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Highlights

Discovering Thoughts, Inventing Future

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Occurrence of *Escherichia Coli* and *Staphylococcus Aureus* in Sliced Pineapple and Water Melon Fruits Sold in Akpan Andem Market, Uyo, Akwa Ibom State, Nigeria

By Dr. Ikon, Grace M. & Jackson, Roseline Idorenyin

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Abstract- The aim of this study was to carry microbiological assessment of the prevalence of *Staphylococcus aureus* and *Escherichia coli* associated sliced ready-to-eat water melon and pineapple fruits. A total of 10 sliced pineapple and water melon fruits were purchased from different vendors at Akpan Andem Market, Uyo. The fruit samples were aseptically transported to the laboratory and processed using standard techniques. Enumeration of total heterotrophic bacteria and fungi, and isolation, characterization and identification of *Staphylococcus aureus* and *Escherichia coli* were all done using standard microbiological techniques. Antibiotic sensitivity test was carried out using the Kirby Bauer disk diffusion test. The results of the aerobic bacterial and fungal counts indicated that the highest bacterial count was 1.85 x 10^4 for water melon and 1.80 x 10^4 cfu/g for pineapple while those of the fungi were 1.3 and 1.4 x 10^3 cfu/g, respectively. For *E. coli*, the least and highest zones of inhibitions were 6.00±1.05 and 23.37±1.20 mm for isolates WS5 and PA3, respectively against CEP and CPX which were *Ciprofloxacin* and *Ceporex.* A total of 4 out of 10 isolates showed multi-drug resistance (resistance to at least 2 antibiotics) and these were isolates PA4, PA1, WS4, and WS5.

Keywords: water melon and pineapple fruits, bacteria, prevalence of staphylococcus aureus, escherichia coli, heterotrophic bacteria, and fungi.

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Keywords: water melon and pineapple fruits, bacteria, prevalence of staphylococcus aureus, escherichia coli, heterotrophic bacteria, and fungi.

I. Introduction

a) Background of study

In developing countries like Nigeria, millions of people on a daily basis consume ready-to-eat fruits and vegetables that are sold by vendors on the streets of major cities and towns (Mahale *et al.,* 2008; Okechukwu *et al.,* 2016). These fruits include oranges, pineapples, water melons, cucumber, and apples to mention but a few. The fruits are often desired and consumed fresh by the populace. Studies have shown that fruits are an excellent dietary source of nutrients, micronutrients and vitamins, water, and fibre which are needed for healthy and proper living (Mahale *et al.,* 2008; Okechukwu *et al.,* 2016). Fruits have been shown to help balance vitamin A and C deficiencies and also lower the risk of some diseases (Kalia and Gupta, 2006).

Due to increased knowledge of the health benefits of these fruits, their consumption is on the increase across the length and breadth of Nigeria. This is due to the accessibility, modern lifestyle of consumers, availability and affordability (Nielsen, 2006; Okechukwu *et al.,* 2016). Furthermore, due to the high costs of the whole fruits as seen with water melons and pineapple, some of these fruits are sold in small sliced pieces so as to make the fruits affordable to many Nigerians. These are usually sliced with stainless knives and then packaged in transparent nylons or polythene bags. Because they are preprocessed by the vendors, when purchased by consumers, they are consumed without rinsing or further processing (cutting and peeling) (Okechukwu *et al.,* 2016).

The processing of these fruits by vendors comes with potential risks of contamination and transmission of pathogens from the fruits and vendors to consumers. In an earlier study, it was shown that enteric pathogens are readily transmitted from ready-to-eat fruits (Mensah *et al.,* 1999). Bacteria and fungi are the common contaminants of fruits and they could be easily transferred from the vendors to the processed fruits through mishandling. The consumption of ready-to-eat fruits directly from street vendors or hawkers potentially increases the risk of food-borne diseases and poisoning caused by a wide variety of pathogens. It is also difficult to attest to the hygiene of these vendors or to the sanitary conditions at points of processing as well as those of the packaging materials. Frequently isolated bacteria from ready-to-eat fruits include *Escherichia coli*, *Salmonella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Shigella sp*, *Mucor sp,* viruses and even parasites have been associated with the transmission of enteric pathogens (Mensah *et al.,* 1999).

Amongst these microbial contaminants, the presence of *Staphylococcus aureus* and *Escherichia coli*

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stand out from a public health point of view. Their presence in foods and fruits indicates recent fecal contamination and overall poor hygiene in the handling and processing of such foods. *E. coli*, a normal flora of the small intestine of healthy humans and animals has been linked to several cases of food poisoning and diarrheal illnesses (Prescott *et al.,* 1999). One strain, *E. coli* O157: H7 has been linked to several food-borne illnesses around the world (Yang *et al.,* 2017).

On the other hand, *Staphylococcus aureus* is a normal flora of the skin of human (Prescott *et al.,* 1998). Thus, their presence in food indicates poor hygiene in the processing steps. Unlike *E. coli*, the genus *Staphylococcus* is very diverse and contains over fourty known species (Brooks *et al.,* 2010). Among these species, *Staphylococcus aureus* is the most commonly isolated. It is also frequently associated with food poisoning cases and the bacterium has outstanding ability to secrete enterotoxin that causes food poisoning (Hennekinne *et al.,* 2012; Brooks *et al.,* 2010).

Furthermore, these sliced fruits are sold by unlicensed vendors whose hygiene and general health status are questionable and unascertained by health authorities. In Nigeria as a whole, there is shortage of data on the incidence of *E. coli* and *Staphylococcus aureus* in street vended sliced fruits.

II. Literature Review

a) Classification of water melon

Watermelon (*Citrullus lanatus*) botanically considered as the fruit is belonging to the family Cucurbitaceae (Edwards *et al.,* 2003). Cucurbitaceae family ranks among the highest of plant families for number and percentage of species used as human food (see Figure 1). The common name of watermelon is Tarbooz (Hindi and Urdu), Tarbuj (Manipuri), Kaduvrindavana (Marathi), Eriputccha (Telegu), Kallangadiballi (Kannada), Tormuj (Bengali), Indrak (Gujarati). Watermelon is originated from Kalahari Desert of Africa but nowadays cultivated abundantly in tropical regions of the world. It has great economic importance with 29.6 million tones estimated production worldwide (Reetu and Tomar, 2017).

b) Physical Characteristics

It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is grown for its edible fruit, which is a special kind of berry botanically called a pepo. The watermelon fruit has deep green smooth thick exterior rind with grey or light green vertical stripes. Inside the fruit is red in colour with small black seeds embedded in the middle third of the flesh (Wehner *et al.,* 2001). Watermelons range in shape from round to oblong. Rind colours can be light to dark green, with or without stripes. Flesh colours can be dark red, red or yellow. India grows approximately 25 commercial varieties, a few of which have delightfully interesting names: New Hampshire Midget, Madhuri 64, Black Magic, Sugar Baby, Asahi Yamato, Arka Jyoti, Arka Manik, Improved Shipper, Durgapura Meetha and Durgapura Kesar to name a few. Watermelon varieties fall into three broad classes based on how the seed was developed: open-pollinated, F1-hybrid and triploid or seedless (Reetu and Tomar, 2017).

Figure 2.1: Water melon fruit

c) Cultivation of water melon

Watermelon is grown in sandy loam soil rich in organic matter with good drainage and pH range for 6.5-7.5 (Kumar *et al.,* 2013).In North Indian plains, watermelons are sown in February-March whereas in Northeastern and Western India best time of sowing is from November to January. In South and Central India, these can be grown almost round the year. Watermelon is a warm season crop grown mainly in sub-tropical and hot-arid regions. Temperature range of 24-27°C is considered as optimum for the growth of the vines. Cool nights and warm days are ideal for accumulation of sugars in the fruits. The seed germinates best when temperatures are higher than 20°C. High humidity at the time of vegetative growth renders the crop susceptible to various fungal diseases.

i. *Planting and transplanting*

Watermelon can be direct seeded in the field or grown as transplants seedling in pots and then transplanted to the field. Before sowing seeds are soaked in warm water for 12 hours. Normally 3.5 kg of seed of watermelon is required for planting one ha area. The hills are usually spaced 1 to 1.5 meters apart in the rows also 2 to 2.5 meters apart. A variation of spacing hills 4meters apart in the rows 1.5 meters apart are also commonly used in the tropics. Apply FYM 20 t/ha, P 55 kg and K 55 kg as basal and N 55 kg/ha 30 days after sowing.

ii. *Weeds and insect control*

Depending upon the season about 2-3 weeding operations is required. The first weeding should be done 20-25 days after sowing while subsequent weeding is done at an interval of one month. The biggest watermelon pest is the leaf-eating beetles, they damage the flowers. The other main problem with growing watermelons is mildew, a fungus that makes the leaves look as if they were coated with white powder.

iii. *Yield and yield components*

The total yield of watermelon is a function of marketable yield, fruit count, percent cull, percent early fruit and fruit size (Dia *et al.*, 2012a; Dia *et al.,* 2012b; Dia *et al.,* 2012c). Marketable yield ranges from a high of 80.44 to a low of 27.43 Mg/ha. Total fruit count ranges from 1.61 to 6.31 thousand fruits/ha. Similarly, percent cull fruit, percent early fruit and fruit size range from 23.42-20.55%, 49.9-17.4%, and 01.72-14.56 kg/fruit, respectively (Dia *et al.,* 2016). Among quality traits, lycopene and sugar range from 8.76 to 52.15 mg/kg and 8.47 to 14.02 mg/kg, respectively (Dia *et al.,* 2016). Variation in watermelon yield and quality is governed by fluctuation in the external environment (Dia *et al.,* 2016c).

iv. *Harvesting and storage*

The crop is ready for harvest in about 75-100 days after sowing. For local market, harvesting should be done at full maturity while for transporting to distant markets, it is done slightly earlier. Watermelons can be stored for 14 days at 15°C. Watermelons should not be stored with apples and bananas as the ethylene produced during storage from these fruits hastens softening and development of off flavour to watermelons (Retu and Tomar, 2017).

d) Nutritional value of fresh watermelon

Watermelon is one of the commonly consumed fruits in many countries. Watermelon contains more than 91% water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon rind contains more amounts of citrulline then flesh. Additionally, watermelon has a number of essential micronutrients and vitamins (Retu and Tomar, 2017). See more nutritional details in the table below.

e) Health Benefits of Watermelon

i. *Heart health*

Watermelon contains high levels of lycopene that is very effective in protecting cells from damage and lowers the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. Watermelon fruit is also a good source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Thus, it prevents stroke and coronary heart diseases (Le *et al.,* 2005).

ii. *Anti-inflammatory and antioxidant support*

Anti-inflammatory foods can help with overall immunity and general health. The lycopene in watermelon makes it an anti-inflammatory fruit. Lycopene is an inhibitor for various inflammatory processes and also works as an antioxidant to neutralize free radicals (Edwards et al., 2003). It also contains a good amount of vitamin-B6 (pyridoxine), vitamin C and manganese. Consumption of food rich in vitamin C helps the body develop resistance against infectious agents and scavenge harmful oxygen-free radicals. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase. Watermelon is an excellent source of Vitamin A, which is a powerful natural antioxidant. It is one of the essential vitamins for vision and immunity.

Table 2.1: Nutritive value per 100g of flesh

Components	Nutritive value	% Recommended daily allowance
Energy	30 Kcal	1.5%
Carbohydrates	7.6 g	6 %
Protein	0.6 g	1%
Total Fat	0.15 g	0.5%
Dietary Fiber	0.4 g	1%
Vitamins		
Niacin	0.178 mg	1%
Pantothenic Acid	0.221 mg	4.5%
Vitamin A	569 mg	19%
Vitamin C	8.1 mg	13.5%
Electrolytes		
Potassium	112 mg	2.5%
Iron	0.24 mg	3 %
Manganese	0.038 mg	1.5 %
Zinc	0.10 mg	1%
Phytonutrients		
Carotene-alpha	303 µg	-
Lycopene	4532 µg	-

Adapted from USDA National Nutrient Database cited by Reetu and Tomar (2017)

iii. *Hydration and digestion*

Watermelons are the perfect example of a food that can help you stay hydrated. Watermelons are nature gift to beat summer thirst due to rich in electrolytes and water content. The watermelon contains fibre, which encourages a healthy digestive tract and helps keep you regular (Retu and Tomar, 2017).

iv. *Skin and hair benefits*

Vitamin A helps keep skin and hair moisturized and it also encourages healthy growth of new collagen and elastin cells. Vitamin C is also beneficial in this regard, as it promotes healthy collagen growth.

v. *Cancer prevention*

Like other fruits and vegetables, watermelons may be helpful in reducing the risk of cancer through their antioxidant properties. According to the National Cancer Institute India, Lycopene help in reducing prostate cancer cell proliferation. Consumption of natural fruits rich in vitamin-A is known to protect from lung and oral cavity cancers (Le *et al.,* 2005).

vi. *Edible seeds and rind*

Most people throw away the watermelon rind and seeds. Rind not only contains plenty of health-promoting and blood-building chlorophyll, but the rind actually contains important amino acid citrulline than the flesh. Citrulline is a non- protein amino acid and was first identified from watermelon. Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles (Rimando *et al.,* 2005). Citrulline improves circulation by reducing muscle soreness and heart rate. Many people prefer seedless watermelon varieties, but black watermelon seeds are quite healthy and edible. They contain iron, zinc, protein, and fibre (Kumar *et al.,* 2013).

f) Watermelon seed antioxidants

Antioxidants can be defined as substances whose presence in relatively low concentrations but significantly inhibits the role of oxidation of the targets (Rakesh *et al.,* 2010). Antioxidants are considered important nutriceuticals with many health benefits (Sharma and Bhat, 2009). Plants are known to be rich in biologically active substances such as the flavonoids, phenolic acids, anthocyanins, ethereal oils, and tannins, many of which exhibit antioxidant activity. Some of the antioxidants found in plants, such as tocopherols (vitamin E), ascorbic acid (vitamin C), and carotenoids are substances of major significance in human physiology. Most of these antioxidants are phenolics. Based on carbon structure, phenolics can either be classified as flavonoid compounds (flavones, isoflavones, flavanones, flavonols, and anthocyanidins) or non- flavonoid compounds (benzoic acid, stilbenes, and hydroxycinmamic acids). Watermelon varieties are said to contain high amounts of antioxidants, including citruline and lycopene. Watermelon seeds contain an antioxidant known as cucurbocitrin, which is extracted and used in lowering blood pressure and improvement of kidney function (Oseni and Okoye, 2013).

g) Pineapple

Pineapple [*Ananas comosus* (L.) Merr. Family: Bromeliaceae] is one of the most important commercial fruit crops in the world. It is known as the queen of fruits due to its excellent flavour and taste. Pineapple is the third most important tropical fruit in the world after Banana and Citrus .Pineapples are consumed or served fresh, cooked, juiced and can be preserved. This fruit is highly perishable and seasonal (Baruwa, 2013; Bartholomew *et al.,* 2003). Thailand, Philippines, Brazil

and China are the main pineapple producers in the world supplying nearly 50 % of the total output. Other important producers include India, Nigeria, Kenya, Indonesia, Mexico, Costa Rica and these countries provide most of the remaining fruit (FAO, 2004; FAO, 2005).

