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Production and Characterization of Bioethanol from Sugar Cane Bagasse Blended with Mormodica Charantia Seed Oil

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Abstract: *Biofuels recently have been developed to partially substitute the use of conventional fossil fuels for the effect of the later on our climate. Climate change and its effect have become the problem of many developed and developing countries, as the whole is working towards adapting and mitigating the effect of climate change to the Environment. The focus of this research is to produce and characterize bioethanol blended with Mormodica charantia oil extracted from Mormodica charantia seeds by varying production parameters. The sugarcane bagasse was source locally from Shika town Giwa local government Kaduna State, Nigeria. In conversion of lignocellulosic biomass such as sugarcane bagasse into bioethanol; four major unit operations were used. The production of bioethanol from the hydrolysate that was obtained from hydrolysis of sugarcane bagasse comprised the fermentation of the hydrolysate with the aid of a catalyst under anaerobic condition (absence of oxygen). It can be inferred from the various analysis conducted on the bioethanol produced that the properties of the bioethanol produced compared favorably with some of the properties. The variation in some of the properties can be attributed to the nature of the feedstock (sugarcane bagasse) used in this study.*

Keywords*:* Characterazition, Bioethanol, Sugar Cane, Bagasse, Mormodica Charantia, Seed Oil

Introduction

It is believed that ethanol production for biofuels began during the 1970s-1980s, however during this period bioethanol production and its usage as a fuel re-entered the industry and product capacity increased. During 1902, in Paris the application of alcohols as fuel to power farm machinery, stoves, heaters and spirit lamps was introduced (Rosillo-Calle and Walter, 2006). When the production of stoves and spirit lamps increased in Germany, the production of

ethanol increased from 38 million litres to 98.5 million litres (Rosillo-Calle and Walter, 2006; Mussatto *et al.,* 2010). This paved the way for the use of ethanol in motor vehicles and in the year 1908, another milestone was reached when Henry Ford built the Quadricycle, also known as the Model-T which was run on ethanol (Rosillo-Calle and Walter, 2006; Mussatto *et al.,* 2010). Henry Ford also stated that, ethanol would be "the fuel of the future" (Chandel *et al.,* 2007) and "the fuel of the future is going to come from apples, weeds, sawdust-almost anything" (Chandel *et al.,* 2007). Since the 1900s and more importantly in present day, this statement is in the limelight towards alternate energy and sustaining the environment. Furthermore, the interest in bioethanol for fuel purposes was provoked by two additional events, the oil crisis during 1973-1974 and the Kyoto Protocol (Jankowski and Sandel, 2003; Alpanda and Peralta-Alva, 2010; Solomon and Krishna, 2011). The oil crisis of the Organization of Petroleum Exporting Countries embargo during the 1970s caused increasing energy prices (Alpanda and Peralta-Alva, 2010) and this called for developing ideas towards energy saving. The Kyoto Protocol was directed towards climate change with an emphasis on strategies for the reduction in carbon dioxide and other greenhouse gas (GHG) emissions, as a 70% rise in GHG emissions was reported between the years 1970 to 2004 (Jankowski and Sandel, 2003; Koh and Ghazoul, 2008; Solomon and Krishna, 2011).

In the United States, bioethanol production dates back to the 1980s and as early as 1975 in Brazil (Balat and Balat, 2009). During 2004 Brazil was the leading bioethanol producer and by the year 2006, the US became the dominant producer. Other countries contributing to bioethanol production are in Africa, the production of biofuels is regarded as an "unexploited resource" (Amigun *et al.,* 2008) mainly due to: inadequate economic and political management; underdeveloped infrastructure for commercial energy production; and the use of biomass as important food sources (Amigun *et al.,* 2008). Sub-Saharan regions of Africa such as Malawi, Swaziland, Zimbabwe and South Africa have taken the initiate to contribute to world biofuel production by developing small-scale bioethanol plants (Balat *et al.,* 2008). South Africa is ranked as the seventh leading bioethanol producer worldwide, with 102 million gallons produced in 2006. Today the energy crisis becomes one of the global issues confronting us. Fuels are of great importance because they can be burned to produce significant amounts of energy. Many aspects of everyday life rely on fuels in particular the transport of goods and people. Main energy resources come from fossil fuels such as petrol oil, coal and natural gas.
Bioethanol has a potential to damage combustible engines in conventional cars and truck (Yossapong, 2010). It is more corrosive than gasoline, highly susceptible and prone to picking up dirt and other contaminants that can damage fuel system engines. Thus, it is essential to blend the bioethanol with other appropriate additives in order to compensate for these shortcomings. The blend of bioethanol and *Mormodica Charantia* seed oil is expected to improve both combustion rate, efficiency and biodegradation properties under appropriate blending conditions through the study of its morphology and other characterizations. The aim of this research is to produce and characterize bioethanol blended with *Mormodica charantia* oil extracted from *Mormodica charantia* seeds.

Literature Review

Biofuel and Bioethanol

Biofuel is a type of fuel whose energy is derived from biological carbon fixation. Bio- fuels include fuels derived from biomass conversion, as well as solid biomass, liquid fuels and various biogases. Although fossil fuels have their origin in ancient carbon fixation, they are not considered biofuels by the generally accepted definition because they contain carbon that has been "out" of the carbon cycle for a very long time. Biofuels are gaining increased public and scientific attention, driven by factors such as oil price hikes, the need for increased energy security, concern over green- house gas emissions from fossil fuels, and support from government subsidies. Biofuel is considered carbon neutral, as the biomass absorbs roughly the same amount of carbon dioxide during growth, as when burnt, (Shala, 2013). The last century has brought unprecedented advances in all of life domains and, along with it, a proportional growth in energy consumption. In the last 40 years, the world energy consumption has doubled, reaching 8978.86 Million tonne in 2012 of which a share of 27.9% is attributed to the transport sector. In the same year, fossil fuels accounted for 81.7% of the world's primary energy supply (31.4% oil, 29% coal and 21.3% natural gas). Because of the continuous increase in energy consumption, environmental impact awareness, high fluctuations of oil market prices and the search for a sustainable fuel supply, biofuels are attracting more and more interest.

Biofuels are renewable energy sources produced from agricultural residues, forest biomass, energy crops, algae/aquatic biomass and other sources of organic matter that can substitute fossil derived fuels. These types of fuels have several advantages over the conventional fossil fuel like: higher combustion efficiency, sustainability and improved fuel security, stimulation of rural development, reduced environmental impact, reduced dependency on petroleum imports, conversion of wastes and residues. There are several factors that need consideration in order to ensure a sustainable, clean and conflict free energy supply competition between food and biomass production for land use and its influences on food prices; land degradability; overall environmental/economic impact – the life cycle assessment and the renewable fuel/fossil fuel ratio (output/input); social impact. Currently, the most common biofuels are ethanol (produced from crops such as corn, wheat, sugar cane and sugar beet) and biodiesel (produced from oil seeds, animal fats and algae).

Ethanol as Biofuel

Ethanol (CH_3CH_2OH) is a clear colorless liquid also known as ethyl alcohol, grain alcohol and EtOH. It is obtained through fermentation of biomass like corn, sugar beet, sugar cane and wheat (also called first generation ethanol). In order to obtain the desired purity, distillation is followed by a dehydration process. Currently, the largest ethanol producers in the world are Brazil and the USA. Ethanol can be used as fuel for internal combustion engines either directly or in blends. Making ethanol available as a vehicle fuel involves several steps: growth, collection and transportation of feedstock; production of first/second generation ethanol; preparation of E10, E15 or E85 and their distribution to the gas stations. Its production is not limited by the feedstock supply (according to Lin and Tanaka, 7-18 billion tons of lignocellulosic biomass are available for use every year) but by technical and economic challenges: due to the resistance of biomass, a relatively harsh pre-treatment process of the feedstock is required,

which causes fermentation problems; production of efficient enzymes to hydrolyze the cellulose at a cost competitive to first generation enzymes hydrolyzing starch; cost of feedstock. In order to assess the environmental performance of all life stages of a product (material extraction, processing, manufacturing, distribution, use and disposal/recycling) a so-called life cycle analysis is performed. Regarding the energy balance analysis of ethanol production, the majority of studies presented a positive value but, there are also some that state the contrary.

Different sources of biofuel

Here are 4 biofuel sources, with some of their application in developmental stages, some actually implemented:

Algae

Algae come from stagnant ponds in the natural world, and more recently in algae farms, which produce the plant for the specific purpose of creating biofuel. Advantage of algae focude on the followings: No CO2 back into the air, self-generating biomass, Algae can produce up to 300 times more oil per acre than conventional crops. Among other uses, algae have been used experimentally as a new form of green jet fuel designed for commercial travel. At the moment, the upfront costs of producing biofuel from algae on a mass scale are in process, but are not yet commercially viable, . (Emad, 2013)

Carbohydrate (sugars) rich biomaterial

It comes from the fermentation of starches derived from agricultural products like corn, sugar cane, wheat, beets, and other existing food crops, or from inedible cellulose from the same. Produced from existing crops, can be used in an existing gasoline engine, making it a logical transition from petroleum. It used in Auto industry, heating buildings ("flueless fireplaces"). At present, the transportation costs required to transport grains from harvesting to processing, and then out to vendors' results in a very small net gain in the sustainability stakes, (Emad, 2013).

Oils rich biomaterial

It comes from existing food crops like rapeseed (aka Canola), sunflower, corn, and others, after it has been used for other purposes, i.e food preparation ("waste vegetable oil", or WVO), or even in first use form ("straight vegetable oil", or SVO). Not susceptible to microbial degradation, high availability, re-used material. It is used in the creation of biodiesel fuel for automobiles, home heating, and experimentally as a pure fuel itself. At present, WVO or SVO is not recognized as a mainstream fuel for automobiles. Also, WVO and SVO are susceptible to low temperatures, making them unusable in colder climates.Biofuel: Sources, Extraction and Determination Dense algal growth in four pilot-scale tank bioreactors fed by treated wastewater from the Lawrence, Kan- sas (USA) wastewater treatment plant (photo by B. Sturm). Each fiberglass bioreactor has an operating volume of ten cubic meters of water, and is operated as an air-mixed, flow-through vessel. Nutrientrich wastewater inflows are pumped in through the clear plastic hose (blue clamp), and water outflow occurs through the white plastic pipe shown at the waterline. These bioreactors are intended to be operated year-round, as the temperature of the inflow- ing wastewater is consistently ca. 10 - 8°C. (Emad, 2013)

Agriculture wastes (organic and inorganic sources)

It comes from agricultural waste which is concentrated into charcoal-like biomass by heating it. Very little processing required, low-tech, naturally holds CO2 rather than releasing it into the air. Primarily, biochar has been used as a means to enrich soil by keeping CO2 in it, and not into the air. As fuel, the off-gasses have been used in home heating. There is controversy surrounding the amount of acreage it would take to make fuel production based on biochar viable on a meaningful scale. Furthermore, use of agriculture wastes which rich with inorganic elements (NPK----) as compost (fertilizer) in agriculture, (Emad, 2013)

Bioethanol production

Generally, the raw materials commonly used for the production of bioethanol are materials with carbohydrate content to which fermentation technology is applied. Degradation of cellulose is the breakdown of cellulose into glucose subunits. Microbiologically, cellulose is mainly degraded by an enzyme known as cellulase which is commonly produced by cellulolytic bacteria and fungi e.g *Saccharomyces cerevisiae, Aspergillus sp,Pleurotus ostreatus* (edible mushroom) e.t.c. Studies have revealed that white paper has abundance of cellulose, hence its choice for the production of bioethanol. Currently, bioethanol production is focused on sugar crops including sugar cane, sugar beets and starch crops, including wheat, potatoes and sweet potatoes, which is often based on excess agricultural production and it is generally recognized that this volume is too small in comparison with the anticipated levels of production required for total conversion of transportation fuel markets from gasoline to ethanol.

Bioethanol production

Bioethanol production from renewable resources continues to attract considerable interest as an alternative to fossil fuel (Rortrup-Nielsen, 2005; van Maris et al., 2006; Lynd et al., 2008). The research and exploitation of bioenergy, such as bioethanol and biodiesel, are not recent topics. In order to cope with the predicted depletion of fossil fuel in the near future, a number of countries have taken steps to reduce their dependence on gas and oil imports, by developing and industrializing new energy forms (de Vries et al., 2007; Groom et al., 2007). Potential energy forms include nuclear energy and some renewable and clean forms, such as solar, hydro, biomass and wind. The research and application of bioethanol for energy purposes were pioneered by the United States and Brazil.

Sugarcane (*Saccharum officinarum*) Bagasse

Energy crisis and environmental pollution that characterized overdependence on fossil fuel as source of energy motivate researchers and government all over the world to search for sustainable and environmentally friendly alternative energy sources**.** Recent upsurge interest in the demand of alternative source of energy and economic meltdown in the price of petrol in world market and advancement in science and technology facilitate the scientific research efforts toward commercial production of bioethanol. The bioethanol is the most promising biofuel from renewable resources, and it is well known that a low-cost feedstock is a very important factor in establishing a cost-effective technology (Mojovic, 2006). The production of ethanol from any lignocellulosic biomass generally involves four process steps—feedstock pretreatment, enzymatic saccharification, fermentation, and ethanol recovery.Pretreatment is one of the most expensive and least technologically mature steps in the process of converting

biomass to fermentable sugars (Saha, 2004 and Sarita, *et al.,* 2014). Sugarcane (*Saccharum officinarum*) bagasse is one of the lignocellulosic materials that composed of up to 75% carbohydrates. Small amounts of pectin, extractives and ashes were also included in biomass composition. Bagasse has high tensile strength crystalline cellulose fibers, embedded in an amorphous matrix of cellulose, hemicellulose and lignin.

Production Methods for Bioethanol

The bioethanol that was produced was produced by the following methods**.**

Biochemical Methods

The production of bioethanol is a two stage biochemical procedure of hydrolysis and fermentation, followed by product recovery via distillation (Hahn-Hagerdal, 2006; Balat and Balat, 2009; Jegannathan et al., 2009).

Hydrolysis

Hydrolysis is defined as a chemical reaction that breaks chemical bonds between the starch molecules of polysaccharides into simple sugars or monomers in the presence of water and a catalyst (Thaker and Kastner, 2004; Chandel *et al.,* 2007; Balat and Balat, 2009; Dwivedi *et al.,* 2009, Guo *et al.,* 2012).

Fermentation In ethanol production, the method of fermentation to convert sugars by microorganisms (MOs) is the oldest and frequently used industrial process (Caylak and Vardar Sukan, 1998; Paul Ross *et al.,* 2002; Malherbe *et al.,* 2007; Balat, 2009).

As early as 1750-4000BC the Egyptians and Sumerians produced dough and alcoholic beverages such as wine and beer by fermentation (Paul, *et al.,* 2002). The fermentation of milk, meat, cereals and vegetables for food products and food preservation dates back to 6000BC in the Middle East During 6000BC, in Iraq it is believed that cheese was the first fermented product.

However, the role of MOs in the fermentation process was unknown (Paul Ross *et al.,* 2002; Blandino *et al.,* 2003).

Materials and Methods

Materials

The following materials were used during the experimental exercises;

1. Sugarcane Bagasse
2. *Mormodica Charantia* seeds
3. Dilute Sulphuric acid
4. Distilled water
5. Calcium hydroxide
6. Sodium Chloride
7. Acetic acid
8. Acetone
9. Acetate buffer
10. Sodium Carbonate

Apparatus

The following apparatus were used in the research during the experimental work;

1. Weighing balance (triple beam balance, model 2016 MB)
2. Hand gloves

3. Specimen Bottles
4. Sieve
5. Stop watch
6. pH meter
7. oven
8. Mechanical grinder
9. Thermometer

EQUIPMENT

The following equipment were used during the research analyses

1. High performance liquid chromatography machine
2. Atomic absorption spectrophotometer
3. Fourier-transform infrared Spectroscopy (FTIR) Machine

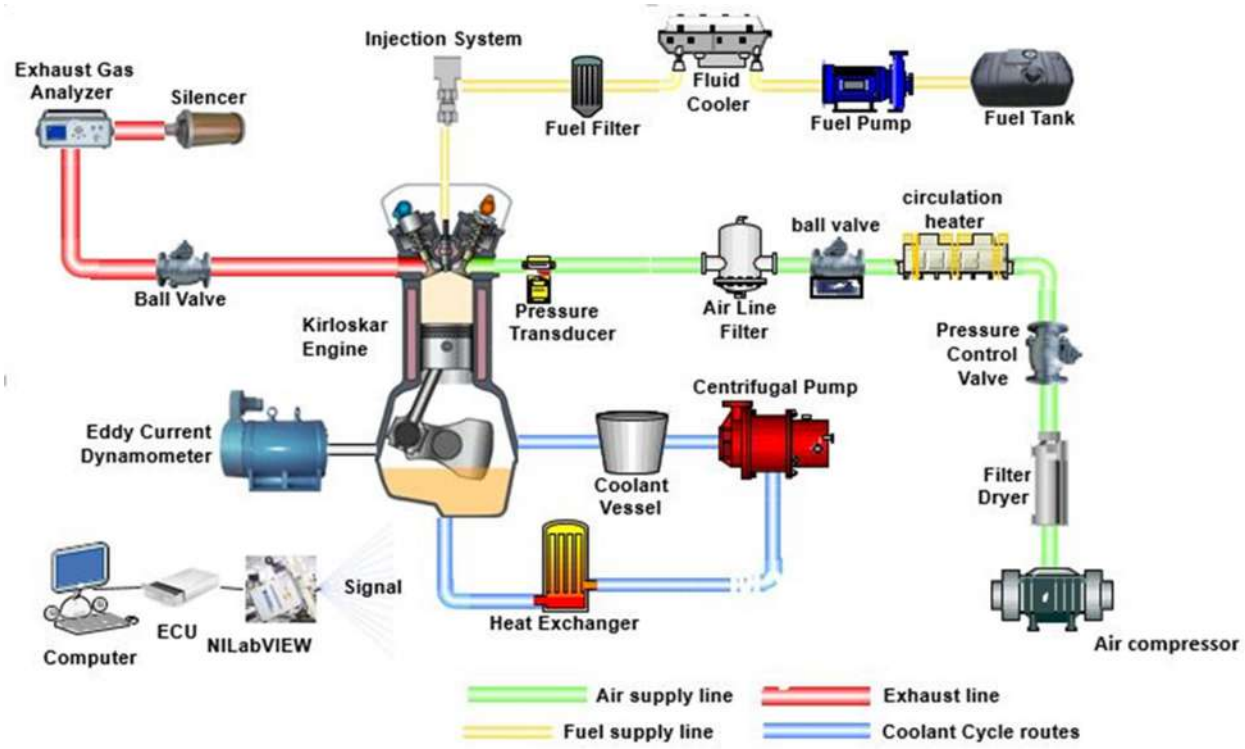

Fig. 2-1 Schematic of the proposed experimental set-up

Table 1- Engine specifications

Item	Specifications
Make	Kirloskar TV1
Type	1-cylinder, direct
Rated power	3.5 kW
Engine speed	1500 rpm
Stroke × Bore	110×87.5 mm

Displaced volume	661 cm^3
Compression ratio	17.1:1
Rated power	3.5 kW
Number of nozzles	3
Number of valves	4
Diameter of nozzle holes	0.3 mm
Injection pressure	210 bar
Injection timing	23° BTDC

Methods

The sugarcane bagasse was source locally from Shika town Giwa local government Kaduna State, Nigeria. In conversion of lignocellulosic biomass such as sugarcane bagasse into bioethanol; four major unit operations was employed. These include: pretreatment, hydrolysis, fermentation and product recovery/ distillation. Pretreatment is the first stage in the production of bioethanol. Pre-treatment as carried out by employing the dilute acid pretreatment method.

Sugarcane bagasse was chipped and grind to powder form; subsequently the dried and weighed bagasse was poured into a 500 ml conical flask and was infused with 250 ml of 4% H_2SO_4 solution.

The conical flask was introduce into a water bath while shaking and stirring for two hours at 60^oC. The pretreated bagasse was collected and wash thoroughly with distilled water to reduce the pH level (Efe *et al.,* 2011). The treated sugarcane bagasse was then be prepared for hydrolysis.

Since lignocellulosic materials contain molecules which are primarily made up of long chains of glucose, it was important to breakdown these glucose chains into smaller chains to free sugars which are fermentable. To this effect, the glucose present in the sugarcane bagasse was hydrolytically converted to monomeric sugars by means of enzymatic hydrolysis. Here 10 ml of the enzyme solution will then be introduce into the broth and allow to remain for 24 hours before fermentation.

The pH level was adjust to 4.5 by adding KOH, which is suitable for enzymatic activity (Verma and Kumar, 2011). This process was then followed by fermentation process, which involves the fermentation of monomeric sugars with the aid of fungi, bacteria or yeast in an oxygen-free environment (Verma and Kumar, 2011). The production of bioethanol from the hydrolysate that was obtained from hydrolysis of sugarcane bagasse comprised the fermentation of the hydrolysate with the aid of a catalyst under anaerobic condition (absence of oxygen). Fermentation was expected to take place for a period of time in which weighed amount of hydrolysate was put in conical flasks with which was properly seal with foil paper and left to ferment. After fermentation, CO_2 is evolved from the mixture which bioethanol was then be distilled out from it at 78^oC.Concentration of Bioethanol bioethanol, glucose and xylose was determined using high performance liquid chromatography (HPLC) analysis (HPLC) as described by (Khattab *et al.* 2013 and 2015).

An experimental design software was employed to optimize the process of production of bioethanol from the sugarcane bagasse. Variables such as was temperature, time, amount of yeast used and the effect of the amount of *momordica chrantia* seed oil on the bioethanol fuel efficiency.

All the experimental analyses was conducted in triplicate and results that was presented was as the average values with average deviation of ±0.00125. The bioethanol that was produced will then be characterize to determine the basic properties such as density, flash point, viscosity, boiling point, refractive index, sulphur content, moisture content, specific gravity, ash content and pour point.

Extraction of the *Mormodica Charantia* Seeds Oil Its Characterization

Extraction of Oil

The *Mormodica Charantia* seeds collected was dried at ambient temperature after which was grinded and weigh. Soxhlet apparatus was use fat was extracted by petroleum ether at maximum temperature of 60 ^{o}C for about 8 h (Yoshime *et al.,* 2016).

The seeds was extracted using the soxhlet extraction methods and then was characterize by gas chromatography. Other parameters were analyze such as the acidity and peroxides values was evaluated by the standard methods of the American Oil chemists' society (AOCS), (Yoshime *et al.,* 2016).

Sugarcane Bagasse and Analytical Methods

The maximum sugar that was produced was then determined using HPLC as described by (Khattab *et al.,* 2013). Liquid hydrolyzates of hemicellulose was collected by filtration, using glass microfiber filter paper GF/A 110 mm diameter. The percent of digestibility was calculated by the following fomula:

Digestibility % = [Total sugar(s) concentration g/l × volume] / [sugar cane bagasse weight (g)] × 100 (Sadat, 2015)

Blend of Bioethanol and *Mormodica Charantia* seeds oil of various composition

From the solution of the bioethanol that was produced 100/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80 volume by volume (in cm^3) was prepared as described by (Shuaibu and Okibe, 2013).

The absorbance of each was taken at 315 nm using the ultra violet spectrophotometrer, this measurement was done in triplicate in each composition and results was taken and calculated as averages. The experiment was repeated for optimal time, amount of catalyst used during the production of bioethanol, acid concentration to determine the best conditions for hydrolysis of hemicellulose (bagasse/sulfuric acid), amount of *Mormodica Charantia* seeds oil blended with the bioethanol, pH, and temperature and graph was plotted accordingly.

Fourier-transform infrared spectroscopy analysis

The solution of the Bioethanol that was produced and the *Mormodica Charantia* seeds oil that was extracted from the *Mormodica Charantia* seeds was subjected to FTIR analysis to find out the functional group (s) present in them.

Results and Discussion

Bioethanol's potential as substitute for conventional petroleum based fuels has been the issue at the tip of everyone's tongue. The focus of this research is to produce and characterize

bioethanol blended with *Mormodica charantia* oil extracted from *Mormodica charantia* seeds by varying production parameters such as fermentation time, temperature, catalyst concentration/enzyme loading and the feedstock ratio. These were considered at two coded levels; high and low levels and the results obtained were presented in Table II as below.

Table II: Variation of Parameters of the Factorial Designs

Level	Time (Hrs)	Temp (^{o}C)	Catalyst conc.(w/w)	Mass of stock
Low	48	48	1	20
High	72	72	2	30

As presented in Table 2 the optimum bioethanol yield of 14.5% was obtained under the operating conditions of 35^{o}C (operating temperature), 72 hours (fermentation time), 2g concentration of catalyst and 30 g (mass of feedstock). The optimum yield of 14.5% obtained in this study conforms to the values of 10-15% reported in literature which is in the ranges of 10-15%. The variation could be attributed to difference in reaction parameters and variation in quantity of sugarcane bagasse utilized as feedstock. From the experimental results, it was noticed that the highest ethanol yields were recorded at temperature values of 35^{o}C.

The combinations of the parameters such as combination of temperature and time, time and catalyst concentration, temperature and catalyst concentration and combination of temperature, time and catalyst concentrations shows negative effects. This implies that an increase in the values of these parameter interactions will bring about a decrease in the yield of the desired product. While other parameters combinations includes combinations of all the factors had positive influence on the yield of bioethanol.

The results as presented in Table III Indicate that the viscosity of the produced bioethanol is 1.30 which is high compared to the set limit of 1.18.

Table III: Properties of Bioethanol Produced

Properties	Units	Experimental values	ASTM Standards
Moisture content	%	0.48	20
Density	g/cm^3	0.965	0.99
Refractive Index		1.299	1.33
Flash Point	^{0}C	19.20	17.89
Viscosity		1.30	1.18
Ash Content	%	0.5	29
Cloud Point	%	20.01	24
Pour Point	%	4.68	5.01

Cloud point which is described as the temperature at which a cloud of crystals will first appear in a liquid that is cooled under prescribed conditions is also an important properties of bioethanol tested for in this study. The results as presented indicate that the pour point of the produced bioethanol from sugarcane bagasse is 20.01^{o}C which is lower than the set limit of 24^{o}C by the ASTM. Also measured is the pour point of the bioethanol produced. Pour point is an important characteristic of the bioethanol that gives the lowest operational temperature of the bioethanol.

The pour point was determined according to ASTM D97 and the value obtained as presented in Table III is 4.68^{o}C which is also lower than the set limit of 5.01^{o}C, which is an indication that the bioethanol produced can be used even in Polar Regions where the atmospheric temperature is not less than 5^{o}C. It can be inferred from the various analysis conducted on the bioethanol produced that the properties of the bioethanol produced compared favorably with some of the properties. The variation in some of the properties can be attributed to the nature of the feedstock (sugarcane bagasse) used in this study.

Conclusion

The quest for alternative energy to either substitute or complement the existing fossil fuel led energy source to the discovery and acceptance of biofuels as a renewable and environmentally friendly energy source. The technology of biofuels recently have been developed to partially substitute the use of conventional fossil fuels for the effect of the later on our climate. Climate change and its effect have become the problem of many developed and developing countries, as the whole is working towards adapting and mitigating the effect of climate change to the Environment. Today the energy crisis becomes one of the global issues confronting us. Fuels are of great importance because they can be burned to produce significant amounts of energy. Many aspects of everyday life rely on fuels in particular the transport of goods and people. Production of Bioethanol/*Mormodica charantia* oil blends of varying compositions was expected to be given out a vital way and open up a window for the research and development of producing a biodiesel oil with excellent engine performance with a no or minimal environmental risk having profitable, feasible and achievable end-used applications. The results obtained from this research study it can be concluded that sugarcane bagasse is a good and sustainable feedstock for production of bioethanol since it is an inedible material.

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Effect of Starch from Ficus Polita (Morecere) Fruit Powder on the Mechanical and Biodegration Properties for Automobile Bumper Application

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Abstract: *The polymer blends composed of Starch have been extensively investigated. However, even though much works have been done on the starch-polyblends, no data were reported on the blends of ficus polita seed powder as filler for poly-blends formulation. This study shows detailed of the physico-chemical properties, physico-mechanical properties, of starch synthesized form ficus polita fruit powder and standard corn starch filled PMMA/PVAc blends in relation to structure properties relationship was conducted to assess its applicability in plastics Industries for automobile bumper application. The research covers the Preparation of PMMA/PVAc blends of varying compositions with and without filler by dry blending and pressing in a compression moulding machine, generation of data analysis of the data obtained, measurement of the mechanical properties. The films were characterized spectroscopically using FTIR which illustrated that the decrease of the intensity of transmission spectra of (50/50 PMMA/PVAc) blend after exposure to UV radiation for 24 hours was lower than that of PMMA and PVAc. Consequently, (50/50 PMMA/PVAc) blend has improved the hardness of its homopolymer. Furthermore, effect of starch from ficus polita (morecere) fruith powder and standard corn starch have improved the hardness of the polymer blends.*

Keywords*: Starch, Fruit Powder, Mechanical, Biodegration, Automobile Bumpe Introduction*

Introduction

Blending procedures had been employed since time immemorial. The principle of blending is geared towards achieving property averaging. A blend is therefore the physical mixture of two or more substances, without a chemical bond. Polymer blends, that is, physical mixture of structurally different polymers which interact with secondary forces such as hydrogen bonding (Abba *et al.,* 2020) with or without the formation of covalent bonding. Polymer blends have been widely used in the industry because of their ability to combine in a unique material the properties of their components, at a relatively low cost when compared to the development of a new polymer. It is well-known that the properties of polymer blends are greatly influenced by the morphology that is developed during the

mixing process. The physical properties of polymer blends are controlled generally by many factors such as the nature of polymer (Mudigoudra *et al.,* 2012), blend composition (Taghizadeh, 2012) and interfacial properties such as interfacial adhesion (Jacob *et al.,* 2019; Abba *et al.,* 2020) and dispersed phase size and shape which are developed during solution blending. Likewise, the morphology of binary blends is also influenced by thermodynamics and kinetic factors. In comparison with single polymer-filler composites, binary polymer system can be viewed technologically as one of the present generation in multiphase polymer system and the fundamental aspect of polymer blends, which have substantial commercial significance as adhesives and coating materials (Chen *et al.,* 2011; Ali *et al.,* 2018). Properties of a given polymer can be improved in various ways such as blending with other polymers or by incorporation of reinforcing fillers (Mamza, 2011). Poly-methyl methacrylate (PMMA) has been widely used in architecture, automobile, air and railway transport systems due to its mechanical and superior optical properties. These wide range of applications of PMMA can be enhance by incorporation of filler into the PMMA matrix, because well dispersed filler may enhance various mechanical and physical properties of PMMA (Abdullah, *et al*., 2015 and Hasan, *et al*., 2017). Blending of polymers provides an efficient way of developing new materials with tailored properties, and thus has received much attention from academia and industry. By blending different polymers, several properties can be improved, while retaining some of the original properties. However, the desire of polymer scientists and engineers to produce improved products by blending a particular pair of polymers is often frustrated by their low compatibility. The incompatibility between polymer pairs and their consequently poor phase morphology are responsible for the poor mechanical properties of most polymer blends. As a result, there is a strong need to enhance compatibility, and the compatibilization of polymer blends by the addition of block or graft copolymer has become an important feature of polymer science and technology, (Siengchin and Road, 2012). The chemical interactions with plastics materials can be categorized into direct chemical attack, preferential chemical attack and environmental stress cracking (ESC), surface attack and swelling (Siengchin and Road, 2012). Combined effects of stress cracking and photodegradation in polystyrene was recently reported by (Siengchin and Road, 2012). Poly(vinyl acetate) (PVAc) is a polymer that is widely used in many applications, such as paints, surface coatings, food additives and adhesives for wood, paper and cloth. PVAc, with its advantageous properties, can be considered a “green” material suitable for overcoming a range of environmental challenges. First, PVAc can be produced from renewable resources. Its monomer, vinyl acetate, can be synthesized from bioethanol, which is extracted from the biomass refining process, and then further treated by dehydration, oxidation and vinylation. Second, PVAc is predominantly synthesized by emulsion polymerization, with the use of water as the dispersion medium during processing (Abba et al., 2020). This type of synthesis is a nontoxic, non-flammable and low-cost system, which is more environmentally friendly than other polymerization methods. Finally, it has been reported that PVAc is principally biodegradable which means that it was modified, hydrolysed, metabolized and finally assimilated by microbial organisms under specific conditions. However, several drawbacks of PVAc, including deficient mechanical properties, a high water or humidity sensitivity and a poor performance at elevated temperatures, pose limitations in some applications. During the last few decades, many studies have focused on improving the properties of PVAc. One of the most famous reports from 1940 by Perrin et al.7 has described in detail, for the first time, copolymerization of ethylene with vinyl-acetate: The product poly(ethylene-co-vinylacetate) has then been widely used in various types of commercial products due to its much higher ductility and lower water sensitivity compared to pure PVAc (Habeeb, 2017). Recently, Studied on the effect of nano-clay and nano-cellulose on the mechanical properties of nanocomposites with improved properties achieved by adding

nanosized reinforcements into PVAc, have received a great attention as (Habeeb, 2017) studied the effects of admixing of nanoclays and cellulose nanocrystals on the adhesive properties of PVAc, especially at elevated temperatures and in wet conditions. According to (Geng *et al.,* 2017) concluded that there was improvement in the adhesive strength and toughness of PVAc dramatically by adding only 0.1 vol% of grapheme in the the PVAcmatrix this was also confirmed by other groups such involved Mathew *et al.,* (2018), investigated the moisture absorption and its effect on the mechanical properties of PVAc reinforced by cellulose nanocrystals, (Geng *et al.,* 2017) studied the viscoelastic properties and toughness of PVAc nanocomposites with cellulose nano-fibers. Biodegradation for materials exposed to natural environment means fragmentation, deterioration of mechanical property or chemical modifications through the action of microorganisms (Kumar, *et al.,* 2016). Biodegradation is a natural process by which organic chemicals in the environment are converted to simpler compounds, mineralized and redistributed through elemental cycles such as the carbon, nitrogen, and sulfur cycles (Kumar, *et al.,* 2016).

Review of the Related Literatures

Over the past 30 years greater attention has been focused on the development of biodegradable blends and composites for the sustained of friendly and healthy environment. In recent times, biodegradable materials have gained importance particularly for the protection of the environment from ever increasing plastic waste (Okada, M. *et al.,* 2000 and 2004 Srabayeeta, B.R. *et al.,* 2015). A number of biological materials may be incorporated into biodegradable polymer. The main characteristic of biodegradable polymers is that they are biodegradable through the action of the microorganism in appropriate environmental conditions. When in contact with the biodegradable polymer, the microorganisms produce enzymes that break the material in progressively smaller segments reduces its average molecular mass, favouring its degradation in the environment. Biodegradable materials can be completely degraded into natural ecosystems such as active sludge, natural soil, lake and marine. Petroleum-based synthetic polymers are widely used in modern society. Many of the mechanical, physical and chemical properties of plastics make them ideal materials for a variety of products and applications. Various approaches to render synthetic polymers biodegradable have been considered. However, the annual worldwide disposal of approximately 150 million tons of petrochemical plastics in commonly used commodities such as polyolefin in packing, bottle and moulding products is a significant environmental problem, especially with the continuously increasing production and consumption of these materials. Most widely used alkane-derived plastics have poor biodegradability and may have lifetime of hundreds of years when buried in typical solid-waste sites. The most attractive renewable natural polymer resource is starch because of its low cost, wide spread availability and potential for mass production from renewable resources. Research on biodegradable plastics based on starch began in the 1970s and continues even today at various laboratories all over the world. Starch satisfies the requirements of having adequate thermal stability with minimum interference in melt properties and negligible disturbance of product quality. Starch is the mixture of amylase and amylopectin. The most important industrial sources of starch are wheat, corn, tapioca, potato and rice. The use of starch to partially replace synthetic plastics will not only reduce the

dependence on petrochemicals but also reduce plastic waste. However, biodegradable plastics from starch cannot compete with conventional petroleum-based plastics because of their poor mechanical properties. It is known that starch must be combined with other materials, like synthetic polymers, to produce satisfactory plastics because starch alone is brittle, moisture susceptible and difficult to process (Liu *et al.,* 2003). Although, various approaches have been attempted to utilize starch commercially for making biodegradable plastics, almost all have involved compounding starch in some form with synthetic thermoplastics. Incorporation of starch into a polyolefin matrix was proposed by Griffin as an effective means of accelerating the deterioration of plastics under biotic environmental exposure conditions (Srabayeeta, B.R. *et al.,* 2015 and Mondal, R. 2016). The development of polymer science and technology adds to traditional disciplines a new knowledge and its application for practical purposes of increasing importance. In one of the previous works, conducted using Scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) by (Gao, J., *et al.,* 2012), poly (vinylacetate) (PVAc), (10 wt%) has enhanced both mechanical and compatibility of PPC and PLA blends . *Ficus polita* is a tropical African evergreen shrub or small tree belonging to the family Moraceae, and usually growing up to 15 metres tall, and sometimes to 40 meters tall. The leaves are occasionally harvested from the wild for food. Traditionally the fruit and young leaf are chewed for dyspepsia (Kuete *et al*., 2011). The young leaves are also edible and the bark and roots infusions are used in treatment of infectious diseases, abdominal pain, dyspepsia and diarrhoea like many of the species of the Moraceae family (Etkin and Ross, 1982; Kamga *et al*., 2010; Kuete *et al.*, 2011). The plant is commonly known as Hartblaarvy, Heart-leaved fig, polish fig, rubber plant, wild rubber fig, wild rubber tree (SANBI, 2015). Locally, it is called durumi in Hausa. Hasan, H.M, (2017), also investigated in his research titled "Black Carbon Incorporation Effect on Optical Properties of Polymethylmethacrylate Films" and concluded that the dielectric constant increased with increasing carbon content compared with the pure sample. This research will focus on investigating the effect of *ficus polita* and standard corn starch on the mechanical and biodegradation properties of PMMA/PVAc blend in order to assess its applicability in industrial and domestic use. The nanotechnology field of study is one of the most popular areas for present researches and improvement in mostly all Science and technical disciplines, this obviously includes polymer science and technology, the investigations cover a broad range of research topics (Paul, 2008). It is a novel science which evolved as it was observed that substances displayed significantly different classification at sizes in a nanometer as compared to the characterization of the same substance at micro-particle sizes. It will make possible the improvement of novel substances providing the basis for the design and development of new characterization and structures which will result in increased performance, reduced cost of manufacturing, maintenance and enhanced functionality (Hind *et al.,* 2019). Presently, assessment on polymer blends filled filler to form nanocomposites have attracted much attention because of their wide spectrum of applications in the field of polymer science and nanotechnology. The polymer nanocomposites heavily rely on geometry, size distribution, aggregation and surface chemistry of organic nanoparticles such as starch from different sources as well as matrix-nanoparticle interactions. The properties of nanocomposites were found to depend on the type of nanoparticles, the content of nanofillers and nature to

bridge chemically and physically with the polymer matrix (Hashim and Basim, 2019). Polymers are largely used in automobile bumper and architectural applications. In previous works, polymers have been used as insulators because of their dielectric properties and high non conducting properties. Polymers have several advantages, such as low cost, secure processing, high strength, flexibility, and excellent mechanical properties. The [(poly-methyl methacrylate (PMMA)/Polyvinylacetate (PVAc)] blend filled standard corn starch and derived starch from *ficus polita* fruit powder with nanoparticles can be considered a quite promising composite material for automotive, environmental and industrial applications such as: vehicular bumper, antibacterial, biosensors, lens, electronics gates, transistors, and transportation.

Materials

The following materials was used

1. Polymethylmethacrylate (PMMA)
2. Polyvinylacetate (PVAc)
3. Standard Corn Starch (SCS)
4. *Ficus Polita* (Morecere) Seed Powder (FPSP)
5. Toluene and dicholoro methylene
6. Dil. Hydrochloric acid, Sodiumhydroxide, and Acetone

Apparatus to be used

The following apparatus was used during the Experimental work

1. Mould
2. Hand gloves
3. Mixing knife
4. Weighing balance (triple beam balance, model 2016 MB)
5. Stop watch
6. Specimen Bottles
7. Density bottle
8. Oven
9. Sam paper
10. Microsrew gauge
11. Sieve
12. Petri dish

Table 1: Equipment and Location

S/N	Equipment	Specification	Location
1.	Two roll-mill	Reliable Rubber and Plastic Machine Company New York Jersey, U.S.A (model 5189)	Nigerian Institute of Leather and Science Technology (NILEST)

2.	Hydraulic press	Carver Inc., Wabah U.S.A (model 3851-0)	Nigerian Institute of Leather and Science Technology (NILEST)
3.	Hardness Tester	Show A hardness tester model number 5019	Nigerian Institute of Leather and Science Technology (NILEST)
4.	Tensometer	Monsator Tensometer model number 9875 type W	Department of Mechanical Engineering Faculty of Engineering Ahmadu Bello University, Zaria
5.	Scanning Electron microscope Machine	Phenom World	Department of Chemical Engineering Faculty of Engineering Ahmadu Bello University, Zaria
6.	X-diffraction machine (XRD)	Shimadzu 6000 X-ray diffractometer	National Steel Raw Materials and Exploration Agency No:18 Rabah Road, Malali Kaduna
7.	Charpy Impactor	Cat. Nr, 15J x 25J	Department of Material Science and Metallurgical Engineering Faculty of Engineering Ahmadu Bello University, Zaria

Methods

Samples Collection and Preparation

Poly (methyl methaacrylate) (PMMA), Polyvinylacetate (PVAc) and Standard Corn Starch was used whereas the local *ficus polita* seeds was collected from Samaru-Zaria dried and kept for filler preparation.

Preparation of filler

The proposed filler *ficus polita* seeds (FPS) was collected from the processing point in Samaru, Sabon Gari Local Government Area, Kaduna State, Nigeria and was dried at room temperature. When it is dried it would then be grounded to powder with a mechanical grinder and sieved through a sieve of mesh size 70µm.The sieved sample was stored separately in plastic container and labeled appropriately after which the hybrid filler shall be prepare according to the following table.

Mixing of the Compound

Two roll was used, it was switched on and the PMMA and PVAc processing temperature was set and that of PMMA at 200°C and PVAc at 150°C respectively. The nip of the rollers was adjusted and material was poured on the nip, after total melting had been attained, both fillers (SCS and FPSP) was incorporated and then cross-mixed until a homogeneous mixture is achieve and the compound was sheeted out for further processing. The compounding was done base on the formulation proposed by Mamza, (2011), according to the following table.

S/No	Mass of Ingredients (g)	1	2	3	4	5	6	7	8	9
1	Poly (methylmethacrylate)	100	90	80	70	60	50	40	30	20
2	Poly (vinylacetate)	0	10	20	30	40	50	60	70	80
3	PMMA/PVAc	100	90	80	70	60	50	40	30	20
4	SCS	0	10	20	30	40	50	60	70	80
5	PMMA/PVAc	100	90	80	70	60	50	40	30	20
6	FPSP	0	10	20	30	40	50	60	70	20

Film Casting and Measurement

Films of PMMA/PVAc, PMMA/PVAc/SCS or PMMA/PVAc/FPSP blends was prepared by solution casting: 6 g of polymethylmethacrylate (PMMA) supplied from Sigma-Aldrich company was dissolved in 20 ml of toluene and dicholoro methylene to obtain films without filler of the blends, then the same amount of the blends solutions was added to 0.1,0.2,and 0.3 of the standard corn starch and *ficus polita* seeds powder to prepare the different filling blends, these homogeneous solutions was spread on a glass plate and allow to evaporate the solvent slowly in air at room temperature for 24h. The thickness of the films was measured with micrometer srew guage in the range of (100 – 470mm), and it was calculated as averages. As proposed by mamza, (2012) and Hasan H.M, (2017)

CHARACTERIZATION

Hardness test.

The hardness test was carried by first placing the specimen on the hard-flat surface of the machine. The indentor for the instrument will then be pressed in to the specimen (6.4 mm) and the hardness readings was manually recorded as the pointer of the hardness tester stop at a ven calibration of the machine under specified force and time. (ASTM D2240-082018).

Summary

Blend films of PMMA and PVAc with different concentrations have been prepared by casting method, and they were exposed to UV and filtered radiation for 24 hours. The films were characterized spectroscopically using FTIR which illustrated that the decrease of the intensity of transmission spectra of (50/50 PMMA/PVAc) blend after exposure to UV radiation for 24 hours was lower than that of PMMA and PVAc. Consequently, (50/50 PMMA/PVAc) blend has improved the hardness of its homopolymer. Furthermore, effect of starch from *ficus polita* (*morecere*) fruith powder and standard corn starch have improved the hardness of the polymer blends.

Recommendation

Further research to be carried out to explore wide varieties of starch sources from food and fruits in polymer blends in identifying wide range of properties for use in various field of application.

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Microbiological Analyses of Drinking Water from Some *Wash Boreholes* in Maiduguri and Jere Metropolis, Borno State, Nigeria

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Abstract: *Water is crucial constituent in the continuance of all forms of life, and generally living organisms can live only for short time without it. Infectivity through water is, therefore, one of the primary concerns for safe and sound water supply therefore, microbiological analyses of drinking water from some wash boreholes in Maiduguri and Jere metropolis were conducted using Total Heterotrophic Bacteria (THB) count and Total Coliform Counts (TCC) the result showed bacterial load ranging from 3 x 10^2 in Mairi Kuwait to 1.2 x 10^2 in House of Assembly Quarters and Mairi Kuwait olala respectively, in which some are within the range of World Health Organization standard (Heterotrophic plate count levels in potable water should be < 500 colony forming unit per millilitre) while few appeared not within the range. Total coliform count (TCC) conducted showed presence in 3 samples Mairi Ustaz 1.0x10^2, Fori, Kakawo 0.50 x10^2 and Mairi Kuwait 0.50 x 10^2 which is a sign of poor source of portable drinking water and perhaps possible source of contamination near the borehole.*

Keywords*: Water, Coliform, Bacteria, Heterotrophic, Borehole*

Introduction

Water is crucial constituent in the continuance of all forms of life, and generally living organisms can live only for short time without it (Shittu *et al.,* 2018). Basis and portability of water reveal on the health conditions of society as microbiological contamination of water is the source of disease occurrence in a lot of society particularly in a lot of underdeveloped countries(Karbasdehi *et al.,* 2017).The infection of disease through water is, therefore, one of the primary concerns for secure water supply (Ahmed *et al.,* 2014). In a lot of underdeveloped countries, availability of potable water becomes a problem when supply is interrupted frequently and shortages become the order of the day (Popoola *et al.,* 2007). The potential of drinking water to transmit microbial infectious agent to great number of people causing

subsequent disease is well documented in a lot of countries at all levels of economic development. The number of occurrence that have been reported throughout the world demonstrates that infection of infectious agent by drinking water remains a significant results in disease (Addo *et al.,* 2019). However, estimate of disease based solely on detected occurrence is likely to underestimate the problem. A significant proportion of water-borne illnesses are likely to go undetected by the communicable diseases surveillance reporting systems. The symptoms of gastrointestinal disease(nausea, diarrhoea, vomiting and abdominal pain) are usually mild and generally last a few days to a week and only a small percentage of those affected will visit a health facility (Addo *et al.,* 2019). Each year greater than 2 million persons, mostly minors less than 5 years of age, die of diarrhoea disease. For minors in this age group, diarrhoea disease accounted for 17% of all death from 2000 to 2013 (WHO, 2015), ranking third among results in death, after neonatal results in an acute respiratory infections (WHO, 2015). Various water borne bacteria can results in significant illness, disease most often results from ingestion of contaminated water or seafood, with gastrointestinal entry of infectious agent or their products. The skin and soft tissue are also common entry point for water borne bacteria. Microorganisms that results in cholera, severe diarrhoea, and other disease are often present in huge numbers in infected human faeces. If drinking water is contaminated with these dangerous microbes, the illnesses can results and these illnesses can spread easily to others (Popoola *et al.,* 2007). Typhoid fever remains a great socio-economic problem in underdeveloped countries, Nigeria inclusive. Perforation of intestines is associated with high mortality with wound infection occurring in 50 — 75% of survivors. Controlling wound sepsis or wound infection with various complications also affected mortality and unsecured drinking water had been the major source of this infection (Adekunle *et al.,* 2004). Water is vital to our existence in life and its importance in our daily life makes it imperative that thorough microbiological and physicochemical examinations be conducted on water. Potable water is the water that is free from disease producing microorganisms and chemical substances that are dangerous to health (Shittu *et al.,* 2008).

