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Antimicrobial Activity of Fruit Extracts of *Xylopia Aethiopica* and its Combination with Antibiotics against Clinical Bacterial Pathogens

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Abstract
The in- vitro antimicrobial activity of ethanol and aqueous fruit extracts of *Xylopia aethiopica*, four conventional antibiotics: gentamycin, ampicillin erythromycin and ciprofloxacin and the combination of each extract with the conventional antibiotics were investigated using the agar diffusion method. Clinically isolated strains of bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* *Streptococcus faecalis* and *Shigella dysenteriae were used for the assay.* The preliminary screening of phytochemical constituents of the fruits of *Xylopia aethiopica* showed the presence of cardiac glycosides flavonoids, phlobatannins, tannins, phenol, anthraquinones, saponin and steroids. The ethanol extract was active against *P. aeruginosa*, *B. subtilis*, *S. aureus*, but showed no activity against *K. pneumoniae* and *E. coli* while the aqueous extract was only active against *S.aureus.* The test organisms showed susceptibility to the antibiotics used except *P. aureginosa* which was resistant to ampicillin. Synergism was obtained in 39.3% of all the combinations investigated, antagonism in 57.1%, and indifference in 3.6% Gentamycin when combined with the aqueous and ethanol extracts had the highest percentage of synergism, Caution should be taken in with concurrent administration of *X.aethiopica* with conventional antibiotics because of the higher percentage of antagonism observed.

KEYWORDS: *Xylopia aethiopica*, antibiotics, plant extract antimicrobial activity, synergism, antagonism.

1. Introduction
Infectious diseases with increasing trends of drug resistant microorganisms have been common global problem posing enormous public health concerns (Iwu *et al*., 1999). The global emergence of antimicrobial resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). According to WHO (2002), the available antimicrobial drugs are costly and beyond the reach of the common man in many poor countries. It was estimated by Anon (1987) that more than two thirds of the world's population relied on plant derived drugs. It is also estimated that local communities have used about ten percent (10%) of all flowering plants on Earth to treat various infections, although only one percent (1%) have gained recognition by modern scientists (Kafaru, 1994). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Ashish *et al.*, 2011). These plant-based systems will continue to play an essential role in health care especially in rural areas around the world.

Xylopia aethiopica is a tree of 20 meter high or more with a clear straight bole to 75cm girth (Burkill, 1985). It is reported, that there are between 100 and 150 species of *Xylopia* distributed throughout the tropical regions of the world, particularly Africa, among them, *X. aethiopica*, *X. brasiliensis*, *X. frutenscens*, *X. grandiflora*, which have been studied more completely, than *X. aromatic.* The various extracts from *Xylopia* spp. have been shown to possess antiseptic and analgesic properties, and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies Various parts of the plant have been traditionally employed in different therapeutic preparations (Konning *et al*., 2004). A fruit extract or decoction of the bark as well as of the fruit is useful in the treatment of bronchitis and dysenteric conditions. In Congo, it is used for the attacks of asthma, stomach aches and rheumatism (Burkill, 1985). *Xylopia aethiopica* has been reported to be recommended to women who have newly given birth as a tonic in the Ivory Coast as a woman remedy, it is taken also to encourage fertility and for ease of childbirth (Burkill, 1985). Sometimes, a combination of *X. aethiopica* with other plant types or a combination of different parts of *X. aethiopica* is used to achieve the desired effects (Fall *et al*., 2003; Ogunkunle and Ladejobi, 2006). Among the conditions treated with *X. aethiopica* in traditional medicine are cough (fruits and roots of the plant) bronchitis, dysentery and biliousness (fruits and stem bark) and boils and sores (leaves and bark) (Ghana Herbal Pharmacopoeia, 1992; Mshana *et al*., 2000). Research on synergism is very limited and few studies have been reported (Nascimento *et al*., 2000).This research intends to justify the ethnobothanical use of the fruits of *Xylopia aethiopica* and to study the in-vitro effect of its concurrent use with conventional antibiotics .

2.1 Preparation of Plant Extract

Fresh fruits of *Xylopia aethiopica* was collected in Ago-Iwoye and Ijebu-Ode in Ogun State and identified at the Elikaf herbarium, Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria, where a specimen voucher was kept. The fruits of the plant were air dried and triturated in a mechanical mill. Soxhlet apparatus was used for extraction. One liter of 80% ethanol was used to extract 200g of the plant material at 78^0C. The filtrate was concentrated on a rotary evaporator at 45^0C and the extract was then kept in sterile bottle under refrigerated conditions at 4^0C until use. Another 200g of plant material was extracted in one liter of water for 4 days with occasional shaking (Harborne, 1998). The aqueous extract was lyophilized to obtain a dry powder extract. The mixtures are then separated using sterilized cotton wool. The extracted liquid was filtered through sterilized Whatman filter paper and the filtrates were then evaporated by concentration. It was then stored at 4 ^{0}C for further use.

2.2 Phytochemical Screening

Photochemical screening for major constituents was undertaken using quantitative methods as described by Trease and Evans (1989) and Sofowora (1993). The plant material was screened for the presence of cardiac glycoside, flavonoids, phlobatannins, tannins, phenol, anthraquinones, saponin, steroids, terpenoids and alkaloids.

2.3 Test Microorganisms

Pure cultures of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Shigella dysenteriae, and Streptococcus faecalis were obtained from Nigeria Institute of Medical Research Centre (NIMR), Lagos, Nigeria. The bacterial isolates were first sub cultured on nutrient agar and incubated at 37 ℃ for 24hrs.

2.4 Antibacterial activity

The agar-well diffusion method was used. 6 wells were made at equidistant positions into previously seeded Mueller Hinton agar plates containing108cfu/ml (0.5 McFarland's standard) of each of the test organism, 0.2ml of the different concentrations of each extracts were introduced into the different holes and allowed to diffuse into the medium for 1 hour at room temperature. The control was set up in a similar manner with ethanol and sterile distilled water. The plates were incubated at 370C for 24hrs. All tests were performed in duplicates and antimicrobial activity was expressed as the mean diameter of the clear zone (mm) produced by the plant extract..

2.5 Effect of antibacterial drugs on test organisms

The antibiotics used were Erythromycin, Ampicillin, Ciprofloxacin and Gentamycin. Sterile swabs were dipped into the standardized bacterial suspensions and then streaked over the surface of the Mueller Hinton plate and allowed to dry for 5mins before the antibiotics were aseptically placed over them and incubated for 24 hours at 370C

2.6 Antimicrobial activity of the combination of extracts and antibiotic discs.

This was carried out using the agar overlay method (Nweze and Onyishi , 2009). Two ml of the least concentration of the extracts was mixed with Mueller Hinton agar and allowed to gel together in the Petri dishes. The Petri dishes containing the media were seeded differently with the different test organisms. The excess broth was pipette off and the cultures were allowed to dry for 30 minutes after which commercially prepared antibiotic discs: gentamycin , ciprofloxacin ampicillin and erythromycin were placed on top of the culture plates and incubated for 24 hours at 37℃. The resulting zones of inhibition were measured and the differences in the reaction of the combination compared to the antibiotics alone were designated as synergism, indifference or antagonism.

3. Results and Discussion

3.1Phytochemical Screening

The preliminary screening of phytochemical constituents of the fruits of Xylopia aethiopica showed the presence of cardiac glycoside, flavonoids, phlobatannins, tannins, phenol, anthraquinones, saponin and steroids but absence of terpenoids and alkaloids (Table 1).Preliminary studies have shown that X. aethiopica fruits contain pharmaceutical constituents such as tannins. Phlobatannins, flavonoids and steroids. These bioactive components which are known to be bactericidal, pesticidal or fungicidal in nature (El astal et al., 2005). It has also been reported that these compounds are mostly secondary metabolites which are capable of producing definite physiological actions on body (Joshi et al., 2009) and are the most important bioactive constituents of natural products (Edeoga et al., 2005). Tannins in plants are known to be astringents, which help in wound healing and are anti-parasitic, they are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003) and are known to show curative activity against several pathogens (Usman and Osuji, 2007).The presence of these metabolites suggests great potential of Xylopia aethiopica as a source of useful phytomedicine.

3.2 Antibacterial activity

Table 2 shows the antibacterial activity of the extracts of *Xylopia aethiopica* at different concentrations. The ethanol extract of the plant had inhibitory effect on B. subtilis and Shigella at all concentrations, P. aeruginosa at 50mg/ml -150mg/ml. It only had effect on S. faecalis and S. aureus at 150mg/ml while no activity was observed against E .coli and K. pneumoniae . The water extract of the plant had no inhibitory effect on the test organisms except S. aureus. The results of the antimicrobial activity ethanol extract of X. aethiopica, shows that the plant extracts was very active against , B. subtilis, and S.dysenteriae at all concentrations used however no observable activity against E. coli and K. pneumoniae . Some authors had reported lack of activity of the plant extracts against E. coli and attributed it to the fact that E. coli, being a Gram negative bacterium, has an extra outer membrane that may be impermeable to the plant extract (Iwu, 1993; Konning et al., 2004; Nweze and Onyishi, 2009). Ezeifeka et al. (2004) and Asekun and Adeniyi (2004) however reported in vitro activities of ethanol extract of this plant against E.coli and K. pneumoniae. From the results, it can be deduced that the antimicrobial activity of the extracts increased with increasing concentrations of the extracts in all the cases

3.3 Effect of antibacterial drugs on test organisms.

The antibiotics used showed inhibitory effect on the microbes except P. aureginosa which was resistant to ampicillin and the highest zones of inhibition ere oberseved with ciprofloxacin with the expection of S. dysentarie (Table 3).All the conventional antibiotics had inhibitory effects on the test organisms except for P. aeruginosa which was resistant to ampicillin. Nweze and Onyishi (2009) also reported the resistance of P. aeruginosa to ampicillin.

3.4 Antimicrobial activity of the combination of extracts and antibiotic discs.

Table 4 shows the in vitro combined activities of the plant extracts from X. aethiopica and the conventional antibiotics against the test organisms , the combinations produced varying zones of inhibition. Gentamycin when combined with the aqueous extract had the highest percentage of synergism, four out of the test organisms were also sensitive to its combination with the ethanol plant extract Table 5 shows the summary of the results of the combinations of the ethanol plant extract and antibacterial drugs and aqueous plant extract and the antibacterial drugs against all the isolates, respectively. Synergism was obtained in 39.3% of all the combinations investigated, while antagonism was observed in 57.1%, and indifference in 3.6%.The combinations of the ethanol extracts of Xylopia aethiopica and the antibiotics gave varying zones of inhibitions. The ethanol extract in combination with erythromycin had the highest percentage of synergism against the test organisms while its combination with the aqueous extract brought about the highest level of antagonism reactions. Gentamycin when combined with the aqueous extract had the highest percentage of synergism, four out of the test organisms were also sensitive to its combination with the ethanol plant extract. Ciprofloxacin which was most active antibacterial drug against the test organisms was the least active when combined with either of the extract. The summary of the combination test showed that synergism was obtained in 39.3% of all the combinations investigated, while antagonism was observed in 57.1%, and indifference in 3.6%. This result differs from the findings of Nweze and Onyishi (2009) who reported synergistic interaction was obtained in 68.4% of the combinations investigated, while antagonism was observed in 26.3%, and indifference in 5.3%. They suggested that the concurrent administration of the plant extract from X.aethiopica with any of the conventional may not necessarily elicit

antagonism as has been a widespread belief. However our result indicated that care should be taken with concurrent administration of X.aethiopica with any conventional antibiotic because of the higher percentage of antagonism observed.

Conclusion

Care should be taken in concurrently taking of X.aethiopica with any conventional antibiotic because of the higher percentage of antagonism observed in this reseach. The ethanol extract in combination with erythromycin and gentamycin with the either of the extract may be used together because of the high levels of synergism observed. However, in vivo studies using animal models will be required for the confirmation of these results.

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Table 1: Phytochemical Constituents of Fruits of *Xylopia aethiopica*

Phytochemical Tests	Results
Cardiac glycoside	+++
Flavonoids	++++
Phenol	+
Terpenoids	-
Phlobatannins	+++
Anthraquinones	-
Tannins	++++
Saponin	++
Steroids	+++
Alkaloids	-

- = absent

++ = slightly present

+++ = present

++++ = strongly present

Table 2: Antibacterial activity of Ethanol and Aqueous fruit extracts *Xylopia aethopica* and antibiotics against bacterial isolates

	Conc	*Sautés*	*P.aeruginosa*	*K.pneumoniae*	*B.subtilis*	*S.dysenterie*	*S.faecalis*	*E.coli*
Xylopia	25mg	-	-	-	12	11	-	-
aethiopica	50mg	-	07	-	24	13	-	-
ethanol extract	100mg	-	10	-	28	17	-	-
	150mg	10	11	-	28	22	15	-
Xylopia	25mg	10	-	-	-	-	-	-
aethiopica	50mg	11	-	-	-	-	-	-
aqueous extract	100mg	14	-	-	-	-	-	-
	150mg	15	-	-	-	-	-	-
Antibiotics								
gentamycin		20	15	15	43	16	32	42
ampicillin		21	-	22	18	12	20	20
ciprofloxacin		40	46	36	43	18	40	49
erythromycin		29	29	21	30	28	23	29
Control								
ethanol		-	-	-	-	-	-	-
Water		-	-	-	-	-	-	-

Table 3: Inhibition zone diameter (mm) of the combined activity of the plant extracts from *X. aethiopica* and the antibiotic against bacterial isolates.