Figure 2.2: Pineapple fruit

h) Aculty of natural and applied sciences: Nutritional value

Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fibre, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. Pineapple is a common fruit in Bangladesh and it has minimal fat and sodium. It contains 10-25 mg of vitamin. Pineapple composition has been investigated mainly in the edible portion. Pineapple contains 81.2 to 86.2% moisture, and 13-19% total solids, of which sucrose, glucose and fructose are the main components. Carbohydrates represent up to 85% of total solids whereas fibre makes up for 2-3%. Of the organic acids, citric acid is the most abundant in it. The pulp has very low ash content, nitrogenous compounds and lipids (0.1%) (Sabahelkhier *et al.,* 2010; Debnath *et al.,* 2012).

i) Uses as Food

Pineapple fruits exhibit high moisture, high sugars, soluble solid content ascorbic acid and low crude fibre. Thus pineapple can be used as supplementary nutritional fruit for good personal health. The pineapple fruits are normally consumed fresh or as fresh pineapple juice. Field ripe fruits are best for eating fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs - dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. In Panama, very small pineapples are cut from the plant with a few inches of stem to serve as a handle. The flesh of larger fruits is cut up in various ways and eaten fresh, as dessert, in salads, compotes and otherwise, or cooked in pies, cakes, puddings, or as a garnish on ham, or made into sauces or preserves. Malayans utilize the pineapple in curries and various meat dishes. In the Philippines, the fermented pulp is made into a popular sweetmeat. The pineapple does not lend itself well to freezing, as it tends to develop off flavours. Canned pineapple is consumed throughout the world. In Africa, young, tender shoots are eaten in salads. The terminal bud or "cabbage" and the inflorescences are eaten raw or cooked. Young shoots, called "*hijos de pina*" are sold on vegetable markets in Guatemala (Debnath *et al.,* 2012; Kader *et al.,* 2010).

j) Medicinal values of pineapple

Pineapple can be used as supplementary nutritional fruit for good personal health. Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. A powerful antioxidant, vitamin C supports the formation of collagen in bones, blood vessels, cartilage and muscle, as well as the absorption of iron. Vitamin C also retards the development of urinary tract infections during pregnancy

and reduces the risk of certain cancers, including colon, esophagus and stomach. Malic acid makes up 13 percent of pineapple juice's acidic content. Malic acid is also beneficial for health. It boosts immunity; promotes smooth, firm skin; helps maintain oral health; and reduces the risk of toxic metal poisoning. Pineapple is also a good source of vitamin B1, vitamin B6, copper and dietary fibre. Pineapple is a digestive aid and a natural anti-inflammatory fruit. Fresh pineapples are rich in bromelain used for tenderizing meat. Pineapple contains a proteolytic enzyme bromelain, which digests food by breaking down protein (Rahmatullah *et al.,* 2009).

Pineapple creates low blood pressure, cure inflammation disease, used for weight loss, control the death rate and prevent diabetes & radical damage. It cures the damaged teeth and makes them strong and healthy. Also help to cure sinusitis and throat problem. Cure different diseases like asthma, obesity, swelling in the body, problems of digestion and heart problem. Pineapples are rich source of manganese which creates strong bones and muscular body. Atherosclerosis and immune disease can be also cured due to high antioxidant content of pineapple. It prevent cancer, heart attack, nausea and gives the long natural hairs. It is use to solve acne, wrinkles, age problem and create strong nails, soft lips and thick hair (Rahmatullah *et al.,* 2009; Debnath *et al.,* 2012).

k) Penetration of micro organisms into fruits

Fruits have an epidermal layer of cells which provides a barrier for penetration of microorganisms. The internal tissues are nutrient rich and many, especially vegetables, have a PH near neutrality. Their structure is comprised mainly of the polysaccharide's cellulose, hemicelluloses, and pectin. The principal storage polymer is starch; microorganisms exploit the host using extracellular lytic enzymes that degrades these polymers. Microorganisms can also enter the fruits during fruits development, either through the calyx cuticle. Fruits possess an outer protective epidermis, typically covered by a natural waxy cuticle layer containing. In addition microorganisms can also penetrate fruits during processing via a number of ways. They can enter via the vendors poor hygiene, water used in washing, packaging materials, and other materials used in processing the fruits such as containers, boards, and even cutleries. Furthermore, the use of dirty utensil, as well as the open display of food encourages sporadic visits by flies, cockroaches, rodents and dusts (Agbo *et al.,* 2017).

l) Staphylococcus food poisoning

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases in the world following the ingestion of staphylococcal enterotoxins (SEs) that are produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly Staphylococcus aureus and very occasionally by other staphylococci species such as *Staphylococcus intermedius* When outbreaks occurred during large social events, chaotic situations resulted requiring the rapid implementation of medical care for a high number of cases (Bonnetain *et al.,* 2003; Do Carmo *et al.,* 2004).

The first description of food-borne disease involving staphylococci was investigated in Michigan (USA) in 1884 by Vaughan and Sternberg. This food poisoning event was because of consumption of a cheese contaminated by staphylococci. The authors stated: 'It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above-mentioned Micrococcus or some other microorganisms which had preceded it, and had perhaps been killed by its own poisonous products. Ten years later, Denys (1894) concluded that the illness of a family who had consumed meat from a cow that had died of vitullary fever was owing to the presence of pyogenic staphylococci.

Table 2.2: Incidence of *Staphylococcus aureus* food poisoning

Year	Location	Incriminated food	Number of cases
1968	School children, Texas	Salad	1300
1971	UK army	Sausages rools, sandwiches ha	100
1975	Flight from Japan to Denmark	Ham	197
1976	Rio to NYC	Chocolate eclairs	80
1980	Canada	Cheese curd	62
1982	North Carolina and Pennsylvania	Ham and cheese sandwich; stuffed chicken	121
1983	Caribbean cruise ship	Dessert cream pastry	215
1984	Scotland	Sheep's milk cheese	27
1985	France, UK, Italy, Luxembourg	Dried lasagna	50
1985	School children, Kentucky	2% chocolate milk	> 1000
1986	Country Club, New Mexico Turkey,	Poultry, gravy	67
1969	Various US states	Canned mushrooms	102
1990	Thailand	Eclairs	485
1992	Elementary school, Texas	Chicken salad	1364
1997	Retirement party, Florida	Precooked ham	18
1998	Minas Gerais, Brazil	Chicken, roasted beef, rice and beans	4000

2000	Osaka	Low-fat milk	420
2006	Ile de France area, France	Coco nut pearls (Chinese dessert)	17
2007	Scouts' camp	Belgium Hamburger	15
2007	Elementary school, Austria	Milk, cacao milk, vanilla milk	166
2008	French district	Pasta salad	100
2009	Nagoya university festival,	Japan Crepes	75

Source: Hennekkinne et al (2011)

m) Escherichia coli

Escherichia coli, one of the 30 members of the bacterial family of *Enterobacteriaceae*, is a coliform bacterium and is one of the 6 types of *Escherichia* species (*E. adecaroxylate, E. blattae, E. fergusonii, E. hermannii* and *E. vulneris*). It is a gram-negative, non-spore-forming, facultative, anaerobic, rod shaped, mesophilic bacterium that grows in 7–45°C. The group of coliform bacteria consists of *Citrobacter, Enterobacter, Klebsiella* and *Escherichia*. While there are bacteria of fecal origin among coliform bacteria, there are also bacteria of plant origin such as *Enterobacter aerogenes, Citrobacter freundii*, and *Klebsiella pneumoniae*. Presence of coliform group in food is indicative of fecal contamination, poor hygienic conditions or existence of enteric pathogens. For instance, the presence of coliform bacteria in raw milk is an indication of poor hygiene in milking or storage conditions.

The presence of coliform bacteria in raw or frozen fruits and vegetables is not important as *Enterobacter, Citrobacter* and *Klebsiella* are naturally present in the microbiota of plants. However, *E. coli* presence in fruits and vegetables is very important in terms of inadequate hygiene. *E. coli* is an important pathogen as it is an indicator of fecal contamination in foods and drinking water. Due to this characteristic, it is considered as an indicator bacterium in food safety and hygiene. Being the prominent bacterium in the facultative anaerobic microbiota of the intestines, *E. coli* is widespread in stool and the environment. Some of its pathogenic strains both cause intoxication by creating toxins and cause gastroenteritis, pathologic kidney and brain damage by causing infection-type food poisoning through cellular increase. Some enterotoxin producing *E. coli* strains are divided into two groups as heat-stable and heat-labile. The heat-stable toxin is known as stable toxin (ST) and the heat-labile toxin is known as labile toxin (LT).

n) Risk groups for Escherichia coli

E. coli is a type of bacteria that normally live in the intestines of people and animals. However, some types of *E. coli*, particularly *E. coli* O157:H7 can cause intestinal infection. *E. coli* O157:H7 and other strains that cause intestinal sickness are called Shiga toxin–producing *E. coli* (STEC) after the toxin that they produce. People with weakened immune systems, pregnant women, young children, and older adults are at increased risk for developing these complications. Most intestinal infections are caused by contaminated food or water. Proper food preparation and good hygiene can greatly decrease your chances of developing an intestinal infection. Most cases of intestinal *E. coli* infection can be treated at home. Symptoms generally resolve within a few days to a week (Prescott *et al.,* 1999).

o) Strains of E. coli

There are six strain of *E. coli* implicated in diarrhea illness in humans. These are briefly explained below.

i. *Enterotoxigenic E. coli (ETEC)*

People living in developing countries have often been reported to have this pathotype in their feces and shown to have developed immunity against this microorganism. Being a cause of mortality in children under 5, the most frequently observed microorganism in childhood diarrhea is ETEC and it is also responsible for 30–60% of travelers' diarrhea. Infection is characterized by watery diarrhea and, depending on the person; its course may range from a normal course to cholera-like defecation with the addition of symptoms such as vomiting and high fever (Prescott *et al.,* 1999; Ekici and Dumen, 2019). Among these potential pathogens, the most common cause of diarrhea in children under five is the ETEC (heat-stable – ST and/or heat-labile – LT type toxin) producing *E. coli* strains. Through the production of fimbrial or non-fimbrial adhesins, ETEC strains cause hypersecretion of fluids by producing enterotoxins that disrupts fluid and electrolyte homeostasis in the epithelial cells of small intestines, leading to watery diarrhea. Without rehydration, moderate or severe diarrhea could lead to dehydration and acute mortality (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

ii. *Enteropathogenic E. coli (EPEC)*

It is known to be the oldest *E. coli* serotype causing diarrhea and its most important characteristic is adherence. In EPEC infections, vomiting and low body temperatures are observed in addition to watery diarrhea. It is known to cause diarrhea in infants and outbreaks can occur in neonatal care units. Humans, pigs and bovines may be infected with this microorganism. EPEC is transmitted from person to person, however; rarely, it is also known to spread through contaminated food and water. The ability to produce attaching and effacing (A/E) lesions is a distinctive phenotype for EPEC. Bacteria cause extensive deterioration on microvilli by strongly adhering

to the host cell membrane. As a distinctive factor, all EPEC strains lack the Shiga toxin (*stx*) producing genes. Among single-pathogen infections, EPEC has the second highest severity score after rotavirus, followed by ETEC (Prescott *et al*., 1999; Ekici and Dumen, 2019).

iii. *Enteroaggregative E. coli (EAEC)*

This pathotype is a food-borne enteropathogen observed in acute and persistent diarrhea cases in children, patients with suppressed immune systems in developing countries and people traveling to endemic regions. Growth disorders and cognitive disorders in children living in developing countries, stem from EAEC infections. In the pathogenesis of EAEC, the first step is the strong adherence to the intestinal mucosa. The second step is leading to the development of enterotoxins and cytotoxins and the third step is known to be characterized with the ability to induce mucosal inflammation (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

iv. *Diffusely-adherent E. coli (DAEC)*

Hep-2 or HeLa cell cultures are called DAEC due to their diffuse adherence characteristics. DAEC serotypes are known to cause chronic diarrhea in children between the ages of 1 and 5. They cause degradation in the intestinal epithelium by binding to proteins that accelerate degradation. Mild diarrhea void of fecal leukocytes is the indication of infection. In France, DAEC strains were found out to be widespread in diarrhea cases observed in inpatients from a hospital with no other enteropathogen. This situation indicates that DAEC strains may be an important diarrheagenic pathogen in developed countries. Recent studies show that some DAEC strains contain virulence factors present in uropathogenic *E. coli* (UPEC) strains (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

v. *Enteroinvasive E. coli (EIEC)*

EIEC strains causing inflammatory damage in intestinal mucosa and submucosa are very similar to those produced by *Shigella*. These microorganisms have the same spreading and reproducing abilities inside epithelial cells. However, clinically, EIEC-related watery diarrhea is much more commonly observed than dysentery caused by *Shigella*. O antigens of EIEC can cross-react with O antigens of *Shigella*. The disease starts with severe abdominal cramping, weakness, watery stool, difficulty urinating and fever. It could rarely aggravate and turn into watery stool containing blood or mucus. The fecal leukocytes observed in shigellosis may also be observed in the mucus smear of a person infected with EIEC. EIEC infections are endemic to less developed countries and are reported to be rarely observed infection in developed countries. The incubation period is observed as 10–18 hours. There is evidence showing that EIEC is transmitted through contaminated foods. Just like in shigellosis, cases of diarrhea with entero-invasive strains can be treated by using antimicrobials effective against *Shigella* isolates (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

vi. *Enterohemorrhagic E. coli (EHEC)*

EHEC are also named Shiga toxin producing *E. coli* (STEC) and also verotoxin producing *E. coli* (VTEC). All strains of EHEC produce Shiga toxins that destroy Vero cells similarly to Shiga toxins produced by Shigella. *E. coli* O157: H7, first defined after the outbreak associated with the consumption of rare cooked minced meat in 1982, is the primary cause of EHEC infection in industrialized countries including the USA, Canada and England. O26, O103, O111 and O145 can be listed among the other EHEC serogroups responsible for food-borne diseases. Even though the O157 strains are the ones that draw the most attention, the strains of other EHEC serogroups, especially O111, are gradually getting reported more and more around the world. Based on the severity of the disease, EHEC is regarded as the most serious *E. coli* strain among foodborne pathogens. *E. coli* O157:H7, differ from the other *E. coli* serotypes because of some of its characteristics, which are: not being able to grow in or above 42°C, not being able to ferment sorbitol, not having β-glucuronidase enzymes and producing enterohemolysins (Prescott *et al.,* 1998; Ekici and Dumen, 2019).