Methodology

Sampling Locations

Samples for this study were collected from 17 different society and from two different local government areas namely; Maiduguri and Jere LGAs respectively, localities include; Mairi behind Ngab oil, Mairi Kuwait, Fori, Kakawo, Galtimari, Mairi Kuwait Olala, Fori Modu Birkila, Fori Bazanna, Mairi Ustaz. Pompomari, Bolori, House of Assemly Quarters, Pinnacle, Barwee.

Sample collection

The water sample were aseptically collected using sterile water container and transported to the lab for further analysis

Serial dilution

1ml of the water samples were individually added to 9 ml of diluents and thorough shaking, further 10-fold serial dilutions were made by transferring 1 ml of the initial sample to freshly prepared normal saline diluents to a range of 10-4 dilutions (Monica, 2006).

Total Heterotrophic Bacteria (THB)

Total Heterotrophic Bacteria was conducted according to Monica (2006), 0.1ml of the diluents at different concentrations were then spread evenly on the surface of the media using a sterile spreader and incubated for 24 hours at 37.2°C colonies were counted and the mean expressed as cfu/ml/ for the samples

Cfu/ml was calculated using $= \frac{number\ of\ colonies}{dilution\ factor\ X\ volume\ plated}$

The colonies formed were further sub cultured on Eosin Methylene Blue (EMB) agar, Salmonella Shigella agar, Thiosulphate Citrate Bile Salt (TCBS) and Blood agar for identification of different bacteria in the water sample

Total Coliform Counts (TCC)

The water samples were cultured on MacConkey agar for 24 hours at 37°C colonies formed were counted (Cheesbrough, 2006).

Microgen tests

Microgen Rapid test was used to confirm bacterial isolates - Microgen™ GNA-ID System for E. coli, Salmonella

Results and Discussion

The results of the microbiological analysis are presented in Tables 1-3. The microbial counts for Heterotrophic bacteria were observed to be high in mairi Kuwait and Mairi Ustaz with bacterial load of 3 x 10^2 in each case.

Table 1 showing Total Heterotrophic Bacteria Count (THBC)

S/NO	Sample	Total Heterotrophic Bacteria Count
1	Mairi behind Ngab oil	2 x10^2
2 .	Mairi Kuwait	3 x 10^2
3.	Fori, Kakawo	2 x10^2
4.	Galtimari	2 x10^2

5.	Mairi Kuwait Olala	1.2 x10^2
6.	Fori Modu Birkila	2.2 x10^2
7.	Fori Bazanna	1.2 x10^2
8.	Mairi Ustaz.	3 x10^2
9.	Pompomari Housing Estate	2.2 x10^2
10.	Bolori layin taya	1.2 x10^2
11.	House of Assembly Quarters	1.2 x10^2

Total Heterotrophic Bacterial count of water samples collected from part of Maiduguri and Jere were analysed and the result showed bacterial load ranging from 3 x 10^2 in Mairi Kuwait to 1.2 x 10^2 in House of Assembly Quarters and Mairi Kuwait olala respectively, according to World Health Organization standard, Heterotrophic plate count levels in potable water should be < 500 colony forming unit per milliliter (WHO, 2019). Count time and again > 500 cfu/ml would indicate a general decline in water quality, in this regard, most of the water analysed are within the range except for Mairi Ustaz and Mairi Kuwait.

Table 2 showing Total Coliform Count (TCC)

S/NO	Sample	Total ColiformCount
1 .	Mairi Kuwait	0.50 x 10^2
2.	Fori, Kakawo	0.50 x10^2
5.	Mairi Ustaz	1.0x10^2

Total coliform count (TCC) conducted showed presence in 3 samples Mairi Ustaz 1.0x10^2, Fori, Kakawo 0.50 x10^2 and Mairi Kuwait 0.50 x 10^2 . TCC according to WHO (2003) should be zero in portable drinking water, this may be because Coliform bacteria are also indicators of contamination with faecal source and it is a measure of degree of pollution and sanitary quality of water. Contamination may be as a result of a layer of bacteria (biofilm) within the well or plumbing system. It is therefore essential to affect strong prevention measures to save ground and surface water system in the location. Sufficient monitoring and inspection of these water sources should be carried out and management of water should be compulsory.

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Effects of Boko Haram Insurgency on the Performance and Retention of Senior Secondary School Students in Mathematics in Adamawa and Yobe States

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Abstract: *This study investigates the effect of Boko Haram insurgency on the academic performance and retention of senior secondary school students in mathematics using Adamawa and Yobe states as case study. The study was guided by two specific objectives and two research questions. The design that was used in this study is descriptive survey. Sample survey based on Purposive and Proportionate sampling was used for data collection in the selected schools in the two states. The data collected was analyzed using descriptive statistics That is Simple Percentage, Mean, and Standard deviation to answer the research questions. The study area as stated in the title of this paper is Adamawa and Yobe states. These two states are among the states in the north east that suffered more as far as Boko Haram insurgency is concerned. Currently, it is a known fact that Nigeria is witnessing high spate of insecurity especially in the northern part of the country by a group known as Boko Haram. The activities of the group have caused negative effects in terms of socio-economic and political developments especially in Adamawa, Borno and Yobe states in the north eastern part of Nigeria. The Jama'atu Ahlus- Sunnah lid-dawad which is popularly known as Boko Haram is a Pseudo – Islamic terrorist group which has its base in Kanamma in Yobe state north eastern Nigeria. Musa, (2011) stated that the Boko Haram has been in existence since 2001 but did not become popular until in 2009 when they participated actively in the sectarian violence which occurred in Maiduguri, Borno state. The name Boko Haram is a Hausa statement which means "Western Education is Sinful". This group is opposed to everything that is of Western Origin, more especially "Western Education". Boko Haram opposes not only western education but also Western Culture and Modern Science as well.*

Keywords*: Boko Haram, Insurgency, Terrorism, Psychological Effects, Trauma and Academic performance*

INTRODUCTION

Terrorism or Insurgency is a multi-dimensional process which needs to be holistically handled in order to understand it systematically. Abiye (2011) noted that domestic terrorism arose in

Nigeria as a result of emergent militants who took advantage of government's inefficient action and inactions in dealing with the fundamental elements of nationhood such as internal security, resource control, injustice, corruption, ethnicity, favoritism and marginalization. These factors have made terrorism to be ethicized in Nigeria. Currently the nation is witnessing high spate of insecurity especially in the northern part of Nigeria by a group of terrorist popularly known as Boko Haram who have been carrying out their terror activities over the past ten years. In addition to Boko Haram, other groups who are also causing serious security threat include Bandits, Gun men, Kidnappers and Fulani Herdsmen etc. The terrorism, conflicts or violence in northern Nigeria are due to high poverty rate in most societies (Rabio, 2000). According to Mohammed (2012), poverty was the major cause of conflicts in Africa, because he subscribed to the notion that poverty helps to extend conflicts once it started. Evidence has shown that when income drops there is tendency for conflicts to surface or re-ignited.(Sanchez and Nunez, 2001) objected to this hypothesis. According to Kwaja, (2009), much of the instability and violence in different parts of Nigeria happens as a result of illiteracy and unemployment; it has clearly shown that, the inability of the state to effectively deliver the basic necessities of life for its people has been identified as the underlining cause of violence, conflicts and insurgency in Nigeria. It is now an obvious fact that unemployment, ignorance, marginalization, corruption, resource agitation struggles, political competition and restiveness are responsible for violence and conflicts (Otoghile and Akov, 2011).

STATEMENT OF THE PROBLEM

The failure to provide effective psychological support to children exposed to the Boko Haram insurgency in northern Nigeria endangers their mental health and reduces the opportunities these children have for educational attainment. Children affected by terrorism suffer poor assimilation weaker school performance (Bloom & Matfess, 2016). Nigerian children especially Borno, Adamawa and Yobe states children affected by the Boko Haram insurgency still face the psychological effects of direct exposure to terrorism. Being victims of terrorism by way of family displacement and loss of family and friends may have long – term broad effects on children's development generally. There are gaps in research policy efforts to respond comprehensively to the Boko Haram insurgency, especially in relation to the psychological effects of the insurgency on children as they pertain to their education. However, this findings aims at investigating the root cause and its psychological effects on Senior Secondary School Students pertaining their education, particularly their performance in Mathematics in Adamawa and Yobe states.

OBJECTIVE OF THE STUDY

The main objective of the study is to investigate on the effects of insurgency on "Student's Academic Performance" in Mathematics in some selected senior secondary schools in Adamawa and Yobe states. The specific objectives are:

1. To determine the effect of Boko Haram insurgency on students towards learning in the two states.

2. To determine the root cause of Boko Haram insurgency in the two states
3. To determine the psychological effects of Boko Haram insurgency on secondary schools students in the two states.
4. To determine whether there is any difference in Mathematics performance of male and female students before and during the insurgency.

LITERATURE REVIEW

Ofongo (2016) conducted a research on the Boko Haram insurgency in Nigeria: What could have been the causes and precursors'? This study will critically examine plausible explanations for the emergence of the Boko Haram conflict. Indeed, there are several underlining factors that led to the emergence and radicalization of the sect. In particular factors as poverty, unemployment and illiteracy are shown to have been responsible for the radicalization of ethnic and religious identities in the country. The Boko Haram group was established in 2001 by Mohammed Yusuf and few members with the stated intention of cutting the injustice sideline by government towards the not privileged . Insecurity, corruption and moral decadence among the youth, unemployment, ignorance and corruption influenced by western education has become immersed in politics given its link to politicians, who are themselves product of Western Education, these posses a contradiction, (Oyeniyi, 2010). The ideology and philosophy of the Boko Haram terrorist have become terribly problematic as the continue to sit between politics and religion, as a result of that many people though had genuine and legitimate reasons to deeply felt the grievances which justify or better still rationalize insurgency (Otoghile & Akov, 2011). Thus, the scars of Boko Haram terrorism may live temporary or permanent injuries and mental health problems with the victims. However, the cognitive reaction to terrorism is uniquely based on individual ecological development. Bananno, Brewin Kaniasty & Greca, (2010) identified life disruption, missed school, weak academic functioning and continuous life stressor as some of the adverse cognitive effects on children exposed to terrorism in the short term and in the long term. A longitudinal study conducted by Halevi et al (2016) examine that exposure to terrorism by children could exacerbate over time and lead to permanent psychopathology and externalize children profile into adulthood. Bloom and Matfess, (2016) found a relationship between exposure to terrorism and poor academic performance among children exposed to violence. Likewise Delaney-Black et al, (2002) revealed that violence exposure is associated with decreased intelligence quotient (IQ) and reading ability of children who were exposed to violence. Amusana and Ejokeb (2017) conducted research on the psychological trauma inflicted by Boko Haram insurgency in the north eastern Nigeria. The divergent views emerge on the rationale behind Boko Haram Islamic insurgency in Nigeria. Some see it as an attempt to Islamatise the secular Nigerian State. While some belief it to be an attempt to change the status quo in order to concretize the perceived dominance of Northerners over the rest of the country. The 2014 invasion of Chibok School Girls in the north eastern part of the country which lead to the kidnap of over 250 girls, continue to generate public trauma and academic curiosity. The psychological effects of this insurgency on various stakeholders are unquantifiable which is going to be our

departure point because its impacts are still unfolding. The Psychological trauma effect will direct our theoretical discussion. Methodically, qualitative and secondary sources of information will dominate our argument. In view of the above underpinnings, this study argues that, Boko Haram insurgency in the North-Eastern Nigeria must be curbed as violence disregards the constitutional principle of universal human rights and has the potential of impacting Psychological Consequences on the people.

METHODOLOGY

Descriptive research design was used in this study. According to Mathiyazhagan and Nandan (2010), survey research designs are procedures in quantitative research in which investigators administer a survey to a sample or entire population of people to describe the attitudes, opinions, behaviors or characteristics of the population. The population of the study comprised of 40,396 students of 10 senior secondary schools in Adamawa State and 38,861 students of 10 senior secondary schools in Yobe State. The population of the study is heterogeneous in nature and comprised of 50,931 males and 28,326 females. The population distribution of the students is shown in Tables 1 and 2 below:

Table 1: List of selected senior secondary schools and population of students in Adamawa State

S/No	Name of School	Population		Total
		M	F	
1	Govt. Girls Sec. School Yola		6,810	6,810
2.	Federal Govt. College Ganye	2,126	1979	4,105
3.	Govt. Sec. School. Shuwa	2,483	-	2,483
4.	Govt. Sec. School Michika	2,768	-	2,768
5.	Govt. Science & Tech. College, Mubi	3,640	-	3,640
6.	Govt. Sec. School. Kiri	3,779	-	3,779
7.	Aliyu Mustapha Academy, Jimeta	3,530	1,245	4,775
8.	Govt. Sec. School. Song	3,725	-	3,725
9.	Special Education Centre, Jada	2,514	1,555	4,069
10.	Baptist High School, Mubi	2,245	1,997	4,242
		26,810	13,586	40,396

Table 2: List of Selected Senior Secondary Schools and Population of Students in Yobe State

S/No	Name of School	Population		Total
		M	F	
1	Govt. Girls College, Damaturu	-	7,952	7,952
2.	Govt. Unity College, Damaturu	3,569	-	3,569
3.	G.G. Sciecne Tech. College, Potiskum	-	3,825	3,825
4.	Fika Govt. Sec. School. Potiskum	3,678	-	3,678
5.	G.S.S. Geidam	3,726	-	3,726

6.	G.S.S. Yunusari	3,959	-	3,959
7.	G.S.S. Gashua	4,296	-	4,296
8.	G.S.S. Karasuwa	2,549	-	2,549
9.	Govt. Girls College, Gadaka	-	2,963	2,963
10.	G.S.S. Jakusko	2,344	-	2,344
		24,121	14,740	38,861

Source: Ministry of Education Yobe State, 2021

The sample size of this study was 319 students using simple random sampling. The sample size was selected based on Krejeic and Morgan (1970) table of sample size selection. A questionnaire has two (2) sections. Section A and Section B. In Section A, general information about the student and school such as type of school and gender was included, while Section B which consist of 10 items questions on Boko Haram insurgency were answered by the students. In this case, each student has decided and carefully selected from the likely given options. The options are: strongly agree (SA), agree (A), disagree (D), strong disagree (SA) and Disagree (D). The questions were drawn in the following areas: root cause of Boko Haram insurgency; its psychological effect on secondary school students performance. The instrument was pilot tested in two of the schools one each from Adamawa and Yobe States with 50 respondents (25 from each state) in order to determine the reliability of the instrument. The reliability of the questionnaire on Boko Haram insurgency (QBHI) calculated using the split Half Spearman Brown method, the reliability coefficient index of 0.88 was obtained. Data collected were analysed using descriptive statistics, frequency counts and simple percentages.

RESULTS

The data obtained from 321 selected students by administering the questionnaires on Boko Haram Insurgency (BHI) were analysed using descriptive statistics such as percentage, mean and standard deviation.

Research Question One: What is the root cause of Boko Haram Insurgency in your State?

Table 3: Frequency, percentages, mean and standard deviation on the root cause of Boko Haram insurgency in Adamawa and Yobe States

S/No	Items	Responses				Descriptive Statistics	
		SA	A	DA	SA	$\bar{x}$	SD
1.	The root cause of Boko Haram insurgency is due to poverty and unemployment	126 (36.5%)	94 (28.1%)	59 (17.2%)	42 (12.3%	3.00	1.06
2.	Boko Haram members took the law into their hands due to illiteracy	112 (32.7%)	120 (34.8%)	43 (12.0%)	46 (12.2%)	3.00	1.04

	and intimidation by security agents						
3.	The root cause of Boko Haram insurgency acts of burning schools, aimed to stop schooling in the State	160 (45.4%)	102 (30.2%)	32 (10.1%)	27 (8.2%)	3.00	0.96
4.	Boko Haram insurgency caused or happened as a result of lukewarm attitude of government	134 (39.6%)	103 (30.4%)	40 (11.2%)	44 (11.6%)	3.00	1.04
5.	The root cause of Boko Haram is due to the favour given to Western education by Nigerian Government against Al-Majiris	60 (20.1%)	8 (4.2%)	161 (43.1%)	90 (24.4%)	2.00	1.06

Results from table 3 revealed that 220 (64.6%) with $\bar{x}$ = 3.00 and SD = 1.06 of the respondents agreed that the root cause of Boko Haram insurgency is due to poverty and unemployment while 101 (39.4%) of the respondents disagreed. Majority 232 (67.5%) with ($\bar{x} = 3.00\ and\ SD\ 1.04$) of the respondents agreed that Boko Haram members took the law into their hands due to illiteracy and intimidation by security agents while 80 (22.2%) of the respondents disagreed. 262 (75.6%) with ($\bar{x}$ = 3.00 and SD = 0.96) of the respondents agreed that the root cause of Boko Haram insurgency acts of burning schools aimed at stopping school system in the region while 59 (24.4%) with ($\bar{x}$ = 2.00 and SD = 1.04) of the respondents agreed that Boko Haram insurgency caused or happened as a result of lukewarm attitude from the side of government while 84 (32.6%) with $\bar{x}$ = 3.00 and SD = 1.04) of the respondents agreed that the root cause of Boko Haram is due to the favour to Western education by Nigerian government against Al-Majiris while majority 251 (67.4%) of the respondents disagreed.

Note: The analyses at the final stage of items 1-5 on the root cause of Boko Haram insurgency revealed that it occurs as a result of poverty, unemployment, illiteracy, intimidation by security agents and as well the lukewarm attitude from the side of government.

Research Question Two: Does Boko Haram insurgency has any psychological effect on the performance of secondary school students in your State?

Table 4: Frequency, percentages, mean and standard deviation on the Psychological effect of Boko Haram insurgency on students performance in mathematics in Yobe State

S/No	Items	Responses	Descriptive Statistics

		SA	A	DA	SA	$\bar{x}$	SD
5.	Boko haram insurgency has affected both psychological and academic performance of students in schools	138 (44.3%)	90 (27.2%)	47 (14.5%)	46 (14.0%)	3.00	1.07
7.	All the students that experienced Boko Haram insurgency are psychologically balance without trauma and low-level retention of studies	32 (8.7%)	35 (9.0%)	120 (35.6%)	134 (46.7%)	2.00	0.93
8.	Government lukewarm attitude towards security cause psychological trauma to students not Boko haram insurgency	72 (21.99%)	75 (22.4%)	82 (26.6%)	91 (29.0%)	3.00	1.10
9.	Students don't want to be called by their status because of Boko Haram phobia, this has affected them in terms of academic performance	158 (457.0%)	102 (39.6%)	42 (13.7%)	30 (9.8%)	3.00	0.99
10.	Students feel shame, disappointed, discrimination and embarrassment psychologically for wearing school uniform during Boko Haram insurgency and this affect their academic performance	102 (29.6%)	90 (29.0%)	69 (20.8%)	60 (20.6%)	3.00	1.11

Result from Table 4 revealed that majority with ($\bar{x}$ = 3.00 and SD = 1.07) of the respondents agreed that Boko Haram insurgency has affected both psychological and academic performance while 93 (28.5%) = of the respondents disagreed. 67 (17.7%) with ($\bar{x}$ = 2.00 and SD = 0.93) of the respondents agreed that all the students that experienced Boko haram insurgency are psychologically balance without trauma while 254 (82.3%) of the respondents disagreed. 145

(44.3%) with ($\bar{x}$ = 3.00 and SD = 1.10) of the respondents agreed that government lukewarm attitude towards security cause psychological trauma to students not Boko Haram insurgency while 173 (55.7%) disagreed. Majority 260 (86.6%) with ($\bar{x}$ = 3.00 and SD = 0.99) of the respondents agreed that students don't want to be called by their status because of Boko Haram phobia, this has affected them socially, academically and psychologically while 72 (23.5%) of the respondents disagreed and finally majority 1982 (58.6%) with ($\bar{x}$ = 3.00 and SD = 1.11) of the respondents agreed that students feel shame, disappointment, discrimination and embarrassment psychologically for wearing school uniform during Boko Haram insurgency while 129 (41.4%) of the respondents disagreed.

Note:

The analyses at the final stage of items 6-10 on the psychological effect of Boko Haram insurgency on students revealed that, it has affected the psychological and academic performance of the school students in so many ways.

CONCLUSION

Based on the findings of this study, the following conclusions were made. From Table 3 the analyses showed that the root cause of Boko Haram insurgency became apparent as a result of security, unemployment, illiteracy and intimidation by security agents.

RECOMMENDATIONS

Based on the findings of the study, the following recommendations were made:

1. Government should ensure that security agents are made available to safeguard all the secondary schools in the North-Eastern states especially Borno, Adamawa and Yobe States.
2. Government should give special attention in terms of security in the region so that this students can be protected from psychological and physical affect by Boko Haram insurgency.

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The Prevalence of *faciolahepatica* in Slaughtered Goats and Sheep (A Case Study of Potiskum Abattoir)

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Abstract: *An investigation was carried out between the months of May and June 2018, to determine the prevalence of faciolahepatica among the breed of sheep and goats slaughtered at Potiskum abattoir. A total of 96 samples of sheep and goats feaces and fresh liver were examined for faciolahepatica egg and adult respectively. The result revealed that 41.7% goats and sheep had faciolahepatica of which goats had 35.4% and sheep 47.9%. The sheep showed the highest rate of infection by faciolahepatica than goats. The health implication of consuming meat contaminated by faciolahepatica was discussed and useful the recommendations were made.*
Keywords*: Faciolahepatica. Goat, Slaughter & Sheep*

INTRODUCTION

Fascioliasis is a highly pathogenic (Valero *et al;* 2003) disease of clinical and Veterinary importance caused by *Faciolahepatica* and Fasciola gigantic (Talukder *et al,* 2010, Shaikh *et al*, 2004 and Ozung *et al,* 2011). Transmission of the fluke and the presence of its infection in any given population is dependent upon and exacerbated by some factors such as; the presence of a substantial reservoir of water parasite and potential host and the presence of the lymneae snail intermediate host, including Fossaria Cubensis, Fossaria bulimoides, Fossaria modicella, Pseudosuccinea columella and Lymneae auricularia, Others are Lymneae viatrix, Padix auricularia and Stagnicola fuscus.

These snail host which commonly measure about 10mm in size, usually occur in areas with high annual rainfall, large areas of poorly drained pastures and moist soil (Afrakhosravi, 2001 and Keiser *et al,* 2007). Other factors which enhance the spread of fascioliasis are opponunity for water source contamination by human and nonhuman hosts and dietary practices that includes the raw, untreated aquatic vegetation or foliage located around water reservoir (Afrakhosravi, 2001, Keiser *et al,* 2007 and Valero *et al,* 2003).
Fascioliasis is cosmopolitan infection. Incidence of the infection has been reported in many countries including Nigeria, Parkistan, China, United States of America and Iran. (Valero et al,

2010 and WHO, 2006). It is commonly reported in ruminants; cattle, goat and sheep. (Okaiyeto *et al,* 2012, Talukder *et al*, 2010 and Ozung *et al,* 2011). Ruminant hosts become infected when forage with metacercarial cyst is ingested. They can also be infected when ingesting cysts suspended in soil and detritus while drinking water. Ingested parasite finds its way to intra hepatic billiary duct or hepatic parenchyma and later to the bile duct where it resides. Infected ruminant liver usually experiences traumatic injury giving rise to diffusely hepatic parenchyma containing haemorrhagic streaks or foci. The animal may experience weight loss, anaemia and general depression. The liver may be enlarged and show abnormal functions. Blood leucocytosis with eosinophilia in response to Cathepsin B (cat 12) antigen secreted by juvenile fluke may be observed (Afrakhosravi, 2011).

Complicated expressions due to synergy with Clostridium noryi and Clostridium haemolyticum result in black diseases referred to as infectious necrotic hepatitis, this infection makes the liver appear black in color. Fascioliasis is a zoonotic disease of public health importance. Man becomes infected when metacercarial of the fluke is ingested along with water Cress Salad and vegetables are grown along banks of water reservoirs inhabited by potential snail hosts. About 2.4 million people are infected worldwide and 180 million are at risk of the infection (Talukder *et al,* 2010 WHO, 2006). In Africa, the infection has been found to be a serious problem in humid and sub-humid zones (Ogunrinade and Ogunrinade, 1980).

The prevalence of fascioliasis differs in different countries. Afrakhosravi (2011) reported a prevalence range of 6.03% to 11.09% among cattle in the Ilam province of Iran. About 14.8% was recorded among buffaloes in Pakistan by Shaikh et al, (2004) and Talukder (2010) reported 21.53% among Black Bengal goats in Bangladesh. A recent report by Ozung *et al*, (2011) reported 1020 (50.52%), 479 (23.72%), and 520 (25.75%) prevalence among cattle, goats, and sheep respectively. Ayana et al, (2009) observed a significant difference at $P < 0.05$ in the prevalence of fascioliasis among cattle, goats, and sheep.

MATERIALS AND METHOD

THE STUDY AREA

Potiskum abattoir is located in the southern part of town near the river of Nahunta with an average temperature of 102 F/38.9 C along latitude 11.709 and longitude 1 1.07. Potiskum abattoir is listed among the largest abattoir in the northeast region of the nation with the largest capacity of an animal slaughtered per day.

MATERIALS USED

The following materials were used to analyze the sample of the infected liver: —

i. Fresh liver sample
ii. Hand lens
iii. Surgical blade
iv. Forceps
v. Dissecting board
vi. Slides and cotton wool

vii. Specimen bottle
viii. Petridishes and beaker
ix. Applicator stick
x. Microscope
xi. Hand gloves Stool sample

Reagents Used

Formaldehyde
Soap
Water
Normal saline

METHOD

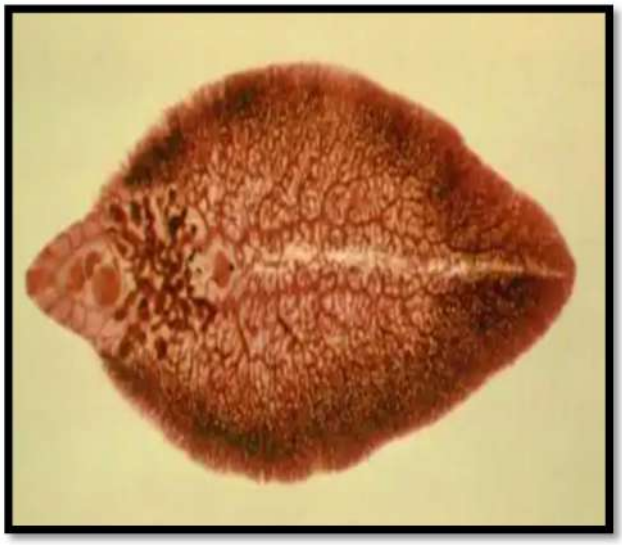
SAMPLING TECHNIQUE

A total 96 liver samples made up of 48 sheep and 118 were sampled randomly for fasciola *hepatica* in the Potiskum) the abattoir at a rate of 6 liver pec week, from the months of May to June 2018. The liver sample where brought to the laboratory for parasitological examination for liver flukes in glass specimen bottle containing 10% 'formaldehyde.

LABORATORY IDENTIFICATION OF THE FLUKES

The fresh liver samples collected were discussed using a surgical blade. The liver flukes were extracted from the liver using forceps. The extracted flukes are observed under the microscope using x10 and x40 objective. Flukes where identified based on the morphological characteristic as described by Groove and Newel (1974); the characteristic are:

i. Leaf like creature
ii. Palabroumen colour
iii. Flattened an oval in shape in a thick layer (cuticle)
iv.



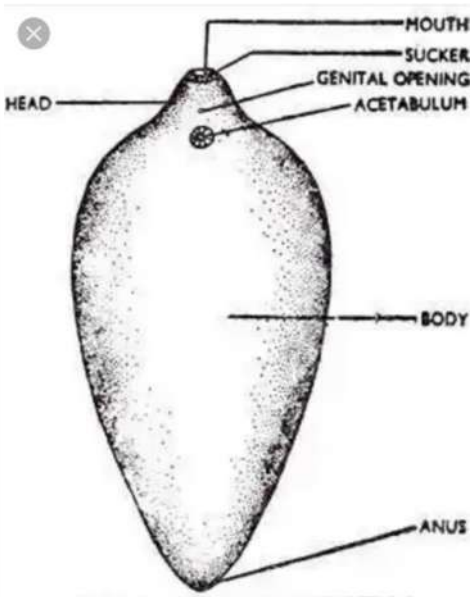


Fig. 185 FACIOLA HEPATICA,

STOOL ANALYSIS FOR FASCIOLA EGGS

Feaces samples collected from the rectum of slaughtered goats and sheep where analyzed by using direct smear methods which is follow;

i. A clean grease-free-slide was placed on a slide holder
ii. Two to three drops of normal saline were placed on the slide. Using the applicator, the feaces was emulsified in to the normal saline on the slide.
iii. It was then placed on the microscope stage for examination blade was dissected using a surgical
iv. It was then examined using x 10 and x40 objectives lens for final identification.

RESULT

PREVALENCE of *fasciola hepatica in* sheep and goats

Throughout the eight (8) weeks period of study 48 goats and 48 sheep have examined for fasciola *hepatica* from the months of May to June 2018. Eggs of fasciola *hepatica* were identified. The feces and fresh liver were examined out of the 96 samples it was discovered that sheep have the higher rate of fasciola *hepatica* as compared to goats. The results were summarized in Table 1, 2, 3, and respectively.

Table 1: Incidence of fasciola *hepatica* among sheep in the months of May 2018

Date of Sampling	No. of Observed	No. of Infected	Condemnation Partially	Level Totally	Condemnation Percentage
04/05/2018	06	03	01	02	50%
11/05/2018	06	04	01	03	66.7%
18/05/2018	06	02	02	-	33.0%
25/05/2018	06	03	01	02	50%
Total	24	12	05	07	50%

Table 2: Incidence of fasciola *hepatica* among sheep in the months of June, 2018

Date of Sampling	No. of Observed	No. of Infected	Condemnation Partially	Level Totally	Condemnation Percentage
05/05/2018	06	02	02	-	33%
12/05/2018	06	03	01	02	50%
19/05/2018	06	02	01	01	33%
27/05/2018	06	04	01	03	66.7%
Total	24	12	05	07	45.8%

Table 3: Incidence of fasciola *hepatica* among goat in the months of May, 2018

Date of Sampling	No. of Observed	No. of Infected	Condemnation Partially	Level Totally	Condemnation Percentage
04/05/2018	06	01	01	-	16.7%
12/05/2018	06	02	02	-	33.3%
19/05/2018	06	01	01	02	16.7%
27/05/2018	06	03	01	-	50%
Total	24	07	05	02	29.2%

Table 4: Incidence of fasciola *hepatica* among goats in the months of June, 2018

Date of Sampling	No. of Observed	No. of Infected	Condemnation Partially	Level Totally	Condemnation Percentage
05/05/2018	06	02	01	-	33.3%
12/05/2018	06	03	02	-	50%
19/05/2018	06	01	01	02	16.7%
27/05/2018	06	04	01	03	66.7%
Total	**24**	**10**	**05**	**05**	**41.7%**

DISCUSSION

Results of the survey revealed that between the month's May and June 2018 a tot number of 96 animal livers were sampled, which is made up of 48 sheep and goats from the Potiskum abattoir.

From table 1 incidence of fasciola *hepatica* among sheep in the month of May 2018. Samples collected and observed on the 11/05/2018 has the highest number of infections with a condemnation percentage of 66.7%; then samples collected and observed on 04/05/2018 and 25/05/2018 have the same number of infections having a 50% condemnation percentage, while sample collected and observed on the 18/05/2018 has the lowest condemnation percentage of 33%. Finally table I shows 12 liver samples were infected with 7 samples totally condemned and having a condemnation percentage of 50%.

Table 2: incidence of fasciola *hepatica* among sheep in the month of June 2018: Samples collected and observed on the 27/06/2018 has the highest number of infection with a condemnation percentage of 66.7%. The samples collected and observed on 12/06/2018 have 50%. Sample collection and observed on 05/06/2018 and 19/06/2018 have the same condemnation percentage of 33%: respectively therefore table 2 shows 11 liver samples were infected with 06 totally condemned and has a condemnation percentage of 45.8%.

Table 3: Incidence of fasciola *hepatica* among goats in the month of May 2018. Samples collected and observed on 25/05/2018 has the highest number of infection and has a condemnation percentage of 50%. Samples collected and observed on 11/05/2018 have a low infection with a condemnation percentage of 33%. Samples collected and observed on 04/05/2018 and 18/05/2018 have the least infection and have condemnation percentage of 16.7% respectively. Therefore, table III shows that samples are infected having 02 totally condemned and has a condemnation percentage of 29.2%.

Table iv: incidence offasciola *hepatica* among goats in the month of June 2018. Sample collected and observed on 27/06/2018 has the highest number of infections and condemnation in the percentage of 66.7%. Samples collected and observed on 12/06/2018 have 3 infected livers with a condemnation percentage of 50%.

Samples collected and observed on 05/06/2018 have 02 infected livers with a condemnation percentage of 33%, while samples collected and observed on 19/06/2018 have only 1 affected liver and a condemnation percentage of 16.7%.

Therefore, Table 4 shows that a total of 10 samples were infected with only 5 totally condemned and has a condemnation percentage of 41.7%. It would be deducted from the table I and II which shows the incidence of fasciola *hepatica* in sheep has the highest condemnation percentage of 50% and 45.8% from their total respectively, while table III and IV shows the incidence of fasciola *hepatica* in goats has low condemnation percentage of 29.2% and 41.79 from the total respectively.

Therefore, from the above tables, it shows that sheep appear to be more susceptible to fasciola *hepatica* disease than goals, this finding is similar to the report of the hall (1988), in which he reported a higher percentage incidence of fasciola *hepatica* in sheep than that of goats. A similar observation was made by Ibrahim et al (1989) in the Maiduguri Borno State of Nigeria.

CONCLUSION

This study revealed that liver fluke's infestation in goats and sheep had a negative effect on both state and the national economy because of the condemnation of liver and carcass at times, it is therefore become necessary to advise that a policy on grazing reserve should go beyond the one existing in the country. If such as done, the rate of animal consumption of infected grass on Madama and ponds would be drastically reduced, thereby reducing, the incidence of the disease.

RECOMMENDATIONS

- Infected liver and condemned carcass should be properly discarded either through burning or burying.
- Regular meat inspection by qualified veterinary doctors to destroy infected liver unfit for human consumption.
- Animal Should be dewormed regularly at a specific interval to prevent fasciola *hepatica* infection.
- Regular infection of the grazing area of these animals to ensure that the intermediates host (snail) are not breading there.
- Liver should be thoroughly cooked before its consumption

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Assessment of Larvicidal Activity of Synthesized Silver Nanoparticles Leaf Extract of *Annona senegalensis* and *Cassia obstufolia* Against 4th Instar Mosquito Larvae

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Abstract: *Silver nanoparticles synthesis has been achieved using plant extract which is ecofriendly. The presents study was carried out to assess the larvicidal activity of aqueous and synthesized silver nanoparticle leaf extract of Annona senegalensis and Cassia obstufolia against fourth instar larvae. It was established that aqueous silver ions can be reduced by the extract of the plant to generate a stable silver nanoparticles. Nanoparticles were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) Spectroscopy analysis. The formation of the silver nanoparticle was monitored through a UV-Vis-spectrophotometer in a wavelength range of 300-900nm. Peaks were revealed at 400nm and 420nm indicating the production of silver nanoparticles. The FTIR analysis strongly supported the capping behaviour of bio-reduced synthesized silver nanoparticles which in turn imparted the high stability of the synthesized silver nanoparticles. SEM micrograph SEM analyses of the synthesized AgNPs were clearly distinguishable. The XRD study revealed the crystalline nature of the nanoparticle with a Face-Centered Cubic (FCC) structure. The fourth instar of mosquito larvae were exposed to different concentration (10-50ppm and 100-500ppm) of the synthesized and aqueous leaf extract. Maximum mortality rate of 90% at concentration of 50ppm and LC_{50} (20.00ppm) LC_{90}(46.0ppm) was achieved for AgNps of C. obstufoliaand the aqueous extract has 86.6% maximum mortality for 500ppm with LC_{50}(223.0ppm), LC_{90}(506.0ppm).while A. senegalensis revealed 75% for AgNPs and 68.3% for aqueous extract. The result of the findings suggests that, synthesized silver nanoparticles of A. senegalensis and C. obstufolia can be used as a rapid, environmentally safer and greener approach for mosquito control.*

Keywords*: Annona senegalensis, C.obstufolia ,Silver nanoparticles ,Characterization, Larvicidal activity.*

1.0 Introduction

Mosquito belongs to the phylum arthropod and is an important vector for many vector-borne diseases, including malaria, filariasis and numerous viral diseases, such as dengue fever, yellow fever, Japanese encephalitis, west Nile, Rift valley fever, zika and chikungunya (Benelli, 2017). In the template climate countries they are important as nuisance pests than as vectors (Abou-Elnaga, 2014). There are about 3000 species of mosquitoes, of which about 100 are vectors of human diseases (Pohlit*et al.,*

2011). Control measures are directed mainly against only one or few of the most important species and can be targeted at the adult or the larval stages (Michigan Mosquito Control Organization, 2013).

For spans of years, several scientists have been engaged in searching for the effective and efficient mosquito control program. The World Health Organization (WHO) expert committee felt that the resistance in vector was probably a major challenge in the struggle against vector borne diseases (WHO, 2017). The conventional insecticides are environmentally non-sustainable and harmful to both human and non-target organism moreover, most mosquitoes species are increasingly becoming physiologically resistance (Karunamoorthi, and Sabesan, 2013).

As the problem of insecticide-resistant mosquitoes to chemical agents is on the rise, natural sources, such as plant are good alternatives to control mosquito vectors.They are harmless to human, target specific, bio-degradable,ecofriendly, and cost-effective (Govindarajan, 2016b).Plants are rich sources of bioactive compounds, which can be used to develop environmental safe vector managing agents. A number of plants have been reported as excellent toxics against mosquitoes acting as adulticidal, ovicidal, larvicidal, oviposition deterrent, and reproduction inhibitors and adult repellents (Govindaranjan, and Sivakumar, 2011).

In recent years, the green synthesis of eco-friendly metal nanoparticles from various plant derived metabolites has increased interests on nanotechnology acting as a good material for vector control. The nanoparticles possess valuable properties such as catalytic, optical, antimicrobial antiviral, antiplasmodial, insecticidal and larvicidal properties (Santhosh*et al.,* 2015). The plant mediated biosynthesis (i.e. "green synthesis")of nanoparticles is advantageous over chemical and physical method, a growing number of plants and fungi have put forward are efficient and rapid extra-cellular synthesis of silver and gold nanoparticles with excellent mosquitocidal properties in both field and laboratory conditions (Dinesh *et al.,* 2015; Amerasan*et al.,* 2015).

Annona senegalensis, commonly known as wild custard apple and wild sour-sop is a shrub or small tree 2-6m tall but may reach 11m under favourable conditions (African Union Scientific, Technical & Research Commission, 2014). The bark is clean to roughish, silver gray or grey-brown, leaves are alternate, simple, oblong, ovate or elliptic, green to bluish green; plants are up to 3cm in diameter on stalks 2cm long, solitary or in corporations of 2-4, bobbing up above the leaf axils(Mustapha*,* Owuna &Uthman, *2013*). The plant is found growing throughout Nigeria and very common in Northern Nigeria, particularly in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja and usually known as (Hausa,Gwándàndààjìì) or (Fulani,dukuu-hi) (Mustapha, 2013).

Cassia obtusifolia family Leguminosae (Fabaceae) is generally distributed in Africa and the Americas. In Sudan it is found mostly on the clay plains of the central rain lands and in the southern regions. *C. obtusifolia* is native to tropical South America but has become widespread throughout the tropics and subtropics. However, the extent of its original distribution in the neotropics is un-known. It grows wild in North, Central and South America, Asia and Africa and is considered a particularly serious weed in many places. The species name comes from the Latin obtus (dull or blunt), and folium ((Devi, Shankar, Femina&Paramasivam, 2012).

From previous literatures, the search for a sustainable natural biodegradable, eco-friendly, and difficult to develop resistance mosquitocide is important and urgent. The current study aimed to assess the larvicidal activity of green synthesized AgNPs using aqueous leaf extract of *A. senegalensis* and *C. obstufolia* against 4^{th} instar mosquito larvae.

2.0 Material and Methods

2.1 Collection of plant materials

The fresh matured leaves of *A. senegalensis* and *C. obstufolia* were collected from Maiduguri Metropolitan Council (latitude $11^0$49' and longitude $13^0$90') Area of Borno State, Nigeria. The plant was identified and authenticated by a plant taxonomist from Lake Chad research institute and a voucher specimen with number 03-458 was deposited at the herbarium of the institute.

2.2 Preparation of plant extracts

The leaf of *A. senegalensis* and *C. obstufolia* were dried in shade and ground to fine powder in a mortar. Aqueous extract was prepared by mixing 50g each of the dried leaf powder with 500ml of water (boiled and cooled distilled water) with steady stirring on a magnetic stirrer (Veerakumar*et al.,* 2013). The suspensions of the dried leaf powder in water were left for 3hrs, filtered through whatman no 1 filter paper, and the filtrate was stored till use.

2.3 Synthesis of silver Nanoparticles

The broth solution of fresh plant leaf were prepared by taking 10g of thoroughly washed and finely cut leaves in a 300ml flask along with 100ml of sterilized double distilled water and then boil the mixture for 5min before finally decanting it. The extracts were filtered with Whatman filter paper no 1. The filtrates were treated with aqueous 1mM silver nitrate ($AgN0_3$) (21.2mg of $AgN0_3$ powder in 125ml of distilled water) solution in a flask and incubated at room temperature for 6hrs. Eighty-eight-milliliter aqueous solution of 1mM of silver nitrate was reduced using 12ml of leaves extract at room temperature for 10 minutes. A resulting dark brown solution indicates the formation of silver nanoparticles (AgNps). The obtained AgNps were centrifuged at 3,000 rpm for 45min and three-repeated wash with distilled water were performed to discard a clear supernatant solution. The obtained pellets were dried and stored for further characterization and bioassays (Veerakumar*et al.,* 2014b).

2.4 Characterization of silver nanoparticles

Synthesis of AgNps solution was observed by UV-Vis spectroscopy. The bio reduction of the Ag^+ions in solution were monitored by sampling of aliquots (1ml) of the aqueous component after 20 fold dilution and absorbance was measured using Jenway 7315 spectrophotometer in 300-900nm range, operated at a resolution of 1nm. The functional groups from synthesized nanoparticles were examined using Fourier Transform Infrared (Shimadzu-8400s FTIR) spectrophotometer at a scan range of 750-4000cm^{-1}. The surface morphology and size of the AgNps were examined using a Scanning Electron Microscope (SEM) and Energy Dispersive X-ray(EDX) spectroscopy for elemental analysis using PhenomProX. The crystalline nature of AgNps were determine using Shimadzu XRD-6100 diffractometer, operating at 45kv and 40mA. CuKr radiation with wavelength of 1.54A^0 and a step size of 0.02^0 in the 2ɵrange 5-80 degrees (Govindarajan, 2016).

2.5 Larvicidal bioassay

The larvicidal activity of the aqueous extract and silver nanoparticles from *A. senegalensis and C. obstufolia* were evaluated according to World Health Organization (WHO), guidelines for testing larvicidal. Aqueous crude extract was tested at the range of 100, 200,300,400, and 500ppm concentrations and silver nanoparticles was tested at the range of 10, 20,30,40 and 50ppm concentrations. Twenty 4^{th} instar larvae were introduced into 500ml glass beaker containing 249ml of distilled water, 1ml of desired concentration of the aqueous leaf extract and silver nanoparticles were added to each. Average of three replicates were recorded. Larval mortality were recorded 24h after exposure. Each test included a set of control groups (1Mm silver nitrate and distilled water) (WHO, 2005).

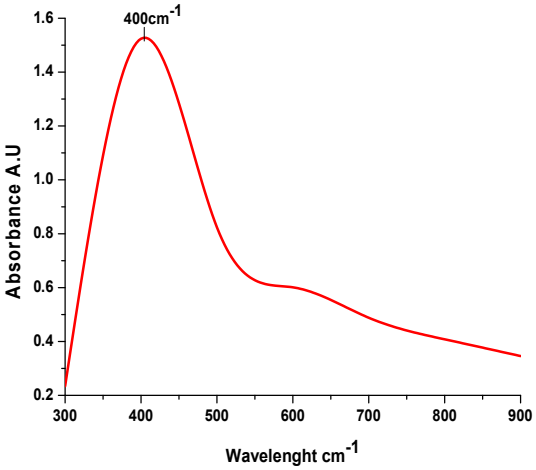
2.6 Statistical Analysis

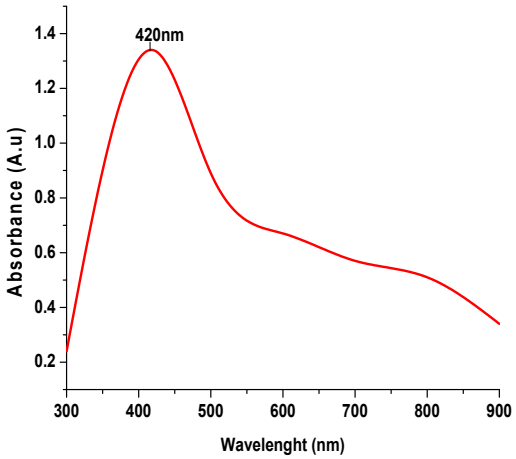
The percentage larval mortality were subjected to log-probit analysis and regression analysis for calculating LC_{50}, LC_{90} statistics at 95% confidence limits of upper confidence limit (UCL), and lower confidence limit (LCL), and chi-square values were calculated using the statistical package for social sciences (SPSS) version 26.0 software. Results with P< 0.05 were considered statistically significant.

