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PERFORMANCE OF TWO TANK RAIN WATER HARVESTING MODELS FOR TROPICAL HOUSES

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ABSTRACT

In the face of inaccessibility to clean and potable water for many households throughout the world, rain water harvesting (RWH) has caught the attention of extensive research in recent decades. Though much progress has been made in optimizing the system component sizes with regard to the maximum achievable water saving efficiencies, attention has not been focused much in providing the collected rain water to individual service points, particularly in multi storey situations where the storage tank is typically placed at the ground or basement level. A two tank RWH model is introduced to address the issue, which will have much smaller tanks positioned at higher elevation to the service points feeding them through gravity with the excess run-off collection cascading to the lower tank pumped back to the upper tank to complete the cycle thereby improving the efficiency of the system. By developing a set of equations based on an algorithm describing the system behavior, the performance of a typical model in a given setting can be analyzed. It will be a significant design tool if the developed equations can indicate the quantity of collected rainwater that can be pumped up based on the demand and the Water Saving Efficiencies (WSE) of individual storage tanks so that the developer can select the storage sizes, make-up water requirement from mains supply and the pump specifications for a given demand. Countries located in the tropical belt has been identified as ideal for RWH, with regular monsoon and convectional rainfall patterns allowing more opportunities to predict the behavior of such models to provide water security to the user.

Keywords: *Rainfall, rainwater, multi storey, water saving efficiency, water security*

1.0 INTRODUCTION

All conventional Rain Water Harvesting (RWH) systems consist of an impervious collection area, a piping system to convey the captured rainwater and a storage tank located typically at or below the ground level. As such, the need arises for the collected rainwater to be pumped up to a given service point at a desired pressure. Though positioning of the storage tank at a higher elevation, i.e. in between the collection area and the service points is a possibility, the space requirement, the need of structural elements to support the elevated tank and aesthetic issues hinder the widespread use of RWH systems, despite research has yielded optimum sizes for storage tanks for a desired Water Saving Efficiency (WSE) Fig.1. This model can be appropriately called the Cascading Two Tank Rain Water Harvesting (CTTRWH) model, where the smaller upper tank enhances the system efficiency, cuts down the quantity of water required to be pumped, reduces the need for supporting structural elements as well as providing the designer the opportunity of concealing it within the building envelope for better aesthetic appearance. A set of generalized curves has been developed (Fewkes, 1999b)**,** based on the Yield After Spillage (YAS) behavioral model (Jenkins, 1978)] for a generic RWH system to predict the WSE (η) for a given set of D/AR and S/AR values where D, S, A and R are the annual demand based on a constant daily demand, the storage capacity, the annual average rainfall and the roof capture area respectively. The curves, validated for tropical countries (Sendanayake et al. 2014)**,** are independent of spatial and temporal variations of A and R and are valid for $0.25 \leq D/AR \geq 2.0$, which can be used to develop and analyze the performance of the proposed RWH model for a two storey building. In the proposed CTTRWH model, two storage tanks are utilized. A smaller capacity tank is positioned at a higher elevation (possibly at the eve level) into which the captured rainwater be directed. This upper tank (S_U) will supply the utility points and feed a bigger tank (S_L) at ground level via the overflow. As such when a rain event occurs, captured rainwater will flow into the upper tank and then cascade down into the lower tank and any excess water to be disposed through the overflow of the lower tank. The total storage capacity of the system consists of the combined capacities of the two tanks and a pump is utilized to transfer collected rainwater from the lower tank to the upper tank when the water level in the latter drops. A schematic diagram of a CTTRWH model is shown in Fig. 2.

3.0 METHODOLOGY

A set of equations are developed based on the WSE of the storage tanks to analyze the behavior of a CTTRWH system for a typical two story building with a constant daily service water demand. The developed equations are validated in a tropical setting, with the use of a prototype in a two storey housing unit in Colombo, Sri Lanka ($6^0$54'N, $79^0$51'E). The equations are then used to determine the possible variations in the system performance with regard to annual demand (D), annual average rainfall (R), roof capture area (A) and the capacity of the upper storage tank (S_U) subject to the operating domain of the generalized curves for WSE.

4.0 SYSTEM DYNAMICS – CTTRWH MODEL

The WSE of a RWH system can be defined by Y/D and denoted by η, where Y and D are the annual yield and demand respectively for a given storage capacity S at a given location with a collection area A and an annual rainfall of R. When the WSE for the upper tank and for the overall system are η_U and η_O respectively, for a given annual demand D in m^3, collection area A in m^2 and annual average rainfall R in m for given storage capacities of S_U for the upper tank and S_P for the parent tank, the yield from the upper tank Y_U and the overall system Y_O are given by;

$Y_U = D^* \eta_U$ and $Y_O = D^* \eta_O$, where η_U and η_O are the WSE of the upper tank and the overall system.

It can be shown that the quantity of collected rain water that is possible to be pumped up (Q) is given by;

$$Q = D(\eta_O - \eta_U) \qquad (1)$$

However, when calculating the storage fraction (S/AR) to obtain the WSE (η) values from generalized curves [2], the values η_O and η_L are almost the same due to the capacity of the lower storage tank (S_L) being significantly larger than S_U and also since AR is much greater than S. For example, if the capacities of upper and lower tanks are 1 m^3 and 5 m^3 respectively, installed in a location where the annual average rainfall is 2000 mm and the roof collector area is 50 m^2, S_O/AR and S_L/AR values would be 0.06 and 0.05. Since the objective of the multi-tank system is to have a smaller upper tank for gravity feeding the harvested rainwater to service points as well as to be accommodated readily into the building structure and a larger lower tank to ensure water security, the above argument holds true. Therefore, without significant errors (1) can be modified as;

$Q = D(\eta_L - \eta_U)$

Therefore, when the system is fully functional, Y_U should reach Y_O, though with reduced pumping due to distribution of the storage capacity between the floor levels.

Based on (1), the following equations can be developed to determine the minimum storage capacities and minimum pumping quantities in a functional CTTRWH system for a constant daily service water demand.

As $S_L > S_U$, for the same A, R and D $\eta_L > \eta_U$

Since for a given demand D,
The shortfall in the upper tank (S_U) is given by $D(1 - \eta_U)$ and
The shortfall in the lower tank (S_L) is given by $D(1 - \eta_L)$

The amount of water that can be pumped up is given by Q;

$Q = D(1 - \eta_U) - D(1 - \eta_L)$, which simplifies to,

$$Q = D(\eta_L - \eta_U) \qquad (2)$$

Additionally, if the total demand for water is D_T, then the amount of water required from the mains is given by M;

$M = D(1 - \eta_L) + (D_T - D)$, which simplifies to,

$$M = D_T - D\,\eta_L \qquad (3)$$

The performance of the CTTRWH model can be studied using the equations (2), (3) and the generalized curves for WSE, varying the parameters A, R, D and S_U.

5.0 RESULTS AND DISCUSSION

Taking Sri Lanka as a case representing tropical countries, where the annual average rainfall is mostly depending on regular and predictable monsoonal and convectional rain clouds, the study was based in Colombo ($6^0$54'N, $79^0$51'E), where the annual average rainfall is 2000 mm (National meteorological department of Sri Lanka). The prototype CTTRWH model consisted of 1 m^3 and 5 m^3 volume storage for the upper and lower tanks which were fed by a roof capture area of 50 m^3. Since the generalized curves for WSE are modeled on constant demand, for a daily demand of 200 L, the yield and the quantity of collected rainwater pumped from the lower tank to the upper tank was recorded on a daily basis for one year. Using a 30 day moving average method, where many data points can be obtained from a limited number of readings, the calculated value of Q (21.9 m^3), can be compared with that of measured values (Chart 1), for a D/AR value of 0.73 and S/AR values of 0.01 and 0.05 for the upper and lower tanks respectively. With the above values, the WSE for the upper and lower tanks for the given D, A and R values are calculated as 90% and 60% respectively. The points corresponding to the measured pumping quantities are coalescing with the calculated annual pumping quantities of 21.9 m^3 confirming the equation (2). Based on the validated equation (2), the system performance of a CTTRWH model is analyzed for the variations of D, R, A and S_U.

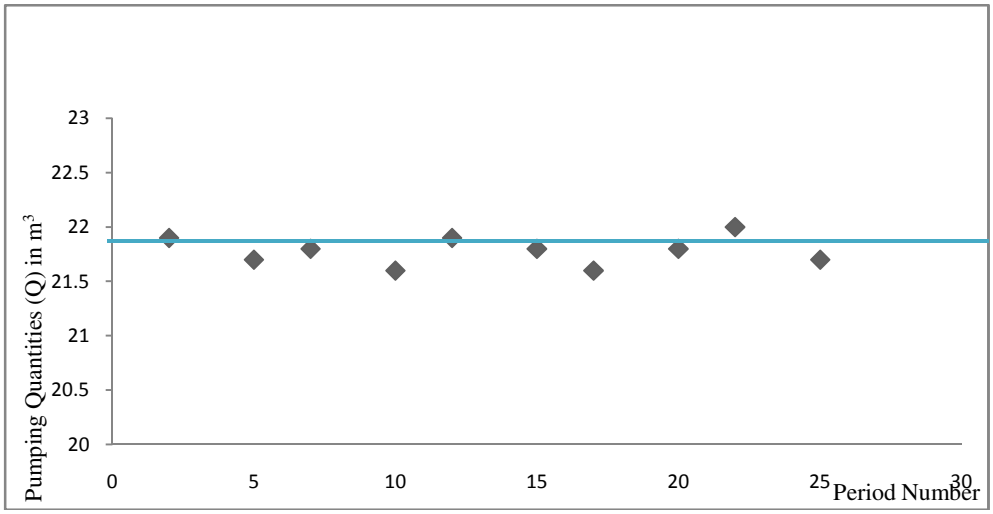

Chart 1: Quantity of collected rainwater that can be pumped up.

5.1 System performance with change in demand (D)

If the demand is reduced by, for example, using water saving devices, the water saving efficiencies η_L and η_U increases rapidly for D/AR > 1.0 and slightly for D/AR < 1.0

This is due to the under-performing of the system for D/AR > 1.0

5.2 System performance with change in rainfall (R)

It can be noted that moving from wet to dry climatic zones, where the minimum annual rainfall (R_{min}) drops, both η_L and η_U dropping and as a result, the dropping of pumping requirement due to lower value for (η_L - η_U)

5.3 System performance with change in capture area (A)

It can be observed that by increasing the capture area A, for a given R, D and S_U that the dimensionless ratio, D/AR, decrease and as a result achieving higher values for η_L. However since S/AR decrease with the increase of A, the difference between the water saving efficiencies of lower and upper tanks, (η_L - η_U), tends to rise, increasing the quantity of water that has to be pumped up.

5.4 System performance with change in upper tank capacity (S_U)

By increasing the size of S_U for a given set of parameters A, R and D, η_U increases reducing the quantity of water required to be pumped up Q, and as a result negating the purpose of a two tank system. It also implies that greater the difference in capacity of the two tanks, the higher the pumping requirement.

The operating domain of the generalized curves dictates that a performing CTTRWH model can be designed only for $0.25 \leq D/AR \geq 2.0$. For values of D/AR beyond this range the behavior of the curves are found to be unreliable, particularly in the critical zone of $S/AR \leq 0.05$. Further, it is noted that for the system to achieve a WSE of over 80% (i.e. $\eta_L \geq 80\%$), D/AR < 1.0

Therefore it can be deduced that, for

$\eta_L \geq 80\%$, D < AR

It can also be observed that when the system parameters are selected so that D/AR > 1.0, when either A or R is increased or the demand D reduced, η_L increases rapidly while the increase in η_U is moderate due to the fixed nature of the upper tank capacity (S_U).

The implications of the above behavior becomes apparent when $R > R_{min}$, which is a usual occurrence since for the reliability of delivery, the minimum annual rainfall, R_{min} is selected in design calculations. It can be shown that when $R > R_{min}$, due to the increase in $(\eta_L - \eta_U)$, the quantity of water to be pumped up Q increases which in turn will increase the demand on the power source. The effect will be more profound if a stand-alone power source is employed to operate the pump. However when D/AR < 1.0, for
$R > R_{min}$ the value $(\eta_L - \eta_U)$ actually reduces, preventing excess loading on the power source.

It can be shown that for tank capacities S_U, S_L and annual demand D, the maximum number of days the system can supply without rain water input is given by,

$$d_{dry} = (S_U + S_L)\frac{365}{D} \qquad (4)$$

In the case of Sri Lanka, from historical data, the average maximum number of non-rainy days (rainfall $\leq$ 0.5 mm) can be taken as, 10, 24 and 45 days for the wet (annual rainfall 1600-4000 mm), intermediate (annual rainfall 600-1600 mm) and dry zones (annual rainfall less than 600 mm) respectively (National Meteorological Department of Sri Lanka). Hence, when selecting a value for S_L, it should satisfy Equation 5.0 for system reliability.

Therefore, from (4),
$d_{dry} \geq$ 10, 24 and 45, for the wet, intermediate and dry zones.

6.0 CONCLUSION

In essence, the two tank model is a RWH system with a distributed storage capacity positioned at two different elevations. Therefore, both storage tanks, individually and collectively, behave according to YAS algorithm and can be described by the generalized curves for WSE. As such, the demand (D) is confined by the limits of validity displayed by the WSE curves. Therefore, for a given A and R, demand D should be in the range of $0.25AR \leq D \leq 2.0AR$. It should also be noted that $S \geq 0.01AR$ so that S/AR value does not fall within the critical zone of the generalized curves and that the daily service water demand is a constant for a given location. However, as human consumption of potable water is habitual, the per capita water usage can be taken as a constant (Hermann, 1999) and hence for household usage situations, annual service water demand can be taken as a constant. Using the data obtained from the prototype CTTRWH system, the measured pumping quantities are almost coalescing with that of the calculated, with the slight shortfall due to system losses. Therefore, the equations developed to describe the performance of the model can be considered as valid. Hence, the two tank RWH model can be effectively integrated into single or multi storey households, with suitable variation in storage sizes and collection areas for a desired WSE, in combination with an effective pumping system. In the study, the effect of the volume of retained water in the piping network to the overall

performance of the system is not considered. However, for typical two storeys housing units it can be of negligible influence taking into account the average pipe lengths and diameters.

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Grazing Intensity Contributes to Cyanogenic Toxicity in Savannah Grasses in Baringo County

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ABSTRACT

The objective of this study was to quantify the influence of grazing intensity on cyanogenic glycosides in Lake Bogoria, Kenya. Field experiments were carried out in ten enclosures. Grazing intensity was varied using simulated grazing method. Grasses were categorized into two age classes; young and old. Cyanogenic glycosides were tested using impregnated picrate paper and concentration determined by hydrolysis and trapping in 1M NaOH. Five of 16 sampled species produce cyanogenic glycosides.There was an inverse relation between Cyanide concentration and age of the plants. Young cuttings yield more Hydrogen Cyanide than older cuttings.Grazing intensity had a significant effect on the concentration of cyanogenic content in some grass species; C. dactylon (P=0.024) and S. laevigatus (P=0.003). This study imply that grazing regime of managed pastures should consider the age of forage while allowing utilization of pastures preferably grazed on mature pastures with low levels of cyanogenic glycosides.

Keywords: Cyanide, grazing, intensity, grasses and glycosides

1.1 INTRODUCTION

Cyanogenic glycosides (CNglcs) are bioactive plant products derived from amino acids. They are a group of plant secondary compounds that contain nitrogen and yield cyanide (cyanogenesis) following their enzymatic breakdown (Møller, 2010). Natural sources of cyanide, include bacteria, plants, and fungi which synthesize and secrete cyanide but the most common sources of cyanide in the environment are from industrial wastes which enter the soil through the solution with rain water and infiltration (Woodrow et al., 2002).

According to Zagrobelny, Bak, & Møller, (2008), Cyanogenesis; the evolution of toxic hydrogen cyanide from endogenous CNglcs—is an effective defense against generalist herbivores but less effective against fungal pathogens). Plants have evolved a plethora of different protection chemicals that covers almost all classes of (secondary) metabolites that represent a significant defense to herbivory: Some are constitutive; others are induced after an attack (Mithöfer & Boland, 2012). According to (Ballhorn Kautz & Lieberei, 2010) many compounds act directly on the herbivore, whereas others act indirectly via the attraction of organisms from other trophic levels that, in turn, protect the plant. An enormous diversity of plant (bio) chemicals is toxic, repellent, or anti-nutritive for herbivores of all types. Examples include cyanogenic glycosides, glucosinolates, alkaloids, and terpenoids; others are macromolecules and comprise latex or proteinase inhibitors. Grasses are also known to produce an array of secondary metabolites, such as hydroxamic acids (Pentzold et al. 2014) and alkaloids, albeit at levels much lower than dicotyledons (Zagrobelny, Bak, & Møller, 2008).Their modes of action include membrane disruption, inhibition of nutrient and ion transport, inhibition of signal transduction processes, inhibition of metabolism, or disruption of the hormonal control of physiological processes.

