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Virus Vectors in Orange

Yield Components of Barley

Highlights

Bovine Clinical Mastitis

Sweetpotato Genotypes Infection

Discovering Thoughts, Inventing Future

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The Role of Virus Vectors in Orange Fleshed Sweetpotato Genotypes Infection - A Case Study

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Abstract- The economic importance of sweetpotato as a carbohydrate food and feed for man and animals is well known in the world all over. Sweetpotatoes are crops vegetatively propagated from vines, root slips (sprouts), and farmers often take vines for propagation from their own field year after year. Therefore, virus diseases are inevitably transmitted with propagation materials to newly planted field, resulting often in a marked decrease in yield. A virus infection is often spread by insects that pierce and suck. As is the case, no living organism such as sweetpotato is absolutely virus-free in its system. Sweetpotato genotypes accumulate viruses and the virus load is the major problem. Sweetpotato virus disease (SPVD) occurs after infection of two viruses: the sweetpotato feathery mottle virus (SPFMV) and the sweetpotato chlorotic stunt virus (SPCSV). These two viruses distort, stunted, cause chlorosis and narrowing of leaves leading to photosynthetic disturbance. SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV - without SPCSV infection – are relatively low and SPFMV resistance of sweetpotato breaks down after the plant is infected by SPCSV. Of all the sweetpotato genotypes, the ones mostly affected by viruses are the orange fleshed sweetpotato genotypes.

Keywords: *orange fleshed sweetpotato, virus disease, yield, SPVD and symptoms.*

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The Role of Virus Vectors in Orange Fleshed Sweetpotato Genotypes Infection - A Case Study

Nwankwo. I. I. M[α] & Opara, E. U[σ]

Abstract- The economic importance of sweetpotato as a carbohydrate food and feed for man and animals is well known in the world all over. Sweetpotatoes are crops vegetatively propagated from vines, root slips (sprouts), and farmers often take vines for propagation from their own field year after year. Therefore, virus diseases are inevitably transmitted with propagation materials to newly planted field, resulting often in a marked decrease in yield. A virus infection is often spread by insects that pierce and suck. As is the case, no living organism such as sweetpotato is absolutely virus-free in its system. Sweetpotato genotypes accumulate viruses and the virus load is the major problem. Sweetpotato virus disease (SPVD) occurs after infection of two viruses: the sweetpotato feathery mottle virus (SPFMV) and the sweetpotato chlorotic stunt virus (SPCSV). These two viruses distort, stunted, cause chlorosis and narrowing of leaves leading to photosynthetic disturbance. SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV - without SPCSV infection – are relatively low and SPFMV resistance of sweetpotato breaks down after the plant is infected by SPCSV. Of all the sweetpotato genotypes, the ones mostly affected by viruses are the orange fleshed sweetpotato genotypes. The slow sweetpotato breeding progress in Nigeria and Africa in general can be attributed to low investments into breeding virus resistant genotypes and selection procedures to farmer needs by formal plant breeding. Control measures in addition to others are put in place to avoid virus disease epidemic: planting of virus free materials, land and crop rotation, thermotherapy, meristem culture, control of vectors by chemical application, soaking of botanical seeds before planting in hot water and others.

Keywords: orange fleshed sweetpotato, virus disease, yield, SPVD and symptoms.

I. Introduction

Sweetpotato [*Ipomoea batatas* (L.)Lam] is cultivated in more than one hundred countries around the globe. It is a dicotyledonous plant belonging to the *Convolvulaceae* family of about 45 genera and 1000 species, with only *I. batatas* of economic importance as food (**Woolfe, 1992**). There are over 1402 varieties of sweetpotato which skin and flesh colour may be almost white, cream, yellow, orange, pink, or deep purple depending on the variety. However, the white/cream and yellow-orange fleshed colours are most common (**Ahmad *et al.*, (2006).**). It is an important staple food crop in some states in Nigeria such as Ebonyi, Nassarawa, Benue and Kaduna where most of the crop is produced.

Wolfgang and his co-workers (2012) reported that the Food and Agriculture Organization (FAO) statistics annual sweetpotato production in Africa has increased moderately from 11.6 million tonnes in 2002 to 12.9 million tonnes in 2006. This production was mainly by smallholders (the majority of whom are women) for home consumption. Typically less than 20% of production is traded in rural and urban markets. Although data on piecemeal harvested sweetpotato crop are difficult to collect.

II. Uses of Sweetpotato

Odebode (2004), reported on the many uses of Sweetpotatoes. He noted that sweetpotato have been consumed as carbohydrate food by man and animals. Many parts of sweetpotato plant, (leaves, roots and vines), are edible. Sweetpotato roots can be boiled, steamed, baked, and fried. Traditionally, sweetpotatoes can be boiled and eaten, fried into chips and eaten, pounded or mixed with yam and eaten with vegetable soup, roasted and eaten with red palm oil or sauce, or made into porridge. Use in preparing "kunnu" drink, processed into fufu and eaten with vegetable soup. Sweetpotato can be processed into flour or mixed with cassava flour to make *amala.* Processed sweetpotato flour can be used as sweetener, eaten boiled with rice, processed into kunuzaki which is oiled and eaten with groundnut cake. The leaves contain up to 4% protein and the fresh Sweetpotato leaves can be used in making vegetable soup and served with Pounded yam, *Eba or Amala*.

In China, sweetpotatoes are also canned or dried and made into flour, cereal, and noodles or sealed in cellophane bags and sold in supermarkets. Sweetpotato roots are often used in making biscuits, cakes, and desserts or processed into products such as chips, Sweetpotato 'Sparri' (toasted 'sparri' granules) soaked in water and eaten with groundnut as a snack or put in boiled water and served with soup, Sweetpotato

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Cake eaten as snacks or main dish to entertain visitors, puff-puff, buns, bread, crisps, chin-chin served as snacks, ketchup (chopped boiled sweetpotato tuber mixed with tomato sugar, onions, vinegar, salt, water) served with bread for breakfast, sweetpotato chips (deep fried sun-dried chips) eaten as snacks, Sweetpotato Jam served with bread for breakfast (ACC/SCN. 1998). To increase more yield for product value additions, breeding work are going on in National and international research centres across the globe.

In the short term, crossing and combining parents with medium to high genetic values across all objectives and traits is all that is required to produce high quality clones for various quality uses. In pre-breeding and medium to long term population improvement, parents are developed by incorporation of new attributes from sources which often only have a high genetic value in one or very few attributes (e.g. excellent disease resistance but poor yield performance and other traits). Within the sweetpotato gene pool, there is an enormous amount of genetic variation for quality attributes. A renowned example is the concentration of pro-vitamin A in storage roots, which ranges from 0 to nearly 1000 ppm on a storage root dry weight basis (dwb). This corresponds to 0 to 20 mg β-carotene in 100 g of fresh sweetpotato storage roots (about 5 mg β-carotene meets the daily requirement of a pre-school child (400 µg/day RAE). Similar magnitudes of genetic variation are found for starch, sugars and probably for dietary fiber. Moderate genetic variation is found for protein and minerals such as iron and zinc. It is quite convenient, however, that the attributes, proteins and minerals are positively correlated genetically with β-carotene in sweetpotato storage roots, so that improvement in pro-vitamin A is linked with an improvement in iron, zinc and other minerals such as calcium and magnesium. Breeders nearly always want to select for several traits concurrently. In practical terms, quality breeding often means to improve quality (three to five traits) and simultaneously maintain sufficient genetic variation for yield, yield stability, adaptability and resistant to major diseases such as viruses.

III. Virus Disease in Sweetpotato

A virus is a microscopic pathogen with a protein structure that is not visible with the naked eye. By rapidly multiplying itself in living plant cells, the virus can damage the sweetpotato and considerably reduce its yield, to the great detriment of the farmer. A virus infection is often spread by insects that pierce and suck. The damage caused by the virus is then usually much greater than the mechanical injury caused by the insect. Of all the sweetpotato genotypes, the ones mostly affected by viruses are the orange fleshed sweetpotato genotypes.

IV. Origin and Spread of Viral Disease of Orange Fleshed Sweetpotato

Viruses are usually spread to orange fleshed sweetpotato crops by insects (vectors) that have sucking mouth parts, especially aphids, plant hopers and whiteflies, but other insect orders and families can also be responsible. These insects can come from the direct vicinity or from far away fields with other types of crops such as cassava, *Telferia occidentalis*. For instance the infamous tristeza virus in citrus trees is spread by an aphid that can be carried hundreds of kilometres by air currents. The infection can thus come from distant places, especially places where wind or tyhoons occurs. Viruses can also be spread by human hands that have come in contact with an infected crop or product. The tobacco mosaic virus is an example of a disease that can be spread in this way. This can sometimes be spread to sweetpotato or tobacco farms through the hands of workers who have rolled cigarettes with infected tobacco. Vegetatively propagated plant material can spread viruses. Soil viruses can be spread by nematodes and certain soil fungi. Orange fleshed sweetpotato field infested with nematodes can equally be attacked by viruses.

Some orange fleshed sweetpotato genotypes can carry a virus without being significantly damaged by it. The farmer may not even notice that the disease is present until it spread to different, more susceptible orange fleshed or white genotypes, where it does cause serious damage. Only then is the presence of the virus clearly evident. This indicated that not all orange fleshed sweetpotato genotypes are equally susceptible. Viruses affecting sweetpotato can be perpetuated and spread between cropping cycles by the use of foliar cuttings taken from infected plants. They are also transmitted from plant to plant by sap soaking insects. So far the only proven vectors of sweetpotato viruses are aphids and whiteflies (Stathers et al., 2005)

The sweetpotato mild mottle virus (SPMMV) and sweetpotato chlorotic stunt virus (SPCSV) are transmitted only by whitefly predominantly Bemisia spp, while sweetpotato feathering mottle virus (SPFMV) and the related sweetpotato virus 2 (SPV2) and sweetpotato virus G (SPVG) are transmitted by aphids. Some weeds such as Morning Glory harbor the viruses but the only economically important source of infection is other infected sweetpotato plants.

Stathers *et al.*, (2005) reported that SPFMV and SPCSV are together the most important viruses affecting sweetpotato in Africa and occur everywhere sweetpotato is growing. Sweetpotato can be infected by more than one virus species and when there is a build up, the viruses help each other to multiply with the result that the disease is even more severe. SPCSV in particular synergizing the multiplication of SPFMV and other viruses Stathers et al., (2005) observed. This severely

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affects the growth of sweetpotato plant. Stathers et al., (2005) emphasized that it is the combination of SPCSV and SPFMV is the most important disease of sweetpotato worldwide and is known as sweetpotato virus disease (SPVD). The SPFMV is transmitted by a wide range of adult winged aphid species including those that do not colonize sweetpotato while SPCSV is transmitted by the mobile adult whiteflies especially *B. tabaci* as they fly from plant to plant. Therefore, since the spread of SPCSV by whiteflies that synergizes SPFMV, whiteflies are the force behind the spread of SPVD.

V. Yield Losses Caused by Viruses

SPMV by itself is sometimes symptomless in sweetpotato, however, Stathers et al., (2005) observed that it can cause vein clearing and purple ring spots on the leaves of susceptible varieties. SPCSV on its own cause dwarfing of sweetpotato plants and either purpling or yellowing of lower leaves. Both viruses cause yield loss when sweetpotato plants are affected. For instance, in South Africa and China yield increases of more than 30% occurred as a result of planting virus-free planting material.

When a sweetpotato plant is infected with both SPCSV and SPFMV, the symptoms become very severe SPVD. Symptoms include severe stunting of the plant and small malformed leaves such as leaf curling and dwarfing, leaf mottling, yellowing of veins. Sometimes with either a chlorotic mottle or vein clearing. These symptoms are most apparent in young sweetpotato plants as they get established although plants can be infected at any age. If the entire oranged fleshed sweetpotato plant is affected by SPVD, the plant produce small storage roots generally of unusable size, resulting in a massive reduction in the yield of individual plants. Stathers et al., (2005) gave the following figures in the Table of effect of viruses on sweetpotato plant:

Comparison of healthy and virus infected sweetpotatoes in some countries as noted by Stathers et al., (2005)

Country	Root yield loss	Cultivar	Comments
Uganda	66%	Bitambi	Severely diseased plants
Uganda	70-99%	7 clones	Virus free clones as negative control
Uganda	57%	Kyebandula	Some controls became diseased
Nigeria	76-78%	TIS 1499	Severe diseased plants
Nigeria	60%	TIB 4	Low level of resistance of SPVD
Cameroon	0-90%	8 clones	Losses varied between clones and field trials

Sweetpotatoes are vegetatively propagated from vines, root slips (sprouts), and farmers often take vines for propagation from their own field year after year. Therefore, if virus diseases are present in the field, they will inevitably be transmitted with propagation materials to the newly planted field, resulting often in a marked decrease in yield. Loebenstein *et al.*, (2009) reported that yields differ greatly in different areas or even fields in the same location. The average yield in African countries is about 7.02 t/ha, with yields of 9.4, 4.4, 2.5 and 3.2t/ha in Kenya, Uganda, Sierra Leone and Nigeria respectively. The yields in Asia are significantly higher, averaging 12.41t/ha. China, Japan, Korea and Israel have the highest yields with about 21.6, 25.8, 16.4 and 44.4t/ha respectively. In South America the average yield is 10.74t/ha with Agentina, Peru, and Uruguay in the lead with 17.2, 16.35, and 13.68t/ha, respectively. For comparison, the average yield in the USA is 20.1t/ha (all data are averaged for 2005 from the FAOSTAT, 2007).

Loebenstein and co-workers (2009) further observed that these differences in yields are mainly due to variation in quality of the propagation material often taken from the previous season of farmer's fields. Often these fields are infested with several viruses, thereby compounding the effect on yields. In China, on average, losses of over 20%, mainly due to sweetpotato feathery mottle virus (SPFMV) and sweetpotato latent virus (SPLV). The infection rate in the Shandong province reaches 5 to 41% . Loebenstein and co-workers (2009) noted that in countries were care is taken to provide virus - tested planting material as amongst others in the USA and Israel, yields increase markedly up to 7 times and more while in some countries, as in Uganda, Kenya and Tanzania virus diseases are a major constraint for sweetpotato production.