3.0 Results

3.1 Uv-vis Spectroscopy

Evidence of reactivity in *A. senegalensis* and *C. obstufolia* leaf extract with $AgN0_3$ solution were visually indicated by a change in colour after 3hrs of incubation. The formation of the synthesized AgNps





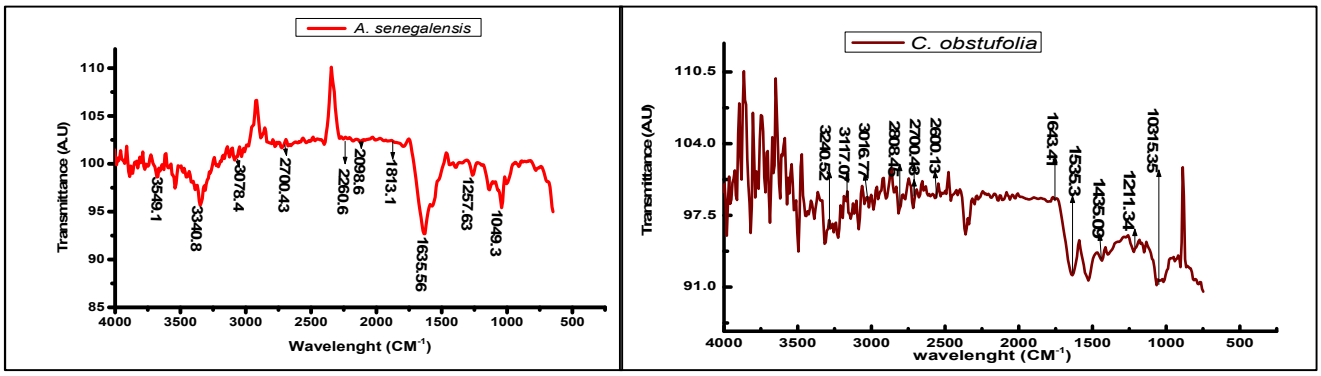
was monitored by scanning the absorption spectra in the range of 300-800nm. The most intense peak was observed at 400nm in *A. senegalensis* and 420nm in *C. obstufolia* (Fig. 1 a & b).

Fig.1 a &b: Uv-Vis absorption spectra of synthesized silver nanoparticles of *A. senegalensis* and *C. obstufolia* leaf extract.

3.2 FTIR Analysis

The FTIR spectrum of AgNPs prepared from *Annona senegalensis* extract showed the presence of different functional groups as reducing agents in the biosynthesis of AgNPs (Fig.4.7). The absorption band at 902.72(N-H) wag, 1049 cm^{-1}(CO-O-CO) streching ,1257cm^{-1}(C-O) stretching, indicates vibration due to the presence of primary amine, secondary amine, anhydride and alkyl aryl ether. Band at 1404.22cm^{-1}(C-F) stretch was due to fluro compound. The band at 1635cm^{-1}(C=C) stretching vibration correspondsto cyclic alkane. The peak at 1813 cm^{-1}(C=C) is an indicative of stretching which can be assigned to acid halide. The peaks observed at 2098cm^{-1}(N=C=S) and 2260cm^{-1}(C=N) indicates the ultraviolet region stretching vibrations assigned to isothiocynate and alkyne respectively. The peak at 2414.96cm^{-1}(O=C=O) indicatesstretchingof carbondioxide. The peak at 2700 cm^{-1}(C-H) corresponds to stretching assigned to aldehyde. Peak at 2793.02 (C-H) was due to stretching corresponding to aldehyde. The peak at 3078 cm^{-1}(C-H) was due to the stretching vibration corresponding to alkene. 3340 cm^{-1}(N-H) was assigned to the amine functional group with stretching vibration and the spectrum from C. obstufolia shows peaks at 833.28cm^{-1}(C-CL) stretch, corresponds to alkyl halides and 941.29(O-H)

bend of Carboxylic acid, 1033cm^{-1}(S=O) stretch, due to sulfoxide and 1211 cm^{-1}(C-O) stretching vibration of vinyl ether. The peak at 1435cm^{-1}(O-H) bending, is due to carboxylic acids. The strong peak at 1535cm^{-1}(N-O)stretching, vibration was due to nitro compounds. The band at 1643cm^{-1}(C=C) stretching, may correspond to alkene. The peak at 1797cm^{-1}(C-H)bending, and 2121cm^{-1}(C=C)stretching, correspond to aromatic compound and alkyne respectively. Bands at 2345.52cm^{-1}(O=C=O) stretching, and 2430.39cm^{-1}(C=C) stretching, are due to carbondioxide and carboxylic acid. The band at 2600cm^{-1}(O-H) indicates a stretching vibration of carboxylic acid. The peaks at 2700cm^{-1}(C-H) and 2808cm^{-1}(C-H) stretching, may both be assigned to the presence of aldehyde. The peaks at 3016 cm^{-1}(O-H), 3117cm^{-1}(O-H) and 3501cm^{-1}(O-H) stretching indicate the presence of alcohol intra-molecular bonding. The medium peak at 3294cm^{-1}(N-H) and 3240cm^{-1}(N-H) stretching vibration corresponds to aliphatic primary amine. (Fig.2a & b).



Fig(2a &b). FTIR spectra of synthesized silver nanoparticles using *G. senegalensis and C.obstufolia* aqueous leaf extract

3.3 Scanning electron microscopy (SEM)

SEM micrograph of the synthesized AgNps of *A. senegalensis* and *C. obstufolia* magnified at X1500 were shown in Fig. 3a&b. The visual observation of the surface morphology of the synthesized *A. senegalensis* nanoparticles showed a spherical, cubic with few cuboidal structures while the SEM representative of C. obstufolia were predominantly spherical withsome uneven structures .

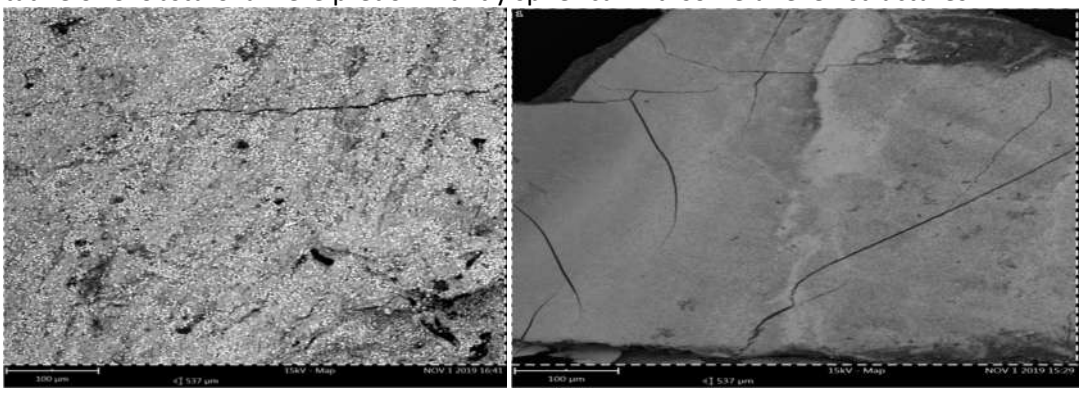


Fig3 a & b. SEM micrograph of synthesized silver nanoparticles of *A. senegalensis* and *C. obstufolia* at X1500

3.4 X-ray diffraction spectroscopy (XRD)

The crystalline nature of *A. senegalensis and C. obstufolia* synthesized AgNps were shown by XRD analysis. Diffraction peaks were observed at 2θ value corresponding to (111), (200), (220) and (311) set of lattice plane and were indexed as face-centered cubic (FCC) structure of silver nanoparticles (Fig.4a & b).

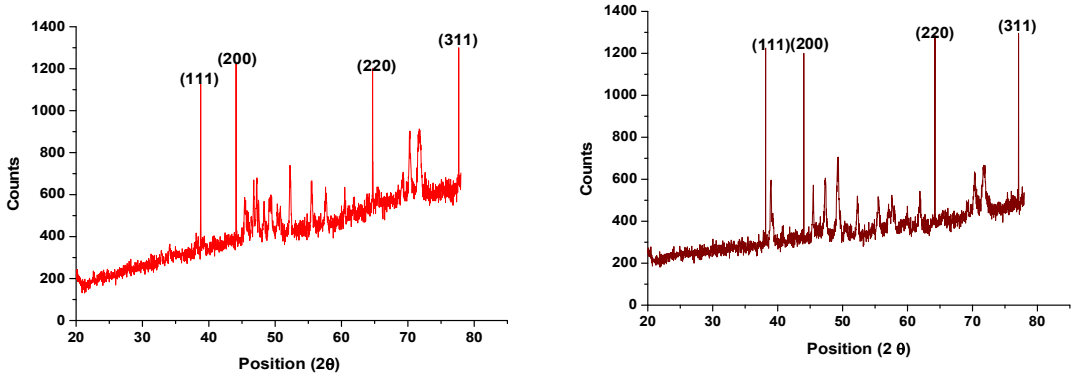


Fig.4a & b. XRD pattern of synthesized from leaf extract of *A. senegalensis and C. obstufolia*

4.0 Larvicidal bioassay

The larvicidal activity of synthesized silver nanoparticles and aqueous leaf extract of *A .senegalensis* and *C.obstufolia*. Data revealed from synthesized AgNps showed mortality rate of 75%±1.53 at a concentration of 50pm, and while the lowest mortality was 28.52±0.58 at 100ppm. The lethal concentration LC_{50} value of 30ppm and LC_{90} 60ppm, were required to kill 50 and 90% larvae respectively. The result of the aqueous extract revealed rate of 68.38% at 500ppm with LC_{50} of 310ppm and LC_{90} of 630ppm respectively. C. obstufolia was highest at 90%, 50ppm AgNPs with LC_{50} 29ppm, aqueous extract was 80.6% at 500ppm and LC_{50} of 223ppm.

Table 1 a: Larvicidal activity of *Anonna senegalensis* Aqueous and Silver Nanoparticles leaf extracts against Fourth Instar Mosquito species (20 larvae) exposed for 24hours

Concentrations (ppm)	% mortality ± SD	LC_{50}(ppm)LCL-UCL	LC_{90}(ppm)LCL-UCL	x^2(df)
	Anonna senegalensis aqueous leaf extract			
500	68.38 ± 0.58			
400	57.40 ± 1.50			
300	48.00 ± 1.53			
200	33.33 ± 1.15	310 (248 - 386)	630(517 - 873)	4.340 (4) ns
100	28.52 ± 0.58			
control	0.00 ± 0.00			
	Anonna senegalensis silver nanoparticles leaf extract			
50	75.00 ± 1.73			
40	60.25 ± 1.00			
30	50.00 ± 0.00	30 (24 - 37)	60(50 - 84)	4.361(4) ns
20	38.33 ± 0.58			

10	30.00 ± 1.53
control	0.00 ± 0.00

SD standard deviation, Values are mean ± SD of three replicates, LCL lower confidence limits, UCL upper confidence limits, X^2 chi-square test, and $p < 0.05$, level of significance.

Table1b. Larvicidal activity of *Cassia obstufolia* Aqueous and Silver Nanoparticles leaf extract against Fourth Instar Mosquito species (20 larvae) exposed for 24hours.

Concentrations(ppm)	% mortality± SD	LC_{50}(ppm)LCL-UCL	LC_{90}(ppm)LCL-UCL	x^2(df)
	Cassia obstufolia aqueous leaf extract			
500	86.67 ± 0.58			
400	76.58 ± 1.15			
300	70.33 ± 0.52	223(161- 279)	506(422 - 663)	7.354(4)ns
200	53.53 ± 0.00			
100	45.33 ± 1.00			
control	0.00 ± 0.00			
	Cassia obstufolia silver nanoparticles leaf extract			
50	90.00 ± 1.00			
40	78.33 ± 1.15	20 (14 - 25)	46 (38 - 58)	8.148(4)ns
30	75.00 ± 1.73			
20	56.67 ± 0.58			
10	48.33 ± 1.53			
control	0.00 ± 0.00			

SD standard deviation, Values are mean of ± SD of three replicates, LCL lower confidence limits, UCL upper confidence limits, X^2 chi-square test, and $p < 0.05$, level of significance.

5.0 **Discussion**

Several investigations were carried out to characterize the biosynthesized silver nanoparticles. The biosynthesis of metal nanoparticles involves the reduction and stabilization potential of plant extract and metabolites (Rajan*et al.,* 2015).

The synthesized silver nanoparticles were incubated at room temperature and within 3hrs of reaction a clear change in colour from brown to dark brown was observed. Such method was well explained by other researchers who worked with different plants (Muthukrishnan*et al.,* 2015; Kalaiselvi*et al.,* 2015). The colour change was due to the excitation of surface plasmonresonance(SPR) in metal nanoparticles (Logeswari *et al.,* 2015). The period of colour change in this study was in conformity with Muthukrishnan*et al.* (2015) and differs with Nithya and Raghavan (2014) who reported colour charge after 24 hours. The variation in bio reduction may be due to difference in Enzymes activities present in the extract of *A. senegalensis and C.obstufolia.* A sharp peak at 400nm and at 420nm were observed (Fig 1a& b). The UV-Vis band is an evidence of the presence of surface plasma resonance (SPR) of AgNps which is ranged from 420-450mm (Ramalingan*et al.,* 2014).

The IR spectroscopy study has confirmed that the carbonyl group of amino residue and peptides of proteins has a stronger ability to bind metal to prevent the agglomeration of particles, and thus stabilization of nanoparticles in the medium (Indhurmthi and Apunprasath, 2019).The identified functional groups and secondary metabolites are responsible for the reducing, capping and stabilization activity of the plant extracts in addition to prevention of aggregation (Suriyakalaa*et al.,* 2013).

The XRD patterns clearly demonstrate that the AgNPs formed in the present study were crystalline in nature. The sharpening of the peaks specifies that the particles be in a nano range. The stronger planes indicate AgNPs as a key element in the biosynthesis. Minor shift in the peak indicates presence of some strains in the crystal structure.. Selvi and Sivanmar (2012) obtained similar reports.

The larvicidal activity of aqueous and silver nanoparticles leaf extract of *A. senegalensis* at various concentrations against 4th instar mosquito larvae was presented in Table 1a & b. *A. senegalensis* and *C. obstufolia* AgNPs exhibited moderate activity than the aqueous extract. The result also revealed that C. obstufolia AgNPs is more potent as a mosquito larvicide which showed maximum mortality even at low concentaration of 10ppm. The findings of this research is consistent with the work of Roni *et al.,* (2012), also disagree with several authors (Parthiban *et al.,* 2018; Bianca *et al.,* 2018). Researchers has reported that synthesized AgNPs may has significant impact on mosquito larvae, findings of the study also in line with (AgalyaPriydarShini*et al.,* 2012, Roni *et al.,* 2012).

Conclusion

The study shows that the aqueous leaf extracts and silver nanoparticles obtained from *Annona senegalensis and C .obstufolia* present a clear larvicidal effect against 4th instar larvae. Furthermore, the effect with the synthesized silver nanoparticles is higher.. The biosynthesis of aqueous leaf extract of *A. senegalensis* and C. obstufolia has the potential to be used as a suitable alternative in mosquito larvae control.

Conflict of interest statement

We declare that we have no conflict of interest

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Construction and Performance Avaluation of an Indirect Solar Dryer for Drying Okro and Pepper

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Abstract: *The solar drying system uses solar energy to heat up air and dry any food substance loaded, which is beneficial in reducing wastage and helps in the preservation of agricultural produce. Based on the limitations of the natural sun drying, for example, exposure to direct sunlight, liability to pests and rodents lack of proper monitoring, and the escalated cost of the mechanical dryer, a solar dryer is therefore developed to cater to this limitation. This work presents the design, construction, and performance of an indirect solar dryer for food preservation. In the dryer, the heated air from a separate solar collector is passed through a grain bed, and at the same time, the drying cabinet absorbs solar energy directly through the transparent walls and roof. The temperature inside the drying cabinet rises to about 59 ºC while that of the ambient rises up to 38 ºC - 74 ºC for about three hours immediately after 12:00h (noon). The dryer is exhibited to the sufficient sun to enable it to be able to dry food items reasonably rapid to a safe moisture level and simultaneously to ensure a superior quality of the dried farm produce. It is able to dry about 1 kg to 2 kg of fresh Okro and Pepper within 12hours while the equal quantity of the Okro and Pepper will probably take up to 24hours in an open-air to dry. Appropriate statistical tool(s) is utilized for performance evaluation.*

Keywords*: Solar, Fabrication, Performance, Dryer, Temperature.*

INTRODUCTION

Drying is one of the methods used to preserve food products for longer periods. The heat from the Sun coupled with the wind has been used to dry food for preservation for several thousand years. Solar thermal technology is a technology that is rapidly gaining acceptance as an energy saving measure in agriculture application. It is preferred to other alternative sources of energy such as wind and shale, because it is abundant, inexhaustible, and non-polluting. Solar air heaters Drying is one of the methods used to preserve food product s for longer period. The heat from the Sun coupled with the wind has been used to dry food for preservation for several thousand years. Solar thermal technology is a technology that is has been in use with simple devices to heat air by utilizing solar energy and it is employed in many applications require to moderate temperature below 80°C, such as crop drying and space heating. Drying is the oldest preservation technique of agricultural products and it is an energy

intensive process. High prices and shortages of fossil fuels have increased the emphasis on using alternative renewable energy resources. Drying of agricultural products using renewable energy such as solar energy is environmentally friendly and environmental impact. Different types of solar dryers have been designed, fabricated and tested in the different regions of the tropics and subtropics. The major two categories of the dryers are natural convection solar dryers and forced convection solar dryers. In the natural convection solar dryers, the airflow is established by buoyancy induced airflow while in forced convection solar dryers the airflow is provided by using fan operated either by electricity/solar module or fossil fuel.

In many parts of the world there is a growing awareness that renewable energy has an important role to play in extending technology to the farmer in developing countries in order to increase their productivity (Waewsak et al., 2006). Traditional drying, which is frequently done on the ground in the open air, is the most widespread method used in developing countries because it is the simplest and cheapest method of conserving food stuffs. Some disadvantages of open-air drying are exposure of the foodstuff to rain and dust, uncontrolled drying, exposure to direct sunlight which is undesirable for some foodstuffs, infestation by insects, attack by animals, etc. (Madhlopa et al., 2002). In order to improve traditional drying, solar dryers, which have the potential of substantially reducing the above-mentioned disadvantages of open air drying, have received considerable attention over the past 20 years (Bassey, 1989). Solar dryers of the forced convection type can be effectively used. They, however, need electricity, which unfortunately is non-existent in many rural areas, to operate the fans. Even when electricity exists, the potential users of the dryers are unable to pay for it due to their very low income. Forced convection dryers are for this reason not going to be readily applicable on a wide scale in many developing countries. Natural convection dryers circulate the drying air without the aid of a fan. They are therefore, the most applicable to the rural areas in developing countries. Solar drying may be classified into direct, indirect and mixed-modes. In direct solar dryers the air heater contains the grains and solar energy passes through a transparent cover and is absorbed by the grains. Essentially, the heat required for drying is provided by radiation to the upper layers and subsequent conduction into the grain bed. In indirect dryers, solar energy is collected in a separate solar collector (air heater) and the heated air then passes through the grain bed, while in the mixed mode type of dryer, the heated air from a separate solar collector is passed through a grain bed, and at the same time, the drying cabinet absorbs solar energy directly through the transparent walls or roof. Therefore, the objective of this study is to develop an indirect solar dryer in which the grains are dried simultaneously by the heated air from the solar collector. The performance of the dryer will also be evaluated.

MATERIALS AND METHODS

Solar drying refers to a technique that utilizes incident solar radiation to convert to thermal energy required for drying purposes. Most solar dryers use solar air heaters and the heated air is which passes through the drying chamber (containing material) to be dried. The air transfers its energy to the material causing evaporation of moisture of the material.

i. The solar dryer was designed using average short-term data obtained from the month of November.
ii. The solar dryer was designed to dry Okro and Pepper.
iii. The collector was positioned at an angle of inclination for the best year-round performance (13º N), which is the latitude of Sokoto, Nigeria.

In the light of the above the following materials which are locally available have been used:

Materials Used

Glass sheet (4 mm), Sheet metal (59 cm/93 cm), Plank wood (51 cm/95 cm), Saw dust, Cast iron (C.I.) pipes, Solar panel (12 volts), Mercury (glass thermometer), Fan (12 volts), Wires (1yard), Masking tape, Net (49/24 cm), Top bond, Ply wood (51 cm/35 cm).

CONSTRUCTION OF THE SYSTEM COMPONENTS

i. Solar Collector

The heat absorber (inner box) of the solar dryer was constructed using 2 mm thick aluminum sheet, painted black, is mounted in an outer box built from wooden plank. The space between the inner box and outer box is filled with saw dust material of about 40 mm thickness and the thermal conductivity of the plate is 200$Wm^{-1}K^{-1}$. The solar collector assembly consists of air flow channel enclosed by transparent cover (glazing). One end of the solar collector has an air inlet vent through a pipe of 2.5cm internal diameter. The overall collector plate has a dimension of 95cm/51cm as shown in Figure 1. It also consists of heater and riser pipes arrangement. The heater and riser pipes were welded before being placed in the collector casing made from wooden plank.

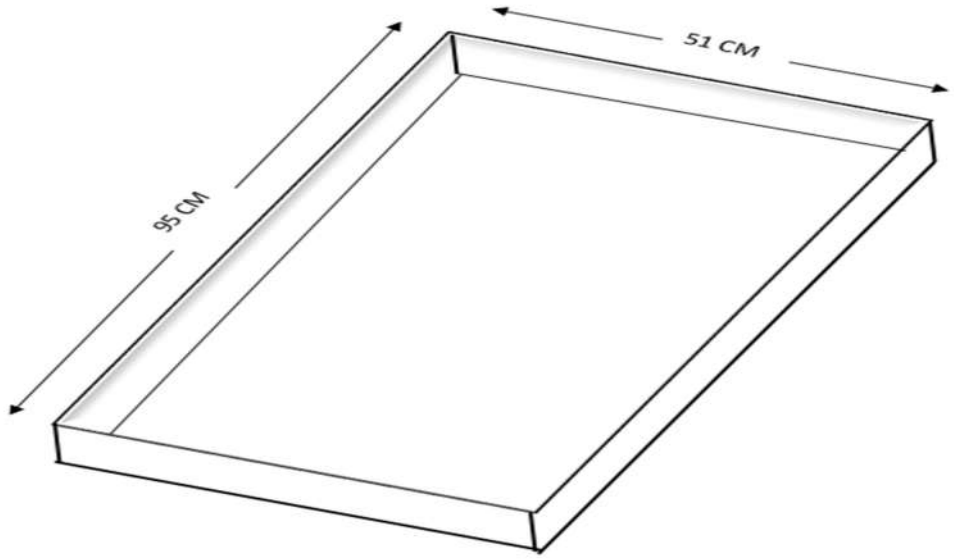

Figure1: Dimension of the solar collector.

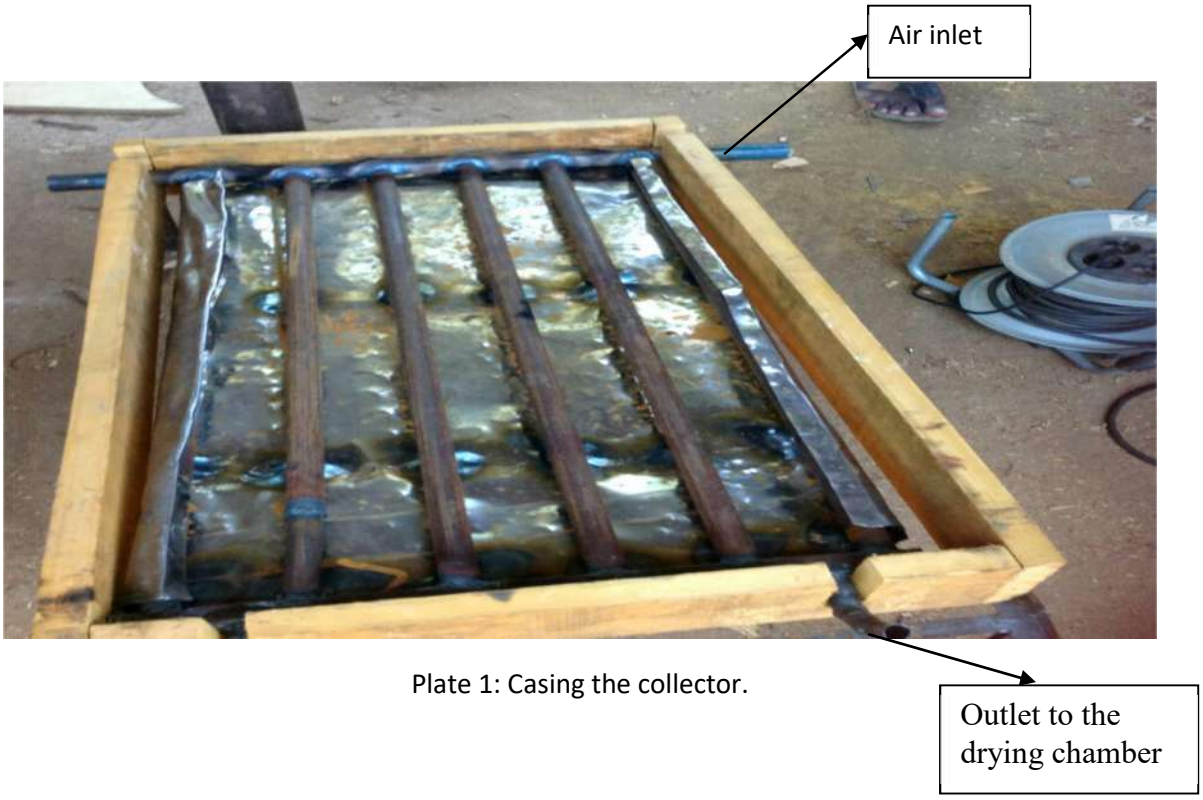

Plate 1: Casing the collector.

ii. Top Cover

The glazing is a single layer of 4 mm thick transparent glass sheet, it has a surface area of 49cm by 93cm and of transmittance above 0.7 for wave lengths in the rage 0.2 – 2.0 µm.

iii. **The Drying Chamber**

The drying chamber together with the structural frame of the dryer was built from wooden plank which could withstand termite and atmospheric attacks. An outlet vent was provided toward the upper end at the back of the chamber to facilitate and control the convection flow of air through the dryer. Access door to the drying rays was also provided at the back of the chamber. The schematic diagram of the drying chamber is shown in Figure 2.

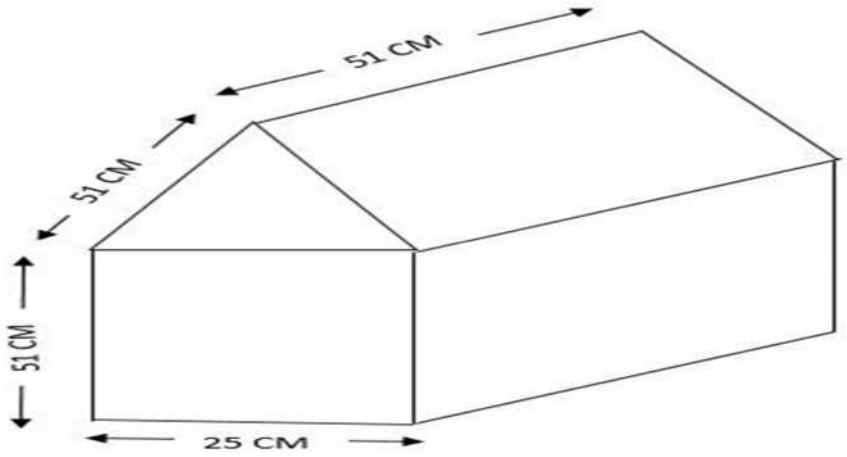

Figure 2: The schematic diagram of drying chamber.

iv. The Blowing Fan

A direct current (D.C.) Fan was connected to the collector pipes to blow the heated air from the heating cabinet to the drying chamber through the pipes in order to make the drying process fast. It is powered by a solar panel.

v. The Solar Photovoltaic Panel

A solar photovoltaic panel of (12 volts) was use to power the fan. the speed of the fan depends directly on the solar intensity, the higher the intensity the higher the speed of the fan, the lower the intensity the lower the speed of the fan.

Finally, both the constructed solar collector and the drying chamber were assembled together as shown in Plate 2 below:

Plate 2: Side view of the Construction.

TESTING OF THE INDIRECT SOLAR DRYER

In the test, solar intensity, ambient temperature, wind speed, glass temperature, air temperature, and temperature of the drying chamber. at 30 minutes intervals were measured for the analyses of the performance of the indirect solar air heater. The Experiments were carried out in front of Physics Laboratory of the Umaru Ali Shinkafi, Polytechnic, Sokoto, Nigeria for two days i.e., 16th, and 17th of November, 2021. To ascertain the performance of the indirect solar dryer by determining how long it will take to dry the Okro and Pepper, and temperature rise in the solar dryer was mounted in the early hours of the day as shown in Plate 3 below:

Plate 3: The Setup of the Experiment.

The readings of inside the cover temperature, air inlet temperature, ambient temperature, glass temperature, temperature of the plate and temperature of the draying chamber, were measured with the use of Mercury in glass thermometer and Digital thermometer with probe every 30 minutes. Average wind speed, insulation (H_S), were also measured using Anemometer and Pyranometer, respectively.

RESULTS AND DISCUSSION

Readings Taken from Solar Dryer

Readings of ambient temperature (t_{amb}), inner glass temperature (t_g), the temperature of the plate (t_p), temperature of the air (t_{air}), the temperature of the drying chamber (t_{ch}), solar radiation (i), and wind speed (v) were taken on the first and second days of the experiment. Results of these readings are discussed by graphical representation in the following section.

The results obtained during the test period revealed that the temperatures inside the dryer and solar collector were much higher than the ambient temperature during most hours of the day-light. The temperature rise inside the drying chamber was up to 74% for about three hours immediately after 12.00h (noon). It was able to dry 2kg and 1kg of fresh Okro and Pepper within 12hours while the equal quantity of the Okro and Pepper was dried within 24 hours in an open air drying. The dryer exhibited sufficient ability to dry food items reasonably rapidly to a safe moisture level and simultaneously it ensures superior quality of the dried product.

VARIATION OF THE TEMPERATURE IN THE SOLAR COLLECTOR, GLASS, AIR AND THE DRYING CHAMBER COMPARED TO THE AMBIENT TEMPERATURE

Figure 3a and 3b, the temperatures show a typical day results of the 30 minutes interval temperature variation in the solar collector and the drying chamber compared to the ambient. The dryer was observed to be hottest about mid-day when the sun was usually at maximum insulation level. The temperatures inside the dryer and the solar collector were much higher than the ambient temperature during most hours of the daylight. The temperature rise inside drying chamber was up to 58 ºC (74%) for about three hours immediately after 12.00h (noon). This indicates prospect for better performance than open-air sun drying.

Also results of solar radiation and wind speed monitored during the tests periods are shown in Figure 4a & 4b and 5a & 5b.

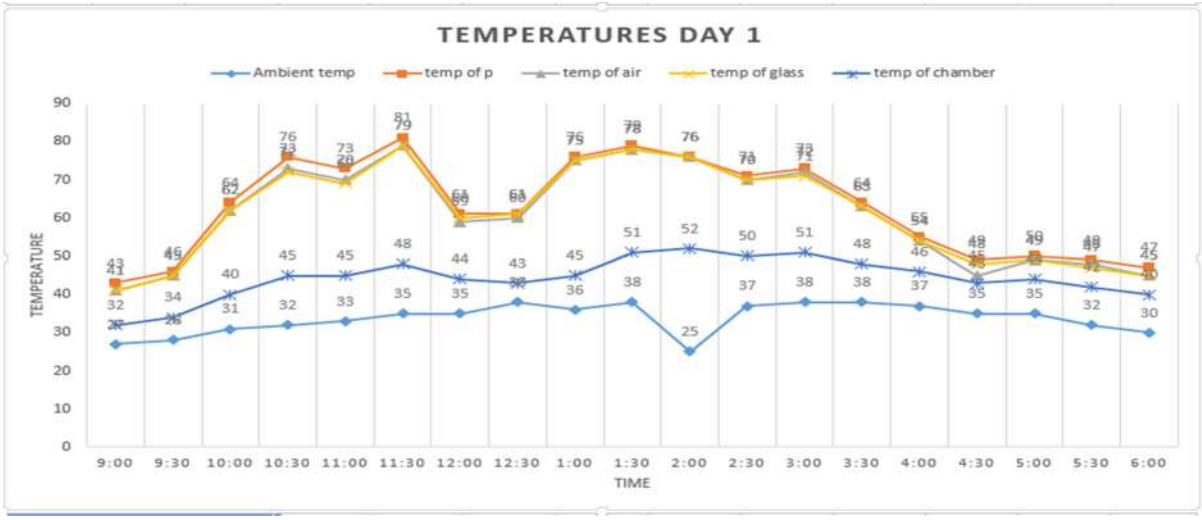

Figure 3a: A graph of temperature against time (day 1).

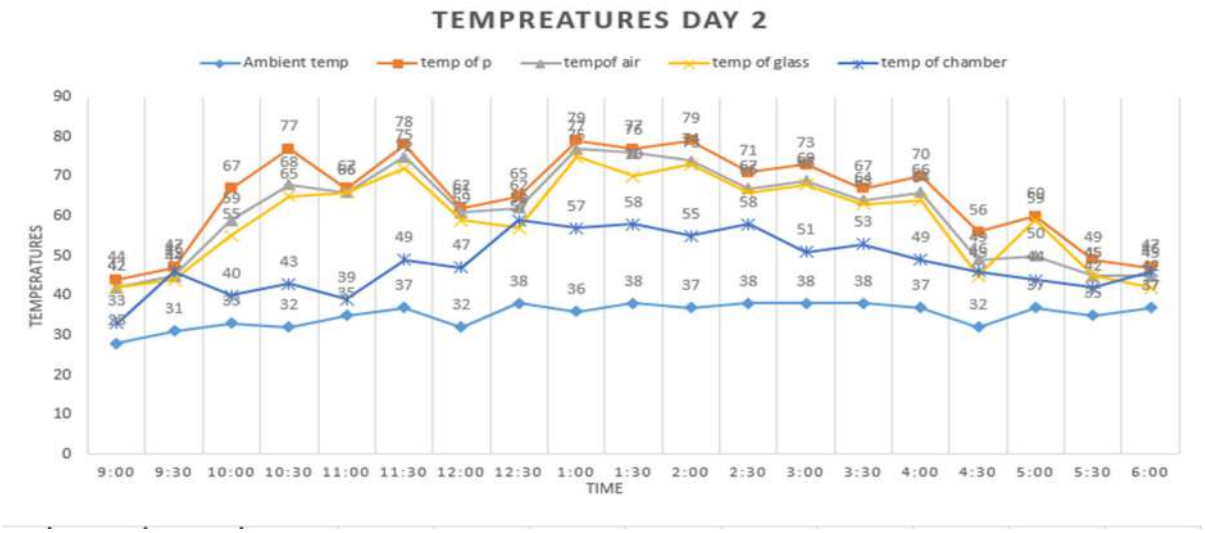

Figure 3b: A graph of temperature against time (day 2).

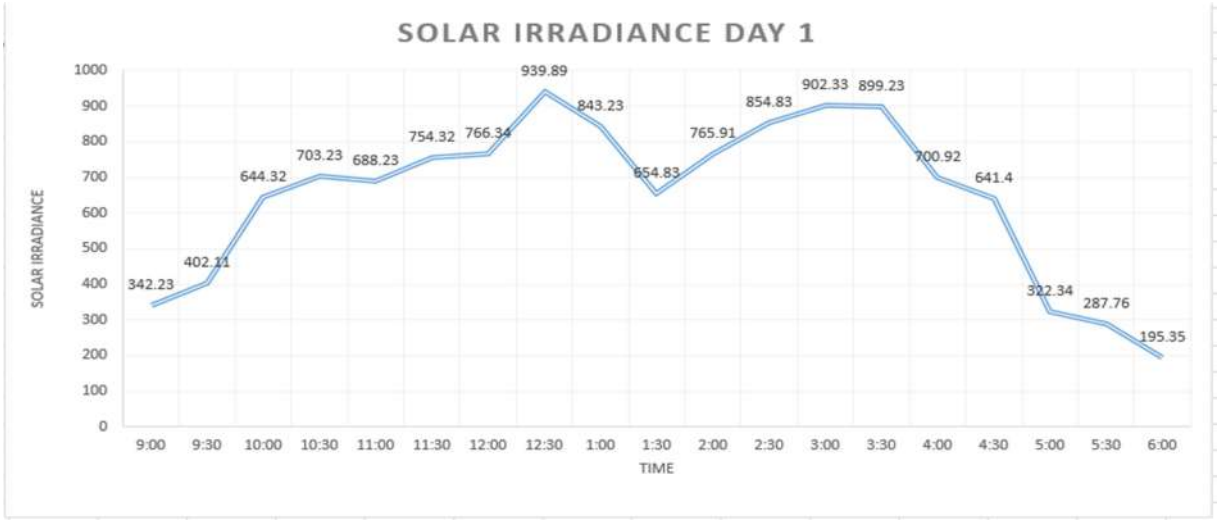

Figure 4a: A graph of solar irradiance against time (day 1).

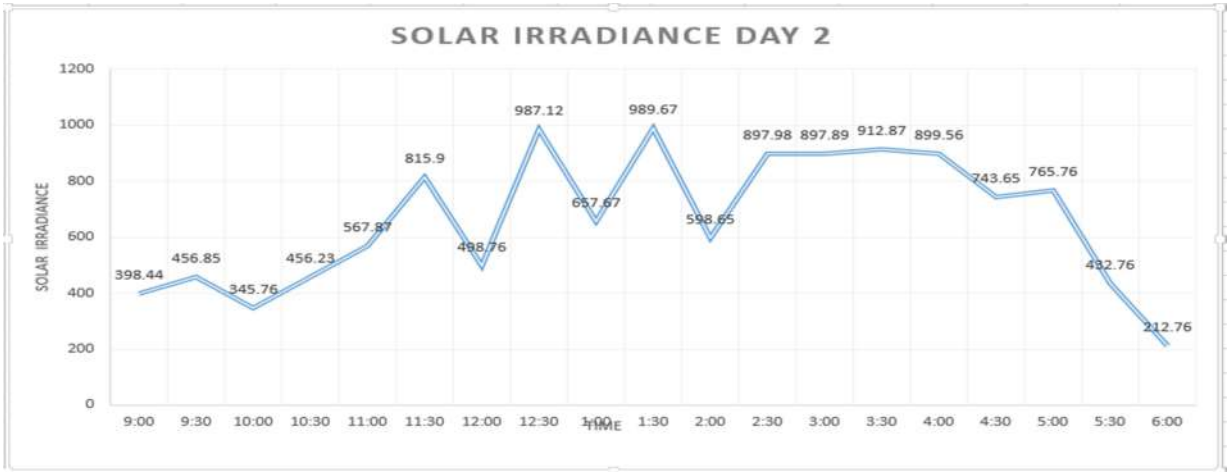

Figure 4b: A graph of solar irradiance against time (day 2).

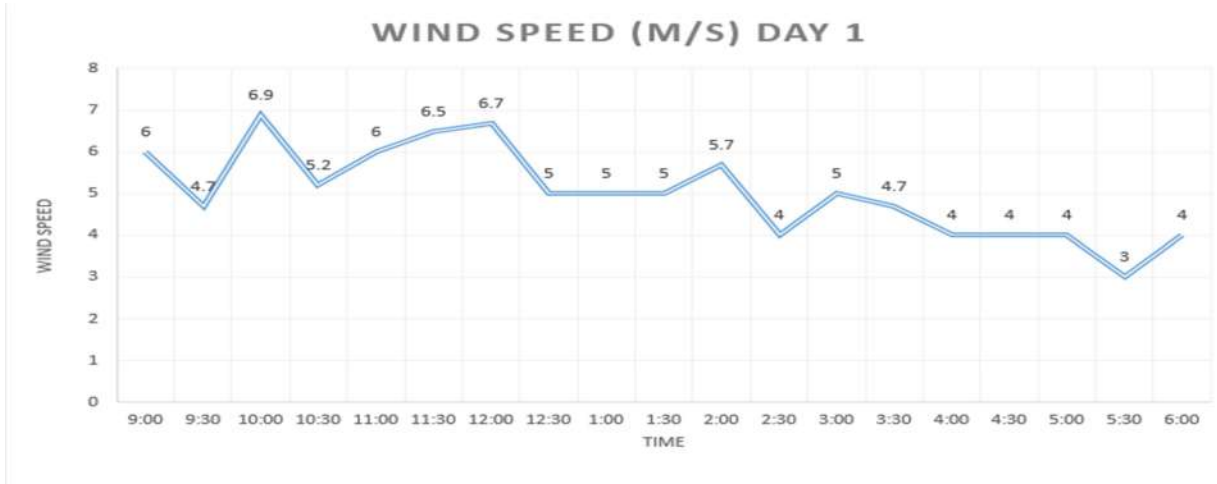

Figure 5a: A graph of wind speed against time (day 1).

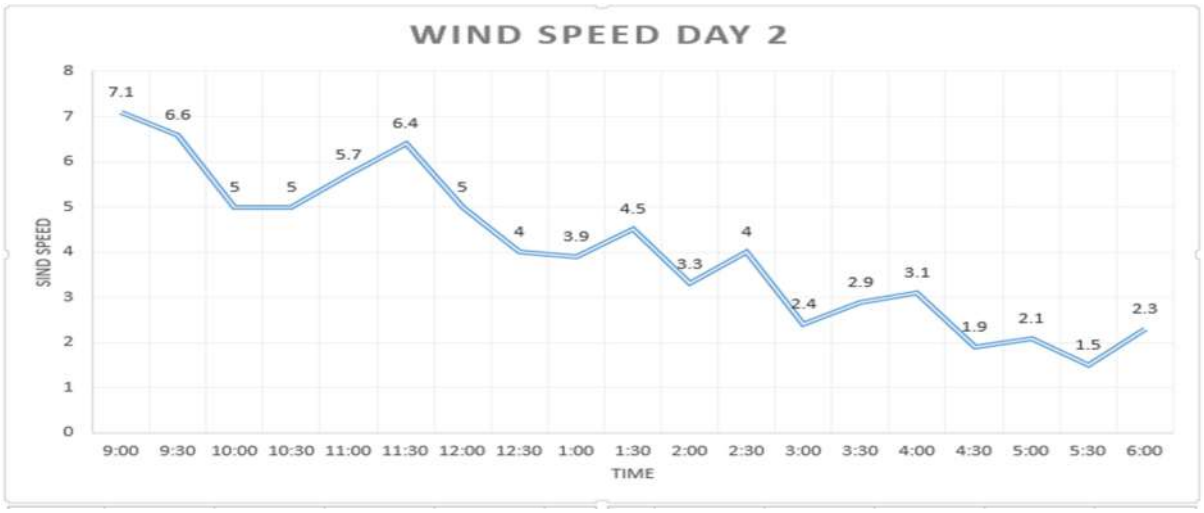

Figure 5b: A graph of wind speed against time (day 2).

CONCLUSION

An indirect solar dryer was constructed and was studied under actual environmental conditions of Sokoto, Sokoto State of Nigeria. It is an economical means to providing long term food preservation for remote areas and small communities in arid zones. Appropriate materials were chosen for constructing the various components of the solar dryer. From the tests carried out, the following conclusions were made.

The solar dryer can raise the ambient air temperature to a considerable high value for increasing the drying rate of agricultural crops. The product inside the dryer requires less attentions, there is ease in monitoring when compared to the natural sun drying technique. The capital cost involved in the construction of a solar dryer is much lower to that of a mechanical dryer.

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Preliminary Studies on the Efficacy of Honey in Milk Preservation

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Abstract: *This work was aimed at analysing the effectiveness of honey to enhance the shelf life of milk sold in some part of Maiduguri. Milk are produce in several part of Borno state, but it could not withstand the test time due lack of proper storage facilities. Samples were collected from Maiduguri Monday market. During this analysis bacterial load and pH value i.e. acidity were used as parameters, test and control were analysed for four days at the temperature of 37^{o}C in triplicate and an average reading was taken. From the results obtained in both preserved and control samples, differences in both pH and microbial load were observed with increase in every increase in time. In day one 4 x 10^{3} colony forming unit (CFU) and 6.9 pH value were observed in both samples, similarly, in day two 5 x 10^{3} CFU and 6.8 pH value were observed while 7 x 10^{3} and pH value 6.5 were obtained in the control sample, in day three 7 x 10^{3} CFU and 6.7 pH value were obtained in the test sample while 9 x 10^{4} and 5.4 pH value were obtained in the control, in day four 9 x 10^{3} CFU and 6.5 pH value were obtained in the test sample while 7 x 10^{5} and 4.8 P^{H} value were obtained in the control sample respectively. At end of this work it is established that pure honey have little effect on the shelf life of the milk as slight changes is observed in both pH and bacterial load in milk with honey and the one that served as control. It was also observed that at pH range of 4.5 to 50 usually get spoiled in this case, At day 3 and 4, the milk has already shown sign of spoilage due odour and texture as indicated by low pH of 4.8 - 5.4 respectively.*

Keywords*: Honey, Milk, Spoilage, Bacterial load, pH, Colony forming unit (CFU)*

INTRODUCTION

Milk and its related products consist of major food products which it provides the sole source of nourishment with rich nutritive values, however, the rich supply of products such as proteins and vitamins in them make it a very good source of growth medium for several pathogenic and spoilage micro-organisms such as *Klebsiella*, *Bacillus*, *Pseudomonas* and *Staphylococcus* are some of the most commonly encountered. Not only in infant, milk serve as one of major in adult, large world population rely on milk as source of source of food and other vitamins such

as vitamins including vitamin A, riboflavin, pyridoxine, biotin, niacin, vitamin D etc. Research findings have revealed that other characteristics of milk also support bacteria growth, such as water availability and dissolved oxygen which supports both aerobic and facultative anaerobic microorganisms (Singh& Anderson, 2004).