The level of cyanogenic glycosides produced is dependent on the age and variety of the plant, as well as environmental factors (Møller, 2010; Ubalua, 2010). Production of Cyanide is thought to be due to the presence of cyanogenic glycosides that release HCN (hydrogen cyanide) when acted upon by enzymes found within plant cells (Ramirez and Barry, 2005). Certain plant species synthesize cyanogenic glycosides and cyano lipids which when disrupted by grazing are hydrolyzed and in the process liberate Hydrogen Cyanide (HCN). Hydrogen cyanide produced has a potential to cause health concerns which include the arrest of the

ATP production and cell death by blocking cytochrome oxidase (Sirikantaramas, Yamazaki & Saito, 2008). Recognizing the herbivore challenge and precise timing of plant activities as well as the adaptive modulation of the plants' metabolism is important so that metabolites and energy may be efficiently allocated to defensive activities.This study seeks to identify savanna the grasses which synthesize cyanide and attempt to elucidate the biological pathways that link mammalian grazing disturbance with cyanogen toxicity associated with these grasses in Kenya. This study also sought to explain on the concentration of cyanogenic glycosides as affected by the age of grasses.

1.2 MATERIALS AND METHODS

The study was conducted in fenced enclosures in Lake Bogoria (00°20'N, 35°59'E) during the months of June-September, 2015.The enclosures (10 in total each 50m×10m) were established in June 2015 and enclosed for one month. In the first experiment, however, grasses were sampled outside enclosures on a random basis and tested for cyanogenic glycosides using picrate-impregnated paper. After identifying the grasses with cyanogenic content, enclosures in sampling points were made to determine the influence of age and grazing intensity on cyanogenic concentration. In total, the experiment consisted of 10 replicates of each of the following factorial treatments: Light grazing (LG + Plot1, p2, p3…), heavy grazing (Hg), and no grazing (Ng) in each plot. Two levels of grazing intensity were applied; light (15cm height) and heavy (5cm). The grazing treatment was begun in late June 2015 by first clipping then second clipping. One control experiment in each sample was established (where no consideration to variation in grazing intensity was established). Sampling units defined by quadrat measuring 0.25m radius was distributed on purposive sampling. Age of grasses was classified into two; young and old pastures using characteristics such as; fluorescence, and leaf blade length. The concentration of Cyanogenic glycosides in grass extracts was measured by hydrolyzing the glycosides and trapping the evolved cyanide in 1M NaOH well a modification of method by Gleadow *et al.,* (2006). Freeze-dried grounded grass tissues (10-15g) was incubated 20h at a temperature of 37^0c with 1ml of 0.1M citrate buffer-HCL pH 5.5, a condition which allowed for the complete conversion of cyanogenic glycosides to cyanide. The cyanide detected using this method is directly proportional to the concentration of cyanogenic glycosides, for example, 1mg CN is equivalent to 11.35mg glycoside prunasin (Gleadow and Woodrow, 2002)

1.3 RESULTS

The results of Experiment 1 showed out of 16 species sampled and tested only five species indicated active on impregnated picrate paper test while other eleven species had no effect on picrate paper illustrating non-cyanogenic. These species which change color on impregnated paper include; *Cynodon dactylon, Cynodon plectostachyus, Digitaria scalarum, Sporobolus spicatus and Cyperus laevigatus*.

The results of age experiment show that younger cuttings of grass had relatively more concentration of cyanogenic content than older cuttings (Table 1). However in some species (*C. laevigatus)* older cuttings had more cyanide content than younger cuttings (1.470Mg CN g^{-1} DW and 1.240 Mg CN g^{-1} DW, respectively). There were higher levels of cyanide in *C. dactylon* (1.89 Mg CN g^{-1} DW–young and 1.74 Mg CN g^{-1} DW-old) than all other species while *D. scalarum* had the lowest level of cyanogenic content(1.210 Mg CN g^{-1} DW young cuttings and 1.130 Mg CN g^{-1} DW older cuttings). However, there was no significant difference in cyanide concentration relative to age of grasses in all species (P>0.05)

Table 1: Cyanide levels and Age of grasses

	Cyanogenic glycosides concentration (Mg CN g^{-1} DW)		
Species	**Young cuttings**	**Old cuttings**	**p-value**
C. dactylon	1.890 (±0.16)	1.740(±0.15)	0.503
C. plectostachyus	1.420 (±0.09)	1.320(±0.11)	0.483
S. spicatus	1.260(±0.09)	1.170(±0.11)	0.538
C. laevigatus	1.240(0±0.06)	1.470(±0.12)	0.103
D. scalarum	1.210(0±0.08)	1.130(±0.07)	0.447

In experiment 3 the general trend shows that cyanogenic content in grasses increases with increase in grazing intensity (Fig 2-5). Two sample t-test, however, showed that there was no significant difference between the two grazing intensities in all species (P>0.05). Nevertheless, one way unstacked-ANOVA test with two grazing intensity and one control experiment showed there was a significant difference in concentration of cyanide as a result of grazing intensity in two species *C. dactylon* (P=0.024) and *C. plectostachyus* (P=0.003) while the other three species, there was no significant difference in levels of cyanogenic glycosides under the two grazing regimes and control.

By Tukey's comparison, null hypothesis H_o: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$ that grazing intensity has no effect on the concentration of cyanide was rejected and concluded that grazing intensity influence the level of cyanogenic content in grasses. The result also showed there was no significant difference in levels of cyanide across all species except *C. laevigatus.* It illustrated that *C. laevigatus* was most susceptible to grazing pressure as compared to other species.

Table 2: Turkey's Pairwise Comparisons of two Grazing Intensities and control treatment

Species	**1st Clipping** ($\overline{X}$ 1- $\overline{X}$ 2)	**2nd Clipping** ($\overline{X}$ 2- $\overline{X}$ 3)	**Control** ($\overline{X}$ 3- $\overline{X}$ 1)	**P-value**
C. dactylon	0.25 (±0.09) $_{Aa}$	-0.36(±0.11)$_{Bb}$	-0.11(±0.064)$_{Da}$	**0.024**
C. plectostachyus	0.19(±0.08)$_{Aa}$	-0.16(±0.09)$_{Ba}$	0.03(±0.052)$_{Da}$	0.202
S. spicatus	-0.04(±0.07)$_{Aa}$	-0.08(±0.07)$_{Ba}$	-0.12(±0.059)$_{Da}$	0.431
D. scalarum	0.06(±0.07)$_{Aa}$	-0.08(±0.06)$_{Ba}$	-0.02(±0.067)$_{Da}$	0.661
C. laevigatus	0.26 (±0.08)$_{Aa}$	-0.31(±0.06)$_{Cb}$	-0.05(±0.054)$_{Dc}$	**0.003**

1.4 DISCUSSION

The results of the second experiment showed that generally, younger plants had a higher concentration of cyanide as compared to older. This result compares favorably with the result in the study done by Ebbs (2004) which showed that cyanide concentrations in tansy were variable, but the plant appeared to concentrate cyanide where soil concentration increased. In his study, Ebbs concluded that the ability to concentrate cyanide may be related to plant age; i.e., younger cuttings tended to yield more HCN than older plants that were taken from the same cyanogenic soil. Also, the higher concentration of cyanide in younger plants has been particularly well documented in sorghum, which is highly toxic to grazing stock when young, but becomes suitable for pasture as plants mature (Ganjewala et al. 2010).

According to Ballhorn et al (2011), CNglc concentrations are higher when growth is limited by environmental factors such as light, temperature, or drought. The study area classified as ASAL is hot and dry throughout most of the year with an average annual mean temperature of about 26.6°C and rainfall is highly variable with a yearly mean of between 635mm. Three explanations are often presented to account for this: (a) CNglcs are concentrated in a smaller amount of plant tissue (Selmar and Kleinwachter, 2013), (b) the plants are phenologically younger owing to delayed growth (Miller et al. 2014), or (c) there is active upregulation at the transcriptional level (Busk & Møller, 2002; Zhu-Salzman et al. 2008). The magnitude of the increase in HCNp in response to low soil moisture depends on the severity and duration of the stress, the ontogenic stage, and the availability of other resources (Gleadow and Woodrow, 2002; O'Donnell et al. 2013; Vandegeer et al. 2013).

In cassava, drought-stressed tubers may become more toxic because of a direct increase in concentration and relocation of linamarin from leaves to tubers. This increased HCNp in drought-stressed cassava is not permanent and decreases after plants are re-watered (Vandegeer et al. 2013).

In general, plants supplied with high levels of nitrogenous fertilizers (ammonia or nitrate) have an increased content of CNglcs. Highly fertilized fields of forage sorghum, for example, can sometimes become toxic to livestock (HCNp > 600 ppm) (Ganjewala et al. 2010; Wheeler et al. 1990). A link between nitrogen supply and CNglc deployment has also been observed in legumes, where the rate of colonization by nitrogen-fixing rhizobia has been associated with higher concentrations of linamarin and lotaustralin and decreased herbivory in both clover (Kempel, Brandl and Schadler, 2009) and lima beans (Ballhorn, Kautz and Schadler, 2013). Not all plants respond to nitrogen in this way. In a study by Busk & Møller (2002), dhurrin concentration did not increase in very young seedlings grown at high levels of potassium nitrate.

In 2014, Miller, Gleadow, and Cavagnaro found out that the higher concentration of cyanide in young plants as compared to the older plants is related to enzymatic activity and adaptive mechanism. They found out that during germination and plantlet development, the cyanogenic potential of the entire seedling declines by 85% as cyanogenic compounds are metabolized to non-cyanogenic substances and negligible amounts of gaseous HCN are liberated during this process. However, since highest levels of the cyanide detoxifying enzyme β-cyanoalanine synthase occur in young seedling tissues, (Webber and Woodrow, 2009), proposed that linamarin is transported from the endosperm via the apoplast to the young, growing tissues for further catabolism.

In further support of this, Ballhorn, Lieberei and Ganzhorn (2005) found out that young leaves exhibit a higher HCNp and HCNc than mature leaves. They concluded thatphenotypic plasticity of cyanogenesis in young leaves of lima bean *Phaseolus lunatus* was based on increased activity of the beta-glucosidase in response to herbivore attack. Similarly, Gleadow & Møller (2014) found out that HCNp varies ontogenetically, phenologically, and chronologically. HCNp is highest in seedlings and decreases with plant age (Gleadow and Woodrow, 2008; Webber and Woodrow, 2009). For example, in *E. cladocalyx*, in the series Sejunctae, seedlings have a high HCNp (Goodger et al. 2006). A similar pattern occurs in lima beans, where only secondary leaves are cyanogenic (Goodger et al. 2006). Newly formed tissues are also nearly always more cyanogenic than older tissues (Gleadow and Woodrow, 2008), as in *E. cladocalyx*, where HCNp is as high in newly formed shoots and young reproductive organs of adult plants as it is in seedlings (Gleadow and Woodrow, 2008). On the contrary, notable exceptions to the pattern described above are the cyanogenic Eucalyptus species from the series *Maidenaria*. They are essentially acyanogenic as seedlings (<10 ppm HCN), becoming cyanogenic only after 6–12 months (Goodger et al. 2006).

Similarly, Webber and Woodrow (2008) concluded that the higher HCNp in younger plants and plant parts is consistent with the optimal allocation theory of plant defense, but as leaves expand, there may simply be a trade-off with leaf toughness and other forms of chemical defense. This may correlate with the transcript levels of the CYP79 genes involved, as in sorghum, where the CYP79A1 transcript levels are higher in young seedlings (Busk and Møller, 2002) and in *L. japonicus,* where expression of the two CYP genes governing the synthesis of lotaustralin and linamarin (CYP79D3 and CYP736A2) is highest in the apical leaves (Takos et al. 2010).

In further support of this finding, Gleadow & Møller, (2014) found out that CNglc concentration is usually higher in young plants when nitrogen is in ready supply, or when growth is constrained by non-optimal growth conditions. All plants produce tiny amounts of HCN as an additional product in the biosynthesis of ethylene, but some plant species can release large amounts from endogenously stored cyanogenic glycosides (CNglcs). CNglcs may accumulate in all parts of a plant [e.g., as in cassava(Wheeler et al. 1990)], only in the aboveground parts [e.g., as in Eucalyptus (Gleadow and Woodrow, 2008) and white clover (Olsenet al, 2013)], or only in vegetative tissues [e.g., as in sorghum (Wheeler et al. 1990)]. This pattern may vary with the reproductive stage as well. Some *T. ulmifolia* populations, for example, lose their cyanogenic capacity around flowering, whereas others do not (Schappert and Shore, 1999). The often-observed location of CNglcs and their catabolic enzymes at the periphery or other entrance sites of plant tissues (peel, epidermis, and vascular bundles) and in young, soft tissues is consistent with a defensive role.

In the third experiment, as predicted, grazing intensity influence the concentration of cyanide, the concentration of cyanide across the species tested varied considerably with some species. Two sample t-test showed that there was no significant difference in cyanogenic concentration in all the species when subjected to both grazing intensities. However, one-way ANOVA shows there was a significant difference in cyanogenic glycosides concentration of two grass species, *Cynodon dactylon* (p-value=0.024) and *Cyperus laevigatus* (P-value=0.003). Cyanogenic glycosides are not toxic and are stored intracellularly in the vacuole, whereas the related glycosidase is present in the cytoplasm. However, upon cell destruction by a feeding herbivore, cleaving off the aglycone moiety is no longer preventable via separation of the enzyme from the substrate. Subsequently, acetone cyanohydrin is released, which can be converted into HCN and acetone either spontaneously or by a *hydroxynitrile lyase* (Ballhorn et al. 2008)

On average the five species that shows cyanogenic trait had relatively low levels of cyanide to be considered toxic (which was highest in *C. laevigatus* (1.580 Mg CN g^{-1} DW) and lowest in *D. scalarum* (1.250Mg CN g^{-1} DW). In animals, the lethal doses of HCN are reported to be between 1.66 and 15 mg/kg body weight (BW) for various species (Ernesto et al., 2002). These varieties, however, could be toxic to grazers if feed exclusively on particular species. In the study area, resources for grazing were limited and depleted, and grazers were considered generalize because all species of grasses were consumed and was the basis for grazers escaping poisoning. Because plants, animals, and fungi all have mechanisms to detoxify and excrete HCN, poisoning occurs only when the rate of intake is greater than the rate of detoxification.

CNglcs are only one of many defenses at a plant's disposal. Defense strategies are likely to vary with different selective pressures (magnitude and type) and with developmental stage (Agrawal, 2011; Ballhorn et al. 2008). CNglcs are effective deterrents to generalist herbivores (Ballhorn et al. 2008; Gleadow and Woodrow, 2002; Zagrobelny et al. 2004), and this is most likely the main evolutionary driver in their occurrence across the plant kingdom (Neilson et al. 2014). CNglcs may also serve as transport forms of carbon and nitrogen (Agrawal, 2011), and endogenous turnover processes may release the nitrogen from CNglcs in the form of ammonia (Neilson et al. 2014). More recently, it has been proposed that CNglcs may also function in modulating oxidative stress (Neilson et al. 2014).

In an extensive survey of the shrub *T. ulmifolia*, Mithöfer & Boland, (2012) found out that in naturalized populations in Jamaica, an inverse correlation was found between mean HCNp and the number of herbivore taxa visiting the plant (Schappert and Shore, 1999). Moreover, 40% of the most highly cyanogenic individuals

were not visited by insects at all. Similarly, only one insect (*Leucopodoptera eumundii*) has ever been found feeding on *Ryparosa kurrangii* (sensu *R. javanica*), a long-lived, highly cyanogenic understory tree from tropical Australia (Webber et al. 2003).

Several studies have detected a correlation between bitterness and HCNp (Lee et al. 2013), as recognized in the common names of highly cyanogenic varieties of Prunus, such as bitter and sweet cherries (*P. emarginata* and *P. avium*, respectively) and almonds (*P. amygdalus* syn .*P.dulcius*). The levels of plant defense chemicals are further influenced by damage (Kadow et al. 2012). Several specialist herbivores not only tolerate CNglcs but also actually sequester them for use in their arsenal of defense compounds against predators (Nishida, 2002; Zagrobelny et al. 2007). For example, larvae of *Euptoieta hegesia* (Lepidoptera) that sequester CNglcs from their host (*T. ulmifolia* L.) are more distasteful to their *Anolis* predators (Zagrobelny et al. 2007; Lee et al. 2013). Larvae of the Burnet moth (*Z. filipendulae*; Lepidoptera) can sequester the CNglcs linamarin and lotaustralin from their cyanogenic host plants, typically bird's-foot trefoil (Zagrobelny, Bak, and Møller, 2008; Zagrobelny and Møller, 2011)

In further support of these, Gleadow & Møller, (2014) documented evidence that demonstrates that factors affecting CNglc concentration can be explained in terms of a resource-based trade-off between plant growth and defense. The difficulty in calculating such costs may arise because the production costs are low and because CNglcs have secondarily acquired important roles in nitrogen transport and storage and offer improved tolerance to oxidative stress, offsetting the direct costs of production. They further postulate that cyanogenesis is an effective defense against generalist herbivores but is not particularly effective against fungal pathogens. Many fungi efficiently convert HCN into ammonia and carbon dioxide. Some insect specialists have evolved mechanisms to sequester or denovo synthesize CNglcs and use them as their defense against predators and as a source of reduced carbon and nitrogen (Webber and Woodrow, 2009).