VI. Virus Disease, a Challenge to Sweetpotato Breeding Work

The main reason for slow sweetpotato breeding progress in Nigeria and Africa in general can be attributed to low investments into breeding virus resistant genotypes and selection procedures to farmer needs (Gibson *et al.,* 2008) by formal plant breeding. This makes successful breeding for yield progress long term in nature and complicated in designing crossing programs. Moreover, sweetpotato as a clonally

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propagated crop can be easily multiplied and maintained, which is an advantage as well as a burden, because many diseases (especially viruses) are transmitted in planting material. The cloning characteristic permits rapid and wide dissemination of successful genotypes and varieties, respectively, and the exploitation of heterosis, an important genetic effect for yield, yield stability and adaptability. The multiplication and maintenance of vegetative propagules in virus loaded environment means multiplication, maintenance and dissemination of virus loaded materials.

Achieving medium to long term yield gains in sweetpotato is a challenge in sweetpotato breeding because the performance of a parent in one generation is not a good indicator for the value of a parent for the next sweetpotato generation. However, the genetic constitution of sweetpotato permits the adaptation of sweetpotato populations to new needs in the broad sense (environments, quality demands, tolerance to pests and diseases) to be achieved quite rapidly from the view point of crop evolution. Examples for this potential abound in the sweetpotato gene pool; it is possible to find many genotypes which are specifically adapted to drought, heat, cold (in tropical highlands), mineral-stress (including acid soils) or extreme salinity. Yield, yield stability and adaptability (including genotype by environment (G by E patterns) of crops are often associated with resistance to biotic and abiotic stress. However, most sweetpotato variety especially the orange fleshed genotypes succumb/broke down to viruses after a few years trial as result of accumulation of the virus disease each year being vegetatively propagated. As a result breeding orange fleshed sweetpotato genotypes with long time virus resistance is a challenge for now.

VII. Sweetpotato Virus Disease (SPVD) Tolerance Clones

The humid tropical low and mid-elevation States in Nigeria (0 to 1200 m.a.s.l.) with only very short dry seasons have high SPVD pressure, which is extreme in regions where sweetpotato is extensively cultivated. As is the case, no living organism is absolutely virus-free in its body system. The same applies to sweetpotato genotypes. Sweetpotato genotypes accumulate viruses in virus prone areas and the virus load is the major problem. SPVD occurs after infection of two viruses: the sweetpotato feathery mottle virus (SPFMV) and the sweetpotato chlorotic stunt virus (SPCSV). SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV - without SPCSV infection – are relatively low and SPFMV resistance of sweetpotato breaks down after the plant is infected by SPCSV. SPCSV resistance has been found in germplasm screening programs and the resistance appears to be conferred by a recessive allele that occurs in low frequency in the sweetpotato gene pool. Field resistance to SPVD has been obtained in East Africa by screening large numbers of sweetpotato genotypes from mainly local germplasm, and open pollinated seed and limited controlled cross progenies, evaluated on-station and on-farm. However, this resistance, still needs to be proven in extensive controlled artificial inoculation with SPVD and field tests under high SPVD pressure locations in Nigeria. It is nearly certain that new sweetpotato varieties with resistance to SPVD will result in significantly higher yields and yield stability in Nigeria, at least for a period of 5 to 8 years. After this period new strains of the sweetpotato chlorotic stunt virus gene pool are expected to emerge. Viruses are serious on sweetpotato. Some of them are briefly discussed below.

VIII. Sweetpotato Viral Diseases in Nigeria

a) Sweetpotato feathery mottle potyvirus (SPFMV)

Sweetpotato feathery mottle potyvirus (SPFMV) This virus disease causes mild or no symptoms in sweetpotato (Nyiira, 1982). It is transmitted by Aphid. In sweetpotato plant or when indicator plant such as *Ipomoea setosa* or *Ipomoea nil* is grafted to the sweetpotato plant, the symptom appear mild and transient vein clearing, vein feathering and chlorotic spots, especially on older leaves, and in roots there is external cracking cork and internal necrosis depending on cultivar and isolate although the symptom is more severe on indicator plants. In indicator plants, the leaves are mosaic, vein clearing, leaf stunting, and distortion (Gibson *et al.,*1998).

b) Sweetpotato chlorotic stunt cloresterovirus (SPCSV)

Gibson *et al., (*1998) reported that in Nigeria, the SPCSV alone causes no symptoms in sweetpotato. He reported that this was proved from a screen house experiment where plants grown from SPCSV infected cuttings produced about 25% of uninfected foliage cuttings and storage root yield was only about 13% of uninfected cuttings. However, Karyeija *et al*., (1998) and Gibson *et al*., (1998), observed in a survey and in an experimental transmission in NAARI that it is the dual infection of SPCSV and SPFMV that is the main cause of Sweetpotato virus disease (SPVD). SPVD is the most destructive disease of sweetpotato in Nigeria and in Africa in general. Gibson *et al*., (1998) also reported that symptoms of SPVD vary with sweetpotato genotype but include stunted plants with small leaves often distorted, narrow strap-like and crinkled, with a chlorotic mosaic and/or vein clearing. Affected sweetpotato plants generally appear pale. Root yield losses due to SPVD vary from 57% - 98% depending on varietal susceptibility. Although Gibson *et al*., (1998) reported that root yield losses vary in other countries. SPVD is most severe in sweetpotato cultivating states in Nigeria

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such as Ebonyi State, Nassarawa State, Benue State and in Umudike Abia State indicating that this disease is wide spread. This disease mostly attack orange fleshed sweetpotato genotypes including the recently released orange fleshed variety UMUSPO/3 by National Root Crops Research Institute, Umudike..

c) Sweetpotato mild mottle ipomovirus (SPMMV)

SPMMV is a virus disease transmitted by whitefly and aphids. Carey *et al*., (1998), noted that SPMMV causes relatively mild symptoms in sweetpotato plants. Carey et al., (1998) and Gibson *et al.,* (1998) noted that no yield loss assessment on *Sweetpotato mild mottle ipomovirus (SPMMV)* has been carried out in sweetpotato in Uganda but the disease was detected in sweetpotato samples in some districts in Uganda such as Mbale, Mpigi, Masindi, Kabale, Tororo and Iganga.

d) Sweetpotato chlorotic fleck virus (SPCFV)

Carey *et al*., (1998) reported that SPCFV have been detected in sweetpotato samples from some districts in Uganda such as Mbale, Mpigi, Masindi, Kabale, Tororo and Iganga. Wambugu (1991) also reported cucumber mosaic virus CMV and sweetpotato latent virus SPLV as common sweetpotato virus disease in sweetpotato field.

IX. Symptoms on Orange Fleshed Sweetpotato

The tissue of Orange fleshed sweetpotato plant damaged by a viral disease does not die off immediately. It does not display any necrotic spots or areas. The most important symptom of viral infections is the light (white and yellow) colour of the leaves or a mosaic pattern of light and darker shades of green on the leaves. Larger spots (sometimes in an oak-leaf pattern) can also appear within which a 'rain-stripe' pattern (with multiple yellow or pale green, narrow, parallel lines and bands) is visible. The spots that form the mosaic pattern can be angular (bordered by the leaf's veins) or rounded and sometimes even ring shaped. The latter example usually involves a soil virus. The leaf veins often also become lighter in colour, appear waxy and have a thin, darker-coloured streak on either side (i.e vein - clearing). Wabungu (1991) observed that the *Psorosis* virus in citrus trees causes their bark to die off and separate above the bud union with the lower trunk. Gummossis then often occurs as well.

In many cases, virus disease leads to dwarfed growth, rosette formation or other strange and leaf abnormalities. Rice, for example, can take on a grassy appearance as its leaves become small and thin. The same in sweetpotato and sometimes with flattened vines with numerous tiny narrow leaves. Cocoa can develop a type of 'witches' broom appearance, in which many small branches grow closely together. Leaf curl in cotton causes deformation of the edges of the leaves, which become curled, wavy or contorted as some parts of the leaf grow faster than others. The same effect can be seen on fruits (e.g. Citrus fruits), which develop shallow grooves, bulges, or other irregularities on their surface (Joep van Lidth de Jeude(2004)..

The symptom of viral infection on sweetpotato genotypes are often not found everywhere in a cultivated sweetpotato field as is usually the case with with fungal or bacterial diseases. However, in orange fleshed sweetpotato genotypes, a whole field can show the symptom of viral infection. It is almost always possible to find a number of sweetpotato plants that show no signs of the disease. Surprisingly, even a plant that is thoroughly infected with a viral disease may only show symptoms on one part, such as one half of a leaf.

Abnormal (lighter) leaf colour, abnormal leaf and stem shape, dwarfed growth and mosaic patterns on leaves can, however, be signs of a nutrient deficiency as well as a viral infection or nematode infestation. A viral disease cannot be diagnosed with any certainty at first glance or without laboratory tests.

X. Tests to Detect Viral Infection in Orange Fleshed Sweetpotato Genotypes

The best approach in this case is probably to conduct a few simple tests to determine whether the anomaly could be caused by a deficiency or nematode infestation (Joep van Lidth de Jeude (2004). This can be done by spraying a nutrient solution of micro- and macro-elements on the affected oranged fleshed sweetpotato plants and applying a nematicide to see if this brings about any improvement in the sweetpotato plant's condition. If not, then it is indeed likely that the damage is caused by a viral infection.

Another test is Sweetpotato virus indexing. This is accomplished by grafting sweetpotato cuttings onto indicator plants (*Ipomoea setosa and Ipomoea nil*), and symptoms are evaluated after 4 weeks (Panta *et al*., 2007). Positive symptom observation is followed by Nitro-cellulose membrane Enzyme Linked Immunosorbent Assay (NCMELISA) with available antisera (sweetpotato feathery mottle virus (SPFMV), sweetpotato latent virus (SPLV), sweetpotato mild mottle virus (SPMMV), sweetpotato chloric fleck virus (SPCFV), C-6 virus sweetpotato chlorotic stunted-virus (SPCSV), sweetpotato caulimo-like virus (SPCaLV) and Cucumber mosaic virus (CMV).

According to Loebenstein and co-workers (2009, Nucleic acid spot hybridization and PCR are optionally used to confirm the presence of some viruses for which antisera are not available, and that after the initial plant health check, infected accessions are

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submitted to virus elimination process and subsequently re-checked.

XI. Control Measures of Viruses in Orange Sweetpotato Field

Viruses spread very fast through the vascular system of a plant to entire plant and plant population. As a result, sweetpotato plants that show symptoms of a viral disease have to be removed from the crop and destroyed as soon as possible. If a virus spread through seeds especially during seedlings evaluation in Breeding Programme, the seeds can sometimes be neutralized by soaking the seeds in warm water before planting. Viruses cannot be treated with chemical agents. The most important way to prevent a viral infection is to use virus-free seeds and vine plant material. It is possible, however, to control the vectors (insects, nematodes) by applying chemicals, or often by adhering to strict periods during which a susceptible crop, or another botanically related crop, is not allowed to be cultivated on a particular field or during a particular period. Burning infected plants, isolation and planting of new fields far away (more than 100m) from old sweetpotato production in the control of viral diseases to maintain or increase production (Panta *et al*., 2007).

Joep van Lidth de Jeude (2004) observed that it is very difficult to disinfect soil that has been infected by a virus. The best approach is to cultivate sweetpotato genotypes that are not susceptible to that particular virus or to initiate a fallow period during which the soil can receive a great deal of sun exposure. Improved, virus resistant orange fleshed sweetpotato are available at the National Root Crops Research Institute, Umudike, Nigeria. Using these resistant orange fleshed sweetpotato genotypes is the easiest way to prevent viral infection.

The production of virus free sweetpotato is achieved almost exclusively by meristem culture *in vitro.* In meristem culture, the essence is to take as large a meristem as possible, while excluding virus infected tissue. Therefore, smaller apical (meristematic) explant might be clean from virus but not a longer apex subtending some leaf primordia (Henderson *et al*., 1984). Virus cleaning of sweetpotato by meristem culture is considered much more effective than by thermotherapy as Kuo (1991) noted and that with an 80% rate of virus clean shoots. Dangler *et al* (1994) reported that virus free sweetpotato propagation material has been produced by heat therapy alone. However, virus cleaning by meristem culture was the basis of the California State programme for sweetpotato improvement (Dangler *et al* (1994).

Therefore for establishing orange flesh sweetpotato field, collect cuttings for new crops from healthy plants. Avoid collecting cuttings for new plantings from very old crops because SPVD may have built up in these crops and SPVD is less easy to see in old plants than in vigorous-growing crops. The following are a summary of control measures in orange fleshed sweetpotato field Stathers *et al*., (2005):

- Remove any diseased plants as soon as they appear.
- Avoid planting new crops where sweetpotato was grown in the last season. This is because roots and cuttings from old surviving diseased plants in the soil will produce disease plants which act as source of inoculums to the new crop.
- Plant new crops far away from old crops so that it is difficult for whiteflies and aphids to reach the new crop.
- Other measures include crop hygiene such as ensuring that crop debris, leaves and roots is completely destroyed by fire or fed to livestock. Viruses attacking plants do not infect animals.
- Plant resistant varieties, which is the most convenient means of controlling SPVD. High yielding resistant varieties have been bred; farmers can use these varieties to produce a crop in areas of high SPVD pressure.
- Farming communities can work together to eradicate SPVD by applying all the control measures since all will benefit from it.