Milk preservation is a major setback especially where refrigeration facilities are a limited or in places where the temperatures go beyond 30^{o}C (Assaf and Khatib, 2021). Maintenance of food products intend in maintaining value both physiochemical and functional properties during their shelf life could be achieved by potential natural preservative which is best and more harmless, it was suggested that antioxidant could be significant contributors in mediated bacteriostatic or bactericidal activity of honey which could be in turn serve as natural preservative for milk (Krushna *et. al, 2007)*. Use of bio-preservative such as whole organism or their products has more advantage as the issue of toxic effect is ruled out, non-immunogenic and enhance the safety of milk products (Ameer *et. al,* 2019). These bio-preservative in microorganisms are produced as bi-products in different forms (Hutcins *et. al,* 2001).

Usually, the pH of cow milk which is commonly used ranges between 6.4 to 6.8, this quality do change due to some conditions, one of such condition is the increase in bacterial population and hence increase in bacterial waste products as a result the pH of the milk changes and the milk become sour and more acidic. Moreover, this is obtained due to the ability of bacteria in the milk to converts the sugar lactose into lactic acid (Helmenstine, 2020). Acidity rises as milk got spoiled consequently, acidity can be quantified to measure milk quality. Which can be articulated in two major ways: titratable acidity,
and hydrogen ion concentration or pH, which indicates acid strength. The natural acidity of milk is 0.16% - 0.18%, and samples with higher figures indicate developed acidity (Ministry of Agriculture, 2013) At lesser pH levels of 4.0 - 5.0, lactic acid bacteria are capable of growth and can produce lactic acid, at the same time as these organisms inhibit the growth of many disease causing bacteria and are also intentionally engaged to ferment milk to create other dairy commodities such as yogurt and cheese, they can also induce undesirable spoilage in certain products (Lowe & Arendt, 2004, Ruiz-Argüeso & Rodriguez-Navarro 1973).

Sample Collection

The samples of milk and honey were purchased from Monday Market Maiduguri Borno State Nigeria in a sterile container and transported to the the lab aseptically.

Preparation of sample

The honey, garlic extract and samples was divided into working or test sample and control, the sample were made in triplicate, to the working sample 10ml of honey were added to 50ml of milk and mixed uniformly, the control was allowed without honey.

pH Determination

Water proof pH meter was employed, the pH electrodes were inserted into a clean water beaker containing calibration reagent and calibrated adequately.the sample to be tested were placed in a clean beaker containing each of the milk samples and the results were read from the monitor (Azeeza *et.al,* 2010).

Microbial Load Determination

The spread plate method was employed in determining the microbial load of the milk samples; ten fold serial dilutions of the sample were made. 1 ml was pippetted into a sterile Petri dish containing growth medium initially prepared and spread evenly, this was incubated at 37^0C for 24 hours. All colonies appeared on the plate were counted using colony counter and recorded approprately (Monica, 2006).

Similarly, odour and texture of the samples were observe every after 24hours

Table 1: Microbial Load of Test and Control

Period	Bacterial load (Test)	Bacteria Load (control)
Day 1	4×10^3	4×10^3
Day 2	5×10^3	7×10^3
Day 3	7×10^3	9×10^4
Day 4	9×10^3	7×10^5

Figure 1: Graphical Illustration of differences in microbial load between test and control samples

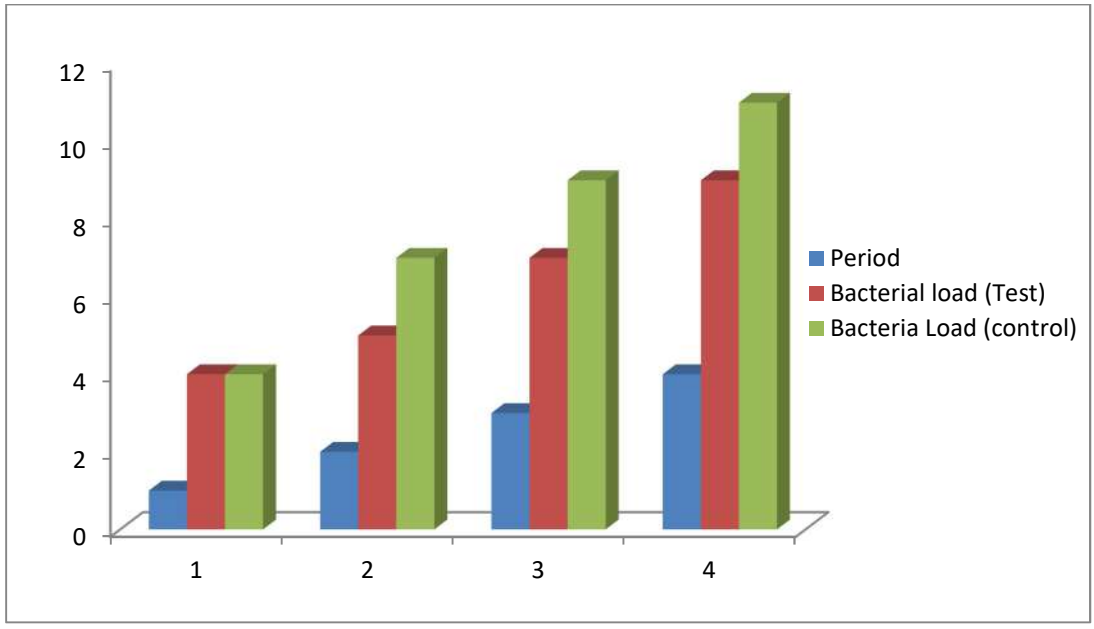
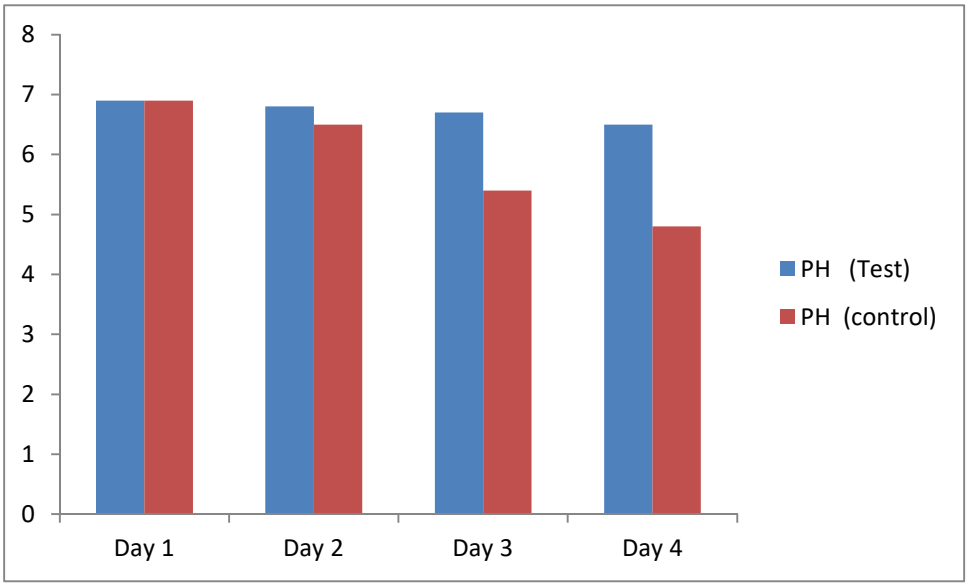


Table 2: pH Values of Test and Control

Period	pH (Test)	pH (control)
Day 1	6.9	6.9
Day 2	6.8	6.5
Day 3	6.7	5.4
Day 4	6.5	4.8

Figure 2: Graphical Illustration of differences in P^H between test and control samples



Discussion

From the result obtained in both preserved and control sample, differences in both pH and microbial load were observed with increase in every increase in time. In day one 4 x 10^3 colony forming unit (CFU) and 6.9 pH value were observed in both samples, similarly, in day two 5 x 10^3 CFU and 6.8 pH value were obtained in test while 7 x 10^3 and pH 6.5 were obtained in the control sample, in day three 7 x 10^3 CFU and 6.7 P^H value where obtained in the test sample while 9 x 10^4 and 5.4 pH value were obtained in the control, in day four 9 x 10^3 and 6.5 pH value where obtained in the test sample while 7 x 10^5 and 4.8 P^H value were obtained in the control sample respectively. It is observed that differences in microbial load and pH value (lower microbial load and low acidity value in test sample when compare to control) this shows that the honey is effectives to a certain extend in the preservation of the milk this is because microbial growths are reduced which in turn reduce acidity and this conforms with work of Aziza *et. al,* 2008. Similarly the microbial activity of the honey, its edible properties as well as its nutritional value combined together to make it a good and harmless preservatives.

As observed on the graph in figure 1, there is rapid increase in bacterial population in controlled sample more than the test sample, this is perhaps due to antibacterial activity of the honey which inhibits the growth of the bacteria and this assertion is in agreement with findings of Cooper *et. al,* (2002). The antibacterial properties in the honey serve as preservative in an indirect manner. Also, the graph in figure 2, which show the relationship between the pH of controlled and test samples, indicates an increase in acidity and in turn lower pH in controlled

and also lower acidity and more pH value in test sample, this is due to the decline in bacterial growth as also clearly stated by Helmenstine, (2020). According to Lowe & Arendt (2004), at pH range of 4.5 to 5.0 milk usually get spoiled.

In conclusion, at day 3 and 4, the milk in control has already shown sign of spoilage due odour and texture as indicated by low pH of 5.4 – 4.8 respectively however, the case is not the same in test sample as pH and microbial load is less and hence odour and texture are acceptable at similar day with controlled

Declaration of conflict of Interest

The authors declare that there is no conflict of interest whatsoever. The authors are responsible for the content and writing of the paper.

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Study of the Comparison between Wind Speeds in a Naturally and Artificially Ventilated Greenhouse

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Abstract: *Winds speed, temperature and humidity are all key factors influencing heat and mass transfer in developed greenhouses. These factors profiles in the entire ventilated greenhouse with tomato crops, were investigated by means of a customized and programmable (TPS- greenhouse-THE SESGREEN) anemometer system, and base on sensor. The experimental results showed that air speed was dependent on both external and greenhouse ventilation flux-φ, together with other factors of humidity and temperature. Under leakage, and due to negligible wind current and environmental temperature effects, air exchange rate remains constant, at a low value (below Maiduguri air velocity ; =4.6 m/s). Therefore, the measured air speed profiles and due ventilation flux, an estimation and mathematical model, calculation gives average internal air speed. This method of using an electronic measurement system, can still be applied to other crops' aerodynamic(s).*

Key words*; Anemometer, Customized, Flux, Greenhouse, SESGREEN*

Introduction

Ventilation, naturally or simulated by vent fans, in greenhouses, processes induce air exchange for the crop's chemistry both within interior air of a greenhouse and its external environment, as a result of air flux flow (wind) and temperature effects.

Ventilation processes induce an air exchange between the interior air of a greenhouse and its external environment due to wind and temperature effects. Air movement provided by natural ventilation influenced the convective heat exchange between the vegetation and the interior air, and thus the microclimate around the vegetation. Most recent studies on natural ventilation have used tracer gas techniques (Bot, 1983; De Jong, 1990; Fernandez and Bailey, 1992; Boulard and Draoui, 1995) and energy balance models (Fernandez and Bailey, 1992; Wang and Deltour, 1996). These two approaches, however, do not allow the air flow patterns in greenhouses to be determined.

Air movement between inside and external greenhouses, provides microclimate around the vegetation of the greenhouse. Most recently, study revealed that ventilation in a surrounding vegetation and energy balance, using balance models are enhanced by balance in the use of proper light shields and good ventilation. However, no data were provided for the support of the hypothesis of the relationships between the greenhouses' air exchange rate and the aerodynamics within.

The objective of this study is therefore to present an enquiry into the anemotry of air speed profiles, in a greenhouse, for external wind speed, buoyancy force and vent opening, humidity-temperature and greenhouse design. The experimental results provide the average air speed for the greenhouse environment's proposal and Gabble-Slopping type greenhouse for Maiduguri Coordinate.

Experimental set-up

The experimental greenhouse-the SESGREEN, was located in Maiduguri ($11^0$5000 N- latitude), and in the North of Maiduguri. This area is characterized by a strong wind of the sub-Sahara, channeled by the Sahara Desert in the East. Data for the anemotry were obtained directly from the SESGREEN-TPS, using computer logging system, in a location at the following coordinates; 11^0 5000 N and 13^0 000E, Altitude; 120.120520 m and Longitude; 130.174035 ^{0}, for all methods used. Cultivation of tomato begins with growing seedlings on a nursery, before transplant. The greenhouse size is ; $118 \times 710\ \mathrm{m}^2$ and outside cultivation is done at an area ; $31 \times 87\ \mathrm{m}^2$.The greenhouse therefore, contained mature tomato crops, growing on a substrate of rock wood-sand(loamy soil) and the crops were planted in twin rows of two.

Crops had 3-4 weeks required to produce a minimal plant size for study, i. e. about 0. 30 m-0.50 m, height. The crops were observed and data recorded, according to leaf size and stem height. Since greenhouse crops are self-pollinated through pollen dehiscence, therefore crops record for the data design are based on yields ,according to fruit weight freshness called its Dry mass. The yield development, during the production cycles were thus recorded.

Therefore, the distance for the vent windows (vent fans) corridors were in rows, separated 0.25 m apart. The leaf indices, during the first 3-4 weeks were 0.071 m and 0.093 m, respectively. The environmental temperature was taken to be the greenhouse (SESGREEN) room temperature at 38^0C, during the summer. Lamp heating device was also used.

Sonic anemotry and climatic measurement

Air, humidity and temperature profiles in the greenhouses were obtained, using the sensor device on Apllic-37, built within the greenhouse (SESGREEN) and base on two dimensional sonic system (Arieh, 2009).

The data were taken at a frequency of 3 Hz and 2-Dimensional resultant air speed in the horizontal plane, at one position and was averaged over 3 times-minutes and recorded on the computer logging (El Hassan, 2000).

The external wind or wind speed and direction, were all based on the coordination of Maiduguri position, and were used for the experiment, found in the data given by the Nigerian Meteorological Agency. Therefore, at Maiduguri flux constant of 5.67 Kw/m^2/day, the path, is at accuracy of about 1.3%, also at 1.68 $\mathrm{m/s^2}$.

Hence, all climatic parameters were then sampled for every 3-4 weeks and recorded on data logger (Fernie *et al*, 2006)

The climatic conditions during measurements, however provided the constants for the various short wave radiation ($11.99 \sim 12.\mathrm{o}\ \mathrm{Wm^2}$), wind speed ($3.0 \sim 2.0 \mathrm{ms^{-1}}$) and relative humidity(for various0 months, between 25 and 83 %), all at optimal temperature of 28 ^{0}C-38^{0}C, variation is at different situations between day and night periods, induced by ventilation (Harrigan *et al*, 2007).

Results and analysis

Values for Specific heat, at constant pressure ($\mathrm{C_{pa}}$)$\mathrm{in\ J/kgk}$, Enthalpy of water repair, at 0^0 C (HW), thermal radiation ($\mathrm{T_p}$),"violation rate" (VR), which is the rate of moisture balance due to perspiration per leaf of crops – in $\mathrm{m^3}$, mass density(ρ) in $\mathrm{Kgm^{-3}}$, Area of greenhouse-(55.5×35.5) in $\mathrm{m^2}$, mass balance (σ) in $\mathrm{Kgm^{-3}}$ and diffusion per leaf area ($\mathrm{R_p}$) in $\mathrm{Sm^{-3}kghr^{-1}}$, were all recorded on a table 1 below.

Table 1: values obtained from experiments

S/No.	Quantity	Value	Unit
1	Enthalpy of water repair/0 ^{0}C (HW)	2.502×10^6	J/Kg
2	Wind Velocity (v)	27.259	M^3/S
3	Violation rate (VR)	2.18	M^3
4	Mass density (ρ)	0.0252	Kg/M^3
5	Area (A)	1970	M^2
6	Diffusion per unit leaf area (R_p)	2.5	S/ M^3 Kg/hr
7	PH-Maiduguri (Damboa road)	6.28	
8	Mass balance (δ)	0.0252	Kg/M^3
9	Humidity ratio of moist air (WI)	0.001470	Kg/Kg
10	Humidity ratio of greenhouse (WP)	0.003767	Kg/Kg
11	Diffusion rate per leaf area (R_p)	2.5	S/m^3 Kg/hr
12	Electric conductivity of moist air $_{maiduguri}$	0.024	W/mK

Therefore, the physical parameters obtained in greenhouse, by weight according to the environmental constants are shown in table 2 below.

Table 2: physical parameters obtained

Name of crop	Fruit dry mass (g)	0ptimal temp. (^{O}C)	R.H. (%)
Tomato	0.048	23-28	50 - 60

Effect of wind speed, under varying humidity and temperature

During the measurements for the various months, the wind effects on internal greenhouse was recorded, the relationship between air velocity, at different heights of crop stem, the internal/external air speed, however continue to vary , January -2.0 ms^{-1}, march -3.0 ms^{-1}, and october – 2.0 ms^{-1}.

Specific calculations and programmes, using programming logger on the APPLIC-37 on SESGREEN uses sensors logger, and have been designed to enable processing. Therefore, environmental physical climatic conditions were recorded at the frequency 0.5Hz, humidity and temperature of between 25^0 C–75^0 C , at accuracy of $\pm$ 0.01^0 C and humidity, ranging between 0 % and 100 % at accuracy of $\pm$ 3%.The wind speed, however was measured at the range of 0 to 40 ms^{-1}, at accuracy $\pm$ 5% (Henderson *et al*,1993).

Mathematical models used

The relationship accounting to the combination of thermal and wind effects was used to calculate the ventilation flux ($\varphi \mathrm{m}^3$/s).

$\therefore \varphi = \frac{L_0 C_d T_e}{3\, g\, \delta T} \times [(\frac{g \delta T}{T_e} h + C_w {U_e}^2) 1\frac{3}{2} - (C_w {U_e}^2) 1\frac{3}{2}]$, where C_d and C_w are empirical discharge and wind effect coefficients, respectively, at between 0.64 and 0.09, g is the gravitational acceleration in ms^{-1}. However the vertical air speed profiles, perpendicular to the air flow can be used to estimate the mean greenhouse air speed, $v_{cal} = \frac{\varphi_v}{A}$, where A is the greenhouse area (Gupta, 2002).

Conclusion

The greenhouse sensors are able to determine the wind flux rate, as velocity vector and temperature, together with that of the humidity.

In this study, the difference in the measured parameters were, therefore determined through censoring measures and in programming device of micro scale, and in ventilation rates of the greenhouse (Mehl, 2005).

This is as a result of buoyancy effects that also generate the difference in both the temperature and humidity levels, and also in evaporation, due to plant transpiration.

Since production of crops in greenhouses are not without challenges. Therefore crops production require adjustments when needed, as they are produced by growers under research, prior to making adjustments , comparisons are made, including all the factors involved , i.e., temperature, humidity and air speeds Since water perspiration in crops and water evaporation provide standard to which evapotranspiration of crops, in a period of the year are, when compared with an outdoor production, then transpiration of water along into humidity, from plant canopy to the surrounding air-water vapour is given by m_t ,and it thus gives the rate of diffusion that provides the rate of moisture transfer in greenhouse (Micheal, 2005).

In order to characterize the impact of the related factors involved in equilibrium with the environment, the crops responses, after monitoring with respect to developed temperatures and humidity recorded revealed a clearest profile in expected metabolic level of growth and crop yield Y_a

However, the yield development is from fruits weight and temperature deficit on plant, due to humidity.

Also, at air conductivity of 0.298 S/m, and light intensity 5.67 Kw/ m^2 /day and PH of 6.26, mass balance M_T was at 4.36 Kg/hr.

Pressure deficit above 3 Kpa in greenhouses are available in greenhouse cultivation in the arid region, due to the climate. Nonetheless, evaporation rates and radiation in outdoor production, in active solar radiation when exposed accompanies reflection of further radiation by other bodies with the effect that there is going to be potential elevation of temperature above a normal growth rate and this deceases yields and quality of crops (Smith,1987).

Therefore, $m_t = 2A_p\rho_p(W_p - W_p) \times \frac{1}{R_p}$

Where A_p is the area of the greenhouse, ρ_p the mass density of air and water mixture, W_p the humidity of greenhouse, W_p the humidity ratio of moist air and R_p is the diffusion rate per leaf area.

$$\therefore M_t = \frac{2\times1970.5\times0.0252(0.000376-0.001470)}{2.5} \text{ and}$$

$$m_{t.=436}\,\mathrm{Kgh^{-1}}$$

This value varies according to crop, but taken as a standard for measurement (Ahammad, 2001).

However, in an open cultivation plant, Eva transpiration is related in 4-steps, ET_c in $\mathrm{mmh^{-1}}$ of crop and T is the mean temperature.

$ET_c = K_c ET_o$, where ET_c is the actual transpiration and K_c is the specific heat of air from the crop canopy. ET_o and ET_c are the relative crop coefficient and reference transpiration, respectively.

Therefore, with TH as greenhouse cover(windows) estimated at 0.03453×10^3 m and considering water vapour is $4.36\ \text{kghr}^{-1}$ and considering air is lighter than dry air, and that soil pressure is P, then the partial pressure of the day air temperature is (P – p), the ratio of the two components, water vapour and dry air is;

$$\frac{\text{kg of water vapour}}{\text{kg of dry air}} = \frac{\text{molar mass of water vapour} \times p}{0.029(P-p)}$$ (Cockshill,1985)

But at room temperature of a greenhouse, (P – p) is nearly equal to P, which at ground level (outdoor) is close to 1.0×10^5 Pa, approximately (Gavin, 2011).

The wind speed is determined by the flux used for ventilation, called the heat flux and is equal to 5.57×10^3 $Wm^{2.}$

$$\therefore \qquad V_{cal} = \frac{5.57 \times 10^3}{1970.5} = 7.83\ ms^{-1}.$$

The value continue to vary according to months of the year, too. The temperature remains between the range of 32^{o} C and above slightly, given enthalpy rate is 19.107 W/m^3 as provided in the greenhouse, thus provides energy that needs no further ventilation for crops. The humidity ratio varies for the crop used, at an average of 60% to 80% and remains within the growth rates of the crop.

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Insecticide Production from Orange Peel Oil

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Abstract: *This paper is aimed at explaining the production of a natural insecticide from natural extracts (orange peel oil and pepper), which are known for their insecticidal repellent properties. The extracts were obtained by extraction using benzene ether and water as solvents. A mixture of 50ml of orange peel, benzene ether oil, and 20ml of dried pepper water extract gave an active insecticide formulation (weight composition) which was determined to be very effective during the test on cockroaches, ants, mosquitoes, and spiders. The formulation obtained was in a liquid form and can be applied using sprays. The long-term effect and the modes of action of the formulation on the insects are not within the scope of this work. The formulation, however, is nontoxic to human health en inhaled but under normal conditions, the effects of ingestion or contact with the skin were not investigated.*

Key words: *Benzene Ether, Orange Peel, Limonene, Citrus Aurantiumdulcis, Citrus Autrantiumrisso and Citrus Maxima*

1.1Introduction

The scourge of malaria and other infectious diseases caused by mosquitoes and other insects alike can be very devastating. More than one million people die from malaria yearly and the majority of them are in Africa, particularly in Nigeria (WHO 2009). The effect on health and by extension the economy can be better imagined due to the fact that it kills the majority of the productive population. Malaria would be the focus of the research work because it has become a global health problem; moreover, it has reached an epidemic level. The work of scientists and researchers of the world health organization has therefore been able to develop a vaccine that completely eradicates the scourge as the parasites have developed resistance to drugs. It is therefore thought of the focus should be shifted from cure to prevention. This prevention can come in two forms. The first involves the prevention by cleaning the environment. The second involves the use of insecticides and treated nets which reduce deaths, especially among children. While we wait for the vaccines to be developed, the production of the insecticides can be from waste materials. The insecticides presently available in the market are too expensive, ineffective, and at times inaccessible because they are imported. This research, therefore,

hopes to lower the prices by producing them locally from waste materials. The effectiveness would however be determined by the end user. The benefit of this research is enormous to society.

1.2Aims and Objectives

This research seeks to investigate the possible use of orange peel in the production of insecticides. The work also tries to show in a limited manner the sustainability of orange as an alternative crop in the production of insecticides.

The specific objective is, therefore:

- To use the orange peel oil in the production of the insecticide.

1.3 Research Statement of Problem

Health and environmental concerns, the scarcity of insecticides, and other pest repellents the people all over the world especially in Africa are driving all research institutes to go locally with the aid of trees and plant herbs in the production of these insecticides. Orange peel could be readily available with its large oil content not put into any economic use as a result this research would explore the possibility of using the vast oil content of this fruit as a feedstock in the production of limonene. In other words, orange peel oil which could be obtained easily could be economically used in the production of insecticides to cut down costs and prevent malaria disease.

2.3 List of Materials

Table 1: List of materials used

S/N	MATERIALS	BOTANICAL NAME	SOURCE
1	ORANGE	CITRUS AURANTIUM RISSO	MAIDUGURI
2	PEPPER	CAPSICUM FRUTESCENT	MAIDUGURI

3.1 Methodology

Method used for the extraction of the oil. Decoating, drying and grinding. A soxlet extractor was used for solvent extraction of the oil. The solvent (benzene ether) was removed from the extract by distillation and the residual oil component was collected and used for the analytical work. A 5 liter capacity soxlet extractor was used in the extraction of the oil from the ground seeds, 200g (w_1) of the ground seeds were packed in a whatman filter paper and inserted in to the soxlet extractor; they together weigh (w_2) 40^0 C 60^0 C benzene ether (BDH analar grade) was used as the extracting solvent. The period of continuous extraction was 16hrs. at the end of the period, the solvent was recovered by simple distillation and the residusl oil was oven dried

at 100^0 C. The oil was then transferred to desiccators; weight (w_3) and allowed to cool, before being weighed. The drying, cooling and weighing was repeated until a constant dry weight was obtained (three cycles of treatment), to within 0.01g. The extracted oil sample was in a well-sealed dark brown coloured glass bottle and kept for analytical test.

3.2 Insecticide formulation

Potency Testing of Extracts

Before the formulation of the insecticides, drops of the extract was tested on insects such as ants, cockroach, weevil and mosquitoes to measure how long each extract takes to have effect on the target insect.

3.3 Derivation of formulation

20ml of the orange peel extract, and 20ml of the pepper extract were mixed in a glass container and tested for optimum concentration.

4.1 Results and Discussion

After conducting some tests on the effectiveness of the formulation on quite a number of insects, the results obtained were discussed briefly as to why the formulation has immediate effect on some insects and slow effect on some other insects.

4.2 Results

Table 2: Target insect and Extraction Results

s/n	Target insect	Extract (oil)	Qty (ml drop)	Results
1	Mosquito	Orange peel	2	Repelled 30 secs
2	Ant	Orange peel	3	Repelled 43 secs
3	Spider	Orange peel	3	Repelled 33 secs
4	Cockcroach	Orange peel	10	Repelled 4 mins
5	Mosquito	Peeper	2	Repelled 50 secs
6	Spider	Pepper	4	Repelled11 mins 43 secs
7	Ant	Pepper	1	Repelled1 secs
8	Cockcroach	Pepper	4	Repelled 3 mins

Table 3: Effects of the formulation (insecticide) on selected insects

Insect	Repellency	Killing
Mosquito	High	After 50 secs
Fly	High	After 40 secs
Ant	High	After 17 secs
Cockcroach	High	After 3 mins
Spider	High	After 1 mins

4.3 Discussion of Results

The results presented in table above showed all the sample insects repelled by the formulation of the orange peel oil and pepper, but variations occurred in terms of the interval of time it takes for a given insect to be knocked out. The variations may be explained by the fact that the individual extracts had different repelling capacities. That is probably why some of the insects likes spiders and cockroaches which are resistant to some chemical insecticides, took longer time to succumb to the formulation. The content of pepper is very active and may affect some of the insects. Ants are very vulnerable to the formulation that is why it took them a shorter time to die in contact with it, while it took the cockroach and spider a longer time to die in contact with it. Another benefit of the formulation is that the odor of the orange peel oil gives the treated room a perfumed smell besides its insecticidal property to overcome the odor of the pepper.

Conclusion

The formulation obtained by mixing 50 ml of orange peel (benzene ether) oil extract with 20 ml of pepper which gave an active natural insecticide with a killing property provided by pepper, while the repelling property provided by the orange peel oil. The cockroach resisted longer with 180 seconds while the fly resisted less with 30 seconds only. The formulation is non-toxic to humans when inhaledand raw materials can be obtained with ease in our environment.

Recommendation

Just as mentioned earlier, the focus of this research work was on the formulation of a natural insecticideswhich will compete with current chemical insecticides but the long term effect has not been investigation, therefore a further study is suggested to find out if there are any drawbacks from the formulation for necessary adjustment.

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Aflatoxigenic fungi and aflatoxin contamination of *Cochlospermum tinctorium* root powder (Kwata) used for soup preparation.

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Abstract: *Cochlospermum tinctorium root powder (Kwata) is commonly used for food and medicinal purposes in West African sub-region. This research evaluated the aflatoxigenic fungi and aflatoxin contamination of C. tinctorium root powder. The fungi were isolated and identified using a standard mycological method. Aflatoxin concentration of the 'Kwata' obtained from different sellers in Sokoto market was evaluated using Enzyme-Linked Immunosorbent Assay technique (ELISA). The total aflatoxin concentration of the different Kwata samples obtained in Sokoto market had concentration from 125 to 580 ppb while the aflatoxin B1 concentrations in the samples were found to be 35.5 ppb, 24.5 ppb, 32 ppb, 23.8 ppb and 45 ppb all of which are above the limits (20 ppb for adult food and 0 ppb for infant food) stipulated by regulatory bodies in Nigeria. The percentage occurrence of fungal species isolated from Kwata samples include; Aspergillus niger-50% in sample A and above 25% in other samples, Aspergillus flavus-50% in sample A and 25% in other samples, Aspergillus fumigatus 33% in samples B and E and 20% in the sample. In conclusion, it was discovered that Kwata sold within Sokoto metropolis is contaminated with aflatoxin and may pose serious public health problems in the long term primarily due to the consumption of soups prepared from Kwata by children population.*

Key words: *Aspergillus flavus, AgraQuant, Aflatoxin, Kwata, ELISA*

Introduction

Kwata (*Cochlospermum tinctorium*) is a bushy plant that is about 50 cm in height with widespread occurrence in Savannah and shrubs land throughout the drier areas of the West African region. It has common names in Nigeria which are: Rawaya or kyamba (Hausa), obazi or obanzi (Igbo) and sewutu (Yoruba). The plant is commonly used for medicinal purposes in West African sub-region for management of various conditions such as pain and inflammation. The roots of the plant are used traditionally to cure fever, hepatitis, and abdominal pain. They are

also used as a remedy for the treatment of gonorrhoea, jaundice and gastrointestinal diseases (Ahmad *et al.,* 2011).

An extract of the root of kwata (*Cochlospermum tinctorium*) is taken to treat malaria in Burkina Faso. In Nigeria, a concoction of the root with tamarind fruits is used to cure snake bites. A decoction is used in a bath to treat urogenital disorders, kidney pain and pain between the ribs. The body is washed with water extract of the root to cure skin diseases. In cote d'voire, powder of the root is applied topically to treat skin diseases; also the root is chewed as a tonic (Burkill, 2000).

Fungi, during their metabolic processes often produce secondary metabolites called mycotoxins; these are poisonous chemical compounds that are capable of causing disease and death in humans and livestock (Marta *et al.,* 2016; Bennett and Klich, 2003). Mycotoxins are not necessary for the growth and development of fungi; they are thought to be used by the fungus to weaken its host as a strategy to make the environment conducive for fungal proliferation (Hussein and Brasel, 2001). The presence of mycotoxins in agricultural products pose severe threats to human health and also cause significant economic losses in several countries (Horn 2003; Wu. F *et al.,* 2014).

Among the several types of mycotoxins, aflatoxins are of major concern with aflatoxin B1 being the most toxic to humans and animals (Olivier *et al.,* 2017). Generally, aflatoxins are genotoxic, carcinogenic, immunosuppressive substances and can cause both acute and chronic toxicity. Worldwide, aflatoxins are estimated to cause 28% of the total cases of the most common liver cancer - hepatocellular carcinoma (HCC) (Wu, 2014). Wu, (2014) suggest that approximately 172,000 cases of HCC per year are caused by consumption of aflatoxin-contaminated diet, and the majority of cases occur in sub-Saharan Africa. In addition to HCC, consumption of aflatoxin-contaminated foods can cause stunted growth in children, acute poisoning and immune-system dysfunction (Groopman *et al.,* 2008). Related health problems are difficult to diagnose, mainly due to cryptic, long-term and chronic exposures. However, as previously shown and recognised by the Kenyan government in 2004 and 2005, hundreds of human death cases can be ascribed to the consumption of aflatoxin-contaminated products (Lewis *et al.,* 2005).

Plants and herbs are used in most households for culinary purposes; they are often used as additives to enhance flavour and aroma. The consumption of plants or herbs contaminated with mycotoxins may cause ill effects rather than improving the well-being of an individual. This scenario can be averted if there is information on the levels of aflatoxin in commercialised plant root powder. This study aimed to isolate the aflatoxigenic fungi and determine the aflatoxin B1 profile of Kwata (*Cochlospermum tinctorium*), a popular root powder used in Nigeria for preparing soups. In addition, a key aflatoxin biosynthesis gene was amplified from some of the isolated aflatoxigenic organisms.

Materials and Methods

Sample collection

Samples of Kwata (*Cochlospermum tinctorium*) root powder were collected at Sokoto central market and then transported to Microbiology laboratory in Usmanu Danfodiyo University Sokoto, for further analysis.

Isolation and culture condition

The fungi associated with the 'Kwata' was isolated following serial dilution and inoculation on Sabouraud Dextrose Agar (SDA) at 25 ^{o}C for seven days and stored as spore's suspension on 20% glycerol for further analysis (Olivier *et al.,* 2017). Morphological and growth characteristics were carried on Sabouraud Dextrose Agar (SDA). The physiological analysis was carried out on Desiccated Coconut Agar (DCA) (Frisvad and Samson 2004), and yeast extract agar (YES) (Pitt *et al.,* 1983).

Identification of isolates

Isolates were identified using cultural and morphological features such as growth pattern, conidial morphology and pigmentation (Tafinta *et al.,* 2013). Microscopic observation was then carried out by placing a drop of lactophenol cotton blue stain on a glass slide, to this a portion of the fungal mycelia from a pure culture was added and covered with a coverslip, avoiding air bubbles in the process. Viewing was then carried out using the x10 and x40 objective lens and organisms were identified using a comprehensive fungi atlas by Samson and van Reenen-Hoekstra, 1988 (Oyeleke and Manga, 2008).

DNA extraction from Isolates

The DNA isolation was performed using the zymoBIOMICs kit (Zymo research). The cultured spores were added into zymoBIOMICs lysis tube, and 750 µl of zymoBIOMICs lysis solution was then added. It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 5 min. The lysis tube was then centrifuged in a microcentrifuge at 10000 x g for 1 min. 400 µl of the supernatant was transferred to a Zymo-spin and centrifuged at 8000 x g for 1 min. 1200 µl of zymoBIOMICs DNA binding buffer was added to the filtrate. 800 µl of the mixture (DNA binding buffer + centrifuged supernatant) was transferred to a Zymo-spin in a collection tube and centrifuged at 10000 x g for 1 minute. The flow from the collection tube was then discarded, and the step was repeated. 400 µl zymoBIOMICs DNA wash buffer 1 was added to the Zymo-spin in a new collection tube and was centrifuged at 10000 x g for 1 min; the flow was discarded. 700 µl of zymoBIOMICs DNA wash buffer 2 was added to the Zymo-spin in a collection tube and centrifuged at 10000 x g for 1 min and the flow through was discarded. 200 µl of DNA wash buffer 2 was added into Zymo-spin and centrifuged at 10000 x g for 1 min. The Zymo-spin was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (50 µl minimum) zymoBIOMICs DNase/RNase free water was

added directly to the column matrix. It was incubated for 1 min, then centrifuged at 10000 x g for 1 min to elute the DNA.

The Zymo-spin IV-HRC spin filter was prepared by removing the base of the Zymo-spin IV-HRC spin filter and placed into a clean collection tube centrifuged at 8000 x g for 3 min and the flow through was discarded. The cap was removed, and 400 µl zymoBIOMICs DNase/RNase free water was added to the Zymo-spin IV-HRC spin filter. The Zymo-spin IV-HRC spin filter was loosely capped and centrifuged at 8000 x g for 2 minutes. The eluted DNA was transferred to a prepared Zymo-spin IV-HRC spin filter in a clean 1.5 ml microcentrifuge tube. The Zymo-spin IV-HRC spin filter was loosely capped and centrifuged at precisely 8000 x g for 1 min.

PCR confirmation of aflatoxigenic fungi

The confirmation of aflatoxigenic fungi was performed as described previously (Medeiros *et al.,*2017). In order to optimise the PCR amplification assay for direct detection of mycotoxigenic fungal species by targeting the aflatoxin biosynthesis gene in the fungi; the primers used for species-specific detection and amplification of the gene involved in mycotoxin biosynthesis were tested and confirmed. The PCR amplification was standardised by empirically varying critical factors that affect amplification such as primer concentration, amount of template and annealing temperature. The set of primers used for amplification are avf723F (5'-ATGGTCACATACGCCCTCCTCGGG-3') and avf1675R (5'-GCCTCGCATTCTCTCGGCGACCGAA -3'), with annealing temperature 58^{o}C. These primers amplify the *avfA* gene that is involved in the conversion of averufin (AVF) to versiconal hemiacetal acetate (VHA). The expected amplicon size is 950 bp (Yu *et al.,* 2000).

The PCR reaction was performed in 0.2 ml thin-wall PCR tubes with flat frosted caps in 17.5 µl PCR reaction volume containing 3µl of template DNA, 1µl each of primer avf723F and avf1675R, 12.5 µl Taq master mix - containing 20 µM of deoxynucleoside triphosphates (dNTP), Taq DNA polymerase and Tag buffer with $MgCl_2$.

The PCR thermocycling conditions include Initial heat activation of DNA polymerase at 95^{o}C for 15 min; followed by 35 cycles of denaturation at 94 ^{o}C for 30 secs, annealing at 58^{o}C for 1 min, extension at 72^{o}C for 90 seconds, and a final extension at 72^{o}C for 10 min. The PCR products were electrophoresed on a 1% agarose gel with a 100bp DNA size marker at 96V for 1 hour (Medeiros *et al.,*2017). The PCR products were then purified using PCR product purification kit, and the purified PCR products were sent to Inqaba Biotech South Africa for sequencing service. The quality of the sequences from Inqaba was analysed using finch TV, and sequence analysis were performed by comparing the sequences against the known sequences in the NCBI database.

Quantification of Aflatoxin in Kwata

The total aflatoxin and aflatoxin B1 was determined by Animal care services Konsult (Nig) Ltd laboratory. The analysis of total aflatoxin and aflatoxin B1 (AFB1) content of the 'Kwata' samples were performed using ELISA kit AgraQuant® ELISA total and Aflatoxin B1 (Romer Labs, Singapore). Five grams (5g) of Kwata was weighed and added to 25 ml of methanol (70%). It was allowed to stand for 10 mins in order to aid aflatoxin extraction, then filtered using a No. 1 Whatman filter paper. 50 µl of sample filtrate and aflatoxin standard were dispensed in separate dilution wells, and each was with 100 µl of the conjugate. 100 µl from the filtrate/standard-conjugate mixture was taken and dispensed in the antibody-coated wells. It was then incubated at room temperature for 15 mins. The content of the wells was discarded, and the wells were washed 3 – 4 times with distilled water. 100 µl of substrate was added to each well and incubated for 5 mins to allow for colour change (different shades of blue to colourless). 100 µl of stop solution was added which converts the blue end-point to yellow, then the mixture was read with an ELISA plate reader at 450nm. The optical densities of standards (0 ppb, 4 ppb, 10 ppb, 20 ppb and 40 ppb) and those of samples were recorded. A standard curve was generated which was used to extrapolate the concentrations of total aflatoxin and aflatoxin B1 of the samples. Samples with high levels of aflatoxin were diluted further with 70% methanol to either 1/10th or 1/20th (or more) of the original concentration in order to obtain readings within the range of the standard curve.

Results

Aflatoxin B1 concentrations in Kwata (*Cochlospermum tinctorium* root powder) obtained from old market Sokoto and its limits for food in Nigeria showed that sample E had the highest concentration of aflatoxin B1(45 ppb), sample A (35.5 ppb), sample B (24.5 ppb), sample C (32 ppb), and sample D has the lowest concentration (23.8 ppb) (**Figure 1**).

The fungi associated with the contamination of Kwata (*Cochlospermum tinctorium* root powder) were determined in this work. Morphological and microscopic identification of the isolated fungi showed that the *C. tinctorium* roots powder were contaminated with four organisms of which three were identified as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The fourth organisms could not be identified based on the chart used for mycological identification (**Table 1**).

The frequency of occurrence of fungi associated with *Cochlospermum tinctorium* root powder obtained from Sokoto market was determined, and the result shows that *A. niger* and *A. flavus* had frequency of 50% in sample A and had more than 25% frequency in all the other Kwata samples (**Figure 2**). *A. fumigatus* had frequency of occurrence of 33.3% in both samples B and E, 20% in sample C, and 20% in sample D. The uncharacterised organism appeared in sample B and D with occurrence frequencies of 16.6% and 20% respectively.

PCR analysis detected the presence of the aflatoxin biosynthesis gene (*avfA*) in *A. flavus* and the uncharacterised organism from the Kwata. The molecular analysis confirmed the presence of the *avfA* gene in the *A. flavus* with 99% identity to *A. flavus* NRRL 3357 and the uncharacterised organism had 100% identity to an *avfA* gene from *A. flavus* isolate AF70 (**Table 2**).

Phylogenetic analysis based on neighbour-joining tree showed that our the aflatoxin biosynthesis genes from our isolates had some degree of difference based on their nucleotide sequences with the divergent from the root at 0.2 confidence limit based on the bootstrap analysis (**Figure 3**).

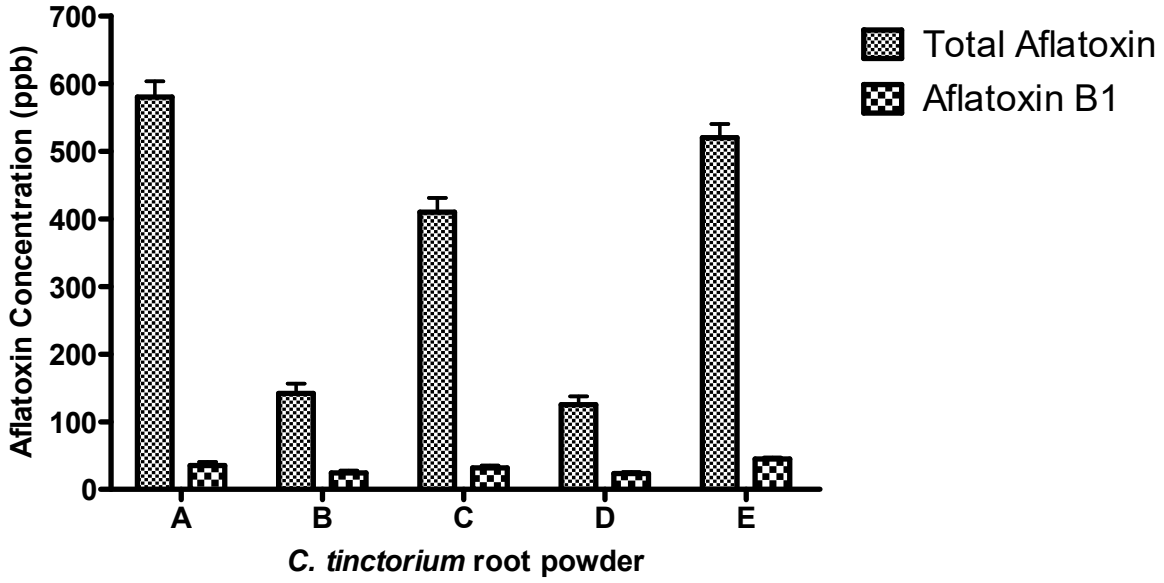

Figure 1: Total Aflatoxin and aflatoxin B1 concentrations in different *C. tinctorium* root powder samples obtained within Sokoto Market, Nigeria. Columns represent means of triplicate measurements and error bars are standard deviations (SD) from the means. The Nigerian limits for AFB_1 concentrations in adults and infants foods are 20 and 0 ppb (Tiffany, 2013) respectively.

Table 2: The Phenotypic identity of fungal species isolated from Kwata (*Cochlospermum tinctorium* root powder) obtained within Sokoto market.

Identified organism	Colony description	Microscopy
Aspergillus niger	It is black having a round shape, and it is powdery. The reverse is yellow.	The conidiophore terminates in vessels, and the conidia are in chains.
Aspergillus flavus	It is green in colour and powdery.	They have hyphae bearing conidiophores
Aspergillus fumigatus	It is blue and smooth	They have hyphae with conidiophores
Uncharacterized	Black in colour with crystals	They have hyphae conidiophores

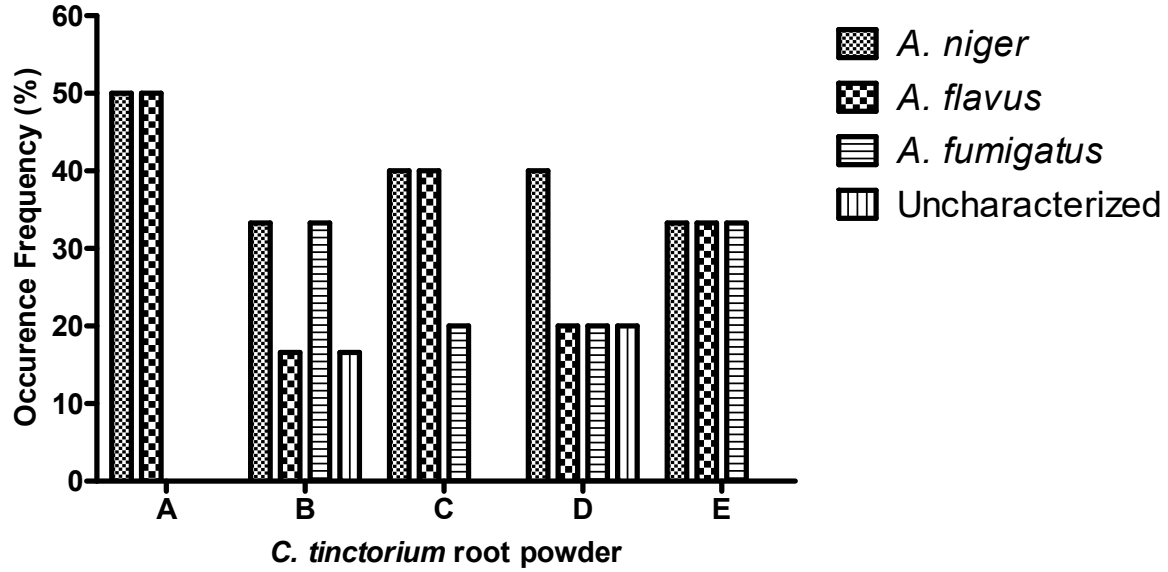

Figure 3: Frequency of occurrence of fungi associated with *C. tinctorium* root powder obtained from Sokoto market, Nigeria.