III. Materials and Methods

a) Materials

The materials used in this study were Binocular microscope, Autoclave, Hot air oven, Incubator, Weighing balance (electric), Bunsen Burner, Refrigerator, Wire loop, Hand gloves, Spatulas, Qubec colony counting chamber, Conical flasks, Petri dishes (disposable), Measuring cylinder, Slides, Cover slips, Durhams tubes, Beaker, test tubes, test tube racks, aluminum foil paper, cotton wool, forcep, masking tapes, and permanent marker. Other include consumables such Eosin Methylene Blue Agar, Nutrient Agar, Saboudraud Dextrose Agar, Mannitol Salt Agar, Muller Hinton Agar, Peptone water, Distilled water, Ethanol (70%), peptone water, nutrient broth, Gram staining reagents, Citrate Agar, Kovac reagent, Oxidase test strip, 3% hydrogen peroxide and MR-VP reagents.

b) Collection of fruit samples

Pineapple and watermelon fruits used in this study were purchased fresh from Akpan Andem Market in Uyo Metropolis, Akwa Ibom State, Nigeria. Five samples each of both fruits were collected from five different fruit vendors. The fruits were purchased as sliced by vendors and packaged in transparent polythene bags. The purchased fruits were then further wrapped with separate aluminum foil papers, placed in sterile polythene bags, and transported within one hour of purchase to the microbiology laboratory for microbiological analyses.

c) *Processing of samples*

This was carried out using a method previously described by Agbo *et al.* (2016) and Abubakari *et al.* (2015) but with a little modification. Each of the samples was placed in a separate sterile beaker and then gently rinsing them with sterile distilled water (50ml). After rinsing the fruits, the edible parts were sliced gently. For each of the fruits, the sliced outer most parts of the fruits together with the water that was used in rinsing them were then homogenized using a sterile electric blender. Following blending, 1ml of the homogenized samples for each of the fruit was then subjected to a ten-fold serial dilution.

d) *Enumeration of total aerobic bacteria and fungi counts*

Following serial dilutions, 10^{-3} dilutions were used for enumeration of total aerobic bacteria and 10^{-2} for fungi. This was done using the pour plate method previously described (Prescott *et al.,* 1999). From each of the selected serial dilution, 1ml was taken and poured into a sterile petri-dish. Then freshly prepared nutrient agar and Saboudraud dextrose agar which were first allowed to reach 45°C were then poured into the plates and allowed to solidify. The plates were then incubated inverted at room temperature for 24 hours and 48 hours, respectively for nutrient agar and Saboudraud dextrose agar. After incubation, the colonies which developed were counted and expressed as colony forming units per ml (cfu/g).

e) *Isolation of Staphylococcus aureus and E. coli*

The *S. aureus* isolates were isolated by pour plating the 10^{-3} dilutions onto freshly prepared mannitol salt agar plates and incubating at room temperature inverted for 24 hours. The discrete colonies (total of 11) were selected and sub-cultured twice in order to purify them. They were stored in freshly prepared nutrient agar slants for biochemical characterization and sensitivity analysis. For *E. coli*, the 10^{-3} dilution were plated onto freshly prepared Eosin Methylene Blue agar plates and incubated overnight at room temperature. Similarly, the selected discrete colonies were sub- cultured twice in order to purify them. After pure colonies were obtained, they were stored in freshly prepared nutrient agar slants for biochemical characterization and sensitivity analysis.

f) *Gram staining*

A bacterial smear was made on a slide, the slides was placed on a staining tray and the smear was covered and allowed to stain for 60seconds, the slide was tilted and gently rinsed with distilled water until the stain was removed. The smear was covered with Gram's Iodine and allowed for 60 seconds, the slide was gently rinsed with distilled water, the slide was tilted and 2 or 3 drops of ethanol run over the slide. It was rinsed with distilled water, the smear was covered with Safranin and stained for 60 seconds and rinsed with distilled water. The slides were allowed to dry and observed using oil immersion. Those that were Gram positive appeared as purple while those that were Gram negative showed red\pink colour.

g) *Biochemical test*

i. *Catalase test*

A small amount of the culture was picked from the agar slopes, using a clean sterile platinum wire loop. This was inserted in drops of 10% H_20_2 on a clean microscopic slide. Production of gas bubbles indicated a positive reaction.

ii. *Oxidase test*

A fresh and sterile filter paper was soaked in 1% oxidase reagent solution, a few colony of the test organism was rubbed on the filter paper, oxidase reagent was poured over the test organism in a culture, positive results gave a deep purple colour after 10 seconds and no colour change if the organism cannot produce oxidase.

iii. *Methylred*

The test organism was inoculated in a glucose phosphate broth and was incubate at 37c for 3 days, then five drops of 0.04% of methyl red were added and was mixed thoroughly. A red colour indicated a positive result while an orange colour indicated a negative result.

iv. *Voges Proskauer test*

A colony of bacteria isolate was transferred in a test tube containing MR-VP medium/broth using a sterile hours. Following incubation, 2 drops of ¤-naphthol was added and 1 drop of potassium hydroxide (KOH) was added into the medium. The medium was shaken at intervals to ensure maximum reaction for 3-5 minutes. Thereafter the medium was observed for colour change from pink to mahogany red within 15-20 minutes which indicates a positive test.

v. *Citrate utilization*

Simmon's citrate agar was dispensed into 2 tubes and allowed to solidify in that position, the tubes were inoculated by stabbing and incubated at 35-37°C for 24hours with a loosely fitted cap. The presence of growth and blue coloration was for a positive test, no colour change indicated inability to utilize citrate.

vi. *Indole test*

Bacterial colonies was transferred into test tube containing MIO medium broth motility indole ornithine medium using a sterile inoculating loop the tube was incubated at 37°cfor 48 hours. Thereafter, 3 to 5 drops of Kovac's reagent was added and shaken to mix properly. On observation the appearance of red ring on the surface of the tube was taken as positive test while orange colour indicated negative.

vii. *Urease test*

Bacterial colonies were inoculated into urea medium. This was incubated at 37°C for 48 hours.

Urease production was shown by a change of colour in the medium from yellow to pink.

viii. *Motility test*

Motility ornithine indole (MOI) medium was prepared. The medium was stabbed, inoculated and incubated with the surface corked. The medium was viewed for bacterial growth at both the edge of the medium as well as the stab line.

h) Antibiotics sensitivity test

Antimicrobial sensitivity test was carried out on the various *E. coli* and *S. aureus* isolates using specific Gram negative and positive antibiotics discs. The antibiotics used for E. coli were CPX, SXT, S, PN, CEP, OFX, NA, PEF, CN, and AU which represent Ciprofloxacin (CPX), sulfamethoxazole+trimethoprim (SXT), Streptomycin (S), Perfloxacilin (PN), Ceporex (CEP), Ofloxacin (OFX), Nalidixic acid (NA), Reflacine (PEF), Gentamycin (CN), and amoxicillin clavulanic acid (AU), respectively. While the antibiotics for Gram negative ERY – Erythromycin, AMX – Amoxil, CHL – Chloramphenicol, LEV – Levofloxacin, CXP – Ciprofloxacin, STR – Streptomycin, APX – Ampliclox, NFX – Norfloxacin, RIF – Rifampicin, GEN – Gentamycin. The test was done as previously described by Kirby Bauer disk diffusion test (CLSI, 2006). Briefly, each of the purified isolates were first inoculated onto freshly prepared peptone water and incubated overnight. After incubation, the turbidity was adjusted to Mac Farland standard before 0.1 ml of the inoculum were picked and streaked onto freshly prepared Muller Hinton Agar and in duplicates for each isolate. The plates were then incubated for 24 hours and the zones of inhibition measured using a meter rule in millimetre.

i) Statistical analysis

Replicate data generated in this study for microbial counts and antimicrobial sensitivity tests were managed and analyzed using Microsoft Excel 2010. The data were analyzed using analysis of variance (ANOVA) with level of significance set at 95%. The data were then presented as mean ± standard deviation and regarded as significant with p-values less than 0.05.

IV. Results

a) Total aerobic bacteria and fungi counts

The results of study are presented in the Tables 4.1 to 4.8. Table 4.1 and 4.2 show the total aerobic bacteria for the various water melon and pineapple samples used in the study, respectively. From the results (Table 4.1), the that the highest count and least counts were 1.60 and 1.31 x 10^5 cfu/g. Table 4.2 shows the mean aerobic counts for the pineapple samples used in this study. The lowest count was 1.23 x 10^5 from pineapple sample 3 while the highest count was 1.56 x 10^5 cfu/g. Tables 4.3 and 4.4 show the total aerobic fungi counts in the water melon and pineapple samples used in this study. Comparatively, the counts for the aerobic fungi were lower than those of bacteria. From the results in Table 4.3, the water melon sample 5 (WM5) had the highest count of 1.3 x 10^3 CFU/g while WM1 had the least count of 5 x 10^2 CFU/g. For the pineapple samples, the total aerobic fungi counts were 6, 8, 11, 13 and 14 x 10^2 CFU/g for samples 1, 2, 3, 4 and 5, respectively. As can be seen, sample s5 and 1 had the highest and least counts of 6 and 14 x 10^2 CFU/g (Table 4.4).

Table 4.1: Total aerobic bacteria counts in WM Samples (CFU/g)

Samples	10^5
WM1	1.30±0.03
WM2	1.60±0.11
WM3	1.53±0.08
WM4	1.31±0.07
WM5	1.51±0.04

Key: WM = Water melon, CFU = colony forming units

Table 4.2: Total aerobic bacteria counts in PA samples (cfu/g)

Samples	10^5
PA1	1.33±0.01
PA 2	1.56±0.10
PA 3	1.23±0.03
PA 4	1.36±0.11
PA 5	1.50±0.12

Key: PA = Pineapple, CFU = colony forming units

Table 4.3: Total aerobic fungi counts in WM samples (cfu/g)

Samples	10^2
WM1	5
WM2	8
WM3	10
WM4	11
WM5	13

Key: WM = Water melon, CFU = colony forming units

Table 4.4: Total aerobic fungi counts in WM samples (cfu/g)

Samples	10^2
PA1	6
PA 2	8
PA 3	11
PA 4	13
PA 5	14

Key: PA = Pineapple, CFU = colony forming units

b) Biochemical and morphological characterisation of the isolates

Although selective media were used to isolate the *Escherichia coli* and *Staphylococcus aureus*, routine morphological and biochemical characterization were carried out and the results are presented in tables 4.5 and 4.6. The results in Table 4.5 indicates that the all the

10 isolates selected for characterization were gram negative, rod shaped isolates, positive reactions for catalase, indole, and gas production. Furthermore, the isolates in Table 4.5 were negative for Oxidase, methyl red, voges proskauer, citrate, urease and H_2S. All the results provided a tentative identification for *E. coli* in addition to the sheen green metallic sheen colour that was obtained on Eosin Methylene Blue Agar.

Table 4.6 the morphological and biochemical testes for the *Staphylococcus aureus* isolates. They were all Gram positive and cocci in shape. Furthermore, they tested positive to catalase, methyl red, voges proskauer, citrate and urease. However, they isolates were negative for motility, oxidase, indole, H_2S and gas production tests. These results provided tentative identification for *Staphylococcus aureus.*

Table 4.5: Biochemical characterization of the *E. coli* isolates

Isolates	GR	Shape	Motility	Catalase	Oxidase	Methyl Red	Voges Proskauer	Indole	Citrate	Urease	H_2S/Gas
Isolate 1	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 2	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 3	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 4	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 5	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 6	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 7	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 8	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 9	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 10	-	Rods	+	+	-	+	-	+	-	-	-/+

Keys: + = Positive, - = Negative.

Table 4.6: Biochemical characterization of the *Staphylococcus aureus*

Isolates	GR	Shape	Motility	Catalase	Oxidase	Methyl Red	Voges Proskauer	Indole	Citrate	Urease	H_2S/Gas	Coagulase
Isolate 1	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 2	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 3	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 4	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 5	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 6	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 7	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 8	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 9	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 10	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 11	+	Cocci	-	+	-	+	+	-	+	+	-/-	+

Keys: + = Positive, - = Negative.

The *E. coli* and *Staphylococcus aureus* isolates were subjected to sensitivity testing and the results are presented in Tables 7 and 8, respectively. A total of 4 isolates showed multi-antibiotics resistance (resistance to atleast 2 antibiotics) and these were isolates PA4, PA1, WS4, and WS5, respectively. Apart from PA5 that was resistant to only one antibiotic Gentamycin (CN), the rest of the isolates were sensitive to the test antibiotics. The highest and the least zones of inhibitions were 6.00±1.05 and 23.37±1.20 mm for isolates WS5 and PA3, respectively for CEP and CPX which were *Ciprofloxacin* and *Ceporex.*

On the other hand, the highest and least zones of inhibitions recorded by the 11 *S. aureus* isolates were 24.10 and 6.00 for norfloxacin and ciprofloxacin, respectively against isolates WM10 and WM 11. As also observed amongst the *E. coli* isolates, a total of 5 isolates showed multi-drug resistance and these were isolates PA1, PA3, PA5, WM77 and WM11.

Table 4.7: Sensitivity of *Escherichia coli* isolates

Antibiotics zones of inhibitions (mm)										
Isolates	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
WS1	22.07±1.01	19.10±0.85	12.13±0.81	12.17±0.76	NG	13.07±0.90	22.10±1.01	16.13±0.81	16.13±0.91	13.50±1.71
WS2	18.07±1.90	21.10±1.85	21.47±1.36	22.17±0.76	19.07±0.90	20.17±0.76	21.10±0.85	22.07±1.01	21.53±1.28	20.57±1.25
WS3	18.20±2.05	18.67±0.58	20.23±1.05	21.10±1.15	19.13±1.11	17.83±1.01	17.13±1.11	16.30±1.11	18.43±1.30	17.33±1.35
WS4	NZ	18.10±0.90	19.07±1.01	18.13±1.12	18.57±1.01	15.10±0.90	16.23±1.01	NZ	10.87±1.24	15.17±1.12
WS5	NZ	NZ	NZ	8.00±1.00	6.00±1.00	NZ	NZ	NZ	NZ	NZ
PA1	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA2	10.37±1.05	14.23±1.27	15.23±1.05	12.33±1.05	12.10±0.90	18.17±1.06	19.80±1.22	20.80±1.30	22.17±1.11	11.57±1.29
PA3	18.00±1.00	18.00±1.91	19.23±0.90	20.10±2.1	21.43±0.81	22.27±0.86	23.37±1.20	20.13±1.03	17.23±1.05	16.20±2.05
PA4	NZ	14.83±0.76	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA5	20.00±2.00	21.00±1.00	20.00±1.00	22.00±2.00	NZ	21.00±2.00	23.00±1.00	20.00±1.00	19.00±1.00	18.00±1.00

Key: NZ = No zone of inhibition, CPX, SXT, S, PN, CEP, OFX, NA, PEF, CN, and AU which stood for *Ciprofloxacin, sulfamethoxazole+trimethoprim, Streptomycin, PN, Ceporex, Ofloxacin, Nalidixic acid, Reflacine, Gentamycin, and amoxicillin+clavulanic acid, respectively.*

Table 4.8: Sensitivity of Staphylococcus *aureus* isolates (mm)

Isolates	CXP	STR	NFX	APX	GEN	ERY	AMX	CHL	LEV	RIF
PA1	NZ	NZ	NZ	14.11±1.05	15.03±0.57	18.00±1.13	10.33±0.11	15.23±2.05	18..13±1.12	20.57±1.23
PA2	18.57±1.01	19.00±1.00	NZ	17.00±0.58	16.00±0.90	18.25±0.58	20.00±2.05	21.00±0.90	22.00±1.57	22.00±1.00
PA3	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA4	14.01±0.58	17.57±0.41	18.00±1.05	18.57±0.58	19.15±1.01	21.00±1.01	23.00±1.00	24.00±2.00	20.00±1.05	18.33±1.33
PA5	17.00±1.01	16.00±0.47	20.00±1.30	NZ	NZ	18.00±0.57	19.00±0.57	23.00±0.57	19.33±0.57	18.33±0.21
WM6	17.04±2.04	16.57±0.58	19.10±1.02	20.12±1.21	21.00±1.25	13.33±1.05	15.10±0.90	19.17±1.08	19.08±1.02	20.08±1.03
WM7	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
WM8	17.00±1.01	16.80±1.02	16.40±0.90	19.00±1.25	20.12±1.25	13.00±0.80	22.50±1.21	18.13±0.71	16.05±0.72	15.50±1.71
WM9	17.00±0.90	22.30±1.21	16.33±0.90	16.13±1.91	18.20±0.57	20.00±1.00	23.20±2.25	18.50±0.31	20.50±1.21	17.80±0.41
WM10	21.00±1.01	23.00±1.00	24.10±2.00	20.00±1.05	18.33±1.33	19.00±1.05	15.01±1.01	18.00±1.00	20.00±1.00	17.00±0.33
WM11	6.00±0.57	7.15±0.90	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ

Keys: ERY – Erythromycin, AMX – Amoxil, CHL – Chloramphenicol, LEV – Levofloxacin, CXP – Ciprofloxacin, STR – Streptomycin, APX – Ampliclox, NFX – Norfloxacin, RIF – Rifampicin, GEN – Gentamycin, NZ = No zone

V. Discusssion, Conclsuion and Recommendation

a) Discussion

In developing countries like Nigeria, street vended sliced fruits are widely consumed by millions of Nigerians in cities and they are a source of affordable nutrients to many of the inhabitants. However, these fruits and the juices are often linked with diarrhoeal diseases largely due to unhygienic handling and processing of the fruits and its juices that are commonly vended on the street in developing countries (WHO, 2002; Baro, 2006 & Tambekar *et al.,* 2009). The major microbial contaminants are usually enteric and skin associated bacteria and fungi (Mensah *et al.,* 1999). However, to a lesser extent, viruses and even parasites have been associated with the transmission of enteric pathogens in foods (Mensah *et al.,* 1999).

