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Full Length Research Paper

Analysis of colourfastness of fabrics treated with dyes extracted from Roselle calyces

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The dyeing potential of roselle (*Hibiscus sabdarifa*) extract variously extracted by boiling, steeping and ethanol solvent were evaluated with three dye fixatives (potassium aluminum sulphate (alum), tannic acid, and citric acid), to study their effects on cotton (100%), stone silk (60% silk:40% polyester) and polyester (100%) fabrics. Colourfastness to sunlight, washing, perspiration (acid and alkali) and rubbing (crocking) were studied on the individual fabrics as 1 × 4 factorial involving three dye extracts (1 dye source × three extraction methods) and three dye fixatives plus a non mordanted control in a completely randomized design (CRD) of three replications. Data on degree of fastness were generated as scores through 1 (excessive colour fade or stain) to 5 (no colour fade or stain) using Gray scale and fastness test rating scale (FTRS) evaluation instruments. Data were analyzed using descriptive statistics (means and standard deviations) and general linear model for factorial experiments of statistical package for social sciences (SPSS) version 16.0. Three null hypotheses were tested using analysis of variance (ANOVA). Treatment means were compared using Scheffe's test at 0.05 probability level. Findings include: Roselle calyces extracts showed reasonable colourfastness on cotton prototypes. The effects of dye extraction procedures did not significantly differ ($p>0.05$) on the colourfastness of cotton prototypes to sunlight ($p=0.33$), washing ($p=0.39$), alkali perspiration ($p=0.06$) and dry rubbing (0.59). The effects of mordant/dye fixatives did not significantly differ ($p>0.5$) on the colourfastness of cotton prototypes to washing ($p=0.95$) and rubbing or crocking ($p=0.68$), but differed significantly ($p<0.05$) on colourfastness to sunlight ($p=0.00$), acid perspiration ($p=0.00$) and alkali ($p=0.00$). Tannic acid and alum mordant were the source of difference. Roselle calyces extracts exhibited much to excessive fade and stain of colour and poor fastness to all parameters tested except rubbing fastness.

Key words: Fabric, colourfastness, dye fixatives, dye extraction, Roselle.

INTRODUCTION

Fabric is a flexible material made up of a network of natural or manufactured fibres formed by any of weaving, knitting or other fabrication techniques (Vanderhoff et al., 1985). Natural fibres are processed from animals' bodies, plants' cellulose and mineral. The major fabrics from animal sources are silk from silk worm and wool from

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sheep. Major plant, vegetable or cellulose fibres are from cotton and linen from flax plants. Asbestos is a mineral fibre from rock formations. Rayon, acetates, triacetates, cupramonium are some regenerated, transitional or cellulosic-based manufactured fibres while purely synthetic fibre fabrics are nylon, polyester, acrylics, olefin, spandex among others. The type of fabric is determined to a large extent by the nature of fibre, fabrication and finishing. Various finishes are applied to fabric to improve its aesthetics and functions. One of such finishing is colour application. Dye is a coloured substance that imparts permanent colour to other substances (Finar, 1973). A good dye is characterized by being soluble in water or dispersible in solvent and transferable to the fabric or other materials to be dyed by the process of absorption and exhaustion. Dye must have affinity to the fabric, be colourfast and organoleptically acceptable to consumers.

Colourfastness is the ability of a dye to resist fading or staining caused by sunlight, washing, perspiration (dilute acids and alkalis), crocking or rubbing and other organic solvents used in laundering and dry-cleaning (Marshal et al., 2000). A single dye may not be fast in all circumstances. However, a dye should remain viable and gracefully age with the product (Weber, 1990). The fastness or stability of a dye in fabric is determined by the type of fabric fibre (natural or synthetic); method of dye application (for instance, conventional exhaust procedure) and class of dye (acidic, basic, direct, vat, fibre reactive, disperse, azoic or mordant dye). A mordant is an element that quickens the chemical reaction taking place between a fibre and a dye. Mordant helps to open up the fibre to enable the dye absorbed and improve the fastness of the dye on the fibre. They also deepen the shade of dye and can change the final colour giving rise to a new colour. The application of dye to a textile with which the dye does not combine readily can sometimes be improved by using a mordant. Some mordant are heavy metals such as chrome and are destructive to fibre and toxic to skin. Aluminum sulphate (alum), ferrous sulphate and other acidifying dye fixing agents including tannic and citric acids act as intermediary between fibre and basic dye (Finar, 1973). Natural dyes are classified as mordant dyes. They do not dye fibres directly but require mordant.

Presently, the global interest in natural dye has increased tremendously. Natural dyes perform very crucial educational, economic, pharmacological, socio-cultural, political, religious as well as psychological roles. They are highly commended for their health and environmental benefits over some synthetic dyes which are toxic, non-biodegradable and carcinogenic. Natural dyes are also valued for the preservation of traditional dyeing arts and crafts. The very high demand for safe dyes in sustainable supply to meet the ever increasing volume required in the wood, food (Obadina and Oyewole, 2007), paper and photography industries, pharmacology (Chenghaiah et al., 2010; Owoade et al., 2015), educational institutions (Spenser, 2011; Bassey et al., 2012), homes, leather and leather product, textiles and clothing industries (Onwualu, 2006; Jothi, 2008), requires more research and development efforts in sourcing dyes from natural sources. No fabric dyeing or printing can be successfully achieved without sustainable supply of quality dyes.

Globally, clothing and textile sector has played major roles in employment and income generation for many nations. In Nigeria, for instance, within the past 15 years, there were up to 180 functional textile mills in the country employing about 800,000 people. Available report showed that out of 13 subsectors in the manufacturing sector, the textile sector comprising cotton textile and synthetic fabrics continued to account for a significant proportion of the overall growth of manufacturing production (Central Bank of Nigeria (CBN) Annual report, 1995). Similarly, textile and apparel sector contributed 34.49% to the rebased nominal GDP in Q1 2014 ranking 3rd after Chemicals and Pharmaceutical sector (41.61%) and Nonmetallic product sector (35.69%) using 2010 as a base year (National Bureau of Statistics Data Release Calendar, 2014).

However, in recent years clothing and textile sector had faced major challenges which precipitated to shutting down of about 155 textiles mills, leaving only 25 mills with low capacity utilization, and 776,000 citizens jobless; Osagie, 2013). In educational institutions, unavailability of non-toxic dyes in sustainable supplies hinder effective teaching and learning and acquisition of skills in arts, craft and science practical classes involving dye utilization. Acquisition of skills predisposes graduates to unemployment, a precursor to poverty, crime and other antisocial behaviours. More so, unemployment leads nations to trade deficit and under development. The Federal Government of Nigeria through the Raw Materials Research and Development Council (RMRDC) has continued to advocate the exploitation of locally available raw materials to substitute or supplement the imported and expensive ones. Dyes, tanning chemicals, cotton, hides and skin are some agro-based raw material inputs into the nation’s textiles and leather industries reported to be imported as local supplies are low (Onwualu 2006). The sector has the potential to grow and achieve competitiveness if there is adequate and sustainable supply of these inputs.

Nigeria is blessed with abundant species of plants capable of yielding dyes. Plant dyes can yield variety of exquisite and interesting colours. Janseen and Cardon (2005) identified a number of 43 local plants with potentials of yielding dyes for fabric colouration which could be found in tropical Africa, including Nigeria yet unexplored. One of such plants includes Roselle.

Roselle (*Hibiscus sabdarifa*) plant, a tetraploid belonging to the family Malvaceae is also known as Sour-sour in Sierra Leone, dab or bissap in Senegal and surrounding countries, oseille de Guinean or Roselle in French, marakwanga in Northern Uganda, Jamaican sorrel or Florida crambery in the Caribean areas (Schippers, 2000). Roselle is also called karkadeh in Arabic (Ali et al., 2005). In Nigeria, Roselle is known as isapa in Yoruba and zobo in Igbo lands. Roselle is a fibre and an ancient crop widely grown in Central and West Africa and South East Asia (Murdock, 1995). Roselle has varieties which differ in colour of calyces ranging from green, red and purple. Roselle is also used for its paper and ornamental values (Schippers, 2000; The Technical Center for Agricultural and rural Co-operation ACP-EU, 2006).

A comprehensive review of different studies of the constituents of different parts of Roselle plant and its phytochemical, pharmacological and toxicological properties has been documented by Ali et al. (2005). Roselle is a rich source of plant polyphenols such as flavonoids and phenolic acids (Ali et al., 2005). A recent study on phytochemical constituents of Roselle calyces extract reveal the following; carotenoid (1.96%), flavonoid (0.02%), lutein (0.03%), polyphenol (0.12%), tannin (0.88%) per 100 g. Roselle calyx contains anthocyanins which are polyphenolic compounds responsible for cyanic colours ranging from salmon pink through red and violet to dark blue of most flowers, fruits, leaves and stems (Peng-Kong, Salmah, Ghazali and Che, 2002), and comprise the largest group of the water-soluble pigments in the plant kingdom (Clydesdale et al., 1990; Owoade et al., 2015).

Raselle is valued in pharmacology in drug and cosmetic productions (Egbujo et al., 2008) Anthocyanins are in recent times regarded as nutraceuticals due to their potential health and possible antioxidant effects, and have been given a potential therapeutic role related to cardiovascular diseases, cancer treatment, inhibition of certain types of virus including the human immunodeficiency virus type 1 (HIV-1), and improvement of visual acuity (Cecchini et al., 2005; Cooke et al., 2005; Talavera et al., 2006 in Owoade et al., 2015). Roselle has also been found useful as food beverage, wine, gel and as food colorant. The production of roselle powder has also been done.

Various studies have been done on Roselle's potentials as refreshing beverages (Clydesdale et al., 1990), food colourants (Hassan and Bakri, 1990), and cosmetics purposes; however, research into fabric colouration using Roselle extract is scarce despite its large anthocyanin property. The organleptic attributes of Roselle dye, including colour hue, value, chroma, odour, texture and evenness of shade on cotton fabric were found to be good and acceptable to consumers. The study on the colourfastness of roselle dyed fabrics is imperative. The focus of this present study was therefore, to analyze fabrics treated with dyes extracted from roselle (*H. sabdarifa*) calyces. Specifically, the study sought to:

1. Extract three different dye extracts from roselle calyces using boiling, steeping and solvent techniques.
2. Mordant samples of cotton (100%), stone silk (60% silk: 40% polyester), and polyester (100%) fabrics with alum mordant, tannic and citric acids dye fixing agents.
3. Dye the mordanted fabrics and no-mordant sample (control) with the roselle extracts using normal dyeing conditions to study their dye ability.
4. Assess the effects of dye extraction methods on the colourfastness of the dyed fabrics (prototypes) to sunlight, washing, perspiration (acid and alkali) and crocking or rubbing.
5. Assess the effects of dye fixatives on the colourfastness of dyed fabrics to sunlight, washing, perspiration (acid and alkali) and crocking or rubbing.

Hypotheses

Three null hypotheses were tested by the study at 0.05 level of significance:

H01: There is no significant difference in the mean rating effects of dye extraction methods and fixatives on the colourfastness of roselle dyed samples of cotton fabrics to sunlight, washing, perspiration and rubbing.
H02: There is no significant difference in the mean rating effects of dye extraction methods and fixatives on the colourfastness of roselle dyed samples of silk fabrics to sunlight, washing, perspiration and rubbing.
H03: There is no significant difference in the mean rating effects of dye extraction methods and fixatives on the colourfastness of roselle dyed samples of polyester fabrics to sunlight, washing, perspiration and rubbing.

MATERIALS AND METHODS

The study adopted completely randomized (CRD) experimental design. Dye extraction and fabric dyeing (prototype development) were done at the University of Nigeria, Nsukka, Enugu State while colourfastness tests were conducted at the International Textiles Industries (ITI), Limited, Lagos State, Nigeria.

Red dry roselle calyces were collected from Ogbete, Enugu. Alum, citric acid, tannic acid, Sodium carbonate (Sal soda) and ferrous sulphate ($FeSO_4$) were collected at Ugo chemical store, UNN. Fabrics studied were cotton (100% natural fibre), stone silk (blend of 60%silk: 40% polyester) and polyester (100% synthetic) fabrics were collected at Enugu. Other materials used include; distilled water, heater, thermometer, rubber hand gloves, Shirley Development Limited (SDLA) America washing machine (auto wash), Thomas Willey milling machine, electronic crock meter, Gray scale, stainless and plastic bowels and spoons, ITI black carbon, transparent glass, ethanol (analytical grade). Their uses were explained following the procedures of the work.

Dye extraction procedures

About 3 kg dried red roselle calyces were further dried under room temperature in the Green House for 40 min at 40°C to facilitate milling. The dried calyces were milled into fine powder using Thomas Willey milling machine.

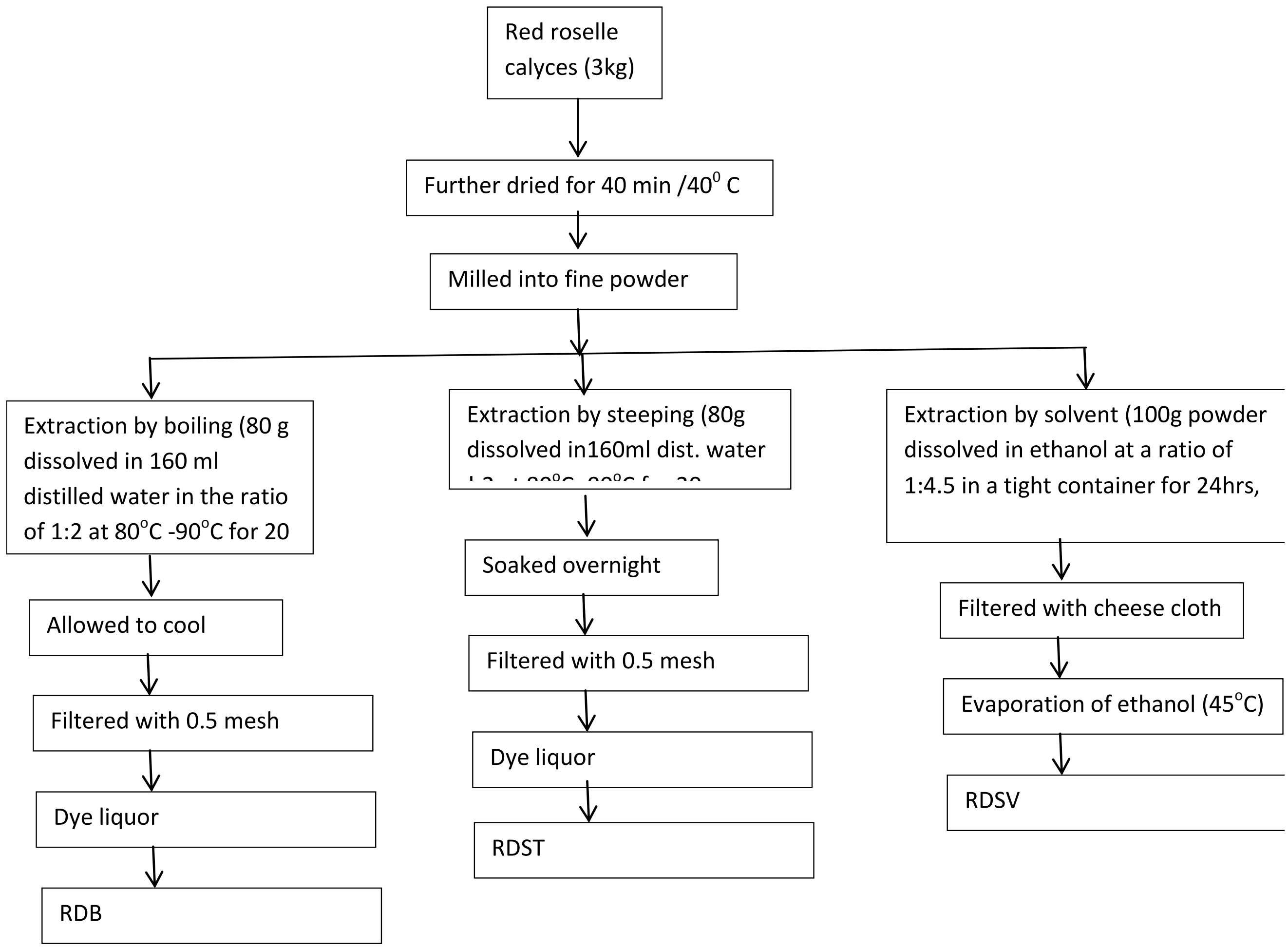

Figure 1. Flow chart for dye extraction from roselle calyces.

Dye extraction by boiling

Extraction of dye from roselle calyces by boiling was done using boiling method as described by Kolender (2003). About 80 g of roselle calyces powder was dissolved in 160ml distilled water in the ratio 1:2 (W/V). It was heated at 80 to 90°C for 20 mins and allowed to cool. The solution was filtered with 0.5 meshes (particle size) to collect the dye liquor and labeled roselle dye extracted by boiling (RDB).

Dye extraction by steeping

About 80 g of roselle calyces powder was steeped in 160ml distilled water in the ratio 1:2 (W/V) and allowed to stand overnight allowing fermentation to take place without heating. The solution was stirred thoroughly and filtered using 0.5 meshes (Particle size) to collect the dye liquor and labeled roselle dye extracted by steeping (RDST).

Dye extraction by solvent

A 100 g roselle calyces powder was dissolved in analytical grade ethanol (98% absolute) in the ratio of 1:4.5 (W/V) in an air tight container. For thorough extraction, the mixture was shaken properly and allowed to stand for 24 h and then filtered with cheese cloth. Ethanol was evaporated to dryness on a rotary evaporator under vacuum at 45°C. Roselle paste extracted was labeled roselle dye extracted by solvent (RDSV) (Figure 1).

Mordanting cotton (100%) fabric samples

Four pieces of cotton fabric samples measuring 24 by 24 (15g) inches each were scoured or washed thoroughly in warm water three times with detergent to remove all sizing. In four different stainless pots containing 1500 ml distilled water and 0.3g sodium carbonate ($NaCo_3$) each, 4g Aluminum sulphate ($AlSO_4$, alum) was dissolved in the first pot, 4g tannic acid was dissolved in the second pot and 4g citric acid in the third. The forth stainless pot containing 0.3g sodium carbonate and 1500 distilled water was not added any dye fixing agent or mordant (control). The four wet scoured cotton fabric pieces were immersed in each of the solution and gently but thoroughly stirred so that the fabrics were opened out in the solution. Each was then heated, held at boil at 80 to 90°C for 1 h and allowed to cool overnight in the solution. The mordanted cotton fabric samples were labeled; alum mordanted cotton (AC), citric acid treated cotton (CC), tannic acid treated cotton (TC), and non-mordant cotton (NC).

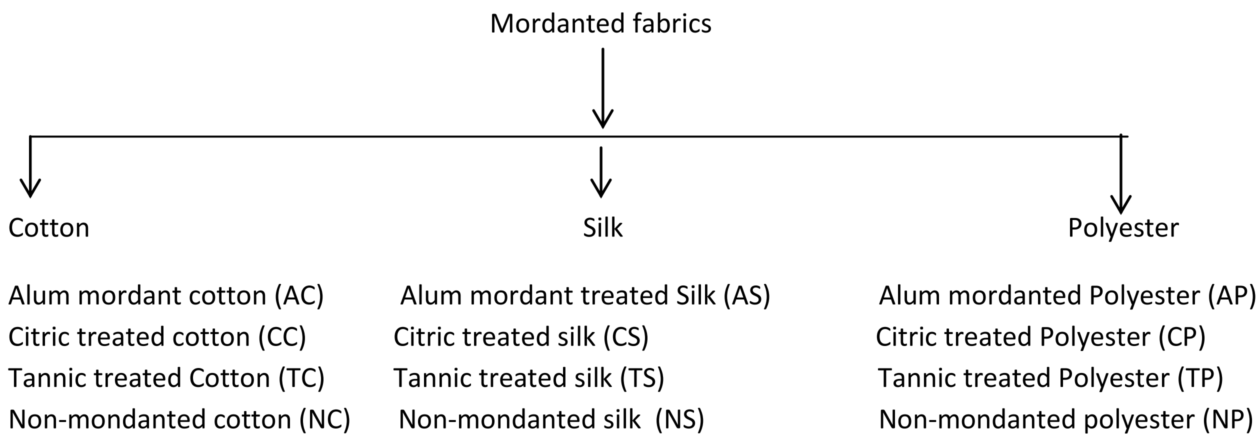

Figure 2. Flow chart for fabric mordanting.

Mordanting stone silk (blend of 60%silk:40% polyester) fabric

Four pieces of stone silk fabric samples measuring 24 by 24 (15 g) each were washed thoroughly in warm water three times with detergent to remove all sizing as in cotton sample above. In four different stainless pots containing 1500 ml distilled water and 0.3g sodium carbonate ($NaCo_3$) each, 4g Aluminum sulphate ($AlSO_4$ alum) was dissolved into the first pot, 4g tannic acid was dissolved into the second pot and 4g citric acid in the third. The forth stainless pot containing 0.3g tartaric acid, 1500 distilled water was not added any dye fixing agent or mordant (control). The four wet scoured stone silk fabric pieces were immersed in each of the solution and gently but thoroughly stirred so that the fabrics were opened out in the solution. They were allowed to stand in the solution overnight without heating. The mordanted fabric samples were labeled: Alum mordanted silk (AS), citric acid treated silk (CS), tannic acid treated cotton (TS), and non-mordant cotton (NS).

Mordanting polyester (100%) fabric samples

Four pieces of polyester fabric samples samples (24" x 24":15g) each were also scoured thoroughly in warm water with detergent three times to desize them. Distilled water (1500ml) was heated and 4g Aluminum Carbonate ($AlCO_3$, alum) and 0.3g Sal soda or sodium carbonate ($NaCo_3$) was thoroughly dissolved in. The four wet scoured polyester fabric samples were immersed separately into each of the pots and gently but thoroughly stirred so that it is opened out in the solution. They were then heated at 80 to 90°C for 1 h and allowed to cool overnight in the solution. They were brought out from the solution drained and labeled thus; alum mordanted polyester (AP), citric acid treated silk (CP), tannic acid treated cotton (TP), and non-mordant cotton (NP) (Figure 2).

Dyeing the mordanted fabrics with the roselle extracts (Developing the prototypes)

Cotton prototypes

Each of the roselle extracts or dye liquor (RDB and RDST) was divided into four portions in different dye pots. About 15 ml additional water was added to the dye bath so the fabric could move freely. The colour of each sample was modified by dissolving 0.5 g ferrous (II) sulphate (Fe_2SO_4) into the dye bath. Each of the alum, citric acid, tannic acid and non mordanted (control) samples of cotton was then immersed separately into a pot containing each of the roselle dye bathes and heated 1 h at 80°C by conventional exhaust dyeing method. Samples were taken out after completion of dyeing. The dyed fabric samples (prototypes) were rinsed to remove excess dyes and dried under a shade. A total of 36 prototype samples made up of cotton (12 prototypes), stone silk (12 prototypes) and polyester (12 prototypes) fabrics, were developed. The dyed prototypes were replicated in triplicates making a total of 108 prototype samples tested for colourfastness.

Colourfastness tests of the prototype fabrics

Fastness to sunlight

Strips of each of the dyed cotton, stone silk and polyester prototypes measuring 2" × 5" were arranged vertically on a flat wooden surface. The fabrics were covered halfway top with ITI industrial black carbon leaving halfway down exposed. All the prototypes were placed behind transparent glass to prevent direct sun rays which might be harmful to the fabric fibres. The specimen was made to stand outside on normal day sunlight for 72 h according to ISO, 105/A03: 1993 after which the samples were collected for rating the degree of fade.

Fastness to washing

Using the Shirley Development Limited, America (SDLA) auto wash electronic washing machine according to ISO, 1993 each of the prototype samples (2" × 5") was covered with plain white fabric, tightly tied and fed into the 4-rack testing pots bit by bit at a temperature of 32 ± 2°C for 45 min. The samples were brought out, allowed to cool and untied. The extent of staining was checked off using the Gray scale and triplicate samples recorded with FTRS.

Fastness to acid perspiration

About 5.5 g sodium chloride was dissolved in a solution of 1 L distilled water and 5 g disodium hydrogen orthophosphate dodecahydrate ($NaH_2PO_4.12\ H_20$) with 5 g histidine. 0.1 N acetic acid was dissolved into the solution to bring the acidic pH to 5.5. The different prototype samples (2" × 5" each) were dipped into the solution, allowed to dry and covered with plain white fabric and tied

Table 1. Colourfastness tests and range of means for taking decisions.

Colourfastness	1.00-1.90	2.00-2.90	3.00-3.90	4.00-4.90	5.00-5.90
Sunlight	Excessive fade	Much fade	Slight fade	Very slight fade	No fade
Dry rubbing	Excessive fade	Much fade	Slight fade	Very slight fade	No fade
Washing	Excessive stain	Much stain	Slight stain	Very slight stain	No stain
Acid Perspiration	Excessive stain	Much stain	Slight stain	Very slight stain	No stain
Akali perspiration	Excessive stain	Much stain	Slight stain	Very slight stain	No stain

strongly as in wash fastness tests. They were fed into the oven and allowed to stay for 4 h at the temperature 32 ± 2°C. The prototypes were allowed to cool; untied and the extent of staining were checked off with Gray scale and recorded with FTRS.

Fastness to alkali perspiration

This test was done using similar procedure with acid perspiration test but alkaline medium was achieved by dissolving 0.I N Sodium hydroxide (NaOH) in 1 L distilled water to bring the pH of the solution to 8.0. The prototype samples were treated as in acidic perspiration test, rated and results recorded with FTRS.

Fastness to crocking/dry rubbing

The different prototypes (2” × 5”) were covered with plain white test cloth, tightly tied and fed into the nozzles of the electronic crock meter machine. The machine scrubbed each sample 15 times for 5 min. The samples were then brought out, untied and the extent of staining was recorded.

Fastness index of the prototypes

Two types of instruments were used to record the fastness index of the prototype fabrics. They include Gray scale, a standardized scale used to rate the degree of colour fade or stain of one prototype fabric due to sunlight, washing, perspiration (acid and alkali) and rubbing or crocking. Fastness test rating scale (FTRS) was developed by the researchers to record the triplicate mean results of the fastness tests done with Gray scale. The fastness and range of means for taking decisions are shown in Table 1.

Data analysis

General linear model for factorial experiment was used for data analysis using statistical package for social sciences version 16. Any sample with mean score 3 or above was regarded as a colourfast sample whereas any sample with score below mean 3 was regarded as a non-fast sample. Analysis of variance (ANOVA) was used to test three null hypotheses while Scheffe’s post hoc test compared the treatment means at 0.05 level of significance.

RESULTS

1. Roselle calyces extracts showed reasonable colourfastness property to rubbing/crocking, sunlight and acid perspiration on cotton prototypes but were generally non-fast to washing and alkali perspiration (Table 2).
2. The effects of dye extraction procedures did not differ significantly ($p>.05$) on the colourfastness of cotton prototypes to sunlight ($p=0.33$), washing ($p=0.39$), alkali perspiration ($p=0.06$) and dry rubbing (0.59) (Table 3).
3. The effects of mordant/dye fixatives did not significantly differ ($p>0.5$) on the colourfastness of cotton prototypes to washing ($p=0.95$) and rubbing or crocking ($p=0.68$), but differed significantly ($p<0.05$) on colourfastness to sunlight ($p=0.00$), acid perspiration ($p=0.00$) and alkali ($p=0.00$). Tannic acid and alum mordant were the source of difference (Table 3).
4. Roselle calyces extracts dye potentials on stone silk and polyester prototypes were generally poor. They exhibited much to excessive fade and stain of colour and poor fastness to all parameters tested except rubbing fastness (Tables 4 and 6)
5. The effects of dye extraction methods did not differ significantly ($p>0.05$) on the stone silk and polyester prototypes colourfastness parameters assessed. Though the effects of dye fixatives significantly differed ($p<0.05$) with tannic acid and alum mordant the source of difference, they were not able to improve the stability or colourfastness of the prototypes (Tables 5 and 7).

Data in Table 1 reveals that six out of 12 cotton prototypes were fast to sunlight. They include - alum and tannic acid mordanted prototypes (RDB-AC, RDB -TC, RDST -AC, RDST - TC, RDSV-AC and RDSV-TC). Alum and tannic acid prototypes dyed with roselle extracted by steeping had the highest fastness index (3.33±0.000). They exhibited slight fade in colour when exposed to sunlight as shown by their mean ratings (3.00±0.000 to 3.33±0.000). For washfastness of cotton prototypes, only RDST-AC and RDST-TC prototypes were fast (3.33±0.000 and 3.00±0.00, respectively) with RDST-AC rating higher. They showed slight stain or bleeding of colour in washing while others exhibited much to excessive stain (2.67±0.58 to 1.67±0.58). Three prototypes were fast to acid perspiration (3.00±0.00 each). They include; RDB-AC, RDST-AC and RDSV-AC. They showed moderate fastness and slight change or fade in colour. None of the prototypes was fast to alkali perspiration as none was rated up to the mean cut off. All the prototypes were fast to dry rubbing except RDSV- NC (2.33±0.155) and RDSV- CC. The fastness ratings range from very slight to slight stain, implying good and fair colourfastness.

Table 2. Mean ratings of the colourfastness of cotton Prototypes to sunlight, washing, acid /alkali perspiration and rubbing.

Dye extraction	Dye fixative	Sunlight		Washing		Acid		Alkali		Rubbing	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
RDB	AC	3.000	0.000	2.666	0.557	3.000	0.000	2.000	0.000	3.667	0.577
	CC	2.667	0.577	1.667	0.577	2.000	0.000	1.667	0.577	3.333	0.577
	NC	2.000	0.000	1.667	0.577	2.000	0.000	1.000	0.000	3.000	0.000
	TC	3.000	0.577	2.667	0.577	2.000	0.000	2.000	0.000	4.000	0.000
RDST	AC	3.333	0.000	3.333	0.000	3.000	0.000	2.000	0.000	4.000	0.000
	CC	2.333	0.577	2.333	0.577	2.000	0.000	1.667	0.577	3.333	0.557
RDST	NC	2.000	0.577	1.667	0.577	2.000	0.000	1.000	0.000	3.000	0.000
	TC	3.333	0.000	3.000	0.000	3.000	0.000	2.333	0.000	3.667	0.577
RDSV	AC	3.000	0.000	2.000	0.577	2.000	0.577	2.000	0.577	3.667	0.577
RDSV	CC	2.333	0.577	2.333	0.577	2.000	0.577	2.000	0.000	2.333	0.155
RDSV	NC	2.000	0.000	1.667	0.577	2.000	0.577	1.333	0.577	2.333	0.155
RDSV	TC	3.333	0.000	2.667	0.577	2.000	0.577	2.000	0.000	3.667	0.000

Key: X-Mean SD-Standard Deviation RDB-AC- Alum mordanted cotton fabric treated with roselle dye extracted by boiling. RDB-CC - Citric acid treated cotton fabric dyed with roselle extracted by boiling. RDB-NC - Non mordanted cotton fabric treated with roselle dye extracted by boiling. RDB-TC - Tannic acid treated cotton fabric dyed with roselle extracted by boiling. RDST-AC- Alum mordanted cotton fabric treated with roselle dye extracted by steeping. RDST-CC - Citric acid treated cotton fabric dyed with roselle extracted by steeping. RDST-NC - Non mordanted cotton fabric treated with roselle dye extracted by steeping. RDST-TC - Tannic acid treated cotton fabric dyed with roselle extracted by steeping. RDSV-AC- Alum mordanted cotton fabric treated with roselle dye extracted by solvent. RDSV-CC - Citric acid treated cotton fabric dyed with roselle extracted by solvent. RDSV-NC - Non mordanted cotton fabric treated with roselle dye extracted by solvent. RDSV-TC - Tannic acid treated cotton fabric dyed with roselle extracted by solvent.

Table 3. F-values and Scheffe's post hoc homogenous main effects of dye extraction methods and dye fixatives on the colourfastness of cotton fabric prototypes to sunlight, washing, acid /alkali perspiration and rubbing.

Treatment	Cotton prototypes	colourfastness to sunlight	Washing	Acid	Alkali	Rubbing
Dye extraction	RDB	2.50^a	2.17^a	2.25^a	1.67^a	3.50^a
	RDST	2.75^a	2.42^a	2.25^a	2.00^a	3.50^a
	RDSV	2.67^a	2.08^a	2.25^a	1.67^a	3.25^a
		p=0.328	p=0.291	-	p=0.06	p=0.059
Dye fixative	Alum	3.00^a	2.87^a	3.00^a	2.11^a	3.78^a
	Citric	2.33^b	2.00^{ab}	2.00^b	1.78^a	3.33^{ab}
	Control	1.89^b	1.58^b	2.00^b	1.11^b	2.98^b
	Tannic	3.33^a	2.87^a	2.00^b	2.11^a	3.78^a
		p=0.000	p=0.953	p=0.000	p=0.000	p=0.678

Key: Means for groups in homogenous subsets are displayed. Values are means of triplicate determinations. Means with the same superscript letter grades are not significantly different from each other at $P \leq 0.05$ using Scheffe's test.

H01: There is no significant difference in the mean rating effects of dye extraction methods and dye fixatives on colour fastness of roselle dyed cotton Prototypes to sunlight, washing, acid and alkali perspiration.

Scheffe's post hoc multiple comparisons interaction main effects of dye extraction method indicate that though steeping extraction technique rated highest (2.75), its effects are not significantly different from those of boiling and ethanol extraction in improving the prototypes fastness to sunlight (p=0.33), Washing (p=0.29), alkali perspiration (p=0.06), and rubbing (p=0.59), respectively.

For dye fixative interaction main effects, tannic acid and alum had comparable positive effects significantly

Table 4. Mean ratings of the colourfastness of stone silk prototypes to sunlight, washing, acid /alkali perspiration and rubbing.

