysis

The data obtained is represented as Mean±SEM and were presented in Tables 1 to 3.

RESULTS

At 14-21 days after introduction of plant powders, untreated seeds had more holes than seeds treated with plant powders and aluminium phosphide (Table 1). Mean number of seeds damaged was quite low at 21 and 28 days for *A. indica* (0.66±0.33 and 0.33±0.33) and *L. inermis* (4.00±0.57 and 2.33±0.33) at 20 g concentration as compared with the control. Furthermore, *A. indica* and *L. inermis* powders were more efficacious in protecting groundnut seeds against damage than powders of *A. senegalensis* and *H. suaveolens.* Seed damaged assessment at 28 days post introduction showed that aluminium phosphide-treated seeds were not damaged.

At 14 days after introduction of plant powders, no live adults were recovered from aluminium phosphide-treated seeds (100% mortality). However, more adults were recovered from untreated seeds (0% mortality) than those treated with plant powders (Table 2). Fewer adults were recovered from seeds treated with *A. indica* (53.3% mortality) and *L. inermis* (33.33% mortality) at 20 g

Table 1. The effect of the plant powders on number of damaged groundnut seeds by *Tribolium castaneum* at 14, 21 and 28 days after introduction.

Treatment (g)	14 days	21 days	28 days
A. indica			
10	8.33±0.33	3.00±0.57	2.33±0.33
15	5.00±0.57	1.33±0.33	0.66±0.33
20	3.33±0.33	0.66±0.33	0.33±0.33
L. inermis			
10	7.00±0.57	4.66±0.33	3.66±0.33
15	5.33±0.33	4.66±0.88	2.33±0.66
20	4.33±0.33	4.00±0.57	2.33±0.33
H. suaveolens			
10	16.33±0.33	9.66±0.33	8.33±0.88
15	13.00±1.15	8.66±0.66	7.33±0.88
20	11.33±0.88	6.00±0.57	4.33±0.33
A. senegalensis			
10	16.00±0.57	8.33±0.33	4.66±0.33
15	9.66±0.88	5.66±0.88	3.66±0.66
20	8.66±0.33	4.00±0.57	3.33±0.33
Phostoxin (ALP)			
10	0.00±0.00	0.00±0.00	0.00±0.00
15	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	0.00±0.00	0.00±0.00
Control	18.66±0.88	14.66±1.45	10.00±1.15

Values are Mean±SEM

concentration which showed the highest % mortality of *T. castaneum* as compared to *H. suaveolens* (16.66% mortality) and *A. senegalensis* (20.0% mortality) at 20 g concentration.

At 6 weeks after introduction of plant powders, there was a drop of 40.89 g (16.36%) in the weight of untreated seeds from the original weight of 250 g compared to seeds treated with *A. indica* and *L. inermis* whose weight loss was 1.88 g (0.75%) at 20 g concentration and 5.05 g (2.02%) at 20 g concentration, respectively. *H. suaveolens* weight loss was 8.75 g (3.75%) at 20 g concentration and *A. senegalensis* powders weight loss was 7.72 g (3.08%) at 20 g concentration (Table 3). Aluminium phosphide-treated seeds suffered minimal weight loss of 0.16 g (0.06%) at 20 g concentration.

DISCUSSION

Recently, post-harvest loss of grain due to insect pests has become a major concern all over the world such that demand for good quality products, which are free from chemical residues, is high and increasing rapidly (Kashi,1981). The significantly fewer number of holes found on groundnut seeds treated with ground leaves of *A. indica* and *L. inermis* compared to untreated groundnut is an indication that these two plants can serve as protectants against *T. castaneum*. It is possible that the plants factors conferring protection on the seeds against *T. castaneum* may have repellent and antifeedant and toxic properties. If repellent and antifeedant, the adult pest would not be inclined to bore into the seeds to feed. If toxic, the adult pest may not do more than scarify the seeds before dying. If death does not occur immediately, the factor(s) may have a debilitating effect on the adult pest, and their life cycle may be unusually prolonged. Whatever is the case, the resultant effect is fewer number of holes and/ or scarifications on the seeds. Epidi et al. (2005) reported that neem leaf extract was efficacious against insect pest. Ivbijaro (1983) report shows the toxicity of neem seed (*Azadirachta indica* A. Juss).

The number of live adults recovered would further demonstrate the efficacy of the different plant powders. Fewer adults of *T. castaneum* were recovered from *A. indica* and *L. inermis* treated lots compared to other plant powders. Further, the number of adults recovered from the control was much higher than that from the ground leaf powder indicating that the materials did not support normal growth and development and caused mortality of *T. castaneum.*

These further show that these plants have great potential in protecting stored grains against *T. castaneum*.

Table 2. Effect of the different plant powders on the recovery of adults of *T. castaneum* at 14 days after treatment of groundnut.

Treatment (g)	Percentage mortality (%)
A. indica	
10	33.33
15	40.00
20	53.3
L. inermis	
10	26.66
15	33.33
20	33.33
H. suaveolens	
10	10.00
15	13.33
20	16.66
A. senegalensis	
10	16.66
15	16.66
20	20.00
Phostoxin (ALP)	
10	100
15	100
20	100
Control	0

Table 3. Effects of the plant powders on weight of groundnut seeds at 6 weeks after introduction of plant powders.