In an earlier study, where the mean bacteria counts for various street vended were examined, they obtained counts for fried yam, fried fish, moi-moi, suya, meat pie and fried plantain were 7.4 x 10^4 cfu/g, 7.4 x 10^4 cfu/g, 1.0 x 10^4 cfu/g, 9.3 x 10^4 cfu/g, meat pie 6.4 x 10^4 cfu/g and fried plantain 7.6 x 10^4 cfu/g respectively (Agbo *et al.,* 2016). In this study, the mean aerobic bacteria counts obtained for water melon and pineapple were 1.85 and 1.867 x 10^4 cfu/g, respectively. In another earlier study, higher counts up to 4.6 x 10^6 cfu/g from juices of pineapple, sweet lime, and vegetables randomly collected from vendors in Nagpur City in India (Titarmare *et al.,* 2009). As expected, the fungal counts were very low compared to their bacterial counterparts. This is in line with earlier studies that showed that fungal abundance always less than those of bacteria in various ecosystems (Edet et *al.,* 2017). In our study, the fungal counts ranged from 0.6 to 1.4 x 10^3 cfu/g for pineapple and 0.5 to 1.3 x 10^3 cfu/g for water melon and these counts were within range of what was obtained in an earlier study where 1.1 x 10^3 – 3.0 x 10^5 and 1.4 x 10^3 – 2.0 x 10^5 cfu/g were obtained for hot and cold food samples, respectively in street vended foods in Abeokuta, Nigeria (Oluwafemi *et al.,* 2013). Furthermore, the fungal species were *Rhizopus* sp; *Aspergillus* spp; *Penicillium* spp. and *Mucor* spp.

Studies abound that have shown several frequently isolated bacteria from ready-to-eat fruits and foods and these include *Escherichia coli*, *Salmonella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Shigella sp*, *Mucor sp,* viruses and even parasites have been associated with the transmission of enteric pathogens (Agbo *et al*., 2016; Mensah *et al.,* 1999). In another study, by Titamare (2009) where 38 street vended fruit and vegetables were evaluated, fecal coliforms, *Staphylococcus* and *Salmonella* were all isolated with total coliform and *Staphylococcus* showing no significant difference.

The main aim of our study was to isolate *Staphylococcus aureus* and *Escherichia coli* that stand out from a public health perspective. The presence of both microbes is a direct indication of poor handling and hygiene. Their presence in foods and fruits indicates recent fecal contamination and overall poor hygiene in the handling and processing of such foods (Titamare *et al.,* 2016; Yang *et al.,* 2017). E. coli is a normal flora of human that resides in the small intestine. One of its strain *E. coli* O157:H7 have been linked to several cases of food poisoning and diarrheal illnesses (Prescott *et al.,* 19998; Yang *et al.,* 2017). On the other hand, *Staphylococcus aureus* is a normal flora of the skin of human (Prescott *et al.,* 1999) and their presence in food is an indication of poor hygiene in the processing steps (Brooks *et al.,* 2010). Like *E. coli,* they are frequently associated with food poisoning cases and the bacterium has outstanding ability to secrete enterotoxin that causes food poisoning (Hennekinne *et al.,* 2012; Brooks *et al.,* 2010).

Apart from clinical and environmental samples that were about the only sources of antibiotics resistance, vegetables and fruits have joined the fray. A recent outbreak of enterohemorrhagic *Escherichia coli* (EHCC) in 2011 resulted in a total of 4321 cases with atleast 50 fatalities (Holzel *et al.,* 2018). In an earlier study, *Staphylococcus aureus* isolated from a total of 53 vegetables (lettuce, penilla leaf and sprouts) cultivated in Korea showed multiple drug resistance and as well as single resistance. This is in line with our findings as 5 out of 10 *S. aureus* isolates showed multi-drug resistance to the test antibiotics used in this study. Two of isolates

PA1 and WM7 isolated from pineapple and watermelon showed 100% resistance to all the test antibiotics. In another study, Agbo *et al* (2016) showed that *Staphylococcus aureus* was highly resistant to Norfloxacin (50.0%), Ampiclox (45.0%), Erythromycin (40.0%), Amoxil (35.0%). Furthermore, Kwaku et al (2016) showed that *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Citrobacter* spp. and *Enterobacter isolated from* carrots and lettuce were resistant to all eight (8) antibiotics in their study.

Tadesese *et al* (2018) isolated *E. coli* from fresh cow milk and fruits juice that were highly resistant to ampicillin (70%), sulfamethoxazole-trimethoprim (60%), clindamycin (80%), erythromycin (60%), chloramphenicol (50%), and kanamycin (50%) susceptible to gentamicin (100%), norfloxacin (100%), tetracycline (60%), polymyxin B (90%), and ciprofloxacin (90%). In this study, most isolates showed resistance to all the antibiotics used.

b) Conclusion

This research project has indeed shown that sliced Water melon and Pineapple that are widely consumed in Uyo and other cities in Nigeria have microorganism that are of public health concern. Isolation of *S. aureus* and *E. coli* is an indication of the poor personal hygiene of the various fruit vendors. The multiple antibiotic resistance shown by the *S. aureus* and *E. coli* isolated from the pineapple and water melon samples are even more worrisome.

c) Recommendation

1. Similar studies should be carried out on other fruits types such as Oranges, Apples, Straw berries that are also vended in the study location.
2. Molecular characterization should be carried out such as Sanger sequencing to identify the various strains.
3. Plasmid profiling of the multi-drug resistant isolates should be carried out to ascertain the resistant genes they might be carrying.
4. Fruits vendors should be made to undergo regular health checks and also be exposed to best and acceptable hygienic practice in handling and processing of the fruits they sell to the public.

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A Look into the Tiny Flat Plates Sheathing the Flimsy Wings of *Amata Passalis* using Light Microscopy

By Nidhi Soman, Surya A. & Sheeba S.

Abstract- *Amata passalis* is a moth popularlyknown assandal wood defoliator, belonging to the family Erebidae under the order lepidoptera. The framework of this study mainly concentrates on the different types of scale cells present on the varied coloured wing areas. The study has been conducted experimentally by scrapping off different coloured scales to a glass slide and fixing it with xylene and observing it under a light microscope. The dimensions of the scales were also studied by measuring it using micrometry. A total of 68 morphologically distincttypes of scales were studied, which includes 48 scales of the dorsal wing and 20 scales of the ventral wing. Analysis of both the dorsal and ventral wing areas revealed the presence of several white, brown and and grey coloured scales. This moth species posess a wing pattern with black coloured region surfaced by white spots. Black and white scales cannot be extracted separately, as the sclaes seen on the white spotted region are small sized ones. Majority of the scales of the dorsal region were point edgedones and they have a length range of about 110.3μm to 149.7μm and width range of 59.1μm to 78.5μm. While examining the ventral wing it was observed that majority of the scales were heart shaped ones.

Keywords: *flimsy wings, light microscopy, micrometry, moth, flat plates.*

GJSFR-C Classification: *FOR Code: 279999*

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Keywords: flimsy wings, light microscopy, micrometry, moth, flat plates.

I. Introduction

Moths and butterflies, diagnosed as insects having scales on their wings are grouped together in lepidoptera, an order which forms part of the class insecta, the dominant division of the sub-kingdom Articulata. The awe-inspiring forms and colouration of the moths and butterflies caused them to be attracted by nature lovers. The colouration and patterns of their wings are formed by thousands of microscopic tiny flat plates known as scales sheathing like tiles on a roof. The scales of these insects were coloured and arranged in innumerable patterns from elusive and cryptic to the bright and showy. The wings which are the most prominent attribute of lepidopterans including moths and butterflies are usually covered on both the veins and membranes with two layers of minute socketted scales. The present study untangles the morphology of different types of scale cells overlaying the translucent wings of *Amata passalis* commonly called as sandal wood defoliator belonging to the family Erabidae.

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II. Materials and Methods

The moth species, *Amata passalis* has been collected from botanical garden of Sree Narayana College Campus, Kollam (Plate 1). The scales that are overlaying the wings of moth species were taken as the experimental sample. Several scales have been scrapped off from different coloured regions of the wing separately and placed on a glass slide. A drop of xylene has been added to the samples that occupies the glass slide. After it has been evaporated the samples were studied under light microscope, so that the photographs of each scale can be taken clearly and separately. Microscopic photography was adopted in this study, for taking the photomicrographs of prepared scale samples. Followed by this, the diameters of each scale under study were measured using morphometrics. It is further preceded for result analysis.

III. Results

Amata passalis is a moth belonging to the family Erabidae. It is often known as the defoliator of sandalwood. This moth species posess a wing pattern comprising of black coloured region surfaced by white spots. The forewing and hindwing have a wingspan of about 5cm and 2cm respectively. A total of 68 morphologically distincttypes of scales were studied, which includes 48 scales of the dorsal wing and 20 scales of the ventral wing. Analysis of both the dorsal and ventral wing areas revealed the presence of several white, brown and grey coloured scales (Plate I.1 to I.2). Majority of the scales of the dorsal region were point edged ones while those of the ventral region were heart shaped ones. The dimensional analysis pinpointed that the scales present on the dorsal and ventral wing areas shows variation both in terms of length and width. Analysis of scales on the dorsal wing area revealed that

the dimension ranges from 110.3μm to149.7μm in terms of length and 59.1μm to 78.5μm width. Majority of the scales of the ventral wing has a length range of about 130μm to 145.7μm and width range of about 55.1 μm to 78.8μm.

IV. Discussion

The flimsy wings of the moths and butterflies are clothed in flattened scales which are actually made from modified hairs. It is these finely ridged, hollow and microscopically perforated scales which gives different colouration and patterns to the wings of this insect as they posess colour pigments. The Lepidopteran scale vesiture of the wings serves a variety of functions. Since these Scales are easily detachable, it helps the insects to free their wing if it is caught in a spider's web (Barish, 1999). In addition to this, the scales enable development of vivid or indistinct patterns which help the organisms protect itself by concealment and camouflage, mimicry and warning. The morphology of scales has been studied by Downey and Allyn (1975) and they classified the scales into three groups namely hair like or piliform, blade like or lamellae and other variable form. In the present study only wing scales are focused. Many morphologically distinct scales has been observed during the present study on sandal wood defoliator. Majority of the scales of the dorsal region were point edged ones while those of the ventral region were heart shaped ones. Scales vary in colour, which can be due to structure or pigmentation (Barish,1999).Analysis of both the dorsal and ventral wing areas of *Amata passalis* revealed the presence of several white, brown and and grey coloured scales. The dimensions of the scales ranges from 110.3μm to 149.7μm length and 55.1μm to 78.8μm width. Similar observations were made by Downey and Allyn (1975) and pointed out that the scales observed from the wings of certain butterflies range in size from 30 μm to 80 μm length and 30 μm to 500 μm width. In the present study it was also found that the scales present on the dorsal wing area has the highest dimension in terms of length. But in terms of width, the values are almost similar on both wing areas of this engrossing moth named *Amata passalis.*

V. Conclusion

The results of this work deciphered that, even though the moths are not as much attractive as other members of the order Lepidoptera including butterflies, their flimsy wings are sheathed with varietyoftiny flat plates known as'scales' on its surface, that are wondrously beautiful, irrespective of their elusive and cryptic markings and patterns.

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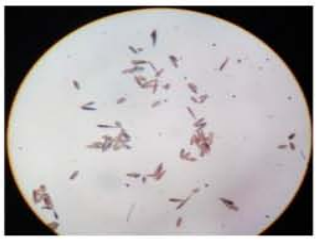
PLATE I

***Amata passalis* (sandle wood defoliator)**

Dorsal wing



ventral wing

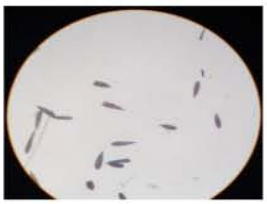


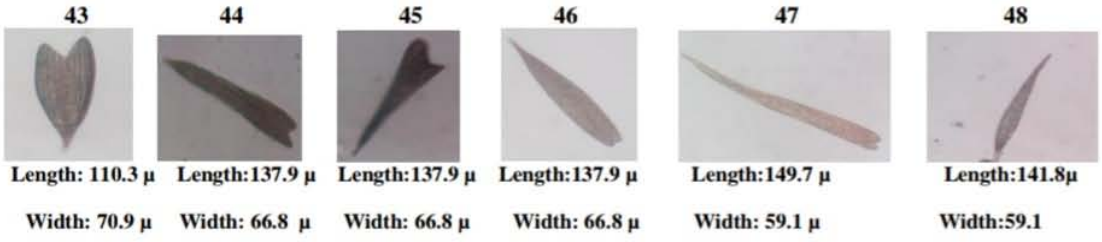
Plate 1: Amata passalis(sandle wood defoliator)

PLATE I.1

DORSAL WING

Plate I.1

PLATE I.2



VENTRAL WING

Plate I.2

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Evaluation of the Results of Introduction Work on the Example of Rare Species Allium Giganteum Regel, A. Isakulii R. M. Fritsch & F. O. Khass

By A. I. Uralov, V. Kh. Turakulova, G. D. Solieva & S. K. Kodirova

Abstract- The terminology used in assessing the results of introduction works has been clarified. Criteria have been developed for assessing acclimatization and adaptation. An adaptation assessment was carried out for Allium giganteum Regel, A. isakulii R. M. Fritsch & F. O. Khass.

Keywords: introduction, adaptation, acclimatization, allium giganteum, a. isakulii.

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Оценка Результатов Интродукционной Работы на Примере Редких Видов *Allium Giganteum* Regel, *A. Isakulii* R. M. Fritsch & F. O. Khass.

A. I. Uralov [α], V. Kh. Turakulova [σ], G. D. Solieva [ρ] & S. K. Kodirova [ω]

Абстрактный- **Уточнена терминология, используемая при оценке результатов интродукционных работ. Разработаны критерии для оценки акклиматизации и адаптации. Проведенаоценгаадаптации для *Allium giganteum* Regel, *A. isakulii* R. M. Fritsch & F. O. Khass.**

Ключевые слова: интродукция, адаптация, акклиматизация, allium giganteum, a. Isakulii.

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I. Вступление

Интродукция как научный метод существует около 500 лет, однако, проблемаобъективнойуспешности роста и развития растений в условиях культуры остается не решенной до сих пор, нет единообразия не только в подходах к интродукции растений, но и в вопросах терминологии. Выделяются следующие аспекты в оценке результатов интродукции: терминология, когда и каким оценкиобразом проводить оценку. Цель данного исследования — уточнить терминологию, используемуюв настоящее время для оценки интродукционнойработы, и разработать критерии для этой оценки.