Nearly all of the variability in the effectiveness of cyanogenic glycosides in defense can be explained by four confounding factors. First, the concentration of the cyanogenic glycosides may be below the threshold toxicity (the concentration are well below the capacity to cause poisoning). Second, the animal feeding on the species under examination may be a specialist that has evolved mechanisms to cope with high levels of HCN in the diet. Third, the cyanogenic plant might be consumed as part of a mixed diet and, therefore, might not be toxic. Fourth, the mode of feeding may be such that the animal does minimal damage to the leaf, thereby limiting the mixing of the cyanogenic glycoside with the degradative β-glucosidases and water.

1.5 CONCLUSION

The level of Cyanogenic glycosides varies phenologically, ontogeneticallyand chronologically. Cyanogenic content decrease with age of plants as influenced by increased activity of the beta-glucosidase; as cyanogenic compounds are metabolized to non-cyanogenic substances as the plant matures. Moreover, the highest levels of the cyanide detoxifying enzyme β-cyanoalanine synthase occur in young seedling tissues a response to higher levels of cyanogenic content. Based on this findings, it is recommended that managed pastures ought to be utilized preferably at mature stages with low levels of cyanogenic content. Additionally, levels of nitrogenous fertilizers (ammonia or nitrate) should be kept low as it increases the content of CNglcs in pastures. Highly fertilized fields of forage should be avoided for grazing at succulent and immature stages. On the subject of grazing pressure, plants respond to herbivory by increasing the defensive chemicals; a proxy of

susceptibility to browsing. Defense strategies are likely to vary with different selective pressures (magnitude and type) and with developmental stage thus grazing regimes should consider intensities as well as grazing frequency.

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No Change: Shapes and Patterns of foliar epicuticularwax found in *Sabal palmetto* not affected byEnvironmental Influences from BP oil spill or Hurricane Isaac

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Abstract

Sabal palmetto, a plant that is common to coastal Louisiana,was studied to assess the damage to its leaves caused by the Deepwater Horizon Oil Spill and Hurricane Isaac. Its leaves are the dominant portion of the plant. The leaves are covered with epicuticular wax. Thisepicuticular wax is the first barrier to environmental stress. The wax has a characteristic shape and pattern. The shape and pattern are due to the genetic make-up of the organism and the environment. The characteristic shape and pattern was viewed under natural conditions using Scanning Electron Microscopy (SEM). While environmental stresses can alter the epicuticular wax shape and pattern, samples of S. palmetto collected in early 2011, show little difference in epicuticular wax shapes and patterns when compared to plants not affected by the Deepwater Horizon BP oil spill. This same area was also affected by strong persistent winds and flooding from Hurricane Isaac. The data show that these environmental factors did not adversely affect the epicuticular wax on the leaves of S. palmettoeither.

1. Introduction

The 2010 Deepwater Horizon BP Oil Spill deeply affected Louisiana. Traces of oil were tracked well into the Louisiana marshes and all along the Gulf of Mexico coastline (Peterson, 2015). Historical losses and impact to the economy and natural ecosystems were recorded (Blum et al, 2015). Even though steps were taken to mitigate the problem, the shores in Alabama still contain traces of oil (Middleton et al, 2015 and Hayworth, 2015).

In Louisiana's coastal ecosystems the dominant organ in shrubby plants like *Sabal palmetto* is the leaf. These leaves are covered by a cuticle and epicuticular wax. These epicuticular waxes have a particular shape and pattern that is unique to the species and the ecosystem (Barthelott, et al., 1998). It has been documented in other palms like that of *Serenoarepens* that species share the same overall characteristics but if individual members of the same species are in different areas they may exhibit differences in the outward appearance of the epicuticular wax (Essig et al., 2000). Epicuticular plant waxes serve as an added barrier for plants. Plants in environmentally stressed areas sometimes exhibit changes in their epicuticularwaxes, therefore if *S. palmetto* is adversely affected by environmental disturbances it should be evident in the epicuticular wax shape and pattern (Baker, 1974 & Shepherd and Griffiths, 2006).

The Wetland Research Center continues to monitor the effects of disturbances. Flooding, nutrient level changes, sediment changes, marsh dieback, and other changes are noted. Studies have not indicated affects at the individual plant level for palms (Mishra et al., 2012). In 2011 leaves of *S. palmetto* were viewed using the SEM to see if there were any lasting changes caused by the Oil Spill. These leaves showed no apparent changes in wax structures and/or patterns. In August 2012, the same coastal area of Louisiana was hit by Hurricane Isaac.In an effort to continue monitoring the health of individual plants along the coastline, this study compares the foliar epicuticular wax of *S. palmetto* taken before Hurricane Isaac with the foliar epicuticular wax of *S. palmetto* taken after Hurricane Isaac using Scanning Electron Microscope (SEM). Plant collections were taken in December 2012 to determine if there were any lasting effects of the hurricane on the epicuticular wax of the leaves of *S. palmetto*. If there is a change this indicates that there may be lasting affects to other parts of the ecosystem as well, this includes plants.

2. Materials and Methods

Plants were collected along the canals in Port Sulphur, LA on December 9, 2012. These canals receive water from the Mississippi River and the Gulf of Mexico. Other studies showed that the Deepwater Horizon Oil spill could not be directly linked to changes in metabolism changes (Ostrom et al, 2014). The locations were the same locations used in the previous 2011 study of the effects of the 2010 BP oil spill (unpublished data). In August 2012, this same area was affected by Hurricane Isaac. The collections were stored in a refrigerator for a few days until they could be placed in a plant press using standard pressing materials and procedures (Anderson, 1999). However, drying procedures varied from traditional procedures in that the vertical plant press was stored in a dry room at approximately 28C with a constant air flow and light source. Plants stayed in the plant press for 25 days. The plants were removed from the plant press and small cross-sections of the leaves were cut. Micrographs were then taken of the leaf cuttings at LSU's Socolofsky Microscope Center

using the Scanning Electron Microscope (SEM). Standard SEM procedures were used (Pathan, et al., 2008). The cut samples were placed onto tabs so that they could be sprayed with platinum using the EMS 550x Sputter coater with 15 nm platinum at approximately 25mA.

Once this process was done the platinum coated samples were viewed using the JEOL JSM-6610LV Scanning Electron Microscope. Only the adaxial surface was viewed. This is the surface that is most exposed to the environment. The micrographs were then compared to micrographs taken in 2011 to see if there were structural and/or pattern differences.

palmetto still show not damage. Note the protrusions of wax and the fungal hyphae, (Fig. 4). This wax, minus the fungal hyphae, is comparable to wax seen on *S. palmetto*in February 2011.It is also comparable to non-disturbed areas. This wax is similar to wax found on other Monocot plants in that the protrusions are vertical and horizontal. The amounts and spacing may differ. These findings imply that even though the habitats were not adversely affected by the Deep Water Horizon BP oil spill and Hurricane Isaac, they have not impacted the epicuticular wax of *S. palmetto leaves* long term. This also implies that the overall long term health of other plants in the area may not be impacted.

These findings were significant because many places are still feeling the effect of the Deepwater Horizon Oil spill. The palms however are hardy and resistant.

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Applying Bivariate Meta-analyses when Within-study Correlations are Unknown

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Abstract

Multivariate meta-analysis can be used to combine several outcome measures instead of only concerning one specific outcome measure. We need to quantify the correlations between outcomes across studies when fitting multivariate models. The model requires an estimate of the correlations between treatment effect for each study. These correlations are known as within study correlations and rarely available in published literature. This limits the multivariate approach in practice. Therefore, we have to either approximate them or ignore correlations between effect estimates within the same studies. In this paper, we discuss some widely applicable ways in which this problem can be resolved. We also examine the use of the Bayesian correlation method as a novel approach for dealing with unknown within-study correlations in bivariate meta-analysis. When there is heterogeneity of effects across studies and high correlation within studies, our approach perform quite well. An application to a meta-analysis of treatments for acute stroke data illustrates the use of the approximated correlation in bivariate meta-analysis with correlated outcomes.

Key Words: multivariate meta-analysis, within study correlations, unknown covariance, Bayesian correlation method, Pearson correlation

1. Introduction

Meta-analysis has become an increasingly important technique and it enables researchers to combine the statistical results of many pieces of research on the same research question. Univariate meta-analysis refers to quantitative methods of synthesizing one statistical result from many studies that examine a certain topic. However, having measures on many variables limit the use of univariate meta-analysis and seek methods that allow combining the results of different studies where each contains a multivariate analysis. There are a variety of multivariate techniques for data synthesis across such studies and one needs to choose a specific technique for a given research problem.

Meta-analysis is typically done on independent studies, and consequently their effect sizes are also independent. However, this is not true for each study. Some studies can use multiple treatments compared to a common control group resulting in correlations between effect sizes. When studies sharing a common control group, the estimate of effect sizes may not be independent and such studies are called as multiple-treatment studies (Gleser & Olkin, 1994). Moreover, studies can have combination of several outcome measures instead of only concerning one specific outcome measure. If such outcome measures come from same individual of interest, they are likely to be correlated; corresponding estimated effect sizes for these measures will be correlated within studies. Studies of this type are called as multiple-endpoints studies (Berkey et al., 1996, Gleser & Olkin, 1994).

Multivariate meta-analysis allows a joint synthesis of the multiple end points or multiple treatment effects and it will produce a pooled result for each end point or each treatment effect simultaneously. This can account for any correlation between end points or treatment effects and the correlation may exist both within studies and between studies. The within-study correlation indicates the association between the summary end points or treatment effect estimates within a study. Same individual in a study contributing towards multiple treatment effects or multiple end points is the reason for that type of correlation. The between-study correlation indicates how the true underlying end point or treatment effect summary values are related across studies and is caused by deference across studies in patient characteristics, such as age, or changes in study characteristics, like the threshold level in diagnostic studies (Riley, 2009).

When the summary data per study are multi-dimensional, practitioners usually choose to perform a separate univariate meta-analysis of each end point or each outcome variable. One reason for this may include the increased complexity of the multivariate approach. The requirement of special statistical software is another problem. Perhaps a lack of understanding as to when multivariate meta-analysis is beneficial or a lack of understanding the consequences of ignoring correlation in meta-analysis (Riley, 2009). Another major barrier is that multivariate meta-analysis models require, from each study, the within-study correlation or individual participant data are available. Unfortunately, within-study correlations or individual participant are rarely reported in primary study publications, and conceivably this must put practitioner off the multivariate meta-analysis approach.

The remainder of this paper is organized as follows. In Section 2 we describe the bivariate fixed effect, bivariate random effect, and bivariate marginal models and then extend the discussion with the detail on how to estimate the pooled estimates, the effect of ignoring within-study correlation, a review of dealing with unavailable within-study correlation needed for the multivariate meta-analysis approach, and a description of a new method called Bayesian correlation approach to approximate this correlation when it cannot be obtained

from alternative sources. In Section 3 we illustrate a simulation study to explore how the statistical properties of the pooled effect estimates are affected by (i) the estimation of unknown with-study correlations using Pearson correlation approach and new Bayesian correlation approach, (ii) strength of the estimated within-study correlations, and (iii) strength of the between study and the within-study variances. The simulation allows us to compare the performance of bivariate fixed and random effect models against separate univariate fixed effect meta-analyses models. In Section 4 we apply the methods to the motivating example. In Section 5, we conclude with a discussion of the benefits of using new Bayesian correlation approach to estimate unknown within-study correlations.

2. Material and Methods

2.1 Bivariate Fixed Effect Meta-Analysis Model (BFMA)

In a bivariate fixed effect model, the two correlated end points or treatment effects of interest are assumed to follow a bivariate normal distribution within studies

$$\mathbf{y}_i|\boldsymbol{\mu}_i \sim N(\boldsymbol{\mu}_i, \mathbf{S}_i), \qquad \mathbf{S}_i = \begin{pmatrix} S_{11i}^2 & S_{11i}S_{22i}\rho_{S_i} \\ S_{11i}S_{22i}\rho_{S_i} & S_{22i}^2 \end{pmatrix}, \tag{2.1}$$

where N denotes a bivariate normal distribution, $\boldsymbol{\mu}_i = (\mu_{1i}, \mu_{2i})$ is the true underlying effect for the i^{th} study and $\mathbf{S}_i$ is the covariance matrix of $\mathbf{y}_i$. The matrices $\mathbf{S}_i$ are referred to as the within-study covariance matrices; their entries are estimated in practice using the individual patient data (IPD) for each study separately but regarded fixed and known when pooling the results to make inferences. The within-study standard errors are usually reported in primary publications and they can be used to obtain the within-study variances (the diagonal entries of $\mathbf{S}_i$). The model (2.1) requires ρ_{S_i} (off-diagonal entries of $\mathbf{S}_i$) to be available in those studies providing both end points, which is unfortunately unlikely. We will review available methods for dealing with unknown within-study correlations in Section 2.6 and also propose a new approach to conduct multivariate meta-analysis in the absent of within-study correlations in the Section 2.6.8.

2.2 Bivariate Random Effects Meta-Analysis Model (BRMA)

The bivariate random effects model allows the μi to vary from one study to the next and further assumes that

$$\boldsymbol{\mu}_i \sim N(\boldsymbol{\mu}, \mathbf{D}), \qquad \mathbf{D} = \begin{pmatrix} D_{11}^2 & D_{11}D_{22}\rho_D \\ D_{11i}D_{22}\rho_D & D_{22}^2 \end{pmatrix}, \tag{2.2}$$

where $\boldsymbol{\mu}$ is the average effect from a normal distribution of study treatment effects and $\mathbf{D}$ is the between-study covariance matrix. The between-study variances D_{11}^2 and D_{22}^2 account for any heterogeneity in μ_{1i} and μ_{2i} across studies, and ρ_D represents their between-study correlation.

2.3 Bivariate Marginal Model

The conventional bivariate random effects meta-analysis model is marginally given by

$$\boldsymbol{y}_i \sim N(\mu, \mathbf{S}_i + \mathbf{D}), \tag{2.3}$$

where the $\mathbf{y}_i$ are further assumed to be independent because they come from separate studies. The model (2.3) reverts to a bivariate fixed effects meta-analysis when $\mathbf{D} = 0$, and to a separate univariate meta-analysis of each end point when $\rho S_i = \rho_D = 0$, i.e. all correlations are 0. Usually the objective of meta-analysis is to estimate $\boldsymbol{\mu}$

and $\mathbf{D}$. Once $\widehat{\mathbf{D}}$ has been calculated, the estimated between-study correlations can be obtained directly as the appropriate entry of $\widehat{\mathbf{D}}$ divided by the corresponding between-study standard deviations, which are obtained as the square roots of the diagonal entries.

2.4 Estimation

A variety of approaches for fitting the random effects model for meta-analysis have been developed. Assuming all studies provide all effects, the pooled estimates $\widehat{\boldsymbol{\mu}}$ are given in terms of $\widehat{\mathbf{D}}$ by

$$\widehat{\boldsymbol{\mu}} = \left(\sum_{i=1}^{n}(\mathbf{S_i} + \widehat{\mathbf{D}})^{-1}\right)^{-1}\left(\sum_{i=1}^{n}(\mathbf{S_i} + \widehat{\mathbf{D}})^{-1}\,\mathbf{y_i}\right), \qquad (2.4)$$

where n is the number of studies.

2.5 The Effect of Ignoring Within-study Correlation

Multivariate meta-analysis models require, from each study, the within-study correlation ρ_{Si} (or equivalently S_{12i}) to be available. But, in practice within-study correlations are rarely reported in primary study publications. In some scenarios the within-study correlations can justifiably be assumed zero or close to zero (Reitsma, et al., 2005, Daniels & Hughes, 1997, Korn, et al., 2005, Thompson & Sharp, 1999), such as in diagnostic studies where sensitivity and specificity estimates are independently derived from separate patients. However, in other settings such as the meta-analysis of longitudinal data (Jones, et al., 2009), or for multiple outcomes such as overall and disease-free survival (Riley, et al., 2007), the true within-study correlations are likely to be non-zero. One study in the literature concludes that if interest lies only in the pooled effects one can fit multivariate model with S_{12i} set to 0 without any significant risk of bias or loss of precision in estimates. However, this recommendation has been questioned by analytically assessing the influence of within-study correlation on the pooled estimates and also by some simulation studies (Riley, 2009).

2.6 Estimating the Within-study Correlation

In BFMA, we assume that (y_{1i}, y_{2i}) follow a bivariate normal distribution as indicated in the model (2.1) and in which ρ_{Si} are the within-study correlations. They indicate the dependency between outcome estimates within a study and which is what we want in implementing a BFMA model. Meta-analysts should not ignore the dependence among study outcomes and should use some procedure to deal with dependence. Some widely applicable ways in which this problem can be resolved are described here and we also propose and evaluate a new method, which we termed as Bayesian correlation approach in the Section 2.6.8.