Treatment (g)	Difference in weight of groundnut $W_0=W_1-W_2$	$W_0/250 \times 100$ (%)
A. indica		
10	4.85	1.94
15	3.27	1.31
20	1.88	0.75
L. inermis		
10	7.38	2.95
15	6.11	2.44
20	5.05	2.02
H. suaveolens		
10	15.53	6.21
15	11.18	4.47
20	8.75	3.75
H. senegalensis		
10	12.48	4.99
15	9.53	3.81
20	7.72	3.08
Phostoxin (ALP)		
10	0.44	0.18
15	0.28	0.11
20	0.16	0.06
Control	40.89	16.36

T. castaneum inflicts serious damage on groundnut kernels, feeding on the embryo and endosperm (Davey et al., 1959). The efficacy of the plants powder as grain protectants against *T. castaneum* was clearly demonstrated by the reduction in weight of the untreated groundnut seeds compared with the treated ones. *A. indica* and *L.*

inermis possibly through a combination of repellency, feeding deterrence and toxicity limited feeding by *T. castaneum* hence recording the least weight loss.

The use of essential oils derived from plant materials in the control of weevil pests of stored products is legendary and it is a practice quite as old as civilization itself. Essential oils are commonly used because they are quite efficacious against all life stages of insects. Moreover, it has been widely reported that terpenes from a variety of essential oils derived from vegetative sources have potent insect pest control properties, which affect the biology of target insects in different ways by acting either as ovicides, repellants, antifeedants, fumigants, contact toxicants or insecticides (Hough-Goldstein, 1990; Watanabe et al., 1993; Rice and Coats, 1994; Tsao et al., 1995). It is therefore, possible that the efficacy seen with the dried leaves of the four botanicals against the pests (*T. castaneum*) could be attributed to these and other constituents.

Aluminium phosphide (ALP) is used as a rodenticide, insecticide and fumigant for stored cereal grains. It is used to kill small verminous mammals such as moles, rabbits, and rodents. The pellets typically also contain other chemicals which evolve ammonia which help to reduce the potential for spontaneous ignition or explosion of the phosphine gas. Pure phosphine is odorless, but technical grade phosphine has a highly unpleasant odor like garlic or rotten fish, due to the presence of substituted phosphine and diphosphine (P_2H_4). As rodenticide, aluminium phosphide pellets are provided as a mixture of food for the consumption by the rodents. The acid in the digestive system of the rodent reacts with the phosphide to generate the toxic phosphine gas. Phosphine is routinely used to kill insects found in stored products.

Conclusion and Recommendations

Botanical control is said to be the best pest control of stored grain pest because it is biodegradable, environmental friendly and does not leave toxic residue. Therefore, plant extracts can be another source of insecticides/pesticides against stored grain pest. The shortcomings of the use of synthetic chemicals which includes resistance by insects, adverse effect on non-target species, pollution of the environment including soil, water and air and hazard of residue necessitated the evolution of natural insecticides of plant origin (Deedat, 1994). It is recommended that the active molecule in *A. indica* and *L. inermis* responsible for its activities be isolated for the development of biopesticides to protect our grains in storage. For more effectiveness of plant extracts, a large amount proportional to the quantity of grains is required for post-harvest control of *T. castaneum* in stored groundnut for planting.

In conclusion, plant extracts have a good potential in serving as alternatives to synthetic pesticides because of their high efficiency and environmental friendliness.

Conflict of interest

The author did not declare any conflict of interest.

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Physiological nutrient use efficiency of banana hybrids across agro ecological regions in Uganda

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Banana is an important source of food and income for millions of people in sub-Saharan Africa and Uganda in particular. This makes it one of the most important food crops in the country. However, production is limited by both biotic and abiotic constraints. Among the biotic constraints, is the decline in soil fertility. In addressing some of these biotic and abiotic constraints, banana breeding programmes are developing and releasing new banana varieties with limited information of their efficiency to capture soil nutrients. This study was to establish the physiological nutrient use efficiency of three new banana varieties namely FHIA17, M9 and M2 at harvesting time. The study was carried out in 8 (eight) districts of Uganda. Soil and tissue samples were collected from already established banana plantations for analysis of N, P, K, Ca and Mg. Tissue samples were collected by destructive sampling. Means of nutrients within district were separated using the Least Significant Difference (LSD) test at 5% level of significance (SAS, 2008). The nutritional status of bananas and the concentrations in various organs were, with respect to N, P, K, Ca and Mg, levels subjected to ANOVA. The relationship between plant content of the five nutrients and their concentration in the soil was determined by coefficient of correlation (r). The results showed no major significant nutrient use efficiencies between cultivars with FHIA 17 having the highest nutrient use efficiency. There were a few positive correlations between soil and plant nutrients.

Key words: Nutrient concentrations, Banana cultivars, destructive sampling, nutrient interactions.