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Quality Characteristics of Candies Produced from Tiger Nuts Tubers (*Cyperus esculentus*) and Melon Seeds (*Colocynthis citrullus. L*) Milk Blend

By Obasi, Nneoma Elechi & Ugwu, Vanessa Chidinma
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Abstract- Tiger nut tubers (*Cyperus esculentus L*) milk and egusi melon (*Colocynthis citrullus*) seeds milk were selected for production of candies. The candies developed from tiger nuts and egusi melon seeds milk and their blends were subjected to sensory and physicochemical analysis. Results showed that the candies had no significant difference ($p<0.05$) in acceptability of sensory parameters viz, appearance, taste, flavour, and mouth feel. Sample 70:30 (70% tiger nuts milk and 30% melon seeds milk candy) was significantly ($p<0.05$) lower than other samples in terms of general acceptability while 100% tiger nut milk candy was most preferred.The candies and the milk had vitamin compositions ranging from 0.04mg/100 to 0.06mg/100 vitamin A and 0.09mg/100 to 0.91mg/100 vitamin C. Protein and minerals like sodium, calcium, magnesium and potassium were significantly increased ($p<0.05$) in 100% tiger nut milk candy while iron was higher in 100% melon milk candy.

Keywords: *tiger nut tubers milk, melon seeds, candy, sensory evaluation.*

GJSFR-D Classification : *FOR Code: 309999*

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Strictly as per the compliance and regulations of :

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I. Introduction

Candy is a type of confectionary which describes a spectrum of sweet goods and takes on different meanings from one country to the other. Among several definitions, candy is defined as a highly cooked coloured and flavoured sugar mass formed into desired shapes. A more or less solid article of confectionery made by boiling sugar or molasses to the desired consistency, and then crystallizing, molding, or working in the required shape. It is often flavoured or coloured, and sometimes contains fruit, nuts, etc... Technically, milk or chocolate can be added to sugars mixtures in candy processing depending on the variety. The utilization of animal milk in candy production results in their unavailability in most African markets and to their high price of purchase. Other milks from plants can be investigated in the production of confectionaries especially candy.

These milks can be manufactured from melon seeds ("Egusi") which are commonly cultivated crops anywhere in the world. "Egusi" (*Colocynthis citrullus L.*) belongs to the species of the genus *Citrullus* of cucurbitaceae family, which usually consists of a large number of varieties that are generally known as melons.

Egusi (*Colocynthis citrullus L.*) is used both as condiment and thickener in Nigerian local soup.This plant family is known for its great genetic diversity and widespread adaptation which include tropical and subtropical regions, arid deserts and temperate locations. Curcubits are known for their high protein and oil contents. Seeds of cucurbits are sources of oils and protein with about 50% oil and up to 35 % protein (Achu, 2005). Specifically for these reasons they are cultivated and consumed world over. Egusi (*Colocynthis citrullus L.*) is among the 300 species of melon found in tropical Africa and it is cultivated for its seeds, which have been reported to be rich in oil and protein. Though the industrial scale production of the oil is yet to be utilized despite its huge potential.

Tiger nut (*Cyperus esculentus L.*) tuber is also another plant crop that its milk can be utilized in candy production. Tigernut (*Cyperus esculentus* L.) is an underutilized crop (family) and was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant. Other names of the plant are earth almond as well as yellow nut grass (Odoemelan, 2003; Belewu and Belewu, 2007). The nut was found to be rich in myristic acid, oleic acid, linoleic acid (Eteshola and Oraedu, 1996). Tigernut is commonly known as earth almond, chufa and chew-fa and Zulu nuts. It is known in Nigeria as Aya in Hausa, Ofio in Yoruba and, Akihausa in Igbo where three varieties (black, brown and yellow) are found. Among these, only two varieties, yellow and brown are readily available in the market. Tigernut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called Horchata De Chufas or tiger nut milk.Tigernut milk is a very nutritive and energetic drink, both for young and old. It is tremendously high in starch, glucose and proteins. Also rich in minerals like Potassium, Phosphorous, Vitamins E and C. Tigernut milk contains a large amount of Oleic acid and it is cardiac preventive. Tigernut milk has never been found to produce allergy (Belewu and Abodunrin, 2008).

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Traditionally, candies are made from cow milk which has led to little or no attention to various sources of imitation milk such as soy bean, melon seed and tiger nut. Therefore, there is need to evaluate the potentials of these plants so as to increase their utilization in the market for candy. Melon seeds and tiger nuts are highly healthful and nutritive. They are consumed as snacks and beverages but due to low awareness on the nutritive and health benefits of these plant crops there is need for increased awareness of their benefits in candy making.

Although previous researches have been made on melon seeds and tiger nuts, there is yet to be a research on the quality of candy produced from melon seed milk and tiger nut milk.

The possibility of candy production from melon seed and tiger nut milk on a large scale would be a good way of utilizing these underutilized plant crops. It will also give an opportunity of purchasing these candies at affordable prices. This work when properly carried out will create awareness on the quality characteristics of melon seed milk and tiger nut milk that will make it possible for them to be incorporated into candy and stabilize this sugar based product.

Main objective is to evaluate the quality characteristics of candy produced from melon seeds and tiger nuts milk blend. While the specific objectives are: to extract milk from melon seeds and tiger nut tubers, then to produce candy from melon seeds milk, tiger nuts milk and their blends. Then finally to determine proximate, mineral, vitamin composition and sensory analysis on candies produced from melon seeds milk, tiger nuts milk and their blends.

II. Materials and Methods

The melon (*Colocynthis citrullus L.*) seeds, tiger nut (*Cyperus esculentus*) tubers, granulated sugar, glucose syrup, and lime were purchased from Ubani market in Umuahia, Abia State, Nigeria. The melon seeds and tiger nut tubers were sorted manually to remove undesirable materials, washed with clean tap water for soil removal, blanched at 60^{o}C for 5 seconds in order to inactivate inherent enzymes and reduce microbial load, then drained prior to utilization.

III. Preparation of Milk from Melon Seeds

Method described by Omole and Ighodaro (2012) was adopted, modified and used. Approximately 800g of melon seeds were toasted in a stainless steel pot for 3 minutes using gas cooker. The toasted melon seeds were boiled in boiling water for 15 minutes. The boiled melon seeds were cooled to 28^{o}C and then milled using a home blender (Evanita-FCL 1731) to obtain slurry which was subjected to filtration using a muslin cloth to obtain melon milk (plate 1). The melon milk obtained was packaged in an airtight plastic container and stored under refrigeration temperature prior to usage. Flow chart for the preparation of melon milk is shown in fig.1.

IV. Preparation of Milk from Tiger Nuts

The already prepared tiger nut tubers (1000g) were soaked for 8 hours in clean tap water, then ground in a ratio of 1liter of water to each 300gram of tiger nuts and the mixture was left to macerate for 10 minutes. The mixture was pressed and filtered using a muslin cloth. The milk obtained (plate 2) was stored in an airtight container prior to usage. Flow chart for the preparation of tiger nut milk is shown in figure 2.

V. Milk Blend Formulation

Tiger nut milk and melon seed milk were mixed at varying proportions; 90:10, 80:20, 70:30, 60:40 and 50:50 to obtain the raw material for candy production, with 100% cow milk serving as the control sample. This was done using a food blender (Evanita-FCL 1731) operated at full speed for 2 minutes.

VI. Milk Candy Production

The method described by Sunny-Roberts (2007) was adopted, modified and used in the production of non-crystalline milk candy. Approximately 100g of sugar, 30g of glucose syrup, 8g of lime juice and specific ratio of milk blend from tiger nuts and melon seeds were combined in a heavy sauce pan over medium heat (45^{o}C) and stirred until the sugar dissolved. A thermometer was inserted into the mixture as it was brought to boiling without stirring until the temperature of the mixture reaches 120^{o}C and this lasted for 60 minutes. The mixture was allowed to cool to about 45^{o}C. The mixture was then poured into suitable molds to form. The resulting candies (plates 4 and 5) were removed from the molds after 30 minutes, cut with a very sharp knife and was left to completely cool for 24 hours. The candies were wrapped in an aluminum foil and stored in an airtight container at room temperature prior to analysis. The same process was repeated for other samples with varying milk blends. The flow chart for candy production is shown in fig. 3.

a) Proximate Analysis

Proximate composition of the milk candy samples was determined in duplicates except for carbohydrate content which was determined by difference.

i. *Moisture content determination*

The moisture content was determined using the conventional method (AOAC, 1990).

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ii. *Ash content determination*

The furnace incineration gravimetric method recommended by AOAC (1990) was used in the determination of the ash content.

iii. *Crude fibre determination*

This was be determined by the Weende method described by James (1995).

iv. *Fat content determination*

The fat content was determined by continuous solvent extraction in a Soxhlet reflux apparatus (James, 1995).

v. *Protein determination*

The micro-kjeldahl method a described by James (1995) was used to determine the protein content of the samples.

vi. *Carbohydrate determination*

The carbohydrate contents of the test samples were determined by estimation using the arithmetic difference method described by James (1995).

VII. Mineral Analysis

The mineral contents of the test samples were determined by the dry ash extraction method following each specific mineral element as described by James (1995). Twenty (20) ml of each sample was burnt to ash on a muffle (as in ash determination) and the resulting ash was dissolved in 100ml of dilute hydrochloric acid (1M HCl) and then diluted to 100ml volumetric flask using distilled water. The solution was used for the various analysis of mineral.

a) Calcium and Magnesium Determination

Calcium and magnesium contents of the test sample were determined by the EDTA complex isometric titration.

b) Potassium and Sodium Determination

The potassium and sodium contents of the samples were determined by photometric method.

c) Iron Determination

AOAC (1990) method was used to determine the iron content.

VIII. Vitamins a and c Determination

a) Vitamin C Determination

The vitamin C content of the beverage sample was determined by the isometric method as described by Pearson (1976).

b) Vitamin A (Retinol) Determination

Vitamin A was determined as described by James (1995).

IX. Sensory Evaluation

The method described by Iwe (2002) was used. The quality attributes such as appearance, taste, flavour, mouth feel, and general acceptability of the candies were tested by 30 panelists randomly selected from the staff and students of Michael Okpara University of Agriculture, Umudike.

a) Statistical analysis

The data obtained were subjected to analysis of variance of a completely randomized design using the SPSS procedure version 16 for personal computers (SPSS 1995), while treatment means were separated using Duncan multiple range test at 95% confidence level.

X. Results and Discussion

a) Proximate Composition Of Tiger Nuts Milk, Melon Seeds Milk And The Candies

The results of the proximate composition of tiger nuts milk, melon seeds milk and the candies are shown in Table1.The moisture content values recorded for milk candy samples ranged from 2.14%-4.31%. 100% melon seeds milk candy had the highest moisture content (4.31%) and it is significantly different ($p<0.05$) from other samples, while samples 60:40 (60% Tiger nuts milk and 40% melon seeds milk candy) and 80:20 (80% Tiger nuts milk and 20% melon seeds milk) had the lowest moisture content (2.14%). This could be as a result of the temperature and length of time the candies were cooked. The range of moisture content values (2.14%-4.31%) observed were lower than the values (5.93%, 4.44%, and 4.37%) reported by Sunny-Roberts (2007) for coconut milk candy, groundnut milk candy and soy milk candy respectively. The difference in values could be attributed to the composition of the candies. Most chemical and biological processes that cause spoilage and deterioration of food which are water dependent would be reduced because of low moisture content of the candies (Sunny-Roberts, 2007).

The fat content of the candies ranged from values 1.31%-2.10%. There was significant difference ($p<0.05$) among the samples. Sample 70:30 (70% Tiger nuts milk and melon seeds milk candy) had the lowest fat content value (1.31%) while 100% cow milk candy had the highest fat content value (2.10%). This could be attributed to the different sources of milk used for production. The fat content value (8.21%) of tiger nuts milk was higher than groundnut milk value (7.86%) which in turn was higher than the value (7.31%) of melon seed milk (Sunny-Roberts *et al.*, 2004) while Omole and Ighodaro (2012) reported the value (3.09%) of melon seed milk to be lower. The decrease in the fat content of the candies is an advantage for the keeping quality of the candies as chances of rancidity would be greatly reduced (Sunny-Roberts *et al.*, 2004).The range of the protein content of the candy samples was from values 1.04%-3.86%. From the result, 100% tiger nut milk candy had the highest value (3.86%) and 80:20 (80% Tiger nut milk and 20% melon seeds milk) sample had the lowest

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protein content value (1.04%). This could be as a result of the ratios of the milk blends as the quantity of tiger nuts milk was higher in all the samples except sample 50:50 (50% Tiger nuts milk and 50% melon seeds milk). There was also significant difference ($p<0.05$) in the protein content of all the samples. The variation in the results is probably due to the method of extraction employed considering the fact that melon seeds have high level of protein content (32.6%) (Oyenuga and Fetuga, 1975).Ash represents the total mineral content of a food material and thus serves as a viable tool for nutritional evaluation (Lienel, 2002). The ash content of the candies ranged from 0.71%-1.25% and were higher than values obtained for candies from other imitation milk which ranged from values 0.23%-1.04% as reported by Sunny-Roberts (2007) while the values for the tiger nuts milk (1.21%) and melon seeds milk (1.49%) were higher than the values reported for imitation milk whose values ranged from 0.04%-0.85%. 100% melon seeds milk candy had the highest ash content value (1.25%) while 90:10 (90% tiger nuts milk and 10% melon seeds milk) candy had the lowest ash content value (0.75%). There was significant difference ($p<0.05$) among some of the samples.

From the result in Table 1, the carbohydrate content of all the samples was very high. 60:40 (60% Tiger nut milk and 40% melon seeds milk) sample had the highest (95.05%) and was significantly different ($p<0.05$) from other samples, while 100% tiger nut milk candy had the lowest level of carbohydrate. The increase in the carbohydrate content of the candy samples could be as a result of the ingredients added to the candies considering the fact that candy is sugar based product.

b) Vitamin Contents of Tiger Nut Tubers Milk, Melon Seeds Milk and the Candies

The results for the vitamin contents are shown in Table 2. Vitamin C is relevant in preventing scurvy and other degenerative diseases (Haliwell, 1996). The vitamin C content of the candies and milk samples ranged from 0.09%-1.19%. The vitamin C content of the tiger nuts milk (1.19mg/100) was significantly different ($p<0.05$) from the vitamin C content of the melon seeds milk and the candies. Vitamin A contents of the candies were very negligible from the results obtained. The vitamin A content ranged from 0.00% - 0.07%. This shows that candies from melon seeds and tiger nuts milk are poor sources of vitamin A.

c) Mineral Contents of Tiger Nuts Milk, Melon Seeds Milk and the Milk Candies

The results for the mineral contents of tiger nut tubers milk, melon seeds milk and the candies are seen in Table 3.