2.6.1 Individual Patient Data Approach

One proposed method for dealing with unknown within-study correlation is individual patient data approach. It involves the collection of raw data from the studies of interest and it allows one to access complete data records from each of the included studies (Dutton, 2011). In multivariate meta-analysis, availability of IPD allows us to calculate the within-study correlation directly in each study. For example, the within-study correlation between the effects of treatment on systolic and diastolic blood pressure can be calculated with the use of individual patient data, by modelling a bivariate regression model between two outcomes jointly in each study (Riley, et al., 2008). Bootstrapping methods may be required to obtain the within-study correlations

using IPD in more complex modelling situations, for instance two different survival outcomes are of interest (Daniels & Hughes, 1997). One issue with this approach is that the individual patient data may not be available in all studies and therefore within-study correlations are still not fully available. In such a situation, one can compute the within-study correlation from a single study where individual data are available and it can be used as a 'likely' within-study correlation estimate for all other studies with unavailable within-study correlations. An average correlation could be used if the IPD is available for more than one study (Kirkham, et al., 2012).

2.6.2 Biological Reasoning (Expert Opinion)

In the absent of IPD, biological reasoning or expert opinion may be used to approximate the within-study correlation. An expert in the clinical field could be asked to suggest a plausible within-study correlation between the estimators for use in all studies. For example, researchers hypothesize that the relationship between all-cause mortality and treatment failure has a positive correlation in the study where beta-lactam is used for the treatment of cancer patients with neutropenia (Kirkham, et al., 2012). In this case even though the direction of the correlation is distinct, the strength might not be determined. However, in some particular situations, an expert can give a numerical value for the correlation based on his prior experience. The incorporation of expert opinion in estimating within study correlation is an alternative in multivariate meta-analysis as it provides a quick and inexpensive alternative when IPD are not available. However, careless use of expert's opinions can result in inaccurate or bias conclusions. Issues such as over confidence, representativeness, translation and linguistic uncertainty can lead experts to provide false or misleading opinions. Hence, the accuracy of the approximation of within-study correlations is an important determinant of the impact of the expert opinion to the outcome of the meta-analysis.

2.6.3 Narrow the Range of Possible Values

In some situations, it is possible to narrow the range of possible values for the unknown within-study correlations. For example, external information has been used for this purpose in the literature (Raudenbush, et al., 1988). Another study has narrowed the range of possible values for the within-study correlation by calculating lower and upper bounds from the 2×2 tables that were available from each study (Berrington & Cox, 2003). The identification of a range of correlation values has similarly helped to inform meta-analysis in other contexts (Abrams, et al., 2005).

2.6.4 Perform Sensitivity Analyses

In the absent of IPD, a sensitivity analysis could be performed by imputing correlations over the entire range of values (i.e. from - 1 to 1), to assess whether and how conclusions depend on the correlation that is imputed. For example, if the eligibility of some studies in the meta-analysis is dubious because unavailability of within study correlations, sensitivity analysis may involve undertaking the meta-analysis twice: first, including all studies and second, only including those that are definitely known to be eligible. In a Bayesian framework, a *uniform (-1, 1)* prior distribution has been used on the within-study correlation and then assessed whether conclusions are robust to change in the specification of this prior (Riley, 2009). However, the use of sensitivity analysis for the estimation of within-study correlations can become problematic in a situation where the dimension is more than two.

2.6.5 Use an Alternative Model

Unavailability of within-study correlations has motivated researchers to build alternative multivariate random effects models for meta-analysis which do not require the within-study correlations. Riley, et al., (2008) has proposed such an alternative model for bivariate random effects meta-analysis and this model includes only one overall correlation parameter ρ which is a combination of the within-study and between-study correlations. This removes the need to know the within-study correlations, and the data that are required to fit the model are the same as those needed for a separate univariate analysis of each outcome, which makes it widely applicable. The alternative BRMA model can be specified as

$$\binom{y_{1i}}{y_{2i}} \sim N\left\{\binom{\mu_1}{\mu_2}, \emptyset_i\right\} \tag{2.5}$$

$$\emptyset_i = \begin{pmatrix} S_{11i}^2 + \varphi_{11}^2 & \rho\sqrt{(S_{11i} + \varphi_{11}^2)(S_{22i} + \varphi_{22}^2)} \\ \rho\sqrt{(S_{11i} + \varphi_{11}^2)(S_{22i} + \varphi_{22}^2)} & S_{22i}^2 + \varphi_{22}^2 \end{pmatrix}, \tag{2.6}$$

where φ_{jj}^2 indicates the additional variation beyond sampling error and ρ denotes the overall correlation. In here, interest lies only in the pooled estimates, or some function of them, and estimation can however become unstable when the estimated correlation ($\hat{\rho}$) is very close to 1 or -1. This alternative model produces pooled estimates that are very similar to those from fitting the general BRMA model where the within-study correlations are known and have better statistical properties than those from separate URMAs, especially given missing data (Riley, et al., 2008).

2.6.6 Pearson Correlation Method

Another approach known as the Pearson correlation method has been proposed with the use of treatment effect estimates in the literature (Kirkham, et al., 2012). In the present of data on both outcomes, the Pearson correlation coefficient can be calculated between the pairs of treatment effect estimates for a bivariate analysis and it can be assumed as a common within-study correlation ρ in each study. Simply, we have to assume that the within-study correlations are the same in each study and that this correlation will be closely reflected in the observed correlation between paired outcome effects across studies. In conclusion, a precise estimate of the Pearson correlation could be obtained with five data points (Abdel-Megeed, 1984).

2.6.7 Use of Formulas

Most imputation approaches have been based on imputing the correlation between treatment effect estimates and assuming this correlation to be identical for every study. Wei and Higgins (2013) propose an approach to approximate the within-study covariance based on information about likely correlations between underlying outcomes instead of treatment effect estimates. In brief, the treatment effect is a quantity that describes the benefit or harm of the treatment, whereas the outcome is the direct measurement on the participants. Evaluating within-study correlation at the outcome level can bring two main advantages. First, these correlations can easily be obtained from external sources, and if not, then plausible values for them could be provided than between treatment effect estimates. Second, these correlations are more natural descriptors of inherent similarities and allow the correlations between treatment effect estimates to vary according to other

measurable features of the study. This paper considers both continuous and dichotomous outcomes, which are the most common in meta-analysis. For instance, the covariance between two estimates of mean differences is given in closed form as

$$COV(MD_1, MD_2) = \frac{n_{12t}}{n_{1t}n_{2t}}\rho S_{1t}S_{2t} + \frac{n_{12c}}{n_{1c}n_{2c}}\rho S_{1c}S_{2c} \quad (2.7)$$

where, n_{1t} , n_{2t} , and n_{12t} denote the number of participants who report outcome 1, of those who report outcome 2, and of those who report both outcome 1 and outcome 2, respectively, in the treatment group. In a similar way, we define n_{1c}, n_{2c}, and n_{12c} for the control group. The correlation between the two outcomes themselves is denoted by ρ and is substituted by a value taken from an external source of information.

2.6.8 Bayesian Correlation Method

In this paper we propose a novel approach and which we call as Bayesian correlation method to deal with unknown within-study correlations in bivariate meta-analysis. The Pearson's correlation approach is quite simple whenever someone wants to get an approximation for the common within study correlation. In practice, Pearson's correlation coefficient is calculated when both variables being studied are normally distributed. This coefficient is highly affected by the outliers, which may exaggerate or dampen the value of the coefficient (Mukaka, 2012). The smaller the number of available treatment effect estimates, the greater the effect of the outliers on value of correlation coefficient. In meta-analysis, the coverage for both outcomes can be slightly worse for the BFMA approach when the true within-study correlation is largely overestimated by the Pearson's correlation approach; this was particularly evident with fewer studies included (Kirkham, et al., 2012).

In Bayesian theory, the estimation of correlation coefficient reduces to estimating the parameters of a bivariate normal distribution given some data. The bivariate normal distribution is not parameterized using ρ, that is, we cannot estimate ρ directly. The model is defined in terms of μ_x and μ_y the means of the two marginal distributions and a covariance matrix Σ which define σ_x^2 and σ_y^2, the variances of the two marginal distributions, and the covariance, how much the marginal distributions vary together. Given correlation as ρ, the covariance can be written down as $\rho\sigma_x\sigma_y$. The model that we want to estimate is

$$[x_i, y_i] \sim N\left([\mu_x, \mu_y], \Sigma\right), \quad \Sigma = \begin{pmatrix} \sigma_x^2 & \rho\sigma_x\sigma_y \\ \rho\sigma_x\sigma_y & \sigma_y^2 \end{pmatrix}. \quad (2.8)$$

In a Bayesian framework, we need to choose suitable prior distributions to implement the model (2.8). Even though the covariance matrix plays the main role in the model, yet modeling a covariance matrix is often a difficult task in practice due to its dimensionality and the non-negative definite constraint. When modeling correlations, a prior distribution is often chosen for the covariance matrix. The most commonly used prior for the covariance matrix is Inverse-Wishart distribution. In order to model a covariance matrix directly, recent interest has focused on broken down it into variance components. Priors are then assigned separately for σ_x, σ_y and ρ. The main advantage of this approach is the greater flexibility (Barnard, et, al., 2000). In order to make our Bayesian correlation estimate more robust, we can replace the bivariate normal distribution to a bivariate t-distribution. Then, it is required to specify a prior for the degree of freedom that both allow for completely normally distributed data or normally distributed data with an occasional outliers. In practice, it is recommended to use the t-distribution because it also estimates the heaviness of the tails of the bivariate t-

distribution and if there is sufficient evidence in the data for normality the estimated t-distribution will be very close to a normal distribution.

In the model 2.8, the pair (x_i, y_i) represents the two treatment effect estimates. Normal priors were assigned for the mean parameters, μ_x and μ_y. The standard deviation S_μ of the prior on μ was seted as 1,000 times the standard deviation of the pooled data to keep the prior distribution broad relative to the arbitrary scale of the data. To keep the prior scaled appropriately relative to the arbitrary scale of the data, the mean M_μ of the prior on μ is arbitrarily set to the mean of the pooled data. A uniform distribution has been assigned as the prior on the standard deviation and in which low value L_σ, set to one thousandth of the standard deviation of the pooled data, to a high value H_σ , set to one thousand times the standard deviation of the pooled data. The degrees of freedom was assigned that is exponentially distributed, which spreads prior credibility fairly evenly over nearly normal and heavy tailed data (Kruschke, 2013).

2.7 Software Implementation

The model 2.8 can be implemented with *R* and the *JAGS* sampler interfaced with *R* using the rjags package. *JAGS* stands for Just Another Gibbs Sampler. It is a program for the analysis of Bayesian models using Markov Chain Monte Carlo (MCMC). *JAGS* is designed to work closely with the *R* language. The *rjags* package works directly with *JAGS* from within *R*. Running a model refers to generating samples from the posterior distribution of the model parameters.

As the number of iterations tends to infinity, the output from an MCMC sampler converges to the posterior distribution of the model parameters. Usually, the MCMC output is divided into two parts: an initial ”burn-in” period, which is discarded, and the remainder of the run, in which the output is considered to have converged (sufficiently close) to the target distribution. Samples from the second part are used to create approximate summary statistics for the target distribution. A reasonable point estimate for correlation coefficient can be obtained from this summary statistics.

3. Simulation Study

A simulation study was carried out to demonstrate the estimation properties from BRMA when a common within study correlation is estimated using Pearson correlation and also using new Bayesian approach. All BRMA simulations results were compared with corresponding UFMA and URMA results.

The within study variances were generated from a 0.25x χ_1^2 distribution. Two set of values (length n) were obtained as one for within study variance of outcome X and one for within study variance of outcome Y. Values outside the range [0.009, 0.6] were disregarded and a new value was generated. Simulated within study variances were then sorted so that the first study has the largest pair of values ($s_{X_i}^2$ and $s_{Y_i}^2$) and so on, until the last study had the smallest pair of values. For each meta-analysis in the simulation, new set of within study variances were generated. This simulation procedure has been used previously and has been shown to simulate a realistic mixture of study sample sizes (Jackson, et al., 2010, Jamie, et al., 2012). The model (2.3) was used to simulate pairs of X_i and Y_i by taking both the true overall treatment effects μ_1 and μ_2 be zero for all simulation scenarios.

The following criterion was used in order to choose suitable parameter values to investigate in the simulation study. The proportion of marginal variation in X and Y due to heterogeneity is given by $I_X^2 = D_{11}^2/(0.056 +$

D_{11}^2) and $I_Y^2 = D_{22}^2/(0.056 + D_{22}^2)$. Three values of these I^2 terms were considered: 0 (no marginal between-study heterogeneity), 0.3 (mild heterogeneity) and 0.75 (notable heterogeneity), giving nine pairs of I^2 values. In order to investigate the special case where all outcomes are independent, both the between-study correlation ρ_D and all within-study correlations ρ_{S_i} set to 0 in simulation runs 1-9. When one or both of the I^2 values is 0 the correlation ρ_D is not defined, and $\rho_D = 0$ is then taken to mean that the covariance $\rho_D D_{11} D_{22} = 0$ in such instances. Runs 10-17 considered situations where the between and within-study correlations are similar, where ρ_D and all ρ_{S_i} were set to 0.7 or to 0.95; only the combinations of $I_X^2 = (0.3, 0.75)$ and $I_Y^2 = (0.3, 0.75)$ were considered when using these correlations, as values I^2 of zero do not permit such a correlation. Runs 18-25 repeated runs 10-17 with the ρ_{S_i} all set to zero.

3.2 Models Fitted to Each Generated Dataset

Different models were fitted to each meta-analysis dataset generated in the simulation study. The UFMA and URMA models were fitted and then the BRMA model was fitted, first using the Pearson correlation approach and then using the Bayesian correlation approach. In the Pearson correlation approach, a common within-study correlation for each study was estimated by calculating the Pearson correlation between the pairs of available treatment effect estimates for the two outcomes. In Bayesian correlation approach, a common within-study correlation for each study was estimated using the posterior distribution of ρ in the model 2.8.

3.3 Assessment of Performance

The performance of the estimates μ_1 and μ_2 were assessed in terms of bias, standard error, mean square error (MSE) and coverage. The BRMA estimates were obtained for both Pearson correlation approximation and Bayesian correlation approximation for each of the 1000 simulation scenarios. The corresponding 1000 UFMA and URMA estimates were also obtained for each of the scenarios (see Appendix Table 4 and Table 5). The comparison was performed by calculating: (a) the average parameter estimates across all the simulations (to estimate bias), (b) the average standard error and MSE of μ_1 and μ_2 (to assess precision) and (c) the coverage of the 95% confidence intervals (CIs) for μ_1 and μ_2. The percentage of simulated datasets for which the 95% confidence interval for an outcome's treatment effect estimate contained the true effect estimate was taken as the coverage.

3.4 Results of Simulation Scenarios

In simulation runs 1-9, both the between-study correlation ρ_D and all within-study correlations ρ_{S_i} were set to 0 and different combinations of between study variances were considered. Applying the UFMA model leads to produce 0.0805 and 0.0790 average standard error for the first and second outcomes respectively. The corresponding statistics when fitting a URMA model were 0.1260 and 0.0877 respectively. Those values indicate that there is a slight increase of standard error of the URMA model over UFMA model. For the same simulation scenario using UFMA model, the MSEs were observed as 0.0291 and 0.0098 for the first and second outcomes respectively. For the URMA model corresponding statistics were observed as 0.0354 and 0.0118 respectively. Further, the UFMA approach had poorer coverage of 73% compared with the URMA approach where the coverage was 93%. Even though both the between-study correlation ρ_D and all within-study correlations ρ_{S_i} were zero, fitting a UFMA gives pooled results with a severely affected coverage. This inconsistency in results could be explained by the deference in between study variances.

In runs 10-17, we considered the situation in which the within-study correlations and the between study correlation are similar, where ρ_D and all ρ_{S_i}were set to 0.7 or to 0.95. The two values (0.024and 0.168) were considered as the between study variances. Under this simulation scenario, applying UFMA model leads to produce mean square errors of 0.0486 and 0.0354 for the first and second outcomes respectively. For URMA model, the corresponding statistics were observed as 0.0572 and 0.0382 respectively. The coverage percentages were observed as 65 and 92 for UFMA and URMA model respectively. When these simulation results were compared with previous simulation (1-9 runs) results, it is clearly evidence a reduction in coverage and an increment in MSE for both UFMA and URMA models. This situation could be explained as an effect of fitting a univariate model when high within study correlation exists between treatment effect estimates.