INTRODUCTION

Bananas are grown on about 1.5 million ha of land and represent 38% of total arable land in Uganda (NARO, 2000). This makes it one of the most important food genomes are reported to be late introductions in East Africa (Simmonds, 1982). The East African highland cooking and juice bananas (*Musa spp.* AAA-EA) and exotic juice bananas (*Musa spp.* ABB) are important staple foods in East Africa, providing more than 25% of carbohydrates and 10% of the calorie requirement for 70 crops in the country (FAO, 2004). The most commonly grown bananas in Uganda are those belonging to the genome AAA, while AAB and ABB million people (Karamura, 2001). The East African highland bananas are by far the most widely distributed cultivar in the region, stretching from Eastern Democratic Republic of Congo to the southern fringes of the Ethiopian highlands and down to Mbeya in Southern Tanzania. Concerns about banana yield decline, have been

voiced so often that it is now considered as an established fact (Baijuka and Wuijsen, 1998). However, most reports are based on farmers' perception (Gold et al., 1993).

In Uganda, a substantial proportion of the bananas are grown near the homestead (Zake et al., 2000). Plots near the homestead generally receive more organic household residues and are often more mulched than fields further away. Bananas are grown primarily for home consumption but are increasingly becoming a source of income for many households. They have a high industrial potential through juice, wine and assorted post-harvest food stuff production. For example, in South-western Uganda, there is a common proverb "Ataine Rutookye n'ente tashwera" meaning "One without a banana plantation and cows does not qualify to marry, for he would not sustain a family" (Tumuheirwe et al., 2003). To most people in the Central region of the country, Matoke, a common recipe from cooking bananas, is synonymous to food (Pekke, 2004). Both the high production and consumption of banana reflects the important role bananas play in food security of the Ugandan people and justifies why it is important to improve and protect the crop.

Whereas, it is the major food crop for at least 30% of the people in Uganda (FAO, 1995), production decline in the country is a reality (Bekunda and Woomer, 1996). This decline has been attributed to low soil fertility, pest and diseases such as Fusarium Wilt, Black Sigatoka and Banana Bacterial wilt (Gold et al., 1993; Wortman et al., 1994). Soil nutrient deficiencies have been caused by continuous cultivation, crop harvests, soil erosion and a host of socio-economic and post-harvest problems (Rubaihayo et al., 1993). The most affected area of the country in banana production decline is the Lake Victoria Basin, a high rainfall region lying within 25 to 30 Km around Lake Victoria (Bekunda and Woomer, 1996).

Uganda is the second largest centre of banana diversity in the world, with a total of 95 banana varieties currently grown among banana producers (Karamura, 1998). A typical household in Uganda grows an average of 7 banana varieties simultaneously with a maximum of 27 varieties (Tumuhairwe et al., 2003). However, banana production has declined over the years from 8.5 to 5.6 tonnes /ha due to low soil fertility as one of the major constraints (Zake et al., 2000). Most farmers perceive continuous cropping and erosion as the main causes of soil fertility decline (Bekunda et al., 1999). Others attribute low banana yields to poor soil fertility, increasing pest pressure [especially the banana weevil (*Cosmopolites sordidus)*, Nematodes (*Radopholus similis)*, *Helicocotylencus multicinctus* and *Pratylechus goodeyi*], disease [black Sigatoka (*Mycosphaerella fijienisis)*, banana bacterial wilt], poor husbandry and moisture stress (Gold et al., 1999; NARO, 2000).

Bananas require large nutrient quantities which must be supplied through fertilizer application in order to obtain optimum yields. Bananas require nitrogen, phosphorus and potassium in large amounts for proper growth and production, and fertilizers not only improve soil nutrient status but also increase plant productivity (Guo et al., 2009). Regular inspection of soil nutrient status plays an important role in diagnosing nutrient deficiencies and sometimes toxicities that can affect crop growth and result in low yield. Plants that are efficient in absorption and utilization of nutrients greatly enhance the efficiency of applied fertilizers, reducing cost of inputs, and preventing losses of nutrients to ecosystems. Inter and intra-specific variation for plant growth and mineral nutrient use efficiency is known to be under genetic and physiological control and are modified by plant interactions with environmental variables. There is need for breeding programs to focus on developing cultivars with high Nutrient use efficiencies (NUE). Identification of traits such as nutrient absorption, transport, utilization and mobilization in plant cultivars should greatly enhance fertilizer use efficiency. The development of new cultivars with higher NUE, coupled with best management practices (BMPs) will contribute to sustainable agricultural systems that protect and promote soil, water and air quality. To deal with nutrition problems and determine the role of management practices, nutrient analysis of soil and plant tissues is an accurate, reliable and quantitative approach to diagnose and precisely correct nutrient deficiencies or toxicities. It is a useful tool in diagnosing nutritional disorders and helps in crop management decisions.

So, it is important to know the nutrient efficiencies of banana plants and soil nutrient status for better plant nutrition management and achieving better production.

This study is specifically on hybrids because, they appear to have higher yields, resistant to some diseases like black sigatoka, they have bigger biomass and so there was need to establish their yield performance, and their nutrient use efficiency. The information would be of great importance during up scaling and information on their growth requirements would be availed and this would help farmers to manage the hybrids well.