The sodium content of the candies ranged from 2.75mg/100-180.50mg/100g. The sodium content value (180.50mg/100g) of 100% tiger nut milk candy was significantly higher ($p<0.05$) than the sodium content value (152.50mg/100g) of 100% melon milk candy while 100% cow milk candy is significantly different ($p<0.05$) from other samples.

Potassium content of tiger nut milk candy was significantly higher ($p<0.05$) than other samples. The potassium content of the samples ranged from 46.00mg/100-172.00mg/100g with tiger nut milk candy showing the highest content and sample 90:10 (90% tiger nuts milk and 10% melon seeds milk) the lowest potassium content value (46.00mg/100). This is an indication that the consumption of the candies can reduce high blood pressure disease.

90:10 (90% tiger nuts milk and 10% melon seeds milk) sample had the highest (5.90mg/100g) iron content while 100% cow milk candy had the lowest content (0.46mg/100g). This could be attributed to the presence of melon seed milk in reasonable amount in that it has higher amount of iron contained in it. Studies have shown that cow milk is a poor source of iron and since it is an essential element in the body, the use of melon seed milk in making candy can help increase its supply to the body. There was significant difference ($p<0.05$) among the samples.

The candies had magnesium content range from values 6.00mg/100g-47.50mg/100g. 100% tiger nut milk candy had the highest magnesium content (47.5mg/100g) while 80:20 (80% tiger nuts milk and 20% melon seeds milk) sample had the lowest (6.00mg/100g). There was significant difference ($p<0.05$) among the samples except samples 70:30 (70% tiger nuts milk and 30% melon seeds milk) and 80:20 (80% tiger nuts milk and 20% melon seeds milk) which had no significant difference.

The values of the calcium content of the candies ranged from 27.15mg/100g- 125.10mg/100g. 100% Tiger nut milk candy had the highest (125.10mg/100g) content of calcium while 80:20 (80% tiger nuts milk and 20% melon seeds milk) sample had the lowest (27.15mg/100g). There was significant difference ($p<0.05$) among all the candy samples. The average value of calcium content of the milk candy samples was higher than the value of 45.70% stated by Manjula and Suneetha, (2014) for pumpkin juice candy. Generally, the presence of these minerals; sodium, potassium, iron, magnesium, and calcium in foods are necessary for bones, tissue repairs, muscles, the blood stream, for body growth and development, and for preventing high blood pressure (Bamishaiye and Bamishaiye, 2011).

d) Sensory Evaluation of the Candy Samples

The sensory scores of the candy samples are shown in Table 4.

As regards to appearance, sample 50:50 (50% tiger nuts milk and 50% melon seeds milk) had the highest (8.13) mean score while sample 70:30 (70%

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tiger nuts milk and 30% melon seeds milk) had the lowest (6.87) mean score. This indicated that 50:50 (50% tiger nuts milk and 50% melon seeds milk) candy and 70:30 (70% tiger nuts milk and 30% melon seeds milk) candy were liked very much and slightly respectively by the panelists and there was no significant difference ($p<0.05$) among the samples. In terms of taste, 100% tiger nuts milk candy ranked highest (7.53). There was no significant difference ($p<0.05$) among the samples. 100 melon seeds milk candy had the highest (7.00) mean score with respect to flavour while 70:30 (70% tiger nuts milk and 30% melon seeds milk) had the lowest mean score (6.13) and there was no significant difference ($p<0.05$) among the samples. The mouth-feel of 100% tiger nuts milk candy had the highest (7.33) mean score while 70:30 had the lowest mean score (5.57). No significant difference ($p<0.05$) existed among the samples. For general acceptability, 100% tiger nuts milk candy had the highest mean score (7.33) which indicated that the sample was moderately accepted by the panelists. 70:30 (70% tiger nuts milk and 30% melon seeds milk) was the least accepted with lowest mean score of 5.97. There was no significant difference ($p<0.05$) among the samples except for 70:30 (70% tiger nuts milk and 30% melon seeds milk) value. From the result obtained in Table 4., 100% tiger nut milk candy had the highest mean score (7.33) in terms of general acceptability which indicates that it is the most preferred by the panelists. This implies that tiger nuts milk can be used for candy production.

Therefore, it has been applied successfully in the making of candy which had comparable nutritional values with candy made from cow milk. This means that the production of such candies on a large scale would be a wise way of utilizing these crops. It would also provide an opportunity of purchasing these products at affordable prices which will in turn make the beneficial nutrients present in the crops available to the consumers through the candies, especially African children.

XI. Conclusion

Aside from animals such as cow, milk can be extracted from other sources such as plant crops, precisely tiger nut tubers and melon seeds. Milk from these plant crops was found to have desirable, acceptable, and relevant physicochemical properties. The results obtained revealed the possibility of using tiger nut milk and melon seed milk as raw materials in food industries. Therefore, it has been applied successfully in the making of candy which had comparable nutritional values with candy made from cow milk. This means that the production of such candies on a large scale would be a wise way of utilizing these crops. It would also provide an opportunity of purchasing these products at affordable prices which will in turn make the beneficial nutrients present in the crops available to the consumers through the candies, especially African children.

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laboratory-prepared fermented groundnut milk. Eur. Food Res. Technol.218:452-45.

Illustrations and Figures

Plate 1 : Melon seeds milk

Plate 2 : Tiger nuts milk

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Plate 3 : Melon seeds milk candy

Plate 4 : Tiger nuts milk candy

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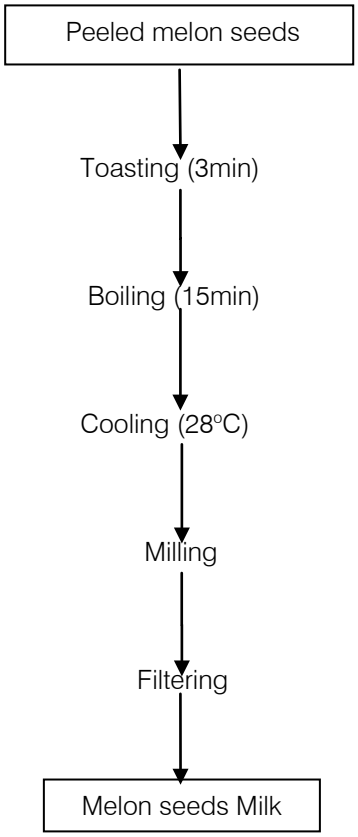

Figure 1 : Flowchart for the preparation of milk from melon seed

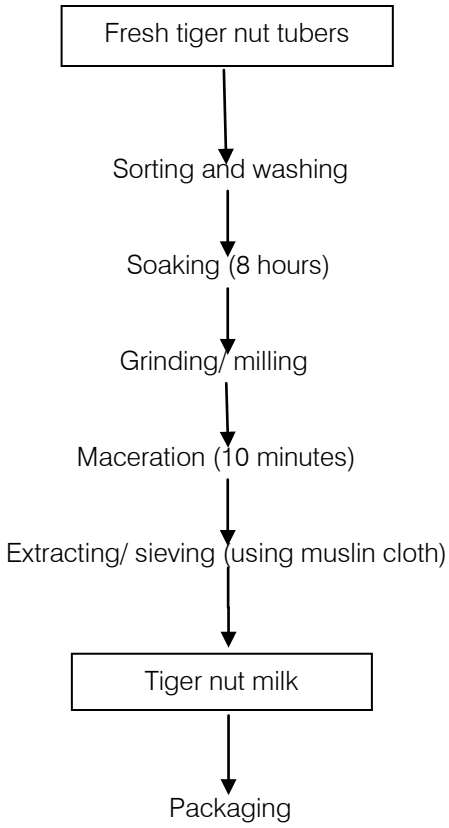

Figure 2 : Flowchart for the preparation of milk from Tiger nut

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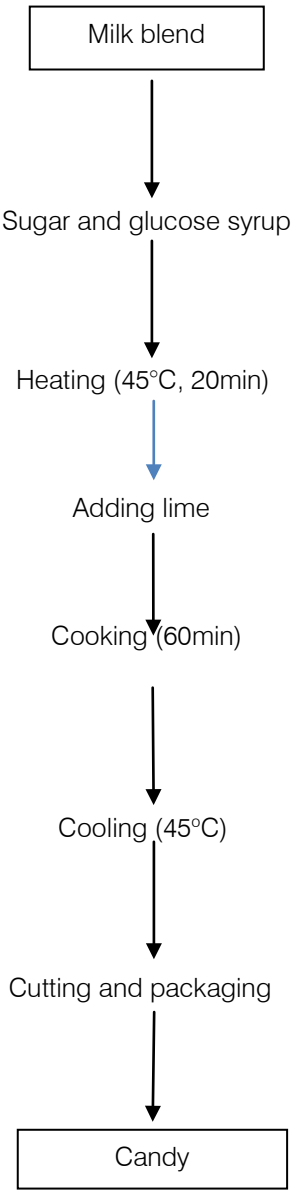

Figure 3 : Flowchart for the preparation of candies from milk blend

Table 1 : Formulation of milk blends

Sample	cow milk (%)	tiger nut milk (%)	melon milk (%)
A	100	0	0
B	0	100	0
C	0	0	100
D	0	90	10
E	0	80	20
F	0	70	30
G	0	60	40
H	0	50	50

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Table 2 : Recipe for milk candy production per sample

Ingredients	quantity (g)
Sugar	100
Lime	8
Glucose syrup	30

Table 3 : Proximate composition of tiger nut tubers milk, melon seeds milk and the candies

*Candy Samples and blends of Tigernut: Melon seeds milk	Moisture content (%)	Crude protein (%)	Ash content (%)	Fat content (%)	Carbohydrate (%)
100% Cow milk candy	2.65f±0.07	2.11^{c}±0.01	1.15^{cd}±0.07	2.10^{c}±0.00	91.73^{c}d±0.14
100% Tigernut milk candy	3.02^{e}± 0.01	3.86^{b}±0.00	0.80^{g}±0.00	1.75^{d}±0.07	90.58^{d}±0.06
100% melon milk candy	4.31^{c}±0.01	1.35^{e}±0.00	1.25^{b}±0.07	1.41^{f}±0.01	91.69^{cd}±0.08
50:50 Tiger nut milk : melon milk	3.65^{d}±0.07	1.32^{e}±f0.00	1.03^{e}±0.00	1.32^{g}±0.01	92.67^{bc}±0.06
60:40 Tiger nut milk : melon milk	2.14^{g}±0.01	1.21^{h}±0.01	1.11^{d}±0.01	1.51^{e}±0.01	95.0^{a}±1.44
70:30Tiger nut milk : melon milk	3.11^{e}±0.01	1.24^{g}±0.01	0.93^{f}±0.01	1.31^{g}±0.01	93.42^{b}±0.00
80:20 Tiger nut milk : melon milk	2.14^{g}±0.00	1.15^{i}±0.00	1.21^{b}c±0.00	1.51^{e}±0.01	93.00^{bc}±1.41
90:10 Tiger nut milk : melon milk	3.13^{e}±0.04	1.04^{j}±0.01	0.71^{h}±0.01	1.73^{d}±0.01	93.28^{b}±0.22
100% Melon milk	83.2^{a}±0.01	1.52^{d}±0.01	1.49^{a}±0.00	7.31^{b}±0.01	8.02^{f}±0.02
100% Tiger nut milk	73.9^{b}±0.01	4.13^{a}±0.01	1.21^{b}c±0.01	8.21^{a}±0.01	12.56^{e}±0.01
LSD	0.08	0.08	0.05	0.01	1.43

**Means in the same column with different superscripts are significantly different (P<0.05).*

Table 4 : Vitamin contents (mg/100g) of tiger nut tubers milk, melon seeds milk and the candies

*Milk and candy samples	Vitamin C	Vitamin A
100% Cowmilk candy	0.84^{d}±0.01	0.060.00
100% Tigernut milk candy	0.91^{b}±0.01	0.040.00
100% Melon milk candy	0.34^{g}±0.01	-
50:50 Tigernut milk: melon milk	0.22^{h}±0.01	-
60:40 Tigernut milk: melon milk	0.09^{i}±0.01	-
70:30 Tigernut milk: melon milk	0.44^{f}±0.01	-
80:20 Tigernut milk: melon milk	0.79^{e}±0.01	-
90:10 Tigernut milk: melon milk	0.84^{c}±0.01	-
100% Melon milk	0.79^{e}±0.00	-
100% Tigernut milk	1.19^{a}±0.01	-

**Means in the same column with different superscripts are significantly different (P<0.05).*

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Table 5 : Mineral contents(mg/100g) of tigernut tubers milk,melon seeds milk and their candies

*Milk and Candy Samples	Sodium	Potassium	Iron	Magnesium	Calcium
100% Cow candy	$152.50^{c}\pm0.71$	$140.0^{c}\pm0.00$	$0.46^{b}\pm0.00$	$32.25^{c}\pm0.07$	$110.00^{c}\pm0.00$
100%Tigernut candy	$180.50^{b}\pm0.71$	$172.0^{b}\pm0.00$	$0.57^{i}\pm0.21$	$47.50^{b}\pm0.71$	$125.10^{b}\pm0.00$
100% Melon candy	$3.20^{i}\pm0.00$	$68.65^{d}\pm0.00$	$5.26^{c}\pm0.01$	$16.25^{d}\pm0.07$	$90.15^{d}\pm0.07$
50:50 melon seeds milk	$6.20^{i}\pm0.00$	$71.25^{e}\pm0.07$	$1.28^{e}\pm0.01$	$12.05^{f}\pm0.07$	$63.25^{f}\pm0.07$
60:40 Melon seeds milk	$11.25^{d}\pm0.07$	$60.35^{e}\pm0.07$	$1.31^{d}\pm0.01$	$8.70^{g}\pm0.00$	$48.25^{g}\pm0.07$
70:30 Melon seeds milk	$9.15^{e}\pm0.07$	$57.30^{f}\pm0.14$	$1.13^{f}\pm0.01$	$6.05^{h}\pm0.07$	$36.25^{h}\pm0.07$
80:20 melon seeds milk	$7.05^{f}\pm0.07$	$50.05^{g}\pm0.07$	$1.03^{g}\pm0.00$	$6.00^{h}\pm0.00$	$27.15^{i}\pm0.21$
90:10 melon seeds milk	$4.25^{h}\pm0.07$	$46.00^{h}\pm0.07$	5.90+±0.00	$12.30^{f}\pm0.00$	$81.20^{e}\pm0.00$
100% melon seeds milk	$2.75^{i}\pm0.07$	$68.00^{d}\pm0.00$	$6.71^{a}\pm0.01$	$13.60^{e}\pm0.85$	$81.00^{e}\pm1.41$
100% Tiger nut milk	$203.00^{a}\pm0.00$	$194.0^{a}\pm00.00$	$0.61^{h}\pm0.00$	$51.50^{a}\pm0.71$	$152.00^{a}\pm0.00$
LSD	0.71	1.02	0.93	0.20	0.03