In runs 18-25, we considered zero the within study correlations and the high between study correlation. The two values (0.024 and 0.168) were considered as the between study variances. Here applying UFMA model leads to produce mean square errors of 0.0379 and 0.0234 for the first and second outcomes respectively. For URMA model, the corresponding statistics were observed as 0.0461 and 0.0270 respectively. The coverage percentages were observed as 71 and 93 for UFMA and URMA models respectively. There is a clear reduction in MSE and an improvement in coverage for UFMA model over the previous simulation scenario and this could be explained as an advantage of UFMA when the within study correlation is actually zero. The improvement of the URMA model over the previous simulation could also be justified as a consequence zero within study correlation.

For all data simulation scenarios, the pooled estimates were approximately unbiased for both $BRMA_{(Bayesian)}$ and $BRMA_{(Pearson)}$ (see Appendix Table 4). Applying the BRMA approach using the Bayesian correlation method ($BRMA_{(Bayesian)}$) appeared to perform quite well comparing with other three approaches. In most of the runs, the $BRMA_{(Bayesian)}$ produces the smallest mean square error for both outcomes. In BRMA (Bayesian) model, the standard errors were an improvement over the other three approaches for both outcomes. For the first outcome, $BRMA_{(Bayesian)}$ has the smallest standard error over all 25 runs and for the second outcome, both $BRMA_{(Bayesian)}$ and $BRMA_{(Pearson)}$ have comparable standard errors. In $BRMA_{(Bayesian)}$ model, the mean coverage for the first outcome is 91.752% and for the second outcome is 94.568%. In BRMA (Pearson) model, the mean coverage for the first outcome is 91.904% and for the second outcome is 94.428%. Thus, in $BRMA_{(Bayesian)}$ model, there is a little improvement over the mean coverage for the second outcome but not for the first outcome. Although URMA approach had poorer standard errors and mean square errors, its pooled estimates were approximately unbiased and coverage was comparable with other two BRMA approaches. Fitting UFMA produces poorer statistical results under all simulation scenarios.

4. Application to Acute Stroke Data

We now apply URMA model and BRMA model to data (see Appendix Table 2) from 21 studies that assess the effectiveness of hypertension treatment for lowering blood pressure (Geeganage & Bath, 2010). A random effects model was chosen a priori because heterogeneity was significant and the value of I^2 index which can be interpreted as the percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, to between-studies variability (Huedo-Medina, et al., 2006) was obtained as 54.4%. The treatment effects on the systolic and diastolic blood pressures are denoted by SBP and DBP respectively. Negative estimates indicate that the treatment is beneficial. The within-study variances corresponding to treatment effects SBP

and DBP are denoted by $Var(MD_{SBP})$ and $Var(MD_{DBP})$ respectively (see Appendix Table 3). The within-study correlations are unknown in this example but expected to be highly positively correlated (Gavish, et al., 2008). Thus, we selected a *uniform(0,1)* prior for the common within study correlation ρ and performed the proposed Bayesian approach to get an approximation for the common with study correlation.

We conducted a BRMA using restricted maximum likelihood and assuming the within-study correlations are equal to the Bayesian approximation 0.6639 gives a mean difference in SBP as -2.5540 and mean difference in DBP as -2.4467. Again a BRMA was performed assuming the within-study correlations are equal to the Pearson approximation 0.6945 gives a mean difference in SBP as -2.5728 and mean difference in DBP as -2.4363 (Table 1).

Table 1: UFMA, URMA, $BRMA_{Bayesian}$ and $BRMA_{Pearson}$ results.

Parameter	UFMA	URMA	$BRMA_{Bayesian}$	$BRMA_{Pearson}$
μ_1(SBP)	-2.2497 (0.9323)	-2.6276 (1.4677)	-2.5540 (1.3612)	-2.5728 (1.3854)
μ_2(DBP)	-2.0175 (0.5009)	-2.4668 (1.0416)	-2.4467 (1.0293)	-2.4363 (1.0295)

When the Bayesian approximation was applied, there was a slight increase in the precision of the estimated treatment effects over the Pearson correlation approach. The correlations between treatment effects were estimated as 1 and 0.9779 across studies for the Bayesian and Pearson approaches respectively. As evidence in here, very high estimated correlations are a common finding in applications of multivariate meta-analysis. When the number of studies is small and/or the within-study variation is large relative to the between-study variation, the between-study correlation is often poorly estimated as +1 or 1 (Riley, et al., 2008). The URMA model produces estimates with generally less precision statistical properties than those from any BRMA models. Applying the UFMA approach has let to poorer treatment effect estimates severely for both outcomes.

5. Discussion

In practice within-study correlations are rarely reported in primary study publications. One of the main challenges of the multivariate approach is knowing how to obtain the within-study correlations to measure the dependence between the outcomes for each study when these are unknown (Riley, 2009). In this paper we proposed the Bayesian correlation method to estimate this quantity to be the assumed within-study correlation across all studies and it was compared with the previously suggested the Pearson correlation approach. Both are convenient approach to obtain a value as the common within study correlation when no other source is available. The simulations show that both method $BRMA_{(Bayesian)}$ and $BRMA_{(Pearson)}$ generally performed well over UFMA or URMA. Comparison results also indicates that $BRMA_{(Bayesian)}$ produces better statistical properties than $BRMA_{(Pearson)}$.

In general, multivariate meta-analysis can offer advantages over a univariate approach. However, the use of multivariate meta-analysis becomes problematic when within-study correlations cannot be specified. We have derived an approximation for the common within study correlation for situations in which individual patient data are not available but correlations between treatment effects can be specified. Our simulation studies assess whether imputing correlation between treatment effect can improve estimation compared with

alternative approaches. We can conclude that when there is heterogeneity of effects across studies and high correlation within studies, our approach perform quite well.

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CALLING A SPADE A BIG SPOON WITH A HANDLE: EUPHEMISMS FOR TABOO WORDS ON SEX IN KIKUYU

By

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Abstract

The present paper reports on the use of euphemisms for taboo terms on sex and sexuality in Kikuyu language. Kikuyu being a majority language in Kenya is comprehended by both native Kikuyu speakers and other Kenyans of different ethnic groups. The aim of this paper is to guide both native and non-native Kikuyu language users on how to use this language by euphemizing it on the central theme of sex. Both primary and secondary data collection methods have been used in this study. The present research has used Politeness theory. The result of the study shows that the Kikuyu people guard against saying things the way they are supposed to be said, especially in polite company.

Key Words: *Taboo, Euphemisms, Sex, Kikuyu, Politeness.*

1.1 Introduction

Euphemisms for taboo words in any language including Kikuyu help one to say things as if they are not, and by so doing is seen as being polite(Leech 1974, Lyons 1981). This paper looks at euphemisms for taboo words in Kikuyu language that relate to matters of sex. We have chosen to research this topic because sex is guarded in polite company and yet it is very important for procreation and health (Njoroge 2014).

Sex is one of the strongest providers of taboo words. Interestingly, sexuality is the provider of arguably the most popular swear words today, namely the four letter word
(F***) and it's many off-springs. One would think however, that something completely natural such as sexual intercourse, would not elicit anything remotely taboo. Instead, one would think that this activity, which hinges on sex, would prompt positive and happy connection. Unmistakably, this is not the case. Pinker (2007) argues that there are several reasons why sex is tabooed, even today.

> Has everyone had fun? Not necessarily. One partner might see the act as the beginning of a lifelong relationship, the other, as one night stand. One may be infecting the other with a disease. A baby may have been conceived, whose welfare was not planned for in the heat of passion. If the couple is related, the baby may be susceptible to a genetic detect (Pinker 2007:347).

In addition, jealousy might be a dangerous opponent should other interested parties get to know what happened as far as sex is concerned, and a problem sets in if a woman engaged in a sex act gets pregnant by another man other than her husband. In such a case the husband might end up raising a child sired by another man especially in a partrilinial marriage setting. The worst misdeed is clearly rape in which one of the parties involved violently forces the other into having sex. Pinker (Ibid) observes that there are many differences between attitude of sex between men and women. In this writer's view, in every act of reproduction, females are committed to long stretches of pregnancy and lactation while males get away with a few minutes of copulation. This author further observes that on the whole, men usually pursue the sex act much more frequently than women. This, to this writer is seen as one reason why the male use swear words more than the female and why sexual talk might be seen as offensive for women rather than for men

.

The aim of this paper is to enlighten native Kikuyu speakers and protect non-native Kikuyu speakers from using provocative Kikuyu language on sex. They will learn the meanings of words and expressions prohibited in most contexts and the recommended words and expressions for the words that are supposed to be avoided in the Kikuyu language. This paper therefore, gives euphemisms for sexual words that people will dread mentioning in polite company, in the Kikuyu language.

3.2 Methodology

Data for this paper was sampled from two sources. The two sorces are primary and secondary sources of data. Secondary data was obtained from extensive library research on the topic where books and refereed journal papers from reputable authors were refered to. This library reading formed a basis upon which the topic of euphemisms and taboo words on sex and sexuality in Kikuyu was based.

Primary data for this paper was collected by use of interviews, which lasted for about thirty minutes with each of the respondents. Questions in the interview schedules were carefully constructed eliciting data on socio-democratic characteristics of the interviewee besides wanting to know whether in his or her knowledge there exist euphemisms for taboo words in Kikuyu. Besides respondents ages, other factors like sex, level of education, religious affiliation and income and occupation of respondents were considered as variables upon which use of euphemisms is applied to taboo words on sex and sexuality in Kikuyu language. Even most interesting was the fact that the researchers had native or near native competence of the Kikuyu language by either birth or association.

3.3 Theoretical Framework

Several theories have been appled in research on euphemisms and taboo terms the world over (Halliday's 1987 Socio-Semiotic theory, Austin's Speech Act theory of 2002, and Lakoff's 1973 Politeness/ Face theory that was extended by Brown and Levinson in 1987). The present research has used Brown and Levinson (1987) theory.

Following the lead by Lakoff on Politenes, Brown and Levinson (1987) suggest that everywhere polite behaviour is based on assumption of cooperation because all social groups need to minimize conflict among co-members.

3.4 Results and Discusion

Below, the present study presents results and discussion on the topic of euphemisms for taboo words on sex and sexuality in Kikuyu language.

3.4.1 Lexicon on Sex

There are words of sexual connotation. The present study found out that these are words that are frequently used in male group talk among the Kikuyu speakers. The findings from the study show that female correspondents use less sex offensive words than their male counter parts. Instead, if forced to, they euphemize the words, topics and expressions. The only exception of calling it as it is, is in the hospital contexts where four letter words are openly used by the medical practitioners of both sexes. The researchers encountered a situation in the maternity wing of Kiambu General Hospital where nurses openly use words like "guika" meaning f*** and *"kiino kina* ***nagiko"*** **"**dirty vagina" without euphemizing them. The researchers observed that these terms were mostly used by female nurses. Some settings may not allow for euphemisms and one such setting is that of the hospital. This is the essence of the concept of register which has to do with language according to use and not user.

Female Kikuyu speaking nurses at the above named hospital used taboo words while on duty for two other reasons. One, the study found out that they were envious of those who were about to get their new borns and so would go a long way to discourage them by use of any means including language. Two, some nurses ended up being nurses as a second option and so did not whole heartedly like the proffesion and so they ended up transfering their frustrations on their clients using language as a tool.

In Kikuyu, there are various words used to refer to the act of copulation; consider the following data:

Taboo words	**Gloss**
Guthicana	To have sex
Ngwikwo	To have sex

Euphemism	**Gloss**
• Gukoma na -	To sleep with
• Gutura	To make a hole
• Kuhaica	To mount onto
• Kuheo	To be given
• Guthegetha	To drill

Gutamura mundu	To hit somebody
• Kuria mundu	To eat somebody
• Kuneo indo -	To be give something
• Kuneo ndurume	To be given a ram
• Kunora thaika	To Sharpen an arsenal
• Kugwata	To have sex with a minor
• **Kukanura kinya**	To rinse the gourd
• Guthinga mwatuka	To seal a rack
• Kuhinyia wira	To work thoroughly
• Kwenjera gikwa	To dig up a yam
• Kuhura kimandi	To have group sex
• Kuhinyia wira	To work thoroughly
• Guikia muti	Voting
• Guthititthia kihari	To plough virgin land
• Kwihura mbira	To wipe off shoot
• Kihura - muthanju -	Flogging stick
• Kuhura mbiruri	To twirl a wonder cone

Findings of the research revealled that all these terms are used to describe copulation in Kikuyu language. However, it was noted that most of thses words can never be used in polite company. The above terms can only be used with close friends and age mates or in the olden days, during initiation into adulthood through circumcision. In all, the researchers found out that the youth could only understand a few of them, the reason being that there is the influence of Sheng in their communication. Thus, the youth have developed other terms in Sheng, a Kenyan Swahili based slang, to mean copulation.

Examples of such terms include, but not limited to, kumangana (to eat each other), kuduu (to do), kukatia (going for/having an affair with), and kupiga mti (beating one with a tree). These ingroup youthful Kikuyu language users euphemise the taboo words for sex and sexuality for their language can be used anywhere without annoying the targeted audience. The aged Kikuyu speakers will not get the meant meaning while the youthful Kikuyu speakers will see it as an ingroup language style.

There are other terms describing copulation that one can comfortably use in polite company so as to save his or her face. Consider the following:

Euphemism	**Gloss**
Kuonana kimwili	To meet body to body
Kuhumburia nguo	To uncover one's clothes
Kwihumbira na murengeti umwe	To cover with the same blanket
Gukoma	To sleep
Guthii na mundu	To go with someone

Using the above terms, the hearer will be less embarrassed and FTAs will be minimal or none existent at all. As Njeri (2007) rightly observed, sexual activity in Kikuyu is euphemized and mostly equated to a game or a common activity. Consider the following terms that the researchers came across in the course of their research:-

Euphemism	**Gloss**
Kuhura ndati	To play game of darts
Gutwara muithikiri	To ride a bicycle
Kwanjia mbiruri	Twirling a wooden cone
Guthegethana	To drill one another
Kuhanyahanga	To scratch repeatedly
Kuhinyia wira	To do an activity thoroughly

As the researchers observed, these terms are used mostly by the youth or the ever-green youth who view a sexual act not as a serious activity, which can result to pregnancy or even getting infected by a serious disease, but as an act of enjoyment and passing time. To such respondents, if as a result of the sexual activity the girl involved gets pregnant, they go on describing her as "ni araihurire" meaning getten filled up. This is because to these youthful Kikuyu language users, sex is just a game.

The Kikuyu are notoriously religious in Christianity for some of the earliest mission stations in Kenyan like Thogoto and Tumutumu are in the Kikuyu nation. Thus in the Kikuyu version Bible, euphemisms are used to refer to the act of copulation. Consider the following verses:

- **Gen 16:2**
  Nake Sarai akitiira aburahamu aim, riitri, Jehova aonangira guciara, ngiguiharitha ati utonye, hari miiiritu uyu undungatagira, hihi na gokoruo ndaya kwihanda ni undu wake. Nake abiramu agiitikira kuigua mugambo ucio mumisiri wamutomgatagiru, wetagwo hagai.

And Sarai said unto Abram behold now, the Lord hath restrained me from bearing, I pray thee go unto my maid. It may be that I may obtain children by her And Abram hearkened to the voice of Sarai.

"Utonye hari muiritu uyu ndugatagira" meaning, I pray thee " go unto my maid" is a euphemism for copulation. Ordinarily, going unto somebody can have many meanings that may not necessarilly be related to the act of copulation.

- **Gen 29: 23**
  And it came to pass in the evening that he too took Leah his daughter and brought her to him; and he went in unto her.
  "*Akionana nake"* meaning "he saw her".
  This too is a euphemism used in place of copulation in Kikuyu version Bible.

- **Gen 20:4**
  Nariri, Abimeleku ndakoretwo amuthengerere; agikiuria atiri, mwathani, githi woraga ruriri ruthingu?

But Abimelech had not come near her; and he said, Lord will you slay a righteous nation?
"Ndakoretwo amuthengerere" meaning "had not come near her". This euphemism means that they had not copulated.

The researchers observed that when a Kikuyu speaking priest is preaching around those verses on copulation, he uses euphemisms to save his image and that of the hearers. Thus all these euphemisms used to describe sex are used so to save the face of both the speaker and the spoken to.

Sex and sexuality cannot be accomplished as human acts without the use of male and female sexual organs. Thus, below we give results and discussion on male and female sexual organs as tabooed and euphemised in Kikuyu language respectively.

4.1.2 The Lexicon on Male Sexual Organ

The male organ is used for urinating as well as for ejaculating. Thus, due to sensitivity of the organ being described, the Kikuyu speakers resort to the use of euphemized expressions. For instance, they use words like "*Itimu"* – "spear" and when such is used, the accurate information which is supposed to be conveyed to the hearer or reader is lost. Such definition styles tend to sacrifice precision in meaning for increased acceptance in the society and as highlighted by Lakoff (1989) politeness supersedes clarity. In this case, the speaker must be polite to save his **face** and that of the listeners. Consider the following terms as used to refer to the male sexual organ by speakers of Kikuyu.