MATERIALS AND METHODS

Study area and experimental design

The study was carried out in 8 districts of Mukono, Jinja, Kamuli found in western Uganda, Wakiso and Mityana in the central and Kyenjonjo, Kabarole and Mubende in western Uganda where plantations of the

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studied cultivars had already been established. An augmented incomplete block design was used **i**n already established farmers' fields. The fields were planted with three hybrids namely FHIA17, M2 and M9, including a local check cultivar Mbwazirume as a control.

Data collection

Physiological nutrient use efficiency and plant nutrient concentration

Nutrient use efficiency was computed as the fruit yield produced per kg of plant nutrients accumulation in above ground biomass or product yield per content of nutrient. Total plant biomass (above ground) was defined as the sum of dry weights of the fingers, peduncle, leaves and pseudo stem. Total nutrient uptake was calculated from measurements of N, P, K, Ca and Mg mass fractions in fingers, peduncle, leaves and pseudo stem biomass and dry weights of the plant parts at harvest (Nyombi et al., 2009). In order to calculate nutrient totals in the plant, the following equation was used.

TAGN = 0% MFW*NCF+%MPW *NCP+%MLW*NCL+% MPsW*NCPs+%MCW*NCC

Where,TAGN = Total above ground nutrient in the plant at maturity, 0%, MFW = finger weight at 0% moisture, NCF = nutrient concentration in the finger, MPW = peduncle weight at 0% moisture, *NCP = nutrient concentration in the peduncle, MLW = leaf weight at 0% moisture, NCL = nutrient concentration in the leaf, %MPsW = pseudo stem weight at 0% moisture, NCPs = nutrient concentration in the pseudo stem, %MCW = corm weight at 0% moisture, NCC = nutrient concentration in the corm.

The samples were then moved to the laboratory for analysis of total N, P, K, Ca and Mg. Handling and preparations were based on procedures as described by Okalebo et al. (2002). From the bunches of each variety, a few fruits were picked, their peel and pulp separated and total sample fresh weights taken. These samples were later oven dried at 70°C for 48 h and the dry matter weight recorded.

The dry matter of the whole plant was also calculated using the following equation:

$$(Sdf*tff/fsf)+(sdl*tfl/fsl)+(sdp_s*tfp_s/fsp_s)+(sdp*tfp/fsp)+(sdc*tfc/fsc)$$

Where, Sdf = Sample dry matter of the fingers, Tff = total fresh weight of the finger, Fsf = fresh sample weight of fingers, Sdl = sample fresh weight of the leaf, Tfl = total fresh weight of the leaf, Fsl = fresh sample weight of the leaves, sdp_s = sample fresh weight of the pseudo stem, tfp_s = total fresh weight of the pseudo stem, fsp_s = fresh sample weight of the pseudo stem. Sdp = sample fresh weight of the peduncle, Tfp = total fresh weight of the peduncle, Tsp = fresh sample weight of the peduncle, Sdc = sample fresh weight of the corm, Tfp = total fresh weight of the corm, Fsp = fresh sample weight of the corm.

Destructive sampling was carried out and three (3) sub-samples taken from the corm, pseudo stem, peduncle, functional leave and fingers on the banana plants. The samples were obtained from the upper, middle and lower parts of the plant parts, that is, leaves, pseudo stem, corm, fingers and peduncle; Total fresh weight of the whole bunch, pseudo stem, peduncle, leaves and fingers were measured and recorded. The samples were oven died at 70°C for 48 h and dry weight recorded. The harvest index (fruit yield) as a proportion of above ground was determined from the sub sample.

Soil and plant nutrient analyses

Composite soil samples (of 5 sub- samples per plot) were taken from 45 fields where the plant samples were collected at a depth of 30 cm. These were plantations where fertilizers had not been applied. The samples were then taken to the laboratory and preparation and handling was based on procedures as described by Okalebo et al. (2002). Samples were oven-dried at 40°C for two consecutive days, and were thoughly mixed and ground using a mortar and a pestle to pass through a 2 mm sieve. The soils were analyzed at the National Agricultural Research Laboratories, Kawanda in central Uganda. Soil pH was determined using deionised water at sediment to water ratio of 1:2.5. Soil texture was determined using the hydrometer method (Bouyoucos, 1936). Soil organic carbon was determined by oxidation with excess aqueous potassium dichromate mixed with sulphuric acid, followed by titration against ferrous ammonium sulphate (Okalebo et al., 2002).

Total N was determined by Kjeldahl oxidation and semi-micro Kjeldahl distillation (Bremner, 1960). Exchangeable bases (K, Mg and Ca) were extracted using excess 1M ammonium acetate solution. Exchangeable K was then determined by flame photometry, while Mg was determined using atomic absorption spectrophotometry.

Phosphorus in the extract was determined using the molyb-denum blue colorimetric method (Okalebo et al., 2002). (LSD) test at 5% level of significance (SAS, 2008).

The nutritional status of bananas, with respect to N, P, K, Ca and Mg, was evaluated by comparing the analytical data with established critical levels. The relationship between plant content of the five nutrients and their concentration in the soil was determined by coefficient of correlation (r). All laboratory analyses were done in duplicate to increase precision of results.

Above-ground dry matter was computed as the sum of fruit, peduncle, corm, leaves and pseudo stem. All directly measured plant parameters were based on oven-dried plant material.