**Means in the same column with different superscripts are significantly different (P<0.05).*

Table 6 : Sensory Quality Scores of the Candy samples

*Candy samples	Appearance	Taste	Flavour	Mouthfeel	General acceptability
100% cow	$7.21^{bc}\pm1.20$	$6.73^{abc}\pm1.66$	$6.37^{ab}\pm1.22$	$6.33^{bc}\pm1.67$	$6.53^{ab}\pm1.20$
100% Tigernut	$8.10^{a}\pm0.89$	$7.53^{a}\pm1.01$	$6.93^{a}\pm1.34$	$7.33^{a}\pm1.58$	$7.33^{a}\pm1.09$
100% melon	$7.77^{ab}\pm1.07$	$7.30^{ab}\pm1.53$	$7.00^{a}\pm1.51$	$7.00^{ab}\pm1.53$	$6.97^{ab}\pm1.07$
50:50 Tiger nut : Melon milk	$8.13^{a}\pm0.82$	$6.50^{bc}\pm1.43$	$6.87^{ab}\pm1.25$	$6.43^{abc}\pm1.63$	$6.67^{ab}\pm0.96$
60:40 Tiger nut : Melon milk	$7.37^{bc}\pm1.16$	$6.93^{ab}\pm1.66$	$6.87^{ab}\pm1.41$	$7.23^{ab}\pm1.55$	$7.17^{ab}\pm1.29$
70:30 Tiger nut : Melon milk	$6.87^{c}\pm0.97$	$5.97^{c}\pm1.45$	$6.13^{bc}\pm1.07$	$5.57^{c}\pm1.83$	$5.97^{c}\pm1.19$
80:20 Tiger nut : Melon milk	$7.03^{c}\pm1.27$	$6.67^{bc}\pm1.42$	$6.83^{ab}\pm1.32$	$6.67^{ab}\pm1.69$	$6.73^{ab}\pm1.26$
90:10 Tiger nut : Melon milk	$7.47^{bc}\pm1.04$	$7.00^{ab}\pm1.58$	$6.80^{ab}\pm1.67$	$6.90^{ab}\pm1.65$	$7.03^{ab}\pm1.47$
LSD Tiger nut : Melon milk	0.78	0.75	0.69	0.83	0.61

**Means in the same column with different superscripts are significantly different (P<0.05).*

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Minimum Inhibitory and Bactericidal Concentrations (MIC & MBC) of Honey and Bee Propolis against Multidrug Resistant (MDR) *Staphylococcus Sp.* Isolated from Bovine Clinical Mastitis

By Aamer, A. A., Abdul-Hafeez, M. M. & Sayed, S. M.
Assiut University, Egypt

Abstract- With the emergence of antibiotic-resistant Staph. sp., search for antimicrobial agents other than antibiotic is of great concern. The study aimed to determine both MIC and MBC of different honey samples against these strains. The study was conducted with 64 different Staph sp. isolated from bovine mastitis and tested in vitro against 11 antimicrobial agents. The most MDR strains (19) were tested in vitro against six honey batches; marjoram, cotton, two fennel samples and two different trefoil samples as well as against 10% propolis-fennel honey mixture. Both MIC & MBC of the tested honey samples against every tested strain were determined. Propolis-fennel honey mixture showed the lowest both MIC & MBC values against all Staph sp. all over the study with highly significant differences, while against different Staph sp., also it had the lowest MIC and MBC values against S. intermedius followed by S. aureus.

Keywords: *MIC, MBC, apitherapy, antimicrobial, staphylococcus, mastitis.*

GJSFR-D Classification : *FOR Code: 300599p*

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Minimum Inhibitory and Bactericidal Concentrations (MIC & MBC) of Honey and Bee Propolis against Multidrug Resistant (MDR) *Staphylococcus Sp.* Isolated from Bovine Clinical Mastitis

Aamer, A. A. [α], Abdul-Hafeez, M.M. [σ] & Sayed, S.M. [ρ]

Abstract- With the emergence of antibiotic-resistant Staph. sp., search for antimicrobial agents other than antibiotic is of great concern. The study aimed to determine both MIC and MBC of different honey samples against these strains. The study was conducted with 64 different Staph sp. isolated from bovine mastitis and tested in vitro against 11 antimicrobial agents. The most MDR strains (19) were tested in vitro against six honey batches; marjoram, cotton, two fennel samples and two different trefoil samples as well as against 10% propolis-fennel honey mixture. Both MIC & MBC of the tested honey samples against every tested strain were determined. Propolis-fennel honey mixture showed the lowest both MIC & MBC values against all Staph sp. all over the study with highly significant differences, while against different Staph sp., also it had the lowest MIC and MBC values against S. intermedius followed by S. aureus. The study revealed that among the different Staph. sp., S. aureus was the most sensitive species to the honey antimicrobial action with highly significant differences. The study concluded that all tested Staph. sp. –despite of being MDR- were sensitive to the antimicrobial activity of all tested honeys where S. aureus was the most sensitive one, while adding 10% propolis powder would maximize its antimicrobial activity significantly.

Keywords: MIC, MBC, apitherapy, antimicrobial, staphylococcus, mastitis.

I. Introduction

As the traditional knowledge about the use of natural products or substances should be scientifically investigated[25] and the antimicrobial application requires safe preparations, knowledge of the composition of antibacterial factors and standardized antibacterial activity[15], the in vitro study of honey therapeutic action is of great necessity for its applicability. Honey possesses therapeutic potential and its antimicrobial activity is widely documented as a large number of in vitro studies of MIC and MBC confirmed its broad- pectrum antimicrobial properties either in solo use [27,29,30,38]or in combination with other agents as royal jelly[9], bee propolis[17], ginger starch[24], garlic extract[25] or rifampicin[33]even on MDR such as S. aureus methicillin resistant (MRSA)[22] or vancomycin-resistant enterococci (VRE)[10]. Propolis extract also proved to possess antimicrobial activity[31,23,34,36,37]. Moreover, subinhibitory concentration of honey in combination with oxacillin restored oxacillin susceptibility to MRSA[22]. The present work aimed to investigate the in vitro MICs & MBCs of different honey batches and propolis powder against different MDR Staph. spp. isolated from bovine clinical mastitis.

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II. Material & Methods

a) Bacterial isolation

Out of 101 milk samples from clinical mastitic cows through a previous work for the same author[40], 64 Staph. sp. strains were recovered and be the baseline of the present study where the most MDR strains (no 19) as Staph aureus (6), Staph intermedius (3), Staph saprophyticus and Staph epidermedis (5 for each)were tested against all honey patches.

b) Antimicrobial sensitivity testing

All these 64 isolated Staph. sp. strains were tested against 11 antimicrobial agents [Oxacillin (OX) 1 μg, Ampicilin (AM) 10 μg, Cefotaxime (CTX) 30 μg, Doxycycline (DO) 30 μg, Enrofloxacin (ENR) 5 μg, Gentamicin (CN) 10 μg, Lincomycin (L) 2 μg, Oxytetracycline (T) 30 μg, Penicillin (P) 10 μ, Trimethoprim – Sulflamethaxzole (SXT) 25 μg and Cloxacillin (CX) 10 μg.]* to determine the MDR strains using disc diffusion sensitivity method according to Kirby-Bauer as described in the guidelines of the National Committee for Laboratory Standards (NCCLS)[2]. For Oxacillin inhibition zones around the disc were measured after 24 and 48 h using the following breakpoints: susceptible (S) ≥ 18 mm; resistance (R) ≤ 17 mm [3].

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c) Honey batches

Six row full strength different unprocessed honey batches were used in the study; A (marjoram), B (cotton), C (fennel-1)**, D (fennel-2)**, E (trefoil-1)** and F (trefoil-2)** as well as G (10% propolis- Fennel honey mixture) as 10% w/v bee propolis powder*** in fennel honey. To study the synergistic action and to detect the sole antimicrobial action of propolis, 50 mg propolis powder (the added amount in propolis honey mixture) was tested plain for its MIC &MBC against all tested strains.

d) Determination of MIC

Three to six strains of the most MDR strains from each species were chosen for the in vitro MIC & MBC study. Honey batches were investigated for their MIC & MBC against the chosen isolated Staph. sp. strains where 1 ml of the tested honey was used in bifold dilution method[5] with series of 6 tubes containing 1 ml of Mueller Hinton broth (Accumix – Verna, India) to achieve final dilutions of 50, 25, 12.5, 6.25, 3.12 and 1.62 % v/v. Standard bacterial inoculums (5x105) of the chosen isolated Staph. spp. were inoculated into all 6 dilutions post thorough honey mix. The inoculated tubes were over night incubated at 37°C. The highest dilution of the tested honey to inhibit growth (no turbidity in the tube) was considered as the MIC value of this honey batch against the tested bacterial species.

e) Determination of MBC

From all tubes showed no visible signs of growth / turbidity (MIC and higher dilutions), loopfuls were inoculated onto sterile Mueller Hinton agar (Accumix – Verna, India) plates by streak plate method. The plates were then overnight incubated at 37°C . The least concentration that did not show any growth of tested organisms was considered as the MBC value of the tested honey against the tested bacterial species.

f) Statistical analysis

Mean values, standard deviation (SD) and ANOVA analysis were adopted by means of PASWV.18 (2010, spss Inc, Chicago, Illinois, USA). Results were considered statistically significant when $P > 0.05$ and highly significant when $P > 0.01$.

*Antibiotic sensitivity discs were purchased from Bioanalyse - Turkey.

**Fennel or Trefoil 1 & 2: honey batches were collected from two different pasture locations.

***Chinese bee propolis provided kindly from Plant Protection Research Institute (PPRI)- Assiut unit.

III. Results

The present study was conducted with 64 Staph. sp. strains isolated from bovine mastitis, where the most MDR strains which showed MDR pattern > 6 antimicrobials were chosen and be prepared for MIC & MBC study as shown in Table (1). Against Staph. sp., all tested strains - which showed at least 6 MDR pattern - were sensitive to all tested honey batches with MICs ranged from 20.83% (trefoil-2) up to 33.33% (fennel-2) (Fig 1) and MBCs from 37.92% (cotton) up to 45.83 % v/v (for both fennel-1 & trefoil-1) (Fig 2). However, 10% propolis fennel honey mixture showed the most favorable results as the lowest both MIC and MBC (13.96% & 28.26 % v/v respectively) with highly significant differences $p>0.01$ (Fig 1&2). Propolis powder alone gave no any bacterial inhibition. S. aureus showed the lowest MIC (13.3%) & MBC (27.1%) v/v with highly significant differences $P > 0.01$ (Fig. 3&4) among all tested Staph. sp. By the statistical analysis for the antibacterial activity of different honey batches against different Staph. sp., it was found that propolis honey mixture had the lowest MIC value against both coagulase positive Staph. sp. (S. intermedius and S. aureus) allover the present study as 6.2% & 7.25% v/v respectively with highly significant differences $P >0.01$ (Fig. 5), while MBC values were 12.5 & 14.58% respectively (Fig. 6).