II. Материал и Методы

Работа проводилась на базе коллекции « Редкие и исчезающие виды растений Узбекистан» в Центральном Ташкентском Ботаническом саду (г. Ташкент). Для проведения оценки адаптации нами взяты два вида рода *Allium* L: *Alliumgiganteum* Regel, *A. isakulii* R. M. Fritsch & F. O. Khass.,

Allium giganteum— Красно книжной вид, включен в список Красную книгу Узбекистан (2016). Луковичный геофит с ранневесенним эфемероидным ритмом развития. Генеративный побег в условиях Ботанического сада достигает 180 см высоты, несет 4-8 (9) листьев. Нижний лист длиной до 70 см, шириной до 16 см. В соцветии образуется до 2550 цветков. Регулярно плодоносит. Вегетативно неразмножается. Распространение в Узбекистане. Гиссарскийхр. Кугитангский хр. Бабатагский хр.[1,2.]

A. isakulii— Красно книжной вид, включен в список Красную книгу Узбекистан (2016).Луковичный геофит с ранневесенним эфемероидным ритмом развития. Генеративный побег в условиях Ботанического сада достигает 80 см высоты, несет 1-3 листьев. Нижний лист длиной до 30 см, шириной до 3 см. В соцветии образуется до 255 цветков. Распространение в Узбекистане.Нуратинский хр.[1,2.]

Для исследований использовали классические методы работы. Исследование ритма развития проводили по фенологическому методу (Бейдеман, 1974). Процент плодоцветения определялся как отношение образованных плодов к общему количеству цветков (30-2000 цветков с популяции).Семенную продуктивность изучали по методике В.И. Вайнагий (1974) (определяли семенную продуктивность, процент семинификации). Для обработки полученных результатов использовали метод вариационной статистики. Определяли среднее арифметическое, его ошибку, коэффициент вариации. Для размножения растений использовали лабораторно-грунтовый метод (Дюрягина, 1982), посев в грунт или деление особи. Посев в грунт проводили осенью. В зависимости от количества семян, посев проводился в 2повторностях по 100 или 400 семян, или однократно имеющимся количеством семян. Выявляли самые благоприятные сроки посева. Для оценки процента плодоцветения, семинификации, грунтовой всхожести учитывали максимальные показатели в годы исследований и считали их высокими при значении более 50 %, средними — 30-50 %, низкими — менее 30 %. При оценке устойчивости к болезням и вредителям,

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засухоустойчивости, морозоустойчивости и зимостойкости руководствовались рекомендациями В.Н. Былова и Р.А. Карписоновой (1978).

III. Результаты и Их Обсуждение

В настоящее время принято понятие интродукции в трактовке Лапина (1972). Интродукция — целеустремленная деятельность человека по введению в культуру в данном естественно-историческом районе новых родов, видов, сортов и форм растений или перенос их из природы в культуру. В трактовке П.И. Лапина понятие интродукции включает два уровня исследования. Во-первых, интродукция внутри ареала, во-вторых, за пределами ареала. В первом случае определяется отзывчивость растений на условия культуры, которые включают освобождение от конкуренции, применение агроприемов, сохранение полезных свойств растений в культуре, изучение биологических особенностей и условий размножения. Во втором случае изучаются процессы перестройки фенотипа и генотипа к более широкому комплексу новых условий. Для отображения процессов реакции растений на окружающую среду используют термины «адаптация» и «акклиматизация». Лапин в той же работе дает определение акклиматизации. Это сложный комплекс явлений, происходящих в растениях под действием природных факторов и созданных человеком условий, изменяющих ход формообразовательных процессов, т.е. суммарная реакция растений на изменившиеся условия среды или воздействие человека при интродукции (Лапин, 1972). Адаптациюрассматривают как совокупность морфофизиологических, популяционных и других особенностей данного биологического вида, обеспечивающую возможность специфического образа жизни в определенных условиях внешней среды. УЛапина (1972) кроме этого рассмотрен термин «интродукционная адаптация» — фенотипические изменения в ритме развития и роста, в обмене веществ и строении растений, происходящие в растениях под воздействием условий новой среды и в результате применения методов интродукции. Этот термин схож с «акклимацией», который не прижился в научной литературе. «Акклимация» — экспериментальная адаптация — приспособление организма к искусственно созданным условиям, чаще этот термин употребляют как синоним акклиматизации. Адаптацию рассматривают на двух уровнях — генетическом и фенотипическом. В первом случае, приспособление генетически детерминированное явление, возникающие в ходе естественного отбора по данному ведущему фактору, и представляет собой длительный исторический процесс, затрагивающий ряд поколений. Во втором случае, приспособление является результатом непосредственной фенотипической или поведенческой реакций, возникающейв ответ на экологический фактор в течение короткого времени. Адаптация на фенотипическом уровне в течение жизни растения и есть акклиматизация. Под акклиматизацией обычно понимают процесс активного приспособления организма к непривычным для него климатическим условиям. Это комплекс быстрых фенотипических реакций, связанных в первую очередь с изменением показателей обмена веществ, а степень изменений определяется индивидуальной нормой реакции.

В современной литературе интенсивно используются понятия «оценка интродукции», «оценка успешности интродукции», «оценка акклиматизации», «оценка успешности акклиматизации», «интродукционный прогноз», «интродукционная устойчивость». Интродукционный прогноз — оценка возможности переноса растений в иные условия в процессе отбора материала. В данном случае используются ряд методов: климатических аналогов, эколого-исторический, флорогенетический, родовых комплексов и т.д. В остальных случаях исследуют визуальные признаки состояния растений в условиях культуры (зимостойкость, засухоустойчивость, фенoритм, размножение), то есть те признаки, которые отражают процесс акклиматизации растений при переносе в условия культуры. Таким образом, оценивается процесс акклиматизации, а не перенос растений (интродукция). В.И. Некрасов (1980) выделяет первичный очаг интродукции и вторичный очаг, при последующем переносе в новое место культивирования. Соответственно можно говорить об оценке акклиматизации при первичной интродукции, при вторичной интродукции и т.д.

Наиболее вероятно изменение генотипа при семенном размножении. Существует также возможность мутаций при акклиматизации у вегетативно подвижных растений и у отдельных интродуцированных индивидуумов на соматическом уровне, без изменения репродуктивных клеток. В первые годы интродукции такие маловероятные явления, как мутации невозможно отследить на морфологическом уровне, поэтому мы допускаем, что в это время у интродуцированных растений мы оцениваем акклиматизацию. Для травянистых растений с небольшим прегенеративным периодом эту оценку можно провести на 1-2 поколениях.

При подведении итогов интродукции растений используют либо визуальную, сравнительно описательную оценку. Для травянистых растений можно отметить разработанную сравнительно-описательную оценку

Семеновой (2007). Описательная характеристика вида и часто используемые шкалы не отражают разнообразие явлений, наблюдаемых при интродукции, и их сложно применять как универсальный метод для оценки растений, у которых выражено вегетативное размножение. Часто используются критерии, которые возможно оценить только после длительного культивирования растений (изменение габитуса по сравнению с природными популяциями, биология прорастания семян). Новая среда обитания оказывает влияние на заложение генеративных органов, динамику их формирования, на степень развития плодов и семян. Это приводит к смещению сроков прохождения различных фаз морфогенеза, влияет на качество пыльцы, число развивающихся семязачатков и во многом определяет степень развития зародыша и эндосперма.

Для оценки акклиматизации было выделено 13 критериев, которые можно объединить в 3 группы: характеристика феноритма, размножение и жизнеспособность в культуре.

При оценке феноритма мы отмечаем наличие цветения (1) и диссеминации (2). Для характеристики оценки размножения определяли процент плодоцветения (3) и семенную продуктивность (4), грунтовую всхожесть семян (5), а также наличие и обилие самосева или способность к вегетативной подвижности (6). Для характеристики жизнеспособности вида в культуре мы выделили 7 критериев: продолжительность жизни особи (7), способность к натурализации: выход за пределы делянки за счет распространения семян или вегетативной подвижности (8), способ размножения в коллекции (9), устойчивость к болезням и вредителям (10), засухоустойчивость (11), морозоустойчивость и зимостойкость (12) и устойчивость к уплотнению почвы (13). Каждый критерий целесообразно оценивать по 3-балльной системе. Оценка адаптации включает кроме данных по оценке акклиматизации — устойчивость феноритма (14), интенсивность отпада особей в прегенеративном периоде (15), жизнеспособность семян при длительном хранении (16), лабораторную всхожесть семян: поиск условий, необходимых для прорастания семян, часто оказывается очень длительным и требует много семенного материала (17), продолжительность жизни популяции в коллекции (18), сравнительную характеристику с природными популяциями по вегетативной сфере (20): число побегов на особь, размеры вегетативных побегов и листьев, число листьев, степень ветвления и сравнительную характеристику с природными популяциями по генеративной (21) сфере: число генеративных побегов, доля генеративных побегов на особь, размеры генеративных побегов, цветков, плодов, процент плодоцветения, процент семинификации. Сравнение с природными популяциями проводится либо на оригинальном материале, либо по литературным данным. Формы связи и характер зависимости между изучаемыми критериями могут быть самыми разными. Н.А. Кохно (1980) и А.А. Лаптев (1980) предложили использовать коэффициент весомости признака в зависимости отего значимости. Однако определить точное значение этого коэффициента, как характер зависимости между критериями, затруднительно. Для этого требуется большой фактический материал, использование метода многофакторного анализа. Для работы интродуктора необходим реальный удобный и универсальный метод оценки роста и развития растений, поэтому мы допускаем, что все критерии равнозначны по влиянию для жизни растения. Каждый критерий целесообразно оценивать по 3-балльной системе.

Итогом оценки акклиматизации и адаптации является распределение видов на основе суммы баллов по перспективности для интродукции. При оценке акклиматизации, используя 13 критериев, выделяются: перспективные (34-39 баллов), среднеперспективные (27-33 балла), малоперспективные (21-26 баллов) и неперспективные (13-20 баллов) виды, а для оценки адаптации на основе 21 критерия виды распределяют на перспективные (53-63 балла), среднеперспективные (42-52 балла), малоперспективные (31-41 балл) и неперспективные (21-30 баллов).

Для проведения оценки адаптации нами взяты шесть видов рода *Allium* L. *A. giganteum* интродуцирован из Сурхандаринской области., размножается луки и семенами, которые сохраняют всхожесть при комнатной температуре в течение 60 дней. В Луковичный сад можно размножать и лукивица. Для размножения в Луковичный сад требуются определенные агротехнические мероприятия: в период от посева до начала плодоношения растений —прополка[3,4,5.].

A. isakulii интродуцирован из Джиззакской области., размножается луки и семенами, которые сохраняют всхожесть при комнатной температуре в течение 45 дней. В Луковичный сад можно размножать и лукивица. Для размножения в Луковичный сад требуются определенные агротехнические мероприятия: в период от посева до начала плодоношения растений — прополка[3,4.].

В связи с этим, допустимо проведение оценки адаптации для этойЛуковичный сад после 5 лет интродукции. Результатом балловой оценки адаптации изученных видов (табл. 2) является их распределение по перспективности. Для

интродукции перспективен вид — *A. giganteum*, среднеперспективные—*A. isakulii*.

Таблица 1: Оценка адаптации редких и Красно книжний видов род *Allium*

Критерий	*A. giganteum*	*A. isakulii*
Цветение	2	2
Диссеминация	3	3
Устойчивость	3	2
Процентсеминификации	3	3
Процент плодоцветения	3	3
Грунтовая всхожесть, %	3	2
Лабораторная всхожесть, %	3	3
Самосев или вегетативное размножение	2	1
Интенсивность отпада особей в прегенеративном периоде, %	3	3
Жизнеспособность семян при длительном хранении	3	2
Продолжительность жизни особи	3	3
Способность к натурализации	1	1
Внедрение в естественные сообщества	2	1
Способ размножения в коллекции	2	2
Устойчивость к болезням и вредителям	2	2
Засухоустойчивость	3	3
Морозоустойчивость, зимостойкость	2	2
Устойчивость к уплотнению почвы	2	2
Сравнительная характеристика с природными популяциями	3	3
	3	3
Продолжительность жизни популяции в коллекции	3	3
	50	46`

Примечание: показатели высокие при значении более 50 %, средние — 30-50 %, низкие — менее 30 %.

Таким образом, используемые в литературе для оценки интродукционной работы термины отражают процессы акклиматизации растений и адаптации популяций. Поэтому наиболее правильно использовать термины «оценка акклиматизации» в первые годы интродукции растений и «оценка адаптации» после длительного их культивирования, на растениях последующих репродукций. На основе разработанной оценочной таблицы –2 для оценки акклиматизации и адаптации проведена оценка адаптации для 6видов. Среди изученных видов*A. giganteum*— является перспективной, вида *A. isakulii*— среднеперспективным.

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Bacteriological Quality of Garri and Ogi Sold in Markets in Keffi Metropolis Nasarawa State Nigeria

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Bacteriological Quality of Garri and Ogi Sold in Markets in Keffi Metropolis Nasarawa State Nigeria

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I. Introduction

a) Background of the Study

Food security both in developed and developing countries has been a growing concern that has led to an unprecedented global interest in Agriculture (Orji *et al.*, 2016) because of the alarming concern of disease outbreak caused by consumption of contaminated food and food products. Market vending has become an important public health issue and a great concern to everybody. This is due to the widespread of food borne disease associated with garri and ogi handlers who lack adequate understanding of the basic food safety issues (Ghosh *et al.,* 2007; Hussain, 2013; Almeida, 1994).Open bowl display of this fermented cassava and maize grain products (garri and ogi) is perceived to be a major public health risk due to lack of basic infrastructure (Rane, 2011; Hussain, 2013). Various studies have identified the source of food safety issues involved in market sales of garri and ogi to be microorganisms (Ghosh *et al.,* 2007). The processing of cassava tuber and maize grain to garri and ogi and its handling involves different stages, at each stage there is a level of contamination (Adejumo and Adebayo 2015). The quality of the product depends on the management of each stage of the processing and handling. The processing of cassava and maize grain into garri and ogi usually takes three to five days both at household and factory level. The unhygienic practices carried out in local markets in Nigeria is associated with practices that lead to microbial contamination due to deposition of bio aerosols on exposed products, which may cause food poisoning and may lead to disease outbreak as a result of these contaminated food products .The practices associated with the production process and handling includes drying on the floor, mat, rock, road side etc. After frying and displaying in open bowls or basins, bags, and mats during packaging at a point of sales increases solid and microbial contamination (Ogiehor and Ikenebomeh 2005).Today Cassava remains one of the most common source of dietary food energy of people especially in Africa. Ogi is a popular traditional infant food and a major staple food in West Africa. (Tsegai and Kormawa 2002).

Recently in Nigeria, the Federal Government launched an agricultural transformation agenda to promote agriculture as a business integrate, the agricultural value chains and as a possible key way of driving Nigeria's economy (Agah *et al.*, 2016). Cassava supplies about 70% of the daily calorie to over 50 million of people worldwide (Oluwole *et al.*, 2014). It can be processed into bread, garri, flour etc. (Orji *et al.*, 2016) (Adejumo and Adebayo 2015). Most preparation of pap meal is from cereals, namely, maize, guinea corn or millet readily available in all parts of the country. Among the proceeds of cassava, garri is an important by product that is commonly consumed in Nigeria because of its ready to eat nature (Orji *et al.*, 2016) (Adejumo and Adebayo 2015), it is the major source of energy and fiber (Ogiehor *et al.*, 2007). Garri can be produced locally by fermentation of peeled cassava tuber in

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Nigeria and other parts of the world (Ray and Sivakumas 2009).

The unhygienic handling and poor sanitary measure that obviously are been observed between the last stages of production could constitute serious health implication as many chances have been given to contaminate the food products (Arasi and Adebayo 2000).The dust being raised by the breeze, storm, passing by vehicles and every other form of air movement bring solid particles and heavy metals into the fermented cassava and maize grain products (Garri and Ogi). The traditional fermentation method employed in ogi production is an untamed process and microorganisms are not controlled (Cheesebrough, 2000). Heavy metal contents in food has a limit, if these limit are exceeded, it could cause harm to the human body.