Data Analysis

Response variables for growth parameters and yield components were checked for normality using t test and found to be relatively normally distributed, necessitating no data transformation. Exploring the effect of cultivar, agro ecological zone and farm soil texture on genotype performance was done by exposing the data to Analysis of Variance (ANOVA) using the general Linear Model (GLM) in SAS software. Means within each agro ecological zone and later by district were separated using the Least Significant Difference (LSD).

The nutritional status of bananas, with respect to N, P, K, Ca and Mg, was evaluated by comparing the analytical data with established critical levels. The relationship between plant content of the five nutrients and their concentration in the soil was determined by coefficient of correlation (r).

RESULTS AND DISCUSSION

Tables of mean Nutrient element concentration in different organs of various banana cultivars

Generally, most of the nutrient elements in the various organs were not statistically significant except for a few. The major differences were observed in organs of FHIA 17 and M2. For example, Ca in the peduncle and in the fingers of FHIA17 was significantly different from those of M2 (Figures 1-6). Ca in the peduncle is said to have the potential to reduce finger drop, since its major purpose is

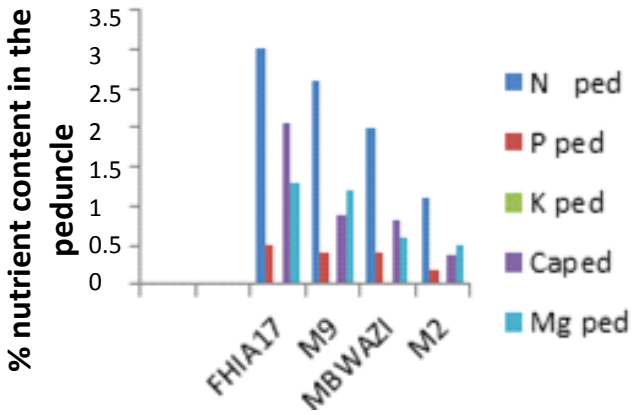

Figure 1. Percentage nutrient content in the peduncle.

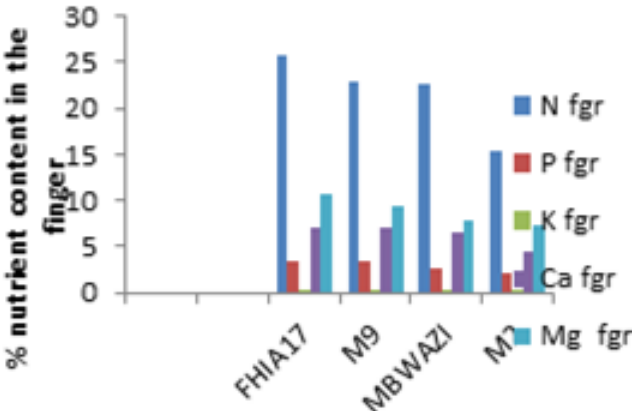

Figure 2. Percentage nutrient content in the fingers.

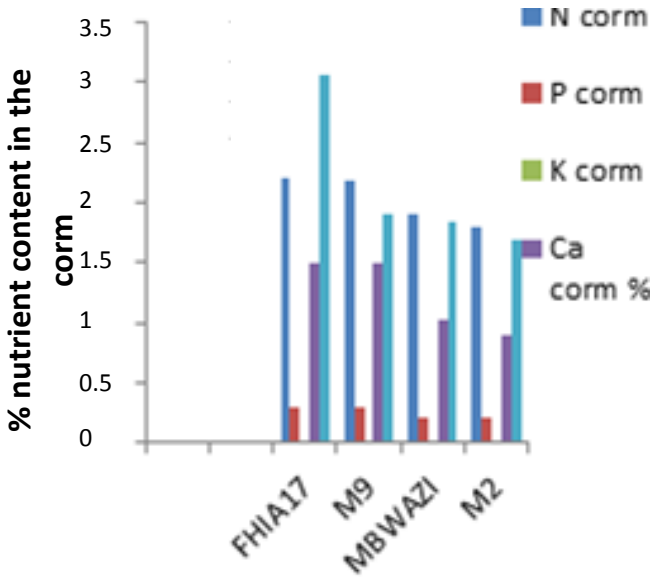

Figure 3. Percentage nutrient content in the corms.

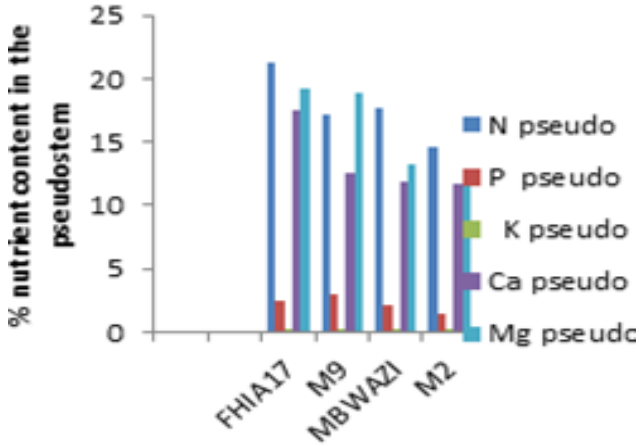

Figure 4. Percentage nutrient content in the pseudostem.