Table 1: *Staph. sp.* isolated from bovine clinical mastitis and MDR pattern of the honey tested strains

Isolates	Antimicrobial testing		Honey tested strains	
	No.	MDR ≥ 5 antimicrobials	No.	MDR pattern
S. aureus	35	30	6	9 antimicrobials
S. intermedius	9	5	3	(6 - 7) antimicrobials
S. saprophyticus	11	8	5	(7 - 9) antimicrobials
S. epidermidis	9	8	5	8 antimicrobials
Total	64	51	19	

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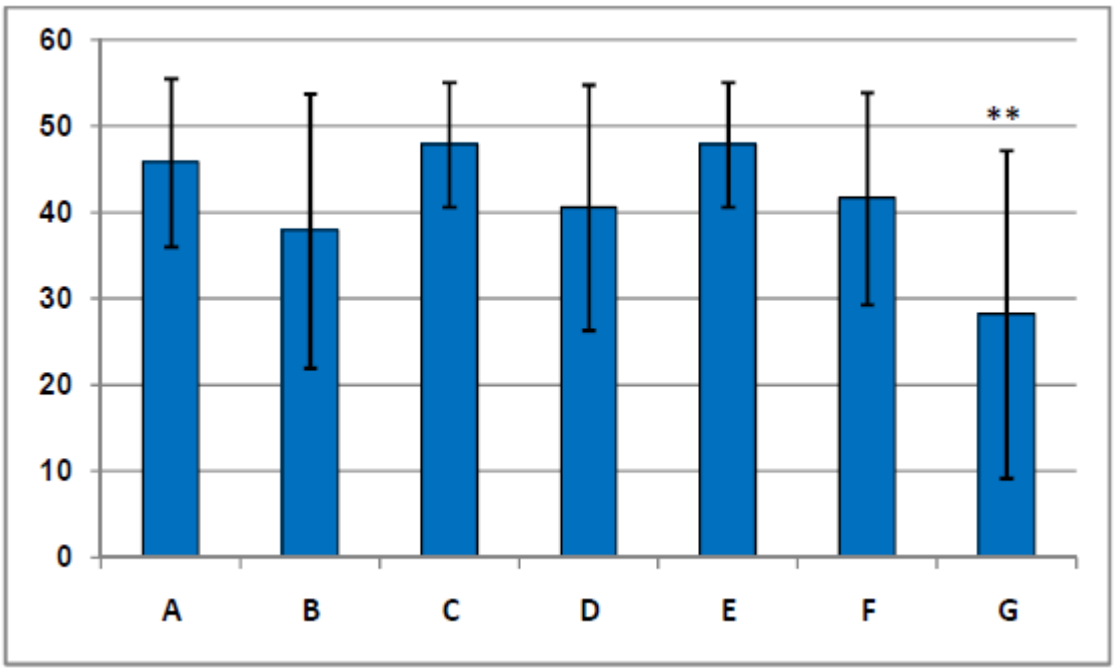

Figure 1 : MIC values of different honey batches against *Staph. Sp*

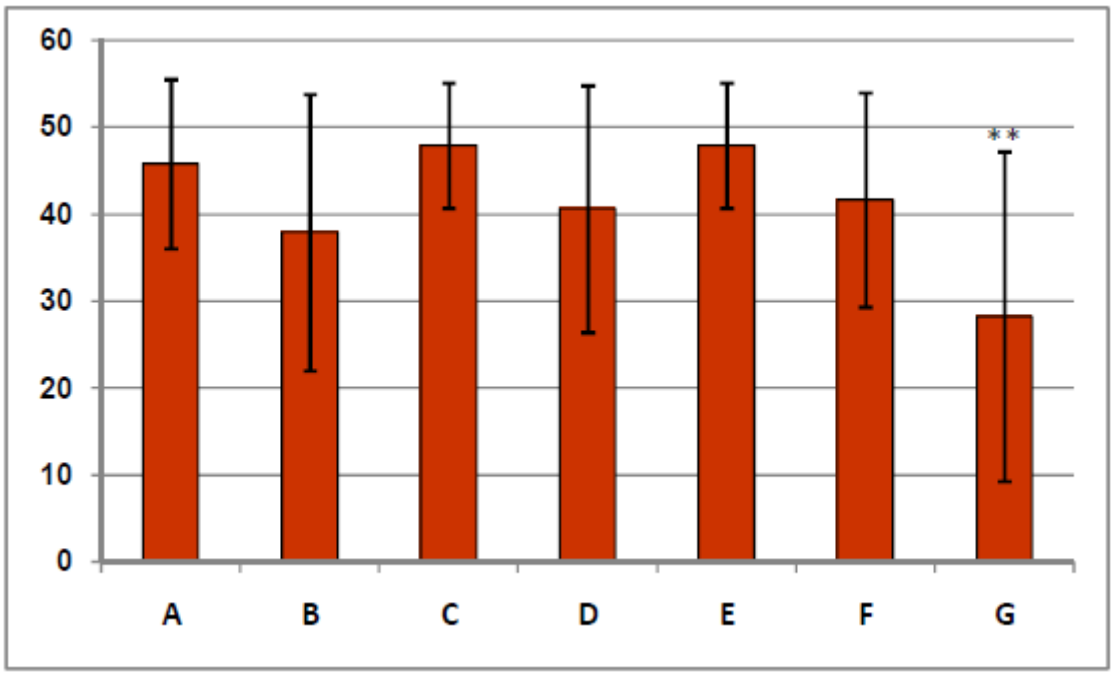

Figure 2 : MBC values of different honey batches against Staph. Sp

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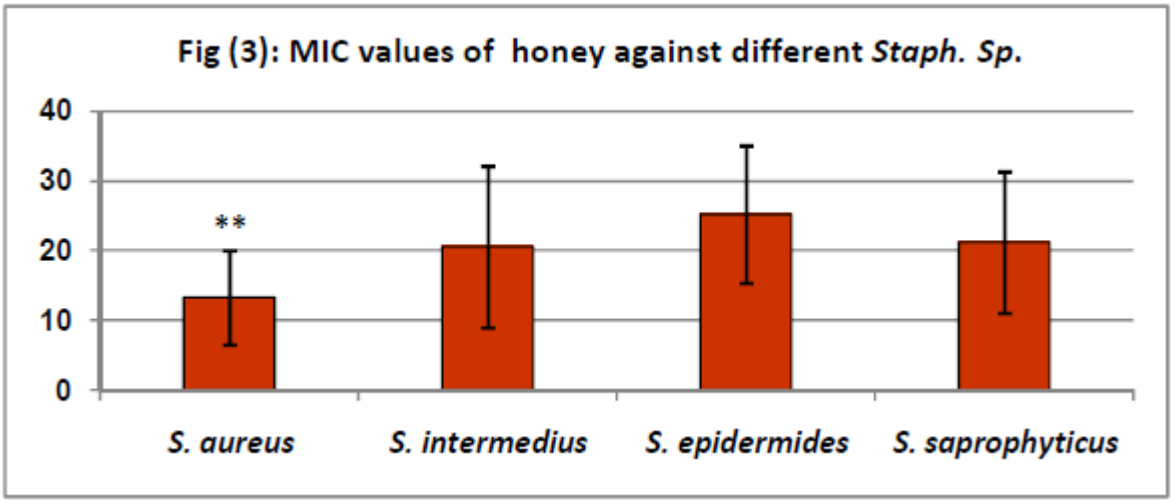

Figure 3 : MIC values of honey against different *Staph. Sp*

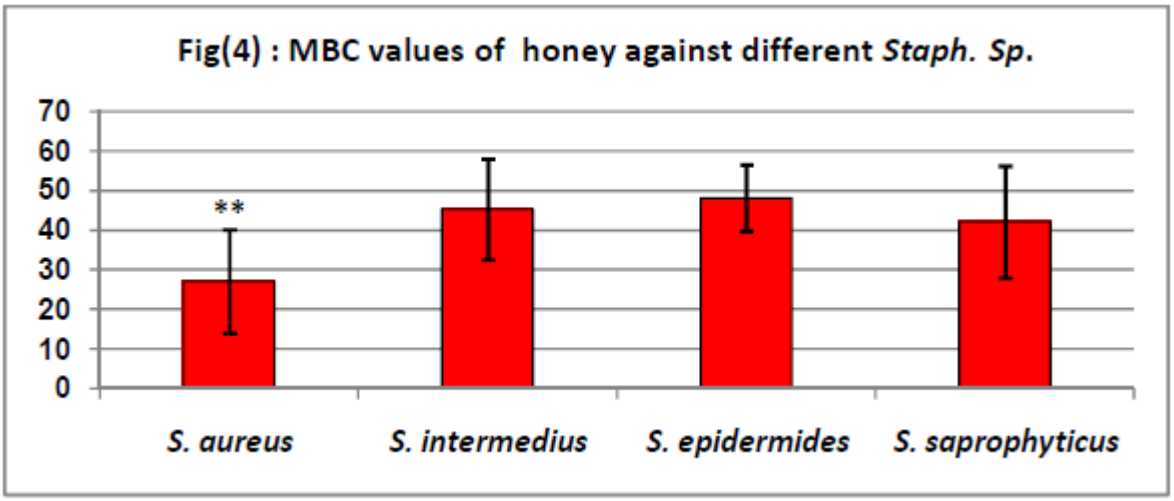

Figure 4 : MBC values of honey against different Staph. Sp

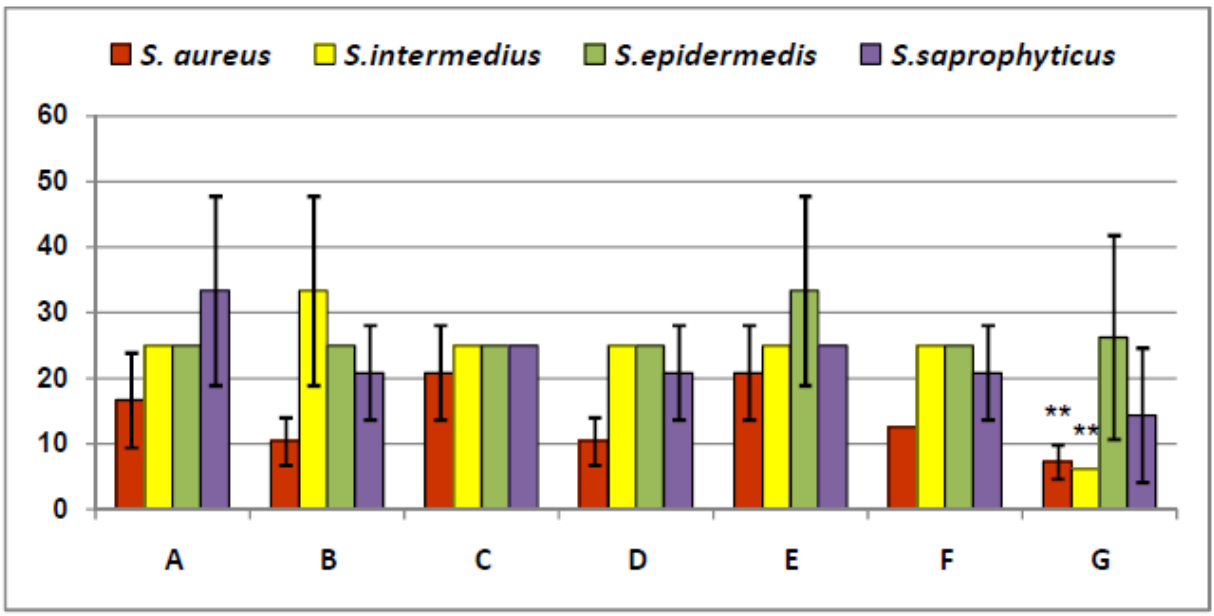

Figure 5 : MIC values of different honey batches against different Staph. Sp

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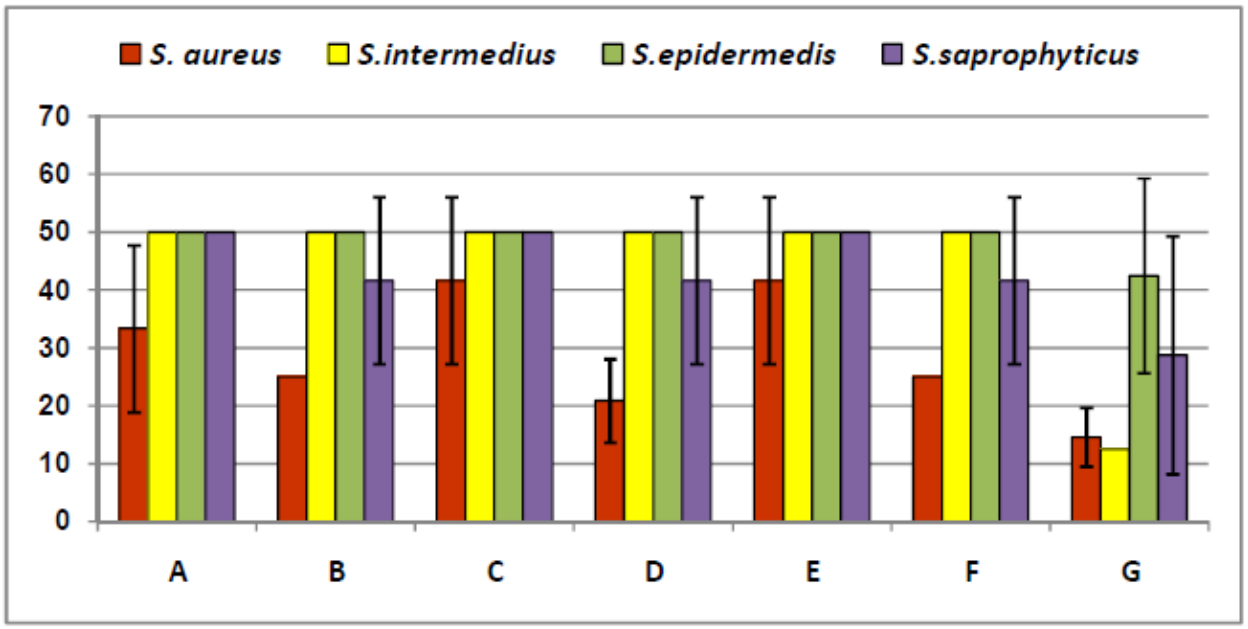

A: marjoram B: cotton C: fennel-1

D:fennel-2 E: trefoil-1 F: trefoil-2

G:propolis/honey mixture

Figure 6 : MBC values of different honey batches against different *Staph. Sp*

IV. Discussion

Veterinary apitherapy nowadays is documented either in dairy [6,16] or broiler[39] farms rather than in immunomodulation performance[12]. Concerning to apitherapeutic antimicrobial activity, it is widely documented as mentioned in the above premise. MRSA contribute the most predominant isolated species from bovine mastitic milk [40] and is widespread pathogen. It is of great concern for human public health hazard threatens transmission among dairy farm workers or their environments [32]. The emergence of antibiotic-resistant bacteria leads to the re-examination of earlier remedies such as honey [9] or propolis [26]. The antibacterial potency differences among different studied honey samples could be attributed to the natural variations in floral sources of nectar and the different geographical locations since honey micro components possess physicochemical and phytochemical characteristics resulting in its potency that differs associated with botanical and geographical origins [18]. Different honey samples of different botanical or geographical origins; Egyptian honey had MIC & MBC values as 12.5 & 50% v/v [7], Malysian honey as 5% & 6.25% w/v [38], UK Manuka honey had MIC as 6% w/v [22] and Ethiopian honey as 6.25% w/v[27]. Honey antimicrobial action involves several mechanisms but mainly the presence of bacteriostatic and bactericidal action is due to production of hydrogen Peroxide [28]. H2O2 alone may not be sufficient to the full activity [21], since it is in conjunction with other unknown honey components produce bacterial cytotoxic effects and DNA degradation. The concentration of polyphenols and H2O2 in different honeys may be of critical importance for bacterial cell survival [20]. Another mechanism of honey antimicrobial activity may be due to its lysosomal contents [35] or micro components as polyphenols, phenolic acids and flavonoids [14] or due to increase in cytokine release [19]. On the other hand, the mechanism of propolis antimicrobial activity is more complex and might be attributed to the synergistic activity between its various potent biological ingredients[17] that more than 300 compounds mainly phenolics and flavonoids [8]. It was found that propolis affects bacterial cytoplasmic membrane, and it inhibits motility, enzyme activity, cell division, and protein synthesis through inhibition of RNA-polymerase which can explain partially the synergism of propolis with drugs[1]. Moreover, galagin and caffeic acid derived from propolis are enzymatic inhibitor agents for bacteria[4]. Since the synergistic action might be detected when the MIC of the combination of both

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studied antimicrobial agents is lower than the MIC of each alone[17], the present study was designed to test the added propolis powder (50 mg) alone where did not inhibit the tested Staph. sp. The present study chose Egyptian fennel honey for propolis mixture as our previous studies [7,12] recommendations. Although fennel showed low results for both MIC & MBC through the present study, its antimicrobial action was maximized giving highly significant difference ($P > 0.01$) when propolis be added 10% w/v. The synergy of honey antimicrobial activity when be added to another antimicrobial was fully studied [25,9,17,24,33] and for propolis, the added flavonoids and phenolic acids - have antibacterial, antifungal and antiviral properties[11] - might maximize the action of these micro components present in honey resulting in synergy of its antimicrobial action. Fortunately, S. aureus (either MRSA or methicillin sensitive) which is the most predominant and virulent pathogen was the most sensitive Staph. sp. to honey antimicrobial action with highly significant. It is documented and proved that S. aureus was the most sensitive species to the antimicrobial activity of honey among all tested bacterial species studied [25,15,13].