Taboo word	**Gloss**
• Muthiita	Penis
• Mucuthi	Penis

Euphemism	**Gloss**
• Itimu	Spear
• Muti	Stick
• Cuma	Metal bar
• Jogoo	Cock
• Mirigo	Load
• Indo	Wealth
• Rwenji	Shaver
• Thiaka	Arsenal
• Icembe	Hoe
• Murao	Plough
• Mucinga	Gun
• Muthi	Pestle

A careful look at the euphemisms of the male sexual organ in Kikuyu shows the societal expectation of Kikuyu man. The men are supposed to be strong, daring, insensitive and having an intense forceful sexual

desire that demands immediate gratification. Spears, guns and arsenals do just that and that is why the pens is refered to as such.

The male sexual organ is represented in positive politeness since it creates a sense of pride and solidarity and portrays men as the "active" partner in sexual matters. This shows that the relationship of people who use such terms is strong enough to cope with what is seen as naturally impolite language (Brown & Levinson, 1987:83).

The testicles are the male sexual organs, which contain seeds of manhood. The use of this term testicles requires politeness; from the study, the researchers discovered that the term testicles is not to be uttered in polite company. Most of the respondents prefer to use euphemisms, in which, the euphemisms of the word give a description of what it looks like and this does not help in explaining it directly. This indicates the Kikuyu societal disapproval of uttering this term in public or in polite company as this violates the moral codes of the Kikuyu. The use of euphemisms indicates the sensitivity that is associated with the term testicle. The most essential element of the meaning is lost. The Kikuyu term or word "*waru"* which is one of the euphemisms, meaning "Irish potato" sacrifices precision for politeness in meaning.

Consider the following data:

Taboo word	**Gloss**
Nyee	Testicles
Heke	Testicles
Ndendera	Testicles

Euphemism	**Gloss**
Waru	Potatoes
Mirigo	Goods

Having looked at the male sexual organ, below we give results of the study's findings and discussion on the female sexual organ as euphemized by the Kikuyu.

3.4.2 The Lexicon on Female Sexual Organ

The researchers observed that in most cases, female genitals in Kikuyu are euphemized for fear of being considered rude and are mostly used by male to male talk and very low female talk. However, the respondents observed that in the event of acquaintance male to female and vise- versa, talk can still use the terms freely. Below are the taboo words and their euphemisms for female genitals in Kikuyu language.

Consider the following terms:

Taboo word	**Gloss**
Kiino	Vagina
Giti	Vagina

Euphemism	**Gloss**
Mugunda wa kianda	The lower garden
Indo	Wealth
Jiko	Jiko
Ndiri	Mortal
Murigo	Goods
Mboco	Beans
Kibuyu	Thermosflask

A closer look at the euphemisms used to represent the female sexual organ present a level of passiveness in sexual matters. Most of the words indicate "to serve." This means that a woman is there to serve the owner who in this context is a man. The owner of the goods being a man, if the owner does not use the 'goods', they will stay unused. For instance, if the owner does not use the thermos flask, it will just be there unused. The euphemisms also reveal that a woman's sexuality is 'goods' "*mirigo"* and that it is the property of a man. As Njeri (2007) observes as a matter of fact, Kikuyu men have been heard in many occasions introducing their wives or girlfriends as

> *uyu niwe mundu wakva*
> literally meaning, meet my personal thing or
> *uyu niwe mutirima wakwa* meaning
> this is my walking stick.

There are terms in Kikuyu language on female sexual organs that are potentially offensive mainly because of the topic and also the profane intent of the expressions. For instance, a woman's behind is referred to as "*mutungi"* meaning "Jerrycan." This is a way of making this body part trivial or mocking it and so can be highly offensive to the woman who is being referred to as this threatens her self-image. Kikuyu women do take offence when men apply such terms to describe their bodies in what they think are euphemisms. Women breasts and buttocks have been assigned terms to describe them. Below are the terms used to refer to these women body organs in Kikuyu.

Consider these terms for breasts**:**

Taboo word	Gloss
Nyondo	Breast

Euphemism	**Gloss**
Makorobia	Avocados
Tuzo	A milk processing plant in Kenya

The researchers observed that the euphemisms for breasts are mostly used by the Kikuyu youth aged 18 – 35 years. The term "*tuzo"* is used when a woman's breasts are seen to be extra big thus seen to be in a position to produce a lot of milk able to sustain a child or children during breasdfeeding. The term avocado is used to describe the youthfulness and stiffness of the breasts hence attracting men for sexual acts.

Consider the following words used for a woman's behind:

Taboo world	**Gloss**
Itina	Buttock

Euphemism	**Gloss**
Thutha	Behind
Mutungi	Jerry can
Githurai	A crowded estate in Nairobi
Njikariro	Sitting apparatus

The researchers observed that the word "*murigo"* which can apply both to a woman's vagina and her buttocks is common with the youth aged 18-35 years, while the other terms are common with people aged 35 years and above and they all point to the ability of a woman attracting men for sex.

A big woman, who also attracts many Kikuyu speaking peoples, is also described with terms like;

Euphemism	**Gloss**
Ngari nene	A big vehicle
Momo	A big woman

On the whole, the researchers observed that most of these words on female sexual organs in Kikuyu are used in male to male talk and when used to describe a woman, they can be insulting or sexually appetizing to men. Some Kikuyu men have roaring appetites for fat women hence the use of terms like *ngari nene* and *momo*.

1.5 Conclusion

From the above discussion on tabooness in relation to sex and sexuality in Kikuyu, it clearly comes out that this important topic is avoided if not greatly guarded by by Kikuyu speech community. Not many respondents used in the study were free to tell the researchers what exactly were the names of the organs that form the totality of sex as a phenomenon besides sex being a natural human act. The Kikuyu culture does not apparently approve of saying matters of sex the way they are supposed to. One reason for this could be the fact that female circumcision that taught youthful Kikuyu women some of these so called taboo words has since been stopped by the government of independent Kenya or due to Western culture influences. Besides, globalization has made many Kikuyu speakers expossed to universal culture which jealously guards against careless and carefree talk on sensitive issues that can offend other people in society. In all, however, men among the Kikuyu speakers are not as keen as are women in euphemizing matters to do with sex and sexuality.

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Effect of Plant age and environment on foliar epicuticularwax in *Sabal minor* and *Sabal palmetto*

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Abstract:

The foliar epicuticular wax is located on the outside of the leaf cuticle. Epicuticular wax is important because it is an added barrier of protection from the environment. Epicuticular wax protects the plant leaf from salt spray from the ocean, insects, water, and any other foreign objects that may damage or penetrate the leaf. The epicuticular wax may contain different patterns, shapes and structures. The structure and composition depend on both genetic and environmental factors. There should exist a correlation between the age, structure, pattern and abundance of epicuticular wax between Sabal palmetto and Sabal minor leaves.

In order to analyze the structure of the foliar epicuticular waxes, micrographs were taken using a Scanning Electron Microscope (SEM), JEOL JSM-6010LA. This particular SEM used fresh material, whereas most other SEMs required dried specimens. The youngest leaf on each plant and the oldest leaf on each plant were used for analysis. The data show us that not only does the environment of the plant influence the amount of wax a leaf may have, but that the age of the leaf also determines how much wax a leaf will have. As the plant ages, the wax does not leave. If there is less wax in older leaves this indicates that a major environmental event has taken place.

Keywords:
foliar, epicuticular wax, micrographs, cuticle, structure, composition

1. Introduction:

The *Sabal minor* and Sabalplmetto palms both thrive in warm humid climates, but the *Sabal palmetto* is only native to southern east coast, while the *Sabal minor* is native to the gulf coast and the southern east coast. This is especially surprising because both palms are native to similar climates and the *S.palmetto* is able to thrive in a variety of soils as opposed to the *S.minor* that prefers moist soil. While the *S.palmetto* is native to the southern east coast, it can only survive in other regions if it is planted in its native region, then transported to a foreign region. Through the analysis of the foliar epicuticular wax on the adaxial side of the leaves, a set of methods could be established that could genetically modify the *S.palmetto*, so that it could be planted and raised in various regions. Foliar epicuticular waxes were chosen for analysis because the most photosynthetictissues are located there and compared to the rest of the plant they are the most susceptible to environmental damage (Jenks et. al,1995). The adaxial side of the leaf is analyzed because it is the side of the leaf that is naturally exposed to sunlight (Buschhaus,et.al, 2007).

The epicuticular wax is composed of superimposed lipids and cutin and is located on the very surface of most complex plant structures.(Gonzalez andAyerbe, 2009; and Koch, and Ensikat,2007). Epicuticular wax serves as a barrier against any foreign articles that could potentially penetrate the plant's surface. Epicuticular wax is hydrophobic, and therefore increases the hydrophobicity,wettability, and self cleaning behavior of the plant's surface. (Koch, and Ensikat,2007). Environmental changes and conditions influence the epicuticular wax as well. For example, a study revealed that the amount of epicuticular wax produced increases as it is exposed to terminal water stress.(Gonzalez andAyerbe, 2009)

Research has revealed that there are many intricate structures located in the epicuticular wax . This was determined through scanning electron microscopy(Jenks et. al,1995). There are many methods that could be used but the most effective while being the least invasive is the use of hexane for solvent extraction. (Morrison et.al, 2006). The results of both of these methods from previous research revealed results that could be used to aid various plant in their resistance to pathogenic and environmental stress. Though aspects of our research are derived from previous studies, we would like to branch out and determine if there is a consistent correlation between the composition and the structure of epicuticular wax. If certain structures can be matched with results from chemical analysis, and a consistent correlation is found, the structures of the epicuticular wax could be changed with genetic modification.

2. Methodology:

2.1 Chemical extraction of the waxes using hexane:

Samples of both palms from the youngest and oldest leaf were collected and labeled. Test tubes were labeled and filled with 10 mL of hexane. The leaves were folded so that the ends of the leaves were not exposed to the hexane. The samples were capped and sealed with parafilm to prevent the hexane from evaporating. The samples were placed in a chemical fume hood and left to sit for various periods of time. One test tube was filled with solely hexane was the control.

2.2 Wax analysis using the GENESYS 20 visible Spectrophotometer:

A blank hexane solution was placed in a cuvette. The cuvette was placed in the spectrophotometer. The spectrophotometer was set certain wavelengths to analyze the absorbances of the waxes. The previous steps were repeated with each sample.

2.3 Structural analysis using the Scanning Electron Microscope JEOL JSM-6010LA

The leaves were cut to fit around the gold plate. The leaf was tapped with dual sided tape so that the adaxial side of the leaf was visible. The gold plate was placed in the sample holder. The sample holder was placed on the stage of the SEM. The chamber was evacuated to make a vacuum. Images were taken at 100x, 500x, and 1000x.

3. Results and Conclusion:

Age	Absorbance	Wavelength
Youngest leaf	**-0.016 A**	**340 nm**
Oldest Leaf	**0.011 A**	**340 nm**
Youngest Leaf	**0.0 A**	**350 nm**
Oldest Leaf	**0.013 A**	**350 nm**

Figure 1.The spectrophotometer gives a quantitative reading of how much light was absorbed by the epicuticular wax in *S. minor* at certain wavelengths. The more wax present the higher the reading.

Age	Absorbance	Wavelength
Youngest Leaf	**0.026 A**	**340 nm**
Oldest Leaf	**0.087 A**	**340 nm**
Youngest Leaf	**0.030 A**	**350 nm**
Oldest Leaf	**0.066 A**	**350 nm**

Figure 2. The spectrophotometer gives a quantitative reading of how much light was absorbed by the epicuticular wax in *S. palmetto* at certain wavelengths. The more wax present the higher the reading.

.

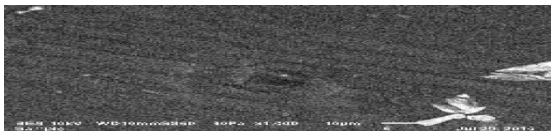
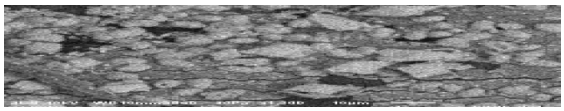
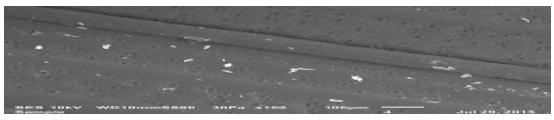
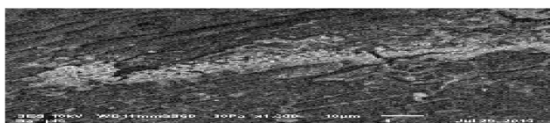
Plant Type	Micrographs	Sheets of waxes	Vertical Plates	Horizontal plates	Globular Units
Sabal palmetto **(Youngest leaf)**		+	-	-	-
Sabal palmetto **(Oldest leaf)**		+	+	+	+
Sabal minor **(Youngest leaf)**		+	-	-	-
Sabal minor (Oldest leaf)		+	+	+	-

Figure 3. The plus sign indicates that a structure is present and the minus sign indicates that a structure is not present

The results display that there is a correlation between the complexity of the wax structures and the amount of wax that is present on the leaf of the palm. There is also a correlation between the absorbance readings and complexity of wax structures between the two types of palms. The *S.minor* has the least complicated wax structures and the lowest absorbance readings and the *S. palmetto* had the most complex wax structures and the highest absorbance readings. This data reveals that the *S.palmetto* has more protection from adverse conditions. This is because the epicuticular wax serves as a barrier and the larger quantity of the wax indicates that there is a stronger barrier.

The evidence shows that there is epicuticular wax present on the young and old leaves. As the leaf gets older more wax is added. It does not lose wax as it gets older it gains wax. Young leaves do not have globular units of wax. This tells us that not only does the environment of the plant influence the amount of wax a leaf may have, but that the age of the leaf also determines how much wax a leaf will have. As the plant ages, the wax does not leave. If there is less wax in older leaves this indicates that a major environmental event has happened to take away the wax. This event could be severe wind damage or an animal etc. Age and environmental cues determine foliar epicuticular wax structure/pattern and abundance.

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Time Comparison Algorithm for University Examination Scheduling

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ABSTRACT

Every educational institution needs to create either lecture or examination time tables for their students. Almost all institution prefers to perform this task with the help of software. The difficult and complex part involved during the development of suchtype of software's are comparing times. Programmer should come across a situation to find logic for time comparison and if it is not performed properly, it may leads to an improper output. For example, one student may get schedule for two different coursesin the same time or in other scenario, same examination hall will be allocated for two different courses in the same time. Both the situation leads to a mess.

This paper discusses about the time comparison techniques for such software's. There are various methods available in modern day programming languages for comparing two timings. But in some cases, we have to performthe comparison up to four or more timings. The reason for this paper is, the same situation was faced by me and browsed through different articles and blogs for a possible solutions. But most blogs remains unanswered or answered with some complex solutions. So, it was an inevitable situation for me to find a new solution for the problem. This paper proposes an algorithm for comparing up to four timings.

In this paper, we discussed about the multiple instances where the time comparison is required while developing such software's and the various methods available inprogramming environmentfor addressing the same. The implementationdifficulties for the specific requirement and the outputs acquired through the above methods are also discussed. Also, the comparison of outputs from the Dotnet methodsand our algorithm is also addressed.

Keywords: Course or Examination Scheduling, Timetabling, Time Comparison, Timeslots, University

1- INTRODUCTION

In every educational institution, always there is a need to prepare course or exam schedules for their learners. In a small to medium size institutions with less number of learners, it is easy to prepare the schedulemanually. But, it is a difficultand tedious task when comes to a large scale educational institutions. Moreover, there are more possibilities of error in manual scheduling, which results into the disaster. So, there comes a need for software which should capable of preparing schedules in a single click.

When developing scheduling software's, the programmers may require comparingtimes in their logics. There may be a situation for performing a comparison between two timings or more. For example, if we consider two times ***TIME-A*** (Start Time) and ***TIME-B*** (End Time), it requires the following comparisons.

a. ***TIME-A*** is greater than ***TIME-B***
b. ***TIME-B*** is lesser than ***TIME-A***
c. ***TIME-X***(User Time) is greater than ***TIME-A*** and ***TIME-B***
d. ***TIME-X***(User Time) is lesser than ***TIME-A***and ***TIME-B***
e. ***TIME-X***((User Time) falls between ***TIME-A***and ***TIME-B***

There are several functions and methods available in modern day languages to compare two timings, like ***DateTime.Compare*** method in visual studio.NET, which can be used to compare two **DateTime** instances. But, when there are cases, where more than two time comparisons are required, then it is inevitable to find a new logic or algorithms.

Even though there are multiple methodsand functions available to compare timings, we would like to explainour own time comparison algorithm in order to perform the task efficiently and quickly. This algorithm has been already tested in Dotnetenvironment. Also, this can be customized based on other IDE's or database applications.

2- SCENARIOS IN TIMETABLING

For instance, if we develop a project which schedules examinations for an educational institution, the programmer may come across many situations to compare times in his program. For better understanding, we will proceed with an example of examination scheduling.

Now, consider the case, there are five labs in an institution (as shown in *table1*)***2037, 2039,2013,2001,2002*** which can be used for scheduling the exams. These labs will be used for all departments' examination within the institution. Under this condition, the programmer needs to check the below scenarios in his program.