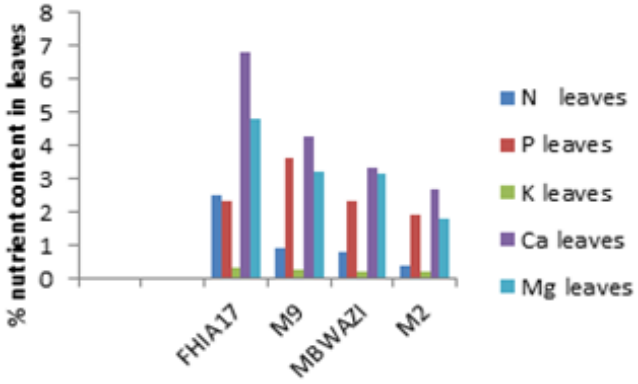

Figure 5. Percentage nutrient content in the pseudostem.

N Ped= Nitrogen concentration in the Peduncle; P ped = phosphorus concentration in the Peduncle; K ped = potassium concentration in the Peduncle; Caped = calcium concentration in the Peduncle; Mg ped = magnesium concentration in the Peduncle; N fgr = Nitrogen concentration in the fingers; P fgr = Phosphorus concentration in the fingers; K fgr=Potassium concentration in the fingers; Cafgr = Calcium concentration in the fingers; Mg fgrs= Magnesium concentration in the fingers; N pseudo = Nitrogen in the pseudo stem; P pseudo= phosphorus in the pseudo stem; K pseudo =potassium in the pseudo stem; Ca pseudo= Calcium in the pseudo stem; Mg pseudo= Magnesium in the pseudo stem; N corm =Nitrogen concentration in the corm; P corm = Phosphorus concentration in the corm; K corm = Potassium concentration in the corm; Ca corm= calcium concentration in the corm; Mg corm = magnesium concentration in the corm; N leaves = Nitrogen concentration in the leaves; P leaves=Phosphorus concentration in the leaves; K leaves= Potassium concentration in the leaves; Ca leaves = Calcium concentration in the leaves; Mg leaves = Magnesium concentration in the leaves; Mbwazi= Mbwazirume.

for desert, this reduces post-harvest losses (Nowakunda et al., 2000). Most of the differences were in FHIA17, this could be because of its genetical make up because it is

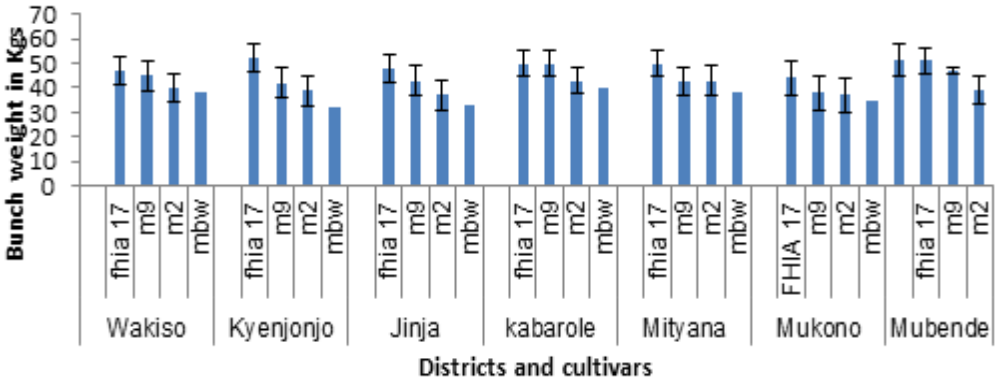

Figure 6. Bunch weights (kgs) for the various cultivars in the different districts.

an AB while the rest are not. M9, M2 and Mbwazirume did not have major differences, this is probably because, they are genetically related since the two hybrids were bred from the east African highland bananas (AAA) and Mbwazirume is one of the AAA cultivars (Barekye et al., 2002).

The few significant differences, could probably be because the mechanism of nutrient uptake is similar for all the cultivars. The results showed that FHIA 17 had the highest nutrient concentrations meaning that its nutrient uptake is higher than the rest of the cultivars. Total nutrient uptake and concentration does not depend on the cultivars but other factors which may include, soil moisture, management practices and soil ph. Most nutrients in the plant were found to be below average, average or just slightly above average, probably because sampling was done at harvest stage.

Generally, the concentration of plant nutrients fell with age. It was also established that all nutrients reach the same proportion in the fruits irrespective of the amounts supplied, (Esguerra, 2009). Insufficient potassium supply reduces the total dry matter production of Banana plants and the distribution of dry matter within the plant. The bunch is the most drastically affected organ and hence the importance of potassium in banana growing. Turner and Barkus (1980) found that while low potassium supply halved the total dry matter produced, the bunch dry matter was reduced by 80% and the roots were unaffected. It was suggested that of the various organs competing for potassium, those nearest to the source of supply is the most successful in obtaining their requirements. Studies of the ontogenic course of potassium uptake under field conditions have shown an overall decrease in whole plant concentration of potassium in the dry matter from sucker to fruit harvest. The potassium uptake is proportionally greater than dry matter accumulation early in the life of the plant. Under restricted potassium supply, the highest potassium uptake rate occurs during the first half of the vegetative phase. It is redistributed within the plant (Vorm and Diest, 1982) to allow further accumulation of dry matter.