Table 4: Molecular confirmation of aflatoxigenic fungi based on PCR amplification of aflatoxin biosynthetic gene (*avfA*)

Seq ID	Best Hit	% Query coverage	% Identity	Accession
AF1	*Aspergillus flavus* NRRL 3357 SAGA Complex component (sgfl3)	69	99	XM002382146.1
SB	*Aspergillus flavus* isolate AF70 aflatoxin biosynthesis gene cluster complete sequence	100	100	XM002382146.1

Figure 3: Neighbour-joining tree showing the relationship of the aflatoxin biosynthesis genes of identified isolates to their closest relatives available on the NCBI database.

Discussion

In this study, we have detected the presence of aflatoxins and isolated aflatoxigenic fungi in Kwata obtained from Sokoto market, northwestern Nigeria, a root powder widely known for its culinary importance. High concentrations of aflatoxin B1 (AFB_1) were found in all the *Cochlospermum tinctorium* root powder samples; the concentrations were in the range of 23.8 ppb to 45 ppb which are all higher than the maximum acceptable limits of aflatoxins in foods. Therefore, the Kwata obtained from Sokoto market is not safe for human or livestock consumption. Aflatoxins are correlated to adverse health effects such as cancer; the most toxic amongst them is aflatoxin B1 (AFB_1), it is a potent carcinogen and has been directly related to liver cancer in several animals. AFB_1 is carcinogenic because it is metabolized by the liver to the highly reactive and electrophilic epoxide intermediate which causes hepatotoxicity (Dohnal and Kuča, 2014).

The mycotoxin producing organisms associated with Kwata (*Cochlospermum tinctorium* root

powder) were isolated and characterized. These organisms include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and another uncharacterized organism. The presence of these organisms is not surprising as they could come from soil or field (preharvest) or during storage (postharvest) (Marta *et al.,* 2016). *Aspergillus spp*. are widely distributed and are the major sources of mycotoxins. *Aspergillus* is found in food storage places and produces mycotoxins at suitable moisture and temperature conditions (Surekha *et al*., 2011). Amongst all the *Aspergillus spp*., *A. flavus* is the major producer of AFB_1 and AFB_2. Other species such as *A. parasiticus* also synthesizes AFB_1 and AFB_2 alongside other aflatoxins such as AFG_1 and AFG_2 (Bennett and Klich, 2003). *A. niger* and *A. fumigatus* have not been reported to produce aflatoxin; however, some strains were found to produce ochratoxin A. and gliotoxin respectively (Schuster *et al.,* 2002; Nieminen *et al.,* 2002). The biological, chemical and physical conditions of *Aspergillus* influence the production of aflatoxins (Kumar *et al.,* 2017).

Amongst the four organisms isolated, *A. niger* has the highest frequency of occurrence in all the samples. Interestingly, *A. niger* has been shown to inhibit the biosynthesis of AFB_1 in *A. flavus* through down-regulation of the expression of major biosynthetic genes. Remarkably, 19 out of 20 aflatoxin biosynthetic genes were reported to be down-regulated by *A. niger* (Xing *et al.,* 2017). Despite this crucial biological role played by *A. niger* in inhibiting AFB_1 biosynthesis, the concentration of AFB_1 in our samples is alarming as it is at least 4-fold higher than the acceptable limit.

PCR analysis detected the presence of a critical aflatoxin biosynthesis gene (*avfA*) in our isolated *A. flavus* and the uncharacterized organism from the Kwata. The molecular analysis confirmed the presence of the gene in our *A. flavus* isolate with 99% identity to that from *A. flavus* NRRL 3357 and the uncharacterised organism showed 100% identity to aflatoxin biosynthesis gene from *A. flavus* isolate AF70. This finding further confirms that our isolates are indeed aflatoxigenic organisms.

Although it is difficult to prevent aflatoxin formation in food before harvesting due to heavy rainfall, temperature and moisture content; however, it is possible to reduce their level by good hygienic conditions during transport and storage (Marta *et al.,* 2016; Zinedine and Maes, 2009). Decreasing or controlling fungal growth and eliminating aflatoxins formation in foods for human consumption and animal feed is essential for food security and health.

Conclusion

In conclusion, our findings show that kwata (*C. tinctorium* roots powder) obtained within Sokoto metropolis harbours fungal species that are capable of producing aflatoxins. Four different fungal species were isolated; three Aspergillus spp (*A. niger, A. flavus* and *A. fumigatus*) and one uncharacterized organism. Furthermore, PCR and molecular analysis of the key aflatoxin biosynthetic gene (*avfA*) confirmed that two of our isolates (A. flavus and uncharacterised) are indeed aflatoxigenic organisms. The 100% identity of the *avfA* gene from

the uncharacterised isolate to *A. flavus* isolate AF70 suggests that the uncharacterised organism is highly likely to be an *A. flavus* strain. The concentrations of total aflatoxins and aflatoxin B1 determined in all the samples exceeded the acceptable limits. Therefore, these results show that *C. tinctorium* root powder is not safe for consumption and it can be used in establishing public health awareness on the consumption of contaminated foods.

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Application of Cell Suspension Culture in Plant & Animal Biotechnology

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Abstract: *Cell suspension culture is a type of culture in which single cells or small aggregates of cells multiply while suspended in an uptight liquid medium. The establishment of single cell cultures through suspension cultures provides an exceptional chance to investigate the characteristics and potentialities of plant cells as well as animal cells for several reasons such as growth and characteristics in different cell lines, physiology and morphological presentations of such cell undergoing finite and continuous growth, studies for industrial research as well as Agricultural purposes. Such systems contribute to our understanding of the interrelationships and corresponding influences of cells in higher organisms. In addition, free cells in cultures permit quick manipulation and withdrawal of diverse chemicals/ substances thereby making them easy targets for mutant selection. Therefore, both plants and animal cells are in direct or indirect participation in suspension cultures where different varieties of crops and cell lines are grown for research and industrial purposes.*
Keywords: Cell, Culture, Suspension, Plants, Animal

Key words: *Aspergillus flavus, AgraQuant, Aflatoxin, Kwata, ELISA*

INTRODUCTION

Suspension culture or otherwise cell suspension culture as the name implies is a type of culture in which single cells or small aggregates of cells multiply while suspended in an uptight liquid medium. The establishment of single cell cultures provides an exceptional chance to investigate the characteristics and potentialities of plant cells. Such systems contribute to our understanding of the interrelationships and corresponding influences of cells in higher organisms. Many plant biotechnologists recognized the merits of applying cell cultures over an intact organ or whole plant cultures to synthesize natural products. Generally, substantial numbers of differentiated cells obtained from diverse tissue origin lose their specialized features and dedifferentiate when grown under conventional two-dimensional cell culture conditions (Hammond; 2001). Suspension culture is introduced as the most popular way of

preventing this problem and maintaining specialized features of cells. The rich and diverse range of available culture vessels for suspension culture makes the selection of specific culture devices baffling (Hammond; 2000) First attempt to perform cell suspension culture was performed by Haberland in 1902. However the process failed to achieve divisions in free cells, but his detailed paper in 1902 stimulated further studies in this area. Similarly, Steward and Shantz; 1996, performed far-reaching work on carrots and reported tremendous success in suspension cultures from carrot root explants and obtained a very large number of plantlets from the culture. More than half a century has passed since the concept and practice of plant cell culture was rest introduced. Unlike most animal cells, plant cells can change from one differentiated state, representing a committed developmental program, to an entirely dissimilar one through a transition by a dedifferentiated state typical of callus tissue. This process is attained by changing concentrations and relative size of two major plant growth regulators (auxin and cytokinin) in the culture medium. Under proper conditions, callus cells can continue to grow in ‘‘immortalized’’ suspension cultures, which can be maintained continuously without differentiation. Plant cells grown in culture showed extraordinary levels of genetic and epigenetic instability. Through reform exhibited in gene activity, plant cells are able to counter to the challenges presented by tissue culture conditions and continue to grow according to internal and external cues. Epigenetic regulation plays an important role. For example, hormone habituation is a process during which plant cells in culture swing their needs for exogenous growth regulators. (Tanurdzic; 2008)

Importance of Cell Suspension Culture Cell suspension culture is performed to obtain single cell clones. Through cell suspension culture the morphological and biochemical changes during plant growth and development phases are studied. Free cells in cultures permit quick administration and withdrawal of diverse chemicals/ substances thereby making them easy targets for mutant selection. Single cells derived from medicinally important plants can be studied for the production of secondary metabolites like alkaloids, glycosides. Suspension is now involved in mutagenesis study. The mutagens can be added directly in the liquid medium. After the mutagen treatment, cells are plated on agar medium for the selection of mutant cell clones (Encina *et al.,* 2001). The hope is that permanent changes in the DNA patterns of some of the cells would be achieved by such treatments. Plant-suspension cells are an in vitro system that can be used for recombinant protein production under carefully controlled certified conditions. Plant-suspension cells can be grown in shake flasks or fermenters to produce secondary metabolites, like vincristine and vinblastine, and to produce recombinant proteins after transformation. Plant cell suspension cultures have been used in various applications in research and discovery, as well as commercial production of the plants by means of micropropagation. They endow with suitable in vitro studies for genetic manoeuvring, mutant initiation, and protoplast production. The cultivation of these cultures can be done at any time in a suitable laboratory environment without being affected by natural conditions such as weather or seasons. Consequently, suspension culture proved promising in the field of plant biotechnology and therefore could help in increase in food production for the rapid increasing global population (Encina et al., 2001). In an upgrading arrangement three-dimensional

suspension culture have established to be an favourable alternative to monolayer techniques for major expansion of cells, suspension methods have been widely adopted: (1) for scalable and controlled extension of stem cells as well as cancer cells ; (2) for channelling stem cell differentiation; (3) for the fabrication of cellular spheroids well as tissue-like constructs . The stipulation of a 3D suspension culture atmosphere, imitating the microenvironment of the cellular niche, has confirmed to be advantageous, encouraging cell survival and maintaining cell functional properties *in vitro* (Massai *et al*, 2016).

Animal Suspension Cultures

As practice in the field of agriculture in particular plant propagation, suspension cultures is also applicable in field of animal as well as microbiological cell propagation. In animal tissue culture three major ways of cultivation were adopted: i. Organic culture ii. Explants plant culture iii. Organotypic culture In organic culture, the architectural properties are of the tissue is maintained in the culture, although not complete. Liquid gas interface are utilised for this purpose which enabled the maintenance of three dimensional structures. On the other hand, explants culture involved the utilization fragments of cell aggregates are placed at the glass – interface in which subsequent attachment enhances passage unto the solid substrate. Similarly, cell culture exhibits that the tissue extrusion from initial explants is dispersed by means of mechanical process or chemical process in to a cell suspension which are usually grown as adherent monolayer on growth suspension media (Lan, 2006)

Applications of pluripotent stem cells in therapeutic and industrial require outsized cell number produce in defined conditions. Single cell-inoculated suspension cultures of human pluripotent stem cells (hPSCs) counting human induced pluripotent stem cells (hips) and human embryonic stem cells (hESC) to stirred tank reactors . These systems allow uncomplicated increase and monitoring of input process. (Haverich *et al*, 2012)

Characteristics and Distribution of Suspension Cultures

The suspension cultures could be categorised into two (2) each containing subdivision: A) Batch Culture: a. Slowly rotating culture b. Shake culture c. Spinning culture d. Stirred culture B) Continuous Culture: a. Chemostats b. Turbidostats. Batch culture Batch culture is a type of suspension culture where the cell components separate in a finite volume of agitated liquid containing medium, such as cell material in quantity range of 10 ml or 20 ml or 30 ml liquid medium in each course constitute a batch culture. Batch suspension cultures are us in most cases maintained in conical flasks incubated on orbital platform shakers at the speed range of 80-120 revolutions per minute (rpm). Single cells and cell aggregates are grown in a specially designed flask; each flask contains about eight nipples like projections. The volume of each flask measured 250 ml. Ten flasks were loaded in a spherical manner on the big horizontal disc of a vertical shaker. The rotation of the flat disc at the speed of 1-2 rpm, the cell within each nipple of the flask are alternately covered in cultured medium and exposed to the air environment. The older medium is constantly replaced by the fresh liquid medium to stabilize the

physiological conditions of the multiplying cells (Biodiscussion, 2015). Under normally circumstances, the liquid medium is not replaced until the exhaustion of some nutrients available in the medium and the cells are kept in the medium for some time. As a result active growth phase of the cell declines the depletion of nutrient. In continuous culture system, nutrient depletion does not occur due to continuous flow of nutrient medium and the cells always remain in the steady state of active growth phase. In this system, culture vessels are generally cylindrical or circular in shape and possess inlet and outlet pores for aeration and the introduction of and removal of cells and medium (Biodiscussion, 2015)

Shake Culture: It is very straightforward and efficient system of suspension culture. This method involves single cells and cell aggregates in preset volume of liquid culture medium are positioned in conical flask. Conical flasks are mounted with the aid of clip on a horizontal or parallel large square plate of an orbital platform shaker. The plate moves by a circular motion at about180 rpm. Spinning Culture: Large volume of cell suspension may be cultured in 10L,bottles which are rotated in a culture spinner at 120 rpm at an angle of 45 0.

Stirred Culture: This system is also used for large scale batch culture. In this method, the large culture vessel is not rotated but the cell suspension inside the vessel is kept dispersed continuously by bubbling sterile air through culture medium. The use of an internal magnetic stirrer is the most convenient way to agitate the culture medium safely. Magnetic stirrer revolves at 200-600 rpm. The culture vessel is a 5-10 litres round bottom flask (Gregory and Susan, 2016).

Continuous Culture System: In this system, the old liquid medium is continuously replaced by the fresh liquid medium to stabilize the physiological stage of the growing cells. Normally, the liquid medium is not changed until the depletion of some

nutrients in the medium and the cells are kept in the same medium for a certain period. As a result, the active growth phase of the cell declines the depletion of nutrient. The cells passing through out flowing medium are separated mechanically and reintroduced in the culture. **Chemo stats**: In this system, cultures vessels are generally cylindrical or circular in shape and posse's inlet and outlet pores for aeration and for introduction of and removal of cells and medium. The liquid medium containing the cell is stirred by a magnetic stirrer. The introduction of fresh sterile medium, which is pumped in at a constant rate into the vessel is balanced by the displacement of an equal volume of spent or old medium and cells. Such a system can be maintained in a steady state so that new cells are produced by division at a rate which compensate the number lost in outflow of spent medium (Narges *et al*, 2015) Turbostats: In this system, the input of medium is intermittent as it is mainly required to control the rise in turbidity due to cell growth. The turbidity of a suspension culture medium changes rapidly when cells increase in number due to their steady state growth. The changes in turbidity of the culture medium can be measured by the changes of optical density of the medium. In Turbostats an automatic monitoring unit is connected with the culture vessel and such unit

adjusts the medium flow in such a way as to maintain the optical density or PH at chosen, present level (Astrid Catalina et al, 2016).

Characteristics and Features of Suspension cultures in Plants

The concept of plant suspension cultures has soon started for about half a century when the idea and practice of animal cell suspension cultures was also introduced (Tanurdzic et al., 2008). The most striking and fascinating about the plant suspension culture is its ability to change from one differentiated state to an entirely different one through a transition via a dedifferentiated state typical of callus tissue. This process is attained by different concentrations as well as relative proportions of two most important classes of plant growth regulatory hormones (auxin and cytokinin) in the growth medium (Osborne and Mcmanus, 1986). Establishment of suspension cultures of plant cells in liquid medium, similar to microbes, in the mid-1950s prompted scientists to apply this system for the production of natural plant products as an alternative to whole plant. The first attempt for the industrial production of secondary metabolites in vitro was made during 1950-1960 by the Pfizer Company and the first patent was obtained in 1956 by Routien and Nickell. However, not much progress in this area was made for many years. Apparently, the industrial production of secondary metabolites required large scale culture of cells (Bhojwani and Razdan,1996).

Flow diagram showing cell suspension culture and regeneration of plant by embryogenesis

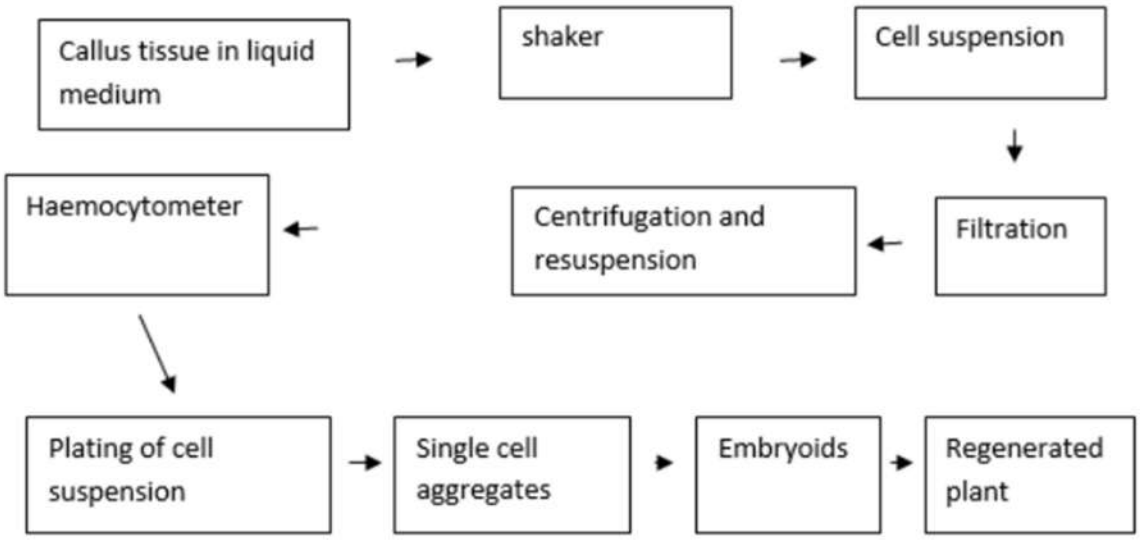

Figure 1; Flow diagram showing cell suspension culture and regeneration of plant by embryogenesis

Culture medium for suspensions

The medium used for raising fast growing friable callus should generally prove suitable for initiating suspension cultures of that species provided, of course, agar is omitted from it. Manipulation of the auxin/ cytokinin ratio to achieve better cell dispersion is desirable. For tobacco, increasing the concentration of 2,4-D from 0.3 mg 1-1 to 2 mg 1-1 and supplementing the callus medium with additional vitamins and casein hydrolysate have been recommended In actively growing suspension cultures the inorganic phosphate is rapidly utilized and, consequently, it soon becomes a limiting factor have demonstrated that in tobacco suspension cultures maintained in a medium with standard MS salts the phosphate concentration declines to almost zero within 3 days of the initiation of culture. When the phosphate concentration in the medium was raised three times the original level, it was completely utilized by the cells within 5 days. B5 and ER media given in Table 3.1 were developed for suspension cultures of higher plants. These and other synthetic media are normally suitable only if the initial population density is around 5 • 104 cells m1-1 or higher. With a lower cell density the medium needs to be enriched with various other components (Bhojwani and Razdan,1996)

In a nutshell, both plants and cells are in direct or indirect participation in suspension cultures where different varieties of crops and cell lines are grown for research and industrial purposes.

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Statistical Analysis on People Affected with Malaria Fever in Rumirgo Askira Uba Local Government, Borno State using Chi-Square Test

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Abstract**:** *The main aim of this research is to assess people with malaria fever according to sex and age in Rumirgo Hospital, Askira Uba local Government, and Borno state. Based on the finding it is revealed that the result of the analysis of chi-square test statistics in Table 4.1 2017 at a 5% level of significance the result show that is3.289318<4.575, and table 2020 is 8.648452<4.575 s both the two table H1 is rejected and H0 is accepted. Concluded that malaria fever does not depend on age and sex in Rumirgo hospital in Askira Uba local Area in Borno state, Table 2018 At a 5% level of significance the result show that that is 81.13969>4.575, table 2019 show that that is 27.26504 >4.575, and show that that is 8.648452<4.575 in both the three tables the result obtained reveal that H0 is rejected and H1 is accepted. Concluded that the malaria fever depends on age and sex in Rumirgo hospital in Askira Uba local Area in Borno state, From the result of the analysis, there are no means significant differences in tables 2017 and 2020 the result shows again malaria fever does not depend on age, while in the analysis from tables 2018, 2019, and 2021 there is a means significant difference it shows malaria is fever depend on age and sex. The government would provide enough hospitals for collecting drugs to eradicate malaria and Provision more laboratory equipment to enhance testing for malaria parasite*

Key words: *Chi-Square, Fever, Malaria, and Rumirgo*

INTRIODUCTION

The Chi-square statistic is a non-parametric (distribution-free) technique used to analyze group differences. Like every non-parametric statistic, to be more precise, this statistic can be used to see if the proportions of the relevant risk factor differ between the study groups. Karl Pearson created the chi-square test and the logic of hypothesis testing (Rakesh, 2015) and (Mary, 2013). One- way ANOVA and chi-squared technique are algebraically related and the null hypothesis

asymptotically the same (Ralph, 1972). According to Cengiz , (2009), Chi-square test statistic is given by the equation:

$$\chi_p^2 = \sum_{ij} \frac{\left(o_{ij} - e_{ij}\right)^2}{e_{ij}}$$

Chi-square test statistics were used in determines the degree of association between malaria prevalence and other factors influencing its transmission. The obtained showed that malaria prevalence depends on risk factors that promote transmission (Faga *et al*, 2010).
Chi-square tests were used in testing the mean of a population or comparing the means from two continuous populations (Teshome, 2019).
Nureni *et al* (2020) investigate that the Logistic regression method was used for the simple analysis of the dataset and it was revealed that people the age of 38-47 years are commonly affected with malaria and that females are the most infected gender species with headache being the most significant symptom based on its Wald statistic value.
Based on the results obtained in the analysis using Chi-Square statistical test, (8.409) > (3.841). Then > and p-value (0.000 <α 0.05) means that H0 is rejected, concluding that there is a statistically significant relationship between parental education level and malaria incidence (Lia, 2017).

Lawrence (2016) Revealed that children who visited rural areas were 6 times more to be expected to have malaria than those who did not Visit the rural areas.
The results reveal that patient symptoms cannot be used to forecast the patient's position, and additional information is required on medical history (AL-NAJJAR *et al*, 2020).
Asogwa (2015) carry out a study to confirm whether patients' diagnosed with the aliment have any relationship established on their gender, years, and age of diagnosis. The observation from the hypotheses agreed in this study using chi-square statistic, was that none of the variables of interest measured were dependent on each other.

Chi-square statistics is one of the usual statistical tests, used for the fit of measurement models and is also sensitive to sample size (Daniel, 2015).
Scholars have employed chi-square tests for more than one hundred years. This research addresses the question of how one would follow a statistically significant chi-square test result to determine the source of that result. Four methods were assessed: calculating residuals, comparing cells, ransacking, and partitioning (Donald, 2015).

RESULT OF THE ANALYSIS

Computational on the effect of malaria fever on sex and age using chi-square statistical technique that Employed for the test of independent of the data at 5% level of significance

H_0: malaria does not depend on age and sex

H_1: malaria does depend on age and sex

Table 1.1: Effect of Malaria Fever on Sex (2017)

S/N	MALE	FEMALE	TOTAL
1	95	105	200
2	93	84	177
3	100	90	190
4	152	148	300
5	125	125	250
6	117	113	230
7	150	160	310
8	128	112	240
9	140	130	270
10	141	143	284
11	136	124	260
12	149	141	290
TOTAL	1526	1475	3001

Table 1.2: Contingency Table

O	E	o-e	(o-e)^2	(O- e)^2/e
95	101.6994	-6.69943	44.88241	0.441324
105	98.30057	6.699434	44.88241	0.456583
93	90.004	2.996001	8.976024	0.099729
84	86.996	-2.996	8.976024	0.103177
100	96.61446	3.385538	11.46187	0.118635
90	93.38554	-3.38554	11.46187	0.122737
152	152.5492	-0.54915	0.301566	0.001977
148	147.4508	0.54915	0.301566	0.002045
125	127.1243	-2.12429	4.512616	0.035498
125	122.8757	2.124292	4.512616	0.036725
117	116.9543	0.045651	0.002084	1.78E-05
113	113.0457	-0.04565	0.002084	1.84E-05
150	157.6341	-7.63412	58.27982	0.369716
160	152.3659	7.634122	58.27982	0.382499
128	122.0393	5.96068	35.5297	0.291133
112	117.9607	-5.96068	35.5297	0.3012
140	137.2942	2.705765	7.321163	0.053325
130	132.7058	-2.70576	7.321163	0.055168
141	144.4132	-3.4132	11.6499	0.080671
143	139.5868	3.413196	11.6499	0.08346
136	132.2093	3.790736	14.36968	0.108689
124	127.7907	-3.79074	14.36968	0.112447

149	147.4642	1.535821	2.358747	0.015995
141	142.5358	-1.53582	2.358747	0.016548
				3.289318

DECISION RULE

At 5% level of significance the result show that $\chi_{cal} < \chi_{tab}$ that is 3.289318<4.575 H_1 is rejected and H_0 is accept. Concluded that the malaria fever does not depend on age and sex in the year 2017 in Rumirgo hospital in Askira Uba local Area in Borno state,

Table 1.3: Effect of Malaria Fever on Sex (2018)

S/N	MALE	FEMALE	TOTAL
1	157	210	367
2	201	246	447
3	196	172	368
4	189	199	388
5	138	149	287
6	165	83	248
7	158	106	264
8	178	143	321
9	204	197	401
10	165	200	365
11	165	191	356
12	250	153	403
TOTAL	2166	2049	4215

Table 1.4: Contingency Table

O	E	o-e	(o-e)^2	(O- e)^2/e
157	188.5936	-31.5936	998.1552	5.292625
210	178.4064	31.59359	998.1552	5.594839
201	229.7039	-28.7039	823.9147	3.586855
246	217.2961	28.70391	823.9147	3.791668
196	189.1075	6.892527	47.50692	0.251217
172	178.8925	-6.89253	47.50692	0.265561
189	199.3851	-10.3851	107.8493	0.54091
199	188.6149	10.38505	107.8493	0.571796
138	147.4833	-9.48327	89.93249	0.609781
149	139.5167	9.483274	89.93249	0.6446
165	127.442	37.55801	1410.604	11.0686
83	120.558	-37.558	1410.604	11.70062

158	135.6641	22.33594	498.8944	3.677425
106	128.3359	-22.3359	498.8944	3.887409
178	164.9552	13.04484	170.1678	1.031601
143	156.0448	-13.0448	170.1678	1.090506
204	206.0655	-2.06548	4.266209	0.020703
197	194.9345	2.06548	4.266209	0.021885
165	187.5658	-22.5658	509.217	2.714871
200	177.4342	22.56584	509.217	2.869892
165	182.9409	-17.9409	321.8768	1.759458
191	173.0591	17.94093	321.8768	1.859924
250	207.0932	42.90676	1840.99	8.889668
153	195.9068	-42.9068	1840.99	9.397277
				81.1399

DECISION RULE

At 5% level of significance the result show that $\chi_{cal} > \chi_{tab}$ that is 81.13969>4.575 H_0 is rejected and H_1 is accept. Concluded that the malaria fever depends on age and sex in the year 2018 in Rumirgo hospital in ASkira Uba local Area in Borno state,

Table 1.5: Effect of Malaria Fever on Sex (2019)

S/N	MALE	FEMALE	TOTAL
1	165	151	316
2	168	134	302
3	207	171	378
4	185	176	361
5	159	160	319
6	197	203	400
7	169	169	338
8	203	199	402
9	160	136	296
10	195	109	304
11	166	161	327
12	232	169	401
TOTAL	2206	1938	4144

Table 1.6: Contingency Table

O	E	o-e	(o-e)^2	(O- e)^2/e
165	168.2181	-3.21815	10.35647	0.061566
151	147.7819	3.218147	10.35647	0.070079
168	160.7654	7.234556	52.3388	0.32556

134	141.2346	-7.23456	52.3388	0.370581
207	201.223	5.777027	33.37404	0.165856
171	176.777	-5.77703	33.37404	0.188792
185	192.1733	-7.17326	51.4557	0.267757
176	168.8267	7.173263	51.4557	0.304784
159	169.8152	-10.8152	116.9676	0.688793
160	149.1848	10.81515	116.9676	0.784045
197	212.9344	-15.9344	253.9039	1.192405
203	187.0656	15.93436	253.9039	1.357299
169	179.9295	-10.9295	119.4548	0.663898
169	158.0705	10.92954	119.4548	0.755706
203	213.999	-10.999	120.9788	0.565324
199	188.001	10.99903	120.9788	0.643501
160	157.5714	2.428571	5.897959	0.03743
136	138.4286	-2.42857	5.897959	0.042607
195	161.8301	33.16988	1100.241	6.798742
109	142.1699	-33.1699	1100.241	7.738919
166	174.0738	-8.07384	65.18692	0.374479
161	152.9262	8.073842	65.18692	0.426264
232	213.4667	18.5333	343.4833	1.609072
169	187.5333	-18.5333	343.4833	1.831585
				27.26504

DECISION RULE

At 5% level of significance the result show that $\chi_{cal} > \chi_{tab}$ that is 27.26504 >4.575 H_0 is rejected and H_1 is accept. Concluded that the malaria fever depend on age and sex in the year 2019 in Rumirgo hospital in Askira Uba local Area in Borno state,

Table 1.7: Effect of Malaria Fever on Sex (2020)

S/N	MALE	FEMALE	TOTAL
1	138	153	291
2	152	172	324
3	188	190	378
4	109	101	210
5	167	154	321
6	155	134	289
7	148	162	310
8	127	123	250
9	167	162	329

10	170	177	347
11	191	158	349
12	189	173	362
TOTAL	1901	1859	3760

Table 1.8: Contingency Table

O	E	o-e	(o-e)^2	(O-e)^2/e
138	147.1253	-9.12527	83.27048	0.565984
153	143.8747	9.125266	83.27048	0.578771
152	163.8096	-11.8096	139.466	0.851391
172	160.1904	11.80957	139.466	0.870627
188	191.1112	-3.11117	9.67938	0.050648
190	186.8888	3.11117	9.67938	0.051792
109	106.1729	2.827128	7.992651	0.07528
101	103.8271	-2.82713	7.992651	0.07698
167	162.2928	4.707181	22.15755	0.136528
154	158.7072	-4.70718	22.15755	0.139613
155	146.1141	8.885904	78.95929	0.540395
134	142.8859	-8.8859	78.95929	0.552604
148	156.7314	-8.73138	76.23705	0.486419
162	153.2686	8.731383	76.23705	0.497408
127	126.3963	0.603723	0.364482	0.002884
123	123.6037	-0.60372	0.364482	0.002949
167	166.3375	0.6625	0.438906	0.002639
162	162.6625	-0.6625	0.438906	0.002698
170	175.438	-5.43803	29.57219	0.168562
177	171.562	5.438032	29.57219	0.17237
191	176.4492	14.5508	211.7257	1.199924
158	172.5508	-14.5508	211.7257	1.227034
189	183.0218	5.978191	35.73877	0.195271
173	178.9782	-5.97819	35.73877	0.199682
				8.648452

DECISION RULE

At 5% level of significance the result show that $\chi_{cal} < \chi_{tab}$ that is 8.648452<4.575 H_1 is rejected and H_0 is accept. Concluded that the malaria fever depends on age and sex in the year 2020 in Rumirgo hospital in Askira Uba local Area in Borno state.

Table 1.9: Effect of Malaria Fever on Sex (2021)

S/N	MALE	FEMALE	TOTAL
1	159	118	277
2	221	146	367
3	117	157	274
4	124	177	301
5	163	168	331
6	165	144	309
7	187	115	302
8	176	164	340
9	189	138	327
10	157	186	343
11	160	109	269
12	160	171	331
TOTAL	**1978**	**1793**	**3771**

Table 1.10: Contingency Table

O	e	o-e	(o-e)^2	(O-e)^2/e
159	145.2946	13.70538	187.8375	1.292804
118	131.7054	-13.7054	187.8375	1.426195
221	192.5023	28.49775	812.1215	4.218764
146	174.4977	-28.4977	812.1215	4.654052
117	143.721	-26.721	714.0134	4.968051
157	130.279	26.72103	714.0134	5.48065
124	157.8833	-33.8833	1148.079	7.271695
177	143.1167	33.88332	1148.079	8.021982
163	173.6192	-10.6192	112.7674	0.64951
168	157.3808	10.6192	112.7674	0.716526
165	162.0796	2.920446	8.529002	0.052622
144	146.9204	-2.92045	8.529002	0.058052
187	158.4078	28.59215	817.5111	5.160799
115	143.5922	-28.5922	817.5111	5.693285
176	178.34	-2.33996	5.475426	0.030702
164	161.66	2.339963	5.475426	0.03387
189	171.5211	17.47892	305.5126	1.781195
138	155.4789	-17.4789	305.5126	1.964978
157	179.9136	-22.9136	525.0308	2.918239
186	163.0864	22.91355	525.0308	3.21934

160	141.0984	18.90162	357.2711	2.532071
109	127.9016	-18.9016	357.2711	2.793328
160	173.6192	-13.6192	185.4826	1.06833
171	157.3808	13.6192	185.4826	1.178559
				67.1856

DECISION RULE

At 5% level of significance the result show that $\chi_{cal} > \chi_{tab}$ that is 8.648452<4.575 H_0 is rejected and H_1 is accept. Concluded that the malaria fever depend on age and sex in the year 2021 in Rumirgo hospital in Askira Uba local Area in Borno state.

CONCLUSION

Table 1.1 at a 5% level of significance the result shows that that is3.289318<4.575, and table 1.7 that is 8.648452<4.575 that s both the two table H1 is rejected and H0 is accepted. Concluded that malaria fever does not depend on age and sex in Rumirgo hospital in ASkira Uba local Area in Borno state. Table 1.3 At a 5% level of significance the result show that that is 81.13969>4.575, table 1.5 show that that is 27.26504 >4.575, and show that that is 8.648452<4.575 in both the three tables the result obtained reveal that H0 is rejected and H1 is accepted. Concluded that the malaria fever depends on age and sex in Rumirgo hospital in ASkira Uba local Area in Borno state. From the result of the analysis, there are no means significant differences in the tables 1.1 and 1.7 the result shows again malaria fever does not depend on age, while in the analysis from tables 1.3, 1.5, and 1.9 there is a means significant difference it shows malaria is fever depend on age and sex.

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Absorption and Desorption Heavy Metal in Different Types of Soil in Bade Postiskum and Nguru Local Government Areas of Yobe State, Nigeria

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Abstract: *This study was to evaluate the phytoremediation potentials of Phaseolus vulgaris (Beans plant) and soil for the metals Cd, Cu, Pb, and Zn. Set of laboratory pot experiment were conducted, viable beans seed were planted into 2kg soil spiked with the salt of the heavy metals. The soil received the salt of Zn as $Zn(SO_4)_3.6H_20$, Pb as $Pb(NO_3)_2$, Cd as $Cd(NO_3)_2$, and Cu as $Cu(SO_4)_2$ at a concentration of 1000ppm and 1500ppm respectively. A separated soil with untreated soil was used to serve as a control. Irrigation was done with 500ml of water after every five days in the evening hours for eight (8) weeks. Samples of the soil and beans were collected at the end of the experiment, the plant were wash with water and carefully separated into root, shoot, and seed, dried with the soil ground and sieve. The grounded soil, roots, shoot, and seed of the experimental plant as well as the control were analyzed for heavy metals. Following digestion with aqua-regia using Atomic Absorption Spectrophotometer (AAS). The BCF, EF, and TF were evaluated for the different metals. The Phaseolus vulgaris (Beans plant) may serve as phytostablizers or metal excluders of Cd, Cu, Pb, and Zn in the soil for having higher values of BCF and EF than TF. Phaseolus vulgaris (Beans plant) may also serve as a phytoextraction for metals in contaminated soil for having higher TF values.*

Key words: *Absorption, Heavy Metals, Plant, and Soil*

1.0 Introduction

Heavy metals are considered significant pollutants because they are non-biodegradable and would accumulate in the soil. Furthermore, heavy metals harmful to the environment because they get in creatures and plants and enter the human body through the food chain. With the rapid development of industry, especially mining and smelting, heavy metals cause significant pollution problems. Electroplating, dyeing, tanning, steel and automobile manufacturing, painting, and other chemical industries discharge the toxic substance into the environment

(Dhal *et al.* 2013; Singha and Sarkar 2015; Padmavathy *et al*. 2016). The large number of heavy metals entering into water or soil would break the dynamic balance between soil, water, and creature (Valderrama et al. 2010; Barrera-Díaz et al. 2012). In China, nearly all the concentrations of heavy metals, such as Cr, Ni, Cu, Pb, Zn, As, Hg, and Cd are higher than their background value in soil (Wei and Yang 2010). Chromium (Cr) is a highly toxic pollutant because of its high mobility and toxicity, even with a low concentration (Martí et al. 2013). Cr exists in the environment in different oxidation states, and the two most stable conditions are trivalent (Cr (III)) and hexavalent (Cr(VI)) (Dhaletal.2013). Chromium exists in four compound forms in soil. Two of them are trivalent (Cr(III)) anions: Cr^{3+} and $CrO^{2-,}$ and the other two states are hexavalent (Cr(VI)) anions: Cr_2O^{72-} and CrO^{42-} (Khezami and Capart 2005). The migration and transformation among the four different forms are influenced by organic content, soil pH, redox potential, etc. Cr (III) is more stable than Cr (VI), and the two states can transmit into each other under certain conditions (Hellerich and Nikolaidis 2005). The toxicity of chromium is closely related to the valence state. Cr (III) is one of the necessary micro-nutrients for the creature in a low dosage. However, the Cr(VI) is 100 to 500 times more toxic than Cr(III) (Kanwal 2012; Toma *et al*.2015.) and is carcinogenic, mutagenic, and teratogenic (Flora 2000). Most Cr has accumulated in the shallow soil surface, 0–20cm below the soil surface. Soil can be firmly adsorbed Cr (III), so once Cr (III) gets into the soil through waste, it will be readily adsorbed by soil colloids. Compared with Cr (III), Cr (VI) exists as an ionic state in soil solution with higher mobility in the soil system and aquatic environment. Cr (VI) is hard to be absorbed by soil particles, and it would transport with groundwater. Hence, Cr (VI) takes a significant threat to the groundwater, surface water, and plant ecosystem. Cr (VI) is easy to be absorbed by the human body and accumulated in an organ. Cr plays a crucial role to maintain human health. Still, excessive Cr can produce great harm to health, such as respiratory system disease and gastrointestinal problems, cause allergic contact dermatitis, and even lead to cancer (Dhal et al. 2013). According to the World Health Organization (WHO), the maximum contaminates level for Cr (VI) in drinking water is 0.05 mg/L (Bolan et al. 2003). For those reasons, Cr(VI) is regarded as a high hazardous pollutant.

Cu (II), is one of the most widely used heavy metals in the industry, is considered a micronutrient but is extremely toxic to the living organism under relatively high concentrations A. Oztürk, et al. (2004) Y. Nuhoglu, *et al*. (2002). To reduce the harm caused by heavy metals to soil and plants, the European Union has established maximum heavy metal limits for soil and industrial by-products such as biosolids and composts to be applied to fields. The soil criteria for Cu (II) set by the European Union is 140mgkg−1 Department of Agriculture for Scotland, (1958). Hence, removal of Cu (II) from the soil and groundwater has been the subject of many studies M. Alkan *et al.* (2001), S. Veli et al. (2007). Clay is a typical, highly weathered soil. It is widely distributed all over the world and contains a significant amount of Al and Mg oxides. Such soils have strong physical and chemical adsorption capacity, due to the soil particles with the large surface area, and carry a negative charge. Besides, different from other high permeability media such as sand, the unique mineralogy of clay such as porosity, pore size, and pore

structure must be considered when studying clay's absorbability. Several previous studies have focused on the absorbability of clay.

For instance, Tassanapayak *et al.* (2008) investigated the efficiency of clay in heavy-ion sorption and found that it can be utilized as potential heavy metal adsorbents in wastewater treatment. Hasine *et al*. (2008). Also studied the role of clay properties in heavy metal ion sorption and desorption with a series of experiments and found that soil composition would greatly affect the sorption efficiency. Li *et al*. (2003). Pointed out that once the clay is contaminated, it is very difficult and it will take a very long time to remove the pollutants. Adsorption is usually a primary process for the accumulation of heavy metals in soils while desorption is a straightforward process for the removal. The study of adsorption and desorption processes is of utmost importance for understanding how heavy metals are transferred between the aqueous and solid phases. In soils, heavy metals can be adsorbed as compounds like ions and complexes or exchangeable forms P. M. Huang et al. (1995). Virtual interfaces involved in heavy metal adsorption in soils are predominantly inorganic colloids Shah *et al*. (2006). A heavy metal such as Cu (II) can be absorbed into the soil and desorbed under certain conditions R. Segura et al. (2006). The mobility of heavy metals is often affected by soil characteristics, such as pH, amount of organic matter, temperature, and the types of ions.

Sources of heavy metals pollution

Heavy metals are derived from two major sources: natural and anthropogenic. Anthropogenic contamination of the environment with heavy metals is the most widely distributed and most deleterious. This is probably a result of their instability and solubility and hence bioavailability (Abdu *et al.*, 2011a). Human activities such as smelting, mining, agricultural activities such as mineral fertilizer and sewage sludge application and pesticide use, industrialization, metal-containing waste disposal, and military activities such as weapon testing are varieties of anthropogenic heavy metal contamination sources. Building materials like paints, cigarette smoke, metallurgy or smelting, aerosol cans, and sewage discharge are all anthropogenic sources of heavy metals (Abdu, 2010). Colouring of plastics during manufacturing is achieved through the addition of pigments containing heavy metals. Coating of cutleries, industrial and hand tools, airplane parts, automobile, and truck parts with heavy metals such as Cd are common anthropogenic sources of pollution (Kirkham, 2006). The use of Cd as luminescent dials and rubber curing also contribute to heavy metal pollution (Adriano, 2001). Wearing and tearing of automobile parts is a major exposure route of heavy metals to the environment. Cadmium can be released from automobile tires as it wears which can be transported into the sewage system through run-off (Kirkham, 2006) or as particulate matter into the atmosphere. Weathering and pedogenesis are the major natural sources of heavy metals. Mineral ores like galena, cerussite, cassiterite, and arsenopyrite can undergo dissolution through chemical weathering thereby releasing heavy metals contained in their structure (Abdu, 2010; Abdu *et al.* 2011b). Heavy metals are constituents of primary and secondary minerals through the process of inclusion, adsorption and solid solution formation termed as co-precipitation (Sposito, 2008). Acid rain and dew are also natural sources of heavy metal pollution (Nriagu,

1990). Atmospheric dust storms, wild forest fires, and volcanic eruptions are input routes for natural heavy metal pollution (Naidu *et al.*, 1997). The effect of pedogenic heavy metal pollution may override that of anthropogenic sources especially when the parent material contains a high level of heavy metal (Brown *et al.,* 1999). oxides and hydroxides of iron, aluminum, and manganese are major soil chemical components contributing to heavy metal mobility in the soil (Tack *et al.,* 2006). Large affinity of the crystalline and amorphous form of this metal oxides and hydroxides for heavy metal influences the movement and sorption of metals in soil (Abdu, 2010). The binding effect of organic matter on soil components also influences the availability of heavy metals (Naidu *et al.,* 2003). The diverse functional groups in organic substances which often dissociate easily under alkaline conditions also affect the availability of toxic heavy metals in the soil. Formation of metal-organic compounds in the soil is achieved through the interaction of humic substances with sesquioxides such as oxides of Fe, Al, and Mn. Heavy metals occluded in the oxides of these metals are often referred to as relatively active fractions (Shuman, 1985). Agbenin (2002), however, observed the inhibitory effect of soil organic matter on the crystallization of heavy metal occluded in Mn and Fe oxides in soils of the Nigerian savanna. The chemistry of the aqueous soil phase exerts a profound influence on metal mobility. Acidic conditions tend to increase the mobility of heavy metals as a result of proton competition and decreased negative binding sites (Horckmans *et al.*, 2007). Conversely, at elevated soil pH, heavy metals such as Pb may be precipitated as insoluble hydroxides. However, the functional groups present in organic matter may dissociate under alkaline conditions thereby increasing the bioavailability of organic matter-bound heavy metals (Fine *et al*., 2005). Competition for metal cations by organic complexing ligands and soil colloidal surface especially at elevated pH also increases heavy metal mobility and bioavailability in soil (Abdu, 2010). This might be attributed to the pH-dependent dissolution/precipitation and redox reactions of the hydrated metal oxides in the soil (Tack *et al.,* 2006). Soil pH is often the most important soil chemical properties influencing heavy metal mobility in the soil. It exerts a strong influence on metal solubility, adsorption, and desorption processes and metal speciation in the soil–solution interface. Christensen (1984) observed a twofold increase in heavy metal concentrations in soil solution due to a unit increase in soil solution pH. Bioavailability is a term used to describe the release of a chemical from a medium of concern to living receptors such as plant roots (Adriano, 2001) and microbes. Concerning heavy metals, it is defined as the fraction of metal in the soil that is accessible to the food chain, plants (Misra *et al.,* 2009), and other components of the soil microbial biomass. Mycorrhizal fungi under symbiosis can increase the adsorptive surface area of plant roots thereby influencing heavy metal uptake (Alloway, 1995). Wang *et al.,* (2009) reported modification of heavy metal movement and fixation as a result of root excretion of organic acids that form complexes and chelates with metal ions.

METHODOLOGY

Sample collection

The soil sample was collected using the method recommended by (Petersen, 1994). 100m^2 of the land was divided into ten equal sized grid cells of 10m^2. A steel augur was use to dig the soil

to a depth of 25cm. Samples was collected from all cells and thoroughly air dried, mixed and stored in large plastic bags.

Experimental pot Design

Pot culture experiment was conducted using 2 kg soil treated or spiked with the soluble salt of the metals Zn, Cd, Cu, and Pb based on early research Ahalya *et al.* (2005). The soil will have received the salt of Zn as $Zn(SO_4)_3.6H_2O$, Pb as $Pb(NO_3)_2$ and Cd as $Cd(NO_3)$ at a concentration of 1000ppm, 1500ppm for Cu, Cd, Zn and Pb respectively, whereas, Soil and Beans were planted in to the pots. Separate pots containing the same amount (2 kg) of untreated soil was used to serve as a control. Plastics trays were placed under each pot and the leached was collected and put back in their respective pots in other to prevent loss of nutrients and trace element from the samples (Garba *et al*., 2011).