It has also been reported that garri sold in markets contain high load of microorganism (Ogiehor *et al.*, 2007) which might cause economic loss and food borne illness and public health threat as a result of these contaminations. The patronage of many consumers could constitute serious health implication as many chances have been given to contamination by organism of epidemiological importance such as *Salmonella, Escherichia coli, Klebsiella, Staphylococcus aureus, Staphylococcus epdidermidis Cryptosporidium, Campylobacter, L.grayi, L ivanovii* (Arasi and Adebayo 2000).

Garri and it ready to eat nature has made it a common practice in Nigeria especially among students to eat garri as snacks without considering the bacteriological implication (Egbuobi *et al.,* 2005). Pap meal is served in Nigeria as a weaning food for infants (1-3 year old) and a morning breakfast for children and adults. In Nigeria, the production process of the wet flour paste, a staple food mostly consumed by children in rural communities and also by adults even in the metropolis, requires the use of water which is often not pure because of the high risk of environmental pollution (Amadi and Adebola 2008). People who patronize open roadside sale of these fermented products have been reported to suffer food borne diseases like diarrhea, cholera, typhoid fever and food poisoning. The environment in which cassava root and maize grain products stay when it has been processed (or not processed) goes a long way in contributing to the contamination of the fermented products (garri and ogi).Contamination of this can be traced to the hands and garments of handlers and utensil in processing, sales and bagging. The method of drying on the bare floor or road exposes the product to air borne microorganisms and this type of contamination is deadly (Adams and Moss 1995).

b) Statement of the Problem

The sale of garri and ogi in the local markets in Nigeria is associated with practices such as open display in bowls and trays, open buckets and mats at points of sale and the use of bare hands in handling and selling of cassava and maize grain products (garri and ogi).These unhygienic practices, may lead to microbial contamination and can cause deterioration in food quality and spoilage, food borne illness and may pose a threat to public health.

Food security has been a major challenge to the world populace over the last few centuries because of the alarming concern of disease outbreak caused by consumption of contaminated food and food products.

c) Justification of the Study

There is limited information to the assessment of some bacteria associated with garri and ogi sold in Nigeria (Olapade *et al.*, 2014) and even the study area, studies have not been reported, and hence this study provides information on bacteriological quality of garri and ogi sold in Keffi metropolis, Nigeria.

d) Objectives of the Study

The specific objectives are;

- To isolate and identify bacteria from garri and ogi sold in Keffi metropolis, Nigeria.
- To determine the percentage occurrence of the isolates.
- To determine antibiotic susceptibility of the isolates.

II. Literature Review

a) Description of cassava

Plate 1: Cassava

Cassava (*Manihot esculenta Crantz*) is a woody shrub native to South America of the spurge family, *Euphorbiaceae*. Although it is a perennial plant, cassava is widely cultivated as an annual crop in tropical areas. The cassava root is long and tapered; it has a firm homogenous flesh enclosed in a detachable rind and about 1mm thick, rough and brown on the outside. The fleshy part may be chalk-white or yellow in color on the outer part with woody vascular bundle extended along the root axis. It is the chief source of dietary food energy for the majority of the people living in the low land tropics, and much of the sub-humid tropics of West and

Central Africa (Tsegai and Kormawa 2002). It supplies about 70% of the daily calories of over 50 million people (Oluwole *et al*., 2014) in Nigeria and about 500 million people in the world (Abu *et al*., 2006). The competing needs for cassava cut across both humans and animals. It is fast becoming a popular raw material in industrial production and is now a preferred material for making biofuels. Cassava is God's gift to the tropics because it can grow in poor soil with inadequate rainfall. The starchy roots of cassava are major source of food for more than 700 million people all over the world. It ranks third in order of staple food crops in developing countries after rice and maize. It has universal applications. Nigeria is the world largest producer of Cassava (Adeniji *et al.,* 2005). We produce over 41 million metric tons per annum and we are followed by Brazil, Thailand, Zaire (now Democratic Republic of Gongo), and Indonesia. Nigeria has tried to expand the local cassava business through the Composite Flour Initiative and the Cassava Empowerment Fund but so far, they have attained little or no success (Knipscheer *et al*., 2007).

i. *Description of garri*

Plate 2: Garri

Garri is dry, crispy, creamy-white and granular. It is a dehydrated, cassava product. It is classified or grouped based on texture, length of fermentation, region or place where it is produced and color imparted by the addition/non addition of palm oil (Abu *et a*l., 2006). It has a high swelling capability and can absorb up to four times its volume in water (Osungbaro *et al.,* 2010). Obtainable in the market is the dry form of post processed garri which can be consumed soaked in cold water. Sugar can also be added to the soaked garri and it can be eaten with meat, roasted groundnuts, smoked fish, boiled beans, coconut, palm kernel, groundnut cake (kwuli kwuli), and fermented maize snacks kokoro. Beverages and milk may also be added as complements. Eba is another food prepared from garri. The granules are added into hot water and stirred to form a stiff paste which can be eaten with indigenous soups or stew (Ogiehor *et al.,* 2007).

It is estimated that 70% of the cassava produced in Nigeria is processed into garri**.** It is produced from Cassava Tubers and is the commonest stable food in Nigeria consumed by over 130 million people (Olapade *et al.,* 2014).

Garri is an important by-product of cassava being an important item in the menu of most Nigerians. It is particularly popular because of its ready-to-eat nature (Abu *et al*., 2006). Garri is a good source of energy and fiber. Other nutrients are also present in marginally nutritional significance (Ikegwu *et al*., 2009). Cassava for garri production is harvested manually in the farm with the aid of cutlasses, hoe and flat iron sheet (digger), which occasionally inflicts various degrees of injury on the root tubers. After harvesting, the root tubers are hauled to the market where they are heaped in 20s, 40s, 50s, and 100s for sales under humid and warm topical conditions. These practices predispose the root tubers to contamination and infestation by various microorganisms (Agboola, 1968).

The production of garri is a burdensome and a tasking procedure and its method of production differs from one locality to another, garri is typically produced by peeling the cassava tubers, washing and grating them, which is packed into closely woven bags (Ray and Sivakumas 2009). After fermentation, frying at high temperature dries the fermented pulp to about 10% moisture content and this may result in partial dextrinization of starch (Akindahunsi *et al*., 1999). Also, high temperature destroys both enzymes and microbe's present. The garri market is competitive, sellers or buyers cannot unilaterally impose prices on the market.

In major garri producing areas, garri is produced by numerous smallholder units which sell garri essentially in village markets. Big markets, which are often fewer, act as an assembly center for garri from the numerous surrounding smallholder units. Such assembly markets are generally well attended by traders from far and wide, especially those markets that are well known for the supply of top quality garri. Garri quality can be defined on the basis of its safety and fitness for use by the target consumer (Osho, 2003). Thus in order to satisfy the taste of the consumers a processor needs to integrate quality into the processing operations in order to build quality into the product. In so doing the processor is able to attract more customers and remain competitive in the market place. Both processors and consumers alike have various indices by which they judge the quality of garri. These include taste (acidity or sourness), swelling capacity, color, texture, crispiness, and absence of foreign matter (cleanliness) (Adebayo *et al.,* 2012).

Traditional methods of processing cassava roots can result in poor quality products that contain unacceptable levels of cyanide, as well as being contaminated by foreign matter and disease-causing agents (Tsegai and Kormawa 2002). Following processing garri is spread on bare floor or on mats to allow cooling before final sieving and packaging for marketing. In the open market garri is displayed in open basins, bowls, bags and mats. These practices potentiate contamination by various groups of microorganisms and may predispose public health hazards (Ogiehor *et al.,* 2002). If people eat these kinds of products, they can suffer from acute cyanide poisoning, goiter, and a nerve-damaging disorder that makes them unsteady and unable to walk properly.

ii. *Mechanization of Garri Production*

Fig. 3: Flow chart for Garri production

Mechanization of garri production is the use of machines, either wholly or in part, to replace human or animal labor in the production of garri, unlike automation, which may not depend at all on a human operator, mechanization requires human participation to provide information or instruction. It implies the use of machinery more complex than hand tools and would not include simple devices such as an un-geared horse or donkey mill. Devices that cause speed changes or changes to or from reciprocating to rotary motion, using means such as gears, pulleys or sheaves and belts, shafts, cams and cranks, usually are considered machines.

Equipment for rapid processing of cassava has been used in Nigeria for over 20 years (Amadi and Adebola 2008). The stages in the processing of cassava include: peeling, washing, grating, dewatering, granulating or sieving, roasting, cooling and packaging (Adeniji, 2000, Oyewole *et al*., 1986; Sanni, 1990). Peeling is sometimes done manually because cassava is bulky and irregular in shape with various peel thickness. Mechanical peeling results in heavy losses. Washing are also manual for convenience and to reduce cost. There are many models of grater. Using electricity, diesel or petrol motor, the grating surfaces are made from iron sheet, galvanized iron or stainless steel; the

first two being rust-prone. Low cost and low energy graters are available in the market; women processors use them. Sieving or granulating is manual and is done on raffia or metal sieves. There are metal sieves which can be used while standing or shaken mechanically (James *et al.,* 2012). Rapid removal of water from fermented pulp lasts from 30mins to 2hr and is achieved by using hydraulic jack or screw press. Rotating over heat 22-60^{0}c (Aseidu and Wieneke 1989) is preferably carried out in a cast-iron pan or an assortment of trays (Ayehu *et al.,* 2014). Rotating-drum roasters do not produce garri of good quality because such devices do not mix and roast well (Amadi and Adebola 2008).The cooling of garri after roasting takes place on suitable trays and the product may be packaged in thick polythene bags.

iii. *Nutritional value of Garri*

Garri is highly rich in starch and fiber content. It is also noticed to contain some amount of proteins, calories, sodium, fat, potassium, copper, iron magnesium, manganese, little calcium selenium, zinc and some essential vitamins like vitamins B6, C and E. The fiber content of Garri makes one to feel full when it is been consumed, and it is very helpful in preventing ailments such as constipation and bowel diseases. It provides us with energy because of its high starchy content.

Red or yellow Garri contains fats and oils, which are great sources of additional nutrients and health benefits.

The major health benefits of garri are that it serves as a complementary food to balance our diet. For example, garri (eba) is being eaten with soups such as vegetables, meats, fish, fats and oil, minerals etc., and they provide various nutrients that make the meal to be a balanced diet. (Health and nutrition 2017).

iv. *Factors Improving Contamination of Garri During Production*

Garri processors are involved in practices which contribute negatively to the microbial quality of the processed Garri. Some of the practices include; burying of basin inside the ground to serve as a discharged point for the grinded cassava paste from the machine: This practice enhances soil particles and debris to fall directly into grinded paste thereby enhancing microbial contamination. The grinding machine is also characterized by visibly unwashed left over paste. This serve as a source of contamination to fresh cassava paste (Lawani *et al.,* 2015).

Keeping of dried cassava paste sack on bare ground. There is the possibility of soil microbes finding its way through the sack into the dried cassava paste. The floor of the manual presser also having direct contact with the ground enhances microbial contamination. Unskilled nature of the garri producers introduces contaminants to their products. Sitting of the cassava effluent site close to the processing site (Baine, 2000). Poor source of water and dirty processing environment (Ogiehor and Ikenebomeh 2005). Dirty environments attributed to markets and indiscriminate dumping of refuse around the markets where garri is sold is another major source of contamination (Trickett, 1992).

b) Description of maize

Plate 4: Corn (maize)

Maize also known as corn is a cereal grain first domesticated by indigenous peoples in southern Mexico about 10,000 years ago. Maize has become a staple food in many parts of the world, with the total production of maize surpassing that of wheat or rice (Singh *et al.,* 2001). However, little of this maize is consumed directly by humans: most is used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup. The six major types of maize are dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn.

Maize is the most widely grown grain crop throughout the Americas, with 361 million metric tons grown in the United States in 2014. Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels, while field corn varieties are used for animal feed, various corn-based human food uses (including grinding into cornmeal or masa, pressing into corn oil, and fermentation and distillation into alcoholic beverages like bourbon whiskey), and as chemical feed stocks. Maize is also used in making ethanol and other biofuels. Raw, yellow, sweet maize kernels are composed of 76% water, 19% carbohydrates, (Adeniji and Potter 1978). In a 100g serving, maize kernels provide 86 calories and are a good source (10-19% of the Daily Value) of the B vitamins, thiamin, niacin, pantothenic acid (B5) and folate. In moderate amounts, they also supply dietary fiber and the essential minerals, magnesium and phosphorus whereas other nutrients are in low amounts. Maize has suboptimal amounts of the essential amino acids tryptophan and lysine, which accounts for its lower status as a protein source (Aguirre *et al.,* 1953).

i. *Mechanization of Ogi production*

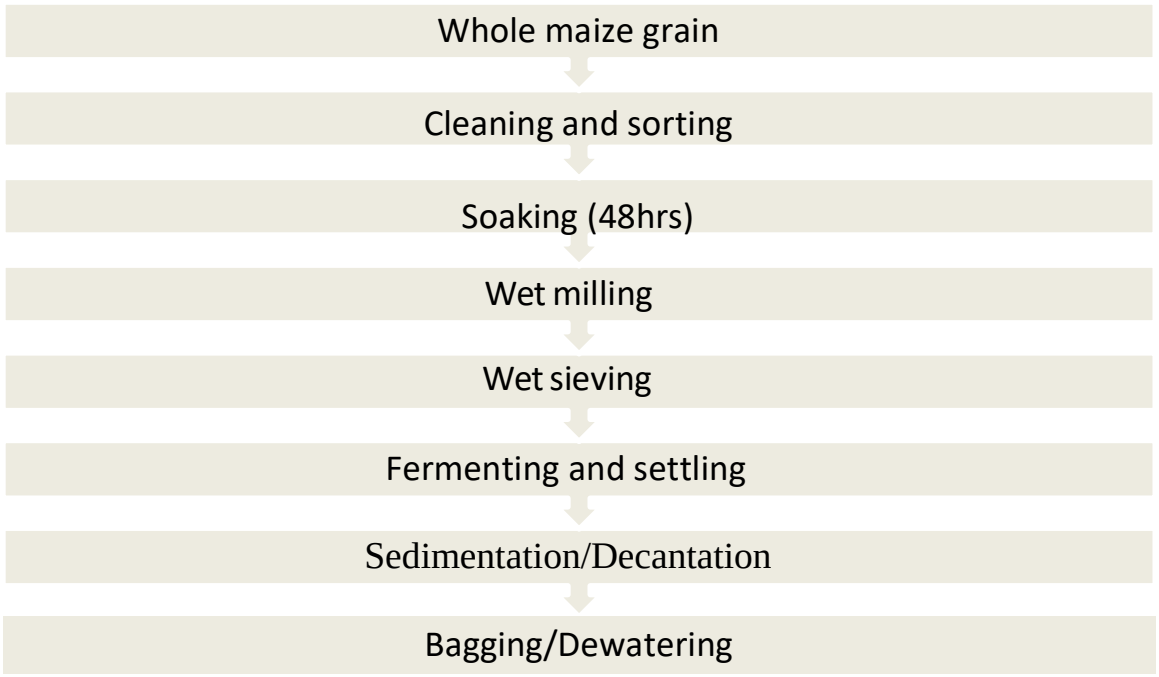

Fig. 5: Flow chart for Ogi production

Akamu (Igbo, ibibio), ogi (Yoruba) or Pap is Nigerian corn meal made from wet corn starch. It has a distinctive sour taste that makes people crave it. It is processed from dry white or yellow corn, millet, guinea corn. After processing it we get the raw akamu, pap or ogi which is then prepared with hot water before serving as a meal. (Akingbala *et al.,*1981)

The dry corn is washed thoroughly and soaked in a generous quantity of cold water for 3 to 4 days. The water in the corn is changed daily, on the 3rd or 4th day; it is washed and blended till smooth. In Nigeria, heavy duty grinders are used for this purpose.