Dye extraction	Dye fixative	Sunlight		Washing		Acid		Alkali		Rubbing	
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
RDB	AS	2.000	0.000	2.000	0.000	2.000	0.000	1.889	0.192	3.333	0.557
	CS	1.667	0.577	2.000	0.000	2.000	0.000	2.000	0.000	2.667	0.577
	NS	1.667	0.557	1.333	0.577	1.000	0.557	2.000	0.000	2.333	0.577
	TS	2.000	0.000	2.000	0.000	2.000	0.000	2.000	0.000	3.333	0.669
RDST	AS	2.000	0.000	2.000	0.000	2.000	0.000	2.000	0.000	3.333	0.577
	CS	1.667	0.577	2.000	0.000	1.000	0.577	2.000	0.000	3.000	0.000
RDST	NS	1.667	0.557	1.333	0.557	2.000	0.000	2.000	0.000	2.667	0.557
	TS	2.000	0.000	2.000	0.000	2.000	0.000	2.000	0.000	3.333	0.515
RDSV	AS	2.000	0.000	2.000	0.000	2.000	0.000	2.000	0.000	3.667	0.577
RDSV	CS	2.000	0.000	2.000	0.000	1.333	0.557	2.000	0.000	3.000	0.000
RDSV	NS	2.000	0.000	1.667	0.577	1.000	0.557	2.000	0.000	2.667	0.577
RDSV	TS	2.000	0.000	2.000	0.000	2.000	0.000	2.000	0.000	3.667	0.622

Key: X-Mean SD-Standard Deviation RDB-AS- Alum mordanted stone silk fabric treated with roselle dye extracted by boiling. RDB-CS - Citric acid treated stone eilk fabric dyed with roselle extracted by boiling. RDB-NS - Non mordanted stone silk fabric treated with roselle dye extracted by boiling. RDB-TS - Tannic acid treated stone silk fabric dyed with roselle extracted by boiling. RDST-AS- Alum mordanted stone silk fabric treated with roselle dye extracted by steeping. RDST-CS - Citric acid treated stone silk fabric dyed with roselle extracted by steeping. RDST-NS - Non mordanted stone silk (control) fabric treated with roselle dye extracted by steeping. RDST-TS - Tannic acid treated stone silk fabric dyed with roselle extracted by steeping. RDSV-AS- Alum mordanted stone silk fabric treated with roselle dye extracted by by solvent. RDSV-CS - Citric acid treated stone silk fabric dyed with roselle extracted by solvent. RDSV-NS - Non mordanted stone silk fabric treated with roselle dye extracted by solvent. RDSV-TS - Tannic acid treated stone silk fabric dyed with roselle extracted by solvent.

Table 5. F-values and Scheffe's post hoc homogenous main effects of dye extraction methods and dye fixatives on the colourfastness of silk prototypes to sunlight, washing, acid /alkali perspiration an rubbing

Treatment	Silk Prototype	Colourfastness to sunlight	Washing	Acid	Alkali	Rubbing
Dye extraction	RDB	1.83^{a}	1.83^{a}	2.00^{a}	1.00^{a}	2.92^{b}
	RDST	1.83^{a}	1.83^{a}	2.00^{a}	1.00^{a}	3.08^{ab}
	RDSV	1.83^{a}	1.92^{a}	2.00^{a}	1.33^{a}	3.25^{ab}
		p=1.00	p=0.720	-	-	p=0.219
Dye fixative	Alum	2.00^{a}	2.00^{a}	2.00^{a}	1.11^{a}	3.44^{a}
	Citric	1.67^{a}	2.00^{a}	2.00^{a}	1.11^{a}	2.89^{ab}
	Control	1.67^{a}	1.44^{b}	2.00^{a}	1.11^{a}	2.56^{b}
	Tannic	2.00^{a}	2.00^{a}	2.00^{a}	1.11^{a}	3.44^{a}
		p=0.141	p=0.001	-	-	p=0.053

Key: Means for groups in homogenous subsets are displayed. Values are means of triplicate determination. Means with the same superscript letter grades are not significantly different from each other at P≤0.05 using Scheffe's test.

different from citric acid and no mordant in their effects on the fastness to sunlight (p=0.00). There was no significant difference in effects of dye fixatives on washfastness (p=0.95) and rubbing fastness (p=0.68) of the cotton prototypes but significant differences existed on acid (p=0.00) and alkali (p=0.00) perspiration of the silk prototypes. Alum was the source of difference for acid perspiration which improved the fastness more than others. For alkali perspiration, the effect of alum was comparable to those of tannic and citric acid dye

Table 6. Mean ratings of the colourfastness of polyester Prototypes to sunlight, washing, acid /alkali perspiration and rubbing.

Dye	Dye fixative	Sunlight		Washing		Acid		Alkali		Rubbing	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
RDB	AP	2.000	0.000	2.000	0.000	2.000	0.000	1.000	0.000	3.000	0.000
	CP	1.667	0.000	2.000	0.000	2.000	0.000	1.000	0.000	3.000	0.000
	NP	1.000	0.577	1.333	0.557	1.000	0.557	1.000	0.000	2.333	1.16
	TP	2.000	0.000	2.000	0.000	2.000	0.000	1.000	0.000	3.000	0.000
RDST	AP	1.667	0.577	2.000	0.000	2.000	0.000	1.000	0.000	3.000	0.000
	CP	1.333	0.557	2.00	0.000	1.000	0.000	1.000	0.000	3.000	0.000
RDST	NP	1.333	0.557	1.333	0.557	2.000	0.000	1.000	0.000	2.333	1.16
	TP	1.667	0.000	2.000	0.000	2.000	0.000	1.333	0.577	3.333	0.000
RDSV	AP	2.000	0.000	2.000	0.000	2.000	0.000	1.333	0.577	3.000	0.000
RDSV	CP	2.000	0.000	2.000	0.000	1.333	0.557	1.333	0.577	3.000	0.000
RDSV	NP	2.000	0.000	1.667	0.557	1.000	0.557	1.333	0.577	2.333	0.577
RDSV	TS	2.000	0.000	2.000	0.000	2.000	0.000	1.333	0.577	3.000	0.000

Key: X-Mean SD-Standard Deviation RDB-AP- Alum mordanted cotton fabric treated with roselle dye extracted by boiling. RDB-CP - Citric acid treated cotton fabric dyed with roselle extracted by boiling. RDB-NP - Non mordanted cotton fabric treated with roselle dye extracted by boiling. RDB-TP - Tannic acid treated cotton fabric dyed with roselle extracted by boiling. RDST-AP - Alum mordanted cotton fabric treated with roselle dye extracted by steeping. RDST-CP - Citric acid treated cotton fabric dyed with roselle extracted by steeping. RDST-NP - Non mordanted cotton fabric treated with roselle dye extracted by steeping. RDST-TP - Tannic acid treated cotton fabric dyed with roselle extracted by steeping. RDSV-AP- Alum mordanted cotton fabric treated with roselle dye extracted by solvent. RDSV-CP - Citric acid treated cotton fabric dyed with roselle extracted by solvent. RDSV-NP - Non mordanted cotton fabric treated with roselle dye extracted by solvent. RDSV-TP - Tannic acid treated cotton fabric dyed with roselle extracted by solvent.

Table 7. Scheffe's post hoc homogenous main effects of dye extraction methods and dye fixatives on the colourfastness of polyester prototypes to sunlight, washing, acid /alkali perspiration and rubbing.

Treatment	Polyester prototypes	Colourfastness to Sunlight	Washing	Acid	Alkali	Rubbing
Extraction method	RDB	1.92^{a}	1.67^{ab}	2.00^{a}	1.00^{b}	2.83^{a}
	RDST	2.00^{a}	1.50^{b}	2.00^{a}	1.00^{b}	2.83^{a}
	RDSV	1.92^{a}	1.92^{ab}	2.00^{a}	1.33^{a}	$2.83^{awashing}$
		p=0.781	p=080	-	p=0.320	p=1.00
Dye fixative	Alum	2.00^{a}	1.89^{a}	2.00^{a}	1.11^{a}	3.00^{a}
	Citric	2.00^{a}	1.56^{a}	2.00^{a}	1.10^{a}	3.00^{a}
	Control	1.69^{a}	1.44^{a}	2.00^{a}	1.11^{a}	2.33^{b}
	Tannic	2.11^{a}	1.89^{a}	2.00^{a}	1.11^{a}	3.00^{a}
		p=0.050	p=0.080	-	p=1.000	p=0.003

Key: Means for groups in homogenous subsets are displayed. Values are means of triplicate determination. Means with the same superscript letter grades are not significantly different from each other at P≤0.05 using Scheffe's test.

fixatives.

Decision rule: The null hypothesis which states there are no significant differences in mean rating effects of dye extraction methods and dye fixatives on fastness of all cotton prototypes to sunlight, washing, acid/alkali perspirations and rubbing are accepted (p>0.05) for dye extraction methods since p-values are greater (p>0.05) than 0.05 probability level. For dye fixatives, null hypothesis was rejected for effects of dye fixatives on fastness of cotton prototypes to sunlight, acid and alkali perspirations (pF<0.05) but accepted for washing and rubbing. P-values are greater than ($p>0.05$) significant level.

Data in Table 3 indicate that majority of the stone silk prototypes were fairly fast only to dry rubbing but were non fast to other silk fastness parameters. Silk prototypes that showed slight stain included RDB-AS (3.33), RDB-TS (3.33±.56), RDST-AS (3.00.58), RDST-CS (3.00±.51), RDST-TS (3.33), RDSV-AS (3.67±.57), RDSV-CS (3.00±.000), RDSV-TS (3.67±.62). All the non mordanted prototypes showed much fades or stain to the different fastness parameters as shown in their low mean ratings.

H02: There is no significant difference in the mean rating effects of dye extraction methods and dye fixatives on colourfastness of roselle dyed cotton Prototypes to sunlight, washing, and acid and alkali perspiration.

The effects of steeping extraction was not significantly different from ethanol and boiling techniques but were comparable to each other in improving the rubbing fastness of silk prototypes. There were no significant differences in the effects of ethanol dye extraction on the colourfastness of silk prototypes to sunlight ($p=1.00$), washing ($p=7.20$), alkali ($p=1.00$), rubbing ($p=0.22$). Though tannic acid and alum had more positive effects on the fastness of silk prototypes, their effects are not significantly different from citric acid and no mordant on fastness to sunlight ($p=0.141$), rubbing ($p=0.053$). Non mordanted silk prototypes were the source of the difference ($p=0.001$) on prototypes fastness to washing. They exhibited the poorest washingfastness ratings.. The P and F- values of acid and alkali fastness of silk prototypes could not be computed with alpha =0.050 of the general linear model for factorial experiments of the SPSS for social sciences version 16.0 used since the means of the different prototypes were homogenous indicating no significant differences.

Decision: The null hypothesis which states there are no significant differences in mean rating effects of dye extraction methods and dye fixatives on fastness of silk prototypes to sunlight, washing, acid/alkali perspirations and rubbing are accepted ($F>0.05$) for dye extraction methods since F-values are greater ($F>0.05$) than 0.05 probability level. For dye fixatives, null hypothesis was rejected for effects of dye fixatives on fastness of silk prototypes to washing, acid and alkali perspirations ($F<0.05$) but accepted for sunlight and rubbing ($p>0.05$). F-values are greater than ($F>0.05$) .05 significant level.

Data on colour fastness of polyester prototypes revealed general poor colourfastness to sunlight, washing, acid and alkali. This is seen in the very low mean ratings of the fastness parameters. Prototypes showed slight fade and stain to rubbing fastness in nine out of 12 prototypes as seen in Table 6.

H03: There is no significant difference ($p\geq0.05$) in the mean rating effects of dye extraction methods and dye fixatives on colour fastness of polyester prototypes to sunlight, washing, acid and alkali perspiration and rubbing.

The Scheffe's post hoc homogenous subsets of dye extraction effects on colour fastness of polyester prototypes reveal no significant differences in prototypes fastness to sunlight ($p=0.78$), washing ($p=0.08$), alkali perspiration ($p=0.32$) and rubbing ($p=1.00$) but significant differences exist in mean rating effects of dye fixatives on prototypes' fastness to sunlight ($p=0.05$) and rubbing ($p=0.00$) but for washing ($p=0.08$) and alkali ($p=1.00$), there were no significant differences ($p>0.05$).

Decision: The null hypothesis stating no significant difference ($p\geq0.05$) is accepted in instances of effects of all dye extraction methods, and dye fixatives effects on prototypes fastness to washing and rubbing fastness. The p-values are greater ($p>0.05$) than 0.05 probability level but rejected for dye fixatives effect on polyester prototypes to sunlight and rubbing since p-values are less ($p<0.05$) than 0.05 probability level.

DISCUSSION

The study assessed the effects of three dye extraction procedures (boiling, steeping and ethanol) on the colour fastness of 100% cotton, silk (60% silk: 40% polyester) and 100% polyester fabrics mordanted with three dye fixatives (alum, tannic and citric acids and no mordant control) to sunlight, washing, perspiration and rubbing or crocking. Findings revealed that cotton prototypes especially those mordanted with alum and tannic acid treated with dyes extracted by steeping exhibited very slight stain in colour when subjected to dry rubbing, slight fade when exposed to normal sun rays for 72 h and slight stain when subjected to acid medium perspiration stressor. This finding indicates that roselle calyces extract has dye potential. This result is not surprising as phytochemical constituents of roselle reveal very high anthocyanin contents as high as 25 g kg^{-1} which is responsible for its red colour (Owoade et al., 2015). The dye ability and stability of a dye on fabric is also determined by dyeing procedure adopted. When a textile material is dyed by a conventional exhaust procedure (gradual heating of the dye bath by a dwell time at the temperature), the dyeing cycle involve three stages: transportation of the dye through the bulk of solution of the dye bath to the surface of the fibre; absorption of the dye molecule from the surface to the interior of the fibre; diffusion of the dye molecules from the surface to the interior of the fibre, How rapidly and evenly stage (1) proceeds depends on the state of heating, and speed of replacement of dye liquor at the fibre surface (Finar, 1973). The frequency of contact per unit time between the material and fresh liquor affects the rate of dyeing and the degree of levelness obtained. Stage II according to Finar is virtually instantaneous. In stage III, the dye originally adsorbed only on the fibre surface penetrate in the interior of the fibre and redistributes itself evenly throughout the fibre cross-section. This phase is dependent upon both time and temperature but is unaffected by the speed of liquor circulation. Migration starts at the same time as diffusion and dye molecule moving from sites with a high dye concentration until an even dye distribution is finally achieved. This may offset any local concentration differences in the material which may have formed at the early stages. The rate of migration varies with the type of dye used and application temperature.

The analysis of variance (ANOVA) results on effects of dye extraction technique on the colourfastness of cotton prototypes indicated no significant difference ($p>0.05$) at 0.05 level of significance. This implies that steeping, ethanol and boiling (temperature not exceeding 90°C) are comparable and did not alter the stability or fastness of the dye. This finding is in line with the findings of Mady et al. (2001), who studied the impact of extraction procedure (cold or hot extraction with or without pasteurization) on the kinetics/stability of anthocyanin and colour degradation of roselle extracts during storage at 4 to 45°C. The results showed that anthocyanin content and initial colour were not modified but the extraction conditions especially temperature greatly affected the stability of the extracts during storage. However, sublimation study on roselle dyed cotton fabric is imperative to investigate the colour degradation during storage.

For the effects of dye fixatives, tannic acid and alum mordant were found to improve the fastness of the prototypes more than citric acid and the control (non mordanted) prototypes. This finding supports those of Ali et al. (2010) and Kulkarni et al. (2011). They agreed that attachment of mordants to dyes is by means of a covalent bond with a hydroxyl group and coordinate bond with another oxygen (the electron donor). In each case, double bonded oxygen and a hydroxyl group (or carboxyl group) are involved. The covalent bond forms between the hydroxyl oxygen and the metal while the coordinate bond forms between the double bonded oxygen and the metal (Llewellyn, 2000), thus bringing about stability of dye and fibre. The high fastness ratings of cotton prototypes to sunlight could be attributed to the substitution pattern of dyes which plays vital role in determining their light fastness and the formation of a complex with transition metal thereby protecting the chromophore of the dye from photolytic degradation (Jothi, 2008). This is the case with prototypes that showed good light fastness.

The colourfastness of stone silk and polyester prototypes to sunlight, washing and perspiration (acid and alkali), was generally poor as seen by their mean ratings in Table 2. The only fastness property which alum and tannic acid mordanted stone silk and polyester prototypes showed slight stain of colour was crocking/rubbing fastness. However, the finding on very poor fastness of silk and polyester prototypes is contrary to $Len_{72}88$ (2009), who affirmed that mordant could be used to improve the stability of dyes on fabrics. Though a fabric may not be colourfast in all circumstances, stability of a dye in fabric to only one fastness parameter cannot qualify a substance to be a good dye. This finding supports Finar (1973), who emphasized that most natural dyes have more affinity to natural fibres while synthetic dyes do better in synthetic fibres. The fibre content of locally available stone silk fabric used in this study comprising 60 percent silk and 40 percent purely synthetic polyester had probably, explained the reason for the non-fastness of stone silk. Moreover, the rationale for selecting polyester fabric for this study was based on the fact that application of a natural dye to a type of fibre (natural or synthetic) with which dye does not combine readily can sometimes be improved by using a mordant as suggested by Finar (1973).

The poor fastness of stone silk and polyester prototypes in this study failed to agree with Finar (1973), while the finding on alum mordanted and tannic acid treated cotton prototypes worked out with roselle dye. It could be observed that all the P-values for acid perspiration fastness of cotton, silk and polyester prototypes were not displayed. Subsets could not be computed with alpha = 0.05, indicating homogeneity of subsets as shown in Table 2. The null hypothesis which states there is no significant difference in mean rating effects of dye extraction methods and dye fixatives on fastness of all prototypes to acid perspiration are accepted.

Conclusion

It could be deduced from the present study that roselle calyces extract has dye potential on cotton fabric when mordanted with alum or treated with tannic acid since prototypes showed fastness to rubbing, sunlight and acid perspiration with. Though steeping extraction method rated highest its mean effects were not significantly different from boiling and ethanol solvent in improving the colourfastness of cotton fabric prototypes to sunlight, rubbing, washing and alkaline perspiration. Stone silk and polyester fabrics treated with roselle dyes showed poor fastness to parameter except rubbing which alone cannot qualify it to be a good dye. It is concluded therefore that with good and acceptable organoleptic attributes, roselle dye could be a veritable colourant for fabric colouration for textile industries, dyers, home makers, teachers and students in educational institutions and other dye utilizing sectors if improved.

RECOMMENDATIONS

The following recommendations are made:

1. The effects of different ratios of alum and tannic acid or other non-harmful but human and eco friendly mordant on the colourfastness of fabrics dyed with roselle calyces extract or other natural dyes should be studied by students and teachers in related fields who either produce or utilize dyes. This will contribute to ensuring sustainable supply of dyes for both private and commercial dyeing purposes.
2. Follow up study should be carried out to investigate the sublimation and shelf life properties of the dyed fabrics by teachers and their students or textile chemist.
3. The large scale textiles and clothing industries should through their textile chemist find ways of exploring and improving the quality of natural dyes for maximum utilization.
4. Traditional dyers, home makers, small and medium scale entrepreneurs should explore plant dyes from the environment not only for economic gains but for the preservation of the prestigious traditional dyeing arts and crafts.

Conflict of Interests

The authors have not declared any conflict of interests.

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Improving the competitiveness of dairy production via value chain approach: The case of Lemu-Bilbilo district in Arsi highlands of Ethiopia

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This study was initiated with the purpose to assess the dairy value chain in Lemu-Bilbilo district of Arsi zone, Ethiopia. This study made use of both qualitative and quantitative data collected from primary and secondary sources. Different Participatory Rural Appraisal (PRA) techniques were utilized to collect primary data in July 2012. The dairy value chain in Lemu-Bilbilo district involves six distinct value adding activities including input supply, production, gathering, processing, transportation and retail trading. The proportion of milk off-take rate by dairy producers was only 15%. Some of the challenges for dairy production at input, production, processing and marketing stage include low quality and poor timeliness of Artificial Insemination (AI) service, information gap on credit services, feed shortage, lack of awareness and knowledge regarding improved feed formulation, unavailability of government and private ranches and multiplication centers for the supply of improved dairy animals. Therefore, to create knowledge based commercial dairy development in the area, there is an urgent need to encourage the introduction and development of improved forage seed, organize recurrent training for dairy producers on improved forages, feed conservation, formulation and feed preservation techniques.

Key words: Dairy; market channel; gross margin; forage; artificial insemination.

INTRODUCTION

The global production, trade and consumption of livestock products in developing countries have increased rapidly in the last two decades and are expected to continue to rise (Delgado et al., 1999; Delgado 2003; Hall et al., 2004). This trend has been termed as the ‘livestock revolution’ (Delgado et al., 1999). The livestock revolution has brought both opportunity as well as risk to smallholders. Farmers would benefit from the created market opportunities arising from change in consumption habits of consumers. At the opposite extreme, consumers have started to attach more weight to the safety and standardization of dairy products than ever before.

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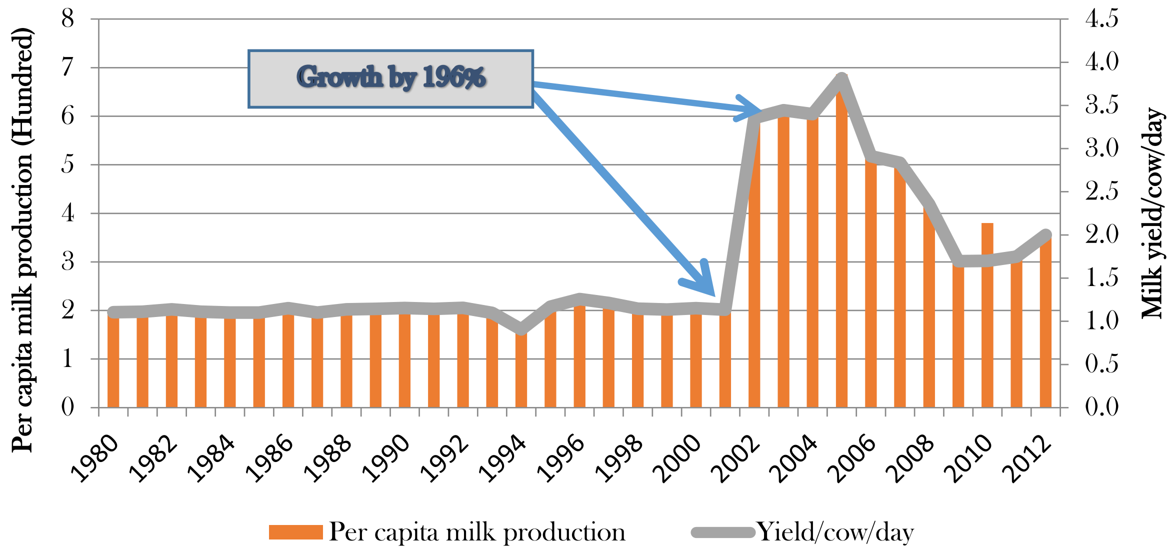

Figure 1. Per capita milk production and milk yield in Ethiopia (1980-2012). Source: FAOSTAT (2014).

Consumers are demanding food products with certain characteristics, such as products perceived to be safe, healthy, and convenient or produced in ways that are beneficial to the environment and take animal welfare and equitable labor concerns into consideration (Hall et al., 2004). According to Negassa (2009), small dairy farmers are also facing threats of losing traditional domestic market outlets. This is because an increasing number of urban consumers might depend on supermarkets rather than traditional markets as their main food sources. All these issues pointed out the necessities for the existence and functioning of a vibrant dairy sector whereby every player in the supply chain are aware of the standards and quality traits requirement of end-users. In order to capitalize the created opportunity and respond to the dynamic consumer demand, small-scale livestock farmers need to get appropriate technical and policy support.

Ethiopia holds large potential for dairy development due to its large livestock population and the favorable climate for improved, high-yielding animal breeds (Mohammed et al., 2004). Milk represents an important livestock product and makes a significant contribution to the nutrition as well as income of the livestock owner. In Ethiopia, the whole fresh cow milk production trends have been increasing since 2000. The country produced 4, 3.3 and 3.8 million liters of cow milk in 2010, 2011 and 2012, respectively (FAO, 2014). Per capita milk production as well as milk yield/cow/day was stagnant for four decades (1960 to 2000). During these years, on average the milk production was 200 L/cow/annum and 1 L/day/cow. These figures arrived by taking into account the 6 months lactation length of a milking cow in Ethiopia. However, this trend have shown significant improvement and twist since 2002. Milk yield has improved substantially from 1.1 L in 2001 to 3.4 lites/cow/day in 2002 indicating an improvement by 196%. That is the per capita milk production increased from 204 L per cow per annum in 2001 to 604 L/cow/year in 2002 (Figure 1). Increase coverage of livestock extension service, use of improved inputs (improved breeds and feed) and introduction of market liberalization policy in early 1990s have jointly contributed to the dramatic growth of the dairy sector. Although the performance of the sector so far are positive when compared to the past, the historical performance of the dairy sector in Ethiopia has been disappointing given its immense potential. Therefore, interventions targeting improvement of the traditional dairy sector are crucial for the development of the livestock sub-sector. This in turn needs identification of root causes for the slow development of the dairy sector and specification of leverage points that could be used as entry points to bring about the desired developments in the sector. This study is initiated to assess the dairy value chain of Lemu-Bilibilo district of Arsi zone with the purpose of identifying major constraints and opportunities in the milk and milk products value chain. The results from this study can provide points of entry for research, policy and development interventions to revitalize the dairy sector of the study area. The rest of the article is organized as follows. Section two describes the data source and analysis approaches followed in the study. Section three presents results of the study. Finally, section four gives recommendations of the study.

METHODOLOGY

The study area

The study was conducted in Lemu-Bilibilo district, located in Arsi

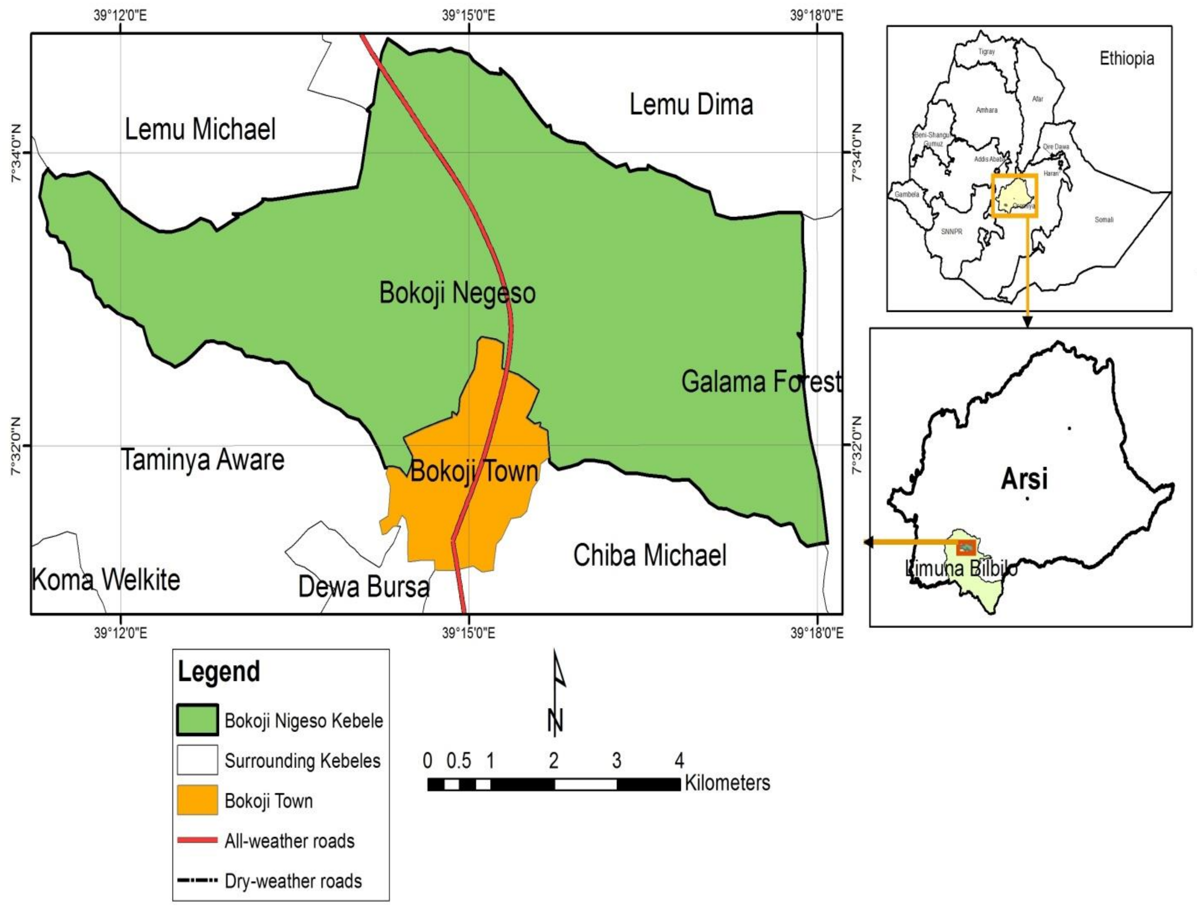

Figure 2. Map of the study area. Source: GIS unit of Ethiopian Institute of Agricultural Research (EIAR)

zone, Oromia Regional State of Ethiopia. The study district was purposively selected based on its dairy production potential as well as its inclusion into the Agricultural Growth Program (AGP). This area is part of the former Bekoji district and located about 235 km southeast of Addis Ababa. The area is characterized as bimodal rainfall pattern with yearly average rainfall of 940 mm. The average annual temperature ranges from 6°c to 26°c. This area is part of the highland area delineated as a dairy shed district due to its comparative potential for improved dairy production. The major crops grown in the area are malt and food barley, fababean, field pea and wheat. In the past, the study area benefited from sustained developmental effort by the Chilalo Agricultural Development Unit (CADU) and Arsi Rural Development Unit (ARDU) in the late 1970s financed by Ethio-Swedish integrated rural development project. As livestock and cattle in particular play an important role in the smallholder economy in Arsi, efforts to improve livestock production formed a natural and important part of the project. Activities included crossing local and exotic cattle and sheep breeds; conducting feeding and management experiments; investigating new fodder crops to level out seasonal fluctuations in pasture production; carrying out vaccination campaigns; and implementing milk collection and marketing programs. Specifically, the study was conducted in Bokoji Negesso Peasant Association (PA) with an altitude of 2876 masl. Bekoji Negesso PA was purposively selected based on its accessibility from the 25 PAs available in the district. Out of a total 2678 ha (91.8%) is allocated for crop production, 5.8% is for grazing and about 2% of the PA land is covered by forest (Figure 2).

Data collection and data sources

This study used qualitative and quantitative data collected from primary and secondary sources. Different Participatory Rural Appraisal (PRA) techniques such as Focus Group Discussions (FGD), Key Informant Interviews (KII) and Personal observation were utilized to collect primary data. Secondary data was collected using a wall-to-wall fieldwork.

Secondary data collection and desk reviews

Before primary data collection, reviews were made from different published and unpublished document that are relevant for the study. Such reviews were made to know previous works in dairy value chain studies in Ethiopia and elsewhere as well as to know the existing information gap. Moreover, secondary data were collected from reports of CSA, different organizations including government institutions such as agricultural offices (regional, zonal and district) and primary dairy cooperatives.

Focus Group Discussions (FGD)

Focus group discussions were held with two farmer groups for two days on 26 and 27th of July 2012. Each FGD has a minimum of 15 to a maximum of 21 farmers. Checklists were used in order to guide the FGD with farmers.

Key Informant Interviews (KII)

Information gathered from FGD with farmers was cross-checked and complemented with the existing secondary sources and through KII. A wide range of stakeholders including cooperative managers, input suppliers, processors, private traders and collectors, staff from different offices (office of district livestock development, cooperative promotion office, partner NGOs) and union managers were used as key informants.

Observation

In order to grasp the business practices and transactions in dairy value chain of the study area major milk products (butter and cheese) market at Bekoji town was visited on the major market day (Saturday). During field observations, discussions were made with butter and cheese collectors and traders, private veterinary drug venders, grain milling factories that supply wheat brans and nouge cake to the surrounding dairy producers.

RESULTS AND DISCUSSION

The dairy value chain in Lemu-Bilbilo district involves six distinct value-adding activities (core functions) from the inception of milk production through reaching to the final consumer. These activities include input supply, production, gathering (bulking), processing, transportation and retail trading.

Input supply and services

The major inputs required for dairy production include purchased feeds like concentrate feeds (industrial by-products) and green fodder, Artificial Insemination (AI), veterinary and credit services, land and labor.

Feed supply

The study area is characterized as mixed crop-livestock farming system. In mixed farming system, crop residues are mainly used as source of livestock feeds together with natural pastures. The dominant crop residues available and used as feeding options for dairy production includes straws of wheat, barley, linseed, faba bean and field pea. The main source of crop residues is from own harvest, but in some cases, farmers also buy from market or other farmers. Preferences for crop residues differ for different crops. Due to its relative palatability of the straw, most of the farmers prefer barley straw to feed their dairy animals.

Purchased feeds

During the rainy seasons, farmers rely mainly on natural pasture to feed their dairy animals. As a result, demand for concentrate feeds and their associated prices decrease during such seasons. However, farmers start seeking concentrate feeds as their natural pasture dwindles. They get these concentrates from flour and oil mills at Bekoji town. According to the response of farmers, the price of concentrate feeds increases from year to year. For instance, the price of oil seed cakes increased from 600 ETB[1]/100 kg in 2010 to 900 ETB/100 kg in 2012 and price of wheat bran increased from 140 ETB /100kg in 2010 to 360 ETB /100 kg in 2012. This is becoming unaffordable for farmers and has a negative bearing on the milk supplied by smallholder farmers. Farmers tend to reduce the amount of concentrate feeding to livestock as its price increases. Moreover, most farmers provide supplementary concentrate feed only to oxen and lactating cows because of the high price, which made them costly to feed to other animals. Most farmers did not have access to training on ration formulation and improved feeding techniques.