	Ethanol extract with antibiotics				Aqueous extract with antibiotics		
Organism	Erythromycin	Ciprofloxacin	Ampicillin	Gentamycin	Erythromycin	Ciprofloxacin	Ampicillin
				Gentamycin			
S. aureus	32	32	21	28	29	31	19
				27			
P. aeruginosa	13	34	-	36	-	35	19
				23			
K. pneumoniae	24	32	13	17	-	39	24
				29			
B. subtilis	38	27	25	23	-	35	20
				29			
S.dysentarie	12	32	12	23	25	40	11
				21			
S. faecalis	30	32	15	25	-	36	19
				29			
E. coli	32	30	29	29	-	35	16
				22			

Table 4: Summary of the plant extracts from *X. aethiopica* and antibiotic combination against all isolates.

	Ethanol extract with antibiotics				Aqueous extract with antibiotics			
Organism	Erythromycin	Ciprofloxacin	Ampicillin	Gentamycin	Erythromycin	Ciprofloxacin	Ampicillin	Gentamycin
S. aureus	synergism	antagonism	indifference	synergism	indifference	antagonism	antagonism	synergism
P. aeruginosa	antagonism	antagonism	antagonism	synergism	antagonism	antagonism	synergism	synergism
K. pneumoniae	synergism	antagonism	antagonism	synergism	antagonism	synergism	synergism	synergism
B. subtilis	synergism	antagonism	antagonism	antagonism	antagonism	antagonism	synergism	antagonism
S.dysentariae	antagonism	synergism	antagonism	synergism	antagonism	synergism	antagonism	antagonism
S. faecalis	synergism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	synergism
E. coli	synergism	antagonism	synergism	antagonism	antagonism	synergism	antagonism	synergism

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Study of Bacterial infection associated with male infertility in Hillah city-Iraq

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Abstract

Objectives: To identify bacterial species present in the lower genital tract of males and to investigate the relationship with semen quality and male infertility. **Methods**: The microscopic analyses, cultures and ELISA technique of 175 semen and serum specimens, collected over 9 months from males investigated for infertility, were prospectively assessed. **Results**: One hundred and seventy five seminal fluid, blood and serum specimens were collected from men investigated for infertility over a period of 9 months (from April 2011 to December 2011) were analyzed. The seminal fluids and serum of patients mentioned to the laboratory from the fertility clinics of Babylon maternity and children Hospital and outer clinics. The results had shown that from 17 microbial species there are, *Ureaplasma urealyticum* 4.938272 %, *Ureaplasma parvum* 2.160494 %, *Mycoplasma hominis* 2.469136 %, *Mycoplasma genetalium* 5.864198 %, *Chlamydia trachomatis* 9.876543 %, *Streptococcus pyogenes* 8.641975 % , *Staphylococcus aureus* 11.11111 %, *Staphylococcus epidermidis* 12.03704 %, *Staphylococcus saprophyticus* 0.925926 %, *Escherichia* coli 20.06173 %, *Proteus mirabilis* 1.234568 %, *Proteus vulgaris* 2.469136 %, *Klebsiella pneumoniae* 0.925926 %, *Pseudomonas aeuroginosa* 1.54321 %, *Neisseria gonorrhoeae* 2.777778 %, *Toxoplasma gondii* 6.17284 % and *Candida* 6.790123 %. Also the infection with microorganisms revealed that it is higher in azoospermic patients than normospermic group (control).

Keywords: Male infertility, ELISA technique, Bacterial infection.

1. Introduction

There is difference as to the influence of certain microbial infection on male infertility. Several investigators have reported difference types of microorganisms in seminal fluid (Ajabor *et al.* 1999). It was reported that detection of bacteria in semen does not essentially suggest infection because bacterial isolates in seminal fluid may signify colonization of the urethral, contamination, or infection. Enterobacteriaceae, *Chlamydia*, *Ureaplasma* and some gram positive bacteria are the most frequently isolated organisms in industrialized countries (Keck *et al.* 1998). In some parts of the world, oligospermia and azoospermia are most common causes of male infertility which has been reported due to bacterial infections (Ajabor *et al.* 1999 & Megafu 2007).

Urinary tract infections are common in men, and clinicians working with infertility frequently encounter patients with these diseases. Infections include either cystourethritis, caused by trivial urinary bacteria or by sexually transmitted pathogens affecting fertility. The possible relationship between infection and infertility has been the subject of controversy since the second half of the 1970s2, and several therapeutic trials have been initiated since then. The criteria for infection-associated infertility have been laid down in the World Health Organization (WHO) manuals, and several studies of the pathogenesis of reproductive disturbance in infected men have been published in the past decade (Rowe 2000).

An understanding of the link between infection of the ‘accessory sex glands’ and reduced male fertility has been scientifically acquired and diagnostic tools are available, but the results of antibiotic treatment in terms of fertility remain disappointing. The last is probably due to the irreversibility of functional damage caused by chronic infection/inflammation. Therefore, prevention, early diagnosis and correct treatment of infections of the male tract,

both trivial and sexually transmitted, are of pivotal importance (Sergio *et al.* 2007).

According to (WHO), seminal fluid infection was defined as the presence of significant bacteriospermia (⩾103 bacteria/ml ejaculate), detection of *Neisseria gonorrhoeae, C. trachomatis, U. urealyticum*; significant leukocytospermia (106 peroxidase positive leukocyte/ml ejaculate). It therefore follows that if some or all the conditions above are not met, the isolation of bacteria in semen are often regarded as contaminants by most practitioners (Okon *et al.* 2005).

2. Patients and Methods

One hundred and seventy five seminal fluid specimens from men were investigated for infertility over a period of 9 months. These seminal fluids of patients submitted to the laboratory from the fertility clinics of Babylon maternity and children Hospital and outer clinics the practical work was done in the college of science for women in bacteriology, virology and biotechnology labs while serum samples were collected from patients in outer clinics "Ibn Al Nafees laboratory and The Specialist Medical laboratory". Each specimen was collected by patient himself into sterile bottle. The subjects were instructed on how to collect the specimens and submit to the laboratory within one hour of collection. They were told to first pass urine and then wash their hands and penis with soap, then rinse with water prior to masturbation and ejaculation into sterile container. The semen was collected after the patient had abstained from coitus for at least three days. The semen was them cultured on Nutrient, MacConky, Mannitol salt, Thayer martin, Eosin methylene blue, blood, kligler, peptone water, MR-VP, citrate, Sabouraud and chocolate agar media then incubated for 24-48 hours at 37oC, these media used for *Streptococcus pyogenes*, *Staphylococcus* aureus, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Eschirishia coli*, *Proteus mirabilis*, Proteus vulgaris, *Klebseilla pneumoniae*, Pseudomonas aeuroginosa, *Neisseria* gonorrhoeae. Also to confirm the culture results we used Microbial Identification BIOMÉRIEUX VITEK® 2 SYSTEM.

The infective microorganisms were identified by Gram stain and cultivation on media for the cultivable microorganisms, others which are not cultivable microorganisms was detected by ELISA technique such as *Chlamydia*, *Ureaplasma* and *Mycoplasma*. The sperm density, volume, viscosity (liquefaction), the percentage of actively motile sperms, the percentage of abnormal forms, the presence or absence of pus cells were assessed. Analysis was carried out immediately they were received.

3. Results and Discussion Workforce Sizing Plan (WOZIP)

The results are summarized in Tables 1 also in figure 1. Table 1 shows that from 17 microbial species, *Ureaplasma urealyticum* consist 4.938272 %, *Ureaplasma parvum* 2.160494 % , *Mycoplasma hominis* 2.469136 %, *Mycoplasma genetalium* 5.864198 %, *Chlamydia trachomatis* 9.876543 %, *Streptococcus pyogenes* 8.641975 %, *Staphylococcus* aureus 11.11111 %, *Staphylococcus epidermidis* 12.03704 %, *Staphylococcus saprophyticus* 0.925926 %, *Escherichia* coli 20.06173 % , *Proteus mirabilis* 1.234568 % , *Proteus vulgaris* 2.469136 % , *Klebsiella pneumoniae* 0.925926 %, *Pseudomonas aeuroginosa* 1.54321 %, *Neisseria gonorrhoeae* 2.777778 %, *Toxoplasma gondii* 6.17284 % and *Candida* 6.790123 % .

It is estimated that 15% of male infertility is related to genital tract infection (Keck *et al.*1998). From many infectious microorganisms, U. urealyticum is one of the most common species (Wang *et al.* 2006). Since 1967, the ureaplasmas have been shown as an aetiology of male infertility (Radhouane *et al.* 2007), and especially when Friberg and Gnarpe first demonstrated a higher frequency of ureaplasmas in the semen of men with unexplained infertility (76%) compared with fertile men (19%) (Emokpae *et al.* 2009).

In 1999, U. urealyticum biovars 1 and 2 were classified into U. parvum and U. urealyticum, respectively (Kong *et al.* 1999). Most of the previous reported studies have discussed the role of ureaplasmas in male infertility without discriminating between *U. parvum* and *U. urealyticum* (Radhouane *et al.* 2007). In our study, we have used the ELISA assay that can facilitate the identification of *U. urealyticum*, *U. parvum*, *M. hominis* and *M. genitalium* in semen specimens. Our results demonstrated that genital mycoplasmas and ureaplasmas seem to be widespread among infertile male patients, as shown respectively by the frequency of 13% and 15.2%. These data are comparable with those reported in previous studies (Andrade-Rocha 2003). *U. urealyticum* was the most prevalent species of *Ureaplasma* genus detected (9.2%) in this study. (Rosemond *et al.* 2006 & Kong *et al.*1999). This wide range might be explained by the diversity of detection methods used for characterizing the studied populations and the technique

used in detection. In our study, *U. parvum* was detected in 3.8% of serum samples. The frequency of this species was lower than that reported by Knox et al. (3.8% vs 19.2%) (Reichart *et al.* 2001). *M. hominis* has been associated with bacterial vaginosis, pelvic inflammatory disease, postpartum fever, and postabortal fever, as well as a number of gynaecological infections (Yoshida *et al.* 2003).

However, its role in non-gonoccocal urethritis (NGU) and in infertility is rarely investigated (Pannekoek *et al.* 2000). The frequency of *M. hominis*, in our study 4.5%, it was comparable to that reported by Andra-Rocha et al. and Radhouane Gdoura et al. but higher than that reported by Rosemond (Deguchi & Maeda 2002). *M. genitalium* was first isolated in urethral cultures from two men with NGU in 1981, although *M. genitalium* has been suggested as a cause of human NGU, the precise role of this mycoplasma in the etiology of NGU remains not established because of the immense difficulty in isolating it from clinical samples (Jensen *et al.* 2004).

Hitherto, *M. genitalium* has seldom been investigated in semen of infertile men. In our study, the frequency of *M. genitalium* 5.864198 % it was higher than that reported by Kjaergaard et al. (Kong *et al.* 1999) (5.864198 % vs 0.9%).

This difference might be explained by the use of different methods for the detection of this bacterium. We have used ELISA that is more sensitive than culture and that can facilitate the detection of *M. genitalium* in clinical samples (Deguchi & Maeda 2000).

In the present study, the frequency of the U. urealyticum was higher than that of *M. hominis. U. urealyticum* was also detected more often than U. parvum; these findings were consistent with other studies (Jensen *et al.* 2004).

Previous studies had reported that the presence of mycoplasmas and ureaplasmas in sperm specimens has no real effect on the semen quality, or on the leukocyte count (Emokpae *et al.*2009 & Kong *et al.*1999). Other investigations seem to show that the presence of mycoplasmas reflects a silent infection rather than infection in infertile patients (Reichart *et al.* 2001), even though when the attachment and invasiveness towards human sperm cell has been demonstrated in vitro (Yoshida *et al.* 2003).

In the present study, the comparison of the sperm seminological variables of *U. urealyticum*-positive and *U. urealyticum*-negative infertile men demonstrated no significant differences in sperm seminological variables, which confirms previous findings. Conversely, a relationship between *U. urealyticum* and semen characteristics was observed in some literature (Emokpae *et al.* 2009 & Lackner *et al.* 2006).

The influence or the lack of influence of mycoplasmas and ureaplasmas on seminology may come from the capability of bacterial species to attach to spermatozoa and to affect directly via cellular interactions their vitality, motility, morphology, cellular integrity and their molecular structure or the development of protective immunity to genital infection by the host (population sensitivity to microbial agents) or other host factors (Lackner *et al.* 2006). Semen with *M. hominis* presented a higher mean of leukocytes than semen with negative *M. hominis*. In contrast, the means of leukocyte count of the positive ELISA for *U. urealyticum, U. parvum* and *M. genitalium* were nearly same than the reference value of the WHO manual. These findings indicate that the presence of mycoplasmas and ureaplasmas in semen is not necessary associated with leukocytospermia, and thus, in spite of potentially pathogenic species. Our results are consistent with previous reports (Radhouane *et al.* 2007 & Yoshida *et al.* 2003). Infection with microorganisms revealed that it is higher in azoospermic patients than normospermic group (control) as shown in figure 1 *Ureaplasma urealyticum* (62.5%, 0) U. *parvum* (100 %, 0), *Mycoplasma hominis* (62.5%, 0) *M. genetalium* (63.16%, 0) *Chlamydia trachomatis* (46.8%, 0) respectively. Interestingly, Staphylococcus aureus as causative organism accounted for 20.3% of seminal fluid infection in this study. This ratio is lower than reported by Okon et al. (Radhouane *et al.* 2007 & Yoshida *et al.* 2003) in Maiduguri, where Staphylococcus aureus was isolated from 62.5% of the seminal fluids. Most practitioners dismiss this infection as mere contamination which is assumed to be of no significance. The WHO definition of seminal tract infection does not clearly differentiate between infection, contamination and colonization of the genital tract. Semen that passes through the genital tract is routinely contaminated with Gram positive cocci such as *Staphylococcus*, *Streptococcus* and Diphteroids. It is generally accepted that *Staphylococcus* aureus which is coagulase positive is regarded as pathogenic and should be treated. The presence of this microorganism can no longer be ignored. The longer the infection persist, the greater the damage and loss of germ cells. The rate (percent) of infection increases from normospermic to azoospermic males (Rosemond *et al.* 2006).