SAMPLE PREPARATION

Sample Preparation

The sample of the plant and soil was collected at the end of the experiment; the plant was wash thoroughly in the laboratory with distilled water, carefully separated in to; root, shoot, and seed. These were dried at room temperature to a constant weight, ground and sieved through a 2 mm nylon sieve according to Lombi *et al.* (2001). The soil sample were dried at 105^{O}C to a constant weight, ground and then sieved through a 2 mm mesh, subjected to further analysis. The dried soil sample was characterized for some physicochemical properties (Lombi *et al*., 2001). $20cm^3$ of concentrated nitric acid was carefully added to 1g of pre-treated soil in a $250cm^3$ beaker. The mixture was allowed to stand for 1hour. Then $15cm^3$ of concentrated per chloric acid was added. The mixture was digested on a sand bath to the appearance of white fumes. The digest was dissolved in $0.10moldm^{-3}$ hydrochloric acid, filtered into a $50cm^3$ volumetric flask and made to mark. A blank was prepared by heating a mixture of $20cm^3$ concentrated nitric acid and $15cm^3$ of per chloric acid to almost dryness and then diluting to $50cm^3$ with $0.10moldm^3$ hydrochloric acid. The sample and blank solutions was stored at low temperature before analysis (IITA, 1979).

Digestion of plant Sample

The sieved samples were digested by weighing 0.5g into an acid washed porcelain crucible and placed in a muffle furnace for about 4 hour at 500^{O}C. The crucible was removed from the furnance and cooled; 10ml of 6M HCl acid was added to the sample in the crucible and heated for about 15minute. A drop of the acid was added to the mixture and heated to dryness. This will be allowed to cool. Additional 1ml of the 6M HCl was added and swirled gently followed by the addition of 10ml distilled water and heated on steam bath to complete dissolution. The mixture was then be allowed to cool and filtered through a Whatman filter paper into a 50 ml volumetric flask and make up to the mark with distilled water (Radojevic and baskin, 1999). A blank was equally be prepared following the same procedure but without the sample. Analysis of the digested samples was done using atomic absorption spectroscopy (AAS).

Digestion of Soil Sample

One gram (1.0 g) of the dried and sieved soil samples was placed in a 100 ml volumetric flask. Fifteen millilitre (15 ml) of concentrated HNO_3, H_2SO_4, and $HClO_4$ acid in a ratio of (5:1:1) was added and heated at 80^{O}C until colourless solution is obtained. This was then being filtered through a Whatman filter paper no. 42 and diluted to 50 ml with distilled water (Allen *et al.,* 1986). Analysis of the digested samples for the metals was carried out using Atomic Absorption Spectroscopy

Determination of Soil pH

Procedure

Twenty grams of the sieved soil sample was placed in a 50 cm^3 beaker; 20 cm^3 of water was added, stirred with glass rod and allowed to stand for 10 minutes. Then, stirred again and the pH meter was immersed into the water/soil suspension and the reading noted. Three replicate analysis was done and the average taken as the final pH. The pH meter was rinsed with water for each soil sample in order to obtain the accurate result that is required (Bodeck *et al.,* 1988).

Particle Size/ Soil Texture

Procedure

Fifty gram (50 g) of the soil was pulverized and placed in a tall, slender jar (35 cm height and 6 cm in diameter) to about a one-quarter full of soil. De-ionized water was added until the jar is three-quarters full. A teaspoon of powdered, non-forming dishwasher detergent was also added. The jar was shaken for 15minutes to break apart the soil aggregates and separates the soil into individual mineral particles. The jar was kept undisturbed for 3 days. The soil particles that settled after 1 minute according to its size marked on the jar depth as the sand. After 2 hours, the level of silt was marked on the jar, and after 3 days clay particles was settle and marked on the jar (Agbenin, 1995).

Calculation

% Sand = $\frac{\text{Thickness of sand x 100}}{\text{Total thickness}}$

% Clay = $\frac{\text{Thickness of clay x 100}}{\text{Total Thickness}}$

% Silt = $\frac{\text{Thickness of silt x 100}}{\text{Total Thickness}}$

(Agbenin, 1995).

Cation Exchange Capacity

Determination by $BaCl_2$ Compulsive Exchange Method:

Preparation of Reagents Barium chloride (0.1M $BaCl_2.2H_2O$) solution: 24.428g of $BaCl_2.2H_2O$ was dissolved in a 1L standard volumetric flask containing 800 cm^3 of water then diluted to the

mark. Barium chloride (2mM $BaCl_2.2H_2O$) equilibrating solution: Dilute 20 cm^3 of the 0.1 M $BaCl_2$ solution to 1 L with water. Magnesium sulphate (0.1 M $MgSO_4.7H_2O$) solution: 24.648 g of $MgSO_4.7H_2O$ was dissolved in a 1L standard volumetric flask that contains about 800cm^3 of water and diluted to the mark with distilled water. Magnesium sulphate (1.5mM and 5mM $MgSO_4.7H_2O$) solution: 15 cm^3 and 50 cm^3 of the 0.1M $MgSO_4$ solution was diluted separately with water and made up to 1L in a standard volumetric flask respectively. Sulphuric acid (0.05M H_2SO_4) solution: 2.8 cm^3 of concentrated H_2SO_4 (98% v/v, sp.gr. 1.84) was poured into a 1L standard volumetric flask almost filled with water, shaken thoroughly and allowed to cool before it is made to the mark with distilled water (Gillman and Sumpter, 1986).

Procedure

Two grams (2.0 g) of soil was weighed into a 30 cm^3 centrifuge tube and 20cm^3 of 0.1M $BaCl_2.2H_2O$ solution was added, shaken for 2 hours, centrifuged at about 4,000 rpm and decanted. Then 20 cm^3 of 2mM $BaCl_2.2H_2O$ solution was added and shaken for 1 hour vigorously at first to disperse soil pellet; it was then being centrifuged and the supernatant discarded. The pH of the slurry was determined. To the slurry, 10.00 cm^3 of 5mM $MgSO_4$ solution was added and shaken gently for one hour. The conductivity of the 1.5 mM $MgSO_4$ solution was determined (this should be ~300 umhos). The conductivity and the pH of the sample solution was adjusted (as necessary) using 0.1 M $MgSO_4$ and 0.05 M H_2SO_4 solutions respectively until the solution conductivity and pH were that of the 1.5 mM $MgSO_4$ solution (Gillman and Sumpter, 1986).

Calculation

CEC (meq/100g) = [Total Mg added(meq) - Mg in final solution (meq)] x 50; where Total Mg added (meq) = 0.1 meq [meq in 10 cm^3 of 5mM MgSO4 solution] + meq added in 0.1 M $MgSO_4$[cm3 of 0.1 M $MgSO_4$ x 0.2 meq/cm3 (0.1 M $MgSO_4$ solution has 0.2 meq/cm^3)] and Mg in final solution (meq) = total solution (cm^3) x 0.003 (meq/cm^3) [1.5mM $MgSO_4$ solution has 0.003 meq/cm3]. The value 50 is to convert the dilution factor from 2 g of soil to 100g (Gillman and Sumpter, 1986).

Determination of Soil Organic Carbon

The percentage carbon was determined from a previous study, conducted by Erik Beiegrohslein, using the same soil samples (Beiergrohslein, 1998). The percentage organic matter in the sample was determined from the percentage carbon based on the relation OM % = C % × 1.732 (Zhang, 2004). OM% represents the percentage organic matter in the soil and C% is the percentage carbon in the soil.

Determination of Organic Matter

Preparation of Reagent

Potassium dichromate solution (0.2M): 49.04 g K_2CrO_7 was dissolved in water and made up to 1L in a standard volumetric flask. Ferrous ammonium sulphate solution (0.4 M): 156.90 g of the sulphate salt was dissolved in water, 20 cm^3 concentrated H_2SO_4 was added and shaken; the

solution was made up to 1L. Concentrated phosphoric acid (85% v/v) and Ferrous was serves as an indicator.

Procedure

One gram of the soil sample was weighed into 250 cm^3 conical flask and 10 cm^3 of 0.2 M K_2CrO_7 solution was added and then swirled gently to disperse the soil in solution. Then 20 cm^3 of concentrated H_2SO_4 was added quickly and then thoroughly mixed. The mixture was allowed to cool for 30 minutes after which 200 cm^3 of water was added, followed by 10cm^3 of concentrated phosphoric acid and 2-3 drops of ferroin indicator. The solution was titrated against 0.4 M ferrous ammonium sulphate solution. A colour change from bluish green to brilliant green indicated the end point. A blank titration was carried out using deionized water without the soil sample (Walkley and Black, 1973).

Calculation

Organic matter (OM) = 10(B – S) x 12 x 1.72 x 100 x 3.10 ZB x 12/4000 x 0.77

where B = titre value for blank, S = titre value for sample, Z = weight of soil sample used, 1.72 = factor for organic matter from carbon, 0.77 = Walkley's recovery factor, 12/4000 = meq weight of carbon, 10 = conversion factor for units (Walkley- Black, 1973).. Therefore,

$$\% \text{ OM} = \frac{(B-S) \times 6.7}{ZB}$$

The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005a) and is calculated using the formula: BCF=Root/Soil

DETERMINATION OF THE MOVEMENT OF METALS FROM ROOTS TO PLANTS

To evaluate the potential of plants for phytoextraction the translocation factor (TF) was used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol et al., 2004). and is calculated using the formula:

TF=Shoot/Root

The enrichment factor (EF) is calculated as the ratio between the plant shoot concentrations and sediment concentrations (metal concentration in shoot/metal concentration in sediments or soil) by Branquinho et al. (2007).

$$\text{EF} = \frac{metal\ concentration\ in\ the\ shoot}{metal\ concentration\ in\ the\ soil}$$

Statistical data Handling

All statistical data handling was performed using SPSS 12 package. Difference in mean concentration of the heavy metals among the different samples was detected using one-way

ANOVA, followed by multiple comparisons using Turkey test. A significant level of ($P \leq 0.05$) was used throughout the study.

4.4 Expected Outcome: The result of this study is expected to indicate the uptake and accumulating ability of soil and plant for the heavy metals; Cu, Zn, Cd and Pb

Results and Discussion

Physicochemical Properties of the Experimental Soil

The physicochemical properties of the experimental soil are as shown in Table 1 below. The taxonomy classification of the soil was found both to be sandy loam with pH of (6.25 and 7.39). The less acidic nature of the soil is generally within the range for soil in the region; soil pH plays an important role in the sorption of heavy metals, it controls the solubility and hydrolysis of metal hydroxide, carbonate and phosphates (Garba *et al*., 2011). A very low organic carbon was observed in both the soil sample (0.53 and 0.37). Low organic matter content in both the soil samples was observed (0.90 and 0.64) as well as low cation exchange capacity (CEC) (4.09 and 3.87 mol/100kg soil). CEC measure the ability of soil to allow for easy exchange of cations between it surface and soil. The low level of clay and CEC indicate the permeability and leachability of metals in the soil. Appreciable amount of silt was observed in both sample i.e. (20.70 and 23.20), silt improves the soil, resulting in better plant growth.

Table 1: The Physicochemical Properties of the Experimental Soil

Parameters	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
pH	6.27±0.004	6.66±0.021	6.70±0.002	7.39±0.012	5.02±0.006	6.01±0.016
EC (dsm^{-1})	0.38±0.006	0.84±0.002	0.76±0.015	0.92±0.021	0.78±0.008	0.13±0.002
CEC (mol/100kg soil)	4.09±0.007	3.87±0.005	5.89±0.008	6.00±0.006	5.91±0.004	5.02±0.008
Organic Carbon (%)	0.53±0.005	0.37±0.003	0.49±0.012	0.57±0.007	0.41±0.023	0.22±0.017
OMC (%)	0.91±0.005	0.64±0.007	0.84±0.004	0.93±0.005	0.74±0.004	0.62±0.003
Silt (%)	20.70±0.006	23.20±0.021	22.32±0.032	21.04±0.014	20.50±0.065	22.02±0.01
Sand (%)	14.70±0.004	12.20±0.006	10.64±0.008	13.002±0.004	14.57±0.012	14.02±0.06
Clay (%)	64.60±0.003	64.60±0.007	64.65±0.016	63.65±0.008	63.89±0.019	63.02±0.07
Textural Class	Clay	Clay	Clay	Clay	Clay	Clay

KEY: Soil 1=Gashua, 2=Dawayo, 3=Potiskum, 4=Mamudo, 5=Nguru, 6=Garbi

Uptake and Translocation of Heavy Metals by *Beans Plant*

Table 2 below shows the uptake, accumulation and translocation of the metal copper, zinc, cadmium and lead by the beans plant at different level of the elements spiked in the soil along with the control. The results show that, the highest level of zinc (421.3±36.6) was found in (table 6 B^1) the Root, and it corresponds to the pot spiked with highest level of zinc in A^1 (1500ppm). The level was observed to increase proportional to the concentration spiked. The control has the lowest or not detected level of the element absorbed, translocated and accumulated in both the root, shoot and the seed. The result show that the value of zinc in shoot was found to be (144.5±2.7) and in seed was found to be (128.1±1.5), the lowest level was found in seed with the value of (-18.13±0.03), (-15.61±0.01) in shoot and in root was found to be (1.05±0.08). Table 3 also the highest level of lead was found in A^I (table 7) with the value of (276.1±54.3) in root, (81.1±30.5) in shoot and seed which content the value of (59.6±5.5). The lowest level was found in seed with the value of (-2.4±0.2), and (-0.3±0.4) in shoot, and root found to be (4.3±0.1). Table 4 Cadmium was found to be (248.4±6.4) in root with the highest value as compare to values in shoot and seed with (102.7±3.9) and (78.2±0.6) respectively. It contents the lowest value as the soil spiked with the lowest 1000ppm with the values of (5.2±0.1), (4.7±0.1), and (4.5±0.1) in root, shoot, and seed respectively. Table 5 Copper has the value of (383.4±33.8) in root and (100.6±18.2) in shoot and (55.7±3.0) in seed, the table showed the uptake and accumulation by the plant; at the different concentration of the element Cd, Pb, Zn, and Cu, spiked into the experimental pots at different concentration. The results showed that, the higher the level of element spiked into the experimental pot, the higher the concentration translocated to the seed. For instance, the control has no value detected in the soil, root, shoot, and seed. The 1000ppm spiked in to the pot the level was found to be (2.4±0.6) in the seed, (11.1±0.8) in shoot and in root with the higher value of (44.8±0.1).

Table 6 shows the level of zinc accumulated in the seed is (128.6±1.5) was higher than what was retained in the when the level in the pot was 1000ppm (15.8±3.2), the value was also higher in root and shoot (421.4±3.6) and shoot (144.1±2.7), when the amount spiked was decrease to 1000ppm the value was reduce to (308.7±29.1) in root and (18.8±1.0) in shoot respectively. Table 7 Lead was found to have the highest value in root (491.7±3.5) when spiked with 1500ppm, (206.1±1.8) in shoot and (116.0±0.8) in seed. When the amount spiked reduced to 1000ppm the value also reduced to (433.5±3.4) in root and in shoot was found to be (31.5±0.7) and in seed (31.5±0.8). Table 8 Cadmium also has the value of (557.1±0.9) in root and (106.1±1.8) in shoot and (98.0.0±0.8) in seed when the amount spiked was increase to 1500ppm, when the amount was reduced to 1000ppm the value also reduces to (36.9±0.3) in root, and in shoot was found to be (21.3±0.2), and (19.0±0.1) in seed. Table 9 Copper has the lowest value in seed when spiked with 1000ppm (13.1±1.8), in shoot been found to be (14.5±0.8) and in root it was (137.1±1.6) but when spiked with 1500ppm the value was increase to (470.6±3.7) in root and in shoot was found to be (171.4±2.0) and in seed was found to have the value of (131.0±1.1) in seed as shown in the Table below. Table 10 Showed the variation in the level of zinc, in experimental pot spiked with the element 1000ppm and 1500ppm. The

uptake and translocation of the element was found to increase as the level spiked in the experiment pot increases. For instance, the level in the root, shoot, and seed of the control was observed at the lowest value. When the soil was spiked with 1500ppm (Zn), the level observed to have the higher value, in the root, zinc was found to be (271.1±5.7), and in shoot was found to be (26.2±2.0) and (2.5±2.0) in seed, as compare to 1000ppm the value were decrease to (-1.62±0.28) in root and (-11.18±0.19) in shoot and (-12.52±0.29) in seed.

Table 11 Lead has the value of (312.4±25.6) in root and in shoot was found to be (47.1±13.7), in seed (37.5±2.2), the lowest value of lead was found to be in seed (-12.52±0.29), and in shoot it was found to be (-10.1±0.19), and in root was found to be (-1.82±0.28). Table 12 Cadmium, the uptake and distribution of the metal Cd in the root, shoot and seed along with its translocation, enrichment and Bioconcentration concentration observed when spiked with 1500ppm the value was found to be higher than what was translocated to the root, shoot, and seed with the values of (128.6±2.8), (57.0±1.7), and (45.1±0.3) respectively. The lowest value was found in the shoot (7.9±0.1), and (10.2±0.1) in seed and (11.6±0.2) in root. Table 13 Copper with the highest value was found in the root with the value of (224.2±15.7), (44.0±8.2) in shoot, and (23.4±0.9) in seed, the lowest value was found to be in seed with (-0.1±0.5), (4.2±0.5) in shoot and (24.0±0.2) in root respectively. The uptake and distribution of the metal Zn in the root and shoot along with its translocation, enrichment and Bioconcentration factors are displayed in table. It shows that most of the metals were absorbed and accumulated in the root with appreciable of translocation to the shoot, and seed. The accumulation in the root was found proportional to the level of the metal spiked into the experimental pots. In another words, the higher the level spiked the higher the concentration in the root. For instance, when the level spiked was 1000ppm, the concentration in the root, shoot, and seed was found to decrease, When the amount spiked was increased to 1500ppm, the accumulation in the root, shoot, and seed equally increases.

Table 2: Levels (ppm) of Zn in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	10.65±0.24	1.05±0.08	-15.61±0.01	-18.13±0.03	0.098	-14.867	-1.466
1500	586.0±0.24	324.7±10.2	63.5±3.8	13.3±2.6	0.554	0.196	0.108
Control	2.09±0.91	0.15± 0.70	0.002±0.18	ND	0.072	0.013	0.001

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 3: Levels(ppm) of Pb in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	20.8±1.6	4.3±0.1	-0.3±0.4	-2.4±0.2	0.207	-0.069	-0.014
1500	520.0±34.7	276.1±54.3	81.1±30.5	59.6±5.5	0.531	0.294	0.156
Control	1.69±0.06	1.02±0.03	0.68±0.01	ND	0.603	0.667	0.402

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation.

Table 4: Levels(ppm) of Cd in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	6.1±0.1	5.2±0.1	4.7±0.01	4.5±0.02	0.852	0.904	0.771
1500	406.6±3.3	248.4±6.4	102.7±3.9	78.2±0.6	0.611	0.413	0.253
Control	3.05±0.082	1.5±0.068	0.65±0.010	0.002±0.001	0.491	0.433	0.213

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 5: Levels(ppm) of Cu in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	102.0±3.5	44.8±0.1	11.1±0.8	2.4±0.6	0.439	0.248	0.109
1500	696.4±18.4	383.4±33.8	100.2±18.2	55.7±3.0	0.551	0.261	0.144
Control	0.35±0.21	0.01±0.20	ND	ND	0.028		

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 6: Levels(ppm) of Zn in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	510.4±4.1	308.7±29.1	18.8±1.0	15.8±3.2	0.601	0.061	0.037
1500	990.1±29.1	421.4±36.6	128.5±2.7	144.1±1.5	0.426	0.305	0.129
Control	4.21±1.15	2.19±0.69	1.69±0.13	0.70±0.02	0.520	0.772	0.401

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 7: Levels(ppm) of Pb in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	628.3±3.6	433.5±3.4	31.5±0.7	31.5±0.8	0.689	0.073	0.050
1500	124.9±1.1	491.7±3.5	206.1±1.8	116.0±0.8	3.937	0.419	1.650
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 8: Levels(ppm) of Cd in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	873.8±4.8	36.9±0.3	21.3±0.2	19.0±0.1	0.042	0.577	0.024
1500	228.9±215.2	157±0.9	106.1±1.8	98±0.8	0.686	0.676	0.464
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 9: Levels(ppm) of Cu in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	522.9±3.6	137.1±1.6	14.5±0.8	13.1±1.8	0.262	0.106	0.028
1500	679.1±15.0	470.6±3.7	171.4±2.0	131.0±1.1	0.693	0.364	0.252
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 10: Levels(ppm) of Zn in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	3.58±1.99	-1.62±0.28	-11.18±0.19	-12.52±0.29	-0.453	6.901	-3.123
1500	476.8±9.1	271.1±5.7	26.2±2.0	2.5±2.0	0.569	0.097	0.055
Control	4.15±1.25	3.05±0.68	2.00±0.16	0.15±0.05	0.735	0.656	0.481

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 11: Levels(ppm) of Pb in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	20.3±0.8	-1.62±0.28	-11.18±0.19	-12.52±0.29	-0.089	5.549	-0.498
1500	507.8±16.2	312.4±25.6	47.1±13.7	37.5±2.2	0.615	0.151	0.093
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 12: Levels(ppm) of Cd in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	15.0±0.1	11.6±0.2	7.9±0.1	10.2±0.1	0.773	0.681	0.527
1500	571.0±0.9	128.6±2.8	57.0±1.7	45.1±3.0	0.225	0.443	0.099
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 13: Levels(ppm) of Cu in Soil, Shoot, Root, Seed of *Phaseolus vulgaris (Beans plant)*. and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	Seed	BCF	TF	EF
1000	55.4±1.8	24.0±0.2	4.2±0.5	-0.1±0.5	0.433	0.175	0.076
1500	534.8±9.4	224.2±15.7	44.0±8.2	23.4±0.9	0.419	0.196	0.082
Control	ND	ND	ND	ND			

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

CONCLUSION

From the result obtained and the translocation factor (TF), Bioconcentration Factor(BCF) and Enrichment Factor(EF) calculated, it can be concluded that, the *Phaseolus vulgaris (Beans plant),* may serve as phytostabilizers or metal excluders of Zn, Pb , Cd and Cu in the soil for having higher values of BCF and EF than TF. *Phaseolus vulgaris (Beans plant),* may stabilize element for having higher value of BCF and EF than the TF and May also serve as Cd Phytoextractor or Metal indicator for having higher value of TF than the EF, whereas *Phaseolus vulgaris (Beans plant),* may serve as a phytoextractor for Cd and Cu or Metal Indicator in soil for having higher TF values.

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Influence of Supplementary Hoe Weeding on the Efficacy of Pre-emergence Herbicide on Groundnut (*Arachis Hypogea* L.) Weed Management in Bauchi, Nigeria

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Abstract: *Field experiments were conducted during rainy seasons of 2018, 2019 and 2020 at Abubakar Tafawa Balewa University Teaching and Research Farm, Gubi, to evaluate the influence of supplementary hoe weeding on the efficacy of pre-emergence herbicide and weed management of groundnut (Arachis hypogea L.) varieties. The trial comprised of ten (10) weed control treatments and three varieties of groundnut which were replicated three (3) times in a split plot design. Data were collected on weed attributes such as weed control index, treatment efficiency index, crop resistance index and weed index as well as on crop parameters such as plant height, number of branches, canopy spread, 100 kernel weight, pod yield, haulm yield and shelling percentage. Findings from the study indicated that weed parameters such as weed control index, treatment efficiency index and crop resistance index were significantly lower under the application of pendimethalin at 2.0 kg a.i.ha^{-1} fb SHW at 6 WAS and hoe weeding at 3 and 6 WAS compared to weedy check that resulted in higher value. The plant height, number of branches, canopy spread, number of pods plant^{-1} were significantly higher with the application of hoe weeding at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} fb SHW at 6 WAS, pendimethalin at 1.5 kg a.i.ha^{-1} fb SHW at 6 WAS. SAMNUT 22 and SAMNUT 23 varieties (P ≤ 0.01) significantly produced taller plants with a greater number of branches, canopy spread and number of pods plant^{-1}. From the findings of the trials, it can be deduced that application of supplementary hoe weeding at 6 WAS increased the efficacy of the pre-emergence herbicide viz-pendimethalin at 2.0 and 1.5 kg a.i.ha^{-1} fb SHW at 6 WAS and butachlor at 2.0 and 1.5 kg a.i.ha^{-1} fb SHW at 6 WAS. Thus, it can be adopted by farmers in the study area in place of the 2-3 manual weeding's that is usually scarce during peak periods alongside SAMNUT 22 or SAMNUT 23 to boost groundnut seed and haulm yield.*

Key words: *hoe weeding, efficacy, pre-emergence herbicide, groundnut, weed management*

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is a major food and oil seed crop grown in West Africa's diverse agro-climatic environments by small-scale, resource-limited farmers (AICC, 2016). Africa

accounts for approximately 90% of global production, with the semi-arid tropics (SAT) accounting for approximately 60% of global production capacity (Vara Prasad *et al*., 2011). Nigeria ranks third in production, trailing only India and China, with 3.0, 6.9, and 13.7 million metric tons, respectively (FAOSTAT, 2016; 2017; ICRISAT, 2019). Groundnut yields in Africa have historically been low due to unpredictable rainfall patterns, insufficient technological innovations available to resource poor and small-scale farmers, prevalence of pest and disease incidence, poor seed varieties, and increased cultivation on marginal land, among other factors (Alemayehu *et al*., 2014; Debele and Amare 2015; Desmae and Sones, 2017). According to Vigueira *et al*. (2013) and Stewart (2017), weed infestation is the most significant constraint causing tremendous reductions in crop yields globally, and they have evolved to exploit croplands through a variety of different mechanisms that confer strong adaptive and competitive abilities. Weeds are estimated to account for one-third of all yield losses (34%), more than animal pests and plant pathogenic organisms (18% and 16%, respectively) (Zimdahl, 2004; Stewart, 2017), resulting in an annual crop loss and weed control cost of USD 33 billion in the United States (Lewellyn *et al*., 2016; Chauhan, 2020). The crop is heavily infested with various weed species as a result of less canopy cover during the first six weeks of its growing cycle, which if left unchecked can result in a 51% reduction in yield potential (Etejere *et al*., 2013). Several scientists have reported various weed control measures in groundnut, including manual hoe weeding, mechanical, chemical, and the integrated approach (Jat *et al*., 2011; Patel *et al*., 2013; Kaur *et al*., 2014). Manual hoe weeding, the most common method used by resource-poor farmers, has a number of drawbacks, including drudgery, crop stand loss, and labor shortages during peak periods (Abbas *et al.,* 2009; Moss, 2019). With the use of herbicide, so many hectares of farm land can be brought under groundnut production. However, pre-emergence herbicide application alone does not provide season-long weed control in most crops, unless supplemented with post-emergence herbicide or hoe weeding at later stages of growth, according to research (Kraehmer *et al*., 2014; Jabran and Chauhan, 2018). It is therefore crucial to assess how supplementary hoe weeding affects pre-emergence herbicides and certain groundnut varieties in order to determine how well they respond to weed control and how best to maximize yield potential.

MATERIALS AND METHODS

The experiment was conducted during the 2018, 2019 and 2020 wet cropping seasons at Abubakar Tafawa Balewa University Teaching and Research Farm, Gubi, (Lat. 10^{o} 45' N and Long. 9^{o}.82' E, 616m above sea level) situated in the Northern Guinea savanna ecological zone of Nigeria. The experimental site is characterized by a unimodal rainfall pattern which has peak in the month of August. The soil of the experimental site is sandy loam with moderate water holding capacity and pH slightly acidic. The trial consisted of ten (10) weed control treatments which comprised of Butachlor at 2.5 kg a.i.ha^{-1}, Butachlor at 2.5 kg a.i.ha^{-1}, Butachlor at 2.0 +

Pendimethalin 1.0 kg a.i.ha^{-1}, Butachlor at 1.5 kg a.i.ha^{-1} *fb* SHW (Supplementary hoe weeding) at 6 WAS (Weeks after sowing), Butachlor at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, Pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, Pendimethalin at 2.0 kg a.iha^{-1} *fb* SHW at 6 WAS, two hoe weeding at 3 and 6 WAS and weedy check and three varieties of groundnut (SAMNUT 14, SAMNUT 22 & SAMNUT 23). These were laid out in a split plot design and replicated three (3) times. The crop varieties were assigned to the main plots while weed control treatments were assigned to the sub-plots. In each year of the trial, the field was harrowed twice to fine tilth and ridged into 0.75m apart using ox-drown ridger. It was then marked into the required number of plots each of gross area of 3 x 4 m (12 m^2) and net plot size of 1.5 x 3 m (4.5 m^2). The ally between main plots, sub-plots and replicates were 1.0 m, 0.5 m and 1.5 m while nutrients at the rate of 20 kg N, 54 kg P_2O_5 and 20 Kg K_2O was applied basally to each plot using 15:15:15 and 34 kg of P_2O_5 using SSP (18% P_2O_5). Sowing was done on the 28th July 2018, 18th July 2019 and 26th of July 2020 seasons using treated seeds of groundnut obtained from Bauchi State Agricultural Development Programme (BSADP) Ministry of Agriculture, Bauchi State. Three seeds were sown along the ridge per hole at a depth of 2 cm and the resultant seedlings were thinned to one plant per stand at 3 weeks after sowing (WAS). The pre-emergence herbicides were applied as per treatment basis a day after sowing using a Cp3 knapsack sprayer set at a pressure of 2.1kg/m^2. Hoe weeding was carried out at 3 and 6 WAS for the hoe weeded plots. Harvest was done on the 15th, 18th and 26th of November 2018, 2019 and 2020 seasons, respectively. Data were collected on weed characters such weed control index, treatment efficiency index, crop resistance index and weed index; crop attributes such as plant height, number of branches, canopy spread, 100 kernel weight, pod yield, haulm yield and shelling percentage. Data collected were subjected to analysis of variance using Genstat (17th Edition) where the 'F' test shows significance. The treatment means were separated using Duncan's multiple range test (Duncan, 1955).

Weed control index (WCI) (%)

This was calculated on dry weight basis as described by Misra and Tosh (1979) using the equation below.

$$\text{WCI} = \frac{WDMc - WDMt}{WDMc} x\ 100$$

Where, WDMc = the weed dry weight (unit/m^2) in control plot;
WDMt = the weed dry weight (unit/m^2) in treated plot**.**

Treatment (Herbicide) efficiency index (TEI)

This is the weed killing potential of herbicide treatment and its phytotoxicity on the crop. It was thus determined as described by Rana and Kumar (2014) follows:

$$\text{TEI (\%)} = \frac{\frac{\text{Yield of treatment - Yield of control}}{\text{Yield of control}} \times 100}{\text{Weed weight in treatment} \times 100}$$

Weed weight in control

Crop resistance index (CRI)

This indicated the relationship between a proportionate increase in crop biomass in treated plots and a proportionate reduction in weed biomass in the treated plots. Thus, it was determined as described by Rana and Kumar (2014) as follows:

$$CRI = \frac{\text{Crop weight in treated plot}}{\text{Crop weight in control plot}} \; X \; \frac{\text{Weed weight in control plot}}{\text{Weed weight in treated plot}}$$

Weed index (%)

This is the percentage yield loss caused due to weeds as compared to weed free check. Higher weed index indicates greater loss. Hence, weed index (WI) was calculated using the formula described by Rana and Kumar (2014).

$$W.I = \frac{Yield\ from\ weed\ free\ check - Yield\ from\ treated\ plot}{Yield\ from\ the\ weed\ free\ check} \times 100$$

RESULTS

Effect of weed control, variety and season on weeds

Weed control index, treatment efficiency index, crop resistance index and weed index.

The mean of combined analysis on the effect of weed control and variety on weed control index, treatment efficiency index, crop resistance index and weed index of groundnut is shown in Table 1. Result shows that weed control index (WCI) and treatment efficiency index (TEI) were significantly ($P \leq 0.01$) influenced by weed control, variety, season and interaction. Application of hoe weeded twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS significantly ($P \leq 0.01$) resulted in higher WCI compared with butachlor at 2.5 kg a.i.ha^{-1} alone

Table 1: Mean of combined analysis across seasons on the influence of supplementary hoe weeding on efficacy of pre-emergence herbicides on weed control index, treatment efficiency index, crop resistance index and weed index of groundnut during 2018, 2019 and 2020 rainy seasons

Treatment	Rate (Kg a.i.ha^{-1})	Weed control index (%)	Treatment efficiency index (TEI)	Crop resistance index (CRI)	Weed index (%)
Weed control (W)					
BUTA	2.5	49.20^{f}	6.70^{h}	2.16^{i}	53.28^{b}
PENDA	2.5	52.95^{e}	7.66^{g}	2.61^{h}	46.38^{c}
BUTA + PENDA	2.0 + 1.0	56.64^{d}	9.37^{f}	3.24^{g}	38.70^{d}
PEND + BUTA	2.0 + 1.0	58.42^{d}	10.73^{e}	4.31^{f}	31.77^{e}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	82.25^{c}	18.84^{d}	11.02^{e}	12.34^{f}
BUTA fb SHW at 6 WAS	2.0	85.81^{b}	21.24^{c}	12.70^{d}	8.38^{g}
PEND fb SHW at 6 WAS	1.5	86.20^{b}	23.13^{b}	14.42^{c}	4.99^{h}
PEND fb SHW at 6 WAS	2.0	88.76^{a}	24.60^{a}	15.11^{ab}	3.06^{i}
Weeding at 3 and 6 WAS	-	88.93^{a}	25.15^{a}	15.39^{a}	2.99^{i}

Weedy check	-	-	-	0.10[j]	91.90[a]
Level of significance		**	**	**	**
SE (±)		0.659	0.210	0.056	0.66
Variety (V)					
SAMNUT 22		73.97[a]	16.86	8.61[a]	28.67
SAMNUT 23		71.90[b]	16.46	8.33[b]	28.98
SAMNUT 14		69.62[c]	16.08	7.34[c]	29.73
Level of significance		**	NS	**	NS
SE (±)		0.439	0.52	0.084	0.45
Season (Y)					
2018		70.65[b]	12.16[b]	7.80	28.34
2019		73.42[a]	24.82[a]	8.26	30.70
2020		72.84[a]	24.38[a]	8.24	29.15
Level of significance		*	**	NS	NS
SE (±)		0.681	0.47	0.150	0.90
Interaction					
W x V		**	NS	**	**
W x Y		NS	**	**	NS
V x Y		NS	NS	NS	NS
W x V x Y		NS	NS	NS	NS

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin. fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing. ** = significant at 1% (P ≤ 0.01); * = significant at 5% (P ≤ 0.05); NS = Not significant.

that resulted in lower WCI. SAMNUT 22 variety significantly (P ≤ 0.01) had higher WCI compared with other varieties. Growing groundnut in 2019 and 2020 seasons significantly resulted in higher WCI and TEI than 2018 season. Interaction effect between weed control and variety on WCI of groundnut was significant (Table 2). Results reveals that hoe weeding twice at 3 and 6 WAS in SAMNUT 22 significantly (P ≤ 0.01) resulted in higher WCI though statistically comparable with two hoe weeding's at 3 and 6 WAS in SAMNUT 23 and SAMNUT 14, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, butachlor at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and butachlor at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS in SAMNUT 22, SAMNUT 23 and SAMNUT 14 compared with the rest of the interaction effects.

Table 2: Interaction effect between weed control and variety on weed control index of groundnut in 2018, 2019 and 2020 (combined season)

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	39.32^{hi}	38.94^{i}	38.24^{i}
PENDA	2.5	43.91^{fg}	42.27^{gh}	39.33^{hi}
BUTA + PENDA	2.0 + 1.0	47.14^{ef}	46.58^{ef}	46.20^{ef}
PEND + BUTA	2.0 + 1.0	48.56^{e}	48.41^{e}	48.29^{e}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	85.72^{bc}	85.57^{c}	75.47^{d}
BUTA fb SHW at 6 WAS	2.0	87.16^{abc}	87.13^{abc}	87.03^{abc}
PEND fb SHW at 6 WAS	1.5	88.32^{abc}	88.22^{abc}	88.04^{abc}
PEND fb SHW at 6 WAS	2.0	89.02^{a}	88.48^{abc}	88.77^{ab}
Weeding at 3 and 6 WAS	-	89.19^{a}	88.82^{ab}	88.79^{ab}
Weedy check	-	-	-	-
Level of significance			**	
SE (±)			1.163	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.

** = significant at 1% (P ≤ 0.01); NS = Not significant.

Table 3 presents the interaction effect between weed control and season on TEI. Results indicates that application of hoe weeding twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS in 2019 season significantly (P ≤ 0.01) resulted in higher TEI compared with the remaining interaction effects.

Table 3: Interaction effect between weed control and season on treatment efficiency index of groundnut in 2018, 2019 and 2020 seasons at Gubi

Weed control	Rate (Kg a.i.ha^{-1})	Season		
		2018	2019	2020
BUTA	2.5	2.91^{w}	14.16^{q}	12.97^{rs}
PENDA	2.5	3.82^{v}	15.24^{q}	13.87^{qr}
BUTA + PENDA	2.0 + 1.0	5.60^{tu}	16.82^{op}	15.64^{pq}
PEND + BUTA	2.0 + 1.0	6.89^{t}	18.53^{kl}	16.93^{op}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	15.06^{nop}	26.27^{g}	25.12^{h}
BUTA fb SHW at 6 WAS	2.0	17.37^{mno}	28.90^{c}	27.41^{ef}
PEND fb SHW at 6 WAS	1.5	18.48^{klm}	32.32^{b}	28.53^{cde}
PEND fb SHW at 6 WAS	2.0	19.21^{ijk}	35.30^{a}	29.26^{cd}
Weeding at 3 and 6 WAS	-	19.63^{i}	36.06^{a}	29.70^{c}
Weedy check	-	-	-	-
Level of significance			**	
SE (±)			0.58	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.
** = significant at 1% (P ≤ 0.01).

Crop resistance index (CRI) was significantly influenced by weed control, variety and interaction (Table 1). The application of hoe weeding twice at 3 and 6 WAS was highly significant (P ≤ 0.01) with higher CRI compared to other weed control treatments while weedy check resulted significantly in lower CRI. SAMNUT 22 variety significantly (P ≤ 0.01) resulted higher CRI compared to other varieties. The interaction effects between weed control and variety on CRI (Table 4) indicates that two hoe weeding's at 3 and 6 WAS in SAMNUT 22 produced highly significantly (P ≤ 0.01) greater CRI compared to weedy check applied to SAMNUT 22, SAMNUT 23 and SAMNUT 14 that resulted in lower CRI.

Table 4: Interaction effect between weed control and variety on crop resistance index of groundnut in 2018, 2019 and 2020 (combined season)

	Rate	Variety		
Weed control	(Kg a.i.ha^{-1})	SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	2.24^{o}	2.20^{o}	2.04^{o}
PENDA	2.5	2.83^{n}	2.68^{n}	2.31^{o}
BUTA + PENDA	2.0 + 1.0	3.29^{lm}	3.29^{lm}	3.02^{mn}
PEND + BUTA	2.0 + 1.0	4.58^{j}	4.26^{jk}	4.09^{k}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	13.58^{f}	11.51^{h}	9.90^{i}
BUTA fb SHW at 6 WAS	2.0	13.62^{ef}	11.59^{h}	9.94i
PEND fb SHW at 6 WAS	1.5	15.27^{d}	15.00^{d}	11.58^{h}
PEND fb SHW at 6 WAS	2.0	16.12^{b}	15.62^{c}	12.98^{g}
Weeding at 3 and 6 WAS	-	16.51^{a}	15.80^{bc}	13.86^{e}
Weedy check	-	0.10^{p}	0.10^{p}	0.10^{p}
Level of significance			**	
SE (±)			0.125	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; GLYP = Glyphosate; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.
** = significant at 1% (P ≤ 0.01); NS = Not significant.

On the other hand, interaction between weed control and season (Table 5) reveals that weeding twice at 3 and 6 WAS in 2019 and 2020 was highly significantly (P ≤ 0.01) which resulted in higher CRI though at par with weeding twice at 3 and 6 WAS in 2018, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS in all the three seasons compared with the rest of the interaction effects while weedy check resulted in lower CRI in all the three seasons.

Result on weed control index (WI) also indicates that weedy check consistently produced higher WI compared to application of hoe weeding twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS which significantly resulted in lower W.I (Table

1). The interaction between weed control and variety on WI was significant (Table 6) which shows that weedy check in SAMNUT 22, SAMNUT 23 and SAMNUT 14 significantly ($P \leq 0.01$) resulted in lower weed index compared with the rest of the interaction effect that resulted in higher weed index.

Table 5: Interaction effect between weed control and season on crop resistance index of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Season 2018	2019	2020
BUTA	2.5	2.02^{o}	2.23^{mno}	2.19^{mno}
PENDA	2.5	2.47^{lmn}	2.68^{kl}	2.62^{klm}
BUTA + PENDA	2.0 + 1.0	3.02^{jk}	3.36^{j}	3.32^{j}
PEND + BUTA	2.0 + 1.0	3.99^{i}	4.47^{i}	4.42^{i}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	10.58^{h}	11.23^{g}	11.11^{g}
BUTA fb SHW at 6 WAS	2.0	12.06^{f}	13.06^{e}	13.00^{e}
PEND fb SHW at 6 WAS	1.5	13.89^{d}	14.68^{c}	14.62^{c}
PEND fb SHW at 6 WAS	2.0	14.74^{b}	15.29^{ab}	15.24^{ab}
Weeding at 3 and 6 WAS	-	15.12^{abc}	15.52^{a}	15.50^{a}
Weedy check	-	0.10^{p}	0.10^{p}	0.10^{p}
Level of significance			**	
SE (±)			0.176	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.

** = significant at 1% ($P \leq 0.01$).

Table 6: Interaction effect between weed control and variety on weed index of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Variety SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	39.84^{f}	48.61^{d}	57.04^{b}
PENDA	2.5	39.84^{f}	48.61^{d}	52.62^{c}
BUTA + PENDA	2.0 + 1.0	38.01^{f}	48.61^{d}	52.62^{c}
PEND + BUTA	2.0 + 1.0	32.28^{g}	31.98^{g}	31.07^{g}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	12.03^{h}	12.18^{h}	12.81^{h}
BUTA fb SHW at 6 WAS	2.0	7.60^{ij}	7.61^{ij}	9.93^{hi}
PEND fb SHW at 6 WAS	1.5	4.13^{k}	4.88^{jk}	5.96^{jk}
PEND fb SHW at 6 WAS	2.0	2.92^{kl}	3.07^{kl}	3.19^{kl}
Weeding at 3 and 6 WAS	-	2.21^{l}	2.46^{l}	2.82^{l}
Weedy check	-	91.23^{a}	92.37^{a}	92.37^{a}
Level of significance			**	
SE (±)			1.17	

The mean of combined analysis on the effect of weed control and variety on plant height, number of branches plant^{-1}, canopy spread, number of pod plant^{-1}, 100 kernel weight, pod yield, haulm yield and shelling percentage of groundnut is shown in Table 7. Results on plant height showed that weeding twice at 3 and 6 WAS was highly significant ($P \leq 0.01$) producing taller plants though at par with application of pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, butachlor at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and butachlor at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS compared to weedy check that produced shorter plants. SAMNUT 22 and SAMNUT 23 varieties significantly ($P \leq 0.01$) produced taller plants than SAMNUT 14 during the sampling period. Plant height did not differ significantly ($P \geq 0.05$) due to season. The interaction between weed control and variety on plant height was significant (Table 8) where result reveals that hoe weeding twice at 3 and 6 WAS in SAMNUT 22 and SAMNUT 23 significantly ($P \leq 0.01$) produced taller plant than the rest of the interaction effects. However, weedy check in SAMNUT 22, SAMNUT 23 and SAMNUT 14 consistently resulted in shorter plants. On the other hand, interaction between variety and season (Table 9) indicates that SAMNUT 22 and SAMNUT 23 in 2019 and 2020 seasons significantly ($P \leq 0.01$) produced taller plants than the rest of the interaction effects.

Result on number of branches plant^{-1} showed that hoe weeded at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS though at par produced significantly ($P \leq 0.01$) higher number of branches plant^{-1} compared with the remaining treatments. Weedy check on the other hand, significantly produced the lowest number of branches plant^{-1}. SAMNUT 22 and SAMNUT 23 varieties significantly ($P \leq 0.01$) resulted in higher number of branches plant^{-1} compared with SAMNUT 14 that had lower number of branches across the sampling periods. Cultivation of groundnut in 2019 and 2020 seasons was highly significant ($P \leq 0.01$) and resulted in producing higher number of branches plant^{-1} compared with 2018 season. The interaction between weed control and variety on number of branches plant^{-1} is shown in Table 10, where weeding twice at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS in SAMNUT 23 and SAMNUT 22 significantly ($P \leq 0.01$) produced higher number of branches plant^{-1} though at par with pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS in SAMUNT 22 compared with the rest of the interaction effects. On the other hand, interaction between weed control and season (Table 11) shows that growing groundnut in 2019 season under hoe weeding at 3 and 6 WAS significantly ($P \leq 0.01$) resulted in higher number of branches plant^{-1} which was also at par with the rest of the interaction effects. Similarly, interaction between variety and season on number of branches plant^{-1} is shown in Table 12, where SAMNUT 22 and SAMNUT 23 varieties in 2019 and 2020 seasons significantly ($P \leq 0.01$) produced higher number of branches plant^{-1} than the rest of the interaction effects.

Results on canopy spread indicated that weeding twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS significantly ($P \leq 0.01$) resulted in higher canopy spread compared with other treatments. However, weedy check significantly resulted in

producing lower canopy spread plant^{-1}. SAMNUT 22 and SAMNUT 23 varieties significantly ($P \leq 0.01$) produced higher canopy compared with SAMNUT 14 which had lower canopy. On the other hand, growing groundnut in 2019 and 2020 seasons significantly resulted in higher canopy spread plant^{-1} than in 2018 season. Interaction between weed control and variety on canopy spread of groundnut was significant (Table 13) where results indicates that application of hoe weeding twice at 3 and 6 WAS in SAMNUT 22 significantly ($P \leq 0.01$) resulted in higher canopy though statistically comparable with the rest of the interaction effects compared to weedy check in SAMNUT 14 that produced lower canopy plant^{-1}. On the other hand, the interaction between weed control and season on canopy spread plant^{-1} at 12 WAS (Table 14) shows that application of two hoe weeding's at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, butachlor at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and butachlor at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS in 2019 and 2020 seasons though at par produced significantly ($P \leq 0.01$) higher canopy spread compared with the remaining interaction effects.