Artificial insemination

There are two categories of AI service providers in the study area: government and community service providers. There is only one government technician who provides AI services in the study area. This AI technician is based at the district health clinic at Bekoji town. The service charge is 6 ETB per conception. A farmer can repeat up to 3 times if conception fails to happen. However, there are cases where the conception fails to happen after 6 times repeated insemination. In the remote areas of the study areas, farmers travel more than 4 kilometers to get the AI service. To expand AI service in the remote areas of the study area, Oriomia Livestock Agency with the financial support of FAO has trained nine secondary school graduate farmers as Community Artificial Insemination Technicians (CAIT). Four out of the nine CAITs were provided with the necessary AI equipment including Liquid Nitrogen container, glove, semen container and inseminating gun. These CAI technicians are also regularly provided with semen and liquid nitrogen. The CAIT service providers charge 12 ETB per conception of one animal. Since they do not have permanent salary from the government, they take 10 ETB for themselves as service charge and pass over the remaining to the government.

Animal health

The most prevalent animal diseases mentioned by key informants and farmers were mastitis, Foot and Mouth Disease (FMD), black leg and parasites. Currently, there are seven animal health posts serving the 27 PAs available in the district. There are also three private drug

[1] *Ethiopian Birr(ETB), during the survey time 1$=17.25 ETB*

stores and one private clinic at Bekoji town. The Regional Government of Oromia has allocated 40,000 ETB as a revolving fund for veterinary drugs for the district. However, there is still shortage of drugs to treat important diseases such as mastitis.

Financial services

Dairy cooperatives and unions in the study area were not involved in the provision of any financial services in a way of credit in kind (inputs) to individual farmers. Dairy producers are appreciative for the efforts of FAO in the provision of in kind credit through distribution of heifers to selected milk producers. However, FAO was unable to continue delivering these services due to unavailability of heifers supplying institutions and had to revise alternative source of AI delivery system through use of CAI model. Even if farmers have access to formal credit sources like Oromia Credit and Saving Institution (OCSI), they tend not to use this service due to short loan repayment periods. Farmers explain that OCSI credit is too small to procure dairy animals and it should be repaid within one year starting from the date the loan is taken. However, dairy production needs at least three to five years to generate return on investment and cannot be done using such very short-term credits.

Market actors

The main actors in dairy and its products market includes smallholder producers, private processor, cooperatives, hotels/cafeteria, individual consumers and farmers.

Dairy producers

The dairy industry in the study area is mainly comprises smallholder farmers. According to the FGD results, the average herd size per household for local breeds is decreasing while that of improved breeds is increasing. This is presumably affected by reduced grazing lands and increase of cultivated lands arising from high population pressure. There is also lack of push factor to relieve pressure on land due to the unavailability of jobs in the urban centers for the young educated farmers. As a result, there is a change in the commercial orientation of farmers towards milk production. In general, smallholder farmers in dairy farming in the study area are characterized as follows:

(1) Most smallholder farmers on average held three local breeds and two improved breed cows. In some cases, exceptional progressive farmer owned as high as 37 heads of improved breeds, mainly Holstein-Friesian and Jersey breeds.
(2) The number of non-lactating cows for local breeds per household was relatively high as compared to the improved ones, which is four and one, respectively. The number of lactating cows per household was two for local breeds and one for improved ones.
(3) The feeding regime of the study area is dominated by communal grazing. Very few farmers practice paddocking, indoor feeding (zero grazing) and cut and carry feeding systems for improved breeds.
(4) Average daily milk yield per local breed dairy cow is 2 L; for improved breeds it is 10 L (morning and evening milk). According to some key informants, some farmers get up to 17 L per day per cross breed dairy cow. This suggests a potential to raise yield per improved dairy cow from the current average of 10 L/day/cow to 17 L, which is equivalent to about 70% increases.
(5) From FGD, the proportion of milk marketed by dairy producers was only 15%. The rest 85% of the produced milk remains within the households either for household consumptions or for processing purposes (butter and cheese). The main reason for not selling milk was low-level of milk production, which is not sufficient enough than home consumption.

Dairy cooperatives

In Arsi Zone, there are 24 primary dairy cooperatives and one zonal level cooperative union established with the support of FAO project at the time of this study. Dairy farmers in the study area supply milk to two dairy cooperatives. Farmers in Bekoji Negesso area supply to Bekoji Zuria dairy cooperative while farmers in Lemu area supply to Lemu Ariya dairy cooperative. Both dairy cooperatives are engaged mainly in bulking raw milk from members and non-members, processing and marketing of processed dairy products. The milk deliveries are received at the collection center only in the morning (60%) and the evening milk (estimated to be 40% of the total production) is not collected. The capacity of the cooperatives was limited in terms of the quantities of milk collected and processed, geographic coverage and number of dairy producers involved. The dairy cooperative's product offerings were limited mainly to butter, skimmed milk[2] and cheese. The direct sale of fresh fluid milk is not common.

Collectors

Collectors are one of the important actors in the dairy value chain. Some of collectors undertake their regular duties for private processor by collecting milk in their rented collection shops at Bekoji, Sagure and Lemu towns. These collectors have monthly salary of about 900 ETB and some commission. They collect about 600 L of

[2] *Skimmed milk is not pasteurized and it is usually sold after it get sour (become Ergo)*

fresh milk per day from individual milk producers of Bekoji, Sagure and Lemu areas. They usually use plastic can to transport the milk to Assela town. In order to detect the milk quality, they mainly use acido meter tests and visual observations for their regular customers.

Private processor

One private dairy milk-processing firm (Dembela private milk processing) is involved in milk marketing in the study area. According to the information obtained from FGD and key informants, Dembela private milk processing commands about 15% of the fresh milk market. The firm collects about 600 L of fresh milk per day from the individual milk producers of Bekoji (250 L), Sagure (200 L) and Lemu (150 L) towns. The main market outlet for this firm was the urban consumers of Adama town.

Hotels/cafeterias

Hotels and cafeterias at Bekoji town directly purchase fluid milk (morning and evening milk) from producers based on contractual agreement. They purchase butter from local butter traders at a price of 140 ETB/kg. The average daily intake for raw milk reaches up to 21 L/day/hotel or cafeteria. According to the information obtained from FGD, hotels/cafeterias command about 70% of the fresh milk market of the study site. They consider quality parameters such as freshness, adulteration with water, taste, hygiene and price in their decision to buy liquid milk.

Individual consumers

There are three main dairy products consumed by individual consumers in the study area: raw milk, butter (edible and cosmetic) and cheese. Smallholder dairy producers are still very important sources of milk for individual consumers of the study area. Smallholder dairy producers sell fresh milk to their neighbor and other individual consumers on monthly contractual basis. In this case, the consumer collects milk from the producer's gate. Since the two parties meet every day, they easily communicate the quality problems so that producers can correct them as much as possible. If not, the consumer looks for better quality milk from other producers usually after finishing the contract. On the other hand, for other dairy products like cheese, edible butter and cosmetic butter, the major points of purchase are town markets and cooperatives shops and the main sellers are traders, individual producers and cooperatives.

Marketing channels

Analysis of information obtained from different sources during the study depicts that there are five main market channels for fresh milk produced in Limu-Bilbilo district (Figure 3). The final consumers of dairy products in the study area are individual consumers and hotels/cafeterias of Assasa, Bekoji and Adama towns.

Channel 1: Milk products supplied to individual consumers and hotels/cafeterias in Bekoji and Assasa towns

This is the first channel through which farmers sell fresh (morning) milk directly to the Bekoji Zurea and Lemu-Ariya dairy cooperatives. The cooperatives process and sell the milk products (butter, cheese and skimmed milk) mainly to individual consumers and traders of Bekoji and Assasa towns. According to the information obtained from FGD, about 7% of the fresh milk produced by farmers goes through this channel via cooperatives. The suppliers to the dairy cooperatives are mainly commercial farmers that have continuously supply milk throughout the year. The cooperatives process the raw milk into butter and skimmed milk and sell mainly to individual consumers and traders of Bekoji and Assasa towns. This channel is the dominant market channel for skimmed milk produced by Bekoji Zurea dairy cooperative. Sale of skimmed milk is virtually made during the dry season. In dry seasons, the supply of fresh milk decreases significantly due to feed shortage, which is exacerbated due to lack of grazing pasture and high price of concentrate feed. During such season, the cooperative process the assembled milk into butter and sell the skimmed milk to Assasa district traders and members at the price of 3.50 ETB/L.

Channel 2: Milk supplied to hotels and cafeterias in Bekoji town

This is the biggest fresh milk marketing channel in which about 70% of the milk produced in the study district is supplied to hotels and cafeterias of the study district. Bekoji town is the hometown of famous long distance runners. Nowadays, there is a growing number of investment on hotel and cafeterias in the study district mainly built with the remittance income obtained from athletics. Smallholder farmers who have little supply mainly opt this channel due to the flexibility of payments. Payments is made based on agreement between supplier and owner of hotels and cafeterias. In most cases, payment is made on weekly and bi-monthly basis, which gives farmers the choice to invest the income on concentrate feed especially during the dry season.

Channel 3: Milk consumed by individual consumers in Bekoji town

Due to change of life style, the per capita milk

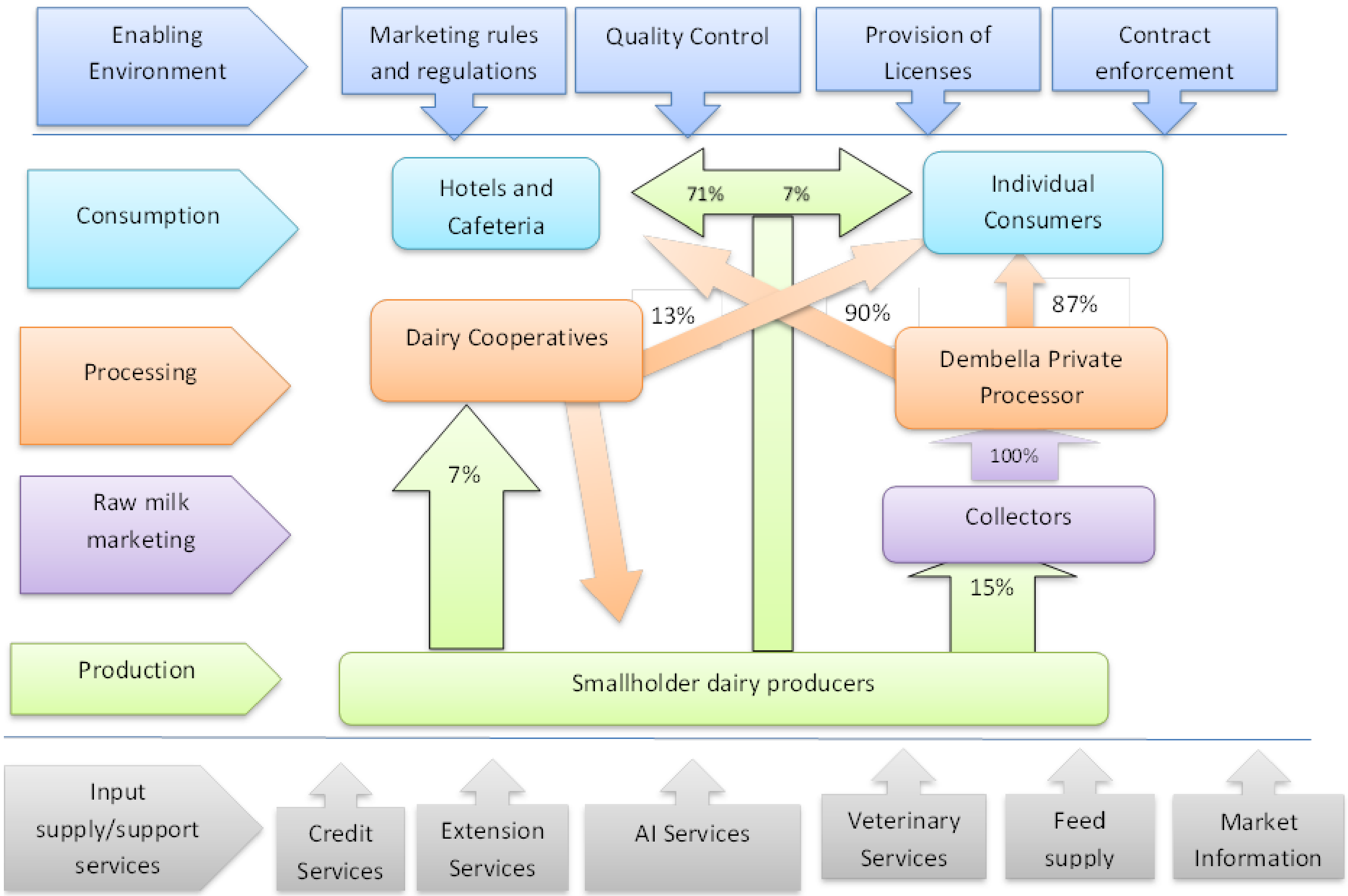

Figure 3. Fresh milk and milk products marketing channel.

consumption in Ethiopia has been increasing over the past few years. Individual households tend to include milk consumption as their daily dietary food intake although the volume and frequency is not enough. According to Central Statistical Authority (CSA, 2011), per capita milk consumption has reached 48 L in 2010/11, which rose by 25% and 29% compared to 2008/09 and 2009/10 seasons. As per the information obtained from FGD and key informants, about 7% of the fresh milk produced by farmers sold directly to individual consumers on contractual basis through this channel. Payment effected in advance on monthly basis. Most of the households consume the whole fresh milk (fresh, pasteurized milk or fermented sour milk). Some households churn the cream that is accumulated over a week to get butter and cheese (*ayib*) for household consumption.

Channel 4: Milk supplied to individual consumers in Adama area

Non-cooperative affiliated and relatively small milk producers usually follow this channel. This is the second biggest fresh milk market channel. About 15% of the morning milk produced by farmers reaches to private processor (Dembella private processor) through milk collectors. In this channel, the milk assembled by Dembella private processor is distributed to individual consumers in Adama town at Dembella selling shop in the form of fluid milk, sour milk (*Ergo*), cheese and butter. Almost 87% of the milk collected from Dembella private processor (i.e., 13% of the morning milk produced in the area) goes to individual consumers at Adama town. During transportation of the assembled milk, some of the collected milk is exposed to sun and becomes too sour for processing, which in turn sold as sour milk (*Ergo*) to individual consumers at Adama town.

Channel 5: Milk supplied to hotels and cafeterias in Adama area

In this channel, 13% of the fluid milk assembled by Dembella private processor (that is, 2% of the morning milk produced in the area) is distributed to hotels/ cafeterias in Adama town on monthly contractual basis.

This channel is relatively better organized in terms of sustainability and trust. In this channel, collectors of Dembella processor and raw milk suppliers have developed strong relationship and trust. In this market channel, the milk rejection rates are relatively lower as compared to the first channel. Producers are allowed to mix the evening and morning milk to supply to collectors which otherwise is the bases for rejection, if they supply to cooperatives. The main reason mentioned by collectors is to attract the milk supply from farmers. Despite the fact that this channel is relatively organized and developed, collectors still use public transportation and Isuzu private trucks for transporting milk from major collection areas to milk processing plant at Assela town which is 56 km far away from the collection centers. Unlike other marketing channels, the most noticeable advantage of this channel is the constant price paid to supplier farmers during dry and wet seasons. This has attracted most farmers to rely on this channel.

Challenges of dairy production and marketing

Technical constraints at input supply

Low quality and untimeliness of AI and animal health service provision: Based on famers' response during FGD, the service rendered by the AI technicians was inadequate and offering low quality services. Due to this problem, nowadays farmers tend to use bull service for breeding, which is more attractive from the point of view of its timely accessibility when service is required. Farmers complained that service per conception was 5 to 6 times and there were cases where they fail to succeed after 6 conceptions. Farmers identified three important reasons for this. The first reason was shortage of technicians. There was only one technician for five kebeles and he cannot be available when the animals were in heat. The second reason was poor semen quality. This could be the result of poor handling, especially because of shortage of liquid nitrogen to maintain the cold chain and keep the semen alive. The third reason was low technical capacity of the technician. Farmers indicated that they do not have confidence on the capability of the technician. However, farmers understand the importance of AI services over natural mating in terms of its proven quality in improving the genetic make-up of the cattle population through access to genes from superior bulls, disease control and cost effectiveness as compared to rearing bulls. Despite this superior advantage, farmers were forced to rely on natural mating due to the above stated inefficiency and inaccessibility of the AI services.

Information gap on credit services: With regard to credit, farmers and dairy cooperatives have limited awareness about the terms and conditions of credit providers. Currently, most farmers do not have good knowledge of how to get credit services, amount of credit and loan repayment periods for dairy farming activities. Farmers refrain from using credit services mainly due to lack of understanding of its terms.

Unavailability of budget for demonstration sites on improved forage production in Farmers Training Centers (FTC): Utilization of FTC as training ground for demonstration of improved forage development was not observed in the study area. The major problem behind this was budget shortage.

Shortage of multiplication centers for the supply of improved dairy heifers and bulls: Farmers stated that there was shortage of ranches that multiply and distribute improved heifers and bulls in the area. The government ranches that used to serve this purpose were privatized and are no longer multiplying and distributing breeding stock. Those ranches that are still multiplying breeding stock under the private ownership such as Gobe are too expensive to be accessible by farmers.

Technical constraints at production stage

Lack of awareness and knowledge regarding improved feed formulation: Despite the efforts of some non-governmental organization such as ACDI/VOCA Ethiopia in provision of trainings for few progressive farmers on improved feed formulation methods by using the idea of proper cost-effective ration techniques, farmers are still lack the basic skills for feed formulation techniques.

Very high price of industrial by-products for feed: Over the last three years, the price of industrial by-products has become sky rocketing. For instance, the price of oil cake increased from 600ETB/100kg in 2010 to 900 ETB /100 kg in 2012 and for wheat bran it increased from 140 ETB/100kg in 2010 to 360ETB /100 kg in 2012. This is becoming unaffordable for dairy producers. Farmers tend to reduce the amount of concentrate feeding to livestock as its price increases.

Technical constraints at processing and marketing stage

Lack of refrigerated trucks: To resolve the fresh milk marketing problems of the cooperatives, FAO had purchased milk cooler machine for the dairy cooperatives union. This was in order to enable primary cooperatives to supply morning and evening milk twice a day to the union at Assela town. However, this facility would be useful only if the cold chain of milk stored in the cooler machine is maintained upon delivery to potential buyers. This needs the availability of trucks with refrigerated tankers. As a result, the cooling machine was left idle in the store. Thus, the union and primary dairy cooperatives have failed to accomplish their intended purposes.

Absence of quality based payments: Since there is no quality based payments for milk producers, milk supplied by farmers were in some cases adulterated with hot water and mixed with evening milk, which was below the required quality standard set by dairy cooperatives.

Weak vertical linkage between cooperatives and potential buyers: The dairy cooperatives have weak vertical market linkages with supermarkets, institutional buyers and private processing plants. Cooperatives immediately process milk into butter and the practice of transporting fresh milk to other regional markets is not common.

Legal and institutional constraints

Existence of too many unlicensed traders: This is the case for butter market, where most of traders are informal while only few of them are licensed. This means the licensed traders are paying taxes and compete with non-tax payers in the market. This in turn distorts the normal working environment of the butter market.

Weak coordination between union, primary cooperatives and farmers: The milk collected by dairy cooperatives of the study area was not delivered to the union. Rather, the primary dairy cooperatives limited their involvement only in processing fresh milk into butter and cheese. The dairy cooperative union of the study area was established at zonal head quarter of Assela town. The linkage of both Bekoji zuera and Lemu Ariya primary dairy cooperatives with that of the union was not strong. The immediate reason was the less involvement of the union in the marketing of fresh milk for which they were initially established. Moreover, the linkage between primary dairy cooperatives and farmers was also not as such strong since farmers are not getting the benefits they are expecting from their cooperatives. Cooperatives are not involved in provision of any capacity building trainings for farmers on milk handling and processing. Furthermore, they even did not supply inputs such as concentrate feeds, veterinary drugs and improved forage seeds. In other parts of the country, unions and dairy cooperatives are serving as major sources of heifers and link farmers to the sources, provide credit services and identify market outlets. For instance, Ada'a dairy cooperative has been sharing dairy related knowledge and information by providing training and advisory services on dairy production and marketing for their member farmers. However, such kinds of relationship and service provision were non-existent in the study area.

Capacity of support service providers

Capacity gap among extension agents and agricultural experts in provision of training for feed formulation techniques: During the FGD, farmers indicated that they were not getting the required support regarding dairy husbandry practices like ration formulation techniques either from extension agents or from experts.

Existing opportunities

The major opportunities available to invigorate the transformation of subsistence dairy sector of the study area into market-oriented dairy farming includes:

(1) Presence of model dairy farmers that have started improved dairy farming in the study area. The availability of few progressive farmers who have adopted the practice of keeping improved dairy cows becomes clear evidence that there is an opportunity to practice dairy farming as profitable farming system in the area.
(2) Good policy road map that aimed at bringing the desired change in the livestock sector. For instance, Oromia Livestock Agency has placed more emphasis on improving the effectiveness and accessibility of AI service through the introduction of oestrus synchronization using mass artificial insemination campaigns.
(3) Good infrastructural facilities: the study area is connected with good asphalt roads to milk deficient Shashemene, Hawassa, Assela and Adama urban towns.
(4) Favorable climate and weather conditions with relatively abundant pasture land.

RECOMMENDATIONS

In order to improve the dairy value chain of the study area, the following recommendations are suggested as intervention options to overcome the constraints of the dairy value chain and make use of the available opportunities.

Training of farmers on improved feed formulation techniques

It is evident that the poor feeding system is partly attributed to the high cost of industrial by-products and less awareness on the part of farmers about the positive gains associated with improved feeding system. Therefore, in order to create knowledge based smallholder dairy development in the area, there is an urgent need for training of dairy producer farmers on feed formulation and feed preservation techniques. This should be accompanied by scaling up the achievements of few progressive farmers on achieving high milk yields through adoption of improved feed formulation techniques.

Improve the effectiveness and efficiency of AI service

Improvement in the current AI service could be made through:

In-service training of local service providers

In order to enhance the technical skills and knowledge of AI technicians, short-term training and refresher courses on relevant areas of AI service would play central role for enhancing the service provision of AI experts in the study area.

(1) Training of farmers on AI service (especially heat detections and reporting) or encouraging the use of farmer AI technicians where it is necessary.
(2) Training of farmers on community bull selections***.***
(3) Expanding different organizational models on AI and animal health services. For instance in the study area, in collaboration with Oromia AI institute and Livestock Agency, FAO provided 30 days training for nine farmers on how to recognize various semen types, early detection of animal heat and proper insemination procedures. In the meantime, necessary equipment was provided for trained AI technicians. Expanding the number of trainee farmers by inclusion of participants from remote areas and improving the provision of facilities (like motor cycles) for trained community AI and animal health technicians could be the right step for transforming the traditional dairy system of the study area into more market oriented dairy sector.

Revitalizing the activity of cattle ranches

Farmers in the study area used to get breeding heifers and bulls from ranches such as Gobe and Abernosa. However, these ranches were privatized with the purpose to increase their efficiency and promote private sector participation in the livestock industry. Despite the intention of the government, these ranches could not provide the intended benefits to the surrounding community. Thus, there is a need to reconsider mechanisms to bring these ranches back to their main objective of supplying better genetic materials to the community. This could be done through a dialogue with the private sector and proper follow up to ensure that they stick to their initial plan of more efficient multiplication and dissemination of these materials.

Awareness creation on credit service terms and conditions

Micro-finance institutions need to create a platform for organizing training on credit service terms and conditions for dairy producers.

Improving the service provision of the union

In order to address the chronic shortage of trained AI technicians and high price of improved feeds, improving the service provision of the union to its members in the form of acting as “Business Hub” by providing those mentioned inputs would alleviate the current problems.

Creating regular stakeholder forum for various value chain actors

Creating regular platforms for dairy producers, dairy co-operatives, union, private traders and potential consumers as a mechanism for creation of market linkage and experience sharing platform is crucial. This initiative would enable them to discuss common problems, find solutions and strengthen networking between important dairy value chain players.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Standardizing germination tests for quinoa seeds

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The standard germination tests have been commonly used on commercial grain crops, such as soybean, field beans, rice and maize. However, there are no standard tests for potential new crops; quinoa being one of them. This work is aimed at evaluating the effect of substrate, temperature and counting time in seed germination of quinoa. The following treatments were used in tests with seeds: on blotter paper, between blotter paper, on sand and within sand, all previously soaked with distilled water. These substrates were combined to the temperatures of 15, 20, 25, 30°C, and alternated between 20 to 30°C. The experiment was conducted on an entirely randomized factorial design 5 × 4 (temperature × substrate) with 4 repetitions. Normal seedlings, abnormal seedlings and dead seeds were counted until stabilized. The germination velocity index and mean time for germination were calculated. From the results, it was concluded that germination test of quinoa seeds should be conducted at 20 to 30°C alternate temperatures, on or between blotter paper, with initial count at 2 days and final count at 4 days.

Key words: *Chenopodium quinoa* willd., substrate temperature, counting time, seed viability.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a plant of the *Amaranthaceae* family. The genus *Chenopodium* comprises of 150 species which are encountered in America, Asia and Europe (Bazile and Baudron, 2014). It is a dicotyledonous plant with ample variability in germplasm, including halophytic ecotypes, soil salinity tolerance, and xerophytes; thriving and reproducing under low moisture conditions (Jellen, 2014). The species originated in around the Titicaca Lake in the Andean mountain range of Bolivia and Peru. This pseudocereal has been largely consumed by local indigenous populations of Bolivia, Peru, Ecuador, Chile, Argentina and Colombia. For centuries, it was one of the main components of daily diet for these peoples. Its cultivation declined during the colonial era, when the conquerors introduced wheat and barley crops. However, in recent times, its popularity has grown in Europe, North America and the Andean region; mainly associated with the needs of those adopting vegetarian diet and those with bias towards gluten and lactose (Jellen, 2014). From all food

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grains, quinoa is the only available grain with natural amino acid balance in its protein. Its high quality is expressed by the content of histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan, valine and, mostly, lysine and methionine, the essential amino acids (Stikic et al., 2012). Its grains are rich in minerals (K, Ca, P, Mn, Zn, Cu, Fe and Na), dietetic fibers and vitamins C and E (Dini et al., 2010). These nutritional qualities have turned quinoa into a reference crop, adaptable to various worldwide growing conditions becoming an option to increase food security (FAO, 2011).

The research has intensified in Brazil with the aim of inserting quinoa crop into production systems. However, it has intrinsic problems relating to seed quality, leading to failure in the field (Sigstad and Garcia 2001; Sigstad and Prado 1999). Seed is the most important component in crop establishment, with direct impact on crop performance and productivity (Azevedo, 2003), and the seed research on quinoa is rather incipient. In Brazil, where the crop has a short history, the first recommended cultivars have shown the seed quality problems and there is no specific standard germination test (Brasil, 2009). Germination test, deterministic of the sowing rate, analyses seeds of different batches and allows comparison. Germination depends on the intrinsic characteristics of seeds and environmental factors, such as temperature, moisture and substrate. The latter can be manipulated and adjusted to optimize germination rate, velocity and uniformity. However, the quinoa seeds have a peculiar structure in regard to the cereals, especially the unique pericarp, an outer layer of the dead cells surrounding it, which is a fruit of achene type (Burrieza et al., 2014). Temperature affects the velocity of imbibition and the chemical reactions occurring in the germination process that influences the uniformity and overall germination rate (Carvalho and Nakagawa, 2012). Seeds of different species have shown unique reaction to temperature, which is optimal when they express the maximum potential of germination in the least period (Borges and Rena, 1993; Popinigis, 1985). Some species respond to alternating temperatures, while others best germinate under constant temperature (Alves et al., 2011). The substrate (physical support in which the seed is placed) functions at maintaining ideal conditions for germination and emergence. The structure, aeration, water retention capacity and presence of plant pathogenic organisms can influence seed performance (Figliolia et al., 1993). The choice of substrate should take into account the seed characteristics, such as size, necessity of water and light, counting facility and seedling evaluation (Popinigis, 1985). The quinoa seed structure and its physiology can also influence storage and germination at higher temperatures than what's prevailing in the high mountains of its origin (Souza et al., 2016). Given the peculiarities of seed plant species that could affect germination, this work aimed at developing methodology to evaluate germination and to establish a standard for routine in quinoa seed quality assessment.

MATERIALS AND METHODS

The work was conducted in the Seed Technology Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAV), University of Brasília, Brazil. The quinoa seeds were of cv. BRS Syetetuba, harvested from a field plot grown in Água Limpa Farm, UnB, between March and August, 2015. The plots were hand harvested when seeds were at physiological maturity and contained 20 to 30% water. After harvest the plants were dried at low air moisture condition; during the dry season, threshed, seeds were cleaned and stored in cold room at 10°C temperature at 12% water content. Before initiating the tests, seeds were immersed in 2% sodium hypochlorite for 10 minutes and rinsed with distilled water to avoid fungal infection. The treatments combined of 5 constant temperatures of 15, 20, 25, 30°C, and alternated 20 to 30°C with 4 substrates: on blotter paper (OP), between blotter paper (BP), on sand (OS) and within sand (WS). For the BP, seeds were distributed over two paper sheets previously soaked using 2.5 times the mass of dry paper, covered with a third sheet of paper and placed in a transparent plastic boxes of 11 × 11 × 3 cm. For the OP, the seeds were placed on transparent plastic boxes, containing two sheets previously soaked in distilled water at the same rate as in BP. The sand germination was evaluated in transparent plastic boxes, keeping the same distance between seeds as in the paper test. Seeds were placed on top of sand (OS) and at 1 cm depth (WS). In both treatments, the sand was watered and kept at 60% field capacity (Brazil, 2009).

The effects of temperatures and substrates on seed germination were evaluated by daily count. Seedlings that had radicle longer than 2 cm were scored and the duration of test was determined by the number of days until germination was stabilized and results were expressed in seedlings percentage. Concomitant to germination test, germination velocity index (GVI) and germination mean time (GMT) were calculated. The normal seedling count was conducted daily, while the indexes were obtained by Maguire (1962) and Labouriau (1983), as follows:

$$GVI = G_1/N_1 + G_2/N_2 + \ldots + G_n/N_n$$

Where, GVI = germination velocity index; G_1, G_2, G_n = number of normal seedlings at first count (G_1), second count (G_2) and last count (G_n);N_1, N_2, N_n = number of days to first count (N_1), second count (N_2) and last count (N_n);GMT: $\sum(\mathrm{ni\ ti})/\sum \mathrm{ni}$, em que; GMT: germination mean time (days); ni: number of germinated seeds at each count interval, and ti: time between first and ith count.

The experimental design was an entirely randomized factorial, combining 5 temperatures and 4 substrates (5 × 4), with 4 repetitions. The germination test percent data were transformed by arc sen $\sqrt{\frac{x}{100}}$, to fit normal distribution and the original means was compared by Tukey test at p=0.05. For the statistical analysis the SISVAR 5.3 Software was used (Ferreira, 2011).

RESULTS AND DISCUSSION

There was significant interaction ($p<0.05$) for all the factors, including temperature × substrate interaction.

Table 1. Germination (%) and germination velocity index of the quinoa seeds (*Chenopodium quinoa* Willd) submitted to standard germination test on different substrates and temperatures.

Substrates	Temperatures				
	15°C	20°C	25°C	30°C	20-30°C
			Germination (%)		
BP	71.5^{Ba}	82.0^{ABa}	77.0^{ABa}	79.0^{ABa}	86.5^{Aa}
OP	71.5^{ABCa}	65.5^{Cc}	68.5^{BCa}	78.0^{ABa}	81.5^{Aab}
WS	73.5^{Aa}	68.7^{ABbc}	73.5^{Aa}	60.0^{Bb}	70.0^{ABc}
OS	78.5^{Aa}	78.5^{Aab}	75.5^{Aa}	78.5^{Aa}	77.5^{Abc}
			Germinationvelocityindex		
BP	17.3^{Ca}	26.5^{Bb}	35.9^{Aa}	37.1^{Aa}	35.9^{Aa}
OP	17.1^{Ca}	16.2^{Cc}	28.3^{Bb}	37.7^{Aa}	30.7^{Ba}
WS	7.9^{Bb}	11.3^{ABc}	16.8^{Ac}	14.5^{Ab}	15.8^{Ab}
OS	18.8^{Ba}	36.9^{Aa}	35.2^{Aa}	38.1^{Aa}	34.2^{Aa}

Means followed by the same capital letter in line and lower in the column do not differ by Tukey test ($p \leq 0.05$). BP: between blotter paper, OP: on blotter paper, WS: within sand e OS: on sand.

The germination rate (%) for between blotter paper (BP) and on blotter paper (OP) was higher for 20 to 30°C alternated temperature (Table 1). However, these results did not differ statistically from those of 20, 25 and 30°C, for BP, and of 15 and 30°C for OP treatment. The within sand seeds had the best germination rate, at 15 and 25°C and, when seeds were placed on sand, there were no statistical differences for temperatures. With the exception of 15 and 25°C, which showed no statistical differences for substrates, the other three temperature treatments had the highest values when the seeds were tested between papers. At 30°C, there was no significant difference and alternated temperature for within sand and on paper tests. Similar results for tests within paper were obtained for malabar spinach (*Basella alba*, Basellaceae) seeds (Lopes et al., 2005).The alternated 20 to 30°C temperature had the best germination, for both blotter paper tests. These results could be explained by the association of fluctuating in temperatures occurring in the environment of origin of plant species (Borges and Rena, 1993). Quinoa originated in the Andean mountains, where low night temperatures alternate with high day temperatures in low moisture environment. The variations in temperature could cause activation of enzymes relating to germination (Vázquez-Yanez, 1984). Moreover, for the higher germination could be the effect of variable temperature on the seed tegument. The seeds then become more permeable to water and oxygen, influencing the balance between promoting and inhibiting substances (Alves et al., 2015).