Where potassium supply is abundant, large amounts of potassium is absorbed during the latter half of the vegetative phase Twyford and Walmsley (1973) and have a special effect on the maturation process (Fox, 1989). Even when potassium supply is abundant, potassium uptake during the life cycle is appropriate to meet the needs of the main plant crop but it is not relevant to ratoons, since in stools the mother plant and followers are present at the same time.

In general, M2 had the lowest nutrient use efficiency (Table 1). This suggests that cultivar FHIA17, which was the best in terms of nutrient use efficiency has a greater ability of converting absorbed nutrients into fruits (Ortiz-Monasterio et al., 2003). FHIA17 was followed by M9 in terms of nutrient use efficiency. FHIA17 nutrient concentration is significantly different from that of other cultivars, it means that, it is able to utilize acquired nutrients into fruits/bunch more than any other cultivar.

The results also showed high N and P use efficiency for FHIA 17 in Kamuli as compared to Kyenjojo, meaning there are less N and P in Kamuli. There was also higher K nutrient use efficient by FHIA17 in Wakiso as compared to Mityana. The results also showed high Ca use efficiency by FHIA17 in Mityana as compared to Wakiso, and high Mg use efficiencies in Kyenjojo as compared to Mityana (Table 1). M2 had higher N nutrient use efficiency in Kamuli than in Kyenjonjo and there was higher P use efficiency in Kamuli than in Mubende.

The results also showed higher K, Mg and Ca use efficiency in Kabarole than in Kyenjojo. M9 had a higher N, K, Mg and Ca use efficiency in Mubende as compared to other districts and higher P use efficiency in Mukono as compared to Kyenjojo. M9 had a higher N and P use efficiency in Kamuli and higher K, Mg and Ca use effiency in Kabarole as compared to other districts. Mbwazirume had a higher N, K, Mg and Ca nutrient use efficiency in Mubende as compared to other districts and

Table 1. Nutrient use efficiencies of different banana cultivars in different districts.

Cultivars	District	Average NUE (N) (%)	Average NUE (P) (%)	Average NUE (K) (%)	Average NUE (Ca) (%)	Average NUE (Mg) (%)
	Jinja	38.1	270.5	10.4	56.5	72.2
	Kamuli	83.8	750.7	18.2	170.1	85.8
FHIA 17	Kyenjojo	30.0	185.4	5.7	41.8	60.5
	Mukono	63.1	430.6	16.1	111.8	117.3
	Wakiso	41.2	499.1	8.9	107.2	114.8
Total average NUE (%)		49.4***	388.5***	12.0***	89.0**	89.0***
CV (%)		11	9	18	14	10.3
	Kyenjojo	33.2	290.5	11.9	53.1	73.9
M2	Mityana	78.7	1009.1	7.3	107.2	34.4
	Mubende	35.9	246.7	9.6	80.7	68.2
	Mukono	38.3	328.3	8.4	100.4	59.9
	Wakiso	31.3	287.2	24.2	60.2	34.3
Total average NUE (%)		39.3*	353.9**	11.0***	79.1**	61.3*
CV (%)		8.5	13.7	18	8	17
	Jinja	43.9	364.2	9.7	107.8	129.3
	Kabarole	46.9	240.9	34.3	179.1	88.9
	Kamuli	55.8	486.8	13.8	117.2	198.5
M9	Kyenjojo	32.0	288.2	8.1	85.9	33.8
	Mityana	33.2	245.0	9.9	141.5	62.0
	Mubende	40.2	239.8	12.8	90.6	111.3
	Mukono	50.5	394.7	12.4	120.0	86.1
	Wakiso	37.1	290.9	10.3	91.3	64.4
Total average NUE (%)		41.9**	316.6*	13.3***	117.8***	87.0***
CV (%)		14	15.4	15	19	12
	Jinja	47.7	359.6	7.0	79.0	68.7
	Kyenjojo	46.8	241.0	10.3	112.2	98.3
Mbwazi	Mityana	47.7	339.2	10.3	178.3	62.7
	Mubende	51.6	299.3	12.1	230.5	119.3
	Mukono	49.1	396.7	9.9	68.6	96.3
	Wakiso	41.7	248.1	9.6	107.9	48.4
Total average NUE (%)		47.8***	331.9**	9.7*	110.6***	85.5**
CV (%)		11.6	17	9.1	9.7	13

*, ** and *** denotes significant differences at p≥0.05, 0.01 and 0.001 respectively, between districts. Mbwazi =Mbwazirume.

higher P use efficiency in Mukono district as compared to other districts. Generally, there was high nutrient use efficiency in the districts of Kabarole, Kyenjojo and Mubende and these are the districts that had bigger bunches, meaning that the farms in the above districts have average nutrients. These three districts are found in the agroecological zone of western mid altitude farmlands, this zone has got a combination of soil types namely Andosols, Histosols and Chernozems, these are all dark soils formed from volcanic materials and rich in organic matter**,** the farms in zone are fairly managed compared to farms in the districts of Wakiso, Jinja, Kamuli, Mukono and Mityana that belong to zones of southern and eastern lake Kyoga and lake Victoria crescent which had plants with low nutrient use efficiency. These agro ecological zones have Acrisols type of soil that has got clays with low cation exchange capacity **(**FAO 1988).