Scenario1

a. All labs should be utilized for ***COURSE- A*** if all the labs are free on ***DATE-A***
b. No labs should be allocated for ***COURSE-B*** on ***DATE-A,*** *since* the labs are already occupied by***COURSE- A on DATE-A***

Scenario2

a. If only three labs(***2037,2039,2013***) are utilized for ***COURSE-A*** on ***DATE-A,*** then, the remaining two labs (***2001, 2002***) should be available for ***COURSE-B*** on ***DATE-A.***

Scenario3

a. If all labs are utilized for ***COURSE-A*** in the time range ***TIME-A to TIME-B*** on ***DATE-A,*** then no labs should be allocated for ***COURSE-B*** in the time range ***TIME-A to TIME-B*** on ***DATE-A***
b. But all labs should be available for ***COURSE-B OR COURSE-C***from ***TIME-C*** onwards on ***DATE-A***

Scenario4

a. If only three labs(***2037,2039,2013***) are utilized for ***COURSE-A*** in the time range ***TIME-A to TIME-B*** on ***DATE-A,*** then the remaining two labs (***2001, 2002***) should be available for ***COURSE-BOR COURSE-C*** in the time range ***TIME-A to TIME-B*** on ***DATE-A***

Scenario5

a. All labs should be available for all courses, if the schedules are generated in different dates.
b. ***Example1:*** All labs should be available for ***COURSE-A***under any time rangeon***DATE-A***
c. ***Example2:*** All labs should be available for ***COURSE-B*** under any time rangeon***DATE-B***
d. ***Example3:*** All labs should be available for ***COURSE-C*** under any time rangeon***DATE-C***

From the *table1*, all labs are already allocated for the ***COURSE-BLUE*** on ***DATE-01/28/2016***in the time range ***08.00:00 to 09:20:00AM***

Table 1 Labs Allocation

Department	Course	Date	Day	From_Time	To_Time	LabsUsed
English	Blue	01/28/2016	Thursday	08:00:00	09:20:00	2037
English	Blue	01/28/2016	Thursday	08:00:00	09:20:00	2039
English	Blue	01/28/2016	Thursday	08:00:00	09:20:00	2013
English	Blue	01/28/2016	Thursday	08:00:00	09:20:00	2001
English	Blue	01/28/2016	Thursday	08:00:00	09:20:00	2002

In our application, we used a relational database and particularly we have taken the table "***LabsAllocation***" from it as shown in the *table1*for addressing the above scenarios. This table has seven attributes as below with 5 tuples.

- Department
- Course
- Date
- Day
- From_Time
- To_Time
- Labs Used

3- EXISTING METHODS

In this section, we will focus the methodswith outputs available for time comparison in Dotnet environment.

3.1- Method1: DateTime.Compare(DateTime, DateTime)

This method compares two instances of DateTime and returns an integer valuewhich indicates whether the first instance is ***earlier than, the same as, or later*** than the second instance.

Syntax (in c#)

Public static int **Compare**
(
DateTime t1, t2
)
t1- the first object to compare *&**t2***- the second object to compare.

Table 2 Output of DateTime.Compare method

Value Type	*Condition*
Less than zero	t1 is earlier than t2
Zero	t1 is the same as t2
Greater than zero	t1 is later than t2

The above method allows comparing two DateTime instances and the requirement for comparing more than two DateTime instances cannot be satisfied by the method.

3.2- Method2: Timespan.Compare(TimeSpan, TimeSpan)

This method compares two TimeSpan values and returns an integer value which indicates whether the first value is ***shorter than, equal to, or longer*** than the second value.

Syntax (in c#)

Public static int Compare
(
TimeSpan t1, t2
)
t1- the first time interval to compare *&**t2***- the second time interval to compare.

Table 3 Output of Timespan. Compare method

Value	*Description*
-1	t1 is shorter than t2
0	t1 is equal to t2
1	t1 is longer than t2

The above method also allows comparing two TimeSpan instances and the requirement for comparing more than two TimeSpan instances cannot be satisfied by the method.

3.3- Using Operators for time comparisons

Generally, while comparison, it is a normal practice to use operators like ***LessThan, LessThanOrEqual GreaterThan, GreaterThanOrEqual***etc. in SQL queries which may not produce the desired outputs all the times. In technical web blogs, we can be find more queries related to using operators for time comparisons which remains unanswered or answered with complex logics. It may be a difficult task for a basic programmer to understand the logics behind the posted solutions or implementing the solutions in his programs. For example, the *table4* is one of the solutions given in the blogs for selecting all dates between two dates.

Table 4 Selecting dates between two dates using SQL

```
Select * from table_name where col_Date between '2011/02/25' AND DATEADD (s,-1,DATEADD(d,1,'2011/02/27'))
```

In *table4*, the logic is to add a day to the current endDate, it will be *2011-02-28 00:00:00*, then subtract one second to make the endDate*2011-02-27 23:59:59*, so that all dates between the given intervals can be retrieved.

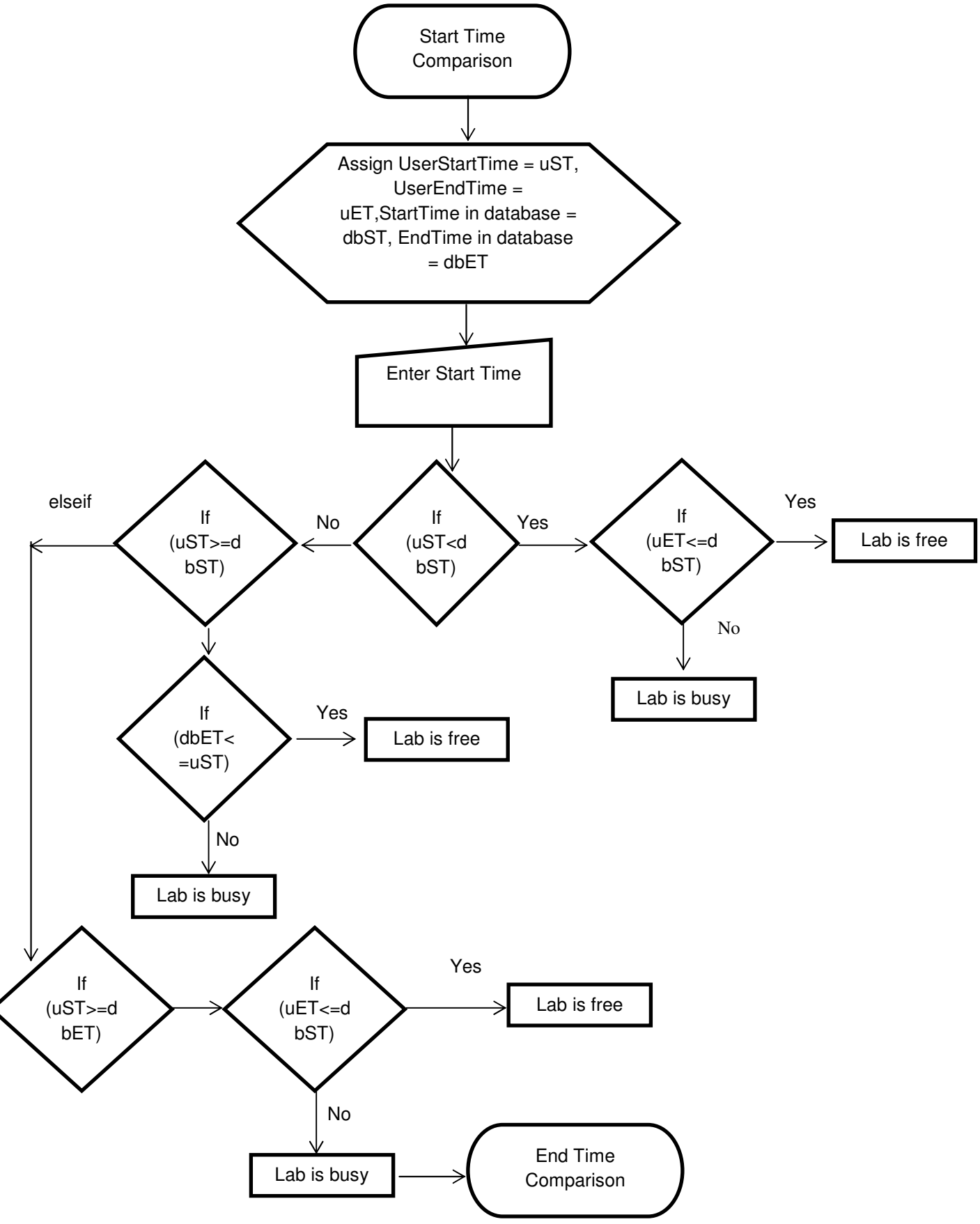
4- PROPOSED ALGORITHM

In the above section, we discussed about the different methods and its limitations for comparing two timings. In this section, we will brief the comparisons techniques of our proposed algorithm. Using this algorithm, we can compare up to four different timings as described in t*able1*.

Table 5 Time Value Descriptions

Serial No	Value Type	Descriptions
1	tsUserStartTime	This is the START-TIME from the user in which he needs to start the exam
2	tsUserEndTime	This is the END-TIME from the user in which he needs to end the exam
3	tsDBStartTime	This is the existing START-TIME available in database(in table Labs Allocation) which has been already allocated for another course
4	tsDBEndTime	This is the existing END-TIME available in database(in table Labs Allocation) which has been already allocated for another course

This algorithm can handle any number of labs and time slots if it is executed through a *for* loop statement. The below section shows the flowchart for the proposed algorithm.


Start Time Comparison
Assign UserStartTime = uST, UserEndTime = uET,StartTime in database = dbST, EndTime in database = dbET
Enter Start Time
If (uST<dbST)
Yes
If (uET<=dbST)
Yes
Lab is free
No
Lab is busy
No
If (uST>=dbST)
elseif
If (dbET<=uST)
Yes
Lab is free
No
Lab is busy
If (uST>=dbET)
If (uET<=dbST)
Yes
Lab is free
No
Lab is busy
End Time Comparison

```
if (tsUserStartTime < tsDBStartTime)
{
	if (tsUserEndTime <= tsDBStartTime)
	{
			//Lab is free
			  //Change Lab status to AVAILABLE

	}
	else if (tsUserEndTime > tsDBStartTime)
	{
	    //Lab is busy
			//Change Lab status to NOT AVAILABLE
	}
}
else if(tsUserStartTime >= tsDBStartTime)
{
	if(tsDBEndTime <= tsUserStartTime)
	{
			//Lab is free
			//Change Lab status to AVAILABLE

	}
	else if(tsDBEndTime > tsUserStartTime)
	{
			//Lab is busy
			//Change Lab status to NOT AVAILABLE
	}
}
else if(tsUserStartTime >= tsDBEndTime)
{
	if(tsUserEndTime <= tsDBStartTime)
	{
			//Lab is free
			//Change Lab status to AVAILABLE

	}
	else if(tsUserEndTime >= tsDBStartTime)
	{
			//Lab is busy
			//Change Lab status to NOT AVAILABLE

	}
}
```

5- RESULTS AND DISCUSSION

In this section, we will consider some scenarios discussed in section 3 and compare the outputs by applying the methods available in Dotnet environment and our time algorithm.

From the *table1*, we can understand that all labs are already allocated for the ***COURSE-BLUE*** on ***DATE-01/28/2016***in the time range ***08.00:00 to 09:20:00AM.***

Now, the user wants to create schedule for another ***COURSE-GREEN***on same ***DATE-01/28/2016***in the time range ***08.15:00 to 09:15:00AM***

Now assign the variables as below

Table 6 Start Time and End Time from user

Variable Name	Value
tsDBStartTime	08.00:00
tsDBEndTime	09:20:00
tsUserStartTime	08.15:00
tsUserEndTime	09:15:00

Since, the labs are already occupied, the software should not allow the schedule generation and stop the process by displaying the message "***Labs are busy. Choose another time***" to the user.

If we apply the ***Timespan.Compare(TimeSpan, TimeSpan)***method for this scenario, wehave to write the code as below

Timespan.Compare(tsUserStartTime, tsDBStartTime**)** = ***Timespan.Compare(***08.15:00, 08.00:00**)**

Timespan.Compare(tsUserEndTime, tsDBEndTime**)** = ***Timespan.Compare(***09.15:00, 09.20:00**)**

The first comparison will return an integer value 1, because the tsUserStartTime is longer than tsDBStartTime and the second comparison will return an integer value -1, because the tsUserEndTime is shorter than tsDBEndTime. Since, we can pass only two parameters to this ***Timespan.Compare***method, we have to perform the comparison twice and with the returned values (1 & -1) around the neck logics should be written to achieve the required output.

Now, apply the time comparison algorithm for this scenario. As it is mentioned earlier, pass the required time slots (as in *table6*) as a parameter for the labs (***2037, 2039, 2013, 2001, 2002***) through a*for* loop to this algorithm.

```
If (08.15:00<08.00:00)  ──────────►  Condition False
{
        If (09:15:00<=08.00:00)
        {       }
        Else if (09:15:00 > 08.00:00)
        {       }
}
Else if (08.15:00 >= 08.00:00)  ──────────►  Condition True
{
        If (09:20:00 <= 08.15:00)
        {       }  ──────────►  Condition Fails
        Else if (09:20:00 > 08.15:00)
        {
                Labs are busy. Choose another time  ──────────►  Condition True
        }
}
Else if (08.15:00 >= 09:20:00)
{
        If (09:15:00 <= 08.00:00)
        {       }
        Else if (09:15:00 >= 08.00:00)
        {       }
}
```

The above algorithm will check the availability of each lab for the time slots given by the user.

6- CONCLUSIONS

Thus, in this paper we have discussed about the time comparison part, the core of any type of scheduling and proposed a method for comparison. All the scenarios discussed above in section3and other scenarios if any can be satisfied with the help of this algorithm. There are methods available in programming languages to compare two DateTime or time span values. But, through this algorithm we can perform comparison up to four timings for any number of labs and time slots.

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Development and Investigate of Palmyrah Fruit Pulp (PFP) Added Yoghurt

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Abstract

The present study was carried out to developed Palmyrah Fruit Pulp (PFP) added yoghurt and investigates the effects of physico-chemical attributes of yoghurt during storage. Result of this study revealed that the physico-chemical attributes like ash, dry matter, total sugar, reducing sugar, pH and titratable acidity were significantly ($p<0.05$) changed among yoghurt made from without PFP and PFP added yoghurt at day one. Further, the results has shown that ash, dry matter contents and titrable acidity were significantly ($p<0.05$) increased during the storage period whereas total sugar, reducing sugar and pH were ($p<0.050$ reduced with storage period. The values of titratable acidity and pH were 0.85±0.06 and 3.97±0.04, respectively at 4th week of storage in 2.5% PFP added yoghurt. Sensory attributes such as flavour, colour, taste and texture of yoghurt made from 2.5% PFP was superior to yoghurt made from all other types of yoghurt. Finally, concentration of 2.5% PFP added yoghurt had the highest overall acceptability compared to other all types of yoghurt.

Keywords: Yoghurt, physico-chemical attributes, titratable acidity, palmyrah fruit, pulp

1. Introduction

Dairy products have generally been considered an excellent source of high-quality protein, calcium, potassium, phosphorus, magnesium, zinc, and the B vitamins (Buttriss, 1997). There has been an unbelievable increase in the production of fermented milks in developed countries and most of the increase is attributed to the healthy image associated with yoghurt (Kamruzzaman *et al.,* 2002; Perdigon *et al.,* 2002; Valli and Traill, 2005). One of the most traditional cultured milk is the yoghurt, which is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tamine and Robinson, 1997; Kumar and Mishra, 2004).

Palmyrah palm (*Borrasus flabellifer)*is widespread in the arid tropic county like India, Sri Lanka and South-East Asia. It is features of the landscape of North- East Sri Lanka where it is called as wishing tree (Jansz *et al*., 2002). It is estimated that at present there about 11 million palms are available in Sri Lanka (Sangheetha *et al*., 2014). Palmyrah has a great capability to produce several products of economic importance, which means a palm that produces anything and everything (Sangheetha *et al*., 2014). Moreover, it was estimated in Sri Lanka that about 20,000 tons of palmyrah fruit pulp is available annually during fruit season but approximately 10000 tons of pulp is not utilized every year because its uses are limited mainly due to the presence of a bitter compound like flabelliferins and lack of trials are done to process into various consumer

attractive value added products ((Jansz *et al* 2002; Janz *et al*., 1994; Sangheetha *et al*., 2014). Palmyrah fruit pulp has a yellow colour due to carotenoids which are precursors of vitamin A and therefore it has potential of being as a source of vitamin A. In addition to that, pulp is contained rich in vitamin C (ascorbic acid) and a good source of pectin which could be used to process the fruits into various products (Theivendrarajah, 2008). Further, pulp is mildly laxative, contains considerable amount of sugars such as sucrose, glucose and fructose (3.4, 3.5 and 3.4 g/100g, respectively) (Ariyasena *et al*., 2001).