There were no significant differences in germination velocity index (Table 1) for 25, 30 and 20 to 30°C alternated, for seeds placed between paper, within sand and on sand. For seeds placed on blotter paper, the highest germination value was at 30°C, followed by 25°C and 20 to 30°C alternated temperatures. These results show a visible influence of high temperature on germination velocity of quinoa seeds, varying between 15°C low and 30°C high. Low temperatures could reduce enzymatic activities involving seed metabolism, reducing rate and delaying germination (Caldeira et al., 2015). The germination velocity index (GVI) and the germination mean time, at all temperatures, within sand substrate had the lowest values (Table 1 and Figure 1). There was a 50% higher reduction in the GVI and a longer period of 5 days was necessary for germination in comparison with other substrates. These results could be explained by the amount of water in sand blocking oxygen around seeds, reducing respiration and delaying or paralyzing germination (Carvalho and Nakagawa, 2012; Borges and Rena, 1993). Therefore, placing the seeds in sand was inefficient to evaluate germination and vigor of quinoa. Similar results were obtained with *Cucumis metuliferus* seeds (Alves et al., 2014). Irrespective of substrate, quinoa seeds germinated in less time when they were exposed to 25, 30 and 20 to 30°C, differing from 15 and 20°C temperatures (Figure 1). For within paper substrate at 15°C 2.1 days was necessary, while at 25, 30 and 20 to 30°C, the quinoa seeds germinated in 1.5 days. When seeds were placed on sand and on paper at 30°C, germination occurred on the first day, whereas at 15 e 20°C two days was needed for paper. Seeds placed on sand at 15°C germinated in 2.2 days.

Velocity of germination can be influenced by temperature. When seeds are exposed to low temperature, in general, there was a delay in germination from reduced metabolic activities. On the contrary, at high temperatures seeds germinated more rapidly as a result of protein denaturation coming from increased metabolic activities (Marcos-Filho, 2015). Daily monitoring

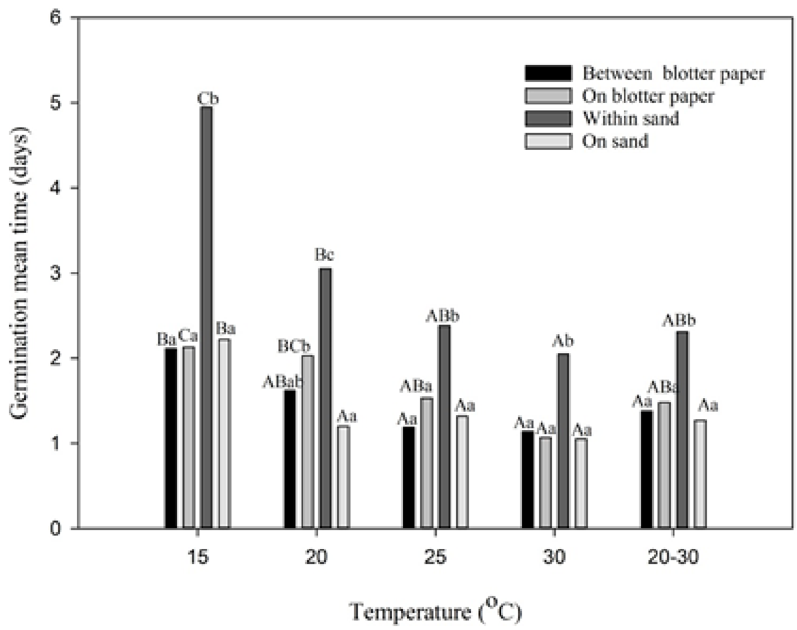

Figure 1. Germination mean time of quinoa seed (*Chenopodium quinoa* Willd.) submitted to the standard germination test on different substrates and temperatures. Means followed by the same letter do not differ by Tukey test ($p \leq 0.05$). Lowercase letters compare substrates at each temperature, capital letters compare the temperature in each substrate.

of quinoa seeds allowed a defined evolution curve for each of the treatments (Figure 2). High germination rates were scored when seeds were placed between paper sheets and on paper sheet for the 20 to 30°C alternated temperature. In general, germination was observed from the first day of the test and on the 2nd day the germination rates were higher than 50%, increasing until stabilized from the 4th day. Therefore, the ideal date for first count should be on the 2nd day and for the second count on the 4th day after sowing. The experiment followed similar criteria to define standard germination test with other crops (Alves et al., 2014, 2015; Caldeira et al., 2015; Oliveira et al., 2014). On soybean and maize, first and second count occurs at 5 to 8 and 4 to 7 days, respectively. On beet root, of the same botanical family as quinoa, first count is done at the 4th day and the second on the 10th day (Brasil, 2009). The more rapid germination on quinoa can be related to the high imbibition capacity of its seeds, causing roots to protrude within 6 to 10 h after being exposed to water (Souza et al., 2016; Makinen et al., 2014). The pericarp consisting of layer of dead cells surrounding the seeds is highly permeable while the tegument and the endotesta are completely consumed during the seed formation, remaining only the exotesta (Burrieza et al., 2014). The external permeability adds up to the water absorption ability of the perisperm formed by large and thin-walled cells, containing highly hydrophilic starch granules (López-Fernandez and Maldonado, 2013). These mechanisms that accelerate seeds germination may have resulted from the environmental conditioners. Around the Titicaca Lake in the high Andes of Bolivia and Peru, the probable center of origin of quinoa, the moisture is reduced. Therefore, the species developed efficient water use during the plant cycle, starting with rapid germination.

Conclusion

The germination test for quinoa seeds using 20 to 30°C, is optimized when seeds are placed in between or on blotter paper, with initial count at 2 days and final count at 4 days from the start of test.

Conflict of Interest

The authors have not declared any conflict of interest.

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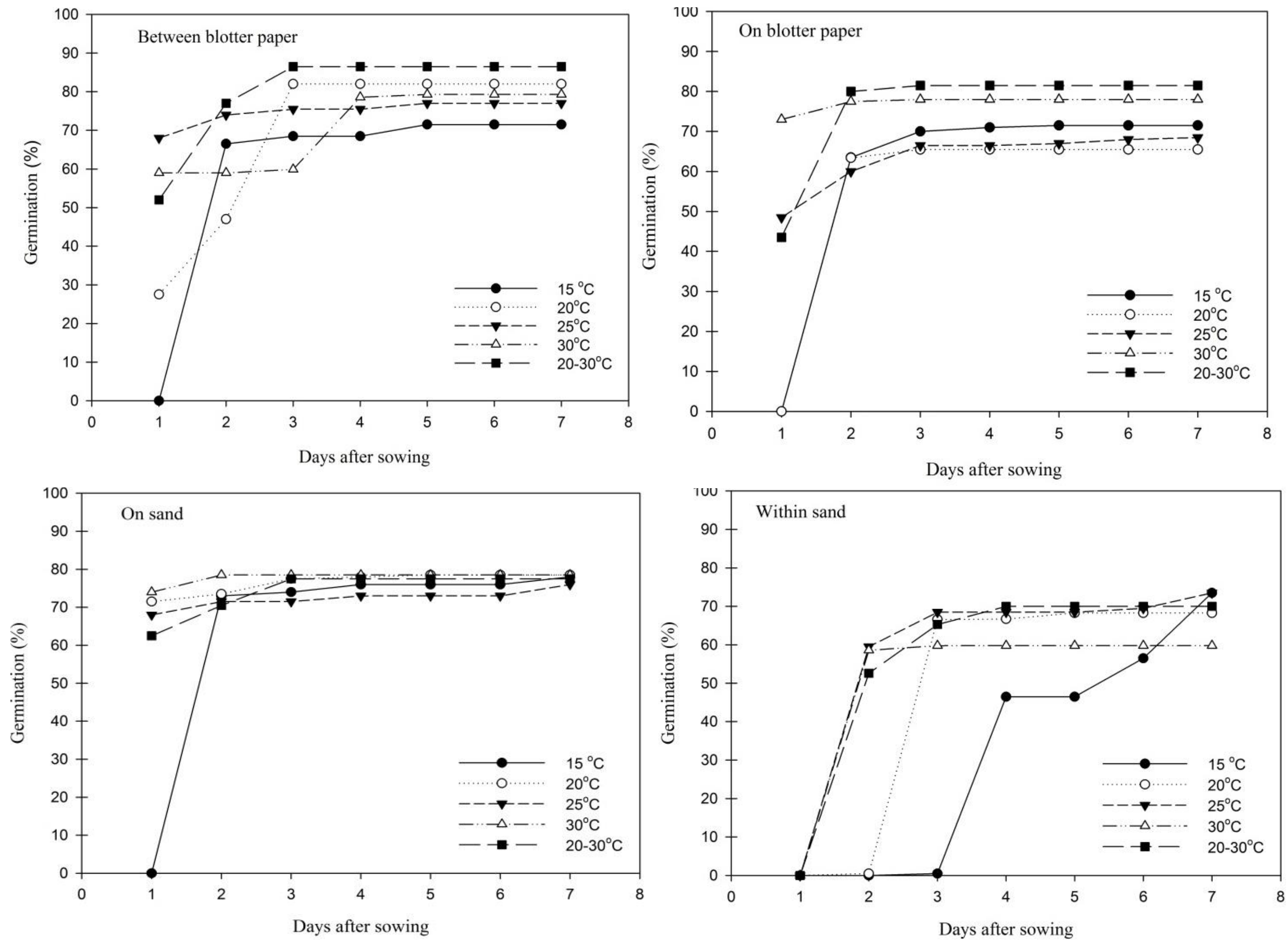

Figure 2. Evolution of quinoa seed germination (*Chenopodium quinoa* Willd.) submitted to the standard germination test on different substrates and temperatures.

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Full Length Research Paper

Study on termite damage to different species of tree seedlings in the Central Rift Valley of Ethiopia

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A high density of termite mounds is common in the Maki-Batu area of the Central Rift Valley of Ethiopia. To determine whether farmers consider termites as one of their problems in growing trees and practices they use to manage termites, short semi-structured questionnaires were distributed to farmers selected randomly. To evaluate the efficacy of some non-chemical control methods, *Eucalyptus camaldulensis* seedlings of five months were transplanted from nursery beds at two experimental sites. The seedlings were planted in three blocks each containing 6 treatments with 3 replicates laid out in randomized complete block design (RCBD). The treatments were: wood ash, cow dung, maize stalk, wood ash + cow dung + maize stalk, Untreated/control and Diazinon 60% EC applied at 2 l/ha as spray on the soil surface and a soil pit drench as a standard check. Plant mortality assessment was recorded once every month for one year. Mortality data were analyzed by analysis of variance (ANOVA) with SAS at 5% probability level. To find out the impact of termites on tree seedlings after transplanting in the area, mortality assessment was conducted at five sites on 11 species of tree seedlings transplanted from nursery beds. Termite damage on the seedlings was recorded once every month. After 12 months, seedlings of each plant species damaged during each month were summed and results were computed as percentages. To assess farmers' perceptions of termites as pests, unstructured questionnaires were distributed to 64 farmers randomly selected. Eucalyptus seedling mortality was very low and there was no significant difference among the treatments ($P > 0.05$) except at one site in which wood ash + manure + maize stover showed higher mortality. Seventy-eight percent of the farmers mentioned that they had planted tree seedlings for different purposes and 72% of them considered termites as the major cause of seedling mortality. Although, higher density of *Macroterems* termite mounds were found in the area and most farmers considered termites as one of the major constraints in growing tree seedlings, this study showed very low termite damage to the tree seedlings studied for one year after transplanting.

Key words: Eucalyptus, farmer, Maki-Batu, mortality, pest.

INTRODUCTION

Termites by no means confine their attentions to dead plant tissues such as wood. Certain species of termites are serious pests of growing crops including living trees (Hickin, 1971) and they are one of the major agroforestry pests in the tropics (Nyeko and Olubayo, 2005). Many other insect pest species cause damage to various parts of the tree, but often they do not cause mortality (Logan et al., 1990; UNEP, 2000). However, termites are seldom primary pests, only damaging the plant, shrub or tree when it has already been affected by fire, disease, drought, mechanical injury, bad planting or other insect pests (Harris, 1971; Hickin, 1971). Although, termites usually appear to be secondary pests, it does not make them of less importance. The initial defect affecting the plant is often of minor importance, after allowing ingress to termites, the effect may be the complete destruction of the plant or, at least, a reduction in its value as a crop (Hickin, 1971).

The extent to which termites are problems to trees and the nature of loss they cause are very much related to the geographic region concerned (Logan et al., 1990). In the tropical and sub-tropical regions of the world where rainfall is low and a dry savannah-type of situation has developed, termite attacks appear most acute and this has caused serious problems in the development of nurseries and young tree plantations. Another phase of the problem is the susceptibility to termite of a popular group of tree species for the tropical planting, the *Eucalyptus* (Hickin, 1971). *Eucalyptus* is preferred as compared to other plants such as *Cassia*, *Albizzia* and *Gmelina* species because of its more rapid growth potential (Harris, 1971). Eighteen termite species are recorded as damaging young trees in forest and plantation nurseries and there are a number of recorded instances of high percentage loss, even complete and total destruction of young trees, particularly *Eucalyptus* (Hickin, 1971). Moreover, Pearce (1997) noted that a high demand for timber in Africa has led to fast-growing trees such as *Eucalyptus*, being planted in poor soil areas where they are under stress and are therefore more susceptible to termites.

The main obstacle to afforestation in the dry areas of Africa is said to be the presence of termites (Harris, 1971; Hickin, 1971). However, not all species of termites present in a locality are destructive to forestry (Nair, 2007). The species ranking as forestry pests vary in different tropical areas. In Africa, the genera, *Macrotermes* and *Odontotermes* are implicated as well as the species *Pseudacanthotermes militaris* in Uganda. Throughout tropical Africa, losses are due exclusively to members of the subfamily: Macrotermitinae, all of which are fungus growers with large nests in mounds or underground (Harris, 1971; Hickin, 1971).

As elsewhere in Africa, termite damage in Ethiopia is generally greater in rain-fed than irrigated crops, during dry periods or drought than periods of regular rainfall, in plants under stress (e.g. newly transplanted forest tree seedlings) than healthy or vigorous plants and exotic (e.g. *Eucalyptus*, maize) than indigenous plants (Wood, 1986, 1991). In western Ethiopia, termite damage on indigenous trees is insignificant. In contrast, serious damage is very common on exotic forestry trees, especially on *Eucalyptus*, one to three years after transplanting (Abdurahman, 1995). Soon after transplanting, seedlings suffer severely from moisture stress, because of soil compaction and low water holding capacity resulting from poor infiltration rates. The roots of such plants begin to dry out and this creates a favorable situation for termite infestation (Gauchan et al., 1998). In some localities up to 100% of *Eucalyptus* seedling loss is common (Abdurahman, 1995). In most areas of Uganda, an average of between 30 and 70% of planted *Eucalyptus* trees are killed by termites (Mazodze, 1995).

Control of termites as forest pests involves both chemical and traditional methods. Current chemical control methods employed are soil treatment, treatment of seedlings before transplanting and baiting techniques and the chemicals currently used include chlorpyrifos, imidacloprid and fipronil. In addition to the current chemical control methods employed, there are a number of alternative, traditional control methods, largely relating to silvicultural practices or plantation management, which are also very important, and should be considered before chemical intervention is attempted. Many traditional methods of control of termites in forest plantations have a sound basis in the principles of ecology (UNEP 2000). Logan et al. (1990), UNEP (2000) and Abdurahman et al. (2010) have reviewed a wide range of control of termites in crops and forestry with non-chemical methods which include cultural and biological control.

Though a high density of termite mounds was reported in the Maki-Batu (Batu formerly called Ziway) area of the Central Rift Valley of Ethiopia (CRVE), no data were available on the extent of termite damage on forestry tree seedlings after transplanting. Therefore, the main objectives of the study were to assess the impact of termites on some species of tree seedlings and test the efficacy of some non-chemical control methods on *Eucalyptus* seedlings after transplanting.

MATERIALS AND METHODS

Study area

The study was conducted between July 2012 and January 2014 in two districts (Bora and Adami Tullu-Jiddo Kombolcha) of East Shawa Zone of Oromia Regional State at six sites (Figure 1). The districts are located in the Central Rift Valley of Ethiopia. Maki is the capital of Dugda district and Batu is the capital of Adami Tulu Jido Kombolcha district. The capital towns of the two districts are about 30 km from each other along Addis Ababa - Shashemene high way. In the context of this study, Maki-Batu area therefore includes the two districts and neighboring districts where high density of

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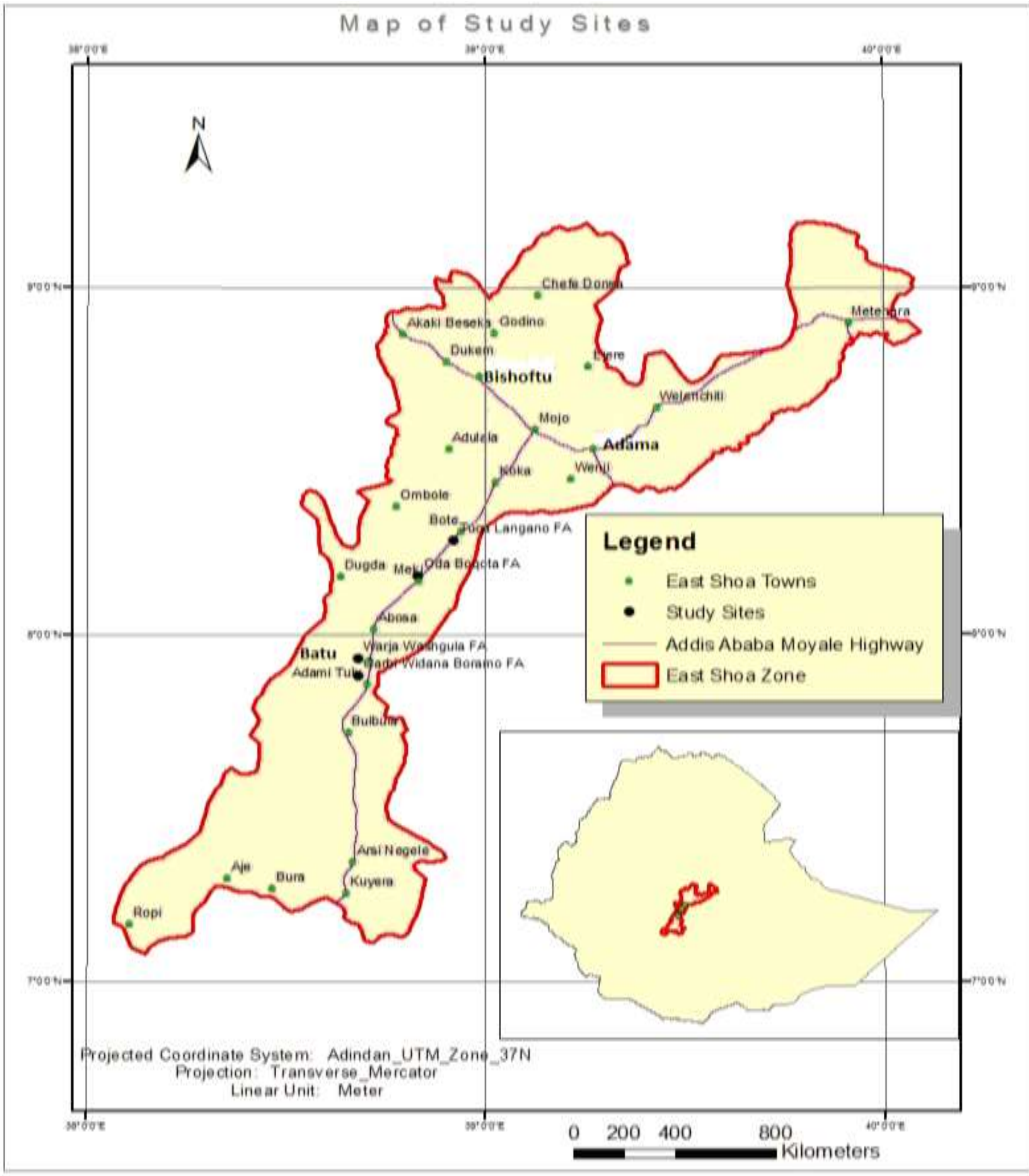

Figure 1. Map showing East Shawa Zone of Oromia Regional State (Source: Daniel and Emana, 2015).

Macrotermes termites are found.

The area has a semi-arid and arid climate and has a bimodal rainfall patterns (Mengistu, 2008). The highest precipitation occurs between July and September with some additional rainfall between February and the end of April, but this usually varies. Rain-fed crops are most often not planted until mid-June because of the unpredictable rainfall pattern in the preceding period (Haimanot, 2002). Coordinates, elevations, mean annual rainfall and temperatures of the capital towns of the three districts are shown in Table 1.

The vegetation in the CRVE is characterized by acacia open-woodland and savanna (Huib and Herco, 2006). The natural vegetation of the area is under high pressure due to the expansion of cultivated land, overgrazing and deforestation and thus the natural flora and fauna are disappearing rapidly (Huib and Herco, 2006; Mengistu, 2008).

Data collection methods and analyses

Farmers' constraints in growing tree seedlings and termite management practices

Short semi-structured questionnaires was dispatched to 64 randomly selected smallholder farmers focusing on general problems they faced in growing tree seedlings (to assess whether they consider termites as one of the problems) and practices they used to manage termites in tree seedlings. Farmers' responses were analyzed and reported as percentages.

To evaluate the efficacy of some non-chemical control methods

Table 1. Coordinates, elevations, mean annual rainfall and temperature at the meteorological stations located in the capital towns of the three districts.

District	Capital towns	Coordinates	Elevation (m)	Mean annual rainfall (mm)*	Mean annual temperature (°C) *	
					Maximum	Minimum
Bora	Bote	8°29'N, 38°91'E	1656	814.5	28.3	13.0
Dugda	Maki	8°15N, 38°82'E	1662	762.8	27.8	14.4
ATJK	Batu	7°56'N, 38°43'E	1650	742.4	27.2	14.0

Source: National Meteorological Agency of Ethiopia; *Average of many years; ATJK = Adami Tulu Jido Kombolcha.

in protecting tree seedlings and for the possible use of the effective method(s) as a management option, a field trial was carried out using *Eucalyptus camaldulensis* seedlings at two sites: Garbi Widana Boramo Primary School (GWPS) (7°53'N, 38°41'E, 1665 m.a.s.l) and Warja Washgula Kebele Administration Farmers Training Center (WWKAFTC) (7°56'N, 38°41'E, 1668 m.a.s.l.). *Kebele* is the lowest administrative level which is below district.

Eucalyptus seedlings mortality under different control options

E. camaldulensis seedlings of five months old were planted in three blocks each containing six plots (2.5 x 2.5 m). Thirty-six seedlings were planted per plot (648 seedlings per site) with a spacing of 0.5 x 0.5 m and 1 m distance between blocks with 3 replications laid out in randomized complete block design (RCBD) based on Mazodze (1995). A total of six treatments were used: T1 (wood ash), T2 (cow dung), T3 (maize stalk), T4 (wood ash + cow dung + maize stalk), T5 (untreated/control) and T6 (Diazinon 60% EC) applied at 2 l/ha as spray on the soil surface and as a soil pit drench around the seedlings as a standard check. Six maize stalks of 40 cm length were buried 20 cm deep in the soil around each seedling in the treated plots; 0.5 kg of cow dung and 0.5 kg of wood ash were added around the base of the treated seedlings. The *Eucalyptus* seedlings raised in plastic bags on nursery beds were provided by Agricultural and Rural Development Bureau of Adami Tulu Jido Kombolcha district and transplanted to the site located at GWPS on July 14, 2012 and to WWKAFTC research site on July 16, 2012.

Eucalyptus seedlings mortality data were analyzed by analysis of variance (ANOVA) with SAS (SAS Institute, 2003). Significant differences between treatments were compared by the least significant difference (LSD) test at 5% probability level. Before analysis data were square root transformed $(X + 0.5)^{1/2}$ to stabilize heterogeneity of variation (Gomez and Gomez, 1984).

Mortality of different species of tree seedlings due to termites

Eleven different species of tree seedlings were transplanted from nursery beds at five sites. *Eucalyptus* seedlings were planted by the researcher on three farmers' fields, while *Sesbania sesban* seedlings were planted on one farmer's field. The tree seedlings at Garbi Widana Boramo Kebele Administration Farmers Training Center (GWBKAFTC) site were planted by the workers of the Farmers Training Center (FTC) and those at Meles Green Park site were planted by the local community.

All the seedlings were raised in plastic bags on nursery beds and were provided freely by Agricultural and Agricultural and Rural Development Bureau of Adami Tulu Jido Kombolcha district. The seedlings were transported to sites by vehicles with care.

Termite damage on the seedlings was recorded starting one month after transplanting and continued every month for 12 months. After 12 months, the numbers of plant dried due to termite infestation of each plant species and site were summed and results were computed as percentages.

During each assessment, wilted and/or dried plants were checked for signs of termite attack above and below the ground. Causes of plant mortality were ascertained by lifting the dried plant and examining the root and stem for typical *Macrotermes* and *Microtermes* damage. The former species cut the base of well-established plants, while the latter species enter and consume the larger roots and prop-roots and continue their excavation into the stem, which can be excavated and packed with soil (Maniania, 2002). Termite species that were found attacking the saplings were collected and preserved in 80% ethanol. Taxonomic identification was done at the genus level using Keys to the Genera of Ethiopian Termites based on soldier characters (Abdurahman, 1991).

Plant mortality assessment was started on the first month after planting, and continued once a month for one year. The numbers of plant that dried due to termite infestation of each plant species and site were summed and results were computed as percentages.

RESULTS

Seventy-eight percent of the respondent farmers mentioned that they had planted tree seedlings for different purposes such as house construction, shade, and for creating good air condition, beauty and environmental rehabilitation. Farmers mentioned that they had faced certain constraints which hinder the establishment of the seedlings after transplanting and their further growth. Of those farmers that planted tree seedlings, 72% considered termites as the most serious problems causing tree seedling mortality followed by livestock (browsing), mole rats and drought (Table 2).

Farmers' termite management practices

Forty percent of those respondent farmers who mentioned termites as problems in growing tree seedlings used different methods of indigenous control, mainly a combination of two or more methods which include good silviculture, cow dung, wood ash and queen removal (Table 3). No one used chemical control method (synthetic termiticide). Application of cow dung and wood ash around base of plants were the major practices used by farmers. Respondents mentioned that termites do not appear in areas where decomposing manure is found.

Table 2. Problems mentioned by farmers in growing tree seedlings after transplanting at Central Rift Valley of Ethiopia.

Response options	Percentage of respondents
Termites	72
Mole rats	28
Drought	20
Domestic animals	60
Salinity	12
Cutworm	4

*The percentages do not sum up to 100 because some individuals mentioned more than one problem they faced in growing tree seedlings.

Table 3. Percentage of respondent farmers that used different indigenous termite management practices against tree seedlings in the Central Rift Valley of Ethiopia.

Management options	Percentage of respondents
Good silviculture and sanitation	17 (6)
Cow dung	56 (20)
Wood ash	61 (22)
Used engine oil	6 (2)
Queen removal and adding hot ash to mound	6 (2)
Opening mound and adding cow dung and wood ash	6 (2)

*The percentages do not sum up to100 because some individuals mentioned that they used more than one management options.

Table 4. *Eucalyptus* seedlings mortality (mean ± SE) one year after transplanting to WWKAFTC site and GWPS site in the Central Rift Valley of Ethiopia.

Treatment	Study sites	
	GWPS	WWKAFTC
Wood ash	0.71 ± 0.00^{b}	0.71 ± 0.00^{a}
Manure	0.71 ± 0.00^{b}	0.71 ± 0.00^{a}
Maize stover	0.88 ± 0.12^{ab}	0.88 ± 0.12^{a}
Wood ash + Manure + Maize stover	1.05 ± 0.12^{a}	0.88 ± 0.12^{a}
Control	0.71 ± 0.00^{b}	0.71 ± 0.00^{a}
Diazinon 60% EC at 2 l/ha	0.71 ± 0.00^{b}	0.71 ± 0.00^{a}
LSD	0.30	0.30

Means within a column followed by the same letter do not differ significantly by least significant difference (LSD) test at 5% of probability level

Eucalyptus seedling mortality under different control options

Seedling mortality due to termites under the different treatments is shown in Table 4. Seedling mortality due to termites under all the treatments was very low. However, higher mortality was recorded in plots treated with combined wood ash, manure and maize stover at GWPS site. There was no significant difference among the treatments at WWKAFTC site.

Mortality of different species of tree seedlings due to termites

The mean percent mortality of different species of tree

Table 5. Mean percent mortality of tree seedlings due to termites after transplanting to different sites in the Central Rift Valley of Ethiopia.

District	Tree seedling species	Site	Number of seedlings transplanted	Percent mortality (n)
ATJK	*Eucalyptus camaldunsis*	FF_1	153	1.3 (2)
		FF_2	54	0.0 (0)
Bora	*Eucalyptus camaldunsis*	FF_3	136	6.6 (9)
ATJK	*Acacia albida* Del.	GWBFTC	53	0.0 (0)
	Jacaranda mimosifolia D.Don	GWBFTC	131	0.0 (0)
	Sesbania sesban (L.)	FF_1	54	0.0 (0)
Bora	*Acacia albida* Del.	MP	171	0.6 (1)
	Acacia senegal (L.) Willd	MP	200	0.5 (1)
	Olea africana Miller	MP	20	0.0 (0)
	Ficus vista	MP	66	0.0 (0)
	Cordia africana Lam.	MP	40	0.0 (0)
	Leacaena leucocephala (Lam.) de Wit	MP	54	0.0 (0)
	Juniperus procera Hochst.	MP	40	0.0 (0)

ATJK = Adami Tullu Jido Kombolcha; FF = farmer's field; GWBFTC= Garbi Widana Boramo Farmers Training Center; MP = Meles Park; n = number.

seedlings due to termites is shown in Table 5. No mortality was recorded in most of the species of the tree seedlings. However, a relatively higher mortality (6.6%) was recorded only on *Eucalyptus* at FF_3. Damage was caused mainly by *Macrotermes* and to less extent by *Odontotermes* and *Microtermes*.

DISCUSSION

Most of the respondent farmers considered termites as one of the major problems in growing tree seedlings and this is basically in agreement with Sileshi et al. (2008) who reported similar results consistent with evidence from literature. For instance, Ugandan farmers ranked termites as the most serious problems in growing trees. However, damage of termites on the tree seedlings, even on the most susceptible exotic plant, *Eucalyptus*, was very low and this was contrary to a wide range of literatures (Hickin, 1971; Harris, 1971; Abdurahman, 1990; Pearce, 1997) and farmers' report in this study. High demand for timber in Africa has led to fast-growing trees, such as *Eucalyptus,* being planted in poor soil areas where they are under stress and are therefore more susceptible to termites (Pearce, 1997). In Cameroon, 100% losses were recorded with *Eucalyptus saligna*, and 60 to 80% with other species; in the drier areas of Uganda between 50 and 70% of transplants were regularly lost; in Northern Nigeria, *E. camaldulensis* had a failure rate of between 68 and 74% in the first eighteen months and 86% after thirty months (Harris, 1971). Cowie and Wood (1989) reported that in some areas in southern, western and eastern Ethiopia, some forest trees are seriously damaged and attack is usually on newly-transplanted seedlings, particularly *Eucalyptus*, and losses could exceed 90%. Further, Abdurahman (1995) noted that in western Wallaga (Ethiopia), termite damage on indigenous trees is insignificant but serious damage is very common on exotic forestry trees, especially on *Eucalyptus*, one to three years after transplanting. In some localities up to 100% of *Eucalyptus* seedling loss is common (Abdurahman, 1995). As per the personal communication made with experts of Asosa Plant Health Clinic, Asosa Zone-, Mana Sibu- and Ayira- districts of Agricultural and Rural Development Offices, termites ranked as number one of all the existing pests. They claimed that there would be total losses in *Eucalyptus* unless chemical termiticides are used. The critical period to prevent termite attack in young plantations of tree seedlings is during the first year in the nursery and a few months after planting out. Later on, as the trees get older, termite infestation may increase, but once a good canopy is formed, attack is often greatly reduced. A positive correlation exists between the presence of drought conditions and incidence of attack (Pearce, 1997).

Tree species like *Acacia senegal*, *Acacia albida, Cordia africana, Ficus vista* and *Olea africana* are all indigenous and the absence of damage be attributed to this factor. Pearce (1997) noted that some of the common tree species seen today in some regions have resulted from selection by termites, that is, the more susceptible ones have been eaten to extinction many years ago. Many of these indigenous trees are therefore more resistant to termite attack and have developed chemical defenses to protect themselves.

The absence of damage or the incident of little damage recorded both on indigenous and exotic tree seedlings may also be attributed to the absence of pest species, low termite population density, and presence of amble food. Abdurahman et al. (2010) reported that in western Wallaga, *Macrotermes subhayalinus, Microtermes, Ancictrotermes, Pseudacanthotermes militaris, Odontotermes* are the most damaging species. However, in the current study, *Ancictrotermes* and *Pseudacanthotermes* spp. were not recorded in the CRVE. All the study sites contained amble wood and grass litter and these might provide sufficient food for termites and thus diverted termites' attention from attacking the tree seedlings. In western Wallaga, where termite damage on *Eucalyptus* seedlings is very high, farmers weed the seedlings and remove the debris out of the fields which leave termites with little food and thus force them to attack the seedlings.

On the other hand, significantly higher termite damage in the plots of *Eucalyptus* seedlings treated with combined wood ash, manure and maize stover than the other treatments at GWPS site might be attributed to the attraction of termites to manure and maize stover. Animal dung and wood are ideal foods for *Macrotermes* termites. In the present study, all the termite species recorded in the area were sampled by using maize stover used as baiting than the other treatments Logan et al. (1990) reported that there are conflicting views on the use of crop residues and other organic matter in termite control. The addition or removal of organic matter from the field has been suggested as methods of reducing levels of attack. Essentially, the conflicting principles are (1) the removal of residues and other debris from the field will reduce potential termite food supplies and hence lead to reduction in termite numbers and subsequent attack; and (ii) that leaving residues in the field or adding further organic matter will provide alternative food to which the termites will be attracted, thereby reducing levels of attack. The respective counter-arguments are (i) that removal of alternative food sources will concentrate termite activity on the crops/trees, leading to increased damage; (ii) that leaving residues and other food sources in the field will provide extra resources and allow termite populations to build up, again to increase damage. There is evidence supporting both views and a generalization may not be possible, different methods being appropriate in different places with different termite species and different crops/trees. The authors have also reported that the use of wood ash is a common practice which demands proper evaluation.