According to Bukharin et al. Opportunistic microorganisms cause classical infections of the urogenital tract and subclinical reproductive tract infections. These infections of the seminal fluid lead to decrease in the number of

spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity. Our result shows that 58.2% of the males had sperm density below 20 million/ml. The sperm morphology deteriorated progressively in oligospermic to severe oligospermic males. In other words it decreased with decreasing sperm density. Azoospermia was also observed in 58.2% of the study population (Radhouane *et al.* 2007 & Rosemond *et al.* 2006).

The results obtained from questionnaire on infertility assessment showed that most of the semen samples from those infertile male patients belong to 20-36 years old category. The idea that bacterial infection may be partly responsible for male infertility arises from the clinical observation of the patients' male reproductive system (Punab *et al.* 2003 & Bukharin *et al.* 2005).

Male Urogenital Tract Infection is one of the most important causes of male infertility worldwide (Keck *et al.* 1998). Infection processes may lead to deterioration of spermatogenesis, impairment of sperm functions, and obstruction of the seminal tract. In the light of the above, there is the need to institute a microbiological intervention to detect the probable microbial agents. In view of our study, it seems that Leukocyspermia is a poor maker to predict bacteriospermia (Table 2, 3). Consequently in presence or absence of the Leukocytospermia, microbiological investigation should be performed on all semen, as a routine test, from infertile male attending infertility clinics. It should be noted that presence of Urogenital Tract Infection and inflammation posed a danger to the fertility profile of male patient and should be eradicated by antibiotics and anti-inflammatory treatment (Rodin *et al.* 2003).

5. Conclusion

Positive seminal fluid cultures were interpreted with caution, taking into account both raised colony counts of single isolates in the semen. Thus the common misdiagnosis of genital tract infection, based on the presence of seminal bacteria, and unnecessary treatment with antibiotics may be avoided.

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Table 1- Types of microbial infection associated with infertile men and control.

Type of microorganism	No.	%
Ureaplasma urealyticum	16	4.938272
Ureaplasma parvum	7	2.160494
Mycoplasma hominis	8	2.469136
Mycoplasma genetalium	19	5.864198
Chlamydia trachomatis	32	9.876543
Streptococcus pyogenes	28	8.641975
Staphylococcus aureus	36	11.11111
Staphylococcus epidermidis	39	12.03704
Staphylococcus saprophyticus	3	0.925926
Escherichia coli	65	20.06173
Proteus mirabilis	4	1.234568
Proteus vulgaris	8	2.469136
Klebsiella pneumoniae	3	0.925926
Pseudomonas aeuroginosa	5	1.54321
Neisseria gonorrhoeae	9	2.777778
Toxoplasma gondii	20	6.17284
Candida	22	6.790123
Total	324	100

Table 2- Relation between microbial isolates, abnormal sperm morphology and total motility.

Bacterial isolate	Abnormal Sperm morphology (>60%)		Total motility ml < 50 % or more with forward progression or < 25 % or more with rapid progression within 60 minutes of ejaculation	
	No.	%	No.	%
Ureaplasma urealyticum	11	10.37736	6	13.63636
Ureaplasma parvum	4	3.773585	1	2.272727
Mycoplasma hominis	2	1.886792	1	2.272727
Mycoplasma genetalium	5	4.716981	1	2.272727
Chlamydia trachomatis	13	12.26415	4	9.090909
Streptococcus pyogenes	9	8.490566	3	6.818182
Staphylococcus aureus	19	17.92453	5	11.36364
Staphylococcus epidermidis	3	2.830189	0	0
Staphylococcus saprophyticus	7	6.603774	1	2.272727
Escherichia coli	22	20.75472	14	31.81818
Proteus mirabilis	1	0.943396	1	2.272727
Proteus vulgaris	0	0	1	2.272727
Klebsiella pneumoniae	0	0	1	2.272727
Pseudomonas aeuroginosa	1	0.943396	1	2.272727
Neisseria gonorrhoeae	4	3.773585	2	4.545455
Toxoplasma gondii	3	2.830189	1	2.272727
Candida	2	1.886792	1	2.272727
Total	106	100	44	100

Table 3- Age classification, number of isolates, leucocytes and sperm count/mL

Age (years)	No. examined			No. of positive leukocyte			Sperm count (x106) mean		
	Azoo-spermia	Oligo-spermia	Normo-spermia	Azoo-spermia	Oligo-spermia	Normo-spermia	Azoo-spermia	Oligo-spermia	Normo-spermia
21-25	20	20	7	11	7	2	0	1.7	32.3
26-30	19	19	6	13	10	4	0	1.1	30.7
31-35	19	19	6	9	12	2	0	1.4	26.6
36-40	19	19	6	12	7	1	0	1.2	22.5

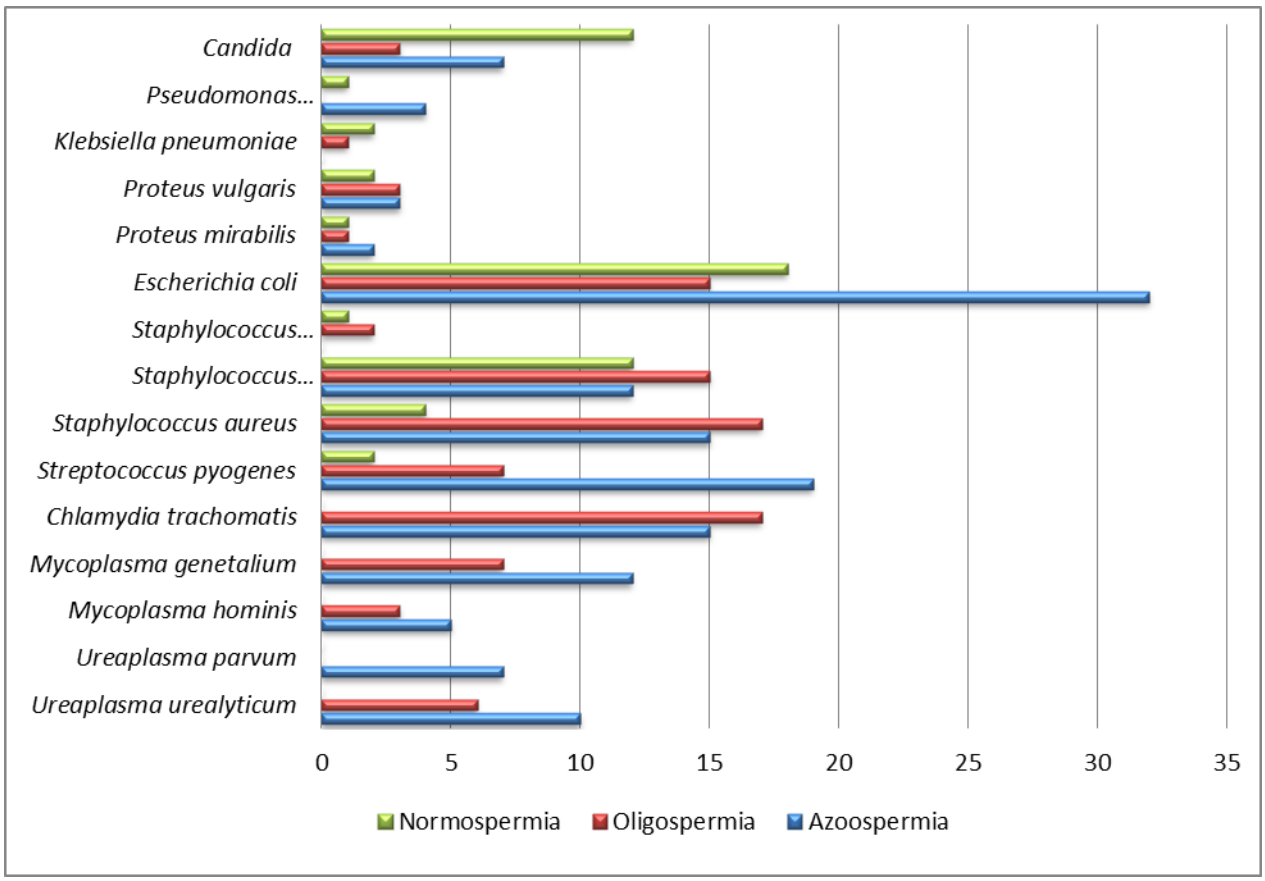

Figure 1: Types of Microbial infection associated with azoospermic, oligospermic and normospermic patients.

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ENVIRONMENTAL TENSIONS AND COMPATIBILITIES IN AGRIBUSINESS SUPPLY NETWORKS: A CASE OF THE FLORICULTURE INDUSTRY IN KENYA

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Abstract
The paper is part of a wider study on supply networks optimisation in the floriculture industry in Kenya. It is an analysis of environmental tensions and compatibilities in agribusiness supply networks addressing the floriculture industry in Kenya. The choice of the floriculture industry is due to the fact that it is currently one of the top contributors to the country's Gross Domestic Product (GDP). The industry has been a subject of debate with regard to environmental sustainability. The principal research question was to find out the environmental consequences of the floriculture industry in relation to its supply networks. The study took both a qualitative and quantitative approach in its methodology. The former involved focused interviews with key stake holders in the floriculture industry in Kenya while the later involved a quantitative approach using mainly principal component analysis (PCA). This identified environmental issues as factor two (2) described as country specific benefits. The paper concludes by making an observation that the floriculture industry needs to apply routine performance measurement and business efficiency techniques to; water, energy and wastewater management as a measure to reduce negative environmental impacts. A recommendation is made for a moving from old cooperate social responsibility to the new sustainability communications. A further recommendation is for the flower farms to engage into research with the Kenya Agricultural Research Institute and other research bodies in Kenya, for water stress tolerant flowers, increase hydroponics agriculture area and invest in rainwater harvesting and treatment systems and, install water water recovery/recycling plant.
Key words: Environmental tensions, Environmental compatibilities, supply networks

Introduction

Discussed in this paper is the background of the study, literature review, the research methodology including a presentation of findings and results. The paper winds up by making a conclusion and recommendations to the industry players and stakeholders on what needs to be done in order to address the environmental issues affecting the floriculture industry.

Background of the study

The paper addresses environmental tensions and compatibilities in the agribusiness supply networks. The issues that need to be considered include: i) extent of environmental pollution caused by the industry; ii) efforts for environmental conservation by industry players. Further studies may be necessary to show that the floriculture industry is neither polluting lakes nor encroaching on wetlands. The drying up of Lake Naivasha, which is situated in one of the most densely populated area with flower farms, has been attributed to the chemical effluents from the surrounding flower farms. It is to be appreciated that high altitude growing at equatorial latitudes produce quality flowers and vegetables without fossil fuels (heating and lighting) – Kenyan produce is grown under the sun.

There is need to examine the extent to which Kenyan growers are reducing carbon footprint by using geothermal and solar techniques for power generation. Carbon footprint stands for certain amount of gaseous emissions that are relevant to climate change and associated with human production activities (Wiedmann and Minx, 2007). Currently, there is a debate on how to measure and quantify a carbon footprint. A survey of definitions, Scopus (2009), defines carbon footprint in terms of how much carbon dioxide emissions can be attributed to a certain product, company or organization. Carbon trading is already gaining foothold into the country, showing how critical it is to environmental conservation.

The financial and social benefits should also be seen to trickle down to the communities where the flower farms are situated. This should be seen in the form of employment creation and improved standards of living. It needs to be observed that most workers in the flower farms are casual laborers earning at least one United State dollar (USD) per day (Fedha, 2009); this has had its consequences in low standards of living, proliferation of slums. The fact that the floriculture industry is at the moment one of the largest single export earner for Kenya as well as a major employer does necessitate a need for improvement on productivity. If each employee in this sector has four

dependants then, the total beneficiaries are 4.8 million people or 13% of the population (Assumption based on average family size in the cut–flower growing areas in Kenya). These therefore necessitate the fact that Kenya has to retain its position in the supply chain network by providing end to end as well as within country optimization. This has to be seen in the backdrop of competition from other countries such as Ethiopia, Uganda, Zimbabwe and South Africa.