Pulp has potential uses apart from traditional products. These include its use in jams and cordials and as a source of pro-vitamin A, pectin and portable alcohol (Balasubramaniam *et al.,* 1999). However the extensive use of PFP is detracted by presence of bitterness in the pulp (Janz *et al*., 1994). Debittering of pulp using naringinase enzyme resulted in a beverage with a pleasant mango cordial like colour, flavour and texture (Janz *et al.,* 1994). Pulp may be used for the production of special kind of fruit flavoured yoghurt as it has unique colour, flavour and texture. However there is lack of information available utilizing palmyrah fruit pulp for fruit flavoured yoghurt. Therefore, this experiment was designed to study the physico-chemical attributes of palmyrah fruit pulp added youghut.

2. Materials and Methods

2.1.Palmyrah fruit pulp (PFP)

Pulmyrah fruit was purchased from super market and brought laboratory. The fruit was heated for 5 minutes at 50^{o}C and cool down to room temperature. Then the washed and dried fruits were peeled, squeezed and palmyrah fruit pulp was obtained.

2.2. Preparation of yoghurt starter culture

Yoghurt culture containing freeze dried Lactic culture (direct vat set) Thermophillic Lactic culture (STI- 12,) was purchased and starter culture was prepared as prescribed by manufacturer. Then culture was stored at 4˚C for the usage.

2.3.Yoghurt preparation

Milks were standardized by using cream separator. The standardised milks were pasteurized at 65^{O}C for 30 minutes and cooled to 37^{0}C. Palmyrah fruit pulp added into the milk at the concentration of 2.5%, 5.0%, 7.5% and 10% at weight basis and sugar was used as a control.

Then the mixtures were mixed well and gelatin was added to the mixture at the level of 0.5% and mixed well. Then the mixtures were heated for 10 minutes at 95 ˚C in stainless steel containers while maintaining constant volume using distilled water. Heated mixtures were cooled to 42 ˚C under tap water. Starter culture (5%, w/v) was added for each mixture at 42 ˚C and equal volume of contents were transferred into the series of plastic container and incubated at 42˚C.

2.4.Proximate Analysis

The cheese samples were analysed in triplicate for moisture content using oven drying at 102^{o} C to get a constant weight according to AOAC method (AOAC, 1990) and percentage moisture was calculated as moisture (%) = 100 - total solid (%). Ash content was determined according to AOAC method (AOAC, 1990. The crude fat content was determined using the Gerber method (Anon, 1972).

2.5. Determination of sugar content

Lane and Eynon method described in Analytical chemistry of food by James (1999) was used to determine total sugar and reducing sugar content. Content was expressed in percentage on fresh weight basis.

2.6. Determination of titratable acidity of yoghurt

The titratable acidity was determined after mixing a yoghurt sample with 10 mL of distilled water and titrating with 0.1 N NaOH using 0.1% phenolphthalein indicator as described by Dave and Shah (1997).

2.7. pH Determination

The pH values of the yoghurt and milk samples were measured at 25°C using digital pH meter (Model -Delta 320, Mettler-Toledo Instruments Co., Ltd, Shanghai) after calibrating with fresh pH 4.0 and 7.0 standard buffers.

2.8. Determination of syneresis of yoghurt sample

Syneresis was defined as the volume of serum that was not retained within the structure on centrifugation. Tubes containing 20 g of yoghurt were centrifuged for 10 minutes at 25^{o}C at 5000 rpm. The amount of serum released from the coagulum was measured in a calibrated measuring flask as described by Domagała, (2009).

2.9. Storage of fermented yoghurt mixture

Starter culture was added for each mixture at 42 ˚C and equal volume of contents were transferred into the series of plastic container and incubated at 42˚C. Then samples were collected from 0 hour to 7th hour with the time interval of one hour during incubation. Before refrigeration of collected samples at 4 ˚C for titratable acidity and pH were measured. Then yoghurt samples were stored at 4 ˚C for further analysis at 1st, 2nd, 3rd and 4th week of storage.

2.10. Sensory analysis

The consumer acceptability studies were carried out using organeoleptic evaluation of yogurt by a jury of 10. The expert panel comprised nonsmokers who were very familiar with dairy products. Five parameters i.e. flavour, taste, color, texture a and overall acceptability were evaluated using a sensory rating scale of 1-7. The panels recognized the yogurt only by codes. Each panel was requested to rinse their mouth by drinking mineral water after assessing each yogurt.

2.11 Microbiological analysis

One gram of yoghurt sample was diluted with 9 mL of 0.15% peptone water diluent and mixed uniformly with a vortex mixer, subsequent serial dilutions were prepared and viable numbers enumerated using the pour plate technique. Counts of bacteria were enumerated on blood agar by incubating the plates aerobically at 37°C for 24 hours (Dave and Shah, 1996). Microbiological count data are expressed as colony forming units (cfu) per gram of yoghurt. Four dilutions were carried out to determine the number of bacteria during storage.

2.12. Statistical analysis

Theresults were analyzed statistically using a computer program ‘‘SAS system for windows Version 9.1.3 for analysis of variance (ANOVA) by one way and comparison of means by Duncan’s multiple comparison test where $P < 0.05$ was considered for significant difference.

3. Results and Discussions

3.1. Palmyrah fruit pulp and milk

The chemical parameters of palmyrah fruit pulp such as total sugar, sucrose and glucose contents were found to be 14.4± 0.5%, 6.3 ± 0.5% and 3.4 ± 0.1%,respectively. The pH and acidity were found to be 4.30 and 0.51%, respectively. Jeyaratnam (1986) has reports that the fresh palmyrah fruit pulp contains total sugars, sucrose and glucose in the rage of 14%-16%, 6.6% and 3.5%, respectively. Chemical composition of fresh milk namely milk fat, ash , dry matter content, titratable acidity, and pH were 3.5±0.06%, 0.75±0.06%, 12.6±0.06%, 0.23±0.01% and 6.7±0.04, respectively. These values were in line with the results of Gösta

Bylund (1995) who reported that cow milk contains of milk fat (4%), ash (0.80%), dry matter content (13.0%), titratable acidity (0.26±0.01%). These values are falling within normalcy.

3.2 Physical and chemical properties of palmyrah fruit pulp (PFP) added yoghurt at day One

3.2.1. Ash and dry matter

The study shows that there were differences ($p<0.05$) among the mean values of ash and dry matter content using different PFP added yoghurts. Ash and dry matter content of different concentrations of PFP added yoghurts has been shown in Table 1. Ash and dry matter content was the highest in 10% PFP added yoghurt and lowest in yoghurt made from without PFP. It could be an addition of PFP in youhurt increases the ash contents. The results were in accordance with Chougrani *et al.,* (2009), who reported similar trends in their study on physico-chemical analysis of fruit yoghurt. Whereas milk fat did not show any significant changes in yoghurt made from different concentrations of PFP.

3.2.2 Total sugar

Total sugar content of yoghurt in different concentrations of PFP has shown in Table 1. The mean value of total sugar content was ($p<0.05$) among the all types of yoghurt. The percentages of total sugar content of 2.5%, 5%, 7.5% and 10% of PFP added yoghurt were 13.14±0.07, 13.45±0.16, 14.09±0.04 and 14.77±0.08, respectively. The 10% PFP added yoghurt had highest mean value than yoghurt made from without PFP. The addition of PFP caused an increase in sugar content of yoghurt. Mean value of total sugar content of PFP added yoghurts were higher than yoghurt made from without PFP.

3.2.3. Reducing sugar

Reducing sugar contents of PFP added yoghurt and yoghurt made without PFP were significantly ($p<0.05$) varied among different types of youhurt. The 10% PFP added yoghurt had higher mean value (2.46±0.01) and the yoghurt made from without PFP had a least mean value (2.17±0.02). It could be indicating that PFP has higher reducing sugar content. Jayathilake and Wijeyaratne (1999) reported in his study that same trends were observed in fruit pulp added yoghurt.

3.2.4. Titratable acidity

The study shows that there was differences ($p<0.05$) among the mean value on the acidity of different PFP added yoghurt samples. The titratable acidity of the PFP added yoghurt was ($p<0.05$) increased from 0.58% to 0.80 % by adding of PFP from 2.5% to 10% as shown in Table 2 The acidity of yogurt was higher in PFP added yoghurt than yoghurt made from without PFP. The addition of PFP increased the acidity of yoghurt due to the PFP contain more sugar and it converted into acid by the fermentation process. These results confirm the results obtained by Rashid and Thakur (2012) who found that the titratable acidity values increase in yoghurt with adding of supplemented sugar.

3.2.5. pH

The pH reduced ($p<0.05$) with the increasing concentration of PFP. The effect of PFP on the pH values of fresh yoghurts is shown in Table 2. The pH was the highest in yoghurt made from without PFP (4.45±0.67) and lowest in 10% PFP added yoghurt (4.01±0.01). This might be increase in acidity, as acidity is inversely proportional to pH. The results generally showed that the higher acidity and lower pH in PFP added yoghurt. Rashid and Thakur (2012) found that there is a corresponding reduction in pH as the acidity increased in honey added yoghurt.

3.2.6. Syneresis

Syneresis percentage has decreased with the increasing level of PFP in yoghurt mixture (Figure 1). At day 1 yoghurt mixture without PFP had significantly ($P<0.05$) lower level of acidity where as mixture with 10% PFP had higher level of acidity. Increase of acidity was increased the curd stability because of the increase in water binding capacity of proteins (Langton, 1991). So that, higher the acidity in yoghurt was reduced the syneresis. Addition of fruit concentrates generally tends to change the consistency of products due to changes in water binding capacity of proteins (Ramaswamy and Basak, 1992). Therefore, this may be another possibility for decreasing syneresis in the yoghurt mixture with increasing level of PFP.

3.3. Fermentation of yoghurt mixture

Resulted values of titratable acidity and pH with time during the fermentation of prepared yoghurt mixtures have been plotted in Figure 2 and Figure 3. Statistically significant ($P<0.05$) model with higher correlation coefficient (R^2) was fixed for each type of yoghurt mixtures using resulted values. Polynomial regression type satisfied the requirements for of titratable acidity of all types of yoghurt mixtures and was selected for interpretation. Correlation coefficients (R^2) for each models selected are displayed Table 3. Rate of titratable acid production of 7.5% and 10% PFP added yoghurt increased with fermentation time and production was gradually reduced when reaching seventh hour. This pattern is exhibited because increasing level of titratable acid gradually inhibits the growth and metabolism of starter. On the other hand, without PFP added yoghurt (0% PFP) had shown lower value of titratable acid compare to other all types of yoghurt mixture. This was indicating that incorporation of PFP started the initial rate of titratable acid production in each yoghurt mixtures during fermentation is being increased with increasing level of PFP in the mixture. Although each type of yoghurt mixture showed the increasing rate of titratable acid production with fermentation time, increasing rate is increased with increasing level of PFP in the mixture. So ultimate result is that amount of total titratable acid produced at a particular time was high at higher level of PFP in the mixture. The pH changes of each mixture with fermentation time produced the pattern, which was the image of pattern produced depends on produced acid

3.4. Physical and chemical properties of palmyrah fruit pulp (PFP) added yoghurt during storage period

3.4.1. Ash

Ash is an indicator of total amount of minerals present in the yogurt and it presented in Table 4. At first week of storage 10% PFP added yoghurt exhibited ($p<0.05$) higher mean value (1.15±0.01%) than yoghurt made from without PFP (0.87±0.02%). At fourth week of storage 10% of PFP added yoghurt showed higher mean value (1.24±0.04%) and yoghurt made without PFP received lower mean value (0.91±0.01%). In this study, the range of ash content varied from 0.87±0.02% to 1.24±0.04% and it increased with storage period. This may due to higher minerals present in PFP.

3.4.2. Dry matter content in yoghurt during the storage period

The increasing trend of dry matter content was observed with increasing amount of PFP. Moreover, it was observed that the dry matter content of yoghurt made PFP were significantly ($p<0.05$) higher than yoghurt made without PFP (Table 4). In general, PFP (10%) added yoghurt obtained higher mean value (25.51±0.10%) than yoghurt made without PFP (19.80±0.10%) at 4th week of storage. The study revealed that the dry matter content of yogurt was increased during storage period is due to the evaporation rate of moisture

content during storage at refrigerated condition. The results are in agreement with the results of Ayer (2014) who reported that increasing amount of fruits tend to decrease the dry matter content.

3.4.3. Fat content in yoghurt during the storage period

Fat contents of yoghurt not only during the storage but also different concentration of PFP added yoghurt did not show any ($p > 0.05$) significant changer among the yoghurt sample (Table 4).

3.4.4. Total sugar and reducing sugar

The result shows in Table 4 that the total sugar and reducing sugar were ($p< 0.05$) decreased throughout the storage period. It might be due to the conversion of lactose into lactic acid with time of storage. At fourth week of storage period, PFP (10%) added yoghurt has significantly ($p<0.05$) high amount of total sugar (12.02±0.16%) and reducing sugar (2.30±0.04%) compare to all other types of yoghurt. Similarly, Goodenought and Kleyn (1976) reported that sugar contents were decreased during storage of yoghurt. It might due to production of lactic acid by fermentation of starter cultures of sugars present in yoghurt (Wedad *et al.,* (2009).

3.4.5. Titratable acidity

Table 5 showed that the result of titratable acidity was ($p< 0.05$) increased throughout the storage period. While titratable acidity was observed at 4^{th} week of storage period that the yoghurt made without PFP has highest ($p<0.05$) mean value (0.80±0.07%) than 10% PFP added yoghurt (1.23±0.07%). The changes in titratable acidity of yoghurt could be fermentation process by microorganism and degradation of lactose. The acidity further increased gradually during storage in all types of yoghurt (Salji and Ismail,1983), who reported significant increase in acidity during storage, it could due to conversion of lactose to lactic acid by lactic acid bacteria.

3.4.6. pH

The pH decreased with during the storage period of yoghurt. Yoghurt made without PFP received higher value (4.38±0.01) than 10% of PFP added yoghurt (3.97±0.01) at first week of storage (Table 5). At 4^{th} week of storage 10% PFP added yoghurt received lowest value (3.62±0.03) compared to other treatments. The changes in pH might due to fermentation process of starter culture organism. During the first week of storage there was a ($p < 0.05$) change among different concentration PFP yoghurt. These results are agreed with results reported by Behrad *et al*. (2003) who mentioned that the pH for all yoghurts reduced from the initial values of 4.5 to 4.09 at 28 days of storage.

3.4.7. Effect of storage on sensorial attributes of different treated yoghurt samples

The sensory attributes analysis of yoghurt samples was evaluated in duplicate in 3 sessions presented in randomized order and coded with three digit numbers using friedman test and category can be graphically presented by a "spider web" in Figure 4 (a-e). Results showed that there were ($p<0.05$) significant differences between the treatments for all attributes of texture, colour, taste, flavour and overall acceptability. All attributes were decreased during the storage period in all kinds of yoghurt. Yoghurt with 2.5 % PFP added yoghurt received higher mean value and yoghurt made with 10% PFP added yoghurt received lowest mean value for texture, taste, colour, and flavour at 4^{th} week of storage. PFP (2.5%) added yoghurt had higher degree of overall acceptability and gained highest scores for other sensory attributes such as taste, flavour, texture and colour. Yoghurt without added PFP yoghurt showed second highest mean value and lowest mean value was obtained in 10% PFP added yoghurt. Based on the sensory analysis, majority of panelist prefer

yoghurt with 2.5% PFP followed by yoghurt without PFP added yoghurt. But more panelists did not prefer 10% PFP added yoghurt due to high concentration of unpleasant sensory attributes.

3.5. Microbiological analysis

It is evident from results (shown in Table. 6) that there was ($p<0.05$) decrease in total viable count (cfu/mg) during storage interval. Yoghurt made from without PFP showed higher bacterial colony count was 1.88×10^6 cfu/mg of 2 week of storage periods and it decreased to 1.30×10^6cfu/mg at 4 week of storage while there was significant ($p<0.05$) effect due to different concentrations of PFP. This result was consistent with the findings of Kailasapathy *et al.,* (2008). Total bacterial colony decreased during storage due to increasing acidity. These findings are in accordance with the results of Samadrita Sengupta *et al*., (2013) who observed that the bacterial colony in yoghurts decreased during the storage period.

4. Conclusion

At day one pH and syneresis decreased with increasing amount of PFP added in yoghurt. Titrable acidity, total sugar, reducing sugar, ash and dry matter contents increased with the increasing of PFP concentration in yoghurt. Nutritional parameters such as total sugar and reducing sugar were ($p<0.05$) decreased with storage period. Whereas, ash and dry matter content were increased in yoghurt during storage period. The titratable acidity was increased during the storage period while pH was decreasing thought out the storage period. Fat did not show any changes among all treatments. In sensory attributes such as taste, colour, texture, flavour and overall acceptability among the different types of yoghurt were differed amount the types of yoghurt. Finally, 2.5% PFP added yoghurt had more preferred than other concentration of PFP added yoghurt.

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