For damage to crops and trees, the first sign of termite presence, especially in seedlings, is wilting. Also, soil runways or sheeting on the soil surface, plants or trees indicates that termites are present. Termites can be collected from the runways and identified. To check for the presence of termites in the field, samples of unhealthy looking plants from the crop should be taken (Pearce, 1997). In the current study, mostly termites of the genus *Macrotermes* were found damaging tree seedlings. Termites of the genus *Macrotermes* are the most common termites in the Central Rift Valley of Ethiopia responsible for lodging of maize plants which cut the plants at ground surface (Daniel and Emana, 2015). Rural houses in the area are mostly damaged by *Macrotermes* (Emana and Daniel, 2014). The genus *Macrotermes* includes several important pests of a wide range of field crops and trees. They attack plants by cutting off young seedlings or mature plants at soil level (Nyeko and Olubayo, 2005).

Conclusion

Although, higher density of *Macroterms* termite mounds are found in the area and most farmers considered termites as one of the major constraints in growing tree seedlings, this study showed very low termite damage to the tree seedlings. The reason for the low damage may be that the termite population is low and/or the termites had enough other food options to eat and thus avoided attacking the seedlings.

Conflict of Interests

The authors have not declared any conflict of interests.

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Determinants of the Brazilian Amazon deforestation

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The Amazon rainforest is the world's largest biome, containing almost 50% of the planet's known biodiversity and is the biggest source of fresh water, which is approximately one-fifth of the global reserves. However, the sustainable use of this ecosystem is threatened by several factors, and deforestation is the main problem. This study addresses the deforestation of the Brazilian Amazon forest, in particular evaluate the determinants of the deforestation process, using for this purpose, time-series of several socioeconomic factors from 1990 to 2015. The methodology applied included statistical analyzes based on the application of multivariate discriminant analysis with the stepwise criteria. The results showed that in order of importance cattle, roads network, population, logging and crop areas were the determinant variables of the deforestation in the amazon.

Key words: Cattle, population, deforestation, Brazilian Amazon.

INTRODUCTION

The Amazon rainforest is the world's largest biome, containing almost 50% of the planet's known biodiversity and its biggest source of fresh water, which is approximately one-fifth of the global reserves. However, the sustainable use of this ecosystem is threatened by several factors, being deforestation its main reason, since it affects natural resources availableness for future generations and jeopardizes a wide range of environmental services, like hydrological cycle, regional climates maintenance and global carbon stocking (Davidson, 2012).

On a regional scale, deforestation promotes ecosystem alterations, such as rainfall decreasing, evapotranspiration reduction, hydric resources contamination (Roulet et al., 2000) and a significant biodiversity loss (Portela and Rademacher, 2001), aggravated by exploration methods used in the Amazon, which increases fauna and flora prejudices and causes a considerable soil productivity loss (Machado and Aguiar, 2001).

Regarding the current deforestation processes, most

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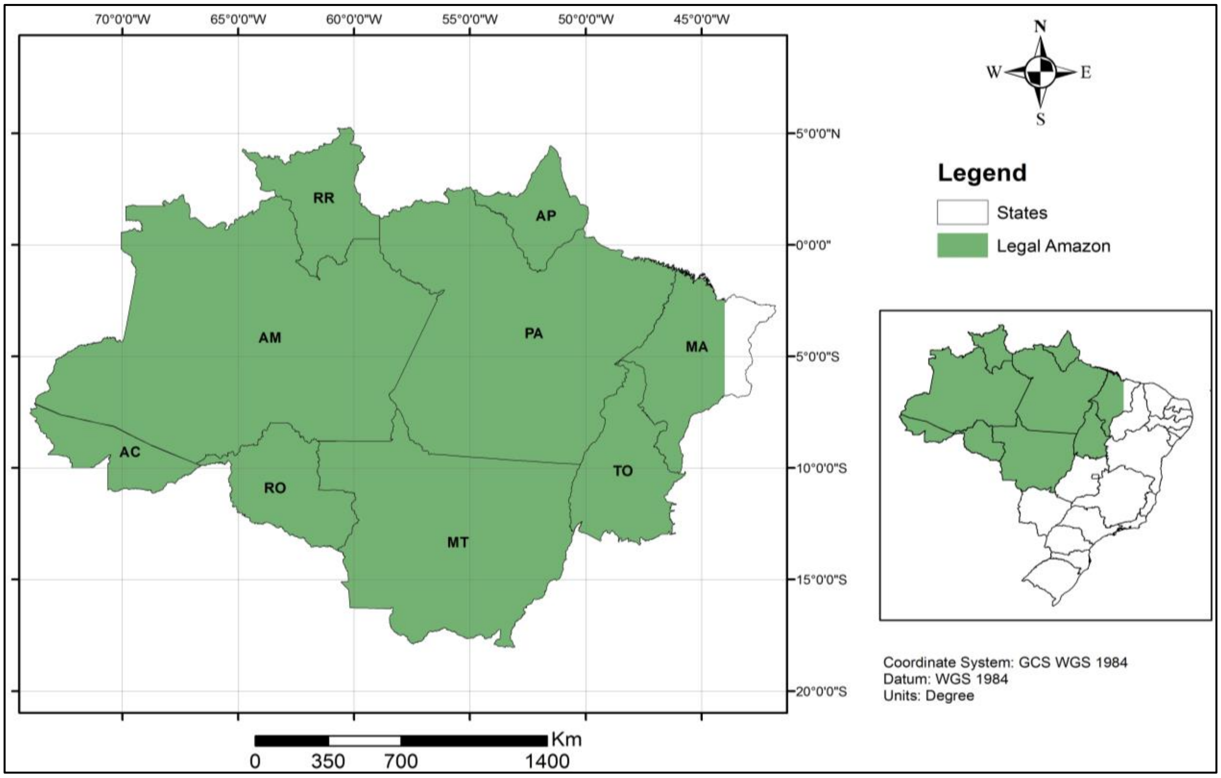

Figure 1. Study area.

researches stated that agricultural activities expansion occurs according to private economic logic (Margulis, 2003). Silva (2006) affirms that most of the Amazon deforestation, until 1997, occurred on lands that presented greater agricultural potential, which was supported by Chomitz and Thomas (2001), who verified that land exploration for agricultural proposes decreases if said area rainfall levels increases, therefore, humid areas are not interesting under an economic perspective, and are least vulnerable to deforestation (Oliveira, 2011). In other researches, major political-economic causes for deforestation in the Amazon are cattle raising (Miragaya, 2008); agriculture of grains (Cattaneo, 2005); logging (Matricardi et al., 2010); and distance to highways (Godar et al., 2012).

Even considering its relevance, between 1990 and 2015, around 358 000 km² of vegetal coverage were destroyed at the Amazon biome. However, in the last few years, deforestation rates decreased, when compared to data collected on early 2000's. In 2004, annual deforestation estimates reached 27.772 km², and from 2005 onwards, a strong decline on the deforestation occurred, reaching 4571 km² in 2015. Nevertheless, even with deforestation reduction in the last few years, the rates are still expressive and can increase again.

Therefore, considering that the Amazon rainforest is indispensable, detailed researches to analyze and comprehend deforestation are necessary in order to support governmental and non-governmental actions to control and reduce deforestation areas.

This research aims to evaluate social-economic variables that influence the Brazilian Amazon deforestation, using as a source, a series of temporal data that covers a period from 1990 to 2015.

MATERIALS AND METHODS

Studied area

The research covers the Legal Amazon area (Figure 1), whose concept originates from Brazilian law number 1.806, January 6th, 1953. With a 5.1 million km² extension, the Legal Amazon included in its domain the seven states of Brazils north region plus the Mato Grosso state and part of Maranhão state (west of the 44º west meridian).

Such official geographic clipping was established considering regional promotion and economic development goals, especially on agricultural frontier areas, influencing the transformation processes of land use.

Studied variables

Variables were chosen based on existing literature focused on studying deforestation that indicated them as causes of forest degradation. Table 1 contains all variables considered in the research and its respective unity of measurement.

Table 1. Variables in the analysis.

Variables	Measures
Deforestation	km²
Cattle	Unities
Logging	m³
Population	Unities
Rural Credit	US$
Road Network	Km
Gross National Product	US$
Crop Area	Ha
Log Price	US$/m³

Database

The database comprehend a period between 1990 and 2015, was collected for every state that constitutes the Legal Amazon, and then the results were organized in a panel; it presented a sampling space whose "n" was equal to 207. Since eight explicative variables was used plus the deforestation as a dependent variable, created a matrix (9 x 207), summing 1863 elements.

Such material was collected using literature focused on that specific matter and pertinent governmental and non-governmental institutes, as well.

The currency used in this study is the dollar (US$), and all monetary values were deflated in order to avoid results distortion caused by inflation.

Statistical analysis

The data set was submitted to statistical analysis, using the multivariate data analysis method, specifically, the discriminant analysis. To perform the statistical analysis the used software were "STATISTICA 8.0" and "SPSS 22".

Discriminant analysis

The discriminant analysis is a statistic technique that enables to identify which variables differentiate the studied groups, and which ones are necessary to improve individuals' classification results in a certain population (Corrar, 2009). Such techniques aim to find a variable that combines others linearly (independents) and that is able to explain, in the best way possible, groups differences. This linear combination is also known as discriminant function:

$$Z_{jk} = a + W_1X_{1k} + W_2X_{2k} + \cdots + W_nX_{nk} \quad (1)$$

In which Z_{jk}= Z discriminant score of the discriminant function j for the k object; W_i = discriminant coefficient for the independent variable i; X_{ik} = independent variable I for the k object.

The (Z) score provides a direct manner for comparing observations in each function. The discriminant function can be expressed by standardized and non-standardized weights and values, considering the standardized version more useful for interpretation purposes (Hair, 2009).

In this research context, the dependent variable was the deforestation, categorized in tree groups (high, medium and low). This categorization was based on states' annual deforestation values quartiles, where lower values regarding the first quartile (lower quartile) were categorized as low, values between the first and third quartile (upper quartile) were characterized as medium and deforestation values whose were above the upper quartile were qualified as high.

The independent variables were chosen according to the literature and data availableness, which was important for discriminant function construction. In other words, while using the discriminant analysis is essential to determine among the sampling elements, variables able to describe population (groups), because otherwise discriminant adjusting quality is compromised (Johnson and Wichern, 2007).

There are automatic selection methods to choose independent variables that might help searching for the most important answer-variables for the discrimination process. Among those methods, the stepwise is one of the most recommendable, being the one chosen for this study.

The step-wise discriminant analysis along the Wilks' Λ method was used to identify which studied variables allow discriminating the tree deforestation intensity groups (high, medium, low). According to this criteria, the variables are included or removed whether its inclusion decreases, or not, the Λ value (Maroco, 2007).

The F value for Λ alteration for when a variable enters or leaves the model is:

$$F = \left(\frac{n-g-p}{g-1}\right)\left(\frac{1-\frac{\Lambda_{p+1}}{\Lambda_p}}{\frac{\Lambda_{p+1}}{\Lambda_p}}\right) \quad (2)$$

In which n is sample global dimension, g is the group number, p corresponds to independents variables number. Λ_p is Wilks' lambda value before adding/removing a new variable and Λ_{p+1} is Wilks' lambda value after adding/removing a new variable. This statistic has an F-snedecor distribution with (g-1) and (n-g-p) liberty rates, and the associated significance probability measures new variable addition/removal significance.

According to Hair (2009), some conditions are necessary to apply the discriminant analysis, such as independent variables multivariate normality, linearity, and variance and covariance matrixes homogeneity and multi-collinearity absence.

In addition, according to Corrar (2009) the two last presumptions are the most relevant, since they affect the discriminant analysis results the most, especially if the analysis goal is identify the characteristics (variables) that most affect the observed groups. To verify the variant and covariant matrixes equality, the Box's M Test is used.

RESULTS AND DISCUSSION

Discriminant analysis

Using the group means equality it was possible to perform a preliminary evaluation aiming to identify which variables are better discriminators for the studied groups. Table 2 shows the results.

According to the Wilks' Lambda the lower the statistic variable, the better its group discrimination capacity, therefore, as seen on Table 2, the variable Cattle is the most capable to define the deforestation groups, since its Wilks Lambda statistics was lower.

On the said table the F-ANOVA test is shown, as well which assists previous test interpretation and evaluation. In this test, the variable Cattle is confirmed as a good option and the variable Log Price is discarded as a

Table 2. Tests of equality of group means.

Variables	Wilks' Lambda	Z	df1	df2	Sig.
Crop Area	0.842	19.092	2	204	0.000
Logging	0.764	31.566	2	204	0.000
Population	0.716	40.447	2	204	0.000
Cattle	0.630	59.816	2	204	0.000
Rural Credit	0.858	16.840	2	204	0.000
Log Price	0.984	1.694	2	204	0.186
PIB	0.684	47.158	2	204	0.000
Road Network	0.699	43.854	2	204	0.000

Table 3. Inserted variables [a,b,c].

Step	Inserted variables	Wilks' Lambda				Exact F			
		Statistics	df1	df2	df3	Statistics	df1	df2	Sig.
1	Cattle	0.630	1	2	204	59.816	2	204	0.00
2	Logging	0.482	2	2	204	44.726	4	406	0.00
3	Population	0.423	3	2	204	36.189	6	404	0.00
4	Crop Area	0.398	4	2	204	29.403	8	402	0.00
5	Road Network	0.373	5	2	204	25.521	10	400	0.00

In each step, the variable that minimizes the Wilks' Lambda is inserted. [a] Steps maximum quantity is 16. [b] F maximum significance to be inserted is 0.05. [c] F minimum significance to be removed is 0.10.

Table 4. Eigenvalues.

Function	Eigenvalues	Variance %	Cumulative %	Canonic Correlation
1	1.325	89.6	89.6	0.755
2	0.154	10.4	100.0	0.365

possible candidate to be included in the discriminant function.

The result of the Box's M Test, the variant/covariant matrixes homogeneity presumption is invalid, based on the test, the null hypothesis was rejected, meaning homoscedasticity absence.

In accordance with study's methodology, the stepwise process was used to select variables that best discriminate the population. The process result is on Table 3.

The stepwise procedure includes variables containing huge discriminant capacity and that were least related among each other (correlated). Therefore, the following variables were included, in this order: Cattle, Logging, Population, Crop Area, and Road Network.

Other variables were not selected (PIB, Rural Credit and Log Price), because, according to the stepwise method the said variables do not contribute to improve the discriminant functions.

Discrimination groups were generated by five explicative variables; they were statistically relevant during proceeding's five steps, according to the p-value. It can be concluded that the discriminant analysis signalized that those five variables are needed to differentiate the groups, with high, medium or low deforestation levels.

Table 4 contains the functions eigenvalues. This statistic indicates that the first function presents a superiority degree when compared to the second function. Each variable explanation capacity is given by the canonic correlation that, in this case, was 0.755 for the first function and 0.365 for the second. By squaring these values, an explanation measure of the variance is obtained: in the first function it is possible to explain 57% of the classification and 13.3% with the second, in other words, functions 1 and 2 are able to explain 70.3% of the total variance.

Analysis next step was to verify if groups' population averages were statistically different from each other, showing that the function is able to define the elements of

Table 5. Wilks' Lambda.

Function tests	Wilks' Lambda	Chi-square	df	Sig.
1 to 2	0.373	199.370	10	0.000
2	0.866	28.951	4	0.000

Table 6. Classification table.

Classification		Deforestation	Predicted Group Association			Total
			Low	Medium	High	
Original	Count	Low	16	36	0	52
		Medium	5	87	12	104
		High	0	7	44	51
	%	Low	30.8	69.2	0.0	100
		Medium	4.8	83.7	11.5	100
		High	0.0	13.7	86.3	100
Cross-validated	Count	Low	15	37	0	52
		Medium	9	82	13	104
		High	0	8	43	51
	%	Low	28.8	71.2	0.0	100
		Medium	8.7	78.8	12.5	100
		High	0.0	15.7	84.3	100

Table 7. Standardized discriminant functions coefficients.

Variables	Function	
	1	2
Crop Area	-0.820	0.864
Logging	0.638	-0.433
Population	-0.053	1.120
Cattle	1.050	-0.749
Road Network	0.577	-0.248

each groups. To testify the discriminant functions the Wilks' Lambda Test was used. The results are on Table 5. The Wilks' Lambda Test showed that is possible to reject the null hypothesis where the groups averages are equal, proving that function 1 and function 2 are significant and can define the groups well.

On Table 6, it is possible to observe the classification efficiency considering the created discriminant functions.

Considering the sample that originated the discriminant functions 1 and 2, it was stated that 30.8% of the samples considered as a low deforestation were classified correctly. As for medium deforestation cases, 83.7% were classified correctly and the high deforestation cases, 86.3% were classified correctly. In relation to the global index, 71% of the deforestation rates were classified correctly.

In the cross validation section each case is classified while leaving it out from the model calculations. The cross-validation global index rate was 67.7%.

By observing the results it is possible to state that the Linear Discriminant Functions presented a satisfactory performance, indicating that the discriminant model is valid and has appropriated statistic levels, because, the success proportion (global index) was higher than the maximum chance criteria, 62.8% and higher than the proportional chance criteria, 47.0%. It is necessary to emphasize that the high deforestation group sample classification, given its elevated accuracy.

In Table 7 it is possible to visualize variables standardized coefficients that are part of the discriminant functions developed with the chosen methodology.

Comparing Table 7 coefficients with Table 8 coefficients

Table 8. Rotated discriminant functions coefficients.

Variables	Functions	
	1	2
Cattle	1.192*	-0.493
Crop Area	-0.993*	0.658
Logging	0.718*	-0.278
Road Network	0.618*	-0.112
Population	-0.304	1.079*

* Higher absolute coefficient among the discriminant functions.

Table 9. Structure Matrix.

Variables	Function	
	1	2
Cattle	0.665*	0.096
Road Network	0.559*	0.322
Logging	0.482*	-0.111
Crop Area	0.369*	0.210
Population	0.469	0.824*

*Higher absolute correlation between each variable and any discriminant function.

Table 10. Potency index.

Variables	Potency Index
Cattle	0.397
Road Network	0.291
Population	0.268
Logging	0.209
Crop Area	0.127

it is possible to observe that after functions rotation, in this case the “Varimax” rotation, a load distribution improvement occurred, which enables a better coefficients evaluation.

It is possible to analyze the structural coefficient matrixes in Table 9, which enabled to correlate the independent variables with the discriminant functions 1 and 2.

The structural coefficients, or discriminant loads, provided functions variables importance. The higher the structural variable coefficient value, the higher its discriminant capacity. Therefore, first function describing variables are Cattle, Road Network and Crop Area. The variable Population is the describing variable of the second function.

Using the obtained values on Table 10, it is possible to affirm that according to the potency Index, the variables that most differentiate whether the deforestation level is high, medium or low were the following: Cattle, Road Network, Population, Logging and Crop Area, respectively.

Thus, the variable “Cattle” that represents the cattle amount existing in Legal Amazon states held an important position in this deforestation phenomenon analysis, showing that the cattle raising during the study period was the major influence over Brazilian Amazon Deforestation.

Several other studies also stated that cattle (Miragaya, 2008), road network (Pfaff et al. 2007), population (Alves, 2010), logging (Asner et al., 2005) and crop areas (Cattaneo, 2005) are determinants for the Brazilian Amazon Deforestation. However, this research is different because identifies determinants’ order of importance and magnitude, in other words, the variables that better discriminate whether the deforestation levels were high, medium, low considering its relevance.

Due the impact caused by the deforestation determinants highlighted in this research, it is recommended take priority on using these factors to elaborate strategies designed to handle deforestation in the Amazon.

It is possible to affirm that according to the results, there are three major forces: agriculture expansion, demographic expansion and logging, these forces are interconnected and cause the mass deforestation. Regarding the population expansion, such phenomenon is accompanied by the infrastructure increasing, mostly the roads, which was highlighted in this research, as well.

Figure 2 demonstrated territorial map and the graphic representation of each group discriminant functions centroids. On the map, it is possible to visualize that function 1 is able to determine the high groups of medium and low deforestation levels, while function 2 separates low and medium groups but it can be observed a substantial overlap among these groups, which affects low deforestation group classification.

In general, the results indicate that an enormous concern regarding forest areas zoning and managed public forests conservation areas should exist. Financing and cost-cutting mechanisms should be established in order to encourage sustainable practices, developing instruments that restrict and raise deforestation costs are some of the public policy strategies that must be implemented.

Angelo (2008) defends adopting economic incentives as and strategy to decrease deforestation rates and encourage sustainable management. The lack of such strategies turns Amazon’s soil exploration more economically interesting for other practices.

Conclusions

The presented research evaluates the deforestation matter in the Brazilian Amazon, using the discriminant analysis and the statistical results obtained from it.

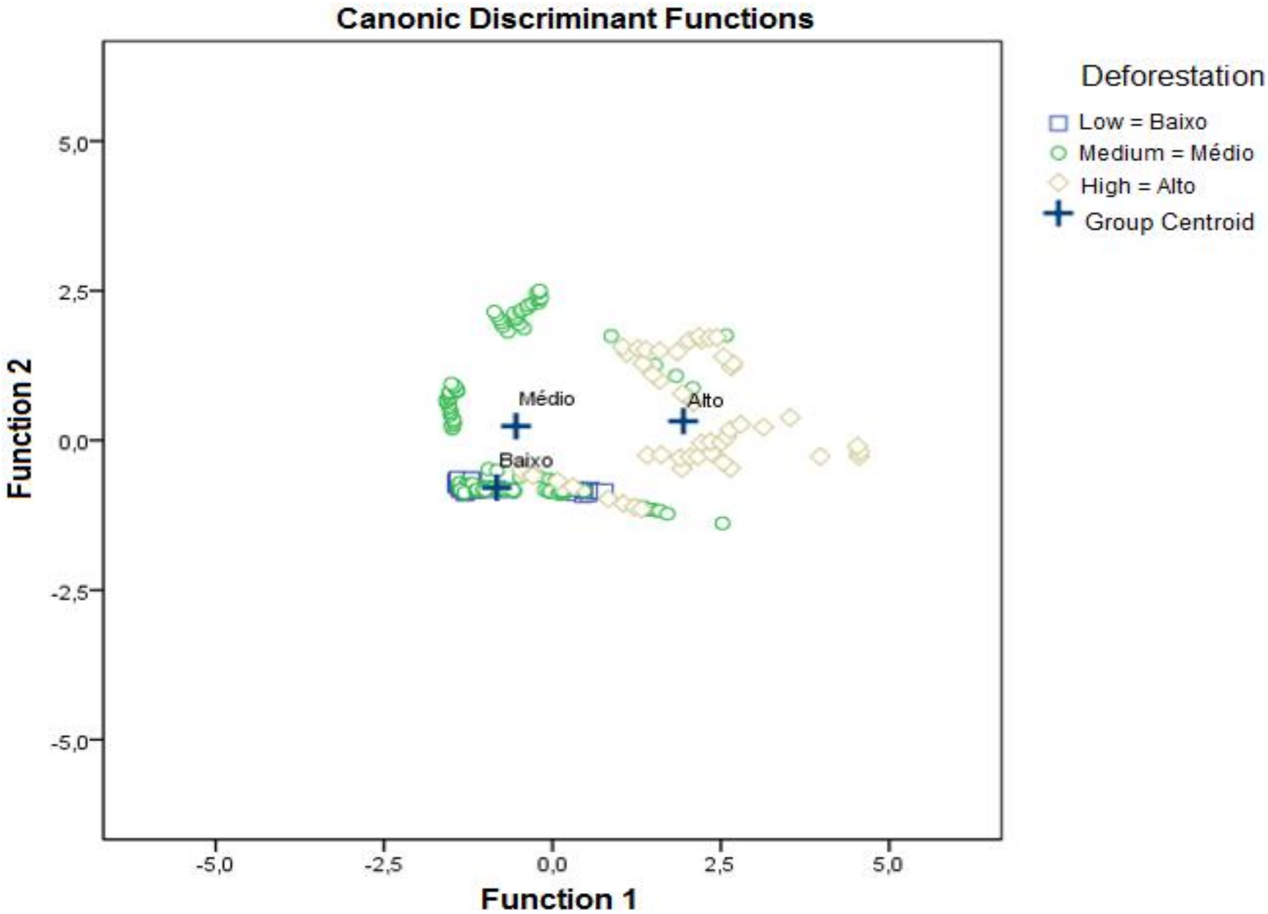

Figure 2. Territorial map.

Therefore, the Linear Discriminant Functions 1 and 2 presented on the results had a classification performance with 71% of success (global index) indicating that the discriminant model is valid and has appropriated statistic levels.

Based on this discriminant model, the deforestation variables were ranked by the potency index that represent the discriminant capacity of the variables. In decreasing order of discriminant power: cattle, road network, population, logging and crop areas. These highlighted variables were defined in this order as the major factors that contribute for the deforestation process and since the deforestation is a complex phenomenon, the research contribution is the statistical support that despite the population and agriculture contribution to the deforestation the major force was the cattle expansion.

In order to reduce the deforestation, this type of information is important to support public policy and strategies aiming to preserve the Amazon biome.

Although, it is imperative to implement public policy mechanisms, that not only fight against the environment aggressions, but also encourage the biome's conservation and sustainable use.

Conflict of Interests

The authors have not declared any conflict of interests.

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Incidence of viruses and virus-like diseases of watermelons and pumpkins in Uganda, a hitherto none-investigated pathosystem

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Common impromptu observations between 2010 and 2012 of apparent virus-like disease symptoms in watermelons and pumpkins in Uganda prompted this study. However, there was no recorded evidence of virus infection in these crops anywhere in Uganda or eastern Africa as a region. Thus, 374 and 522 watermelon and pumpkin plants, respectively, growing in 13 fields were surveyed to record virus-like disease symptoms in Uganda's four districts of Mbale and Kamuli (eastern region), Mpigi and Masaka (central region) during August to November 2013, and January to March 2014. Leaf samples were also collected and tested for four viruses known to commonly infect watermelons and pumpkins worldwide. Symptom severity was assessed using a scale of 0 to 5, while virus incidence was determined by serological methods. The four viruses tested were *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucurbit aphid borne-yellows virus* (CABYV). In both crops, there was a higher incidence of virus-like diseases in central than in eastern region (P = 0.000). All the four viruses were detected with CMV as the most common, followed by WMV, ZYMV, and CABYV. Differences in virus incidences between the districts were not always significant. Single CMV, dual CMV+WMV and triple CMV+WMV+ZYMV were the most common single, and multiple infections in both crops. Watermelons contained more single virus infections than mixed infections (P < 0.001), but this difference was not significant in pumpkins (P = 0.468). In all, single virus infections were significantly higher in watermelons than pumpkins (P < 0.001). This is the first study to report incidence of viruses and virus-like diseases in watermelons and pumpkins in Uganda, and in eastern Africa as a region. The importance of these results with respect to crop production and next steps in virus disease management are discussed.

Key words: Cucurbits, DAS-ELISA, multiple virus infection, TAS-ELISA, virus incidence, Uganda.

INTRODUCTION

Plant diseases caused by viruses are major biotic constraints in crop production worldwide (Anderson et al., 2004, Alabi et al., 2011; Navas-Castillo et al., 2011; Tzanetakis et al., 2013). However, the impact of plant virus diseases is exacerbated in the tropics because tropical conditions favour continuous presence of both primary and secondary hosts and super-abundance of vectors that efficiently transmit the viruses (Fargette et al., 2006; Morales, 2007; Barult et al., 2010; Navas-Castillo et al., 2011; Geering and Randles, 2012; Fereres and Raccah, 2015). Moreover, virus diseases in non-priority crops at local or regional scale when ignored or left to ‘fallow’ may constitute none investigated pathosystems as sources of harmful viruses in surrounding cropping systems (Jones et al., 2010; Lebeda and Burdon, 2013). An example is the watermelon/pumpkin pathosystem in eastern Africa including Uganda that has not been studied, despite pumpkins being common intercrops of other well characterized pathosystems such as cassava (Legg et al., 2011; Ndunguru et al., 2015) and sweetpotato (Valverde, 2007; Clark et al., 2012).

Watermelon and pumpkins belong to the family Cucurbitaceae (cucurbits) which consists of approximately 118 genera, and over 820 species (Lebeda et al., 2006). There are several edible species such as melon (*Cucumis melo* L and *Citrullus lanatus* (Thunb) Matsum and Nakai), cucumber (*Cucumis sativus* L), pumpkin (*Cucurbita moschata* Duchesne) and (*Cucurbita maxima* Duchesne), zucchini and squash (*Cucurbita pepo* L.) (Lebeda et al., 2006). Cucurbits are produced in several parts of the world, of which China, USA, Europe and the Mediterranean countries together account for over 96% of the world’s production (FAOSTAT, 2016). In Uganda and surrounding countries of eastern Africa, watermelons and pumpkins are the only main cucurbits of some economic importance. However, these crops have not been prioritized in Uganda. For example, only 7.0% and less than 1.0% of 3,630 households sampled in 6 rural districts of Uganda were involved in the production of pumpkins and watermelons, respectively in 2012 (Kabunga et al., 2014). Nonetheless, the food and high nutritional security benefits of fruit and vegetable production such as watermelon and pumpkin at household level can be improved if there were deliberate efforts to promote the intensification of smallholder production of these crops (Kabunga et al., 2014).

Worldwide, over 60 species of viruses in different taxonomic groups have been detected and/or characterized from cucurbits (Lecoq, 2003; Akhtar et al., 2012; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah, 2014; Romay et al., 2014). The Mediterranean region is implicated for the highest virus diversity in cucurbits from where at least 28 different viruses have been detected (Lecoq and Desbiez, 2012). This high virus diversity is attributed not only to the genetic and ecological diversity of their hosts, but also to the huge diversity of agro-ecosystems, ranging from highly heated glass houses to traditional rain-fed cultivation providing favourable conditions for vector-mediated transmission of the viruses (Lecoq and Desbiez, 2012). Globally, *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family Bromoviridae) is the most widespread of the cucurbit-infecting viruses, followed by *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) (genus *Potyvirus*, family Potyviridae), and *Cucurbit aphid borne-yellows virus* (CABYV, genus *Polerovirus*, family Luteoviridae) (Lecoq, 2003; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah, 2014; Romay et al., 2014).

However, this hierarchy is variable depending on time, host, and geography. Aphids of different species transmit CMV, WMV and ZYMV non-persistently, or CABYV persistently (Dodds et al., 1984; Kishaba et al., 1992; Lecoq and Desbiez, 2012), but seed-transmission of ZYMV (Simmons et al., 2011, 2013) and CMV (Zitter and Murphy, 2009; Jacquemond, 2012) are also known. The geographical and host range of plant viruses is delimited more by virus-vector than virus-host relations (Power and Flecker, 2003, Power, 2008), which in accordance with its broad vector range makes CMV cosmopolitan infecting over 1200 species in over 100 families of plants worldwide (Zitter and Murphy, 2009; Jacquemond, 2012; Lecoq and Desbiez, 2012).

In Africa, with an exception of a few studies in some northern, western and southern countries (Lecoq et al., 1994, 2001, 2016; Nono-Womdim, 2003; Yakoubi et al., 2008; Mnari-Hattab et al., 2009; Mohammed et al., 2014; Ibaba et al., 2015), studies of plant viruses infecting cucurbit crops are generally limited on the continent, and almost non-existent in eastern Africa including Uganda. Instead, a lot of effort has been invested in viruses of other crops (maize, cassava, sweetpotato, and banana), yet, pumpkins are often intercropped with these crops. In Uganda, watermelons are mainly grown as a single crop in relatively large fields, sometimes in the neighbourhood of other crops or the periphery of wetlands (Turyahabwe et al., 2013). In contrast, pumpkins are grown on a smaller scale usually integrated into the coffee-banana agro-forest production systems where coffee, cassava, sweetpotato, and banana are the main crops (Munyuli, 2011). The complex inter-cropping systems allow the direct exposure of pumpkins to many viruses that infect other crop plants into which they are intercropped in Uganda.

Between the year 2010 and 2012, we inadvertently

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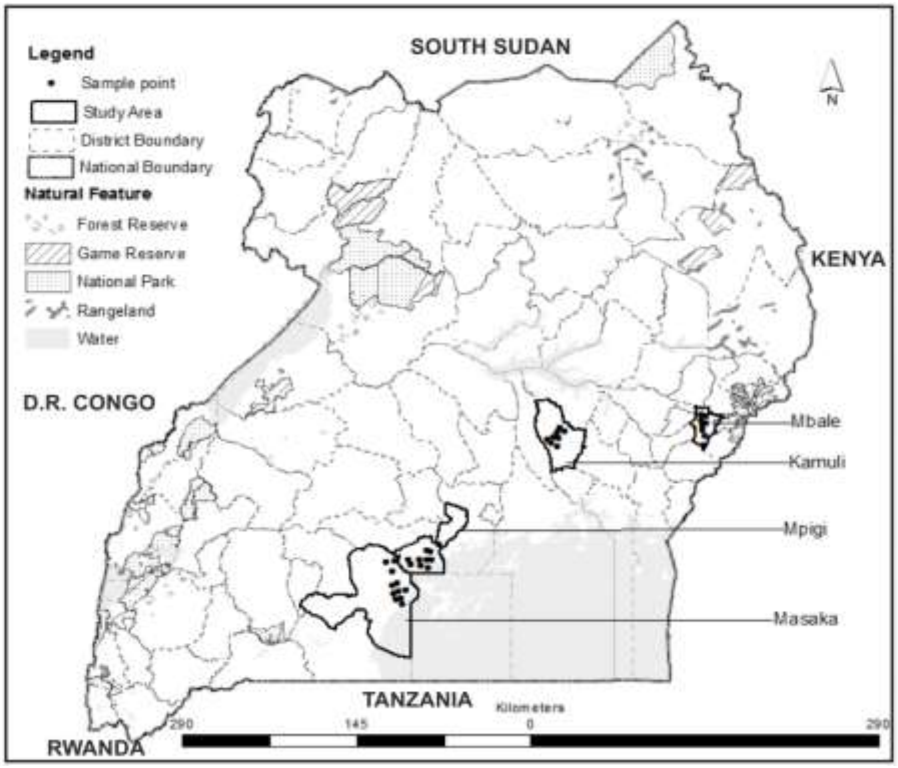

Figure 1. Map of Uganda showing the four districts (Mbale, Kamuli, Mpigi, and Masaka) that were surveyed for viruses and virus-like diseases in watermelons and pumpkins.

observed in various districts of Uganda typical virus disease symptoms (mosaics, yellowing, necrosis, stunting) on pumpkins and watermelons, similar to those described previously for cucurbits (Lecoq and Desbiez, 2012). Consequently, we followed up with systematic studies to assess symptoms and test for viruses in a few districts as a probe into this non-investigated pathosytem.