The social condition is characterised by high poverty rates since most workers earn less than one USD per day. There is also high prevalence of human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS). About 65% of the workers are casual laborers earning about UDD 30 per month. It is also to be noted that 75% of the casuals are women that have single parenthood families (Fedha, 2009). The industry uses many chemicals such as fertilizers, insecticides, fungicides, nematocides etc. Some of these chemicals have potential to cause serious harm to the ecosystem and human health. Studies / statistics on pesticides use and pesticide related health effects in the floriculture industry in Kenya are very rare and incomplete (Fedha, 2009).
Most of the flower farms are situated around Lake Naivasha. Lake Naivasha is a Ramsar site. Implying that it is a protected wetland under the Ramsar convention in which Kenya is a signatory (HCDA, 2008). Therefore, Kenya has the international obligation to protect such a site from ecological damages. However, pesticides and the degradation products find their way into the lake. This has had serious consequences on pollution of the lake. There is also need to develop the road infrastructure in Kenya in order to make the industry more competitive. There is, however, reasonable investment in cold chain facilities at the airports. Liberalization of trade which has resulted in the removal of exchange control and other constraints has been instrumental in the success of the industry. The Kenyan government has tried to give incentives in the form of nil or reduced duties on inputs for the sector.
Some questions of concern in the floriculture industry that exist in Kenya are: who benefits from the industry; is the industry fighting poverty among the poor in Kenya ; what are the long term effect of the pollutants from the industry on the environment; what are the long term effects of the industry on reproductive health, cancer related diseases, child health; where do the elderly workers go, and what health effects may manifest later; and what are the impacts of urbanisation with poor planning which indeed is a social problem? It is necessary for Kenya to stay competitive while addressing the possible problems that may be as a result of the mismanagement of the supply networks end to end and within the country.

Literature Review

The literature in this paper originates from the academic discipline of supply chain management, supply chain networks, organization structure and design, country industry development and triple bottom line benefits. The complexities of the floriculture industry which includes: extremely short shelf life; very specific cycles with extreme peaks; mixing characteristics of service and product dimensions; and the challenge of operating part of 'first world' supply network in a developing economy. This creates the need for end to end optimisation of the supply networks (Awuor, 2012)
The study will be of benefit to supply chain practitioners in the floriculture industry and any other industry with similar peculiarities. It will also be of benefit to the academia interested in the subject of supply chain management and indeed contribute to the body of knowledge in the subject. Existing theory that would be used as a basis for this study is depicted in the Venn diagram in fig. 1.1.

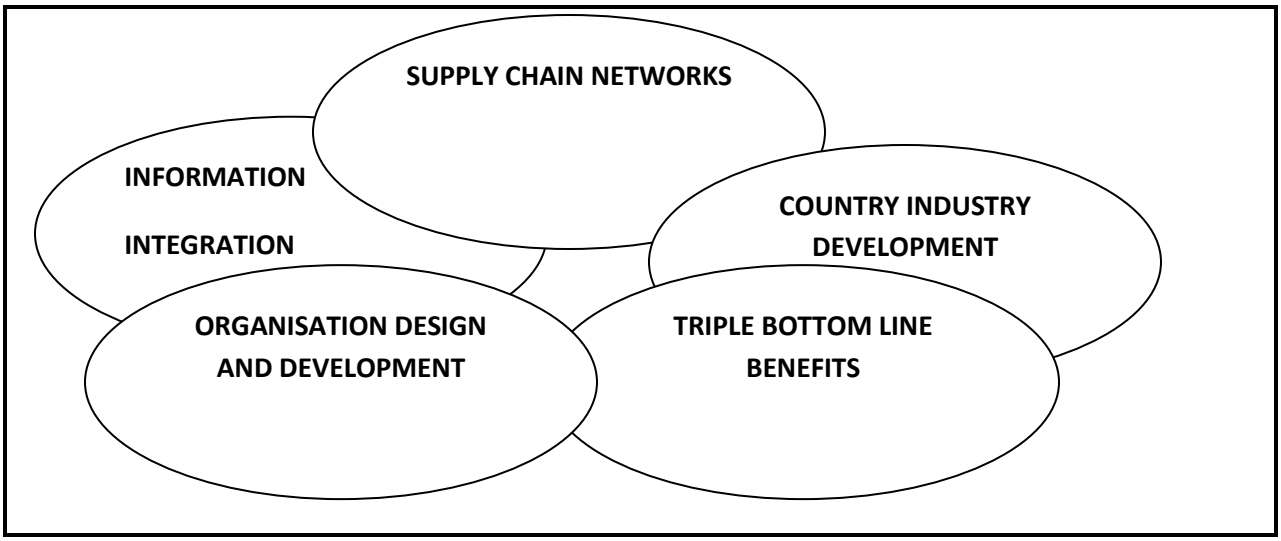

Fig. 1.1: Venn diagram illustrating theory covered
(Source: Awuor, 2012)

In order to consolidate further the research problem, a brief literature overview from the following five fields can be formulated as shown on the Venn diagram on fig. 1.1. These fields are: i) theory relating to supply chain networks; ii) theory on organisation design and structure; iii) issue to be addressed is theory related to Information communication technology; iv) country industry development; and v) triple bottom line benefits.
The supply chain network is the facet of the supply chain that translates organizational goals of the company. The network in this regard refers to the physical movement of the goods from suppliers' supplier to the company and ultimately to the customers' customer (Bolstorff, 2006). The factors considered in setting supply chain network goals include service level, order fulfillment cycle time, supply chain management costs and inventory days of supply.
Supply chain networks links to supply chain process which is in reference to the supply chain operations reference (SCOR) model (plan, source, make, deliver and return). Factors considered in setting supply chain process goals includes transactional productivity for sales orders, purchase orders, work orders, return authorization, replenishment orders and forecasts (Bolstorff and Rosenbaum, 2003).

Use of information communication and technology (ICT) software's such as enterprise resource planning (ERP), electronic data interchange (EDI) and warehouse management systems offer the obvious benefits of better, faster, more accurate information capture and sharing, but there are benefits found also in what it avoids: the human element (Goldsby *et al*. 2005).
Organisation structure and design is important from the point of view of the individual firms and the industry at large. The organisation may refer to elements in a single company or to a bigger part of the complex system itself. It prioritizes organizational performance by balancing customer requirements for delivery reliability, responsiveness and flexibility with the internal needs of cost, profitability and asset utilization (Bolstroff, 2006). Organization structure will also involve determining the influence of power relationships in SCM. According to Bolstroff (2006), the following questions are important with regard to organization design: does your organization structure address centralization, globalization, and functional silos; are all relevant functions in place; are all the functions necessary; is the current flow of inputs and outputs between functions appropriate; and does your organization structure support your suppliers and customers organization structure?

Environmental tensions
Studies suggest that increasingly there are health challenges associated with the floriculture industry in Kenya (Awuor, 2012). There are unknown effects of pesticides and other agro-chemicals used in the industry (Shivoga,

2008). There is also concern that employment is mainly temporal and part time, targeting mainly the youth with gender bias toward women workers at seventy five percent. but the supervisors are mainly men (Shivonga, 2008). This suggests that job security is nearly nil. Hence questions on the economic and social benefits of the industry still lingers.

There are also challenges of environmental concern. The flower farms occupy about 2000 hectares (20 Kilometers Squared) which are mainly concentrated around Lake Naivasha (KFC, 2008). The lake is currently shrinking mainly due to excessive abstraction of water for irrigation, industrial and domestic use. The southern shores of the lake are already blinded with algal bloom. The shrinkage of the lake is also attributable to pollution from pesticides and fertilizer run off. Cut – flowers are grown in greenhouses and the predominant pesticide used is methyl bromide (Shivonga, 2008).This chemical is a health risk when inhaled. Workers are exposed to these chemicals through transplanting, pruning, cutting, packing, spraying / fumigation and dusting. Also, through re-use of pesticide – saturated green house plastics for domestic purposes such as covering houses. Therefore, there is need for close collaboration between researchers and the floriculture farms and industry player to develop home – based solutions.

Environmental compatibilities

The National Environmental Management Authority (NEMA) is the national body mandated with the responsibility of conducting environmental audits before the flower farm are given a go ahead to establish their activities. The Kenya Flower Council (KFC) also has a code of regulations to be observed by its members. The silver standard is laid out in five sections (Awuor, 2012):

i) "Farm management, responsibilities and documentation" requires growers to keep records on health and safety, worker terms and conditions, employee remuneration, wage deductions, and agrochemical stocks, application and training. It also requires growers to pay royalties to plant breeders according to international rules'';

ii) "General Worker Welfare" covers worker wages, labour conditions and health and safety. The section stresses the importance of complying with national labour and health and safety legislation (particularly the Regulation of Wages and Conditions of Employment Act). In only a few instances does the code go beyond the provisions of the law and consequently few responsible flower industry employers fall foul of the code;

iii) "Agrochemicals" covers crop protection strategies, worker protection, and the use, application, storage, transport, and disposal of pesticides. Kenyan law contained in "The Factories Act" and "The Pest Control Products Act" gives some guidance on these issues. However, the code takes most of its provisions from widely recognized principles of best agricultural practice";

iv) "Protection of the natural environment" covers use of fertilizers, water management, soil conservation, disposal of non-hazardous waste, and the protection of wildlife and water sources. Until recently there has been little legal guidance on protecting the environment. However, in 1999 new legislation was enacted to cover a wide range of environmental issues. Implementation of the act has yet to take place and the extent to which it will affect the KFC code has yet to be fully gauged. The Kenyan flower industry continues to come under considerable criticism from environmentalists worried that pollution and over-exploitation of natural resources will permanently degrade the natural environment. The KFCs silver standard code, and more particularly the gold standard, is designed to deflect criticism from KFC members. During the course of the fieldwork for this study, several KFC members expressed the apparently genuine opinion that maintaining a sustainable natural environment was crucial to the long term future of the industry;

v) "Post harvest" covers health and safety, and environmental requirements that are specific to grading, packing houses and cold stores.

It is however, of much concern that there is no strict compliance with this rules as was noted in March 2010 when the fish in Lake Naivasha were virtually dieing as result of pollution of the lake (Awuor, 2012). Lake Naivasha has the highest concentration of flower farms in Kenya. The Lake Naivasha region is the hub of the Kenya's cut flower industry. The region is situated around 100 km northwest of Nairobi in the Great Rift Valley at an altitude of between 1,800 – 2,000m above sea level. The temperature range for the region is between 7.3 – 22.7 degrees Celsius and annual rainfall ranging from 156.0 mm/month to 1134.0 mm/month distributed throughout the year with peaks in April/May for the long rains and October/November for the short rains (HCDA, 2008).

It is estimated that close 70 per cent of the country's total flower production is concentrated around Lake Naivasha. Other than the growers, the Lake Naivasha cluster comprises other key actors in the flower industry including research institutions, breeding farms, quality control and regulatory agencies, input suppliers, credit and finance institutions, trade promotion agencies and other intermediary organizations. The emergence and growth of Lake Naivasha cluster has been attributed to a number of factors. Key amongst these includes:

i. Proximity to Jomo Kenyatta International Airport (JKIA), Nairobi: By its location along the Nairobi – Nakuru highway, approximately one hour from the city center, the Naivasha cluster has easy access to the airport making transportation easier. Nairobi is considered a major hub in the East African region and served by major airlines according Kenya an easy access into Europe and other parts of the world;

ii. Availability of fresh water resources for irrigation: Lake Naivasha is the only fresh water lake in the whole of the Rift Valley region. Flower growing requires a lot of water for irrigation and the presence of Lake Naivasha attracted many farmers to this region. Besides the lake, there are lots of underground water resources (aquifers) which the farms drill to use for irrigation;

iii. Large farms for large-scale commercial production: The availability of large, inhabited tracts of land with suitable soils for flower production around Lake Naivasha was another contributory factor to the development of the cluster. Historically these large tracts of land were owned (through leasehold) by white settlers such as Lord Delamere Estates which owns most of the land around Naivasha town. Both the white settlers and the government therefore leased out the fallow land to the large scale commercial flower growers; and

iv. The soils and climate are conducive for horticultural production: Both the soils, temperature and annual rainfall range around Lake Naivasha are favourable for cut flower production.

The case of March 2010 in which a lot of fish died from the lake has gone further to confirm that environmental audit is not strictly observed. The heavily polluted and shrinking, Lake Naivasha is in dire trouble. Environmentalists say the cause is clear: flower farms. Some 60 flower farms line the entire lakeside, growing cut flowers for export largely to the EU. While the flowers industry is Kenya's largest horticultural export (405.5 million last year) it may have also produced an environmental nightmare.

Interviews with environmentalists revealed that flower farms have taken water from the lake for irrigation and then dumped pesticide-waste back into the lake. Long-ignored by policymakers, the situation has recently reached a head due to thousands of fish and other freshwater organisms perishing in the lake. Fishing, once common in the lake, has since been banned. A preliminary inquiry linked the flower farms to the lake's troubles stating that the fish mortality was likely caused by low levels of dissolved oxygen. The lake is also shrinking due to a variety of factors: over-irrigation from the farms, water requirements for nearby Naviasha town, and climate change. However, it is to be noted that Lake Naivasha is a Ramsar site and Kenya has the international obligation to protect it from environmental degradation.

Research Methodology

The study thus makes use of a two-phased design. This according to Lee (1999), is a study in which a quantitative approach is followed by a qualitative approach (or the reverse), and this sequencing implies comparable standards for methodological rigor. First, there was need to identify the key success factors in the floriculture industry. This necessitated the qualitative approach. Secondly, there was need to understand the level of significance of the key success factors and the ultimate contribution to performance of the floriculture industry.