High nutrient use efficiency for elements suggests deficiency for those various elements in the different areas, while low nutrient use efficiency suggests sufficiency of these nutrients (Ortiz-Monasterio et al., 2003).The variations in the elements use efficiency is probably generally due to the different soil types, soil management, altitude and climate among others.

The bunch weights for the various cultivars between the districts were not significantly different. These results support results for the nutrient use efficiencies of various cultivars in the different districts**.**

Table 2. Correlation matrix of soil properties and plant nutrient concentration.

Variables	N (%)	P (ppm)	K (cmolc/kg)	Ca (cmolc/kg)	Mg (cmolc/kg)	pH	OM	Total N	Total P	Total K	Total Ca	Total Mg
N (%)	1											
P (ppm)	0.41*	1										
K cmolc/kg	0.34 *	0.521	1									
Cacmolc/kg	0.63*	0.383	0.602	1								
Mg cmolc/kg	0.64 *	0.411	0.53	0.848	1							
pH	-0.19	-0.03	0.265	0.368	0.24	1						
OM	0.73*	0.34*	0.29*	0.60*	0.62*	0.04*	1					
Total N	0.004	0.171	0.095	0.089	0.0059	-0.03	0.070	1				
Total P	0.116	0.15	0.20	0.23	0.11	0.15	0.15		1			
Total K	-0.317	-0.17	-0.00	-0.13	-0.17	0.14	-0.2	0.55	0.46	1		
Total Ca	-0.05	-0.00	-0.12	-0.04	-0.05	-0.17	-0.0	0.64	0.44	0.61	1	
Total Mg	-0.07	-0.08	-0.14	0.00	-0.03	-0.11	0.3	0.450	0.283	0.372	0.338	1

OM= Organic matter; NS=not significant.

Correlation between soil nutrients and plant nutrient concentrations in banana plants

The results showed a negative correlation coefficient of total Potassium in the plant and Nitrogen in the soil -.0.317 ($p<0.012$) (Table 2) suggesting that high Nitrogen in the soil leads to maximum dilution of Potassium in the plant (Bolvin et al., 2004). All the nutrient concentrations in the plant were significantly positively correlated to each other. Similarly, the entire nutrients in the soil were positively correlated to each other, this is because, different nutrient elements affect the uptake of others as reflected in (Table 2).

On the other hand, N and P in the plants are also positively correlated to all the other studied nutrients in the soil but the correlation is not significant, whereas K, Ca and Mg in the plants are negatively correlated with all the studied nutrients in the soil, except for Mg in the plant and Ca in the soil which are positively correlated, It was observed that pH was low in most places. At acidic pH values, phosphate ions react with aluminum (Al) and iron (Fe) to again form less soluble compounds. The uptake, translocation and perhaps assimilation of cations and anions by plants depend not only on the concentration and availability of these ions in the nutrient medium but also on the presence of other cations and anions. The absorption of ca, for instance, may be depressed by excessive amounts of potassium or magnesium but favored by nitrate. The mechanism of these interactions is not always clear; in many cases it depends on ion competition on colloidal surfaces either at the surface of a cell or within the cytoplasm.

Organic matter was shown to be significantly positively correlated with all the nutrients. The higher the organic matter, the higher the nutrient elements in the soil and vice versa. This is because organic matter serves as a revolving nutrient bank account, meaning that it holds nutrients (Cations Exchange Capacity). Organic matter also improves on soil structure, maintains tilth and minimizes erosion; it prevents soil compaction, improves water infiltration and releases nutrients in an available form (Rietberg, 2008).

Conclusion and Recommendations

It was observed that nutrient use efficiency depends on other factors such as soil type, altitude, farm management and nutrient interactions (antagonism) and many others other than cultivar.

Fhia 17 was the best in terms nutrient use efficiency. M2 proved to be the poorest in terms of nutrient use efficiency, this means that it does well in areas with fertile soils and good management like Kabarole and Kyenjojo districts that are found in agroecological zone of Rwenzori footslopes and fortportal. Cultivars with high nutrient use efficiency like FHIA 17 do better in marginal land compared to the rest of the cultivars because FHIA 17 has the potential to convert the available nutrients into fruit unlike the other cultivars and farmers should be provided with suckers for those cultivars.Total nutrient uptake and concentration does not depend on the cultivars but other factors which may include, soil moisture, management practices and soil ph.

All the nutrient concentrations in the plant were significantly positively correlated to each other. Similarly, the entire nutrients in the soil were positively correlated to each other and therefore better soil management practices are recommended.

Conflict of interest

The authors did not declare any conflict of interest.

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Appendix 1. Source of funding and time frame.

Activity	Time frame	Item	Amount	Source of funds
Data collection	20012 February- June	Fuel (L)	600,000	BECANET
		SDA	200,000	BECANET
		Labor	100,000	BECANET
Sample analysis	2012 July-August	Sample analysis	550,000	Becanet
		Total	1,450,000	

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