V. Conclusion

It was concluded that all tested MDR Staph. sp. were sensitive to the antimicrobial activity all tested honey samples, where S. aureus was the most sensitive one among the four tested Staph. sp. It was concluded that adding 10% w/v propolis powder to the chosen honey patch would maximize its antimicrobial activity with highly significant difference. The promising results encourage the utilization of propolis extract in combination with the chosen honey patch for
treatment of subclinical bovine mastitis to achieve the synergistic antimicrobial action.

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Assessment of the Calorific Value of Charcoal from *Gmelina Arborea (Roxb), Tectona Grandis (Linn)* and *(Bentham)*

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Abstract- This study was carried out to determine the calorific values of charcoal produced from the wood of *Gmelina arborea*, *Tectona* grandis and *Pentaclethra macrophylla* (Bentham). The purpose of this study is to find out the calorific values of charcoal from these different species at different levels and positions in the tree. The wood species were subjected to three different treatments. The results shows that there were significant differences ($p<0.05$) between the treatments and parameter measured (Top sapwood, Middle sapwood, Base sapwood, Top heartwood, middle heartwood and Base heartwood). T_3 gave the highest calorific value of charcoal produced, while middle heartwood gave the best result for all treatments. Based on this investigations T_3 (*Pentaclethra macrophylla* (Bentham) can be used for charcoal production. Forest depletion by man is one of the causes of climate change. Bearing this in mind, we need to exploit our forests in a sustainable manner. Households, especially in the rural settlements rely very much on fuel wood for cooking. This has contributed in no small measure to the depletion of our forests. Extension agencies need therefore to take advantage of the result of this study toencourage households to use charcoal, especially that derived from *Pentaclethra macrophylla.*

Keywords: *calorific value, charcoal, gmelina arborea, tectona grandis, pentaclethr macrophylla.*

GJSFR-D Classification : *FOR Code: 620399*

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Keywords: calorific value, charcoal, gmelina arborea, tectona grandis, pentaclethr macrophylla.

I. Introduction

Resources of the tropical forest are vast and these resources vary (Etukudo, 2000). Throughout the tropics, people have depended on these (indigenous) flora and fauna for food security and a host of daily needs from medicine to fibre (Etukudo, 2000). Trees are the dominant component of tropical forest and they produce timber which is termed the major forest produce (Akande *et al,* 1998).

In 1969, the FAO panel of experts on forest Genetic resources assigned priority for improved utilization and conservation to *Gmelina arborea*. This reflected the fact that many tree planters considered *Gmelina* to be a very promising species due to ease and cheapness of establishment, rapid early growth, expectations of early returns and promising wood characteristics, including high durability and good yield and quality of pulp.

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The African oil bean *Pentaclethra macrophylla* (Bentham) is a tropical tree crop found mostly in the Southern and Middle Belts regions of Nigeria and in other Coastal parts of west and Central Africa. It belongs to the family *leguminosoe*, and sub-family *Minmosoidea* (Keay, 1989) with no varietal characterization. The tree is recognized by peasant farmers on these parts of the country for its soil improvement properties and as a component of an agro-forestry system (Asoegwu *et al, 2006*).

Oka for (1982) reported that *Pentaclethra macrophylla* is a food tree species for outlying farms. In the forest zone presently, they grow either wild or semi-wild with no organized cultivation in plantations or Orchards in Nigeria.

Tectona grandis (*Teak*) is a woody tree species from the family *verbenaceae*, has a high proportion of heart wood, which trends to be dark and of a uniform golden brown colour. Teak is easy to tend, has fast growth rate, it is a tree that has been found to possess good quality of timber for construction, furniture work, building, and as a sources of pulp for the manufacture of paper generally. Teak is generally good for any work which involved wood. Past research works have revealed that positive responses have been obtained in some of the pioneering fertilizer experiments conducted by (Nwoboshi, 1973, Kadeba 1978).

Half of the world's population uses biomass fuels for cooking. In 1992, 24 million tones of charcoal were consumed worldwide. Developing countries account for nearly all of this consumption, and Africa alone consumes about half of the world's production. Charcoal production has increased by about a third from 1981-1992, and is expected to increase with the rapidly growing population in the developing world. Charcoal is produced by a partial chemical reduction of wood under controlled condition. The yield of charcoal by weight is usually about 20%-30% of the dry weight of the wood used and the yield by volume is 50% (Earl, 1975). The techniques for making charcoal range from simply covering and burning wood with soil and fuel to the use of very sophisticated automatic retorts and furnace. In countries with surplus manpower and plentiful forest resources, the use of portable steel Kiln to make charcoal offers a low cost investment opportunity.

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According to Earl (1975), the physical and chemical property of charcoal depends on those of the original materials from which it was made and on the carbonization process. Most users prefer charcoals that do not break easily and will continue to emit heat for a long time.

A lot of economic benefits may be derived from the managed use of forest energy resources directly, indirectly and intangibly. According to FAO (2000) the most obvious direct benefits is that obtained from home production of valuable fuel. Equally important is the effect which charcoal production may have in reducing the cost of sivicultural operation by reducing the cost of removal of cut-down wood which therefore increases the total profitability of forest resources. Indirect benefits are in the areas of employment, provision of employment is a benefit not only because of the extra production obtained from more efficient use of human resources but also because of the possibility of collecting taxes for government projects which will in turn generate more employment. The intangible benefits are difficult to quantify but include such benefits as the encouragement of self reliance, conservation of the world fossil fuel resources and reduction of environmental pollution, and reduction of waste.

The charcoal has energy value of 7.1 calories which is higher than that of wood which stands at 3.5 calories (Earl, 1975). This means that charcoal cooks food faster than fuel wood when used domestically by rural households. In spite of this superior quality over fuel wood people feel it does not produce charcoal of various calorific value**.** It is necessary to correct these perceptions on charcoal especially with regards to the ones that will prove to have higher and lower calorific values. Because charcoal produces more heat than wood, it needs to be investigated to find out what species, what part of the tree produces the highest so that efforts can be made to mass produce them in efficient ways.

II. Objectives of the Study

The major objective of this study is to determine the calorific values of charcoal produced from the wood of *Gmelina arborea, pentaclethra macrophylla (bentham) and Tectona grandis*. Specifically, this study aims at determining:

i. The calorific value of charcoal from the axial direction
ii. The calorific value of charcoal from the horizontal direction.
iii. The calorific value within species variation.
iv. The calorific value among species variation.

III. Materials and Methods

a) Study area

The study was carried out in Delta State University, Asaba campus and Sapele, both in Delta State. The study area is in the rainforest zone. The climate is the equatorial type with two seasons in a year, the wet and dry seasons.

The mean annual rainfall is 1250mm with a bio-modal distribution. The wet seasons starts from April and ends in October. Annual temperature ranges from an average minimum of 21.3°C to an average maximum of 31.2°C. The study area in Delta State lies between latitude 06°05^{1}N of the equator and longitude 08°02^{1}E and 06°07^{1}E of Greenwich meridian.

b) Experimental Layout

Experiment I was arranged in 3*x*3*x*2 factorial in a completely randomized design (CRD). With three factors experiment (three species; *Gmelina arborea, Tectona grandis, Pentaclethra macrophylla*), three positions (base, middle relative to the top of the tree),quality of the wood (sapwood or heartwood).

Experiment II was arranged in a one-way analysis of variance

c) Source of wood collection

Samples were purposely selected from the plantation of the Delta State University Asaba Campus for the study.

d) Materials used

1. Motor saw
2. 50m distance measuring tape.
3. Haga altimeter
4. Diameter girth tape
5. Ballistics bomb calorimeter

e) Parameters measured

The parameters collected in this experiment include:

i. Total height of the tree
ii. Length of the felled tree
iii. Diameter at breast height of the tree

f) Procedures for the experiment

The height measurements of each of the three trees species were taken with the aid of the Haga altimeter. Felled trees were cut into three discs i.e. at diameter at breast height (DBH), middle and top with the motor saw. Samples of wood were removed from the disc for the two experiments.

i. Axial: Two (2) specimens from the heartwood and sapwood were collected each at three (3) different positions i.e. at breast height, middle and top.
ii. Horizontal: From the diameter at breast height, five (5) different positions from the pith to the bark.

The discs were replicated three (3) times wrapped and marked separately and were taken to Sapele for charcoal production in earth kiln.

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The charcoal was taken back to Asaba campus for measurement of the calorific value by using ballistic bomb calorimeter. It was re-burnt in the ballistic bomb calorimeter to determine the calorific values.

IV. Determination of Calorific Values of the Charcoal

Gross calorific values of the charcoal specimens (segments) were determined by using ballistic bomb calorimeter. Three determinants were carried out from each segment and the mean values were computed. This was done as applied by (Akachukwu, 2005) in his experiment on calorific values of various wood species.

V. Data Analysis

Data collected was been subjected to analysis of variance (ANOVA) and significant means separated using least significant difference (LSD).

VI. Results

Table 1 shows the results of calorific values of charcoal produced from the wood of *Gmelina arborea, Tectoria grandis* and *Pentaclethra macrophylla*. The parameters considered include: Top sapwood, middle sapwood, base sapwood, top heartwood, middle heartwood and base heartwood. Result on top sapwood indicated significant ($p<0.05$) difference among treatment means (Table 1).

Top sapwood in treatment 1 were lower than treatment 2 while treatment 3 were higher.

Result on middle sapwood revealed that treatment means were significantly ($p<0.05$) different. Middle sapwood for treatment T_1 were lower while T_2 and T_3 were higher numerically.

Result on Base sapwood collected were significantly ($p<0.05$) affected. The base sapwood in T_3 was significantly ($p<0.05$) higher in value than other treatments.

Table 1 : Calorific Values Of Charcoal Produced From The Wood Of *Gmelina Arborea, Tectona Grandis* And *Pentaclethra Macrophylla*

Parameter	1	2	3
Top sapwood	7124.33^c	7216.33^b	7402.00^a
Middle sapwood	6772.00^c	7351.67^b	7616.67^a
Base sapwood	6939.00^c	7323.00^b	7599.67^a
Top heartwood	7250.67^c	7496.00^b	7575.67^a
Middle heartwood	6788.33^a	7043.33^a	7121.33^a
Base heartwood	6960.00^b	7419.00^a	7591.00^a

abc, means with different superscript within same row are significantly ($p<0.05$) different.

Result on Base sapwood collected were significantly ($p<0.05$) affected. The base sapwood in T_3 was significantly ($p<0.05$) higher in value than other treatments.

Result obtained for Top heartwood shows that the treatment means were significantly ($p<0.05$) different. However, top heartwood from T_3 have higher numerical values than other treatments.

The middle heartwood shows that treatment means were not significantly ($p>0.05$) different. Middle heartwood from T_2 and T_3 were significantly higher ($p<0.05$) than those of T_1 which has a lower value of 6788.33.

Result obtained for Base heartwood shows that treatment means were significantly ($p<0.05$) different. Base heartwood from T_2 and T_3 were found to be higher than those of T_1.

Table 2 : calorific value of charcoal produced from different pith position of *Tectona grandis, Gmelina arborea, Pentaclethra macrophylla*

Pith to bark	Gmelina	Tectona	Pentaclethra
1	5852^b	6724^a	6985^a
2	5765^b	7123^a	7250^a
3	6183^c	7245^b	7433^a
4	6053^c	7400^b	7951^a
5	7231^b	7622^b	8212^a

abc, means with different superscript within same row are significantly ($P<0.05$) different

Result on pith to bark on position indicated significant ($P<0.05$) difference among treatment means. Result on pith to bark on position 2 indicated significant ($P<0.05$) difference among the treatment.

Result on pith to bark on position 3 revealed that treatment means were significantly ($P<0.05$) different, for treatment T_1 were lower while T_2 and T_3 were higher numerically.

Result on pith to bark on position 4 shows that the treatment means were significantly ($P<0.05$) different, however, T3 have higher numerical values than other treatments.

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Result on pith to bark on position 5 shows that treatment means were significantly ($P<0.05$) different. T_3 was formed to be highest in term of numerical value than those of T_1 and T_2.

VII. Discussion

The better performance in the calorific values of charcoal produced from the experiment I and II, these species of woods *Gmelina arborea* (T_1), *Tectona grandis* (T_2) and *Pentaclethra macrophylla* (T_3).