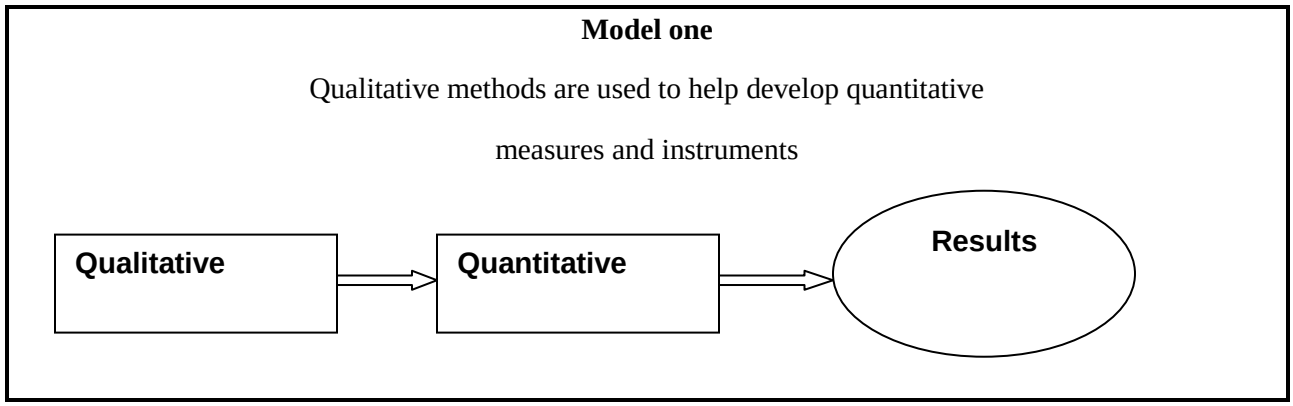

Fig. 1.2: Integrating qualitative and quantitative research
(Source: Steckler *et al.* 1992)

The first phase of the study makes use of the phenomenological approach. Focused interviews were done targeting members of the civil society, regulatory bodies, and farm and industry players. This initial qualitative study was beneficial in identifying the emerging issues in developing a conceptual model for simultaneous optimisation of the supply networks in the floriculture industry in Kenya. The instrument for data collection in the phase two of the research process is a questionnaire. The questionnaire has mainly closed ended questions to facilitate the processes of quantitative analysis. Kothari (2005), argues that before administering questionnaires, it is always advisable to conduct 'pilot study' (pilot survey) for testing the questionnaire. This indeed serves as the replica of the main study and it brings into light the weaknesses (if any) of the questionnaire and also of the survey technique. Questionnaire piloting thus assisted in making the necessary improvements in the research instrument. Figure 1.3 below gives a summary of the research design showing how the findings of phase one of the study is fed into the phase two of the study.

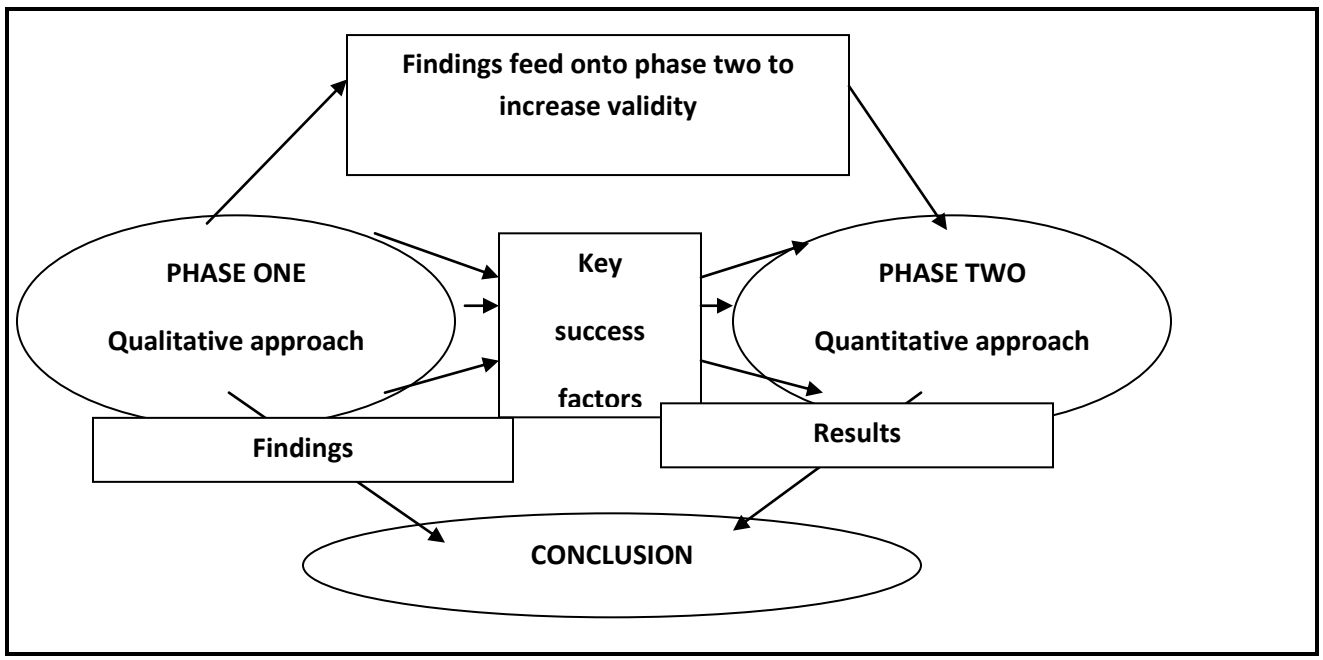

Fig. 1.3: Framework for this research process

(Source: Awuor, 2011)

The findings of the qualitative phase of the study feed onto the quantitative phase in order to increase the validity of the results. The findings and results of the two phases of the study were used in making the conclusions and recommendations of the study. The recommendations were addressed to both the flower growers and the government.

Discussion of findings and results

The proposed conceptual model shows that there are four main factors of interest in developing the conceptual model which were identified from phase one of the study (Awuor, 2012). The factors identified through phase one of the study are: country specific benefits; end to end benefits; key success factors and constraints. The constraints form a negative factor in the model and are identified as: access to finance; information integration; and ability to integrate into the supply networks of large firms. This explains the reason for forcing principal component analysis to produce four factors with loadings greater than 0.49. Environmental issues emerged as factor 2, described as country specific benefits.

Table 1.1: Summary of main factors from proposed conceptual model

Factor	**Description**
Factor 1	**Key success factors** – country development, research and development, financing, operational cost, customer responsiveness and information integration.
Factor 2	**Country specific benefits-** environmental audit, social audit and financial audit.
Factor 3	**End to end benefits-** operational cost, financing, country development, research and development.
Factor 4	**Constraints-** access to finance information integration, ability to integrate into supply networks of large firms

(Source: Awuor, 2012)

Principal component analysis only forced for four factors with loadings higher than 0.490 revealed results outlined in table 1.1. This was in an attempt to raise the factors previously identified through analysis data captured in the phase one of the study. By performing PCA and having five factors extracted was in attempt to identify the presence of any significant factors left out in the proposed model.

Table 1.2: PCA with verimax rotation forced to four factors reflecting factor loadings greater than 0.49

	Component			
	1	2	3	4
VAR00001				
VAR00002				
VAR00003				
VAR00004				

VAR00005				
VAR00006			.563	
VAR00007			.564	
VAR00008			.621	-.504
VAR00009			.609	
VAR00010				
VAR00011				
VAR00012				
VAR00013				
VAR00014	.602			
VAR00015	.647			
VAR00016	.569			
VAR00017		.798		
VAR00018		.820		
VAR00019		.841		
VAR00020		.815		
VAR00021		.856		
VAR00022		.812		
VAR00023		.728		
VAR00024				
VAR00025				
VAR00026				
VAR00027				
VAR00028				
VAR00029				
VAR00030				
VAR00031				

VAR00032				
VAR00033	.560			-.502
VAR00034	.549			-.545
VAR00035				-.522
VAR00036	.546			-.500
VAR00037				
VAR00038				
VAR00039			.527	
VAR00040			.513	
VAR00041				
VAR00042				
VAR00043				
VAR00044	.498			
VAR00045				
VAR00046	.490			
VAR00047				
VAR00048	.569			
VAR00049	.531			
VAR00050				
VAR00051				

(Source: Awuor, 2012)

The result of PCA forced for four factors with loadings greater than 0.490 resulted in four factors being identified based on information provided by the research instrument on appendix H. For instance VAR0017 to VAR0023 formed a distinct grouping isolated as factor 2 whereas VAR006 to VAR009 and VAR0039 to VAR0040 also formed a distinct grouping isolated as factor 3.

Conclusion and recommendations

Need for all players in the industry to be members of the Kenya Flower Council to ensure that there is compliance with the silver code of practice which emphasizes on (Awuor, 2011): farm management responsibilities and documentation; general worker welfare; use of agrochemicals; protection of the natural environment and post harvest treatment. Kenya flower growers have been granted an international label that recognizes good environmental and social practices. Growers who have a Kenya Flower Council Silver Certification or higher can

now register with Fair Flowers Planters (FFP) that partners with more than 4,000 European flower vendors (Doughman, 2010);

The industry need to apply routine performance measurement and business efficiency techniques to water, energy and wastewater management as a measure to reduce negative environmental impacts; moving from old cooperate social responsibility (CSR) reporting to the new sustainability communications; engage into research with KARI for water stress tolerant flowers; increase hydroponics agriculture area and invest in rainwater harvesting and treatment systems and, install water recovery/ recycling plant;

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ASSESSMENT OF GRAIN STORAGE TECHNOLOGIES FOR EFFECTIVE MARKETING IN SUSTAINING FOOD SECURITY PROGRAMME BY TRADERS IN SOUTHWEST NIGERIA

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Abstract

Nigerian Food Security Programme is centred on three-tier grain storage with active participation of traders in storage of 85% of grain requirements through the On-Farm Storage Programme. The study assessed grain storage technologies to determine suitable ones for storage and marketing by traders in Southwest Nigeria. A pre-data survey of recommended grain storage technologies was followed by multi- stage sampling of Oyo, Ondo and Ogun States for 120 rural and urban traders. Data were analysed with descriptive and inferential statistics at p = 0.05. Traders preferred recommended storage technologies except silo. Only sacks were preferred out of the indigenous storage technologies. Technology attributes and communication factors are essential for use of recommended storage technologies. There is no significant relationship between age (r = 0.86), income (r = 0.78) and use of recommended storage technologies while quantity of grains stored (r = 0.94), years of experience in grain storage (r = 0.93) and educational status (X_2 = 9.51) were significantly related. Rural and urban traders were not significantly different in their levels of use of recommended grain storage technologies (tc = 0.20). Traders' storage extension through the use of various channels of communication, trainings and adult education programme were recommended.

Key words: Recommended technologies, indigenous technologies, determinants, use.

1. Introduction

Food security has a long history as an organizing principle for social and economic development (Maxwell and Frankenberg, 1992). Over time, this concept has been operationally defined in a number of ways. In most cases, the definitions include elements of availability (supplies of food), accessibility (both physical and economic), and utilization (physiological ability to absorb and utilize consumed nutrients) (USAID, 1997). Food security connotes access by all people at all times to safe and nutritious food needed to maintain a healthy and active life (FAO, 2005). Idachaba (2004) posited that one of supply side causes of food insecurity is food marketing problem. He argued further that the dwindling agricultural production in Nigeria is a confirmation of the unattractiveness of agriculture as a result of low returns and compensation being paid to farmers which tend to discourage increased production.

The food marketing problems are evidenced when farmers (who are the primary producers and who reside mostly in rural areas) could not get their produce to the market at the right time (thereby incurring considerable post-harvest losses). This perceived cheating causes discouragement and leads to loss of interest in farming and consequently a reduction in food production. The post harvest policy of the Nigerian Food Security Programme is centered on three tier grain storage; Strategic Grain Reserve, Buffer Stock and On-farm storage. The On-farm Storage Programme is supposed to hold 85% of the grains required for food security (Olumeko, 1998). To achieve this, farm level storage is to be complimented with private sector storage stocks which include grain merchants and consumers (Talabi, 1998). Muhammad-Lawal and Omotesho (2008) posited that cereals provide 34% of the farming households total calorie intake and 47% of protein supply respectively and therefore recommended increased cereal production. According to FAO (1997), if available food could be evenly distributed (through efficient national and international markets) each person would be assured of recommended 2700 calories a day. Grain merchants play a prominent role in food storage through their activities as middle-men between producers and consumers hence they store grains throughout the year. According to Shelton (2007), the grain crop is a major investment that needed to be protected. Grain quality does not improve in storage, but the initial quality must be maintained. According to Ladele and Ayoola (1997), efficient food marketing system would reduce post-harvest losses, ensure adequate returns to farmers' investment and stimulate expansion in food production thereby enhancing the level of food security in Nigeria. Food marketing is a very important but rather neglected aspect of agricultural development. Traders therefore had to critically embrace effective storage procedures so as to make their grains acceptable to consumers. In Nigeria, food marketing by farmers and their families mostly in the immediate post-harvest period usually involves a lot of costs. These costs are so high that lowering the costs through efficient marketing system

may be as important as increasing agricultural production.