Results on number of pods plant^{-1} was highly significantly influenced by weed control, variety, season and interaction. The application of two hoe weeding's at 3 and 6 WAS and pendimethalin at 3.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS resulted in significantly ($P \leq 0.01$) higher number of pods plant^{-1} compared with weedy check that had lower pods plant^{-1}. SAMNUT 22 and SAMNUT 23 varieties significantly ($P \leq 0.01$) produced higher number of pods plant^{-1} compared to SAMNUT 14 that had lower number of pods. Growing groundnut in 2019 and 2020 seasons significantly ($P \leq 0.05$) produced higher number of pods plant^{-1} than 2018 season. Interaction between weed control and variety on number of pods plant^{-1} is shown in Table 15 which reveals that weeding at 3 and 6 WAS in SAMNUT 23 significantly ($P \leq 0.05$) produced higher number of pods plant^{-1} which is comparable with weeding twice at 3 and 6 WAS in SAMNUT 22 and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS in SAMNUT 22 and SAMNUT 23 compared with the rest of the interaction effects. The interaction between weed control and season on number of pods plant^{-1} indicates that hoe weeded twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS in 2019 and 2020 seasons resulted significantly ($P \leq 0.05$) higher pods number plant^{-1} compared with the remaining interaction effects (Table 16). On the other hand, interaction between variety and season on number of pods plant^{-1} (Table 17) reveals that SAMNUT 22 and SAMNUT 23 varieties in 2019 and 2020 seasons produced significantly ($P \leq 0.05$) higher number of pods plant than the rest of the interactions.

Result of 100 seed weight was significantly influenced by weed control, variety, season and interaction. Application of weeding twice at 3 and 6 WAS and pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS significantly ($P \leq 0.01$) produced higher 100 seed weight which was at par with pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, butachlor at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and butachlor at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS compared to weedy check that had lower seed weight. The SAMNUT 23 variety was highly significant ($P \leq 0.01$) and resulted in heavier 100

seed weight compared with other varieties. Growing of groundnut in 2019 and 2020 seasons significantly had higher 100 seed weight compared to 2018 season. The interaction between weed control and variety on 100 seed weight (Table 18) indicates the superiority of hoe weeding at 3 and 6 WAS in significantly ($P \leq 0.01$) producing heavier 100 seed weight though at par with other interaction effects.

Table 7: Mean of combined analysis across seasons on the influence of supplementary hoe weeding on efficacy of pre-emergence herbicides on plant height, canopy spread plant^{-1}, number of branches plant^{-1}, number of pods plant^{-1}, 100 kernel weight, kernel yield, haulm yield and shelling percentage of groundnut during 2018, 2019 and 2020 rainy season

Treatment	Rate (Kg a.i.ha^{-1})	Plant height (cm)	Number of branches plant^{-1}	Canopy spread plant^{-1} (cm)	Number of pods plant^{-1}	100 kernel weight (g)	Pod yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	Shelling percentage (%)
Weed control (W)									
BUTA	2.5	50.77^{f}	12.41^{f}	42.60^{f}	22.81^{f}	29.58^{d}	734.5^{g}	1974de	48.59^{g}
PENDA	2.5	52.07^{e}	14.15^{e}	43.99^{e}	25.41^{e}	30.01^{cd}	771.9^{f}	2059^{cde}	48.59^{g}
BUTA + PENDA	2.0 + 1.0	52.12^{e}	14.33^{e}	46.93^{d}	26.81^{d}	30.14^{cd}	836.7^{e}	2126^{cde}	48.70^{g}
PEND + BUTA	2.0 + 1.0	53.25^{d}	15.78^{d}	46.93^{d}	27.19^{d}	30.84^{c}	894.0^{d}	2248^{bcd}	48.81^{e}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	55.33^{c}	17.37^{c}	51.14^{c}	28.67^{c}	35.71^{ab}	1073.7^{c}	2321^{a-d}	59.11^{cd}
BUTA fb SHW at 6 WAS	2.0	56.11^{bc}	17.74^{c}	51.60^{c}	30.22^{b}	35.92^{ab}	1118.7^{b}	2396^{abc}	59.85^{bc}
PEND fb SHW at 6 WAS	1.5	56.74^{b}	18.85^{b}	52.23^{b}	30.63^{b}	36.14^{ab}	1160.4^{a}	2597^{ab}	60.52^{ab}
PEND fb SHW at 6 WAS	2.0	58.94^{ab}	19.48^{ab}	53.25^{a}	34.11^{a}	37.10^{a}	1167.9^{a}	2649^{ab}	60.78^{a}
Weeding at 3 and 6 WAS	-	60.42^{a}	20.04^{a}	53.27^{a}	34.85^{a}	37.43^{a}	1173.6^{a}	2709^{a}	61.15^{a}
Weedy check	-	45.03^{g}	11.14^{g}	34.28^{g}	13.37^{g}	19.82^{e}	465.6^{h}	1431^{f}	40.37^{f}
Level of significance		**	**	**	**	**	**	**	**
SE (±)		0.357	0.285	0.217	0.347	0.652	12.11	131.3	0.2794
Variety (V)									
SAMNUT 22		57.20^{a}	18.30^{a}	50.13^{a}	29.98^{a}	30.81^{b}	941.5^{b}	2660^{a}	58.82^{b}
SAMNUT 23		57.68^{a}	18.80^{a}	50.28^{a}	30.83^{a}	34.71^{a}	986.8^{a}	2374^{b}	61.08^{a}
SAMNUT 14		45.63^{b}	11.47^{b}	43.96^{b}	22.61^{b}	32.18^{b}	920.8^{b}	1839^{c}	51.82^{c}
Level of significance		**	**	**	**	**	**	**	**
SE (±)		0.771	0.440	0.423	0.504	0.535	12.85	76.9	0.619
Season (Y)									
2018		52.20	11.47^{b}	30.43^{b}	23.51^{b}	26.77^{b}	787.1^{b}	1704^{c}	56.82^{b}
2019		54.11	18.80^{a}	56.97^{a}	28.96^{a}	37.97^{a}	1031.0^{a}	2685^{a}	60.63^{a}
2020		53.87	18.30^{a}	56.55^{a}	27.98^{a}	35.78^{a}	1011.0^{a}	2504^{b}	59.71^{a}
Level of significance		NS	**	**	*	**	**	**	*
SE (±)		0.767	0.496	0.769	1.136	0.684	32.88	75.1	0.965
Interaction									
W x V		**	**	**	**	**	NS	NS	**
W x Y		NS	**	**	**	NS	NS	**	**
V x Y		*	**	NS	**	NS	NS	NS	NS
W x V x Y		NS	NS	NS	NS	NS	NS	NS	NS

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; GLYP = Glyphosate; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing. ** = significant at 1% ($P \leq 0.01$); * = significant at 5% ($P \leq 0.05$); NS = Not significant.

Table 8: Interaction effect between Weed control and Variety on plant height of groundnut at 12 WAS in 2018, 2019 and 2020 combined seasons

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	54.83^{fhi}	53.09^{ijk}	44.59^{n}
PENDA	2.5	55.17^{fhi}	54.79^{g-j}	45.02^{n}
BUTA + PENDA	2.0 + 1.0	55.62^{f-i}	56.16^{fgh}	46.06^{mn}
PEND + BUTA	2.0 + 1.0	56.03^{fgh}	57.63^{def}	47.53^{lm}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	57.53^{efg}	59.63^{cde}	47.94^{l}
BUTA fb SHW at 6 WAS	2.0	60.29^{bcd}	59.68^{b-e}	51.91^{k}
PEND fb SHW at 6 WAS	1.5	60.51^{bc}	60.29^{b-e}	52.47^{jk}
PEND fb SHW at 6 WAS	2.0	60.90^{bc}	61.37^{b}	53.31^{ijk}
Weeding at 3 and 6 WAS	-	66.37^{a}	67.57^{a}	54.02^{h-k}
Weedy check	-	42.70^{o}	41.98^{o}	41.86^{o}
Level of significance			**	
SE (±)			0.969	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing. **= significant at 1% (P ≤ 0.01).

Table 9: Interaction effect between variety and season on plant height of groundnut at 12 WAS in 2018, 2019 and 2020 seasons

Season	Variety		
	SAMNUT 22	SAMNUT 23	SAMNUT 14
2018	55.37^{b}	55.33^{b}	41.13^{d}
2019	57.93^{a}	56.99^{a}	49.42^{c}
2020	56.88^{a}	55.85^{a}	48.73^{c}
Level of significance		*	
SE (±)		1.334	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing. * = significant at 5% (P ≤ 0.05).

Table 10: Interaction effect between weed control and variety on Number of branches plant^{-1} of groundnut at 12 WAS in 2018, 2019 and 2020 combined seasons

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	15.33^{hij}	13.89^{j-m}	10.22^{q}
PENDA	2.5	16.56^{fgh}	15.22^{g-k}	10.22^{q}
BUTA + PENDA	2.0 + 1.0	17.00^{fg}	15.67^{ghi}	10.78^{q}
PEND + BUTA	2.0 + 1.0	18.56^{de}	17.22^{ef}	11.56^{pq}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	19.78^{cd}	19.78^{cd}	12.44^{m-p}
BUTA fb SHW at 6 WAS	2.0	21.33^{bc}	20.22^{cd}	12.56^{m-p}

PEND fb SHW at 6 WAS	1.5	21.89^{ab}	22.22^{a}	12.67^{m-p}
PEND fb SHW at 6 WAS	2.0	22.11^{a}	22.44^{a}	13.89^{i-n}
Weeding at 3 and 6 WAS	-	22.78^{a}	23.11^{a}	14.22^{i-l}
Weedy check	-	13.67^{k-o}	13.22^{l-p}	8.33^{r}
Level of significance			**	
SE (±)			0.643	

Table 11: Interaction effect between weed control and season on number of branches plant^{-1} of groundnut at 12 WAS in 2018, 2019 and 2020 seasons

	Rate	Season		
Weed control	(Kg a.i.ha^{-1})	2018	2019	2020
BUTA	2.5	12.26^{l}	12.78^{l}	12.67^{l}
PENDA	2.5	14.00^{k}	15.00^{j}	15.00^{j}
BUTA + PENDA	2.0 + 1.0	15.14^{k}	15.22^{j}	15.20^{j}
PEND + BUTA	2.0 + 1.0	16.65^{hi}	17.67^{gh}	17.66^{gh}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	19.63^{fgh}	19.67^{fg}	19.65^{fgh}
BUTA fb SHW at 6 WAS	2.0	20.67^{df}	20.67^{ef}	20.65^{df}
PEND fb SHW at 6 WAS	1.5	21.08^{bcd}	22.11^{bcd}	22.10^{bcd}
PEND fb SHW at 6 WAS	2.0	23.15^{abc}	23.22^{abc}	23.21^{abc}
Weeding at 3 and 6 WAS	-	23.68^{ab}	23.78^{a}	23.76^{ab}
Weedy check	-	10.50^{mn}	11.33^{m}	11.31^{m}
Level of significance			**	
SE (±)			0.682	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; GLYP = Glyphosate; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.
** = significant at 1% (P ≤ 0.01).

Table 12: Interaction effect between variety and season on number of branches plant^{-1} of groundnut at 12 WAS in 2018, 2019 and 2020 seasons

	Variety		
*Season	SAMNUT 22	SAMNUT 23	SAMNUT 14
2018	13.00^{b}	12.40^{b}	10.02^{d}
2019	22.00^{a}	21.63^{a}	11.73^{c}
2020	22.00^{a}	21.63^{a}	11.63^{c}
Level of significance		**	
SE (±)		0.795	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test.
** = significant at 1% (P ≤ 0.01).

Table 13: Interaction effect between weed control and variety on canopy spread plant^{-1} of groundnut at 12 WAS in 2018, 2019 and 2020 (combined season)

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	44.12^{mn}	45.63^{lm}	38.85^{pq}
PENDA	2.5	45.99^{kl}	47.13^{jk}	40.87^{o}
BUTA + PENDA	2.0 + 1.0	49.06^{hi}	48.51^{hi}	41.44^{o}
PEND + BUTA	2.0 + 1.0	50.85^{g}	48.89^{hi}	42.85^{n}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	53.56^{b-f}	52.93^{f}	46.68^{kl}
BUTA fb SHW at 6 WAS	2.0	53.72^{a-f}	53.82^{a-f}	47.51^{ijk}
PEND fb SHW at 6 WAS	1.5	54.51^{a-e}	54.24^{a-f}	47.94^{ij}
PEND fb SHW at 6 WAS	2.0	54.97^{abc}	54.94^{a-d}	49.78^{gh}
Weeding at 3 and 6 WAS	-	55.03^{a}	55.00^{ab}	49.85^{gh}
Weedy check	-	40.28^{op}	38.05^{q}	36.70^{r}
Level of significance			**	
SE (±)			0.553	

Table 14: Interaction effect between weed control and season on canopy spread plant^{-1} of groundnut at 12 WAS in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Season		
		2018	2019	2020
BUTA	2.5	33.14^{op}	48.67^{jk}	48.65^{ik}
PENDA	2.5	33.23^{op}	50.85^{i}	50.83^{ij}
BUTA + PENDA	2.0 + 1.0	33.31^{o}	55.01^{h}	55.08^{h}
PEND + BUTA	2.0 + 1.0	33.31^{o}	55.14^{h}	55.13^{h}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	37.47^{mn}	61.20^{fg}	61.20^{fg}
BUTA fb SHW at 6 WAS	2.0	37.53^{mn}	62.24^{b-g}	62.22^{b-g}
PEND fb SHW at 6 WAS	1.5	37.53^{m}	63.10^{a-e}	63.08^{a-f}
PEND fb SHW at 6 WAS	2.0	37.64^{mn}	64.76^{abc}	64.74^{a-d}
Weeding at 3 and 6 WAS	-	38.01^{m}	64.86^{a}	64.84^{ab}
Weedy check	-	30.14^{q}	43.85^{l}	43.81^{l}
Level of significance			**	
SE (±)			0.848	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.

** = significant at 1% (P ≤ 0.01).

Table 15: Interaction effect between weed control and variety on number of pods plant^{-1} of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	23.56^{lm}	25.78^{jk}	19.44^{o}
PENDA	2.5	28.44^{fgh}	27.89^{g-j}	19.78^{o}
BUTA + PENDA	2.0 + 1.0	28.56^{efg}	29.33$_{efg}$	22.33^{mn}
PEND + BUTA	2.0 + 1.0	28.78^{fg}	30.11^{ef}	23.00^{lm}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	30.56^{de}	32.00^{cd}	23.44^{lm}
BUTA fb SHW at 6 WAS	2.0	32.67^{c}	32.78^{c}	24.67^{kl}
PEND fb SHW at 6 WAS	1.5	33.22^{c}	32.89^{c}	26.33^{hjk}
PEND fb SHW at 6 WAS	2.0	36.67^{b}	37.33^{ab}	27.22^{g-j}
Weeding at 3 and 6 WAS	-	38.44^{ab}	39.00^{a}	28.22^{f-i}
Weedy check	-	20.67^{no}	19.11^{o}	12.00^{p}
Level of significance			**	
SE (±)			0.761	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb^{1}= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.
** = significant at 1% (P ≤ 0.01).

Table 16: Interaction effect between weed control and season on number of pods plant^{-1} of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Season		
		2018	2019	2020
BUTA	2.5	22.89^{no}	25.15^{h-p}	24.71^{h-p}
PENDA	2.5	22.78^{m-q}	25.56^{g-o}	25.16^{g-n}
BUTA + PENDA	2.0 + 1.0	25.33^{h-p}	27.44^{f-k}	26.72^{e-l}
PEND + BUTA	2.0 + 1.0	25.56^{h-p}	27.78^{fgh}	26.68^{e-i}
BUTA fb^{1} SHW^{2} at 6 WAS^{3}	1.5	26.00^{h-m}	30.33^{cde}	30.13^{c-f}
BUTA fb SHW at 6 WAS	2.0	26.67^{e-m}	32.00^{bcd}	31.82^{bcd}
PEND fb SHW at 6 WAS	1.5	27.44^{e-j}	32.22^{b}	32.01^{bc}
PEND fb SHW at 6 WAS	2.0	27.89^{efg}	37.22^{a}	37.12^{a}
Weeding at 3 and 6 WAS	-	28.33^{b-g}	38.11^{a}	37.97^{a}
Weedy check	-	19.89^{qr}	16.18^{r}	16.11^{r}
Level of significance			**	
SE (±)			1.272	

Table 17: Interaction effect between variety and season on number of pods plant^{-1} of groundnut in 2018, 2019 and 2020 seasons

	Variety		
Season	SAMNUT 22	SAMNUT 23	SAMNUT 14
2018	26.53^{bc}	27.37^{b}	20.40^{e}
2019	31.70^{a}	32.57^{a}	22.63^{d}
2020	30.71^{a}	31.77^{a}	22.60^{d}
Level of significance		**	
SE (±)		1.341	

Table 18: Interaction effect between weed control and variety on 100 seed weight of groundnut in 2018, 2019 and 2020 seasons

	Rate	Variety		
Weed control	(Kg a.i.ha^{-1})	SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	32.40^{f-k}	32.48^{f-k}	30.09^{klm}
PENDA	2.5	32.48^{f-k}	34.14^{e-j}	30.75^{kl}
BUTA + PENDA	2.0 + 1.0	34.31^{e-j}	34.44^{e-i}	31.26^{h-k}
PEND + BUTA	2.0 + 1.0	34.65^{b-g}	34.52^{c-h}	31.98^{g-k}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	35.83^{b-e}	36.14^{b-e}	34.49^{c-h}
BUTA fb SHW at 6 WAS	2.0	36.19^{b-e}	36.50^{a-e}	35.36^{b-f}
PEND fb SHW at 6 WAS	1.5	36.69^{a-e}	37.43^{a-e}	35.73^{b-f}
PEND fb SHW at 6 WAS	2.0	37.74^{a-d}	37.97^{ab}	36.14^{b-e}
Weeding at 3 and 6 WAS	-	37.82^{abc}	39.66^{a}	36.52^{a-e}
Weedy check	-	28.05^{m}	27.83^{mn}	25.57^{o}
Level of significance			**	
SE (±)			1.197	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.

** = significant at 1% ($P \leq 0.01$).

Table 19: Interaction effect between weed control and season on haulm yield of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Season		
		2018	2019	2020
BUTA	2.5	1238^{h}	2127^{ef}	2011^{ef}
PENDA	2.5	1322^{h}	2293de	2217de
BUTA + PENDA	2.0 + 1.0	1337^{h}	2393^{cde}	2326^{cde}
PEND + BUTA	2.0 + 1.0	1393^{gh}	2581^{b-e}	2500^{b-e}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	1581^{fgh}	2885^{a-d}	2772^{a-d}
BUTA fb SHW at 6 WAS	2.0	1598^{fgh}	2933^{abc}	2881^{abc}
PEND fb SHW at 6 WAS	1.5	2011^{efg}	3153^{ab}	3160^{ab}
PEND fb SHW at 6 WAS	2.0	2011^{efg}	3204^{ab}	3100^{ab}
Weeding at 3 and 6 WAS	-	2355^{cde}	3265^{a}	3205^{a}
Weedy check	-	1195^{hi}	1538^{fgh}	1457^{fgh}
Level of significance			**	
SE (±)			228.4	

Table 20: Interaction effect between weed control and variety on shelling percentage of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Variety		
		SAMNUT 22	SAMNUT 23	SAMNUT 14
BUTA	2.5	48.22^{ijk}	48.00^{ijk}	47.33^{k-n}
PENDA	2.5	48.22^{ijk}	48.56^{ijk}	47.33^{k-n}
BUTA + PENDA	2.0 + 1.0	48.22^{ijk}	48.89^{ijk}	47.56^{klm}
PEND + BUTA	2.0 + 1.0	48.22^{ijk}	49.33^{ij}	47.56^{klm}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	59.33^{e-h}	59.33^{e-h}	58.00^{gh}
BUTA fb SHW at 6 WAS	2.0	60.67^{def}	60.67^{def}	59.33^{f-i}
PEND fb SHW at 6 WAS	1.5	60.78^{cde}	62.00^{cd}	59.67^{efg}
PEND fb SHW at 6 WAS	2.0	62.22^{bc}	62.22^{bc}	60.67^{def}
Weeding at 3 and 6 WAS	-	64.00^{a}	64.00^{a}	63.22^{ab}
Weedy check	-	35.67^{o}	35.67^{o}	35.33^{op}
Level of significance			**	
SE (±)			0.771	

Table 21: Interaction effect between weed control and season on shelling percentage of groundnut in 2018, 2019 and 2020 seasons

Weed control	Rate (Kg a.i.ha^{-1})	Season		
		2018	2019	2020
BUTA	2.5	46.67^{l-s}	57.56^{f-q}	56.61^{f-r}
PENDA	2.5	46.67^{l-s}	57.78^{e-o}	56.83^{e-p}
BUTA + PENDA	2.0 + 1.0	46.89^{k-u}	58.89^{d-m}	56.79^{d-n}
PEND + BUTA	2.0 + 1.0	46.89^{k-u}	58.89^{d-k}	56.92^{b-l}
BUTA fb[1] SHW[2] at 6 WAS[3]	1.5	53.00^{j-t}	60.21^{b-i}	59.18^{b-j}
BUTA fb SHW at 6 WAS	2.0	53.00^{j-t}	61.33^{b-g}	59.57^{b-h}
PEND fb SHW at 6 WAS	1.5	54.89^{g-s}	62.22^{a-e}	61.63^{a-f}
PEND fb SHW at 6 WAS	2.0	55.89^{g-s}	62.33^{abc}	62.10^{abc}
Weeding at 3 and 6 WAS	-	55.89^{g-r}	63.22^{a}	62.88^{a}
Weedy check	-	36.00^{uv}	36.33^{r-v}	36.00^{uv}
Level of significance			**	
SE (±)			1.069	

Means followed by the same letter (s) within a column are not significantly different at 5% level of probability using Duncan Multiple Range Test. BUTA =Butachlor; PENDA = Pendimethalin.fb[1]= Followed by; SHW= Supplementary hoe weeding; WAS= Weeks after sowing.
** = significant at 1% (P ≤ 0.01); NS = Not significant.

DISCUSSIONS

Effect of weed control, variety and season on weed parameters

The significantly higher weed control index obtained by such treatments might be attributed to the treatments' low weed index values as a result of season long weed management. This backs up the findings of Sah *et al*. (2017) and Sahoo *et al*. (2017) who found that pendimethalin and oxyfluorfen supplemented with hoe weeding each, reduced weed density and weed index when compared to sole application for weed control in ginger and groundnut, respectively. The improved vigour of crop plants due to weed control strategies could explain the higher CRI and TEI obtained. The hand weeding at 3 and 6 WAS together with pendimethalin at 2.0 kg a.i. ha^{-1} *fb* SHW at 6 WAS recorded the highest CRI and TEI. The unweeded control, on the other hand, had the lowest CRI, showing that weeds were the most damaging to the crop. Siddhu *et al*. (2018) found similar results in pigeon pean and onions, respectively. Similarly, in rice and soybean, Mishra *et al*. (2016) and Lal *et al*. (2017) observed higher CRI and TEI in herbicidal treated plots followed by SHW at intervals due to season-long weed control, which was statistically comparable to hoe weeding twice. Higher WI obtained in weedy check could be to unrestricted weed growth throughout the season resulting in lower CRI as well as growth and yield penalty. Our findings corroborate those of Prashanth *et al*. (2016) and Chandu *et al*. (2018) who discovered higher weed index in rice due to uncontrol weeds resulting in yield penalty. The ability of SAMNUT 22 variety in producing significantly

higher WCI and CRI could be attributed to the variety's genetic makeup, which develops more branches and a higher canopy, smothering the growth of weeds. Higher WCI, and TEI values in the 2019 and 2020 seasons compared to previous season could be attributed to favorable environmental circumstances that resulted in higher treatment efficacy in reducing the weed population, letting the crop to use the available growth resources for assimilate production. This conclusion supports the findings of Siddhu *et al*. (2018) and Rana and Rana (2018) who separately found that herbicidal treated plots with SHW at intervals had greater WCI and TEI due to the treatments' season-long weed control.

Effect of weed control, variety and season on crop performance

Higher growth and yield attributes such as plant height, number of branches, canopy spread, number of pods, 100 were found to be higher in plots that received weed control treatment. This could be attributed to year-round weed control, which resulted in better weed management during the early stages of crop growth, and later weed growth was checked by hoeing, resulting in low weed density and weed dry weight, allowing the crop to take full advantage of available growth resources for optimum growth and development. Plant growth characteristics increased due to low weed density per square meter of crops, according to Priya et al. (2013) and Ferdous *et al.* (2017). Similar findings have been confirmed by Wadafale *et al*. (2011) on increased number of branches of soybean due to season long weed control. Higher canopy spread obtained could be due to higher number of leaves and greater number of branches resulting from the efficacy of the treatments in smothering the growth of weeds. Our findings are similar to those of Sangeetha *et al*. (2012) and Smita *et al*. (2014), who found that an increase in the number of leaves, larger leaves and number of branches plant^{-1} resulted in a higher canopy spread; which in turn increased dry matter production. It is clear that employing preemergence herbicides + SHW at 6 WAS to control weeds eliminates weed interference and increases podding due to optimal nutrient uptake. This assertion was in line with Adhikary *et al*. (2016) who discovered that a larger number of pods plant^{-1} and mean pod weight collected were associated with improved nutrient accretion due less weed infestation, which translates to increased dry matter and CGR from agricultural plants. The higher pod yield, 100 seed weight, haulm yield and shelling percentage produced demonstrates the treatment's effectiveness in causing less crop-weed competition throughout the crop's growth period, less weed count, and less dry weight of weeds enhanced water and food intake, which might have increased the availability of carbohydrates by speeding up photosynthetic activity, led in cell division, multiplication, and elongation, resulting in an increase in cell size for growth and yield production. This current finding is consistent with those of Bhale *et al*. (2012) and Nikhil Reddy *et al*. (2016), who found that effective weed control strategies increased groundnut pod production. Furthermore, Amaregouda *et al*. (2013) further confirm that effective weed control strategies boost soybean growth and pod yield. Olayinka and Etejere (2015) and Kalhapure (2013) also confirmed an increase in groundnut yield components due to effective weed

management. Similarly, Abouziena *et al*. (2013) and Sinha *et al*. (2018) found an increase in 100 kernel weights of groundnut and transplanted kernel yield of rice in Bangladesh due to effective weed control. However, due to continuous competition for growth resources (space, light, nutrients, etc.) with the crops, weedy check considerably reported the shortest plants, number of branches plant, canopy spread, number of pods plant^{-1}, 100 seed weight, pod yield, haulm yield. Due to the negative effect of weed competition, plant growth aspects as reported by Tyagi et al. (2011) and Ferdous *et al*. (2017) were drastically reduced. The much taller plants, number of branches, canopy spread and number of pods generated by SAMNUT 22 and SAMNUT 23 varieties could be attributed to the genetic make-up to produce taller plants with a broader canopy spread under favorable climatic conditions offered by the 2019 and 2020 cropping season could be attributable to better environmental conditions and nutrient uptake, which allowed for the development of more pods per plant^{-1}, which is directly related pod yield, kernel yield, and 100 seed weight than what was obtained in previous season. The SAMNUT 23 variety's dominance in producing significantly heavier 100 kernel weight, pod yield and shelling percentage could be the explanation for the higher 100 seed weight, which was ascribed to the variety's genetic makeup to produce larger and heavier seed sizes due to larger pods. Our findings corroborate those of Parthipan (2020), who found that crops perform better when weeds are efficiently managed. Similarly, Pereira *et al*. (2015) asserted that the number of pods generated by plant^{-1} favored mean pod weight, pod yield, kernel yield, and 100 kernel weight, and that the number of pods produced was also influenced by a variety of environmental factors and management practices adopted.

Interaction effects of weed attributes

The significant interaction obtained between weed control and variety on WCI and CRI demonstrated the efficacy of pre-emergence treated plots that were supplemented with hoe weeding at 6 WAS across SAMNUT 22, SAMNUT 23 and SAMNUT 14 could be aided by season long weed control which reduces crop-weed rivalry for limited growth resources which simultaneously increasing the ability of the varieties ability to establish additional branches and canopy capable of inhibiting weed growth. This finding corroborates those of Meena *et al*. (2011), who reported higher WCI pigeon pea due to maximum weed management resulting in pod yield gain.The significant interaction obtained between weed control and season on TEI and CRI might be ascribed to decreased weed density and weed index, together with prevalent weather conditions, which improves season-long weed control achieved with supplementary hoe weeding applied to the pre-emergence herbicide at 6 WAS, as well as the second weeding applied to the hoe weed treatment that controls the second flush of weeds. As a result, improves the weed control treatments broad-spectrum efficacy. Omisore *et al*. (2016) and Kashid (2019) reported the lowest weed cover score, weed density, and weed index as a result of pre-emergence herbicide application when paired with one hoe weeding, consequently

enhancing WCI and TEI of cowpea and rice, respectively, in Nigeria and India. The significant interaction discovered between weed control and variety on weed index showed that SAMNUT 14, SAMNUT 22 and SAMNUT 23 in weedy check significantly resulted in producing the highest weed index was aided by uninterrupted crop-weed competition for limited growth resources which decreased the ability of the varieties to develop more branches plant^{-1} and canopy spread capable of suppressing weed growth. Our findings corroborate those of Prashanth *et al*. (2016) and Chandu *et al*. (2018) who discovered higher weed index in rice due to uncontrol weeds resulting in yield penalty.

Interaction effects of crop attributes

The significant interaction discovered between weed control and variety on crop attributes such as plant height, number of branches plant^{-1}, canopy spread, number of pods plant^{-1} and shelling percentage could be attributed to higher weed control index, treatment efficiency index, crop resistance index achieved by the treatments in SAMNUT 22 and SAMNUT 23 varieties that enable the varieties to explore their growth and yield potential compared to plots that weeds were kept unabated and resulted in lower crop attributes. Our findings are in line with the findings of Kanatas *et al*. (2020) who reported that herbicide application and cultivar significantly decreased the density of weeds compared to weedy check treatment. Similarly, significant increase in the growth attributes of groundnut due to season and varietal effect could also be attributed to favorable season enjoyed during 2019 and 2020 that led to release of nutrients due to effective weed control that enables the SAMNUT 22 and 23 to effectively utilized the available nutrients to explore their genetic make-up in producing higher growth and yield attributes of groundnut. This corroborates with the earlier reports of Omisore *et al*. (2016) and Kashid *et al*. (2019).

CONCLUSION AND RECOMMENDATIONS

Findings from the field experiments indicated that weed parameters such as weed control index, treatment efficiency index and crop resistance index were significantly lower under the application of pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS and hoe weeding at 3 and 6 WAS compared to weedy check that resulted in higher value. The plant height, number of branches, canopy spread, number of pods plant^{-1} were significantly higher with the application of hoe weeding at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS. Similarly, 100 kernel weight, haulm yield and shelling were significantly higher with the application of hoe weeding at 3 and 6 WAS, pendimethalin at 2.0 kg a.i.ha^{-1} *fb* SHW at 6 WAS, pendimethalin at 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS, butachlor at 2.0 and 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS. The SAMNUT 22 and 23 varieties produced taller plants with a greater number of branches, canopy spread and number of pods

plant^{-1}. On the other hand, SAMNUT 23 variety significantly produced higher 100 kernel weight, pod yield and shelling percentage while SAMNUT 22 variety produced higher haulm yield, respectively. From the findings it can be deduced that application of pendimethalin at 2.0 and 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS and butachlor at 2.0 and 1.5 kg a.i.ha^{-1} *fb* SHW at 6 WAS in place of manual weeding at 3 and 6 WAS with SAMNUT 22 or SAMNUT 23 can be adopted by farmers towards boosting groundnut yield in the study area to avert scarcity of manual labour during peak periods.

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Isolation, Phenotypic Characterization, Antimicrobial Resistance and Susceptibility of ESBL Producing-*Klebsiella pneumoniae* from Urine Specimen Collected from Patients in Maiduguri

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Abstract: *Klebsiella pneumoniae is one of the Enterobacteriaceae strains that can produce Extended-Spectrum Beta-Lactamases Enzymes (ESBLs) and become highly effective against different Beta-lactams antimicrobials. It is associated with different diseases which can cause high mortality and morbidity. This study aims to elaborate on the isolation, phenotypic characterization, antimicrobial resistance and susceptibility of ESBL Producing-K. pneumoniae from urine specimen collected from patients in Maiduguri. The study was Hospital-based, descriptive, and cross-sectional in design. Two hundred and twenty (220) clinical samples were collected for this study from both in and outpatients with clinical symptoms of urinary tract infections attending Umaru Shehu Ultra-Modern Hospitals Hospital and State Specialist hospital Maiduguri respectively. There is no significant association between hospitals and the status of the ESBL Producing-K. pneumoniae (P = 0.2789; DF = 1; Chi-squared = 1.172). The results obtained from urine culture revealed 122 (55.45%) positive and 98 (44.55%) negative samples of lactose fermenting organisms from both State Specialist Hospital Maiduguri and Umaru Shehu Ultra-Modern Hospital, out of which, 65 (29.55%) were positive and 45 (29.55%) were negative from State Specialist Hospital Maiduguri and 57(25.91%) were positive and 53(24.09%) were negative from Umaru Shehu Ultra-Modern Hospital. A total of 19 (8.46%) positive and 46 (20.91%) negative samples were K. pneumoniae and these were obtained from State Specialist Hospital Maiduguri and 11(5%) positive and 46(20.91%) negative samples of K. pneumoniae were obtained from Umaru Shehu Ultra-Modern Hospital. All the positive ESBL samples were resistant to all the antimicrobials with the exception of Amoxiclav and Imipenem. Furthermore, the non ESBL Producing-K. pneumoniae were all susceptible to all the antimicrobials except Ampicillin which was resistant. Therefore, there is need to use more antibiotic and antimicrobial agents. It is likewise imperative to identify the resistance genes associated with ESBL producing K. pneumoniae and the virulence genes associated with the ESBL genes.*

Keywords: *lactose-fermenting, ESBL-producing Klebsiella pneumoniae, In and Out patient, Antimicrobial agents, Maiduguri.*

Introductions

Klebsiella pneumoniae is one of the most significant multidrug-resistant (MDR) opportunistic Gram-negative bacteria. It is associated with different diseases which can cause high mortality and morbidity due to nosocomial and non-hospital acquired infections such as pneumonia, urinary tract infection (UTI), burns infections, and bacteraemia (Zhong *et al.,* 2013).

In recent times, *K. pneumoniae* became more resistant to antimicrobial mainly to third-generation cephalosporins. Many studies focused on the isolation of this pathogen from patients infected with different infections (Chili *et al.,* 2016; Singh *et al.,* 2017; and Karlowsky *et al.,* 2017).

K. pneumoniae is one of the Enterobacteriaceae strains that can produce Extended-Spectrum Beta-Lactamases Enzymes (ESBLs) and become highly effective against different Beta-lactams antimicrobials. These enzymes have the ability to hydrolyse these cephalosporins compromising the efficacy of these antibiotics, ESBLs-producing bacteria are resistant to various antimicrobial classes, leading to difficult-to-treat diseases called multi-drug resistance (MDR) (Nathisuwan *et al.,* 2001). Multidrug-resistant bacteria and ESBL producing *K. pneumoniae and* other Gram-negative bacteria have worldwide distribution with high degree of prevalence in both hospitals and communities (Leverstein-van *et al.,* 2002, Aljanaby, 2013, Legese *et al*., 2017). Extended spectrum beta lactamases are plasmid-mediated enzymes that are capable of conferring resistance to penicillins, first, second and third generations cephalosporins and carbapanems (Johann *et al.,* 2008). ESBL producing *K. pneumoniae* strains isolated from both in and outpatients can cause treatment failure with different antimicrobial therapy such as beta-lactams, cephalosporins, aminoglycosides and others (Kim *et al*., 2016; Tang *et al*., 2017). The ESBL are enzymes produced by different types of bacterial species as a means for defence against *β. Lactam* drugs with the genes encoding for those enzymes being mainly located on mobile genetic elements (Pfeifer *et al.,* 2016). The rapid emergence of ESBL producing Gram-negative bacteria like *K*. *pneumoniae* has significantly increased the risk of developing serious nosocomial infections worldwide (Brinkworth *et al.,* 2015; Latifpour *et al.,* 2016).

K. pneumonia has recently gained recognition as an infectious agent due to rise in the number of severe infections. These pathogens showed more resistance in response to treatment of pneumonia among neonates, elderly and immune-suppressed individuals within the healthcare-associated settings (Paczusu *et al.,* 2016; Quan *et al.,* 2016). Treatment of these infections depends heavily on effective antimicrobial therapy and delaying treatment may lead to a higher mortality ratio. Therefore, the presence of MDR genes in the infecting pathogen could negatively affect the treatment outcome (Lin *et al.,* 2016).

The prevalence varies worldwide and in closely related regions. In Nigeria, a study conducted by Yusha'u *et al* 2010 in Kano revealed 9.25% ESBLs production amongst isolates of *Enterobacteriaceae.* Also, a study conducted by Oluwe *et al.,* 2010, on the determination of ESBL prevalence recorded 5% prevalence of *K. pnuemoniae* and 2.5% prevalence of *Escherichia*

coli. There is no routine laboratory detection of ESBL producing isolates in most health care facilities and due to the high treatment failure associated with ESBL-producing organisms, it is imperative to ascertain the phenotypic characterization, their antimicrobial resistance profile and select the most appropriate drug for managing ESBL infection in Maiduguri Metropolis.

2 Materials and Methods

Study Area and Period.

The study was conducted at Maiduguri from January to March, 2022. Maiduguri which is located in the capital city of Borno stated.

Research Ethics and patient consent

Ethical clearance and protocol will be obtained from State Specialist Hospital and Umaru Shehu Ultra Model Hospital Maiduguri to enable the collection of samples. Patient consent was requested to collect swabs from wound.

Sampling Method.

A non-probability |(convenient) purposive type of sampling method was used.

Study Design

The study was Hospital-based, descriptive, and cross-sectional in design.

Sample Collection and Transportation: Two hundred and twenty (220) clinical samples were collected for this study. One hundred and ten (110) urine samples were collected were collected from both in and outpatients with clinical symptoms (Urinary Tract Infections UTIs) attending Umaru Shehu Ultra-Modern Hospitals (USUMH) Hospital and State Specialist hospital Maiduguri (SSHM) respectively.

Urine samples were collected in a well labelled universal containers indicating the source of the sample that is the Hospital in which the samples were collected. Samples were immediately transported to the laboratory for bacteriological procedures and analysis.

Bacterial Isolation

The urine sampled was inoculated on MacConkey agar (Himedia- India) surface and incubated aerobically over night at 37^0C. All isolates were preliminarily screen by their colony, morphology, colour (pinkish mucoid colonies) and gram staining techniques (Gram negative rod shaped, non-capsulated and non-sporing). Further identification included Biochemical test methyl red negative and indole negative, citrate positive, and non-motile. In addition, all *K. pneumoniae* isolates were streaked and inoculated on Hicrome selective *Klebsiella pneumoniae* agar (Himedia-media) which was used for final identification of *K. pneumoniae*

ESBL Detection Methods: Hicrome selective *Klebsiella pneumoniae* agar (Himedia) was used for the primary isolation of ESBL *K. pneumonia* prior to confirmatory screening as per clinical and laboratory standard institute guidelines 2014 (|CLSI, 2014|)

ESBL Screening and Confirmation

The ESBL test screening test was performed by the standard disk diffusion method using

cefotaxime (30µg), ceftriaxone(30µg) and ceftazidine (30µg) (Oxoid, UK). More than one antibiotic disk was used for screening to improve the sensitivity of ESBLs detection as per CLSI standard procedure (CLSI, 2014). Pure isolates were suspended into normal saline and the turbidity of the suspension was adjusted at 0.5 MacFarland's standard. The suspension was then inoculated onto Mueller-Hinton Agar (Himedia-India) with a sterile swab. Cefriaxone (30µg), ceftriaxone(30µg) and ceftazidine (30µg) were placed at a distance of 20mm and incubated at 37^0C overnight (16-15 hours). The isolates that were less sensitive to cefriaxone, (inhibitory zone ≤ 23mm) cefotaxime (inhibitory zone ≤ 27mm) and ceftazidine (inhibitory zone ≤ 22mm) around the disc were suspected to be ESBL-producing (CLSI, 2014). For the confirmatory test, the suspected ESBL-producing *K. pneumoniae*, a double disc synergy method on Mueller-Hinton agar was done according to 2014 CLSI guidelines. used to sscreened for ESBL production using the modified Kirby Bauer method. A disk of amoxicillin/clavulanic acid (20/10 µg) was placed at the middle of Mueller-Hinton agar plate and then ceftazidime and (30µg), cefotaxime (30µg) were placed at a distance of 20mm putting the amoxicillin/clavulanic acid in the middle of the plate. The inoculations were then incubated at 37^0C for 24 hours after which it was examined for an expansion of inhibition zone of the axyimino-β-lactamscaused by the synergy of clavulanate in the amoxicillin-clavulanate disk which was interpreted as a positive ESBL production.

Results

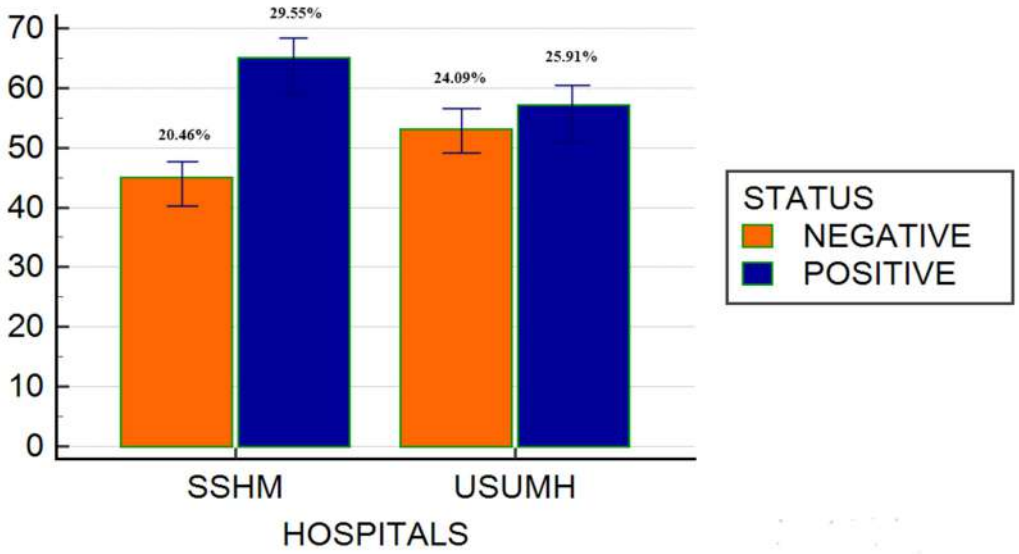

Figure 1: Growth of lactose fermenting organisms on MacConkey Agar

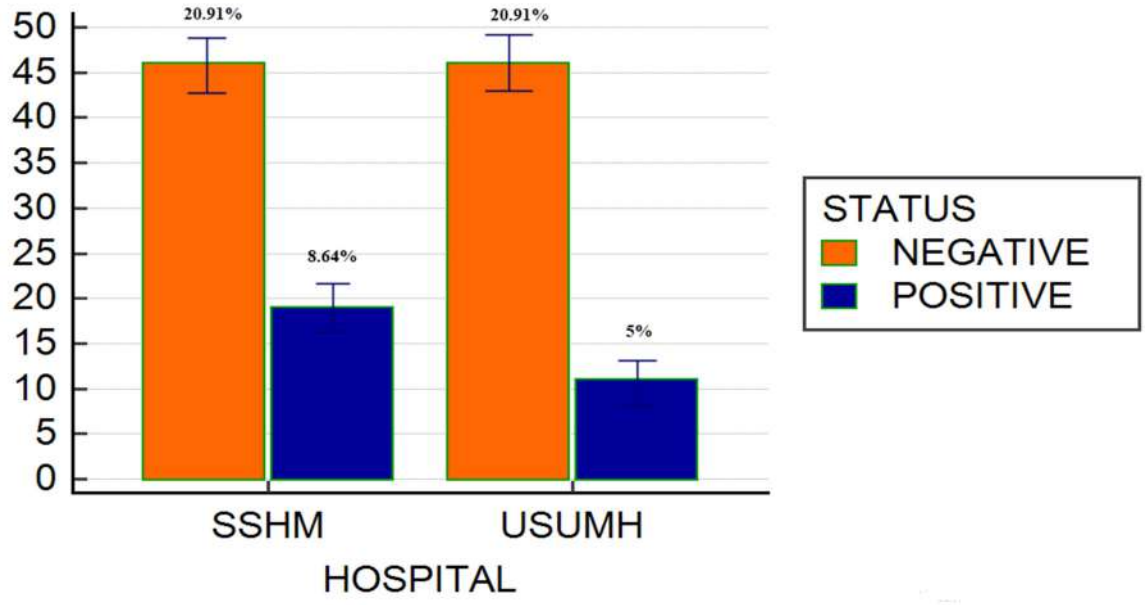

Figure 2: Isolation of *K. pneumoniae* from positive lactose fermenting organisms

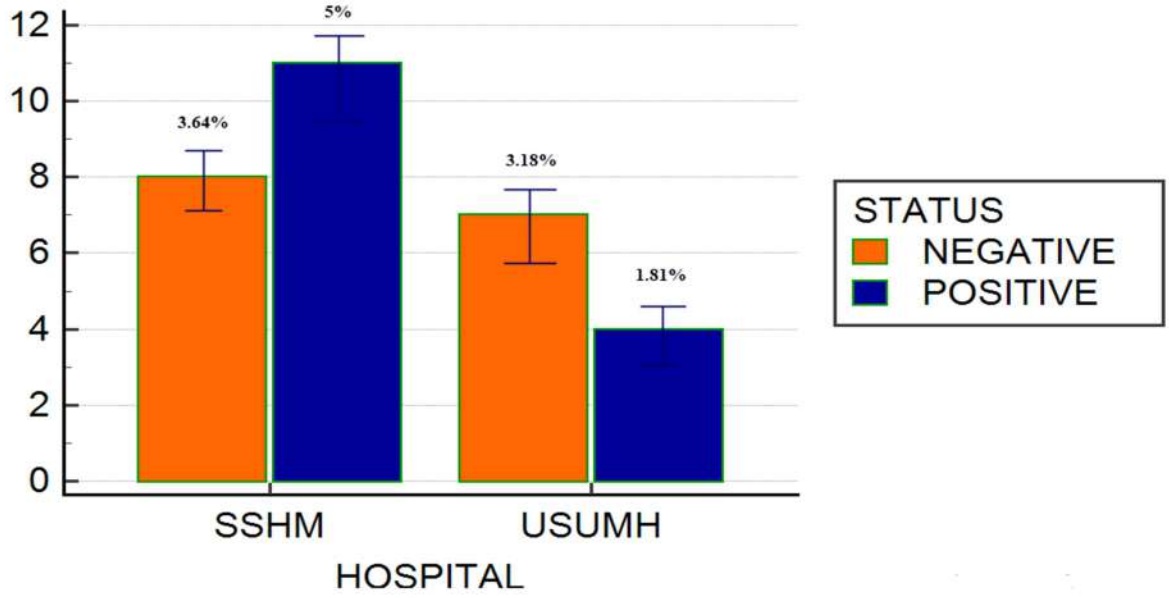

Figure 3: Positive ESBLs Producing-*K. pneumoniae*

The results obtained from urine culture revealed 122 (55.45%) positive and 98 (44.55%) negative samples of lactose fermenting organisms from both State Specialist Hospital Maiduguri (SSHM) and Umaru Shehu Ultra-Modern Hospital (USUMH), out of which, 65 (29.55%) were positive and 45 (29.55%) were negative from SSHM and 57(25.91%) were positive and 53(24.09%) were negative from USUMH. A total of 19 (8.46%) positive and 46 (20.91%) negative samples were *K. pneumoniae* and these were obtained from SSHM and 11(5%) positive and 46(20.91%) negative samples of *K. pneumoniae* were obtained from USUMH.