Therefore, the aims of this study were to determine the prevalence of virus-like diseases on watermelons and pumpkins in four districts located in different agro-ecological zones of Uganda, and to use serological methods for determining incidence of four viruses, namely, CMV, WMV, ZYMV and CABYV in the plants. The study was carried out in two surveys, during which 374 and 522 watermelon and pumpkin plants, respectively were assessed for the viruses and virus-like diseases.

MATERIALS AND METHODS

Watermelon and pumpkin fields surveyed

Two of the agro-climatic zones of Uganda, namely the central (Lake Victoria basin), and eastern zone that reflect different rainfall patterns and diversities of cropping systems (Mubiru et al., 2012; Dixon et al., 2014), were surveyed for pumpkins and watermelons and four viruses (CMV, WMV, ZYMV, and CABYV). The Lake Victoria basin (1174 to 1235 meters above sea level) experiences two relatively dry periods (December to March and June to July), while peak rainfall (1250 to 1500 mm per annum) periods are in March to May and October to November, and a minimum temperature of 12°C (NEMA, 2009). Eastern zone has a mean altitude of 1075 meters above sea level) also receives high rainfall greater than 1200 mm twice a year during (April to June and July to September). The minimum temperature is 15°C (NEMA, 2009). The micro-climates, rainfall patterns and cropping regimes in these zone are traditionally influenced by Lakes Victoria and Kyoga (Huxley, 1965; Phillips and McIntyre, 2000; Herrman and Mohr, 2011).

From each agro-climatic zone, 2 districts that commonly grow pumpkins and watermelon (Mpigi and Masaka in the Lake Victoria basin; Kamuli and Mbale in Eastern zone) (Figure 1) were chosen and surveyed for plants of these crops displaying virus-like disease symptoms and those healthy-looking. The surveys were carried out in August to November 2013, and repeated on the same fields in January to March 2014. In each district, fields surveyed (located 20 to 40km apart) were chosen from areas known to most grow watermelons or pumpkins. Sampling of plants in field was conducted as described by Tugume et al. (2008). The plant samples were identified in fields (gardens) of watermelon, and pumpkins, or in fields where watermelon or pumpkins had been intercropped with cassava, banana, millet, maize, sorghum, or sweetpotato. The number of fields surveyed per district ranged from 3 to 4. Overall, 9 to 13 fields were surveyed throughout the study.

Sampling and virus disease assessment in watermelons and pumpkins

Leaf samples from 15 to 20 plants of watermelon, pumpkin or both, expressing virus-like symptoms were sampled from each field. In addition, leaf samples were also collected from some symptomless plants. The leaf samples were picked by moving diagonally across the field from one individual plant to the next as described by Aamir et al. (2010). Because of the creeping growth habit of watermelons and pumpkins, care was taken not to sample the same plant more than once. Virus-like symptom severity was scored visually in the field on a scale of 0 to 5, based on extent of leaf damage; 0 = non-symptomatic; 1 = mild yellowing, mild rugosis or mild necrosis; 2 = yellowing symptoms, rugosis or necrosis; 3 = severe yellowing, severe rugosis and severe necrosis; 4 = severe yellowing, severe rugosis, severe necrosis and mild stunting; 5 = stunting, severe yellowing, severe necrosis as described previously by Coutts et al. (2011). The sampled leaves were placed in plastic polythene bags, placed in a cool box and transported to the laboratory at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) for serological detection of the viruses.

Serological detection of four viruses in watermelons and pumpkins

A total of 374 and 522 plants of watermelon and pumpkins, respectively, were each tested for four viruses; CMV, WMV, ZYMV, and CABYV. Leaf discs (3 to 5 discs, each 2-cm in diameter) were excised from leaves of a plant, combined, and tested for CMV, WMV, and ZYMV using Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA). In contrast, CABYV was tested using Triple Antibody Sandwich-Enzyme Linked Immunosorbent Assay (TAS-ELISA). DAS-ELISA and TAS-ELISA procedures were as described by Clark and Adams (1977), with minor modifications using polyclonal antibodies supplied by DMSZ Braunschweig (Germany) and monoclonal conjugate antibodies supplied by BIO-RAD (Hercules, California, United States). Each sample was tested in triplicate.

Briefly, DAS-ELISA plates were coated with WMV-, CMV- and ZYMV-specific polyclonal antisera (first antibody) in a coating buffer (pH 9.6) for 12-16 hours at 4°C. The excess unbound antibodies were washed off three times using 0.05 % Tween-20 in PBS buffer (Clark and Adams, 1977) after which sap of test plant samples (extracted using the sample extraction buffer) were added and plates incubated overnight at 4°C. After washing three times with 0.05 % Tween-20, a 3000-times diluted alkaline phosphatase conjugated monoclonal antibody (BIO-RAD) (the second antibody) was added, and the ELISA plates incubated for 4 h at 30°C. The plates were washed three times for the third time with 0.05% Tween-20.

In TAS-ELISA for testing CABYV, the addition of alkaline phosphatase conjugated monoclonal antibody (BIO-RAD) was preceded with a second CABYV-specific monoclonal antibody (DMSZ Braunschweig, Germany). These antisera were supplied with lyophilized leaf samples of pumpkins infected with CMV, WMV, CABYV, or zucchini infected with ZYMV, which were used as positive controls in the ELISA assays. The presence or absence of CMV, WMV, ZYMV, and/or CABYV was confirmed through color development following the addition of *p*-nitrophenyl phosphate (1 mg/mL) in substrate buffer after incubating at room temperature in the dark for 60 to 120 min. Absorbance values were measured at 405 nm using a microplate reader (BIO-RAD Laboratories, USA). Samples were considered to be positive only when their absorbance values were greater than three times that of the blank/negative control (Wang and Gonsalves, 1990).

Data analysis

Field virus-like disease incidence (here defined as the extent of disease infection of the field) was calculated by expressing the number of infected plants as a percentage of the total number of plants in the field, estimated as percentage infection; in this case, 1 to 20%, 21 to 49%, 50 to 100% infection is considered as low, moderate, and high incidences, respectively (Nono-Womdim et al., 1996). Prevalence of disease in a given district was determined by expressing the total number of fields with plants showing virus-like symptoms as a percentage of all fields surveyed in that district. Data generated from DAS- and TAS-ELISA tests was recorded in binary form as 0 or 1 to imply absence or presence of a specific virus and analysed using StataCorp LP 4905 (Lakeway Drive College Station, Texas USA).

RESULTS

Fields of watermelon and pumpkin sampled

In all, 171 and 267 plant leaf samples of watermelon and pumpkins respectively were observed and collected for virus testing during August to November 2013. Testing was repeated on additional 176 and 255 leaf samples of watermelon and pumpkins respectively collected during January to March 2014. Thus, 347 plants of watermelon and 522 plants of pumpkins were assessed throughout the study. The plant samples were collected from fields where plants were found in varied cropping systems in close proximity (10 to 20 meters) to other crops or wild vegetation. For example, most watermelon samples were obtained from fields that had been planted as a monoculture crop (Figure 2) and in rare circumstances where watermelon was intercropped with maize or beans (*Data not shown*). In some cases, watermelon fields were neighbouring other crops such as banana, cassava, or sweetpotatp (Figure 2A and B) while in other cases, isolated fields had been established far from other crop stands (Figure 2C). No watermelon samples were collected from Kamuli because watermelon fields were not found in the areas surveyed of this district in the two consecutive surveys. In contrast, pumpkin plants in all districts were either intercropped in maize, beans, sweetpotato, or banana (Figure 2D), although in some cases, the plants grew out of accidental seed drops on rubbish heaps near homesteads (Figure 2E and F). No field was found with watermelon and pumpkins intercropped with each other.

Three fields of watermelon in each district (except Kamuli; total of 9 fields) (Table 1) were observed and assessed in August to November 2013, and then repeated in January to March 2014 for incidence of virus-like diseases and plants tested for viruses. In contrast, a total of 13 pumpkin fields were assessed with 3 fields from each district, except Kamuli from where 4 fields were assessed (Table 1). On average, disease prevalence was lowest in fields from eastern Uganda than central Uganda (Table 1). For example in watermelons, lowest prevalence was recorded in Mbale (15.1 %) and highest in Mpigi for which almost two thirds of all plants in the fields showed virus-like diseases (57.8 %) (Table 1). This same trend was observed in pumpkins

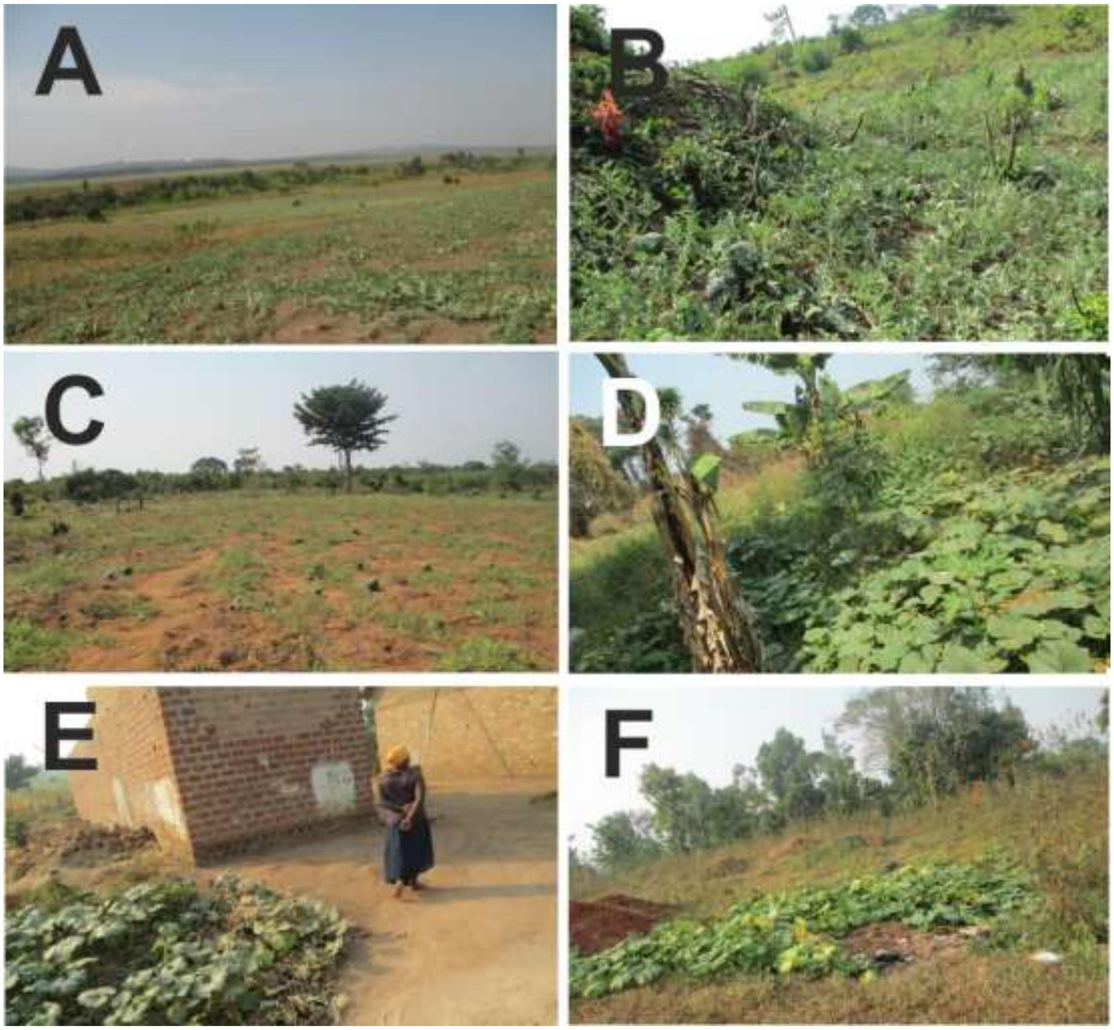

Figure 2. Examples of crop fields of watermelon (A, B and C) and pumpkins (D, E and F) in which the plants were observed for virus-like symptoms and collected for virus testing. Fields of watermelons near cassava and banana fields on the left in Masaka district (A); fields of watermelon planted in a newly cleared area growing next to sweetpotato in the background in Mbale district (B); watermelon field established in a new field not neighbouring any other crops in Masaka district (C). Watermelons were usually grown in relatively large monocultures (A, B and C) as compared to pumpkins which were commonly intercropped with other crops like banana, cassava and other crops (eg., D, taken in Masaka district) and near homesteads (for example, E, in Kamuli district). None of the watermelon plants were found in isolation from other watermelon plants, as opposed to some pumpkin plants found growing in isolation well detached from other crops (e.g., **F**, in Mpigi district).

with prevalence of 13.0 (Mbale) to 55.3% (Masaka).

Incidence of virus-like disease symptoms in watermelon

Of the 171 watermelon samples collected in the first survey (August to November 2013), 136 plants (79.5 %) were from symptomatic plants displaying various disease symptoms typical of virus infection in plants. In the second survey (January to March 2014), 143 of 176 samples (81.3 %) collected were from plants showing virus-like symptoms. There was no statistically significant difference in severity of virus diseases symptoms between the two surveys (χ^2 = 8.9459, df = 5, P = 0.111). In all, 68 plants (19.6 %) had no virus disease symptoms. Plants displayed various virus-like symptoms such as yellowing, rugosis, necrosis, blistering and stunting while some few showed a mixture of different symptoms (Figure 3). On average, virus-like disease incidence of 30.9, 45.7 and 52.9% was observed in Mbale, Mpigi, and Masaka, respectively. The most common virus-like disease symptoms observed in watermelon were yellowing and rugosis (24.8%), mild yellowing (22.5%),

Table 1. Prevalence of virus-like diseases in fields of watermelon and pumpkins from four districts of Uganda across the two surveys in 2013 and 2014. No watermelon fields were surveyed in Kamuli district because no watermelons were found grown there for the two consecutive surveys. Virus-like diseases incidence was 0.0% in watermelon in Mbale field 1 in 2013 and field 2 in 2014 because these fields were too young/newly planted (field 1 in 2013) or had just gotten harvested (field 2 in 2014).

District	Field	Incidence of virus –like diseases in the field					
		Watermelons			Pumpkins		
		Aug-Nov 2013	Jan-Mar 2014	Average	Aug-Nov 2013	Jan-Mar 2014	Average
Mbale	1	0.0	30.2	15.1	20.1	15.6	17.9
	2	60.7	0.0	30.4	10.6	15.4	13.0
	3	55.2	39.1	47.2	15.5	20.7	18.1
	Average	57.9	34.7	-	15.4	17.2	-
Kamuli	1	-	-	-	50.3	30.1	40.2
	2	-	-	-	40.2	20.1	30.2
	3	-	-	-	25.6	20.4	23.0
	4	-	-	-	30.2	25.8	28.0
	Average	-	-	-	36.6	24.1	-
Mpigi	1	60.0	55.6	57.8	70.9	30.1	50.5
	2	45.1	42.7	43.9	60.8	25.7	43.3
	3	50.2	20.3	35.3	40.7	60.0	50.4
	Average	51.8	39.5	-	57.5	38.6	-
Masaka	1	55.0	45.2	50.1	50.5	30.0	40.3
	2	60.0	50.3	55.2	70.6	25.4	48.0
	3	52.1	30.0	41.1	80.3	30.2	55.3
	Average	55.7	41.8	-	67.1	28.5	-

Figure 3. Some of the virus-like symptoms observed in watermelon plants in the field. Symptomless young (A) and old (B) plants which tested positive for CABYV and WMV, respectively. Rugosis in young (C) and leaf chlorosis in old plants (D). Many watermelon plants were found infested with aphids (E). Sometimes clarity of symptom description was hampered by the total destruction of watermelon shoot system by the virus-like diseases in which case only tinny leaf areas were left as is in (F, taken in Masaka district, central Uganda). In such cases, the diseases wiped out premature watermelon plants.

Table 2. Incidence of virus-like disease symptoms in watermelon and pumpkin plants in the districts of Mbale, Mpigi, Masaka, and Kamuli. Numbers in parentheses are the total number of samples showing a given virus-like disease symptom(s) while those outside the parentheses are their respective percentages in a given district. P values were deduced by comparison of virus-like disease severity between districts. Statistically significant values of P are indicated with an asterisk (*).

Symptom severity/District	Watermelon plants							Pumpkin plants							
	Mbale	Mpigi	Masaka	Total	Df	X2	P	Mbale	Kamuli	Mpigi	Masaka	Total	Df	X2	P
0-Symptomless	61.7 (42)	10.3 (7)	27.9 (19)	19.6 (68)	10	42.659	0.000*	37.3 (53)	26.8 (38)	13.4 (19)	22.5 (32)	27.2 (142)	15	45.976	0.000*
1-Mild yellowing	25.6 (20)	33.3 (26)	41.0 (32)	22.5 (78)	-	-	-	21.5 (37)	31.4 (54)	26.2 (45)	20.9 (36)	33.0 (172)	-	-	-
2-Yellowing, rugosis	29.1 (25)	38.4 (33)	32.6 (28)	24.8 (86)	-	-	-	12.1 (11)	39.6 (36)	24.2 (22)	24.2 (22)	17.4 (91)	-	-	-
3-Severe yellowing	30.5 (18)	49.2 (29)	20.3 (12)	17.0 (59)	-	-	-	12.2 (9)	24.3 (18)	27.0 (20)	36.5 (27)	14.2 (74)	-	-	-
4-Mild stunting	20.0 (10)	45.0 (18)	30.0 (12)	11.5 (40)	-	-	-	16.7 (6)	16.7 (6)	30.6 (11)	36.1 (13)	6.9 (36)	-	-	-
5-stunting	18.8 (3)	50.0 (8)	31.3 (5)	4.6 (16)	-	-	-	28.6 (2)	14.3 (1)	42.9 (3)	14.3 (1)	1.3	-	-	-

and severe yellowing (17.0%).

Most of the disease symptoms on watermelons were observed in Mpigi (94.2%) and Masaka (82.4%) as compared to Mbale (64.4%) (Table 2). Some of the symptoms in watermelon plants are shown in Figure 3. Most plants displaying severe yellowing and stunting were mostly encountered in samples from Mpigi (46.4%) and Masaka (30.4%) as compared to Mbale (23.2%). There was a higher incidence of virus symptoms in central Uganda districts (Mpigi and Masaka) than in the eastern district of Mbale (Table 2). These differences were statistically significant (χ^2 = 42.659, df = 10, P = 0.000) (Table 2). In addition, virus-like diseases were present in all (100 %) of the twelve watermelon fields surveyed in central Uganda (Masaka and Mpigi), compared to 66.7% disease prevalence in watermelon fields in Mbale.

Incidence of virus-like disease symptoms in pumpkin

A total of 522 pumpkin plants (267 in the first, and 255 in the second survey) were assessed under this study in the four districts of Mbale, Kamuli, Mpigi and Masaka. Plants that displayed no symptoms were 142 (19.6%) as compared to 380 plants (72.8%) which displayed various kinds of symptoms. Like for watermelons, the observed virus disease-like symptoms on pumpkin plants includes yellowing, rugosis, necrosis, blistering and stunting or a mixture of these symptoms (Table 2, Figure 4). The most common virus symptoms observed in pumpkins were mild to severe yellowing (33.0%), yellowing and rugosis (17.4%), and stunting (7.0%) (Table 2). More symptomatic pumpkin plants were observed in the central districts of Mpigi (32.6%), and Masaka (32.6%), as compared to the eastern districts of Mbale (18.6%) and Kamuli (16.3%), making the eastern districts with low disease incidences (Table 2). These differences were statistically significant (χ^2 = 45.976, df = 15, P = 0.000) (Table 2). Prevalence of virus-like diseases was higher in central region (Masaka and Mpigi) where in each case 83.0% of the pumpkin fields had virus-like disease symptoms as compared to 50.0% in Mbale and 75.0% in Kamuli (eastern region). Considering watermelons and pumpkins together, higher disease incidence was recorded in the central than eastern districts surveyed, and watermelon showed a higher disease incidence (80.4 %) than pumpkins (72.8 %).

Incidence of four viruses in watermelons and pumpkins

In the 347 watermelon plants assayed, CMV was the most frequently detected virus, found in 74.1% of the plants. The other three viruses; WMV, ZYMV, and CABYV were detected in 61.4, 21.6 and 19.0% of watermelon plants respectively (Table 3). These viruses were detected in at least one watermelon plant from each of the three districts of Mbale, Mpigi, and Masaka. With the exception of WMV for which there was a significant difference in the incidence between districts (χ^2 = 24.896, df = 2, P = 0.000), the other three viruses (CMV, ZYMV, and CABYV) showed no significant difference in incidence between the districts (Table 3). Most of the watermelon plants (36.2 %) infected with CMV were found in Mpigi (Table 3). Apart from CABYV, there were significant differences in the incidence of viruses between the two survey periods (Table 4).

Thus, CMV was more frequently detected in the first survey (August to November 2013) than in the second survey (January to March 2014) (χ^2 = 18.071, df = 1, P = 0.000) (Table 4). Also, more WMV incidence was recorded in the first survey (57.3 %) than the second survey (42.7%) (χ^2 =

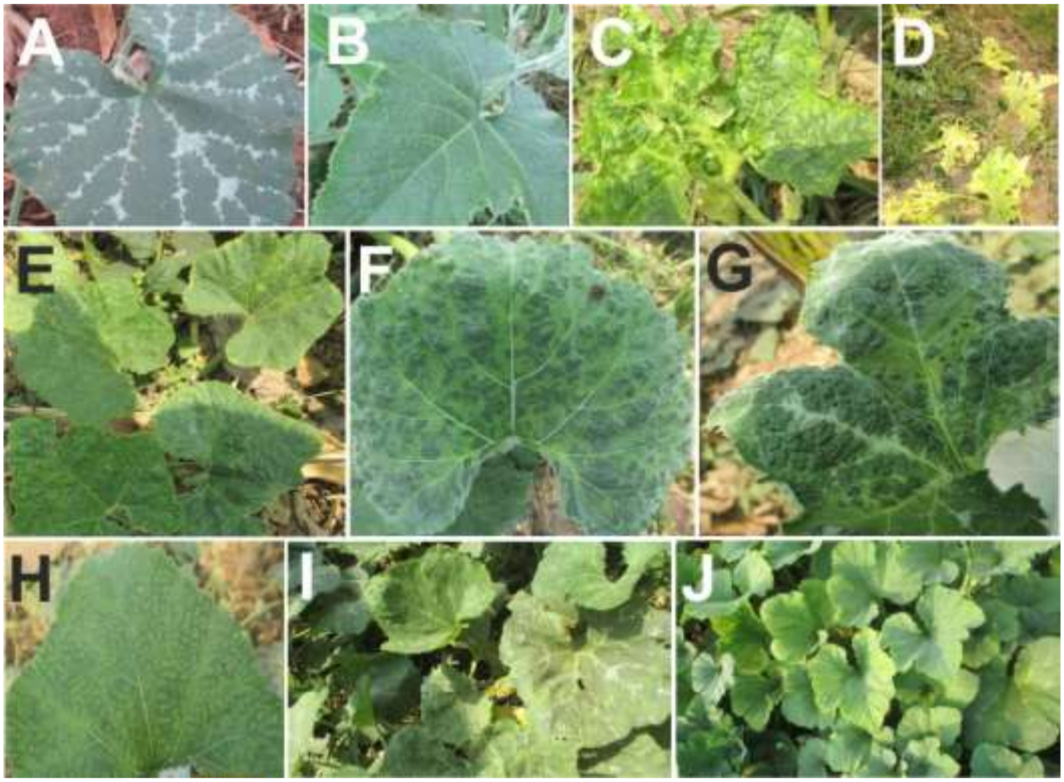

Figure 4. Some of the virus-like disease symptoms observed in on leaves of pumpkin plants in the field. Many symptomless leaves (for example, A) tested negative for all the viruses tested, while some other symptomless leaves tested positive for some viruses (e.g., B tested positive for CMV and WMV). Severe blistering of leaves (C), and blistering associated with severe chlorosis (D) in plants tested positive for all the four viruses tested. Mild rugosis as observed in leaves of whole plants (E) or when in advanced stages characterized by dark green islands (F) or severe in which case leaf starts to reduce in area (G). Mottling observed in plants testing positive for ZYMV (H). Plants with mild rugosis (I) or chlorosis and stunting symptoms (J) growing in close association with healthy looking plants; such a close association allows the ease of mechanical transmission of viruses in the field.

14.114, df = 1, P = 0.000) (Table 4). Furthermore, a higher number of more samples tested positive for ZYMV in the first survey (65.8 %) as compared to the second (34.2 %) (χ^2 = 10.612, df = 1, P = 0.001). Five (5) samples (7.4%) from symptomless watermelon plants tested positive for at least one of the four viruses assayed. Contrastingly, 12 (4.3 %) samples from symptomatic watermelon plants were consistently negative for all the viruses tested under this study.

The order of occurrence of the four viruses in pumpkins followed similar pattern to that for watermelons. Thus, CMV was also the most frequently detected virus in 59.9% of 522 pumpkin plant samples tested, compared to 46.4, 20.1, and 16.9% of samples testing positive for WMV, ZYMV and CABYV, respectively (Table 3). Differences in the incidences of WMV and ZYMV between in the districts were not statistically significant (P ≥ 0.092; Table 3). In contrast, incidences of CMV and CABYV in the districts were significantly different: CMV was highest in Masaka (30.7%) and lowest in Mbale (17.2%) while CABYV was highest in Kamuli (37.8%) and lowest in Mbale (15.3%) (P ≤ 0.024, Table 3). Incidence of CMV was significantly higher during the first survey as compared to the second survey (χ^2 = 8.594, df = 1, P = 0.003) (Table 4). On the other hand, more samples tested positive for ZYMV and CABYV in the second survey than the first survey (Table 4). These differences were statistically significant (χ^2 = 10.352, df = 1, P = 0.001 and χ^2 = 10.176, df = 1, P = 0.001) respectively (Table 4). Eleven (11) symptomless pumpkin plants (7.7 %) tested positive for at least one virus compared to 23 symptomatic plants (6.1 %) that consistently tested negative for all the four viruses under this study.

Single and multiple virus infections

Of the 338 watermelon samples that tested positive for viruses, 233 samples (68.9 %) were singly infected with only one of the four viruses as compared to 105 samples (31.1 %) in which two or more (multiple) virus infections were detected (Figure 5). This difference was statistically

Table 3. Incidence of viruses infecting watermelons and pumpkins in the different districts. Numbers in parentheses are the total number of samples tested negative or positive while those outside the parentheses their respective percentages in a given district. P values were deduced by comparison of virus incidence in the different districts. Statistically significant values of P are indicated with an asterisk (*).

Virus	Incidence of viruses watermelon plants							Virus	Incidence of viruses watermelon plants						
	District	**Negative**	**Positive**	**Total**	**Df**	**X2**	**p**		**District**	**Negative**	**Positive**	**Total**	**Df**	**X2**	**p**
	Mbale	42.2 (38)	31.1 (80)	34.0 (118)	2	3.681	0.159		Mbale	30.8 (64)	17.2 (54)	22.6 (118)	3	29.959	0.000*
CMV	Mpigi	31.1 (28)	36.2 (93)	34.9 (121)	-	-	-	CMV	Mpigi	16.8 (35)	27.1 (85)	23.0 (120)	-	-	-
	Masaka	26.7 (24)	32.7 (84)	(31.1(108)	-	-	-		Masaka	16.8 (35)	30.6 (96)	25.1 (131)	-	-	-
-	-	-	-	-	-	-	-		Kamuli	35.6 (74)	25.2 (79)	29.3 (153)	-	-	-
	Mbale	50.0 (67)	23.9 (51)	34.0 (118)	2	24.9	0.000*		Mbale	26.1 (73)	18.6 (45)	22.6 (118)	3	6.453	0.092
WMV	Mpigi	26.1 (35)	40.0 (86)	34.9 (121)	-	-	-	WMV	Mpigi	21.8 (61)	24.4 (59)	22.9 (120)	-	-	-
	Masaka	23.9 (32)	35.7 (76)	(31.1(108)	-	-	-		Masaka	21.8 (61)	28.9 (70)	25.1 (131)	-	-	-
									Kamuli	30.4 (85)	28.1 (68)	29.3 (153)	-	-	-
	Mbale	35.1 (95)	30.3 (22)	34.0 (118)	2	1.52	0.465		Mbale	23.3 (97)	19.8 (21)	22.6 (118)	3	5.59	0.133
ZYMV	Mpigi	35.4 (96)	32.9 (25)	34.8 (121)	-	-	-	ZYMV	Mpigi	23.8 (99)	19.8 (21)	22.9 (120)	-	-	-
	Masaka	29.5 (80)	36.8 (28)	31.1 (108)	-	-	-		Masaka	22.8 (95)	33.9 (36)	25.1 (131)	-	-	-
-	-	-	-	-	-	-	-		Kamuli	30.1 (125)	26.4 (28)	29.3 (153)	-	-	-
	Mbale	34.4 (100)	32.1 (18)	34.0 (18)	2	0.104	0.949		Mbale	24.1 (102)	15.3 (15)	22.5 (117)	3	9.453	0.024*
CABYV	Mpigi	34.7 (101)	35.7 (20)	35.7 (20)	-	-	-	CABYV	Mpigi	21.8 (92)	28.6 (28)	23.0 (120)	-	-	-
	Masaka	30.9 (90)	32.1 (28)	31.1 (108)	-	-	-		Masaka	26.7 (113)	18.4 (18)	25.1 (131)	-	-	-
-	-	-	-	-	-	-	-		Kamuli	27.4 (102)	37.8 (37)	29.4 (153)	-	-	-

significant (χ^2 = 96.447, df = 1, P < 0.001). In pumpkins, out of the 457 samples that tested positive for viruses, single virus infections were detected in 234 samples (51.2 %) while 223 plants (48.8 %) were found with two or more (multiple) virus infections (Figure 5). However, this difference was not statistically significant (χ^2 = 0.526, df = 1, P = 0.4683). In both crops, CMV was the most frequently detected single virus infection, followed by WMV (Figure 5). While ZYMV and CABYV were the third and fourth most common single virus infections, respectively, in watermelon, this order was reversed in pumpkins (Figure 5). In both crops, the two most common multiple infections were dual infections of CMV and WMV, and triple infections of CMV, WMV, and ZYMV (Figure 5). Overall, the difference in proportions of single virus infections was significantly higher in watermelon than pumpkins, whereas multiple infections were significantly more frequent in pumpkins than watermelon (χ^2 = 25.082, df = 1, P < 0.001).

DISCUSSIONS

Several viruses and virus diseases infecting cucur-bitaceous crops including watermelons and pumpkins have been reported in many parts of the world (Lecoq, 2003; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamiam and Abou-Jawdah 2014; Romay et al., 2014). However, such reports are unavailable in Uganda or limited in eastern Africa as a region, with an exception of a recent report of CABYV, and *Pepo aphid-borne yellows virus* (PABYV, genus *Polerovirus*) in two watermelon samples from Tanzania (Desbiez et al., 2016). Therefore, this study becomes the first comprehensive report of viruses and virus-like diseases in watermelons and pumpkins in Uganda and the region. Our interest for the study was provoked by our frequent impromptu observations

Table 4. Variability in the incidence of the four viruses infecting watermelons and pumpkins across the two surveys conducted in August-November 2013 (survey 1) and January-March 2014 (survey 2) in Uganda. Numbers in parentheses are the total number of samples that tested negative or positive while those outside the parentheses are their respective percentages for each virus in a given district. P values were deduced by comparison of virus incidences between the two surveys. Statistically significant values of P are indicated with an asterisk (*).

Virus	Incidence of viruses infecting watermelons across the two surveys							Virus	Incidence of viruses infecting watermelons across the two surveys						
	Surveys	**Negative**	**Positive**	**Total**	**Df**	**X2**	**P**		**Surveys**	**Negative**	**Positive**	**Total**	**Df**	**X2**	**P**
CMV	1	30.0 (27)	56.0 (144)	49.3 (171)	1	18.071	0.000*	CMV	1	43.3 (90)	56.4 (177)	51.2 (267)	1	8.594	0.003*
	2	70.0 (63)	44.0 (133)	50.7 (176)	-	-	-		2	56.7 (118)	43.6 (137)	48.8 (255)	-	-	-
ZYMV	1	36.6 (49)	57.3 (122)	49.3 (171)	1	14.114	0.000*	WMV	1	52.1 (146)	50.0 (121)	51.2 (267)	1	0.238	0.625
	2	63.4 (85)	42.7 (91)	50.7 (176)	-	-	-		2	47.9 (134)	50.0 (121)	48.8 (255)	-	-	-
ZYMV	1	44.6 (121)	65.8 (50)	49.3 (171)	1	10.612	0.001*	ZYMV	1	47.6 (198)	65.1 (69)	51.2 (69)	1	10.352	0.001*
	2	55.4 (150)	34.2 (26)	50.7 (166)	-	-	-		2	52.4 (218)	34.4 (218)	48.8 (91)	-	-	-
CABYV	1	47.1 (137)	60.7 (34)	49.3 (171)	1	3.493	0.062	CABYV	1	54.6 (231)	36.7 (36)	51.2 (267)	1	10.176	0.001*
	2	52.9 (154)	39.3 (22)	50.7 (176)	-	-	-		2	45.4 (192)	63.3 (62)	48.8 (255)	-	-	-

made in some fields in Mpigi and Masaka (central Uganda) of the seemingly rampant virus-like diseases, first in pumpkins and later in watermelons between 2010 and 2012.