Proper storage begins with the condition of the harvested grain, including moisture level and how it leaves the harvester and then is transported and handled. Grain bins should receive a thorough check up and cleaning, including removal of old grains. Ideally it is better to store grains in several small bins rather than a few large ones (Shelton, 2007). Long term grain storage is profitable (Beranek, 2010) and one of the major factors in determination of grain sales is storage structures. Addition of storage facilities is anticipated to increase marketing flexibility thereby strengthening marketing position. Importance of storage structure in grain marketing is highlighted by Oelke *et al* (2008), who stated that much grain is damaged during storage and can result in reduced profits. Good storage management is essential to prevent spoilage which is caused by mould growth and insect activity. A properly managed aeration system greatly improves the storability of grains by maintaining a cool, uniform temperature throughout the storage to reduce mould growth, insect activity and prevent moisture migration.

This study is intended to assess the storage structures that are used by grain traders for marketing in Southwest Nigeria. In doing this the following specific objectives were addressed.

(a) Enumerate recommended grain storage technologies in Southwest Nigeria.
(b) Assess awareness, use and preference of storage technologies by traders.
(c) Investigate respondents' scale of preference of storage technologies in use.
(d) Determine factors that affect the use of modern grain storage technologies by traders in Southwest Nigeria.

Due to differences in demographic characteristics of respondents the following hypotheses stated in Null form were tested at 5% level of significance.

Ho_1: There are no significant relationships between socio-economic characteristics of respondents and use of recommended grain storage technologies.

Ho_2: There is no significant difference between rural and urban traders' levels of use of recommended grain storage technologies.

2. Materials and methods

2.1 Study Area and Data Collection

The Southwest zone of Nigeria lies between latitudes 6^o and 9^o north of the equator and longitudes 2^o to 6^o east of the Greenwich Meridian. A pre-data survey was carried out to enumerate recommended grain storage technologies. Three States; Oyo, Ondo and Ogun were purposively sampled for data collection based on geographical locations as well as social and economic ties with other States of the zone. Multi-stage sampling was carried out as follows; half of agricultural zones of States' Agricultural Development Programmes (ADPs) were purposively sampled based on ADP's recommendation of grain production and handling. Strata of local government areas were sampled in the agricultural zones based on grain production and handling. Four rural communities with less than 5,000 people and four urban communities with more than 5,000 people were purposively sampled in each State based on ADPs' recommendation. In each community a purposive sampling of 5 grain traders was made making a total of 40 traders for each State and 120 for the study. The communities of study were; Ibadan, Shaki, Igbetti, Iddo as urban communities and Ikereku, Olorunda-Aba, Ilua and Egbeda as rural communities in Oyo State. In Ondo State urban communities selected were; Ikare, Akure, Owo, Oke-Agbe and rural communities of; Obasoto, Ijoka, Ise-Akoko and Akunu, Communities of study in Ogun State were; Iperu, Obafemi-Owode, Abeokuta and Odeda as urban and; Akinside, Eruku, Ogunmakin and Simawa as rural.

3.1 Recommended Grains Storage Technologies

The Crop Storage Unit (CSU) of the Federal Department of Agriculture developed the following technologies for grain storage.

(i) Modified oil drum with storage capacity of 175 kg.
(ii) Metal bins with galvanized iron sheet of capacities 1000 kg, 600 kg, 400 kg, 300 kg and 150 kg.
(iii) 2-metric tonne and 5-metric tonne indoor structure.
(iv) Reinforced concrete bin of 10-metric tonne capacity.

The Nigerian Stored Products Research Institute (NSPRI, 1982) stated that for grains to be stored effectively, the following procedures must be followed.

(a) Sorting of grains to remove damaged and infested grains.
(b) Determination of moisture content so as to store at safe level.
(c) Pre-storage treatment of grains.
(d) Storage in recommended structures.

In line with these, the following structures were recommended by NSPRI for storage.

(i) Hermetically sealed containers – metal drums with tight-fitting screw caps tops, plastic containers, tins and bottles.
(ii) Polyethylene bags or polyethylene-lined sacks.

(iii) Ventilated crib.
(iv) Stores and warehouses.
(v) Inert atmosphere silo.

2.2 Data Analysis

Variables measured included storage technologies respondents were aware of, use and their preferences for such technologies. Preference scale was measured on a 3 point scale of high, average and low with scores 3, 2 and 1 respectively (Table 1).

Storage method preference index was measured by equation 1;

$$Index = \frac{Total\ score\ of\ Individaul\ Respondent}{Total\ score\ obtainable\ fro\ the\ variables(12)} \qquad (1)$$

The Index ranged from 1 to 0.33.

Cut off index was taken as 0.67 which is the difference between the highest and the lowest score obtainable. Respondents were then categorized into three groups based on their preference for the method used as follows;

Greater than 0.67 implies highly preferred.
Equal to 0.67 implies moderately preferred.
Less than 0.67 implies not preferred.

In identifying factors that are favourable for use of food grains storage technologies, respondents were asked to rate the factors as very high, high, average, low and very low. The descriptive units were converted to normalized standard scores by finding the proportion of each level, determining the cumulative proportion as well as cumulative proportion at mid-point. The sigma (Z) score of each cumulative proportion at mid-point was found from the table of normal deviates Z corresponding to proportions P of a dichotomized unit normal distribution. The lowest sigma score was added to sigma score of all descriptive units. These scores were then rounded up to the nearest sigma. Determinants with Z rounded progressively from 0 to 2 and up to 3 were adjudged favourable. Hypotheses were tested with Chi-square, Pearson Product Moment Correlation and students't-test.

3. Results and discussion

3.1 Socio- economic characteristics of Respondents

Table 2 shows the Socio-economic characteristics of respondents. Very few of the traders (3.3%) fall between 20 to 30 years age range while less than one-quarter (24.2%) were between 31 to 40 years of age. More than one third (35%) were between the age range of 41 to 50 years and more than one quarter (26.7%) between 51 to 60 years. Few (10.8%) were between 61-70 years. He and Deng (2005) contend that age has a positive impact on adoption, suggesting that the probability of adoption is higher among older clienteles than younger ones. However age in traditional agriculture is significant in two ways. The first is in productivity while the second has to do with increased rate of adoption of technologies. Hamidu *et al* (2006) cited that many studies revealed that old farmers often tend to be more conservative (traditional) and afraid of taking risk which the adoption of new technology entails and that young farmers are more dynamic and more willing to take risk connected with the adoption of new agricultural technologies.

Few of the respondents (18.3%) had no formal education, more than one third (25.8%) had primary education and less than one third (31.5%) had secondary education. Few (15.8%) had tertiary education. Issa *et al* (2011) and Oyesola and Adeboye (2011) have pointed out the importance of education in the use of agricultural technology. The necessity therefore arises for training on modern storage technologies as well as adult education programme. Less than half of the respondents (46.7%) had income of between ₦100, 000 and ₦ 200, 000 per annum while more than one third (37.5%) had between ₦201, 000 to ₦400, 000. Few (13.3%) had ₦ 401, 000 - ₦ 600, 000 while very few (2.5%) had ₦ 600, 000 to ₦ 800, 000 as annual incomes. There is need for provision of credits as well as subsidies on storage inputs so that traders could access modern storage technologies. Vishwanath and Goldhaler (2003) listed income as one of the socio demographic variables that indirectly influence adoption intention. Few respondents (12.5%) had 1 to 5 years of experience on grain storage while one 25.8% and 31.7% had 6 to 10 years and 11 to 15 years respectively. Scott *et al* (2008) posited that experience is a factor influencing adoption of an innovation, it is therefore necessary to train traders with longer years of experience on the benefits of the use of modern storage technologies. Majority of traders (85.0%) stored between 1 and 20, bags of 100 kg grains. This has shown the subsistence level of grain trading in the study area. There is need for provision of storage facilities at subsidized rates and encouragement of group action processes so as to promote effective grain storage for more profits.

3.2 Use of Recommended Grains Storage Technologies in the study Area

The awareness, use and preference of storage technologies in the study area are presented in Table 3. The result shows that sacks and bowls recorded 100% awareness. This is because they are cheap and are useful for short time storage. Sacks are easy to move around, and can be used as they are needed. However, bowl storage pre-exposes

grains to pests, unfavourable weather and dirt. Majority of the respondents were aware of recommended storage technologies such as improved cribs (70.8%), stores and warehouses (95.0%), drum and hermetic containers (80.8%), polythene lined bags (65.0%). Silo is the only recommended technology that recorded low awareness (20.8%). Apart from local crib, awareness of other indigenous technologies was low. They were mainly limited to rural communities. Use of all recommended technologies was very low. There is need for aggressive extension services for traders on grain storage. All indigenous technologies except bowls (44.2%) recorded very low use also. This can be attributed to certain deficiencies like exposure to destructive agents like pests, rain, wind and unsuitability for pre-storage treatment.

The preference of the grain storage technologies by respondents shows that majority (70.0%) preferred stores and warehouses; while more than two thirds (68.3%) preferred improved crib and 65.0% hermetic containers. Silo is not preferred as a recommended structure (3.3%). However, sacks were preferred as an indigenous structure (53.3%) based on total number of respondents. Sacks were preferred due to subsistence level of grain trading leading to short transaction periods. Silos are expensive to construct and have problems of moisture migration and condensation (NSPRI, 1982). The use of polythene-lined sacks and inert atmosphere silo as recommended by NSPRI should therefore be encouraged.

The use of recommended technologies for effective grain storage was nearly at equal levels between rural and urban traders (Table 4). However, recommendations of pre-storage treatment and storage of grains in modern structures had low scores. Extension should reach traders on importance of these levels of storage so as to have good quality grains throughout the year thereby sustaining the national food security programme.

Considering all the recommended technologies; (silo, modern crib, stores and warehouses, drum and hermetic containers, polythene-lined sacks), only silo was not preferred by traders (Table 5). This finding is in line with NSPRI (1982) contention that in Nigeria, problems of condensation and moisture migration have militated against the use of conventional silos for grain storage. Temperature fluctuations between day and night have resulted in this. Also, there is pressure build up and the resultant effect is cracking and caking of stored grains. Of all the indigenous technologies (platform, rhumbu, local crib, ceiling top, hanging over fire places, sacks and bowls), only sacks were preferred. This has shown that extension has a lot of work to do in introducing modern storage technologies to traders. Aggressive extension efforts like demonstrations, Small Plot Adoption Techniques (SPAT) and activities of Non-Governmental Organisations (NGO's) should be incorporated into extension policies for traders.

3.3 Determinants for Use of Modern Grain Storage Technologies by Traders

Determinants for use of modern grain storage technologies were categorized into situational factors, communication factors, technology attributes, perceived incentives and perceived disincentives. Communication factors; extension agent contact, adoption by peers, media presentation, cooperative society initiative, local leader presentation are favourable determinants among traders. There is need for uses of interpersonal communication to get traders adopt relevant technologies (Adekoya and Ajayi 2000 and Torimiro *et al.*, 2000) after which print and electronic media can be used for diffusion. Technology attributes favourable are; technology cost, efficiency of technology, accessibility of technology, flexibility of technology and stored quantities. Situational factors of storage duration and need based technology are also favourable. These factors are necessary for considerations by researchers when developing grain storage technologies for the use of grain merchants.

Extension system presently operated in Nigeria does not give the required attention to the activities of traders. The necessity arises therefore to use many channels of communication to reach traders as well as proper feedback to researchers on the type of storage technologies desired by traders. Quaddus and Hofmeyer (2007) posited that external influence raise small business awareness of an innovation. It is therefore very necessary for extension services to consider the roles of traders in the use of recommended grain storage technologies so as to sustain the food security programme, especially in areas of all year round availability of food.

The Correlation analyses of the socio-economic characteristics of traders on use of recommended storage technologies shows that there is no significant relationship between age (r_{cal} = 0.86,) and income (r_{cal} = 0.78,) and use of recommended grain storage technologies while years of experience (r_{cal} = 0.93) and quantity of grains stored (r_{cal} = 0.99,) are significantly related. Furthermore, Chi-square analysis also shows that educational status (X^2_{cal} = 9.51) is significantly related to use of modern grain storage technologies. The implications are that educational status and experience encourage use of modern technologies for storage of larger quantity of grains for a lot of reasons. Extension should focus on traders in terms of grain storage for sustainability of food security. Trainings as well as adult education programmes are necessary to encourage the use of recommended grain storage technologies.

Analysis of the differences in use of recommended grain storage technologies between rural and urban traders shows that there is no significant difference between rural and urban traders' levels of use of recommended grain storage technologies (t_{cal} = 0.20). The implication is that the use of various levels of recommended grain storage technologies need more emphasis in both rural and urban communities. This is because when conditions are

favourable traders, no matter the community of abode will be favourably disposed to use of recommended technologies.

4. **Conclusion and Recommendations**

Few modern and improved grain storage technologies were adopted out of the total developed by research institutions and faculties in the study area. There is average level of awareness of recommended grain storage technologies which are generally accepted for use by traders in preference to indigenous technologies. The use of recommended technologies is not cosmopolitan biased. Traders considered the attributes of technologies as well as communication factors as very relevant in the use of recommended grain storage technologies.

Based on the findings from this study, the following recommendations are made.

(1) Extension should prioritize the activities of traders in food storage to sustain grain availability all the year round.

(2) Importance of various levels of grain storage should be demonstrated through the use of various channels of communication.