Charcoal produced by *Pentaclethra macrophylla* (T_3) yield better results compared to those produced by *Gmelina arborea* (T_1) and *Tectona grandis* (T_2), T_3 has more heat locked up than T1 and T_2 and this is in agreement with FAO (2001), report that the gross calorific value of a substance is the number of heat units that are liberated when a unit weight of that substance is burnt in oxygen, carbon dioxide, sulplur dioxide, nitrogen, water and ash. And these also in line with the finds of Foley (1986) which state that, the energy efficiency of a charcoal is dependent upon many factors: with type, moisture content, wood species, wood arrangement and the skill of the producer. All these factors is what resulted to why T_3 yield more result than T_1 and T_2

VIII. Implication for Forestry Extension /Advisory Service

Forest depletion by man is one of the causes of climate change. Bearing this in mind, we need to exploit our forests in a sustainable manner. Households, especially in the rural settlements rely very much on fuel wood for cooking. This has contributed in no small measure to the depletion of our forests. It is also observed that people hardly plant trees. This has contributed to the depletion of our forests. Instead of continuing to exploit the forests indiscriminately for cheap source of fuel, charcoal is a promising alternative source of cheap fuel. These can be purchased from saw mills where they are prepared for sale at cheap rate. Charcoals are also environmental friendly unlike kerosene which is also very costly. Forestry extension agencies need therefore to take advantage of the result of this study to encourage households to use charcoal, especially the one derived from *Pentaclethra macrophylla.*

IX. Conclusion and Recommendation

The results obtained showed that there were significant ($p<0.05$) differences in treatments (T_I, T_2 and T_3 for experiment I and II) which could be related to the age of the wood.

On the other hand, there were no significant ($p>0.05$) differences in Middle heartwood among species in different treatments. Thus, it could be concluded that calorific values of charcoal produced from the wood of *Gmelina arborea, Tectona grandis* and *Pentacletura macrophylla* (*bentham*) gave the same result means that the age and species of the wood had no influence on the calorific values at T_3 (*pentaclethra macrophylla*).

Therefore, calorific values of charcoal produced from pentaclethra wood species are therefore recommended for both Experiment I and II.

Forestry extension advisory service should encourage the use of charcoal through advocacy and legislation. This will save our forests.

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The Effect of Cutting Time under Different Amounts of Nitrogen on Yield and Yield Components of Barley

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Abstract- The present research was aimed to study the effect of different levels of inorganic fertilizer N on the yield and yield components of barley varieties at Gorgan Research Station, Iran in 2014-2015 year. A split plot layout within randomized complete block design with 3 replications was used. Main plot were different level of nitrogen fertilizer (35,70 and 105 kg N ha^{-1}) from urea source, and sub plot were different varieties (Line 3, Line 7 and Line 17). Condition represented the effect of nitrogen was significant on feed and grain yield, Protein yield, Plant height, HI. Maximum Plant height, HI and grain yield was recorded in sterling. The highest Feed yield, grain yield was observed in Line 5 variety. Nitrogen applied at the rate of 105 kg N ha^{-1} resulted in maximum Plant height, Harvest Index, feed yield, grain yield, Protein yield.

Keywords: *barley, forage, grain yield.*

GJSFR-D Classification : *FOR Code: 070199*

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The Effect of Cutting Time under Different Amounts of Nitrogen on Yield and Yield Components of Barley

Alireza Alazmani

Abstract- The present research was aimed to study the effect of different levels of inorganic fertilizer N on the yield and yield components of barley varieties at Gorgan Research Station, Iran in 2014-2015 year. A split plot layout within randomized complete block design with 3 replications was used. Main plot were different level of nitrogen fertilizer (35,70 and 105 kg N ha^{-1}) from urea source, and sub plot were different varieties (Line 3, Line 7 and Line 17). Condition represented the effect of nitrogen was significant on feed and grain yield, Protein yield, Plant height, HI. Maximum Plant height, HI and grain yield was recorded in sterling. The highest Feed yield, grain yield was observed in Line 5 variety. Nitrogen applied at the rate of 105 kg N ha^{-1} resulted in maximum Plant height, Harvest Index, feed yield, grain yield, Protein yield.

Keywords: barley, forage, grain yield.

I. Introduction

Barley (*Hordeum vulgare* L.) is the major cereal in many dry areas of the world and is vital for the livelihoods of many farmers. Barley is an annual cereal crop and grown in environments ranging from the desert of the Middle East to the high elevation of Himalayas (Hayes *et al.,* 2003). It is the major food source in many North African countries. In Iran, it is mainly grown for grain and straw for small ruminants during winter, with green fodder sometimes used for winter grazing. Barley can replace wheat as the dominant crop due to its tolerance to drought and salinity. Barley assumes fourth position in total cereal production in the world after wheat, rice, and maize. Barley is more productive under adverse environments than other cereals. Barley serves as a major animal fodder, base malt for beer and certain other distilled beverages.

Excess nitrogen increased leaf area, tiller formation, leaf area index and leaf area duration and this increasing is led to much greater production of dry matter and grain yield (Ryan et al., 2009). Sylvester et al.(1990) reported that plant height of cereals increased significantly and linearly with increased nitrogen application. in an experiment on the effects of nitrogen on barley cultivars concluded the biomass-related trait of leaf area was also increased by the application of N fertilizer. Also, percent increase in lodging incidence over the unfertilized treatment was assessed. the lodging data was so variable, and it was not statistically different between treatments (Ahmad and Rashid, 2004). in a similar experiment on seed yield of barley stated seed yield is a complex character depending upon a large number of environmental, morphological and physiological characters. Grain yields also depend upon other yield components (Ryan et al., 2009), in an experiment on barley stated as expected, the main factors N and variety were significantly affected either on the yield parameters, but The interactions were less consistent.

The amount of nitrogen that a barley crop needs to maximize yield and quality will depend on the seasonal conditions, soil type, and rotational history of the soil as well as the potential yield of the crop. Nitrogen is needed for early tiller development of barley to set up the crop for a high yield potential. Cantero et al.(1995) reported that spilt N application had little effect on yield, but decreased lodging and spike population with increased grain weight. Singh and Uttam (2000) recorded increased grain yield with increase in nitrogen level. However, increasing N fertility beyond a certain limit induced lodging and ultimately decreased grain yield and its components. The aim of this study is to determining yield on cultivars of barley in different levels of nitrogen.

II. Materials and Methods

An experiment was conducted on the basis of split plot layout with completely randomized block design with 3 replications. Main plot were different level of nitrogen fertilizer 35,70 and 105 Kg N ha^{-1}) from urea source, and sub plot were different Genotypes (G1), (G2) and (G3). This research was conducted in 2014-2015 year, at research farm of farming building of Gorgan Research Station, Iran. A plot size of 2.5 m x 2 m having 6 rows, 30 cm apart was used. Phosphorus at the rate of 30 kg N ha^{-1} was applied as basal dose. All other input and agronomic practices was carried out uniformly. Nitrogen as urea (46.6% N) was applied at the above mentioned levels. It was added into three equal portions, the first part was applied in planting time and the second part was applied in double ridge Stage, and third part in booting stage. Other normal agronomic

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practices for barley production were followed. feed and grain yield, Protein yield, Plant height, Harvest Index was measured. All data are presented as mean values of three replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by (Gomez, 1994). SAS computer software was used to carry out statistical analysis. The significance of differences among means was compared by using Least Significant Difference (LSD) test.

Table1 : Pedigree genotypes at different levels of nitrogen

Genotypes	Pedigree
G1	SAHRA
G2	GLLU/ Rusewll//Caeuva
G3	FIBERDA/STE//L.527//SAwsom/GC

III. Results and Discussion

N fertilizer had significant influence on feed and grain yield, Protein yield, Plant height, Harvest Index (Table 1). Our results are in line with Le Gouis et al. (1999) and Moselhy and Zahran (2002) who reported that nitrogen application had little or no effects on days to emergence. Pervez et al. (2009) in an experiment on the effects of nitrogen on barley cultivars concluded the biomass-related trait of leaf area was also increased by the application of N fertilizer. Cultivar had significant influence on feed and grain yield, Protein content (Table 1). Singh and Uttam (2000) in an experiment on barley stated as expected, the main factors N and variety. were significantly affected either on the yield parameters, but The interactions were less consistent.

The highest of feed and grain yield, Protein yield, Plant height was achieved in 105 kg N ha^{-1} fertilizer treatment. The lowest of them related to control (Table 2). Haudhary and Mehmood (1998) reported that wheat varieties with spikes are larger and longer than the smaller and shorter grains, have greater power-sharing for photosynthetic material. Demotes and Jeuffroy (2001) showed that the highest levels in flag leaves of barley plants, the use of 35 kg N ha^{-1} respectively. The highest of feed and grain yield, Plant height, HI achieved in Line7 cultivar but the highest of Protein yield, related to Line 17 cultivar (Table 2). N fertilizer and cultivar interaction had significant influence on ear length (Table 1). The maximum of feed and grain yield, Protein yield, Plant height achieved in 105 kg N ha^{-1} and Line7 cultivar (Table 2).

More feed yield (2793 kg ha^{-1}), grain yield (4238 kg ha-1) Plant height (84/2 cm), HI (37/5 %), Protein yield (97/3 kg ha^{-1}) was produced by the application of 105 kg N ha^{-1}. Oweis et al. (1999) who observed that nitrogen application significantly affected productive tillers m^{-2}. Zeidan (2007) observed similar results for grain spike-1 in barley. Weight and number of grains $spike^{-1}$ was significantly increased with increasing N fertilization as reported by Moselhy and Zahran (2002). They further revealed that application of nitrogen fertilizer significantly increased spike length, number of grains $spike^{-1}$, 1000 grain weight, grain yield and N uptake by the crop (Demotes and Jeuffroy., 2001).

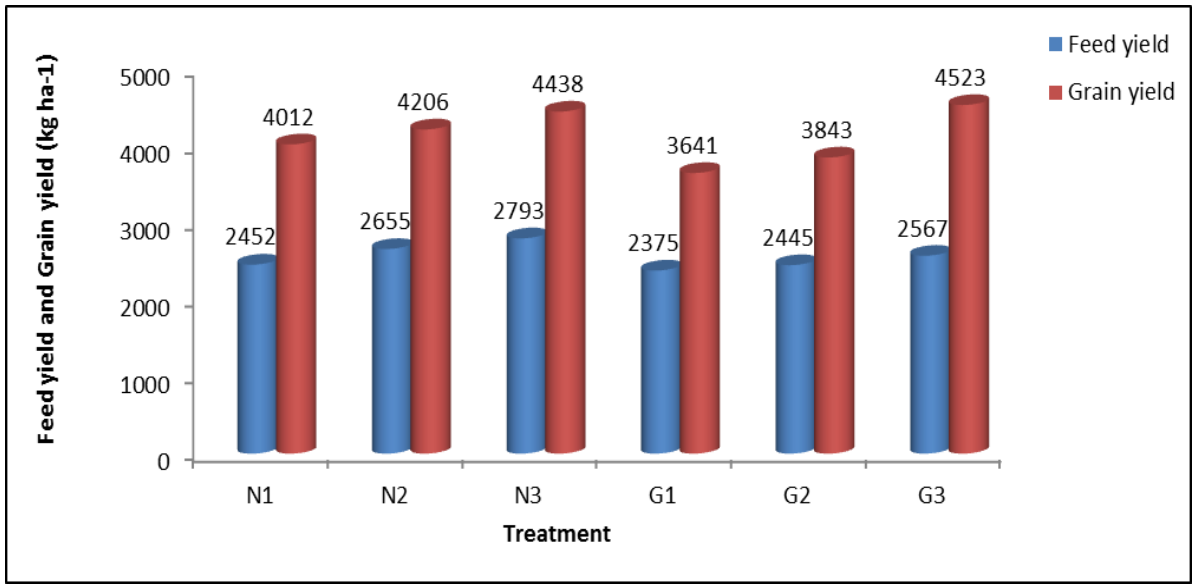

Figure 1 : The effect of nitrogen on the yield of forage and grain yield of barley

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IV. Conclusion

The results showed that, with increasing in nitrogen fertilizer, the feed and grain yield, Protein yield, Plant height was increased and led to increased production of seed yield too. So, the results show that consumption of 105 kg N ha^{-1}, is sufficient for the plant needs and produce maximum yield. also reported similar results for barley and stated Excess nitrogen increased leaf area, tiller formation, leaf area index and leaf area duration and this increasing is led to much greater production of dry matter and grain yield. Also, the maximum of seed yield, related to Line7. Then, on the basis of the results obtained, the fertilizer treatment 105 kg N ha^{-1}.

Table 1 : Analysis of variance (mean squares) for yield of barley genotypes 2012-2013, 2013-2014

s.o.v	Df	Feed yield (Kg ha^{-1})	Grain yield (Kg ha-1)	Protein yield (Kg ha-1)	Plant height(cm)	Harvest Index
Error a	6	60786	28654	321/4	20/6	10/3
year	1	595234 n.s	2593987 n.s	2114 n.s	308/3 n.s	73/7 n.s
Nitrogen	2	6008218 **	405431 **	41532 **	2054 **	80/2 **
Genotype	2	9591589**	21234 **	41672 **	5245 **	31/7 **
Interaction	4	397355 n.s	614354 n.s	4871 n.s	43/6 n.s	16/4 n.s
Error b	24	18791	50317	5224	16/6	5/45
CV	-	12/4	11/5	14/6	5/27	7/76

*ns = Non-significant * = Significant at 5% level of probability*

Table 2 : mean compare Nitrogen fertilizer for yield of barley genotypes 2013-2014

Treatment		Feed yield (Kg ha-1)	Grain yield (Kg ha-1)	Protein yield (Kg ha-1)	Plant height(cm)	Harvest Index
Nitrogen	N1	2452 c	4012 c	82/5 c	80/2 c	38/3 a
	N2	2655 b	4206 b	90/9 b	82/6 b	38/8 a
	N3	2793 a	4438 a	97/3 a	84/2 a	37/5 b
Genotype	G1	2375 c	3641 c	85/2 c	80/5 c	38/8 b
	G2	2445 b	3843 b	90/2 b	84/0 a	39/7 a
	G3	2567 a	4523 a	99/5 a	87/5 b	39/4 a

Means followed by different letter(s) in a row are significant at 5% level of probability

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"

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"

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2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

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Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

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