The study found higher severities and frequencies of virus disease symptoms in central than eastern region districts in both crops. Similarly, incidences of WMV (in watermelons) or CMV and CABYV (in pumpkins) were higher in central than eastern districts of Uganda. These results are consistent with previous reports on studies of viruses in different crops in Uganda. For example, virus infection inciting passion fruit woodiness disease showed a low incidence of up to 5% in eastern as compared to a high rate of 100% in central Uganda when 15 districts were surveyed (Ochwo-Ssemakula et al., 2012). Also, cultivated sweetpotato showed low incidences of viruses and virus diseases in eastern region compared to central region in multiple surveys of up to 35 districts of Uganda (Aritua et al., 1998, 2007; Mukasa et al., 2003). Cultivated sweetpotato cropping system in eastern Uganda is characterised by a break in two growing seasons as compared to central Uganda where the crop is continuously grown all year-round which explains low incidence in eastern versus high incidence in central (Mukasa et al., 2003). Whether such a scenario may also hold for watermelons and pumpkins is unknown. Geographical prevalence and distribution of plant viruses often have a strong bearing to virus-vector relations (Power and Flecker, 2003; Power, 2008) implying that availability vectors transmitting the viruses is a key determinant in high virus incidence (Ng and Falk, 2006; Bragard et al., 2013; Whitfield et al., 2015). All the viruses under the current study are known to be transmitted by aphids (Dodds et al., 1984; Kishaba et al., 1992; Lecoq and Desbiez, 2012), but CMV (Zitter and Murphy, 2009; Jacquemond 2012; Sevik and Balkaya 2015) and ZYMV (Simmons et al., 2011, 2013) can also be seed-transmitted in different hosts. A relatively higher number of different aphid species were observed in central than eastern districts (*Our unpublished data*), and this may partly explain the observed low and high incidences in east and central respectively.

Besides involvement of vectors, differences in virus incidences may reflect regional differences in the abundance of susceptible vs. resistant host genotypes grown by farmers, and variabilities in environmental parameters influencing virus transmission and disease development (Fargette et al., 2006; Geering and Randles, 2012). Accordingly, virus infection and symptom development in plants is a function of variable factors of environment, virus strain, host plant species or genotype, age, and the host's physiological state (Cooper and Jones, 2006).

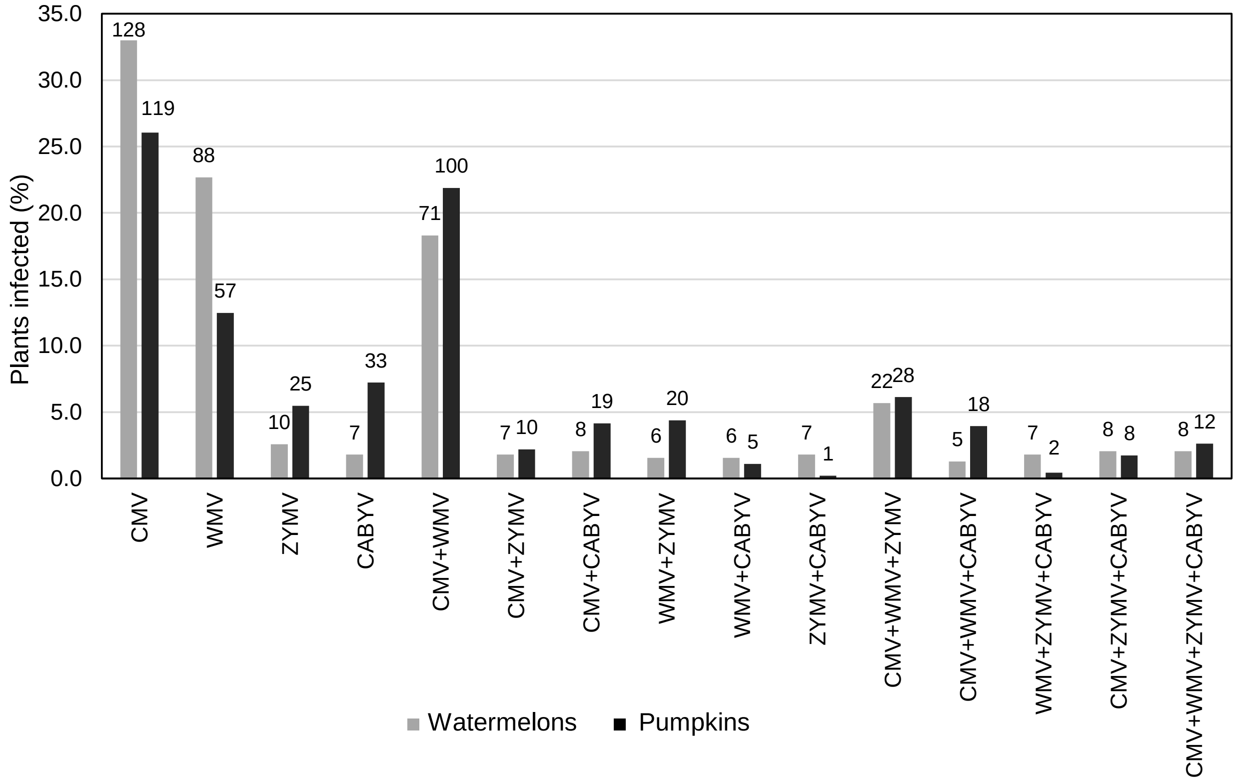

Figure 5. Natural occurrence of single and mixed virus infections in watermelon and pumpkin plants as a percentage of plants found infected with viruses. Number of plants detected with a virus or viruses is indicated above each bar.

Symptomatic plants testing negative for all the viruses tested implies the presence of other viruses not assessed. As many as 66 viruses have been reported in these crops in other parts of the world (Lecoq, 2003; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah 2014; Romay et al., 2014), which is much higher than the pre-selected virus species assessed under this study. For example, some strains or species of *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon chlorotic stunt virus* (WmCSV), *Pepo aphid-borne yellows virus* (PABYV) among others are known to infect these crops (Bananej and Vahdat 2008; Mohammed et al., 2012; Lecoq and Desbiez, 2012; Desbiez et al., 2016). Also, the methods used in the study were limited to serology using a highly stringent threshold of three times or more absorbance of the blank for a plant sample to be considered as virus infected, which might have affected the proportions of plants declared virus-infected with the viruses assayed. In contrast, symptomless virus infections as observed in this study are also common in many pathosytems implying cryptic virus infections (Roossink, 2010). In all, more extensive molecular diagnostic procedures that are independent of prior knowledge of symptoms such as siRNA deep sequencing (Kreuze et al., 2009; Boonham et al., 2014) must be employed on these crops to broaden our understanding of virus infections. CMV was the most frequently detected virus both in single and mixed infections in watermelons and pumpkins, possibly because of a large number of aphid species (up to 60) known to transmit this virus to over 1200 plant host species (Lecoq and Desbiez, 2012). Some of the aphid species implicated in CMV transmission have been reported in Uganda (Orawu et al., 2015), and in watermelons and pumpkins (*Our unpublished data*). Similarly, many surveys in different vegetables susceptible to these viruses have reported high abundance of CMV as compared to the other viruses (Köklü and Yilmaz, 2006; Jossey and Babadoost, 2008; Quemada et al., 2008). Dual CMV+WMV and triple CMV+WMV+ZYMV were the most common mixed infections. It is expected that viruses transmitted by a common vector to the same host should depict higher frequencies of mixed infections than those transmitted by different vector agents (Opiyo et al., 2010; Salvaudon et al., 2013; Tugume et al., 2016; Tollenaere et al., 2016). Because all the viruses under this study are known to be aphid-borne (Lecoq and Desbiez, 2012), and their selection for assay was pre-determined, observed differences in single/mixed infections might be a reflection of variable transmission efficiencies, aphid populations, and/or host preferences by the aphid vectors.

In contrast, short cropping cycles of 3 to 4 months for watermelons compared to long cycles of 5 to 16 months including multiple and extended harvesting dates for pumpkins may result into high single virus infections in watermelons because the crops are exposed for a short inoculation time by viruliferous insect vectors in the field. Indeed, our study demonstrated that single virus infections were significantly higher in watermelon than pumpkins. Conversely, multiple infections were significantly more frequent in pumpkins than watermelon since pumpkins have long cropping cycles that expose them to repeated inoculations in the field.

Although watermelons and pumpkins are both currently widely grown in Uganda, cultivation of watermelons is a more recent activity in Uganda having started not earlier than 15 to 20 years ago (*Our personal observations*). In contrast, cultivation of pumpkins is known for over a century with several subsistence crop fields routinely intercropped with pumpkins and can be found in virtually all districts of Uganda. However, to-date, FAOSTAT database lacks production statistics for Uganda on watermelons and pumpkins (FAOSTAT, 2016) which might imply negligible production and associated economic benefits at a national level. Nonetheless, unlike pumpkins for which local non-improved seed is saved by subsistence farmers from a previous crop, most of watermelon cultivation in Uganda is conducted on relatively large farms for commercial purposes using commercially available (imported) improved or hybrid seed. Due to the nature of pumpkin cropping systems (semi-perennial, and mostly inter-cropped with other crops) in Uganda, it is likely that the viruses studied here might have long existed in pumpkins but remained un-noticed or a non-issue since the crop is not priority in Uganda (MAAIF, 2010; FAOSTAT, 2016).

With more recent arrival of fast-growing and short-lived watermelon – a host for the same viruses into the same cropping system, incidences of the same viruses on the pumpkins may get enhanced, even though these crops are mostly never intercropped with each other. Short-lived plant hosts of vectored plant viruses attract and amplify vector populations, and subsequent high virus incidence in long-lived hosts. A classic example is demonstrated under a natural ecosystem by introducing annual grass species that have overturned the dominance of perennial native bunchgrasses in California grasslands via increased infection with aphid-borne Barley/Cereal yellow dwarf viruses (Malmstrom et al., 2005; Borer et al., 2007; HilleRisLambers et al., 2010).

Also, feeding preferences or feeding behaviour of insect vectors of plant viruses can be altered after exposure to infected plants and acquisition of virus (Stafford et al., 2011; Ingwell et al., 2012; Moreno-Delafuente et al., 2013; Rajabaskar et al., 2014). In these contexts, a much higher abundance of aphids was observed on watermelons than on pumpkins in Uganda (*Our unpublished data*), and whether or not it has implications with increased virus impact on pumpkins is an interesting subject of our future studies. Overall, the current study opens obvious opportunities for research at a number of fronts, considering the favourable tropical environments for virus disease development (Fargette et al., 2006; Thresh 2006; Jones 2009). Watermelons and pumpkins are important fruit and vegetable crops, respectively, in Uganda, but unfortunately, these are not prioritized for research (MAAIF, 2010; FAOSTAT, 2016), hence the several unknowns.

Firstly, how are the distributions of these and other viruses structured in broader geographical areas of Uganda and the region? – This requires diagnostic tools screening several samples but also considering many more virus species in broader areas than those covered under the current study because many more viruses probably infect these plants in Uganda. Secondary, what is the breadth and depth of genetic and genomic variability of the virus isolates, strains and species from watermelons and pumpkins in Uganda and the region? – This requires molecular genetic characterization of isolates from diverse geographical areas for their full or partial genomes and comparison with isolates/species from other parts of the world. It also allows testing of appropriate evolutionary hypotheses on the viruses. Thirdly, what is the breadth and depth biological variability of virus isolates from the different hosts? – This will involve biological characterizations of vector transmissibility between hosts, virus host ranges in common local weeds, virus-virus interactions and functional analysis of unique genomic segments that may exist. Fourthly, how is virus disease epidemiology in watermelons and pumpkins determined by factors of vector population dynamics across germplasm and geographic scales, cropping systems and husbandry practices? Assessing germplasm diversity across regions, the level yield losses due to virus diseases, spatio-temporal scales of disease prevalence will constitute the main activities under this research question. Importantly, East Africa as a region including Uganda is a cradleland and centre of diversification of dozens of important plant viruses decimating important crops there and elsewhere (Ndungruru et al., 2005, 2015; Tairo et al., 2005; Fargette et al., 2006; Tugume et al., 2010a,b, 2013; Mbanzibwa at al., 2011; Legg et al., 2011; Clark et al., 2012).

An in-depth assessment of viruses in watermelon and pumpkins as suggested earlier especially once conducted via regional alliances gives another chance to re-assess the significance of this region as a home of important plant viruses.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Controlled water stress in uniformity of maturity and productivity of conilon coffee

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The aim of this work was to evaluate the uniformity of fruit maturity and the productivity of conilon coffee in function of different periods of water stress in the post-harvest period. The study was conducted in a field from August 2014 to July 2015 in an old coffee crop of conilon coffee, cultivar Vitória 'Incaper 8142', cultivated at a spacing of 3.0 × 1.2 m, located at the Federal Institute of Espírito Santo Campus Santa Teresa, ES, Brazil. The experimental design was in randomized blocks, with 4 treatments and 10 repetitions. Each useful plot consisted of 12 plants. The treatments consisted of the application of different levels of water deficit, being: control (continuous irrigation with humidity close to the field capacity) (T1); water deficit of 20 (T2); 40 (T3); and 60 (T4) days after harvest. Productivity was determined by harvesting and weighing the grains of each useful plot. The maturation uniformity was evaluated by random harvesting of 100 fruits in each plot. The application of the treatment with 60 days of water deficit presented greater uniformity of fruit maturation and water saving when compared to the other treatments. The treatment with estimated water deficit of 33 days after harvest showed higher average grain yield (79.2 sc ha^{-1}).

Key words: *Coffea canephora*, irrigation management, grain quality, water, productivity.

INTRODUCTION

Coffee cultivation is one of the most important economic activities in Brazil. Conab (2016), the country produced in 2015 about 43.24 million sacks of coffee benefited. The State of Espírito Santo (ES), which occupies less than 0.5% of the national territory, is the largest Brazilian coffee conilon state (*Coffeea canephora*), yielding 10.7

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Figure 1. Municipality of Santa Teresa in the state of Espírito Santo where studies were carried out.

million bags of the benefited product in the 2015 harvest (CONAB, 2016). The culture is present in 65 of the 78 municipalities of the ES, which represents more than 80% of the municipalities (Pezzopane et al., 2010).

The improvement of the quality without damaging the productivity of conilon coffee in Espírito Santo is of utmost importance for coffee growers, since the consumer market is increasingly demanding, and there is a trend towards the progressive differentiation of the market in quality (Silva and Guimarães, 2012). One challenge faced by coffee growers in relation to coffee quality is the harvesting of cherry fruits at the same maturation stage.

The quality of the coffee beverage is directly related to the fruit maturation. In regions with good rainfall distribution, the appearance of different bloomies contributes to a disuniform fruit maturation, with a direct effect on the product price (Matiello et al., 2005).

In Espírito Santo State, the practice of selective harvesting of cherry coffee beans is not common due to labor difficulties and increased cost of production of the benefited sack. It is believed that controlled water stress in irrigation may be an alternative to selective harvesting, since such practices have the potential to increase flowering uniformity and, consequently, improve the final grain quality without reducing crop productivity.

After the harvest, the coffee tree goes through a period of physiological rest, keeping its metabolism reduced. This period coincides with the cold and dry period in most of the producing regions, which favors the control of the water supply to the crop. At this stage, the crop is sensitive to climatic elements, especially hydration and/or temperature drops, leading to the growth recovery and eventually flower opening. Under normal conditions of cultivation, the crop not only resists satisfactorily, but also recovers quickly after the end of a dry period. Thus, the control of the irrigation period significantly affects the coffee maturation, since water is the stimulus to the emission of floral buds.

There are few studies comparing controlled water regime with maturation uniformity for conilon coffee, but some studies on arabic coffee (*Coffea arabica*) showed that such management can improve fruit quality. Mera et al. (2011) verified that the interruption of the irrigation for 70 and 109 days, from June, in the region of Planaltina (DF), provided higher fruit percentages for cherry coffee and higher grain yield for 70 days' interruption, and lower grain yield for interrupted irrigation for 109 days. Similar results were also found by Silva et al. (2009). Nascimento (2008) reported that year-round irrigation reduced the emission of flowers, and treatments with controlled water regime obtained superior results to those without irrigation and all year-round irrigation for most of the variables evaluated in arabica coffee.

The objective of this work was to evaluate the uniformity of fruit maturation and conilon coffee yield in function of different periods of water stress in the post-harvest period.

MATERIALS AND METHODS

The study was performed in a field, from August 2014 to July 2015, in an old crop of conilon coffee, cultivar Vitória Incaper 8142, cultivated in spacing of 3.0 × 1.2 m, located at the Federal Institute of Espírito Santo Campus Santa Teresa (Figure 1), at 130 m of altitude and coordinates 19°49'S, 40°40'W. In the classification of Köppen, the climate of the region is a Cwa type (subtropical of dry winter) with temperature and average annual precipitation of 18°C and 845.2 mm, respectively.

The soil of the experiment area was classified as eutrophic argisol and had the following characteristics in the 0 to 30 cm layer in Table 1.

The experiment was arranged in randomized blocks, with four treatments and ten repetitions. Each plot consisted of 20 plants, where only 12 were considered useful. The treatments consisted of different levels of water deficit, being: T1: Control (continuous irrigation with humidity always close to the field capacity); T2: water deficit of 20 days after harvest; T3: water deficit of 40 days after harvest; T4: water deficit of 60 days after harvest. Water stress higher than this time period in the conilon coffee may impair the development of the crop, affecting productivity at the end of the harvest.

The plants were irrigated by a drip irrigation system with 3 × 1.2

Table 1. Soil analysis of the study area.

pH (in H_2O)	6
Al exchangeable (cmolc dm^{-3})	0.0
H + Al (cmolc dm^{-3})	2.5
Ca (cmolc dm^{-3})	2.5
Mg (cmolc dm^{-3})	0.7
P Mehlich (cmolc dm^{-3})	0.8
P remaining (mg L^{-1})	28.0
K (cmolc dm^{-3})	110.0
S (cmolc dm^{-3})	9.0
Organic matter (dag kg^{-1})	2.2
Fe (mg dm^{-3})	85.0
Zn (mg dm^{-3})	9.9
Cu (mg dm^{-3})	3.2
Mn (mg dm^{-3})	218.0
B (mg dm^{-3})	0.31
Na (mg dm^{-3})	40.0
Base saturation (V) (%)	58.2
Effective Cation Exchange Capacity (CTC) (cmolc dm^{-3})	3.5
CTC at pH 7.0 (cmolc dm^{-3})	6.0
Base sum (cmolc dm^{-3})	3.5
Base saturation (%)	58.2
Ca saturation at CTC (%)	41.8
Mg saturation at CTC (%)	11.7
K saturation in CTC (%)	4.7
Clay (g kg^{-1})	300
Silt (g kg^{-1})	118
Sand (g kg^{-1})	582

m spacing, a dripper for each plant, with a flow rate of 20 L h^{-1} and an application intensity of 5.5 mm h^{-1}. The drip irrigation system consisted of buried PVC main and branch lines and lateral lines with the respective drippers, passing over the soil along the crop lines.

The determination of the irrigation uniformity for the measurement of the applied blade was carried out semi-annually using the methodology cited by Bernardo et al. (2005). The field capacity was determined by the field method and the permanent wilting point was obtained at a tension of 1,500 kPa using the Richards Extractor (Mantovani et al., 2009), representative of the layers from 0 to 0.20 m, 20 to 0.40 m deep. The soil density of the two layers was determined by the volumetric ring method, as described by Oliveira and Ramos (2008).

In order to perform irrigation management and characterize the influence of climatic elements on the floral bud behavior of coffee plants, daily temperature (°C) and precipitation (mm) data were obtained in an automatic meteorological station near the experimental area.

Irrigation management was carried out with Irrisimples® program, which determines the water demand of the crop, evaluating the daily water balance using coefficients of adjustments on the reference evapotranspiration (ETo). The program defines the irrigation depth according to the difference between water demand and effective precipitation, generating the irrigation slides. The ETo was determined using the Hargreaves model. To calculate the crop evapotranspiration, the Gesai model was used (Mantovani et al., 2009).

Weekly, soil samples were taken at depths of 0 to 20 and 20 to 40 cm for determination of soil moisture by the standard greenhouse method (Bernardo et al., 2005), to guarantee the measurement of the method. Kc (coefficient of culture) values were obtained from Mantovani et al. (2009). The coefficient, due to soil moisture and total soil water retention capacity were calculated according to Bernardo (2005). The values of Kl (coefficient due to localized irrigation) were obtained by Keller and Bliesner method (Mantovani et al., 2009).

Irrigation was performed when the sum of the evapotranspiration of the crop, subtracted from the effective precipitation, was approximately equal to the value of the actual soil water capacity (CRA), calculated by the formula: CRA = CTA (Total Soil Water Capacity) × f (Availability factor = 0.6). The duration of each irrigation event was calculated by the ratio between the total required irrigation (ITN), which considers the irrigation efficiency, and the intensity of water application of the emitters, determined with an evaluation of the irrigation system.

The fertilizations were performed according to the recommendations of Prezotti et al. (2007), and practices followed the recommendations of Ferrão et al. (2008), and these are the most common recommendations for the conillon coffee in ES State.

The productivity of the crop was determined by manually collecting through sieves all useful plants from each treatment and converting the harvested beans into ha^{-1} sacks. The uniformity of maturation was evaluated by taking 100 fruits, randomly, in each plant harvested, to determine the percentage of green, yellow, cherry and black grains.

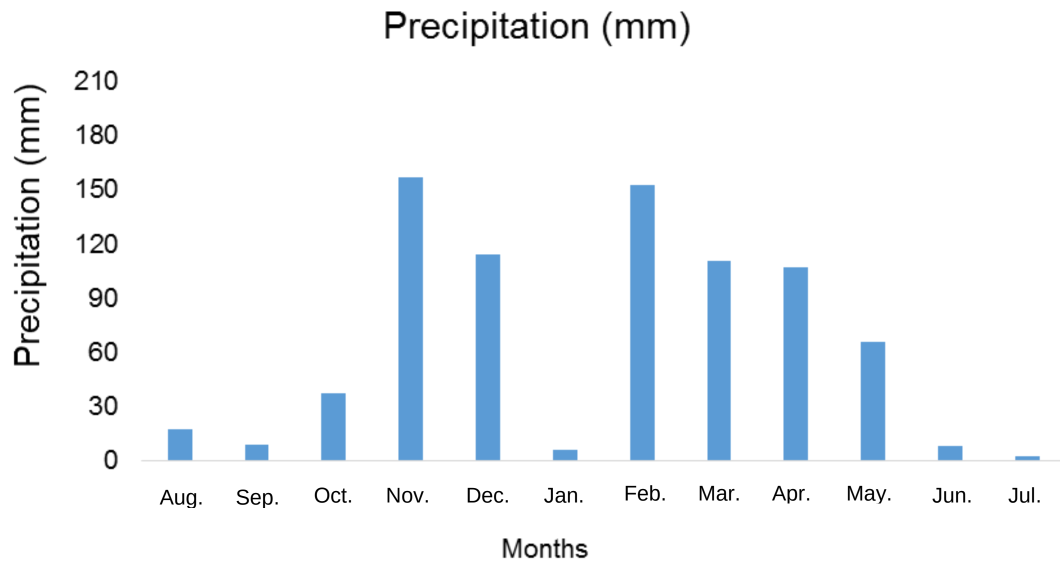

Figure 2. Monthly precipitations occurred in the period from August 2014 to July 2015.

The productivity data were submitted to variance analysis, where the quantitative character factors were compared by the analysis regression. The maturation uniformity data were submitted to the Chi-square test, quantitatively evaluating the ratio between the results of the treatments and the expected distribution for the phenomenon of maturation uniformity. The software SAEG 9.1 (UFV, 2007) was used in the analysis.

RESULTS AND DISCUSSION

In the drier months (August to October 2014 and June and July 2015), where rainfall was lower, there was a higher need of irrigation, as evapotranspiration of the crop was higher in these periods, so a larger slide of water was performed to supply the irrigation. In January, the precipitation was low, but the average temperature and the irradiance indexes were higher, there was a greater need of irrigation, as shown in Figure 2.

The soil of the experimental area has a permanent wilting point of 16.28%, soil water availability factor of 22%, and field capacity of 28%. It was sought to maintain soil moisture always between the safety factor of soil water availability and field capacity, thus ensuring that the plants did not suffer any stress, as shown in Figure 3. Due to technical problems in the irrigation system, there was a sudden drop in soil moisture in September 2014.

The abrupt drop in soil moisture did not affect crop development. In a short period of time, soil moisture has resumed to the level of safety. The uniformity of maturation and the highest proportion of mature grains at harvest were higher in the treatment that was the longest time under post-harvest water stress (T4), as shown in Figure 4.

Uninterrupted supply of water by irrigation can stimulate the opening of new flowers, which generates fruits at different stages of development and desuniform maturity at the time of harvest (Nascimento, 2008). Therefore, it will directly interfere on yield, since smaller grains demand greater volume of coffee to reach 60 kg sack of the benefited product.

Marsetti et al. (2013), evaluating the effects of water deficit, on floral bud dormancy and floral opening of conilon coffee produced under climatic conditions in the northern state of Espírito Santo, verified that the treatment that interrupted the irrigation for 63 days guarantee the uniformity of flowering, which is directly related to the maturation uniformity of grain.

Statistically, it was possible to estimate the behavior of grain maturity uniformity by chi-square analysis at 5% probability, which revealed that the application of the 60-day water deficit provided the harvest of most fruits at the cherry maturation stage (Figure 5).

Usually, the harvesting started when the percentage of mature fruits is above 80%. Green coffee fruits produce defects during fermentation and drying, giving rise to black or black-green grains, while fruits after their complete ripening (blacks/raisins) resulted in black and burned grains. These defects will negatively interfere with the type and quality of the beverage (Fonseca et al., 2007).

Proper management of irrigation in the post-harvest period is of a great importance for producers to add value to their product, since harvesting at the appropriate maturation stage is one of the factors that will influence the final quality of the beverage. Treatment 4 presented a lower proportion of dried fruits, which provides better coffee quality at the end of its processing. It is known that the final quality of green coffee is associated both to its intrinsic characteristics of coffee species and cultivar and post-harvesting processes (Saraiva et al., 2009), in order

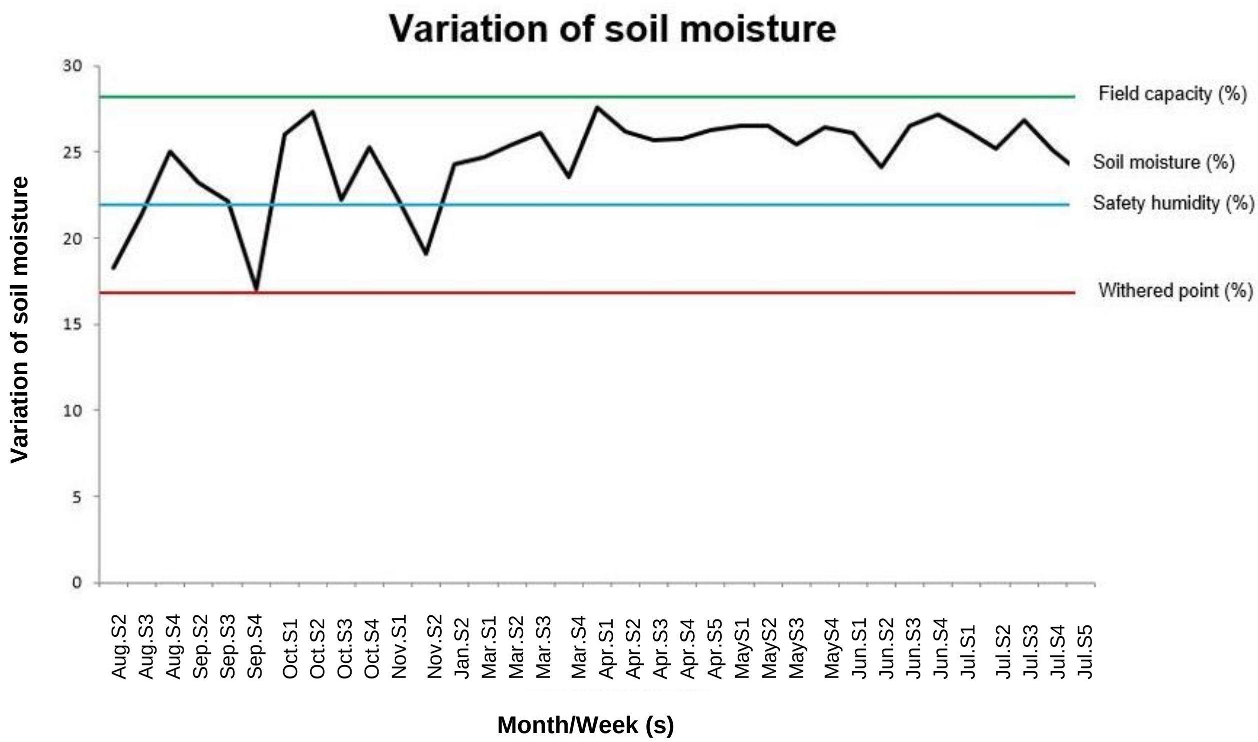

Figure 3. Variation of soil moisture throughout the experimental period.

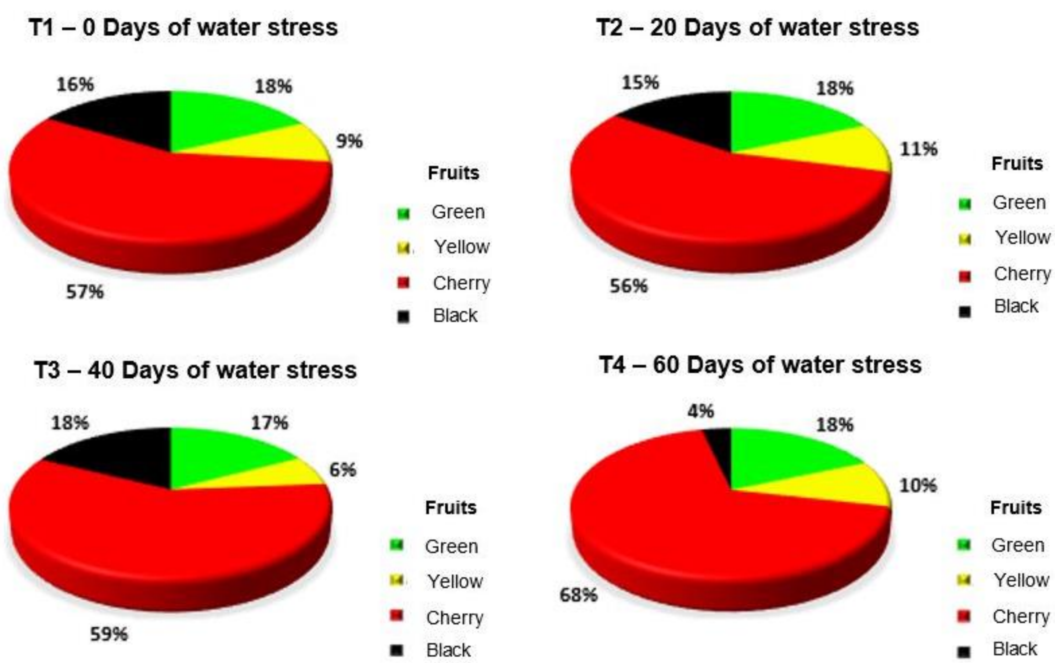

Figure 4. Percentage of maturity of the coffee beans of Conilon Vitória for the 4 treatments.

to achieve a better quality beverage, several factors are involved in the process, from the coffee genotype, the management of the crop up to the post-harvest processing of the coffee fruits.

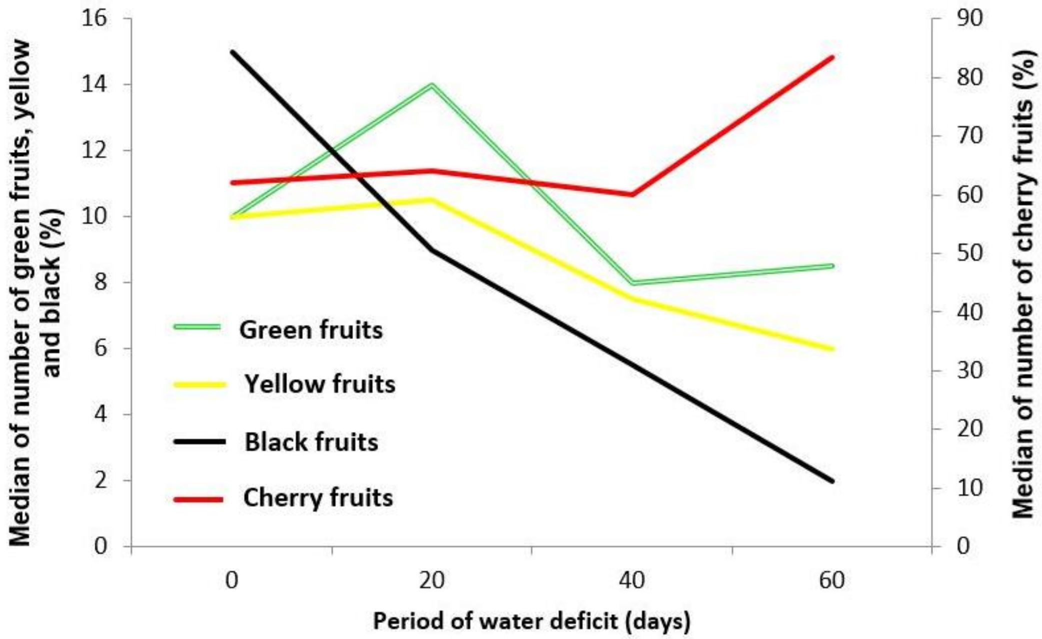

Figure 5. Uniformity of fruits maturation due to periods of water deficit.