Table 1: Chi-Squared test for the growth of lactose fermenting organisms on MacConkey Agar

Chi-squared test

Chi-squared	1.172
DF	1
Significance level	P = 0.2789
Contingency coefficient	0.073

There is no significant association between hospitals and the status of the lactose fermenting organisms (P = 0.2789; DF = 1; Chi-squared = 1.172).

Table 2: Chi-Squared test for the isolation of *K. pneumoniae* from positive lactose fermenting organisms

Chi-squared test

Chi-squared	1.602
DF	1
Significance level	P = 0.2056
Contingency coefficient	0.114

There is no significant association between hospitals and the status of the *Klebsiella pneumoniae* (P = 0.2056; DF = 1; Chi-squared = 1.602).

Table 3: Chi-Squared test for the Positive ESBLs Producing-*K. pneumoniae*

Chi-squared test

Chi-squared	1.249
DF	1
Significance level	P = 0.2638
Contingency coefficient	0.200

There is no significant association between hospitals and the status of the *Klebsiella pneumoniae* (P = 0.2638; DF = 1; Chi-squared = 1.249).

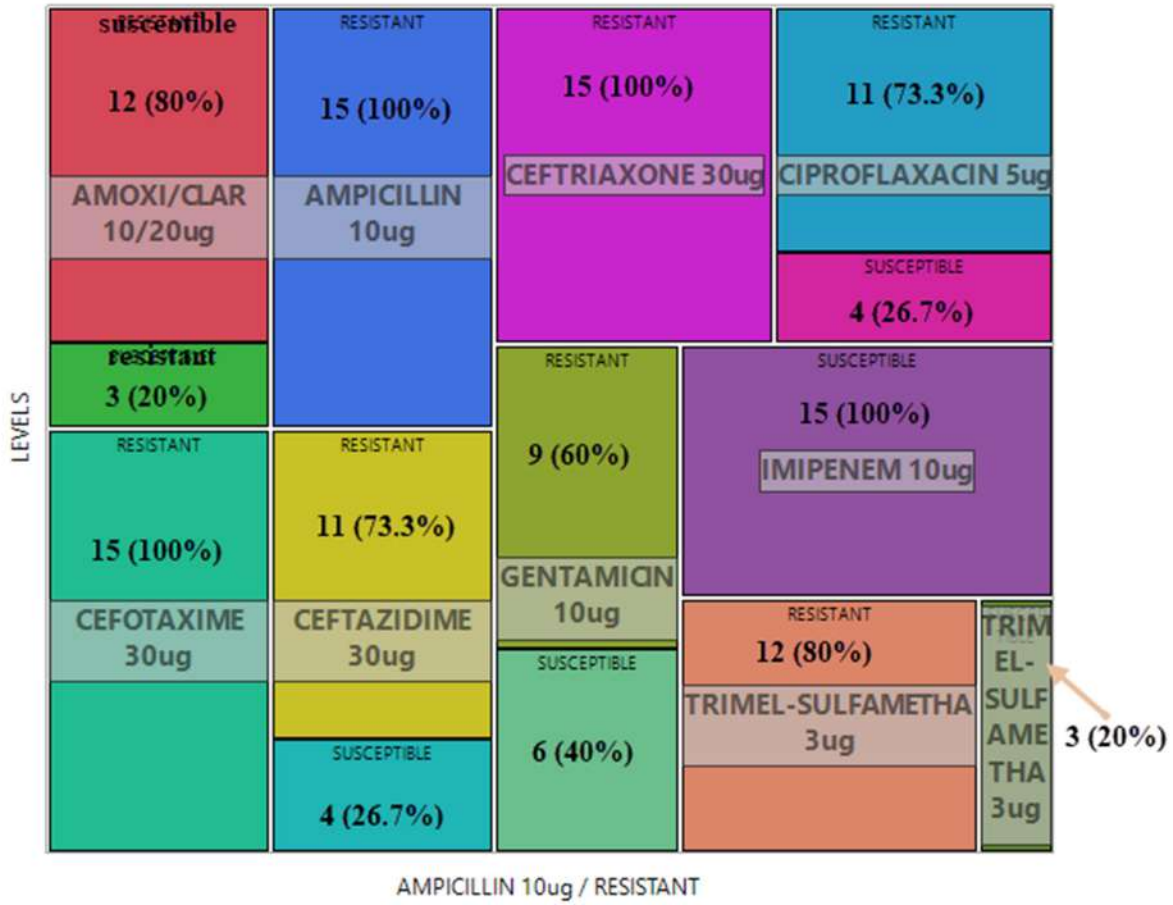

Figure 4: Antimicrobials susceptibility pattern for positive ESBL Producing-*K. pneumoniae*

The positive ESBL Producing-*K. pneumoniae* 12 (80%) were resistant to Amoxi/Clar (10/20ug) while, 3 (20%) were susceptible to Amoxi/Clar (10/20ug). Whereas 15 (100%) of the ESBL

Producing-*K. pneumoniae* were all resistant to Ampicillin (10ug). While 15 (100%) of the ESBL Producing-*K. pneumoniae* were all resistant to Ceftriaxone. Furthermore, the positive ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Ciproflaxacin (5ug) and 4 (26.7%) were susceptible to Ciproflaxacin (5ug). Additionally, 15 (100%) of the ESBL Producing-*K. pneumoniae* were all resistant to Cefotaxime (30ug). The positive ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Ceftazidime (30ug) while, 4 (26.7%) were susceptible to Ceftazidime (30ug). Whereas positive ESBL Producing-*K. pneumoniae* 9 (60%) were resistant to Gentamicin (10ug) while, 6 (40%) were susceptible to Ceftazidime (30ug). Whereas 15 (100%) of the positive ESBL Producing-*K. pneumoniae* were all resistant to Imepenem (10ug). The positive ESBL Producing-*K. pneumoniae* 12 (80%) were resistant to Trimel-Sulfamethan (3ug) while, 3 (20%) were susceptible to Trimel-Sulfamethan (3ug).

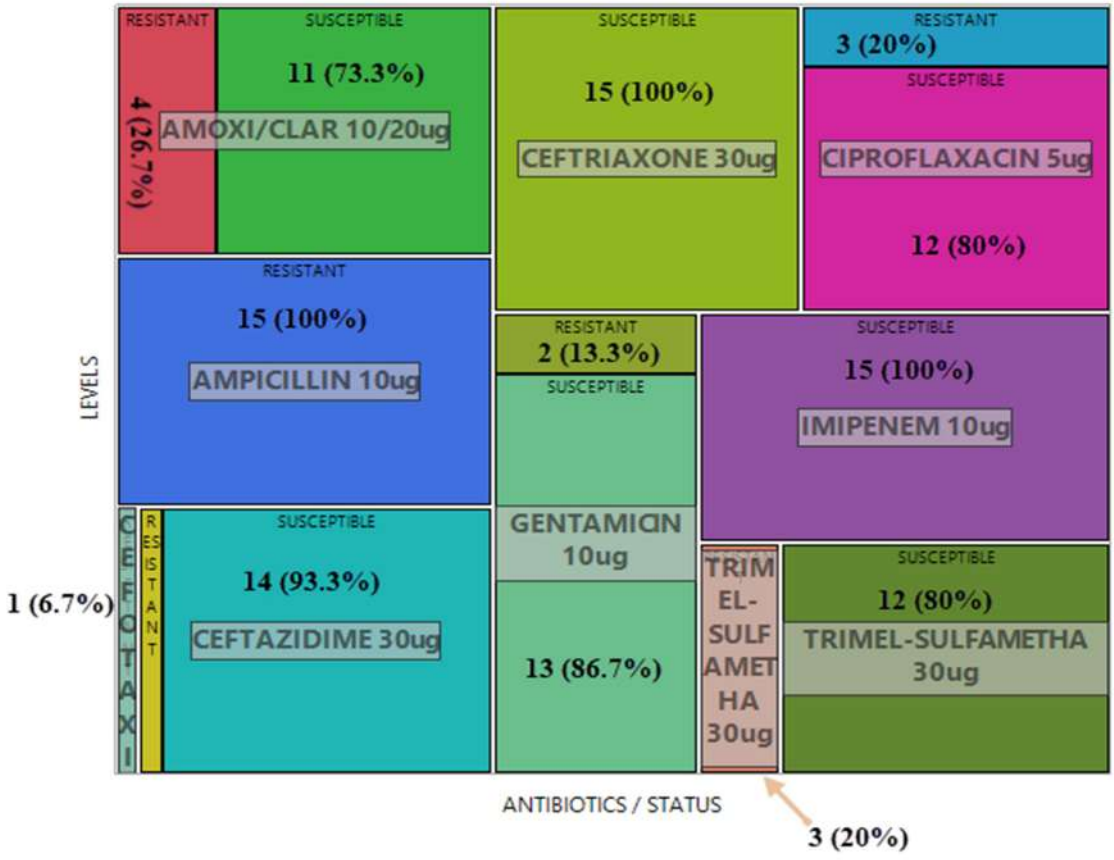

Figure 5: Antimicrobials susceptibility pattern for negative ESBL Producing-*K. pneumoniae*

The negative ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Amoxi/Clar (10/20ug) while, 4 (26.7%) were susceptible to Amoxi/Clar (10/20ug). Whereas 15 (100%) of the negative ESBL Producing-*K. pneumoniae* were all resistant to Ceftriaxone (30ug). While 3 (20%) of the negative ESBL Producing-*K. pneumoniae* were resistant to Ciproflaxacin (5ug) and susceptible to 12 (80%) of Ciproflaxacin (5ug). Furthermore, the negative ESBL Producing-*K. pneumoniae* 15 (100%) were all resistant to Ampicillin (10ug). Additionally, 2 (13.3%) of the negative ESBL Producing-*K. pneumoniae* were resistant to Gentamicin (10ug) and susceptible to 13 (86.7%). The negative ESBL Producing-*K. pneumoniae* 15 (100%) were all resistant to Imepenem (10ug). The negative ESBL Producing-*K. pneumoniae* 1 (6.7%) were resistant to Ceftazidime (30ug) while, 14 (93.3%) were susceptible to Ceftazidime (30ug). Whereas the negative ESBL Producing-*K .pneumoniae* 3(20%) were resistant to Trimel-Sulfamethan (30ug) while, 12 (80%) were susceptible to Trimel-Sulfamethan (30ug).

Discussion

Klebsiella pneumoniae is one of the most momentous multidrug-resistant (MDR) opportunistic Gram-negative bacteria. It is linked to dissimilar diseases which can cause high mortality and morbidity due to nosocomial and non-hospital acquired infections. In the current study, 65 (29.55%) and 57 (25.51%) were positive for the lactose fermenting organisms from both hospitals which were linked to urinary tract infections. These findings were in accord with the study of conducted in various countries (Yusha'u et al., 2010; Yahaya et al., 2016; Ahmed et al., 2017; Mengistu et al., 2018). Our findings also revealed that there was no significant associations between the hospitals and the infective agents.

Similarly, the current study revealed 19 (29.2%) and 11 (19.3%) from SSHM and USUMH respectively for *K. pneumoniae* from positive lactose fermenting organisms. These findings were similar to the findings divulged by Yahaya et al., (2016) in their study on the characterization of Klebsiella species.

Furthermore, result obtained from the double disc synergy method for the confirmation of ESBL producing- *K. pneumoniae* using Ceftazidime, Cefotaxime and amoxicillin/clavulanic acid (20/10 µg) in the present study showed 11 (57.9%) and 4 (36.4%) from SSHM and USUMH respectively for *K. pneumoniae*. These findings were similar to the reports of (Yusha'u et al., 2010; Yahaya et al., 2016). However our findings were slightly higher than their findings this could be due to the differences in the hospital settings.

Additionally, in the current study the susceptibility patterns of antimicrobials showed varying degree of resistance and susceptibilities for positive and non ESBL samples. Thus, all the positive ESBL samples were resistant to all the antimicrobials with the exception of Amoxiclav and Imipenem. Furthermore, the non ESBL producing-*K. pneumoniae* were all susceptible to all

the antimicrobials except Ampicillin which was resistant. These findings were similar to the study conducted by Mengistu et al., (2018) that non ESBL producing-*K. pneumoniae* may be resistant to Ampicillin by other mechanisms.

Conclusion

Carbapem (imipenem) is the most effective antibiotic for infections with *K. pneumoniae* for both ESBL and non ESBL producing-*K. pneumoniae.* This study revealed limited group of antibiotics that are ineffective for ESBL Producing *K. pneumoniae* and most of the antibiotics are effective for non ESBL producing *K. pneumoniae*. Our findings similarly discovered that there was no significant associations between the hospitals and the infective agents.

Recommendations

To ascertain the resistance and susceptibility of the antimicrobial agents against ESBL producing *K. pneumoniae*. There is need to use more antibiotic and antimicrobial agents. It is similarly important to identify the resistance genes associated with ESBL producing *K. pneumoniae* and the virulence genes associated with the ESBL genes.

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Phytochemical and Synthesis of Silver Nanoparticles Using *Citrus Aurantifolia* Leaf Extract

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Abstract: *This studdy investigates synthsis and antimicrobial activity of silver nanoparticles using citrus aurantifolia leaf extract to achieve, the fresh leaf of citrus aurantifolia was collected, authenticated, prepared, extracted with de-ionzed water and concentrated using standard procedures. Four different concentrations of plant extract were prepared in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM AgNO3 metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of Citrus auratifolia leaf extract silver nanoparticles The nano products formed were coded (As, Bs, Cs, Ds).However, a control solution was made by 10 mL of citrus auratifolia leaf extract (solution) without silver nitrate solution in which no colour change was observed. Citrus aurantifolia leaf extract reveals the presences of some phytochemicals such alkaloids, flavonoids, saponins and tannins respectively and it could be served as a potential agent of synthesizing silver nanoparticles.*

Keywords: *Citrus Aurantifolia, Silver Nanoparticles, Phytochemical.*

INTRODUCTION

Nano is a metric measure of one billionth of a meter and covers a width of 10 atoms. In terms of comparison with real objects, an example that hair is 150,000 nanometers may be given. The rapidly developing nanotechnology is the inter-disciplinary research and development field of biology, chemistry, physics, food, medicine, electronics, aerospace, medicine, etc., which examines the design, manufacture, assembly, characterization of materials that are smaller than 100 nanometers in scale, as well as the application of miniature functional systems derived from these materials. It represents the whole of development activities. As far the nanobiotechnology, on the other hand, it is the result of a combination of biotechnology and nanotechnology branches with a common combined functioning (Pearce, 2012). The reason for the intense interest of scientists nowadays in nanoparticles is that nanoparticles can

exhibit different properties and functions than normal bulk materials. The most important factor that enables production of nanostructures in desired size, shape and properties and provides their usage in various fields is that the effects of classical physics are reduced and the quantum physics becomes active. Other reasons for the different behavior of nanoparticles in physical, chemical, optical, electrical and magnetic behavior include the limitations of load carriers, size dependent electronic structures, increased surface / volume ratio, and other factors incurred by the unique properties of atoms (Shah *et al*., 2015).

The process of removing toxic and waste metals in the environment includes microorganisms, plants and other biological structures; achieved by means of oxidation, reduction or catalysis of metals with metallic nanoparticles. Metallic nanoparticles produced by biological methods; are used in the biomedical field for purposes such as protection from harmful microorganisms, bio-imaging, drug transport, cancer treatment, medical diagnosis and sensor construction because of their unique properties such as being insulator, optics, antimicrobial, antioxidant, anti-metastasis, biocompatibility, stability and manipulability (Schrofel *et al*., 2014; Singh *et al*., 2016).

Citrus aurantifolia (christm) swingle (lime) belong to the family rutaceae. It is a dense and irregularly branched tree. The stem is spreading and woody, brown in color, with short stiff spines on twigs. The leaves are acute, entire elliptic, oblong-ovate, dark green above, pale-green blow, altanate with narrowly winged leaf petioles. Flowers are white and stand from lea corners. The fruit is round, greenish-yellow with thin skin, juicy, fragrant and very acidic. The seeds are small, avoid and pale (Aliyu, 2006).

Citrus aurantifolia in its natural state is widely used in West Africa, particularly in Nigeria where it is employed in herbal medicine to treat several illnesses. It forms an essential ingredient in the preparation of most herbal concoctions (Aibinu *et al*., 2007)

This research work was designed to carry out phytochemical and synthesis of silver nano particles using citrus aurantifolia leaf extract will help provide scientific baseline information on phytochemical and plant based silver nanoparticles.

MATERIALS AND METHODS

Sample collection and Extraction

Citrus aurantifolia L. (Lime) leaf: Fresh leaf of *Citrus aurantifolia*(CA) L. was collected at Mashidimami garden along Damboa road, Maiduguri. The plant part was authenticated by a botanist in the Department of Biological Sciences, University of Maiduguri, Borno state and washed several times with water to remove the dust particles and then air dried to remove the residual moisture and pulverized into powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of conical flask. Then the

solution was incubated for 30 min. and subsequently subjected to centrifuge for 30 min. at room temperature with 5000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vaccume filter. Then the solution was used for the reduction of silver ions Ag^{+}) to silver nanoparticles (Ag^{o}).

Synthesis of Silver Nanoparticles

Four concentrations of plant extract were prepared (20, 40, 60, 80 mL) in 20 mL of 1 mM silver nitrate in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM $AgNO_3$ metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of *Citrus auratifolia* leaf extract silver nanoparticles however, a control solution was made by 10 mL of *citrus* leaf extract (solution) without silver nitrate solution.

After incubation, the solution was centrifuged at 12000 rpm for 4 minutes, and the obtained precipitate mass i.e., *Citrus auratifolia* leaf extract silver nanoparticles was washed three times with distilled water and centrifuged at 12,000 rpm for 3min.The mass was collected and oven dried at 42°C. Thereafter *Citrus auratifolia* leaf extract silver nanoparticles so obtained were scraped, coded (As, Bs, Cs, Ds) and kept for further analysis.

Phytochemicals Screening

The plant was dried under shade and pulverized into power. Two hundred grams (200g) of powered plant material was soaked in stopped container with methanol for three day with frequent agitation. The extract was filtered, concentrated. The methanol extract was screen for its phytochemical using standard procedures described by (Brain and Tuner, 1975; Markham, 1982; Sofowora, 1993; Trease and Evans, 2002).

Table 1: The Result of Synthesis of Silver Nanoparticles Using Citrus Leaf Extract

S/NO	Sample	Ratio(v/v)	Colour after 24 hrs	*Citrus a.* leaf extract silver nanoparticle
1	A	1:1	Tan	As
2	B	2:1	Latte	Bs
3	C	3:1	Linen	Cs
4	D	4:1	Off white	Ds
5	E	-	Yellow	-

Key:
A, B, C, D = *Citrus aurantifoloia* leaf extract solution + $AgNO_3$
E= *Citrus aurantifoloia* leaf extract solution
(As, Bs, Cs, Ds) = *Citrus a.* leaf extract silver nanoparticles

Table 2: The Result of Phytochemical screening of citrus aurantifolia leaf extract

TEST	RESULT
Alkaloids	+
Anthroquinones	-
Cardiac-glycosides	-
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	-

Key: + = Present
- = Absent

Discussion

The synthesis of the silver nanoparticles was confirmed by the characteristic colour change from greenish to tan, latte, linen and off white respectively, for the ratios of 1:1, 1:2, 1:3 1:4 of *Citrus a.* leaf extract solution together with AgNO3 as shown in the (Table 1). The nano products formed were coded (As, Bs, Cs, Ds). This result is in consonance with the findings of Sivakumar and Vidyasagar (2014) reported that during the synthesis of silver nanoparticles using Annona reticulata the color of the reaction mixture after 20 min, at room temperature, changed to dark brown, indicating the formation of AgNPs.

The result of phytochemical screening of methanolic leaf extract of *citrus aurantifolia* revealed the presence of alkaloids, flavonoids, saponins and tannins respectively, among the metabolites, alkaloid have been reported to have analgesic properties this shows that the plant part could be considered as a potential pain reducing agent. Moreover, tannins Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran and Mahadevan,1990). In addition to its use in leather processing industries, tannins have shown potential antiviral and antibacterial. (Lin *et al*., 2004; Akiyama *et al*, 2001; Funatogawa *et al*., 2004; Yangand Kun-Ying, 2000). But the metabolites such as terpenoids, cardiac-glycosides and anthroquinones were not found in the extract.

Conclusion

In conclusion, this study reveals the results of phytochemical and synthesis of *Citrus aurantifolia* leaf extract silver nanoparticles. The result of this study shows that *Citrus aurantifolia* leaf extract contain some phytochemicals such alkaloids, flavonoids, saponins and tannins respectively and it could be served as a potential agent of synthesizing silver nanoparticles.

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Evaluation of Heavy Metals and Antimicrobial Efficacy of Hand Sanitizers Sold in Makurdi, Benue State-Nigeria

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Abstract: *Hand sanitizer has become more popular than ever due to its effectiveness during the Corona Virus Disease 2019 (COVID-19) pandemic. Hand sanitizers kill germs on hands and other surfaces on contact, helping to slow the spread of transmissible diseases.This study evaluates heavy metals concentrations and the antimicrobial efficacy of four alcohol-based hand sanitizers used in Makurdi,Benue State. The assessment involved two locally-made hand sanitizers labeled as sample B and K, and two industrial-made hand sanitizers labeled as sample C and D. After digestion with concentrated nitric acid and perchloric acid, the concentrations of cadmium, nickel, mercury and lead were determined using Atomic Absorption Spectrophotometer (AAS).The concentration of cadmium and nickel were found in all the samples analyzed; Cd has0.0109±0.0001, 0.1235±0.0003, 0.1208±0.0002, 0.1136±0.0002 mg/L for sample B, K, C and D respectively while Ni has 0.011±0.000, 0.013±0.000, 0.070±0.000, 0.051±0.000 mg/L for sample B, K, C and D respectively. Mercury and lead were not found in all the samples. It was observed that the concentrations of cadmium and nickel were slightly above the permissible limit byWHO, NESREA and EPA.The results also revealed that the zone of inhibition(antimicrobial efficacies) of hand sanitizers, B, K, C and D on E.coli, S.aureus,P.aeruginosa and candida spp were (17.07±1.36, 12.20±1.3, 15.50±1.01 and 11.67±1.04 mm), (11.30±0.89, 11.10±0.17, 11.06±040 and 6.67±0.29 mm), (10.20±0.17, 11.30±0.30, 10.10±0.85 and 6.33±1.04 mm) and (9.97±0.35, 10.40±0.17, 9.97±0.65 and 6.17±0.58 mm) respectively. All samples had a bacteriostatic effect to all organisms but hand sanitizer B was generally observed to have higher effect on the entire test organism. Control was observed to be effective on all three bacterial strains while it was observed to be none effective on candida spp. The study shows that the hand sanitizers analyzed contained some heavy metals (Cd and Ni) which are toxic to human body. Therefore, manufacturers must observe proper care while producing hand sanitizers.*

Keywords: *Hand sanitizer; Heavy metals; Anti microbial efficacy; Toxicity; AAS*

Introduction

The importance of sanitation cannot be over emphasized. Physical contact between people and objects is a key vehicle for the spread of pathogens (Kampf et al., 1999; Liu et al. 2010).

Hand hygiene is well known as one of the most significant of activities essential for the reduction of transmission of infectious diseases (Aiello et al., 2008; Hassan et al., 2012). Majority of early reports focused primarily on the role of hand washing as an infection control measure (Alvarado et al., 2009; Zapka et al., 2017; Pittet et al., 2006). This changed by the early 2000s, when the Centers for Disease Control and Prevention (CDC) issued a guideline authorizing that alcohol-based hand rub (ABHR) be regularly used for disinfecting hands (Baldissera et al., 2006; World Health Organization 2009). These ABHRs which are the most commonly used hand sanitizers are often composed of alcohol, ethanol, isopropanol or propanol (Chang et al., 2012; Pires et al., 2017). They have a recommended concentration range from 60-95 %. In addition to being useful in the absence of water, other advantages of the use of the hand sanitizers include, high antimicrobial activity in a shorter time, and the lack of requirement for drying of the hand (which could serve as another source of contamination).

The outbreak of corona virus in Wuhan China led to an increased awareness of the role of hand sanitizers in infection control (Garner & Favero, 1986; Centers for Disease Control and Prevention, 2002). and an upsurge of various brands of hand sanitizers into the Nigerian market (Pickering et al., 2010). Most of these products have made numerous claims, notably their ability to eliminate 99.9 % of microorganisms. A number of these claims have not been verified (Reynolds et al., 2006).

Heavy metals are natural elements characterized by their high atomic mass and their high density. Although, typically occurring in lower concentration, they can be found all through the earth crust. Commonly, a density of at least 5.5 g cm^{-3} is used to define a heavy metal and differentiate it from other light metals. Some heavy metals like copper, selenium or zinc are essential trace elements with function indispensable for various biological processes also driving the entire human metabolism (Duffus, 2002). Zinc serves as a pivotal constituent of zinc finger enzymes (Duruibe et al., 2007). On the other hand many of them e.g mercury, cadmium, arsenic, chromium, thallium, lead and others, classically represent the “dark side of chemistry”; they exert toxic effects even at low concentration (Terfassa et al., 2014; Tamas et al., 2014; Florea & Busselberg , 2006).

Materials and Methods

Sample Collection and Preparation

Two industrially made and two locally made hand sanitizers were obtained in Makurdi, Benue State in August 2021. The samples were duly certified by StandardOrganization of Nigeria (SON) and/or National Agency for Food and Drugs Administration and Control (NAFDAC). They were labeled C and D for industrial made and B and K for local made respectively and store at the room temperature in the laboratory prior to analysis.

Sample Digestion

10 mL of each sample was measured in a measuring cylinder, 8 mL of concentrated nitric acid and 4 mL of perchloric acid were added to the conical flask containing the sample. The sample mixture was then placed on the hot plate and heated to boil. The flask was removed from the hot plate and cooled to room temperature. To the cooled sample, deionized water was addedand the content was filtered in a 25 mL volumetric flask with No. 42 whatman filter paper. Subsequently, the solution was made up to volume using deionized water. Similarly, reagent blank was prepared by taking a mixture of the reagents (concentrated nitric acid and perchloric acid) and treating it in the same manner as the sample.

Determination of Heavy Metals

Cadmium,nickel, mercury and lead were analysed after digestion with HNO_3 and $HClO_4$using Atomic Absorption Spectrophotometer (AAS) with appropriate hollow cathode lamps. The whole procedure was done in triplicate.

Microorganisms

The microorganisms used for the study were:
Bacteria: *Staphylococcus aureus*, *Peudomonasaeruginosa,* and *Escherichia coli*
Fungus: *Candida spp.*
These microorganisms were obtained from the Department of Biological Sciences, Benue State University, Makurdi.

Preparation of Media

Nutrient Agar

Nutrient agar powder (T.M Media, 98 % pure) was collected and prepared according to the manufacturer's instruction in Microbiology Laboratory, Department of Biological Sciences, Benue State University, Makurdi. The nutrient agar (11.00 g) was weighed using an analytical weighing balance and dissolved in 500 cm^3 of distilled water and heated till the solution boiled and the heat, turned off. After cooling down, the nutrient agar solution was autoclaved for

about 15 min. The nutrient agar solution was dispensed into a sterile petri dish and allowed to set.

Potato Dextrose Agar (PDA)

Potato Dextrose Agar (T.M Media, 98 % pure) was collected and prepared according to the manufacturer's instruction in Microbiology laboratory, Department of Biological Sciences, Benue State University. The nutrient agar (19.50 g) was weighed using an analytical weighing balance and dissolved in 500 cm^3 of distilled water and heated till the solution boiled and the heat, turned off. After cooling down, the PDA solution was autoclaved for about 15 min. The PDA solution was dispensed into a sterile petri dish and allowed to set.

Determination of Antimicrobial Activity Using Disk Diffusion Method

Antibacterial Testing

The disk diffusion method was performed using nutrient agar. The test organism was transferred onto the petri dishes containing the molten nutrient agar. The inoculums were spread around the plate using a sterile wire loop. This was to give a uniform distribution of the test organism in the agar and allowed to set. Multiple disks were separately impregnated with both industrial made hand sanitizer and local made hand sanitizer. After making sure the disks were completely infused with the industrial made hand sanitizer and local made hand sanitizer respectively, it was aseptically transferred onto the plates containing *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli*. The plates were incubated at 37 ˚C for 24 h. The diameter of the zone of inhibition was measured for both hand sanitizers.

Antifungal Testing

The disk diffusion method was performed using potato dextrose agar. The test organism was transferred onto the petri dishes containing the molten potato dextrose agar. The inoculums were spread around the plate using a sterile wire loop. This was to give a uniform distribution of the test organism in the agar and allowed to set. Multiple disks were separately impregnated with both industrial made hand sanitizer and local made hand sanitizer. After making sure the disks were completed infused with the industrial made hand sanitizer and local made hand sanitizer respectively, it was aseptically transferred onto the plates containing *Candida spp.* The plates were incubated at 37 ˚C for 24 h. The diameter of the zone of inhibition was measured for both hand sanitizers.

Results

The results of the analysis (heavy metal concentrations and antimicrobial efficacy) are as presented in Table 1 and 2 respectively.

Table 1: Heavy Metal Concentrationin Some Selected Hand Sanitizers Sold in Makurdi, Benue State, Nigeria

Sample	Cadmium (mg/L)	Nickel (mg/L)	Mercury (mg/L)	Lead(mg/L)
B	0.0109±0.0001	0.011±0.000	ND	ND
K	0.1235±0.0003	0.013±0.000	ND	ND
C	0.1208±0.0002	0.070±0.000	ND	ND
D	0.1136±0.0002	0.051±0.000	ND	ND
WHO	0.003	0.01	0.01	0.1
NESREA	0.01	0.05	0.01	-
EPA	0.005	0.02	0.002	-

- Values are Mean ± SD in triplicates ND=Not Detected

Table 2: Zone of Inhibition of hand sanitizers in some strains of bacteria and fungus

Hand sanitizers	Zones of inhibition (mm)				
	P. Aeruginosa	S. aureus	E. Coli	Candida Spp	FLSD (P<0.05)
B	15.50±1.01[b]	12.20±1.31[ab]	17.07±1.36[b]	11.67±1.04[ab]	0.945
K	11.06±040	11.10±0.17	11.30±0.89	6.67±0.29	1.000
D	9.97±0.65	10.40±0.17	9.97±0.35	6.17±0.58	4.233
C	10.10±0.85	11.30±0.30	10.20±0.17	6.33±1.04	3.767
CON.	5.55±0.39	5.10±0.17	5.40±0.35	0.00±0.00	NS

- Values are Mean ± SD in triplicate
- NS =Not Significant
- FLSD= Facial Least Significance Difference

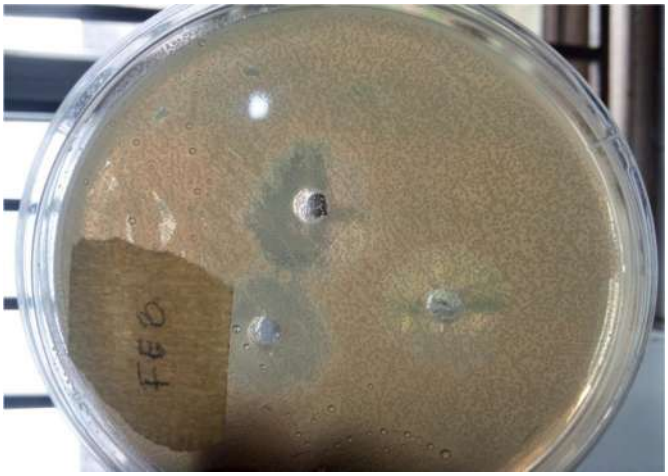

Figure 1: A sample of the sensitivity disc, incubated after 24 hour at 37 °C showing the zone of inhibition against bacteria.

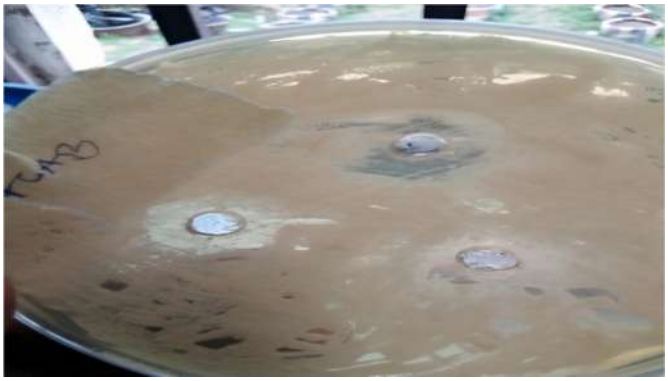

Figure 2: A sample of the sensitivity disc, incubated after 24 hour at 37 °Cshowing the zone of inhibition against fungi.

Discussion

The concentration of cadmium (Table 1) was 0.0109±0.0001 mg/L in sample B, 0.1235±0.0003 mg/L in sample K, 0.1208±0.0002 mg/L in sample C and 0.1136±0.0002 mg/L in sample D. It was observed that the concentration of cadmium in sample K was slightly higher compared to other samples. The concentrations of cadmium in all the hand sanitizers analyzed wereslightly above the permissible limit of WHO, NESREA and EPA as shown in the table1.

The concentration of nickel was 0.011±0.000 mg/L, 0.013±0.000 mg/L, 0.070±0.000 mg/L and 0.051±0.000 mg/L in sample B, K, C and D respectively as shown in Table 1. It was observed that the concentrations of nickel were generally below the permissible limit of NESREA (0.05

mg/L)but slightly higher than those of WHO (0.01 mg/L) and EPA (0.02 mg/L).Mercury and lead were not found in all the test samples exhibiting their safe levels.

From Table 2, the efficacy of different hand sanitizers used in Makurdi on some selected strains of bacteria and fungus was presented.It was observed that hand sanitizer B showed higher effect on E. coli (17.07±1.36mm) than the other test organisms. This effect on *E. coli* was significantly higher than the effect on *S. aureus*(12.20±1.31mm) (p=0.038); and *Candida spp*(11.67±1.04mm) (p=0.018) respectively. However, no significant different was observed between *E. coli* than *S. aureus* and *P*.*aeruginosa*(15.50±1.01mm) (p=0.425). The effect of hand sanitizer K was also observed to be slightly higher on *E. coli* than *S. aureus* and *P. aeruginosa* but with no significant differences between them ($p > 0.05$). On the other hand, a highly significant difference was observed when its effect on the three bacterial strains was compared to its effect on *Candida spp* ($p< 0.05$) with the effect observed to be higher on the bacterial strains.

The effect of the sanitizer D was also significantly higher on the bacterial strains compared to the *Candida spp*(6.17±0.58)(p=0.000). Its effect was however slightly higher on *S. aurues* (10.40±0.17 mm) compared to *E. coli* (9.97±0.35 mm) and *P. aeruginosa* (9.97±0.65 mm) with no significant difference between them ($p > 0.05$). In the same manner, sanitizer C showed a slightly higher effect on *S. aureus* (11.30±0.30mm) than *E. coli* (10.20±0.17mm) and *P. aeruginosa* (10.10±0.85mm) with no significant difference between them ($p > 0.05$). Its effect on the three bacterial strains was however significantly higher than on the *Candida spp* (6.33±1.04mm) (p= 0.000).

The control was observed to have effect only on the bacterial strains with no significant difference between them ($p > 0.05$). Sample B was generally observed to have higher effect on the entire test organism compared to all other samples used in the study (Table 2).This may be due to the type of alcohol formulations used, since alcohol serves as the main ingredient in bacteriostaticeffect (Aiello et al., 2008; Alvarado et al., 2009; Shoge et al., 2021). Ethanol and isopropyl alcohol formulations are popularly used worldwide. Methanol or ethylene glycols are quite poisoning, the metabolites of isopropyl alcohol are considerably less toxic, and treatment is largely supportive (Tamas et al., 2014).

Conclusion

All the tested samples of hand sanitizers showed a satisfactory bacterial reduction to *E. Coli*. The findings of this study indicate that all the hand sanitizer samples had a bacteriostatic effect to*Staphylococcus aureus*, *Peudomonasaeruginosa, Escherichia coli*and*Candida spp.*

The study also showed that some of the hand sanitizers, contain heavy metals which are toxic to the human body, therefore, manufacturers must carefully select gelling agents and other additives during production to safeguard human health.

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Evaluation of Heavy Metals and Aflatoxin Contents of Selected Biscuits Sold in Makurdi, Benue State-Nigeria

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Abstract: *Biscuits are baked, edible and common flour based food products. Metallic elements with a density that is greater than 5 g/cm^3 (Heavy metals) are not easily degradable or metabolized and are usually persistent and may be biologically accumulated in food items, trapped on the outer surface or may be added due to manufacturing or processing of food for consumption. Heavy metals can develop gradually in the body tissue and overtime could exceed tolerable limits which can cause intense toxicity leading to human disease, disorders, defects, illness, malfunctioning and malformation of organs and ultimately death. Aflatoxins are poisonous, carcinogenic by-products of the growth of the molds Aspergillus flavus and Aspergillus parasiticus, and are the most studied and widely known mycotoxins. In this research, five biscuits samples were purchased at Wurukum and Modern Market, Makurdi. The samples were digested and Heavy metal (Zn, Pb, Cu, Cd, Cr, Ni, Fe, Co and Hg) concentrations and aflatoxin content evaluated using Atomic Absorption Spectrophotometer (AAS) and Enzyme-linked immunosorbent Assay (ELISA) respectively. All the metals analysed were in their permissible limits except for Cr which was 0.117±0.0001 mg/Kg in sample B, 0.122±0.0001 mg/Kg in sample C, 0.209±0.0001 in sample D and 0.166±0.0001 mg/kg in sample E against its WHO permissible limit of ≤ 0.1 mg/kg. Hg was only detected in sample D and E but were within the WHO permissible limit of ≤1.0 mg/kg. The aflatoxin in all the samples was undetected indicating that the samples were free from aflatoxin. The entire result showed that the samples were free from heavy metals and aflatoxin contaminations. Efforts should however be made to control Cr concentration as the consumption of these products with time is liable to pose health problems arising from Cr accumulation.*

Keywords: *Biscuit; Heavy metals; Aflatoxin; Toxicity; AAS; ELISA*

Introduction

Biscuits are a form of relatively small, packaged, ready-to-eat fast foods, usually taken not as a regular meal, but as a stopgap. Snacks may be eaten to temporarily hold hunger or to satisfy a craving. Biscuits are often a form of ready-to-eat food, designed to be portable, quick, and satisfying (World Health Organization, 2015). In spite of the fear expressed over foods originating from the informal food production and marketing system, many people in the urban centres cannot help eating packaged, home-made snacks due to their busy schedule (Oyelola *et al*., 2013). Contamination can occur in food due to pick up of metals from equipment, processing or packaging materials. These contaminated food products with heavy metals contribute to human dietary intakes and the levels of these metals need to be regularly observed and controlled. While the health effects of microbial contamination may be noticed within days or weeks, the effects of chemical contamination may take a longer time to manifest. Examples of chemical contaminants are metals like Pb, Cd, Zn, Hg, Mg, Mn, Cu, Co. Though some of these metals (e.g. Zn, Cu, Co, Mn) are classified as essential elements, when present in the body above certain concentrations, they can become harmful causing various health conditions. Metals like Pb and Cd have no known biological functions and may exhibit toxicological problems even at low or trace concentrations (Marsh & Bugusu, 2007; Iwegbue, 2012; Elham-Elshewey *et al.,* 2015). Depending on the type of metal, the health effects of metal poisoning include gastrointestinal disorders, tremor, diarrhoea, paralysis, vomiting, convulsion, diabetes, cancer, anaemia among others (Adefris, 2011; Duruibe *et al.,* 2007). Heavy metals disrupt the physiological functions of the body by accumulating in vital organs and glands such as the heart, brain, kidney, bone, and liver (Ray & Ray, 2009). In order to check exposure to food contaminant toxicity, the Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly released a list of maximum levels for contaminants and toxins in foods (Codex Alimentarius Commission, 2011). Frequent assessment studies are therefore necessary to determine the safety of foods that are consumed regularly. Many studies have found metal contaminants above recommended safe levels in many foods that are regularly consumed in Nigeria and other places (Lanre-Iyanda & Adekunle, 2012; Elham-Elshewey *et al.,* 2015).

Materials and Methods

Sample Collection

Three packs with different batch numbers of the same brand of biscuits each for Sample A, B, C, D and E were bought from Wurukum and Modern markets, Makurdi, Benue State.

Sample Preparation

The biscuit packs were opened and the pieces removed. The pieces were then dried in an oven at 60 ℃ for duration of 30 min for complete crispiness. Each sample was ground to fine powder

using a pre-cleaned agate mortar and pestle. The three batches were mixed and homogenized to constitute a composite sample.

Sample Digestion for AAS Analysis

2 g each of the ground samples was weighed in crucible and then 2 mL of concentrated HCl and 6 mL of concentrated HNO_3 were added (ratio of 1:3) and heated at 140 ℃ and then cooled at room temperature. After cooling, 2 mL of concentrated H_2SO_4 was added and heated at 140 ℃ and then cooled at room temperature. Later, 10 mL of HNO_3 was added and then heated again until the solution became clear. The resulting solution was cooled at room temperature and was then diluted with 25 mL of deionized water and filtered through Whatman No. 42 filter paper and <0.45 1m Millipore filter paper. It was then transferred quantitatively into well labeled sample bottles for AAS analysis.

Sample Digestion for Aflatoxin Evaluation

50 g of prepared sample was taken in a 500 mL conical flask and 25 mL water, 25 g diatomaceous earth and 250 mL chloroform were then added to it. The flask was securely stoppered with masking tape and shaken on a risk action shaker for 30 min to extract the toxin. The mixture was filtered through fluted filter paper and the first 50 mL was collected and kept for aflatoxin determination.

Sample Analysis for Heavy Metal Concentration

The well labeled samples were then analyzed for some heavy metal concentrations at the Federal Ministry of Agriculture and Rural Development, Zaria office, Kaduna State using the Atomic Absorption Spectrophotometer (AAS) model PG990.

Sample Analysis for Aflatoxin

The prepared and well labeled samples were then analysed for aflatoxin at National Agency for Food and Drug Administration and Control (NAFDAC) Laboratory, Kaduna, Kaduna State using ELISA.

Determination of moisture content

The air oven method was used to determine the moisture contents of the biscuits. 3 g of the biscuit samples was weighed into different petri dishes, dried separately at 105 ℃ for 4 hr and cooled in a dessiccator. The samples were finally dried to a constant weight and the percentage moisture content was calculated as follows:

$$\% \text{ moisture content} = \frac{W2-W3}{W2-W1} \times 100$$

Where W_1= Weight of crucible

W_2=Weight of crucible + sample before drying

W_3=Weight of crucible + sample after drying

Results

The results for the analysis are as shown below:

Table 1: Concentrations of Heavy Metals in the Samples

Heavy Metals	Concentration (mg/kg) A	B	C	D	E	WHO
Zn	2.315±0.0046	2.507±0.0036	1.568±0.0007	1.425±0.0021	2.663±0.0027	≤ 99.4
Pb	1.215±0.0004	0.851±0.0004	1.191±0.0008	1.178±0.0005	2.915±0.0005	≤ 3.0
Cu	0.316±0.0004	0.334±0.0003	0.324±0.0002	0.328±0.0003	0.376±0.0002	≤ 73.3
Cd	0.182±0.0014	0.167±0.0009	0.180±0.0014	0.162±0.0016	0.220±0.019	≤ 0.2
Cr	0.154±0.0004	0.117±0.0001	0.122±0.0001	0.209± 0.0001	0.166±0.0001	≤ 0.1
Ni	0.395±0.0006	0.444±0.0002	0.422±0.0007	0.483±0.0018	0.524±0.0006	≤67.9
Fe	2.392±0.0012	1.896±0.0011	1.409±0.0002	1.477±0.0008	7.439±0.0074	≤42.5
Co	0.268±0.0008	0.219±0.0009	0.221±0.0007	0.272±0.0002	0.309±0.0006	≤ 2.0
Hg	ND	ND	ND	0.125±0.0001	0.117±0.0002	≤1.0

- ND=Not Detected

Table 2: Concentrations of Aflatoxin in the Samples

Parameter (µg/kg)	Samples A	B	C	D	E	FAO
Aflatoxin	ND	ND	ND	ND	ND	<0.05

- ND: Not detected

Table 3: Moisture Content of the Samples

Parameter (%)	Samples A	B	C	D	E	WHO
Moisture content	4.95±0.05	5.21±0.04	5.15±0.45	4.98±0.02	5.00±0.04	1-6 %

Discussion

The concentrations of all the heavy metals evaluated in sample A were within WHO acceptable range in food thus indicating no harm to consumers (Table 1). For the rest of the samples, only Cr out of the nine evaluated metals was higher than the permissible limit set by WHO. The concentration of Cr was 0.117±0.0001 mg/kg, 0.122±0.0001 mg/kg, 0.209± 0.0001 mg/kg and 0.166±0.0001 mg/kg in B, C, D and E respectively against the WHO permissible limit of ≤ 0.1 mg/kg.

In general, all the heavy metals detected were within their respective WHO standards except Cr. The reasons for the high levels of the metal in the samples could arise from unsafe storage conditions or at any point in the production chain. High levels of Cr indicates that, continuous intake of sample B, C, D and E can cause serious health problems such as bloody diarrhea, muscle weakness, kidney problems among others.

The aflatoxin in all the samples was not detected indicating that the raw materials were free from this mycotoxin. This also indicates that both the processing and the storage conditions were also appropriate. The moisture content in all the samples were very low and within the WHO standards thus confirming that microorganisms including aflatoxins cannot thrive.

Conclusion

The concentrations of heavy metals in the studied samples were generally low and within the permissible limits set by WHO. Chromium was however higher than the recommended concentrations in sample B, C, D and E. The assessment provides some preliminary evidence that these products contained unacceptable levels of Cr with potential serious consequences for children and adults who consume the products. Regulation on food should be strongly enforced so as to checkmate this ugly occurrence in order to protect human health.

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