A chiffon cloth is draped over a big bowl and tied up. The bowl should be big enough to accommodate the ogi and the water that will be used to rinse. The blend is sieved rinsing as necessary till only the chaff is left.

When done, take off the chiffon cloth and set the mixture of water and ogi aside to settle for at least 3 hours, after about 3 hours or when you notice that the water is clear, decant the clear water and pour the rest of the mixture into the muslin bag. Tie the bag and keep it in such a way as to let the water drain from the ogi.

After 24 hours, bring out the ogi from the bag, cut it up into single-use chunks, place in containers (bowls or plastic bags) and put in your freezer till you are ready to use it. (Akingbala *et al.,* 1981).

ii. *Nutritional value of Ogi*

Traditional weaning foods in West Africa are known to be of low nutritive value and are characterized by low protein, low energy density, and high bulk. Maize pap or *koko* has been implicated in the etiology of protein-energy malnutrition in children during the weaning period. Cereal-based diets have lower nutritional value than animal-based ones. Cereals form the primary basis for most of the traditional weaning foods in West Africa. The protein content of maize and guinea corn is of poor quality, low in lysine and tryptophan. These two amino acids are indispensable to the growth of the young child. (Ankinrele and Basir 1967).

iii. *Factors improving microbial contamination of Ogi*

Akamu is a nutritive food mostly consumed by infants as weaning food, adults also enjoy this delicacy. ogi is often produced locally by local producers and there is risk of high microbial contamination which often makes the food product unfit due to the presence of organisms that cause spoilage, food poisoning or food intoxication in the food product. (Awada *et al.,* 2005).

Food poisoning and infection results to fatal consequences in infected individuals and the main risk factors are channeled towards contaminated raw materials, poor fermentation conditions, poor personal and environmental hygiene and post processing handling (Ezendianefo and Dimejesi 2016). It could also be due to the contaminants from the grinding engine which was only washed before use (Nout, 1994).

Typically, microbial load starts multiplying from the first day (0hr) and attain optimum at 24-48 hrs of fermentation before it starts declining from 72-96hrs. The number of microbes of the family *Enterobacteriaceae* is usually the least during fermentation of maize used in preparing ogi, this growth that can proliferate in MacConkey agar which includes *Escherichia coli, Klebsiella*, *Citrobacter*, *Proteus*, *Salmonella*, *Shigella* e.t.c does not participate in fermentation processes (Sylvester *et al.,* 2016). In a study by Oyelana and Coker (2012), the growth of *E.coli* and *Klebsiella* reduces significantly at the end of fermentation; hence their occurrence could result from the water used in fermentation or as normal flora of the maize before fermentation. Microbes found in food products get in through diverse means which include exposure, handling, and use of contaminated utensils for processing. The processing of maize fermentable food is mostly done by small holders who process it for little and to a larger extent for commercial purposes or sales. The processing of this food product is carried out using rudimentary or undeveloped equipment and seldom in an unhygienic environment majorly during processing and handling. Microbe is known to be wide spread and prevalent, this makes the product prone however to unintentional contamination by microbes found in the environment. Oyelana and Coker (2012), listed *P.aurogenosa, Lactobacillus plantanum*, *Staphiloccocus aureus, E.coli, Klebsiella* e.t.c as microbes found in air, the authors have reported that the organisms can be found in water used for fermentation. A study reported by Izah and Ineyougha (2015) proved *S.aureus, E.coli, Alcallegenes fecalis, Proteus, Micrococcus, Citrobacter, Streptococcus, Vibrio, Shigella, Enterococcus, Flavobacterium, and Chromobacterium* species are commonly found in different portable water source in Nigeria such as lakes, hand dug wells, borehole, rain water e.t.c (Izah *et al.,* 2016) which are also used in domestic activities. Several microorganisms found in fermentation medium are pathogenic and infectious, whereas a few are non hazardous, virulent strains of this microbes lead to the condition such as gastroenteritis, this happens generally but most time unreported especially in rural areas. The storage procedures of fermented maize product are mostly carried out in environment devoid of quality control. Food gets contaminated through the storage material. This may include bagging of milled fermented maize for Ogi production, the bagging of milled maize is however fermented in water prior to use, during this process it could get contaminated by microbes in the water and putting prospective consumers of this food at risk of food borne illnesses (Sylvester *et al.,* 2016). Ogi processed by small holders lack control measures, no laid down step by step procedure for processing such as stating the quantity of water that could be added to certain weight or quantity of maize during fermentation. The fermentation period lies solely on individual and quality. The only quality control often carried out by producers is the organoleptic characteristics and to a great extent depends on the individual (Sylvester *et al.,* 2016).

Fermented foods are affected by temperature, water activity, hydrogen ion concentration (pH), oxygen availability and substrate used for the fermentation process of the food (Oyewale and Isah 2012) and to a large extent are influenced by their environmental factors, this could be controlled by manipulating the environmental factors where possible. Fermentation process for ogi involves the use of naturally occurring microorganisms to produce new products therefore; fermentation could be a functional and effective approach for curbing microbial contamination of this food product (Kohajdova and Karovicova 2007).

c) *Food borne illness*

Food borne illness is defined as diseases usually either infectious or toxic in nature, Caused by agents that enter the body through the ingestion of food (WHO, 2002).Governments all over the world are intensifying their efforts to improve food safety in response to an increasing number of food safety problems and rising consumer concerns (WHO, 2004). According to one report from the United States Department of Agriculture Economic Research Service, "food borne illnesses account for about 1 of every 100 U.S. hospitalizations and 1 of every 500 deaths" (Buzby *et al*., 2001). They also estimated that food borne illness triggered by just five food borne Pathogens - *Campylobacter, Salmonella, E. coli* 0157:H7, *Listeria monocytogenes* and *Toxoplasma gondii* - cause $6.9 billion in medical costs, lost productivity and premature deaths each year in the United States. The presence of bacteria is diverse and may be introduced in majority of post heating procedures. Water used in production of locally made foods has been identified as the major source of contamination (Okeke *et al*., 2000). The World Health

Organization (WHO, 2002) describes important population factors which could result in a high susceptibility to food borne infections. According to the WHO, age is an important factor because those at the extremes of age have either not developed or have partially lost protection from infection. People with a weakened immune system also become infected with food borne pathogens at lower doses which may not produce an adverse reaction in healthier persons.

Seriously ill persons, suffering, for example, from cancer or AIDS, are more susceptible to infections with *Salmonella, Campylobacter, Listeria, Toxoplasma, Cryptosporidium,* and other food borne pathogens. In developing countries reduced immunity due to poor nutritional status render people, particularly infants and children, more susceptible to food borne infections (WHO, 2002). Food borne illnesses can occur as isolated cases or constitute an outbreak, which can involve two to thousands of people and reach different states. Food borne outbreaks in recent years in the United States have been linked to the consumption of such food items as ground beef, cookie dough, peanut butter and jalapeno peppers (WHO, 2004). According to Altekruse *et al* (1999), some of the factors altering food borne disease patterns are the types of food that people eat, the sources of those foods, and the possible declining public awareness of safe food preparation practices.

In cases of suspected food borne illnesses drug resistance is now of serious concern in hospitals. Drug resistance is one of the natures ending process whereby organisms develop tolerance for new environmental condition. They may be due to pre-existing factor in the organisms or it may result from the acquired factors. Some naturally susceptible strains of bacteria may acquire resistance (Onifade *et al*., 2005).

d) Mechanism of pathogenesis of food pathogens

Bacterial Infections

The Majority of all cases of Food Poisoning are due to bacterial infections. These include:

i. *Salmonellosis*

Salmonella is a type of bacteria that cause typhoid fever and many other infections of intestinal origin (Clark, *2002). Salmonella* species are Gram-negative bacilli, most are motile with peritricious flagella, ferment glucose with the production of acid and gas or acid only. Some *Salmonella* produce H_2S (Jawetz and Adelberg 2004). Compared with other gram-negative rods, *Salmonella* is relatively resistant to various environmental factors; grow at temperatures between 8^{O}C and 45^{O}C and in a pH range of 4 to 8 (Adams and Moss 1995). *Salmonella* is often pathogenic to humans and animals. Humans, animals such as rodents and even insects such as flies may play an important role in spread of *Salmonella* bacteria especially from contaminated fecal matters to food. Large scale handling tends to increase spread of trouble and food prone to this bacterial infection are food held unrefrigerated for a long period of time (Center for Disease Control 1978). Infection results from the ingestion of food or water containing sufficient number of these bacteria to reach and invade the small intestine (Adams and Moss 1995). *Salmonella* produce three main types of disease in humans: Enteric fever (Typhoid fever), *Bacteremia* and *Enterocolitis*, but mixed forms are frequent (Jawetz and Adelberg 2004). Nontyphoidal strains of the genus *Salmonella* are estimated to be responsible for over 1.4 million illnesses and, subsequently, to account for 9.7% of total food borne illnesses, 25.6% of total hospitalizations, and 30.6% of deaths caused by known food borne pathogens (Mead et al., 1999) . *Salmonella* are associated with the intestinal tracts of animals and humans, and, although human illness has been associated with exposure to other vehicles of transmission (e.g., pets, and contaminated water), it is estimated that 95% of Salmonellosis cases involve food borne transmission (Tauxe, 1991). Most cases of Salmonellosis are considered to be endemic or sporadic because they are not clustered. The usual explanation for endemic cases is the inappropriate handling in kitchens and restaurants of contaminated food (including improper storage, undercooking, or cross contamination (Blaser, 2004). Salmonellosis is the most frequently occurring bacterial food infection and in some years the most frequently occurring bacterial food borne illnesses. This is from a report from Center for Disease Control within 1984-1986, *Salmonella* infection had highest number of cases and outbreaks from 1978-1982 and over time has not changed in comparism to other bacterial infection such as *Escherichia coli, Staphylococcus aureus*, *Shigella*, *Klebsiella*, *Proteus* e.t.c (CDC 1983b). Symptoms of Salmonellosis include fever, abdominal pain, diarrhea and vomiting caused by contaminated foods, contaminated beef meats, raw poultry; unwashed fruits; Vegetables grown in contaminated soils; Eggs; Rice.

The emergence of antimicrobial-resistant *Salmonella* has been a problem (Dabney *et al*., 1997). Multidrug-resistant (MDR) strains of *Salmonella* are now frequently occurring worldwide and the rates of multidrug-resistance have increased considerably in recent years. The incidence of multidrug resistance (MDR - resistance to three or more antibiotics) in *Salmonella* strains increased from 1.6% in 2005 to 2.1% in 2010 (Gordana *et al*., 2012). Even worse, some species of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (WHO, 2005).

ii. *Typhoid Fever*

Typhoid fever is a life threatening illness caused by *Salmonella typhi*. *Salmonella Typhi* lives only in humans; persons with typhoid fever carry the bacterium in their blood stream and intestinal tract .Typhoid fever is common in most parts of the world except in industrialized regions such as United States, Canada, Western Europe, Australia and Japan. According to WHO "Typhoid fever is still common in the developing world, where it affects about 12.5 million persons each year, (WHO, 2004).Patients with typhoid fever usually have a sustained fever as high as (39-40°C).They may also feel weak or have stomach pain, headache or loss of appetite. In some cases, patients have a rash of flat and rose- colored spots. The only way to know for sure if an illness is typhoid fever is to have samples of stool or blood tested for the presence of *Salmonella typhi*. The disease is prevented by: Avoiding foods and beverages from street vendors; avoiding unpasteurized milk and milk products; cook poultry and egg thoroughly; avoiding unwashed fruits.

iii. *Listeriosis*

Listeriosis is a bacterial infection. Two species of *Listeria* are pathogenic; *L. monocytogenes* infects humans and animals, (Ryan and Ray 2003) and *L. ivanovii* has been considered to infect ruminants only.

Listeria is one of the causes of food poisoning. It's triggered by *Listeria* bacteria that can live in soil, water, dust, animal poop, and other substances. One can get sick if they eat food that carries it. For most healthy people, the infection doesn't pose much of a threat, even if it makes you sick for a day or two. But for some people, the infection can be serious or even life-threatening, particularly pregnant women and their babies. Although a *Listeria* infection may cause only a mild illness in the mother, consequences for the baby may include: Miscarriage, Stillbirth, Premature birth, a potentially fatal infection after birth, people whose immune systems aren't working right, and seniors.

People pick up the infection most often from deli meats that aren't processed properly or from dairy products made from milk that isn't pasteurized in other words; the milk hasn't been heated to kill germs. Other common sources of outbreaks are: Cantaloupes, Hot dogs, Soft cheeses.

When infected with *Listeria*, the signs typically include: Diarrhea, Nausea, Achy muscles, Fever (Mahon *et al*., 2014).

They could appear a few days after one eats the bad food, or they might take a couple of months to show up. If the infection spreads to your nervous system, it's more serious. This severe form is called Listeriosis, it is fatal for 20% of people who have it. This happens most often with the very young, the very old, and people with weakened immune systems. The signs could be: Headache, Stiff neck, Confusion, Loss of balance, Convulsions. *Listeria* is ubiquitous and is primarily transmitted via the oral route after ingestion of contaminated food products, after which the organism penetrates the intestinal tract to cause systemic infections. The diagnosis of Listeriosis requires the isolation of the organism from the blood and/or the cerebrospinal fluid. Treatment includes prolonged administration of antibiotics, primarily ampicillin and gentamicin, to which the organism is usually susceptible (Swaminathan and Gerner 2007).

iv. *Campylobacteriosis*

Campylobacteriosis is an infection by the *Campylobacter* bacterium, most commonly *C. jejuni*. It is among the most common bacterial infections of humans, often a food borne illness. Occasional deaths occur in young, previously healthy individuals because of blood volume depletion (due to dehydration), and in persons who are elderly or immune compromised. The illness can also be caused by *C. coli* (also found in cattle, swine, and birds), *C. upsaliensis* (found in cats and dogs) and *C. lari* (present in sea birds in particular). It produces an inflammatory, sometimes bloody, diarrhea or dysentery syndrome, mostly including cramps, fever and pain.

The prodromal symptoms are fever, headache, and myalgia, which can be severe, lasting as long as 24 hours. After 1–5 days, typically, these are followed by diarrhea (as many as 10 watery, frequently bloody, bowel movements per day) or dysentery, cramps, abdominal pain, and fever as high as 40 °C (104 °F). In most people, the illness lasts for 2–10 days. Complications include toxic mega colon, dehydration and sepsis. Such complications generally occur in young children (< 1 year of age) and immune compromised people. A chronic course of the disease is possible; this disease process is likely to develop without a distinct acute phase. Chronic Campylobacteriosis features a long period of sub-febrile temperature and asthenia; eye damage, arthritis, endocarditis may develop if infection is untreated (Wilson *et al.,* 2008).

e) Factors contributing to the emergence of food borne illnesses

Outbreaks occur wherever pathogenic agents in sufficient number or quantity encounter a susceptible population without effective measures (Holt *et al.,* 1994).

1. *Genetic Variability*

The large genetic variability of microorganisms is the principal reason why so often some microorganisms survive after any unfavorable environmental change. Some strains are hyper mutable, which reinforces the potential for survival and have very short generation times (Holt *et al.,* 1994).

2. *Environment*

"Environmental factors also contribute to emergence of food borne illnesses; hot humid climates favor the growth of fungi and the production of mycotoxins. Human actions and behavior directly affects food safety. People are vectors for disease, traveling from place to another more rapidly than ever before. According to WHO, it is estimated that about 900.10 of all cases of *Salmonella* in Sweden are imported (WHO, 2002).