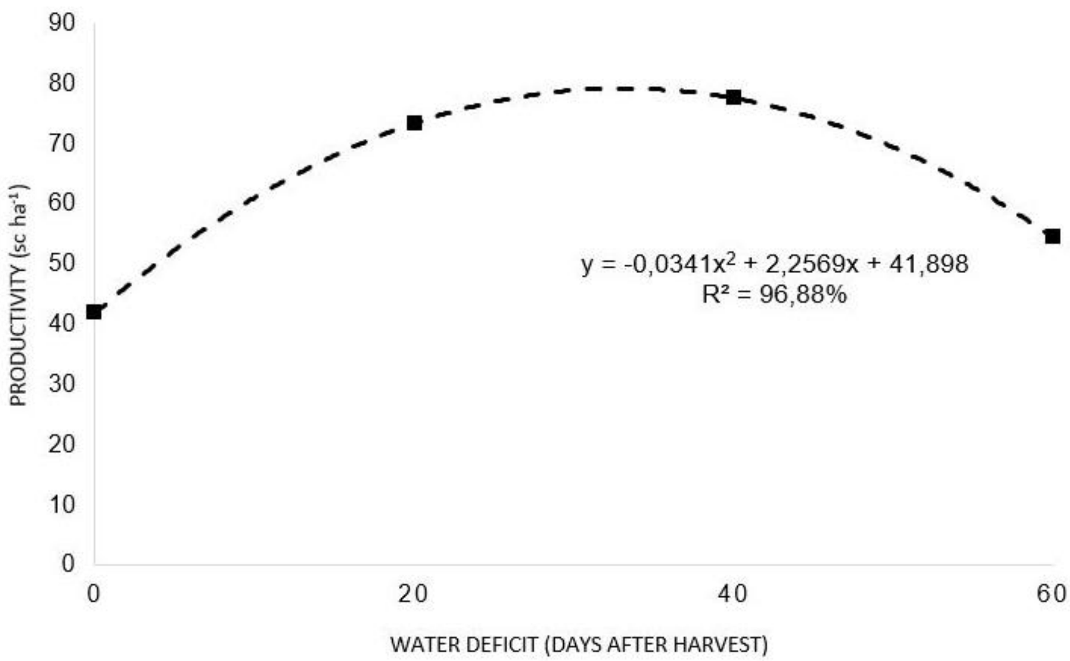

Figure 6. Productivity of coffee Conilon Vitória for the appropriate treatments.

Regarding productivity, it was verified that the control had a lower value (41 sacks ha^{-1}) when compared with the other treatments. The higher estimated productivity (79.2 sc ha^{-1}) in the quadratic regression occurred when there was a water regime of 33 days after harvest, as shown in Figure 6. The T4 treatment produced about 54 sc ha^{-1}, higher productivity than the constant irrigated treatment. In addition, this management can generate savings of water, energy and labor for the coffee farmer, making the production cost of the crop relatively smaller. Silva et al. (2003), observed a similar behavior in arabica coffee, obtaining a great difference between irrigated and non-

Table 2. Water use during the experimental period (sum of irrigation slides applied and rainfall occurred during the period).

Period	T1	T2	T3	T4
	mm			
August	40.7	22.2	17	17
September	40.8	40.8	40.8	8.5
October	78.4	78.4	78.4	78.4
November	156.8	156.8	156.8	156.8
December	121.7	121.7	121.7	121.7
January	35.4	35.4	35.4	35.4
February	157.7	157.7	157.7	157.7
March	161.9	161.9	161.9	161.9
April	146.6	146.6	146.6	146.6
May	75.71	75.71	75.71	75.71
June	20.6	20.6	20.6	20.6
July	11.6	11.6	11.6	11.6
Total	1047.91	1029.41	1024.21	991.91

irrigated coffee, with values of 66, 72, 72.2 and 72.4 sc ha^{-1} in the non-irrigated treatments, irrigated constantly, irrigated with suspension of 30 days and irrigated with suspension of 60 days, respectively. The water deficit for a longer period of time (60 days) is related to producers that aim to produce a higher quality coffee, causing a greater uniformity of maturation in the grains.

The adoption or not of a period of water deficit in the stage of development of the floral bud called "E4", to standardize the flowering of the coffee tree, is perhaps one of the major bottlenecks of irrigated coffee in Brazil. In addition to the synchronization of the flowering, irrigation affects the maturation of the fruits of the coffee tree to make it slower, when compared with the maturation of the fruits without irrigation, which is more precocious (Fernandes, 2011).

As expected, water savings were higher as the period of water stress increased. Treatment 4 presented a smaller total applied slide compared to Treatment 1 (Table 2), generating an economy of 56 mm of water per productive cycle of the crop. There are considerable values for a commercial area since there is a saving of 56 L of water per m^2, and consequently there was greater saving of electricity, labor, and less detrition of the irrigation system since it will be used less times.

Conclusion

The water stress of 60 days after harvesting gives a higher uniformity of maturation of the coffee conilon, obtaining up to 68% of cherry grains. The water stress of 33 days after harvest increases 37.3 sc ha^{-1} the productivity of coffee conilon, when compared with coffee irrigated throughout the year. The water stress of 60 days after the harvest contributed to save 56 L of water per m^2 during a productive cycle of the coffee conilon Vitoria 'Incaper 8142'.

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Full Length Research Paper

Nitrogen and potassium fertirrigation on yield characteristics of Italian Zucchini in protected cultivation

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This study aimed to evaluate the effect of different doses of nitrogen and potassium applied through fertirrigation on yield characteristics of Italian Zucchini, Novita Plus cultivar. The experiment was conducted in a greenhouse at the Irrigation Technical Center of the State University of Maringá, Maringá - PR, adopting a completely randomized design in a 4 x 4 factorial scheme, with three replications. Four doses of nitrogen (0, 90, 180 and 270 kg ha^{-1}) and four doses of potassium (0, 90, 180 and 270 kg K_2O ha^{-1}) were tested. The fertirrigation was applied via micro irrigation through drip emitters in a continuous line, operating at a flow rate of 4 L h^{-1}. Number of leaves, number of male and female flowers, average yield and mass of fruits per plant were evaluated. There was an increase in leaf production at the maximum nitrogen dose, as well as an increase in the number of male flowers at the maximum dose of nitrogen and potassium. The number of female flowers was influenced only by nitrogen, with an increase in the number of flowers at the highest nitrogen levels. Regarding the fruit yield, the best obtained doses were 179.19 kg ha^{-1} of N and 171.25 kg ha^{-1} of K_2O. Finally, the doses that expressed maximum fruit mass gain were 136.92 kg ha^{-1} of N and 184.17 kg ha^{-1} of K_2O.

Key words: *Cucurbita pepo* L., cucurbitacea, sexual expression, production, nitrogen metabolism.

INTRODUCTION

The cucurbitaceae family has more than 120 genera and approximately 800 species, and it appears among the main families of vegetables grown by man (Teppner, 2004). The crops of greater economic importance, belonging to this family, are melons, watermelon, cucumbers and pumpkins. In addition to the food value, cucurbits farming in Brazil, in particular pumpkins, have great social importance. It generates direct and indirect jobs since it demands high amount of manual labor from cultivation to marketing (Resende et al., 2013).

The Italian Zucchini is among the ten vegetables of greatest economic value in the country, where the central and southern regions of Brazil stand out for their production and productivity (Carpes et al., 2008). In 2011,

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3,187,953 tonnes of vegetables were produced in the State of Paraná, Brazil, with 1% of the gross value of production, characterized by the production of the Italian Abobrinha, reaching a productivity of 17.8 tons per hectare (Paraná, 2013).

Despite the fact that melon and watermelon are the most grown vegetables in volume from that family, pumpkin is becoming more popular and occupying a significant portion of the agribusiness. In Brazil, its cultivation is traditionally carried out in the field, however, with increasing farming technification, many producers have conducted its cultivation in a protected environment with excellent results (higher yield and quality), especially in less favorable seasons (Pôrto, 2011).

One characteristic of growing vegetables is the intensity of cultivation, that is, resources such as water, nutrients and pesticides are applied in order to exploit the maximum yield potential of plants (Echer et al., 2014). In protected environment farming, this "intensity of cultivation" aims to increase yield and hence achieve greater economic return, given the high cost of the structures. This is due to the adaptation of this culture to the most diverse conditions, its production is possible all year round. The cultivation in a protected environment is gaining space every year due to the specific conditions of Italian Zucchini cultivation, which can be recreated in these environments, guaranteeing better growing conditions, besides accelerating and homogenizing the maturation process of the crop, facilitating its harvesting and commercialization (Lúcio et al., 2008). This cultivation practice has been widely used in the southern region of Brazil, where producers increase their net yields and reduce their losses due to rigorous cold in winter (Couto et al, 2008).

One of the major obstacles to the production of Italian Zucchini in protected environment is the lack of consistent information regarding the nutrition and fertilization of the plants (Pôrto et al., 2012). In addition, another difficulty for the production of pumpkins in protected environment refers to pollination. As pollination is carried out by means of insects, in the protected environment, it is necessary to use labor to carry out the procedure, considering the absence of insects in this environment (Romano et al., 2008).

In general, absorption of nutrients in vegetables follows the pattern of the growth curve (dry matter accumulation). Among the macronutrients, potassium is commonly the nutrient most absorbed by vegetables (Fontes and Lima, 1993). In a study conducted by Vidigal et al. (2007) to evaluate the progress of the absorption of nutrients in pumpkin cultivation, the authors found that K was the nutrient absorbed in larger quantities by the plant, followed by N and Ca, as it was observed for most vegetables. According to these authors, the order of accumulated amounts of macronutrients was K> N> Ca> P> Mg> S.

Among the nutrients of higher demand by plants, nitrogen is one of the most important. This nutrient influences the processes involved in the growth and development of plants by altering the source-sink relation and hence the distribution of assimilates between vegetative and reproductive organs (Pôrto et al., 2014). In cucurbits, the increase in N dose, to a limited extent, provides increment in plant leaf area, having an effect on the production of photoassimilates and consequently the production of fruits (Queiroga et al., 2007).

Nitrogen fertilization is very important to obtain adequate yield, and the proper dosage varies according to the desired productivity, cultivar, management techniques, nutrient source and edaphoclimatic conditions (Pôrto et al., 2012). When applied in excess, nitrogen fertilization can result in reduced quality and nutritional security due to nitrate concentration and, when applied in small amounts, can cause nutritional deficiency with chlorosis and subsequent necrosis of leaves (Pôrto et al., 2014).

Potassium has a key role in the activation of enzymes and transport of carbohydrates, as well as being part of the photosynthesis processes, respiration, cell growth and elimination of active oxygen. Potassium deficiency symptoms in the plant are expressed through reduced growth, reduced resistance to pathogens and degradation of indole-3-acetic acid (IAA) (Malavolta, 2006).

The objective of this study was to evaluate the performance of the Italian Zucchini crop using different doses of nitrogen and potassium via fertirrigation.

MATERIALS AND METHODS

The experiment was conducted in a protected environment at the Irrigation Technical Center of the State University of Maringá - UEM, located in Maringá - PR (23°23'56.50 '' S and 51°57'7.53 '' W, 542 m of altitude). According to Koppen, the local climate is classified as Cfa, subtropical, with the occurrence of average temperatures below 18°C in the coldest month and temperatures above 22°C in the hottest month, with annual rainfall ranging from 1400 to 1600 mm (Caviglione et al., 2000).

The experimental design adopted was completely randomized (CRD) in a factorial 4 × 4 with three replications. The first factor consisted of four nitrogen doses (0, 90, 180, 270 kg ha^{-1}) and the second factor of four potassium doses (0, 90, 180 and 270 kg ha^{-1}). The environment of cultivation is 30 m long, 5.7 m wide and has a ceiling height of 2.5 m. The environmental structure has arch type roof, covered with 150 µm thick polyethylene film, with anti-UV treatment and anti-aphid screen on the sides. Inside the environment, it was installed a meteorological station, scheduled to record daily temperature data.

The soil is classified as Distroferric Red Nitosol, presenting moderate A horizon with clay texture (Embrapa, 2013). Its chemical characterization showed pH in $CaCl_2$ = 4.9; P = 3.19 mg dm^{-3}; K^+= 0.16 $cmol_c$ dm^{-3}; Ca^{2+}= 2.00 $cmol_c$ dm^{-3}; Mg^{2+}= 0.60 $cmol_c$ dm^{-3}; Al^{3+}= 0.62 $cmol_c$ dm^{-3}; H^+ + Al^{3+}= 3.74 $cmol_c$ dm^{-3}; organic matter = 17.97 g dm^{-3}; CEC = 6.50 $cmol_c$ dm^{-3} and base saturation (V%) = 42.46%. Lime application was performed (0.29 t ha^{-1}) to increase the base saturation up to 80%, according to the recommendation for the crop, developed by Trani (2007). The seedlings were grown

Table 1. Summary of the analysis of variance for the results of leaf number (LN), number of male flowers (NMF) number of female flowers (NFF), average number of fruits per plant (ANF) and average fruit mass (AFM).

Source of variation	Pr>Fc				
	LN	NMF	NFF	ANF	MMF (g fruit^{-1})
Doses of N (A)	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
Doses of K (B)	0.7391^{NS}	0.0000*	0.0155*	0.0249*	0.0008*
A*B	0.1422^{NS}	0.0668^{NS}	0.0906^{NS}	0.1367^{NS}	0.0720^{NS}
Overall average	28.33	51.13	16.02	7.15	225.16
CV (%)	7.69	5.49	6.11	8.08	10.84

*Significant at 5%; NS- non significant; DF- degrees of freedom; CV- coefficient of variation.

in 72 cells polystyrene trays, containing commercial substrate and remained in a protected environment until the time of transplanting, when they had three true leaves.

After transplanting the seedlings, aiming at the establishment of the crop, daily irrigations were performed, keeping the soil at field capacity for ten days. Thereafter, irrigations and fertirrigations were performed according to the reading of tension values recorded in six tensiometers installed in the experimental area, three at the depth of 10 cm and three at the depth of 20 cm.

Nitrogen and potassium applications were performed when the tensiometers recorded tensions of 20 KPa, and this value was considered as critical, indicating the need to carry out a new irrigation according to the methodology suggested by Marouelli (2008). Irrigation management was performed based on the estimate of crop evapotranspiration (ETc), which corresponds to the reference evapotranspiration (ET0), obtained using the evaporimeter of Piché, and multiplied by the crop coefficient (Kc). For the Italian Zucchini, Kc values of 0.15; 0.95 and 0.70 at the initial, intermediate and final cycle were used, respectively, according to Allen et al. (2006).

The irrigation system used was drip micro irrigation. The system operated at a flow rate of 4 L h^{-1}, monitored by means of a glycerine manometer with 10 mwc operating pressure. The uniformity of water distribution in the irrigation system was evaluated through Christiansen coefficient (CUC) according to the methodology described by Keller and Karmeli (1974) and a value exceeding 90% was obtained which is considered "excellent" according to Bernardo (2008).

Nitrogen and potassium levels were established based on the total need of nutrients along the zucchini crop cycle, cultivar Novita Plus, according to Trani (2007). The nitrogen was applied using the calcium nitrate fertilizer, while potassium was provided by the application of potassium chloride. Doses of N and K were injected into the main irrigation system line before the filtration system. A 0.5 hp centrifugal pump was used as injector equipment, installed with suction, which repressed the solution of water + fertilizer from a 500 L capacity tank where the mixture was performed. The system was initialized and concluded with the application of water, in order to stabilize the flow of the drippers and avoid the occurrence of clogging.

The first male and female flowers began to appear 22 days after transplanting. Manual pollination process was started, according Romano et al. (2008), removing the petals from the male flowers and smoothly rubbing them on the female flowers, which then were tied with waxed paper bags in its peduncle. Around two days after pollination, the waxed paper bags were removed.

Harvests began 30 days after transplanting and were performed daily until the depletion of the productive capacity of the plants. For comparison study of growth of Italian Zucchini plants under the different treatments, the following parameters were evaluated: number of leaves emitted per plant, number of male flowers, number of female flowers, average fruit mass (g fruit^{-1}) and yield (t ha^{-1}).

With the acquisition of data, analysis of variance was performed and on the occurrence of significant differences at the level of 5% for the variables doses of nitrogen and doses of potassium, regression analyzes were applied using the SISVAR statistical software, version 5.4 (Ferreira, 2014).

RESULTS AND DISCUSSION

Analysis of variance of the studied variables is presented in Table 1. There was no interaction between nitrogen and potassium for any of the variables. The nitrogen dose factor had a significant effect on the number of leaves, number of male flowers, number of female flowers, average number of fruits per plant and average fruit mass. Significant effect was observed for the dose of potassium factor for number of male flowers, number of female flowers, average number of fruits per plant and average fruit mass.

Regarding the nitrogen doses, it was possible to observe an increase in the number of leaves of Italian Zucchini, by means of an increasing linear function, as shown in Figure 1. It is noted that increasing levels of N provided higher number of leaves. Each 1 kg ha^{-1} of N promoted the increase of 0.0744 leaves per plant. On average, the application of 270 kg ha^{-1} of N represented an increment of 110% as compared to the control treatment.

Possibly, the application of N doses increased the lifespan of the leaves (Garcez neto et al., 2002), allowing them to be able to perform photosynthetic activity for a longer time interval. Consequently, photoassimilates production in each leaf occurred over a longer period, thus favoring the increase of biomass production and *Cantalupensis* melon under protected environment. The authors verified that there was an increase in the number

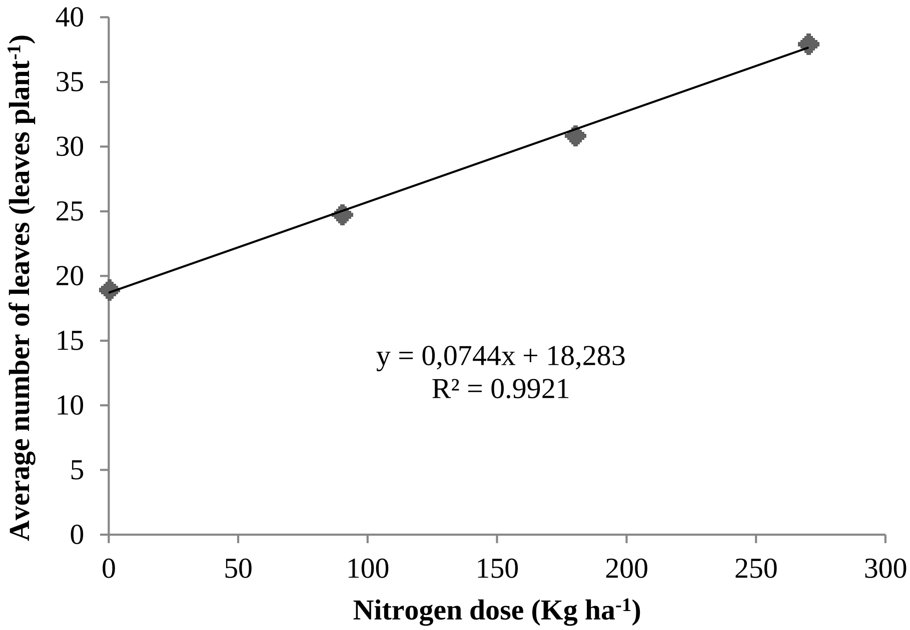

Figure 1. Average number of leaves of Italian Zucchini plants, Novita Plus cultivar, due to the application of different nitrogen doses.

of leaves per plant up to the dose of 180 kg ha^{-1}. Corroborating with the results obtained in this study, Higuti et al. (2010) evaluated the yield of pumpkin seedlings with different doses of potassium and found no significant difference for the number of leaves. The number of male flowers of Italian Zucchini plants showed a significant difference for the N dose factor as well as for the K dose factor (Table 1).

An increase in the number of male flowers is observed through the linear function with the application of nitrogen and potassium, as outlined in Figure 2. It is noted that the highest number of male flowers was 69.8, obtained at the dose of 270 kg ha^{-1} of N. Ng'etich et al. (2013) assessed the number of flowers according to the levels of nitrogen and obtained 67.5 flowers at the dose of 160 kg ha^{-1} of N. According to these authors, the role of nitrogen in increasing the number of male flowers per plant is clear, being the highest number obtained with higher doses. The Italian Zucchini plants possibly absorbed nitrogen in adequate amounts, a fact that reflected in the production of male flowers. Kano et al. (2010) stated that the nutrient stimulates the production of flower buds. The flower buds pass through the cellular differentiation process, which also has the involvement of nitrogen (Cabello, 1996).

Regarding potassium levels, it was observed that the dose of 270 kg ha^{-1} of K_2O allowed obtaining of 53.5 male flowers per plant. Representing a 10% increase as compared to the control treatment. According Shabala (2003), the nitrogen is involved in protein synthesis, which in conjunct composes amino acids, which have a specific function. The potassium acts as ionic regulator being involved in processes such as the activation of transport enzymes of various types of solutes and opening and closing of stomata. Thus, these two major nutrients are concentrated in vegetable organs of intense metabolic activity, such as flower buds. The nitrogen doses and potassium doses presented a significant difference in the number of female flowers (Table 1). Increase in the number of female flowers of Italian Zucchini was observed with the application of increasing doses of N, and it was possible to adjust a crescent linear regression model. Regarding the potassium doses, a quadratic regression model was adjusted, as shown in Figure 3.

The highest number of female flowers (20.17 female flowers $plant^{-1}$) was obtained with the application of the dose of 270 kg ha^{-1} of N. The number of female flowers in the dose of 270 kg ha^{-1} was 51% higher than what was obtained with the application of 0 kg ha^{-1} of nitrogen. Ng'etich et al. (2013) assessed the number of Italian Zucchini flowers and obtained 24 flowers per plant. According to these authors, the maximum number of flowers occurred at the dose of 120 kg ha^{-1} and was 63.5% higher than the number of flowers at the dose of 0 kg ha^{-1}. According to these authors, doses above 120 kg ha^{-1} did not result in increase in the number of flowers. This result differs from that obtained in this experiment, where it is apparent that the number of flowers has a linear increase as a function of nitrogen. No reduction in the number of flowers was observed in doses greater than 120 kg ha^{-1}.

It can be verified in this experiment that the doses were

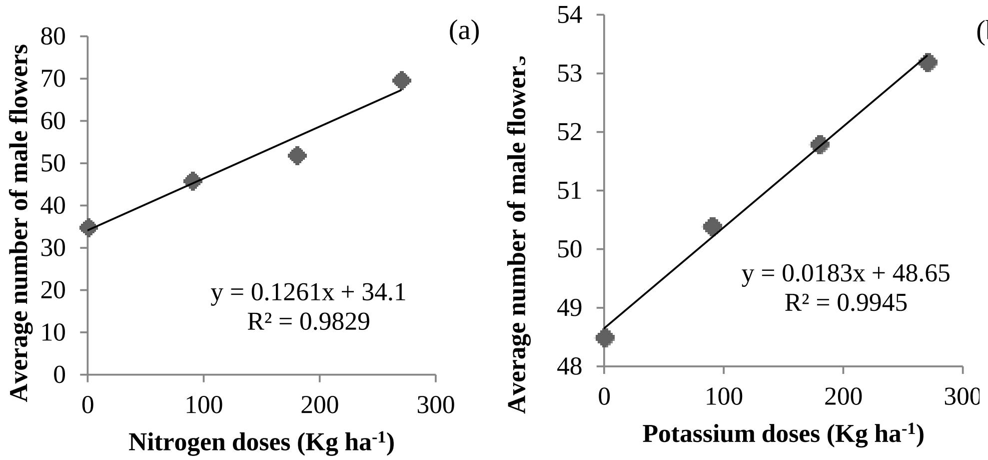

Figure 2. Average number of male flowers of Italian Zucchini plants, Novita Plus cultivar, due to the application of different nitrogen (a) and potassium (b) doses.

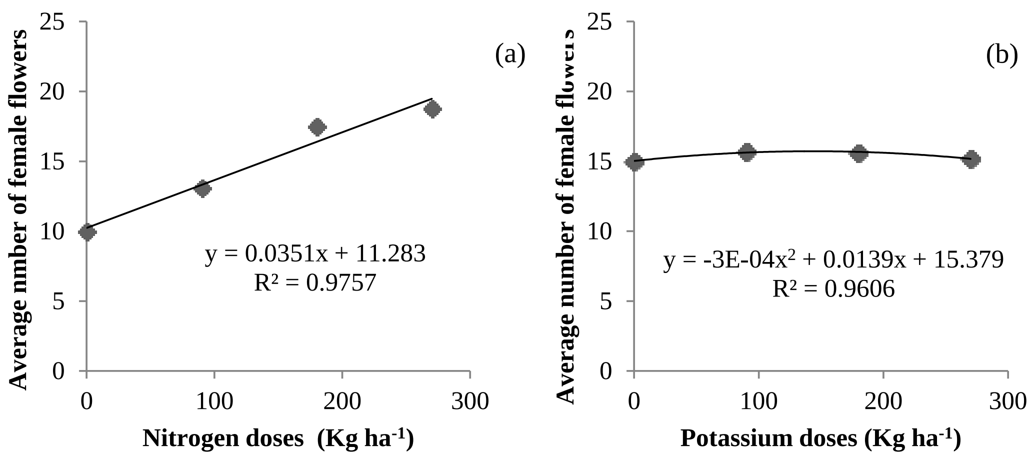

Figure 3. Average number of female flowers of Italian Zucchini plants, Novita Plus cultivar, due to the application of different nitrogen (a) and potassium (b) doses.

adequate, not causing toxicity by excess of the nutrient. Both the deficiency and the excess of nitrogen can reduce the formation of female flowers. Exceeding doses of nitrogen at the flowering stage in vegetable crops, like the Italian Zucchini, favor abortion of female flowers (Silva and Marouelli, 2002).

Nitrogen, besides favoring vegetative growth and leaf size, also plays a key role in the expression of female flowers. Nitrogen fertilization, especially with the use of nitrate, induces an increase in the number of female flowers on the plant (Menezes, 1994). According to Amer et al. (2009) the growth of pumpkin plants at higher nitrogen doses leads to a significant decrease in the sexual ratio (number of male flowers/number of female flowers), signifying an increase in the number of female flowers. The maximum number of female flowers (16.6 female flowers plant^{-1}) was provided by the application of the dose of 173.75 kg ha^{-1} of K_2O (Figure 3B). Potassium did not have great influence in the production of female flowers. Possibly this is due to the fact that the nutrient has qualitative effect on the fruits, or even contributes to the resistance of shoot to fungal diseases (Filgueira, 2008). However, there is no connection, so far, between the production of female flowers and potassium doses.

Nitrogen and potassium doses significantly influenced the number of fruits per Italian Zucchini plant (Table 1). It was possible to adjust a quadratic regression model for the N dose factor, as well as for the K dose factor, as shown in Figure 4.

The maximum fruit yield was 12.1 fruits per plant,

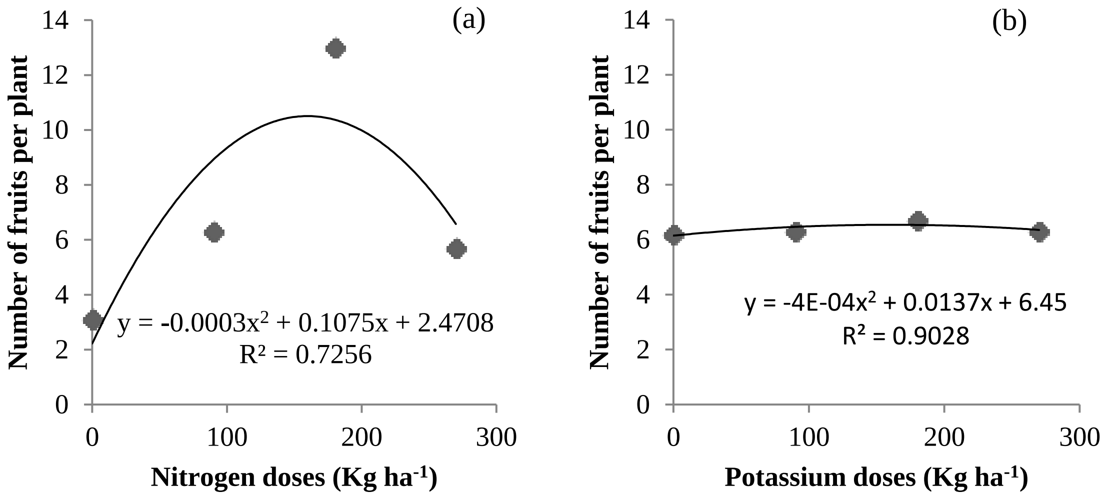

Figure 4. Average number of fruits of Italian Zucchini, Novita Plus cultivar, due to the application of different nitrogen (a) and potassium (b) doses.

obtained with the application of 179.19 kg ha^{-1} of N. As compared to the control, it corresponds to a 363% yield increase. Doses above 179.19 kg of N resulted in decrease of the number of fruits. According to Queiroga et al. (2007), in cucurbits the increase in N doses, to a limited extent, provides increment in plant leaf area, having an effect on the production of photoassimilates and thus in fruit yield. Pôrto et al. (2012) evaluated the effect of different doses of nitrogen (0, 50, 100, 200 and 400 kg ha^{-1} of N) on the number of fruits per plant of zucchini (Caserta cultivar). The authors concluded that the dose of 323 kg ha^{-1} of N provided the maximum number of fruits per plant (7.7 fruits $plant^{-1}$).

Regarding the doses of potassium, the Italian Zucchini plants produced the maximum number of fruits per plant (7.6 fruits $plant^{-1}$) with the application of 171.25 kg per ha of K_2O. The achieved results may be related to the finding of an optimal dose, considering that potassium can contribute to the improvement of chemical and physical characteristics of fruits, due to the optimization in the process of transpiration and formation of carbohydrates (Taiz and Zeiger, 2012). However, if nutritional imbalances take place, caused by excessive applications of potassium doses, physiological disturbances such as drop in yield, fruit quality and increase in osmotic pressure may occur (Marschner, 1995).

Research carried out by Grangeiro and Filho (2004) showed that the highest fruit indexes in watermelon crop were obtained up to the dose of 300 kg ha^{-1} of K_2O. Doses above 300 kg ha^{-1} K_2O caused decrease in fruit indexes for this crop.

The average fruits mass was influenced by the application of nitrogen and potassium doses (Table 1), and it was possible to adjust quadratic regression models for both factors, as shown in Figure 5.

The application the dose of 270 kg ha^{-1} of N caused a drop in average fruit mass. The yield obtained with this dose is lower than that achieved with the control, indicating that the excess of nitrogen is equally or more harmful than the nutrient deficiency. The maximum average fruit mass (240.17 grams $fruit^{-1}$) was obtained with the application of the dose of 136.92 kg ha^{-1} of N. This represents approximately 11.5% increased as compared to the control. According to Ng'etich et al. (2013), the increment in vegetative and reproductive characteristics due to the increase in the supply of nitrogen could be attributed to the increase in nitrogen uptake and its role in chlorophyll synthesis and consequently in the process of photosynthesis and carbon assimilation.

Pôrto et al. (2012) studied the effect of different doses of nitrogen (0, 50, 100, 200, 400 kg ha^{-1} of N) and found significant differences in average fruit mass of zucchini, Caserta cultivar. These authors found quadratic responses with the application of the maximum estimated dose of 265 kg ha^{-1} of N, which provided the production of fruits with the maximum average fruit mass of 240 g per fruit. This result represents an increase of 54% as compared to the control, being higher than the values obtained in the present work.

The application of 184.17 kg ha^{-1} of K_2O provided fruit mass of 227.71 grams $fruct^{-1}$. Higher doses resulted in the drop of the fruit mass, because the excess potassium ends up inhibiting the absorption of calcium, an essential element for fruit formation.

Esmaielpour and Hokmalipour (2014) verified that 150 kg ha^{-1} of potassium allowed maximum yield. According to these authors, potassium increases the transport of assimilates to the fruit, increasing the "sink" power of the fruit.

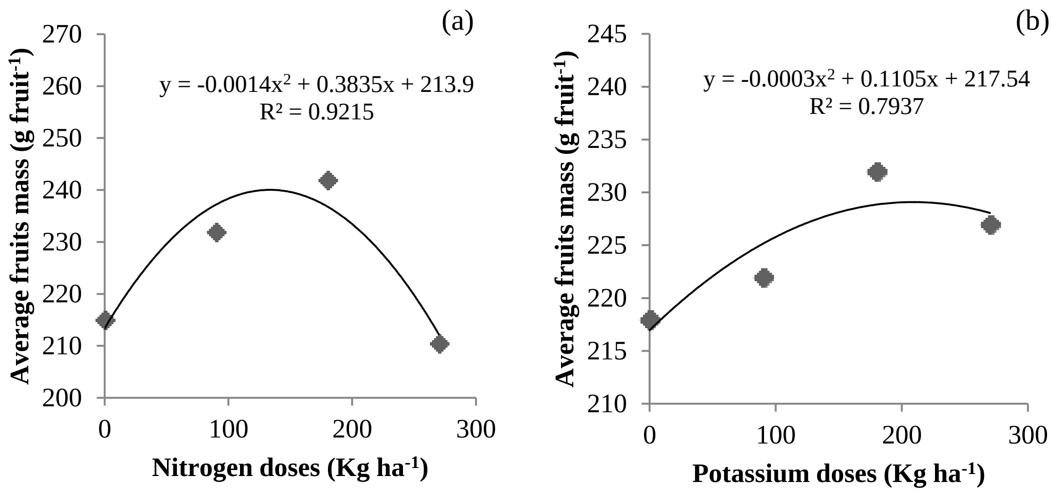

Figure 5. Average fruits mass of Italian Zucchini, Novita Plus cultivar, due to the application of different nitrogen (a) and potassium (b) doses.

Conclusions

The number of leaves of Italian Zucchini crop has a direct relationship with increasing nitrogen doses, unlike potassium doses that did not exert any effect.

Regarding floral crop production, the studied nutrients had significant effect on the production of male and female flowers, giving more emphasis to the nitrogen that allowed maximum sexual expression of the crop in male flowers through the maximum nitrogen dose.

The maximum doses of the studied nutrients resulted in losses in average number of fruits and average fruit mass, due to the inhibition of absorption of other nutrients as well as cause physiological disturbances to the plant.

Conflict of interests

The authors have not declared any conflict of interests.

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