(3) Trainings as well as adult education programmes are necessary for traders in the areas of food storage.

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Table 1: Measurement of Preference of Storage Technology in use

Preference for method use	**Scoring**		
	High	Average	Low
	3 points	2 points	1 point
Better than other methods			
Cost effective			
Suitable for my need			
Other methods not understood			
Total	12 points	8 points	4 points

Maximum score obtainable is 12 points and minimum score obtainable is 4 points.

Table 2: Socio-economic Characteristics of Respondents

Factor	Frequency	Percentage
Age (Yrs)		
20 – 30	4	3.3
31-40	29	24.2
41-50	42	35.0
51-60	32	26.7
61-71	13	10.8
Educational Status		
No Formal Education	22	18.3
Primary Education	41	35.8
Secondary Education	38	31.7
Tertiary Education	19	15.8
Income (₦000)		
1- 200	56	46.7
201-400	45	37.5
401-600	16	13.3
601-800	3	2.5
Experience in Grain Storage (Yrs)		
1-5	15	12.5
6-10	31	25.8
11-15	38	31.7
16-20	15	12.5
21-25	10	8.3
26-30	6	5.0
31-35	1	0.8
36-40	4	3.3
Quantity of grains stored (100 kg bags)		
1-20	102	85.0
21-40	4	3.3
41-60	7	5.8
61-80	1	0.8
81-100	4	3.3
> 100	2	1.7

Table 3: Awareness, Use and Preference of Storage Technologies

Storage Structure	Awareness Freq. %	Use Freq. %	Preference Count Freq. %	Preference Rank
Platform	41(34.2)	5(4.2)	5(4.2)	10th
Mud Rhumbu	9(7.5)	0(0.0)	3(2.5)	12th
Local Crib	96(80)	10(8.3)	43(35.8)	6th
Ceiling Top Under Roof	43(35.8)	2(1.7)	11(9.2)	8th
Hanging Over Fireplaces	33(27.5)	1(0.8)	6(5)	9th
Sacks	120(100.0)	53(44.2)	64(53.3)	5th

Bowls	120(100)	6(5)	16(13.3)	7th
Improved Crib	85(70.8)	4(3.3)	82(68.3)	2nd
Stores and Warehouses	114(95)	14(11.7)	84(70.0)	1st
Drum and Hermetic Containers	97(80.8)	11(9.2)	78(65)	3rd
Polythene lined bags	78(65)	12(10)	71(59.2)	4th
Silo	25(20.8)	2(1.7)	4(3.3)	11th

N=120

Table 4: Use of Recommended Grain Storage Technologies

	Urban Traders		Rural Traders		Total	
Recommended Technology	**Frequency**	**%**	**Frequency**	**%**	**Frequency**	**%**
Sorting of Grains	54	(45.)	58	(48.3)	112	(93.3)
Determination of Moisture Content	59	(49.2)	57	(47.5)	116	(96.7)
Pre-storage Treatment	34	(28.3)	32	(26.7)	66	(55.0)
Storage in Modern Structure	26	(21.7)	17	(14.2)	43	(35.8)

Table 5: Preference of Grain Storage Technologies among Traders

		Index Category			
Technology	**Use**	**Not preferred <0.67**	**Moderately preferred =0.67**	**Highly preferred >0.67**	**Perception**
Silo	2	2	-	-	NP
Modern Crib	4	-	1	3	P
Stores and Warehouses	14	1	4	9	P
Drum and Hermetic Containers	11	-	2	9	P
Polythene-lined Sacks	12	-	4	8	P
Platform	5	3	1	1	NP
Rhumbu	-	-	-	-	-
Local Crib	10	7	2	1	NP
Ceiling Top Under roof	2	2	-	-	NP
Hanging Over Fireplaces	1	1	-	-	NP
Sacks	53	10	15	28	P
Bowls	6	4	2	-	NP

P = Preferred NP = Not preferred

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Growth and Performance as affected by inclusion of *Moringa oleifera* leaf meal in Broiler chicks diet

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Abstract

The experiment was carried out to investigate the inclusion of *Moringa oleifera* leaf as feed additive in broiler chicks. Eighty Anak 2000 Strains of two weeks old were allotted to four treatments with five replicates of four birds each in a completely randomized design. Four different diets with metabolizable energy levels ranging from 2800 to 2900kcal/kg diet were formulated and fed to the chicks for a period of four weeks. The level of inclusion of *Moringa oleifera* leaf meal ranged from 0% which served as the control, 1%, 2% and 3% in the diet. Inclusion of *Moringa* significantly ($P < 0.05$) enhanced weight gain of birds at 2% level of inclusion. The inclusion of *Moringa* did not significantly ($P < 0.05$) enhance feed intake and feed conversion.

Keywords: Broiler chicks, *Moringa oleifera*, growth, feed intake, feed conversion

1. Introduction

One of the practical solutions to some of the problems of poultry in the tropics is to pay attention to the areas of nutrient requirements of birds for maintenance and production and the nutrient composition of the available feed stuffs. The most logical step to take in solving the shortage and dwindling raw material supply is to direct efforts towards utilizing plants by-products and wastes for feeding poultry birds.

Moringa oleifera is one of the plants that can be utilized in the preparation of poultry feeds. The plant apart from being a good source of vitamins and amino acids, it has medicinal uses (Makkar and Bekker 1999; Francis *et al* , 2005). *Moringa oleifera*, otherwise regarded as a "miracle tree" has been used in the treatment of numerous diseases (Pal et al, 1995; Makomen *et al*, 1997; Gbasi *et al*, 2000 and Matthew et al, 2001) including heart disease and obesity due to its hypocholesterolemic property (Gbasi *et al*, 2001; Olugbemi *et al* 2010) also reported this quality. *Moringa oleifera* leaves have the calcium equivalent of 4 glasses of milk, 3 times the iron of spinach, 4 times the amount of vit A in carrot, and 2 times protein in milk (Loren, 2007). The leaves of *Moringa* are good source of protein, vitamins A, B and C and minerals such as calcium and iron (Dahot, 1988). The leaves of *Moringa* has high protein content which is between 20 – 33% on a dry weight basis, the protein is of high quality having significant qualities of all the essential amino acid as reported by Foidl and Paull (2008). Murro *et al* (2003) reported that the leaves contains a high level of vitamins A, B, C and calciuim.

Kakengi *et al* (2003) reported that *Moringa oleifera* leaf meal was substituted for sunflower seed meal as a protein source for layers. The effects of substitution on feed intake, dry matter intake, weight, laying percentage and feed conversion ratio were investigated and they suggested that *Moringa* leaves could completely replace SSM up to 20% without detrimental effect on layers. However the crude fibre content if high can impair nutrient digestion and absorption (Aderemi, 2003; Omu 2011).

This study therefore considers the utilization of *Moringa oleifera* for improving the nutritional value of broilers and also to investigate the level of inclusion that will yield optimum performance of birds (broilers).

2. Materials and methods

The experiment was carried out at the Teaching and Research Farm of Tai Solarin University of Education, Ijagun, Ogun State, Nigeria. Feed ingredients were purchased from F.A feed (Nig) Limited, Ijebu – Ode, while *moringa oleifera* leaves were harvested from a farm in Ijebu – Oru, Ogun State. The *moringa oleifera* leaves were harvested and air dried under shade for 4 days and milled, after which the leaf meal was added into the diets at 0% to 3% level. A total of eighty unsexed two weeks old Anak 2000 strain of broiler chickens were allotted to four treatments with five (5) replicates of four birds each. The birds were assigned in a completely randomized design (CRD). Each

treatment group was fed one of four experimental diets containing 0, 1, 2 and 3% Moringa Oleifera leaf meal. The experimental birds were raised using deep litter systems, which was divided into experimental units. The records of growth rate and feed intake were taken weekly four a period of six weeks. The data collected were subjected to analysis of variance (ANOVA). Durcans's multiple range (DMR) Test was used to separate means where significant different were observed.

3. Results and discussion

The result of the performance characteristics is shown in Table 3. The inclusion of *Moringa oleifera* leaf meal in the diet of the broilers significantly ($P<0.05$) enhanced their weight gain at 1% level which was significantly higher than the control.

The birds fed T2 recorded significant, ($P<0.05$) higher weight gain then T1 while those fed T3 diet recorded significantly ($P<0.05$) the highest body weight gain. The reason for the improved weight gain can be attributed to high protein content of *Moringa* leaf meal as claimed by (Danol, 1986) (Kakengi *et al* 2003) and (Olugbemi *et al.*, 2010).

The decrease in weight gain of birds fed T4 diet as compared to T2 and T3 despite the higher crude protein content may be due to higher crude fibre content which may impair nutrient digestion and absorption as claimed by (Otuma and Onu, 2008), while the reduced weight gain of broilers fed the control diet (T1) may be ascribed to low crude protein content of the diet compared to other diets.

The feed intake significantly ($P<0.05$) varied among the treatments. The reduced intake of diet T4 may be due to high crude fibre content which may invariably reduce palatability (Kakengi *et a*l 2008).

The feed conversion ratio of the birds was significantly improved in all the treatments, while the diets produced no significant ($p>0.05$) impact on the protein efficiency ratio of the broiler birds.

4. Conclusion

The effect of *Moringa Oleifera* leaf meal used in this study was pronounced in the weight gain of the birds and it is also concluded that broilers can tolerate *Moringa oleifera* leaf meal up to 3% birds of inclusion without adverse effect on their growth.

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Table 1: Composition of experimental diets (%)

Ingredients	Treatments			
	T1	T2	T3	T4
Maize	40	40	40	40
Soya meal	25	25	24	23
Groundnut cake	5	5	5	5
Moringa oleifera	-	1.0	2.0	3.0
Wheat offal	5	5	5	5
Orzel shell	5	5	5	5
Bone meal	2.0	2.0	2.0	2.0
Corn bran	14	13	13	13
Fish meal	2.5	2.5	2.5	2.5
L. lysine	0.25	0.25	0.25	0.25
Dl. Methionine	0.35	0.35	0.35	0.35
Salt	0.4	0.4	0.4	0.4
Broiler premix	0.25	0.25	0.25	0.25
Mycofix	0.25	0.25	0.25	0.25
Total	100	100	100	100

Table 2: Calculated nutrient

	Diet 1	Diet 2	Diet 3	Diet 4
	Control Moringa	Moringa oleifera (1%)	Moringa oleifera (2%)	Moringa oleifera (3%)
Me (kal/kg)	2680.2	2506	2506.562	2506.563
Crude protein (%)	18.61	18.66	18.82	18.85
Crude fibre (%)	5.14	5.16	5.17	5.20
Calcium (%)	3.50	3.43	3.45	3.47
Phosphorus (%)	0.05	0.82	0.83	0.85
Methionine (%)	0.40	0.50	0.50	0.50
Lyshe (%)	0.75	0.80	0.80	0.80

Table 3: Performance of broiler chicks fed *Moringa* leaf meal

	T1	T2	T3	T4	S.E
Mean initial body weight (g)	145.25	140.60	150.20	148.50	1.36
Mean final body weight (g)	110.23^{c}	1415.124^{ab}	1521.56^{a}	1255.76^{b}	48.32
Mean body weight gain (g)	954.98^{c}	1274.52^{a}	1371.36^{a}	1107.46^{b}	46.55
Mean daily weight gain (g)	51.63^{c}	62.45^{a}	65.14^{a}	53.92$^{b1.92}$	
Mean total feed intake	2415.20^{c}	2822.40^{a}	28.73^{a}	22.046^{b}	45.73
Feed conversion ration	2.26^{b}	1.90^{a}	1.85^{a}	1.86^{a}	0.61
Protein efficiency ratio	2.08	2.58	2.46	2.57	0.14

Means having the same letter(s) in a column are not significantly ($p<0.05$) different

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In situ degradability of dry matter of browse forages consumed by ruminants in the semi-arid region of northern Nigeria

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Abstract
Three ruminally cannulated bulls were used to determine variations in dry matter (DM) degradability of forage consumed by ruminants in the semi-arid region of north Nigeria. Organic matter and crude protein (CP) contents were higher (P< 0 05) in all the browse forages. Higher numerical values neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and cellulose were recorded. DM degradability after 24 and 48 h of ruminal incubation were higher (P< 0 05) in all the browses. Higher values (P< 0 05) in DM bag losses at zero time (a fraction) were recorded for the browses. The insoluble but fermentable DM (b fractions) were higher (P< 0 05) in among browse forages. Numerically lower values of DM c fraction were found in browses, whereas DM potential degradability were higher (P< 0 05) in all the experimental leaves. High (P< 0 05) contents of CP in grazed forage, DM degradability after 48 h of ruminal incubation, and b and a+b, were observed in the browse leaves. Thus, these results may be related to both the better feeding value of forage consumed by the animals and better performance of livestock during in this areas. Then, the DM degradability after 48 h, together with the soluble fraction 'a' and insoluble but fermentable fraction 'b' and the c fraction permit the nutritive value of the forage consumed by grazing goats to be accurately described.

1. Introduction

Most laboratory techniques used in food evaluation are still judged according to their ability to predict the nutritive value of foodstuffs. The *in situ* dry matter (DM) degradability of forage consumed by livestock has been used in this way to determine whether degradation characteristics of individual vegetative species could be used to predict its nutritive value (Kibon and Ørskov, 1993). The chemical components of neutral-detergent fibre (NDF) constitute proportionately 0 30 to 0 60 of DM of forages. The NDF degradability, which is usually low, depends on the quantity and distribution of the lignin component. Poorly lignified plant material such as young grass may be highly degradable while the degradation of straw is low due to extensive lignification (Jung, 1989). Nutritive value of forage is closely related to the rate of disappearance of material from the rumen (Ingvartsen, 1994); thus, the degradability of DM and NDF will directly influence the nutritive value of foodstuffs (Van Soest, 1994). The *in situ* technique permits DM and NDF digestion kinetics in the rumen to be estimated and considerable research has been conducted to compare the degradation and fermentation of these factors. Nevertheless, there is little information concerning the DM and NDF degradability of the forage consumed by grazing goats. Consequently, the objective of this study was to determine the DM degradability characteristics of the diet consumed, during dry and wet periods of the year, by ruminant animals in the semi-arid region of Nigeria.

2. MATERIALS AND METHODS

2.1 Description of site and samples

All forages were harvested from Maiduguri (11.05^{0}N; 30.05^{0}E; 364m above sea level) of Borno State, North Eastern part of Nigeria. The species were *Garderna sokotensis, Khaya senegalensis, Kigalia Africana, Leptadenia lancifolia, Maerua angolensis, Olea hochstteteri*. The browse forages were harvested from at least 10 trees per species selected at random in four locations within the study area at the end of rainy season. The samples were sun-dried, milled and sub- sampled for analysis.

2.2 Sample preparation and chemical analysis

About 500g of the harvested and pooled samples from each plant were oven dried at 105^{0}C for 24hours, cooled and weighed. The samples were analyzed in triplicate for crude protein (CP), according to AOAC (2002) procedures. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined as described in Van Soest et al. (1991). Hemi cellulose was estimated as NDF-ADF and cellulose as ADF-lignin.

2.3 In sacco DM degradability study

The rate of nutrient disappearance in the browse species was determined by the use of nylon bag technique. Milled (<1.0mm) samples were oven-dried overnight (24 hrs) at 70^{o}C prior to weighing into the bags measuring 140x20mm when laid flat. A piece of marble was included in each bag containing 5g of feed sample to prevent the bag from floating in the rumen.The weight of each bag and its content was then recorded. Ten bags containing the sample were incubated at the same time in each animal. A bag was removed from each of the three animals at 3, 6, 12, 24, 48, 72 and 96 hours for observation of nutrient disappearance. The bags were tied using a nylon twine and carefully inserted into the rumen. After each incubation period, the bags were carefully removed and rinsed with tap water until the water was clean and clear. The washing procedure took 30 min and then the bags were oven-dried. The bags were allowed to air-calibrate to room temperature for about three hours in a desiccator before weighing to determine bag plus marble plus feed sample residue weight for dry matter determination. The difference between the initial and final weights of each sample was regarded as degraded material and thereafter expressed as a percentage of the initial weight. After incubation, all the bags were withdrawn from the rumen at the same time and immediately placed under running cold tap water until the rinse water became clear. This was done to wash off ingested feed particles adhering to the bags as well as stop further fermentative processes. The bags with the sample residues were then oven dried at 65^{0}C for 48hours and the weight of the bags plus residues measured and recorded. The zero-hour washing losses that is, losses due to non-incubation, were determined by soaking 5g of each of the samples in triplicates in warm water (37^{0}C) for 1 hour which was followed by washing and drying of the bags as done with the incubated sample residues. Dry matter losses was computed as the difference between the determined dry matter content of the pre- incubated samples and the determined dry matter content of the incubated residues. The rumen degradation parameter of DM was calculated using the equations of Ørskov and McDonald (1979):

P = a + b (1- e-ct)

Where: P = Potential degradability after time 't'

a = Water Soluble Fraction (zero hour)

b = Insoluble but degradable fraction after time 't'

c = Rate of degradation of slowly degradable fraction b

t = Incubation length i.e. 3, 6, 12, 24, 36, 48, 72, 84 and 96 hours

e = exponential. The effective ruminal degradability of DM (EDDM) was calculated according to Ørskov and McDonald (1979): EDDM = a+b $\times (c/(c + k))$ where $k = 0.12h^{-1}$

2.4 Statistical Analysis

Data obtained from the degradation characteristic of the incubated plant species at the different hours were subjected to analysis of variance (Gomez and Gomez, 1984) using the completely randomized design. Treatment means were separated by Duncan's multiple range test.

3. RESULTS AND DISCUSSION

The chemical composition of the browse forage leaves determined in this study is presented in Table 1. Dry matter content ranged from 838.30 g kg^{-1} DM in *Poupartia sirrea* to 983.00 g kg^{-1} in *Garderna sokotensis* on DM basis. Generally, the examined plant leaves had high crude protein content. The values of CP in browse has been shown to be above the minimum level required (7%) for microbial activities in the rumen (Njidda, 2011).Values obtained for organic matter content of the browse forages ranged from 742.60% in *Poupartia sirrea* to 868.70 g kg^{-1} DM in *Khaya senegalensis*. NDF, ADF and ADL contents in the browse forages studied were generally higher and similar to the values reported by Njidda (2011, 2012a, b) and this can limit feed intake (Meissner *et al.*,1991). Cellulose levels in the browse forages were within the range of 131.20 g kg^{-1} DM in *Leptadenia lancifolia* to 187.20 g kg^{-1} DM in *Kigalia Africana* while hemicellulose content of the browse leaves ranged from 189.20 g kg^{-1} DM in *Leptadenia lancifolia* to 432.90 g kg^{-1} DM in *Kigalia africana*. There was an increasing disappearance of DM from the incubated leaves over time. This was generally moderately in all plant leaves. At 48 hours of incubation over 40% of the dry matter in most of the leaves had been degraded. Most of the plant leaves were 60% degraded at 96 hours of incubation. The disappearance of dry matter from the browse plant leaves in this study was observed to be moderate and well above 40% of their reported potential degradability values after 48hrs incubation. According to Ehargavi and Ørskov (1987) high degradability values after 48hrs of incubation imply high digestibility since degradability values at this time are regarded as being equivalent to digestibility.

Degradation characteristics for dry matter in the different browse leaves incubated in the rumen of bulls are presented in Table 2. Significant differences (P<0.05) were observed between the leaves in all the degradation characteristics. Soluble dry matter fraction 'a' was observed to be generally low with the least value being in *Maerua*

angolensis (2.23%). The rapidly degradable fraction 'a' was generally low across the leaves studied. This is possibly an indication of high level lignifications in most of the leaves or may have, according to Adogla-Bessa and Owen (1995), resulted from the accumulation of soluble carbohydrates due to later stages of maturity. The insoluble but degradable DM fraction 'b' was observed to be high in the browse forages. This observation may probably be due to it cell wall content (Wilson, 1994).It was. observed in this study that although relatively close, the potentially degradable DM 'a+b' value was high for all browses. Potentially degradable 'a+b' dry matter in the browse leaves was high, above 60%. However, according to Singh and Makkar (1992) the statistical variations may be associated with their fibrous components such as the structural polysaccharides, which vary in their degradation among forages. The rate of DM degradation 'c' per hour of the potentially degradable portion was slowest in *Ziziphus mauritiana* (0.012/hr) and fastest in *Maerua angolensis* (0.060/hr). The value for the rate of degradation 'c' of the dry matter in the slowly degradable fraction 'b' were generally slower than those reported by Kaitho *et al.* (1997) for some multipurpose tree leaves.Values for effective degradability (ED) of DM at 0.12-outflow rate were found to be highest in *Acacia nilotica* with 29.50 whereas *Prosopis africana* had the least value of 16.30. According to Mupangwa (2003) variations in effective degradability of dry matter in forages closely corresponds with the proportion of potentially degradable dry matter and level of NDF. Llamas-Lamas and Combs (1990) and Njidda *et al.* (2012) have observed forages with low fibre to have high effective dry matter degradability compared to those with high fibre content. This to some extent may explain the low effective degradability reported for the browse forages in this study.

4. CONCLUSION

In conclusion, the leaves of the browse forages showed high potential as a feed supplement to ruminant animal in the semi arid especially in terms of crude protein supply for effective microbial activity in the rumen.

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Table 1. Chemical composition of browse forages of Semi-arid region of Nigeria (g kg^{-1} DM).

Browse Forages	DM	CP	NDF	ADF	ADL	Cell	Hemicel	OM
Garderna sokotensis	983.00^{a}	151.40^{c}	544.20^{d}	219.30^{d}	121.30^{d}	183.20^{b}	324.90^{c}	799.00^{b}
Khaya senegalensis	976.30^{a}	139.60^{d}	486.20^{d}	211.60^{e}	121.00^{d}	182.50^{b}	274.60^{d}	868.70^{a}
Kigalia Africana	946.30^{c}	134.02^{e}	688.10^{a}	255.20^{a}	97.00^{e}	187.20^{a}	432.90^{a}	766.70^{d}
Leptadenia lancifolia	958.30^{b}	163.30^{b}	433.10^{e}	243.90^{b}	152.80^{a}	131.20^{f}	189.20^{f}	782.30^{c}
Maerua angolensis	922.60^{e}	174.30^{a}	586.70^{c}	228.90^{c}	144.70^{b}	164.00^{d}	357.80^{b}	767.60^{d}
Olea hochstteteri	941.30^{d}	138.70^{d}	438.40^{e}	206.80^{f}	96.70^{e}	171.30^{c}	231.60^{e}	801.30^{b}
Poupartia sirrea	838.30^{f}	132.20^{e}	591.20^{b}	230.30^{c}	140.30^{c}	143.00^{e}	360.90^{b}	742.60^{e}
MEANS	**938.01**	**147.64**	**538.27**	**228.00**	**124.82**	**166.06**	**310.27**	**789.74**
SEM	**1.46**	**2.06**	**0.96**	**1.31**	**1.08**	**1.33**	**0.93**	**0.43**

a,b,c,d=mean values along the same column with different superscripts are significantly different P<0.05); DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; Acid detergent lignin; Cell.=Cellulose and Hemi cellulose; OM=Organic matter; SEM=Standard error of means.

Table 2. Dry Matter Disappearance of semi-arid browses (% DM)

Browse forages	0	3	6	12	24	48	72	96
Garderna sokotensis	8.76^{a}	9.63^{a}	19.43^{a}	30.39^{c}	40.04^{b}	57.65^{a}	61.08^{b}	71.25^{c}
Khaya senegalensis	8.33^{b}	9.12^{b}	19.34^{a}	29.70^{c}	39.35^{b}	50.99^{b}	61.82^{a}	72.23^{c}
Kigalia Africana	4.91^{d}	5.67^{d}	17.06^{b}	26.90^{d}	38.03^{bc}	48.87^{c}	59.66^{c}	70.14^{c}
Leptadenia lancifolia	6.26^{c}	7.39^{c}	17.76^{b}	36.67^{b}	39.03^{b}	48.57^{c}	60.16^{c}	69.61^{c}
Maerua angolensis	2.23^{a}	3.70^{e}	13.69^{d}	41.64^{a}	46.39^{a}	47.31^{c}	58.58^{d}	70.45^{c}
Olea hochstteteri	4.22^{e}	5.84^{d}	16.24^{c}	27.52^{d}	37.81^{cd}	48.24^{c}	58.96^{d}	69.82^{c}
Poupartia sirrea	3.24^{f}	5.55^{d}	5.47^{e}	24.57^{e}	29.93^{e}	41.27^{d}	54.48^{e}	65.16^{d}
MEANS	**5.42**	**6.70**	**15.57**	**31.06**	**38.65**	**48.98**	**59.24**	**69.80**
SEM	**0.23**	**0.47**	**1.64**	**0.70**	**0.93**	**2.04**	**0.62**	**1.77**

a, b, c, means in the same column with different superscript differ significantly (P<0.05); SEM=Standard error means; NS=Not Significant

Table 3. Degradation characteristics and Effective degradability of DM of semi arid browse forages incubated in the rumen of bulls

Browse Forages	a	b	a+b	c	Lag T	ED
Garderna sokotensis	8.22^{a}	65.86^{c}	74.19^{c}	0.023^{b}	0.50^{b}	19.60^{a}
Khaya senegalensis	8.33^{a}	68.01^{b}	76.34^{b}	0.023^{b}	0.20^{e}	19.80^{a}
Kigalia Africana	4.91^{c}	67.69^{b}	72.60^{d}	0.026^{b}	0.20^{e}	17.10^{b}
Leptadenia lancifolia	6.26^{b}	61.20^{d}	67.46^{e}	0.033^{b}	0.20^{e}	19.90^{a}
Maerua angolensis	2.23^{e}	59.24^{e}	61.47^{f}	0.060^{a}	0.90^{a}	20.00^{a}
Olea hochstteteri	4.22^{c}	67.28^{b}	71.50^{d}	0.026^{b}	0.30^{d}	16.80^{b}
Poupartia sirrea	3.24^{d}	74.86^{a}	78.10^{a}	0.016^{b}	0.40^{c}	12.90^{c}
MEAN	**5.34**	**66.30**	**71.67**	**0.029**	**0.38**	**18.01**
SEM	**0.66**	**0.87**	**0.86**	**0.013**	**0.02**	**1.36**

a, b, c, means in the same column with different superscript differ significantly (P<0.05); SEM=Standard error means; NS=Not Significant