3. *Urbanization*

Urbanization is a major factor in emergence. Crowding increase human contact and chances for transmission particularly in developing countries where the health services are far away from the villages and farms, so there will be gab in reporting the cases of outbreaks and investigations or disease surveillance will be very low (Holt *et al.,* 1994).

4. *Economics*

War and economic collapse provide opportunities for disease outbreaks. The infrastructure that provides clean water, community medicine, disease surveillance, and food control of these are easily affected by economic disruption (WHO, 2004).

f) Impact of food safety in homes and public

Food safety is important for the people's general health and daily life, economic development, and social stability, and the government's and country's image (Rohr *et al.,* 2005). The potential impact of food safety outbreaks can be devastating. A single event of a food borne disease outbreak can bring unimaginable economic losses (Hussain, 2013).

g) Improving food safety in 21st century

Food is essential to life; hence food safety is a basic human right. Billons of people in the world are at risk of unsafe food. Many millions become sick while hundreds of thousand die yearly. The food chain starts from farm to fork/plate while challenges include microbial, chemical, personal and environmental hygiene (Rajul *et al.,* 2016). To ensure food safety and to prevent unnecessary food borne illnesses, rapid and accurate detection of pathogenic agents is essential. Innovative technology such as Nuclear Magnetic Resonance (NMR) coupled with nano particles can detect multiple target microbial pathogens' DNA or proteins using nucleic acids, antibodies and other biomarkers assays analysis. The food producers, distributors, handlers and vendors bear primary responsibility while consumers must remain vigilant and literate. Government agencies must enforce food safety laws to safeguard public and individual health. Medical providers must remain passionate to prevent food borne illnesses and may consider treating diseases with safe diet therapy under proper medical supervision. The intimate collaboration between all the stakeholders will ultimately ensure food safety in the 21st century (Fung *et al.,* 2018).

III. Materials and Methods

a) Materials

i. *Glass wares/Equipment*

The glass wares used include: Petri dishes, test tubes, conical flask, glass slides and cover slips, pasture pipette, sterile bottles, beakers, measuring cylinder, Bijou bottle.

The equipment used include: autoclave, Incubator, hot air oven, microscope and other laboratory tools such as Bunsen burner, weighing balance, colony counting chamber, spatula, test tube rack, wire loop, foil paper, wire gauze, forceps, and cotton wool.

ii. *Antibiotics*

The antibiotics used in this study include; Gram negative antibiotics - ampicillin, cefurorrixime, ceftazidime, chloramphenicol, sulphamethoxazole (trimethoprim), streptomycin, ciprofloxacin, gentamicin, amoxicillin/clavulanic acid, and ceftriaxone.

Most of this antibiotics act on both gram positive and gram negative bacteria (broad spectrum antibiotics), but are majorly gram negative antibiotics.

iii. *Media and reagents*

The media that were used include:

Nutrient agar: a general purpose nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms.

Salmonella shigella agar: is a selective and differential medium. It is used for the isolation, cultivation and differentiation of gram-negative enteric microorganisms

Deoxycholate citrate agar: It is particularly useful for the isolation of organisms that cause bacillary dysentery, *Salmonella* strains that cause food poisoning and *Salmonella Paratyphi*.

Manitol salt agar (MSA): commonly used, a selective and differential growth medium in microbiology, it inhibits the growth of some microorganisms while encouraging growth of others.

MacConkey agar: is an indicator, a selective and differential medium for bacterial. Designed to selectively isolate gram negative and enteric bacilli and differentiate them based on lactose fermentation.

Nutrient broth: is a general purpose medium used for general maintenance of cultures and routine work.

Eosine Methylene Blue Agar (EMB): a selective media for the isolation of *Escherichia coli*

Mueller Hinton broth: is recommended for dilution of antimicrobial susceptibility testing of all species of most commonly encountered aerobic and facultative anaerobic bacteria.

Mueller Hinton agar: agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Peptone water: is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride, is rich in tryptophan, used as a primary enrichment medium for the growth of bacteria.

Simon citrate agar: It is a defined, selective and differential medium that tests for an organism's ability to use citrate as a sole carbon source and ammonium ions as the sole nitrogen source.

MR.VP agar: used for The Methyl Red and Voges proskauer test.

Urease agar: is used to differentiate between rapidly positive Proteus species and other slower urea positive members of the *Enterobacteriaceae* and urease activity in other gram-negative organisms.

Crystal violet: it is the active ingredient in Gram's Stain, used to classify bacteria. The primary stain.

Lugos iodine: it is the active ingredient in Gram's Stain, used as the mordant.

Safranin: is used as a counter stain in some staining protocols, coloring all cell nuclei red.

Ethyl alcohol: is an important industrial chemical; it is used as a solvent, in the synthesis of other organic chemicals, and as an additive to automotive gasoline. Also used as a decolorizer in gram staining

Hydrogen peroxide: used as a reagent in catalase test.

b) Study Area

This work was carried out within Keffi metropolis of Nasarawa State. The microbiological analysis was carried out in the microbiological laboratory of Nasarawa State University Keffi. Keffi is located in the middle belt of Nigeria. It is geographically situated on the latitude 8°50°N longitude 7°52°E. Keffi town is on latitude 85° above sea level and it is in North West of Lafia, the state capital of Nasarawa State. It is 53km away from Abuja (Capital of Nigeria) in the guinea savannah of Nigeria (Akwa *et al*., 2007).

c) Collection of Samples

A total number of forty (40) samples were collected from Keffi and Angwan lambo markets in Nasarawa State. Ten (10) each of yellow and white garri types was randomly collected from the market and twenty (20) of fresh ogi was collected using sterile sampling bottles. The samples were appropriately labeled to indicate the name of the market, garri type (yellow and white), sample number, date and time of collection. Samples were transported in sterile laboratory box to the microbiology laboratory for analysis within one hour of collection.

d) Media preparation and Sterilization

The laboratory media used were prepared according to the manufacturer's instruction and were sterilized using autoclave at 121°C for 15 minutes when required.

e) Microbiological Analysis

One gram (1.0g) of each sample of Garri was homogenized in 9.0ml of sterile distilled water (10^{-1} dilution), further serial dilution of sample homogenate to 10^{-7} was carried out also in sterile distilled water, transferring 1ml of initial suspension into subsequent tubes used for the serial dilution. Approximately 0.2ml aliquot of appropriate dilution was spread on plates of *Salmonella-Shigella* agar, Eosin Methylene Blue agar (EMB), Mannitol salt agar (MSA) and MacConkey agar. All culture plates were incubated at 37°C aerobically for 24-48 hours. Culture plates were examined for enumeration and identification of colonies after incubation period.

i. *Coliform Test*

Aliquot 1g samples in test tubes with inverted Durham tubes in Lactose broth were incubated at 37°C for 24-48h. Tubes showing gas production and or color change of dye were reported as presumptive coliform test positive. These positive tubes were streaked out. Duplicate plates were made for confirmatory test. Plates were incubated for 24h at 37°C and 44°C respectively. Growth of characteristic colonies constituted a confirmatory test positive. Colonies from confirmatory test were Gram stained and inoculated into lactose broth for completed coliform test. Gas production and or color change of dye plus Gram negative non-spore bearing rod represent presence of coliform (Speck, 1976; Oranusi and Braide 2004). Absence of growth at 44°C indicated absence of fecal coliforms.

f) Identification of bacterial isolates

Bacterial isolates were characterized on the basis of the colonial morphology and biochemical characteristic. The colonial morphology that was observed for each isolate includes color, shape, elevation, colony surface and optical characteristics. The cellular morphology of isolates that were noted included Gram reaction and behavior, shape of cell, arrangement and spore staining where necessary. The biochemical tests that were carried out include citrate test, methyl red, indole test, catalase test, oxidase test, urease test and voges-poskauer test. Representative bacteria were characterized and identified as described by Holt *et al* (1994).

i. *Gram staining*

The gram staining for presumptive identification of the bacteria isolates was carried out as prescribed by Cheesbrough (2006). Briefly, a pure colony of each of the bacterial isolate was smeared on a slide containing a drop of normal saline on a clean grease free slide, and allowed to air dry and fixed by passing it over a Bunsen burner flame. The smear was stained with crystal violet (primary stain) for 1min and rinsed with water, further

staining with iodine (mordant) for 1 minute and rinsed with water. The slide was then briefly decolorized with 95% ethanol and rinsed with water and counterstained with safaranin for another 1 minute after which the slide was rinsed with water and allowed to air dry. The air dried slide was then examined using oil immersion objective lens. Red or pink reaction after gram staining indicated the presence of gram negative bacteria while purple color indicated the presence of gram positive bacteria.

ii. *Indole Test*

The indole test for the bacterial isolates was carried out as described by Cheesbrough (2006). Briefly a colony of each bacterium isolate was inoculated in 5ml of sterile peptone water in Bijou bottle and incubated at 37°C for 24 hours: after which four (4-15) drops of Kovac's indole reagent was added through the bottles side. Formation of red ring indicated indole positive test.

iii. *Citrate Utilization*

The citrate test for the bacterial isolates was carried out as earlier described by Cheesbrough (2006). A colony of bacteria isolate was inoculated by aseptically stabbing into Simon Citrate Agar slant and incubated at 37°C for 72hours. Formation of blue color indicated citrate positive and forest green color indicated citrate negative.

iv. *Catalase Test*

The catalase test for the bacterial isolates was carried out as earlier described by Cheesbrogh (2006). A colony of the bacteria isolates was emulsified in a drop of hydrogen peroxide on a clean grease free slide. Effervescence, indicating free oxygen as bubble signified the presence of catalase whereas no effervescence signified the absence of catalase.

v. *Oxidase Test*

The oxidase test for the bacterial isolate was carried out as earlier described by Cheesbrogh (2006). A piece of filter paper was placed in a Petri dish and 3 drops of freshly prepared oxidase reagent was added. Using a sterile glass rod, a colony of test organisms from a culture plate was removed and smeared on the filter paper. Oxidase positive organisms gave purple color within 5-10 seconds, and in oxidase negative organisms, color did not change.

vi. *Methyl Red/ Voges-Proskauer Test*

The Methyl Red/Voges Proskauer test for suspected organisms was carried out as follows; a pure colony of suspected organism was aseptically inoculated into 10ml of Methyl red/Voges Proskauer medium in Bijou bottles and incubated at 37°C for 48hr.The 48hr culture was divided into two portions. To the 1st portion some drops of Methyl Red indicator was added and formation of red color indicated Methyl Red positive. To the 2nd portion, a drop of 40% (w/v) KOH was added followed by some drops of β-napthol and formation of pink-red color indicated Voges-Proskauer positive.

vii. *Urease Test*

The urease test for the bacteria isolates was carried out as earlier described by Cheesbrogh (2006). The surface of a urea agar slant was streaked with a portion of a well- isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture. The cap was left on loosely and the tube was incubated at 35°-37°C in ambient air for 48 hours to 7 days. It was examined for the development of a pink color for as long as 7 days.

g) Disc diffusion antibiotics susceptibility test

In this test, wafers containing antibiotics are placed on an agar plate where bacteria had been inoculated, and the plate left to incubate at 37°c for 24hrs. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition. Once the zone diameter is measured it must be compared to a database of zone standards according to the CLSI standards to determine if the bacterium being studied is susceptible, moderately susceptible or resistant to the antibiotic in question.

IV. Results

In this study a total of 40 samples were collected from the markets (Main market and Angwan lambo market), 20 of Garri and 20 of Ogi. The microorganisms isolated were *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* species (Table 1). The percentage occurrence of isolates in garri from the markets was *E.coli* 15%, *S.aureus* 5%, *Salmonella* species 25% and *Klebsiella* species 0% (Table 2). The percentage occurrence of garri isolates based on garri type were *E.coli* 30% from white and 0% from yellow, *S.aureus* 10% from white and 0% from yellow, *Klebsiella* specie 0% in both yellow and white, *Salmonella* specie 10% in white and 40% in yellow (Table 3). In ogi, percentage occurrences of isolates were *E.coli* 45%, *Staphylococcus aureus* 30%, *Salmonella* species 65% and *Klebsiella* species 10% (Table 4). This study manifested bacterial contamination of samples of garri and ogi with *Salmonella* species being the predominant organism. A total of eighteen (18) isolates were gotten from both samples, thirteen (13) of ogi and five (5) of garri. The isolates were resistant to ciprofloxacin at 100%, ceftriaxone at 100%, ampicillin at 100%, amoxicillin at 100%, ceftazidime at 100%, cefurixime at 100%, streptomycin at 27.8%/ chloramphenicol at 27.8%, sulphamethoxazole at 61.1%, and gentamicin at 33.3% (Table 5).

Table 1: Cultural, morphological and biochemical characteristic of bacterial isolates of garri and ogi

Cultural characteristics		Morphological characteristics		Biochemical characteristics							
Shape	Pigment	Morphology	Gram Staining	IN	CAT	OX	MR	VP	COG	CIT	Presumptive Organisms
Circular	Greenish metallic sheen on EMB	Rod	-	-	+	-	+	-	-	-	*E.coli*
Circular	Yellow on MSA	Cocci	+	-	+	-	+	+	+	+	*S. aureus*
Smooth	Pink on MacConkey	Smooth	-	-	+	-	-	+		+	*Klebsiella* species
Circular	Blackish on SSA	Rod	-	-	+	-	+	+	+	+	*Salmonella* species

Where: IN-Indole, CAT-Catalase, OX-Oxidases, MR-Methylene red, VP-Voges proskauer, COG- coagulase, CIT- citrate, - = Negative, + = Positive, SSA- *Salmonella shigella* agar, EMB- Eosin Methylene Blue, MSA – Mannitol Salt Agar

Table 2: Percentage occurrence of bacterial isolates of garri sold in markets in keffi

Markets	Samples collected	Number of samples	*E.coli (%)*	*S,aureus (%)*	*Salmonella specie (%)*	*Klebsiella Specie (%)*
Main market (1)	A	5	0 (0.00)	0(0.00)	2(40.00)	0(0.00)
Main market (2)	B	5	2(40.00)	1(20.00)	1(20.00)	0(0.00)
Angwan lambu market (1)	A	5	0(0.00)	0(0.00)	2(40.00)	0(0.00)
Angwan lambu market (2)	B	5	1(0.00)	0(0.00)	0(0.00)	0(0.00)
Total		20	3(15.00)	1(5.00)	5(25.00)	0(0.00)

Key = A- yellow Garri, B- white Garri.

Table 3: Percentage occurrence of bacterial isolates based on the type of Garri from markets

Isolates	Garri types	
Bacteria isolates	White (%)	Yellow (%)
Escherichia coli	3 (30.00)	0(0.00)
Staphylococcus aureus	1(10.00)	0(0.00)
Salmonella specie	1(10.00)	4(0.00)
Klebsiella specie	0(0.00)	0(0.00)
Total	5 (25.00)	4(20.00)

Table 4: Percentage occurrence of bacterial isolates of Ogi sold in markets in Keffi

Markets	Number of samples	*E.coli (%)*	*S.aureus(%)*	*Salmonella* species*(%)*	*Klebsiella* species*(%)*
Main market (1)	5	4(80.00)	3(60.00)	5(100.00)	2(40.00)
Main market(2)	5	4(80.00)	2(40.00)	5(100.00)	0(0.00)
Angwan lambu(1)	5	1(20.00)	0(0.00)	1(20.00)	0(0.00)
Angwan lambu(1)	5	0(0.00)	1(20.00)	2(40.00)	0(0.00)
Total	20	9(45.00)	6(30.00)	13(65.00)	2(10.00)

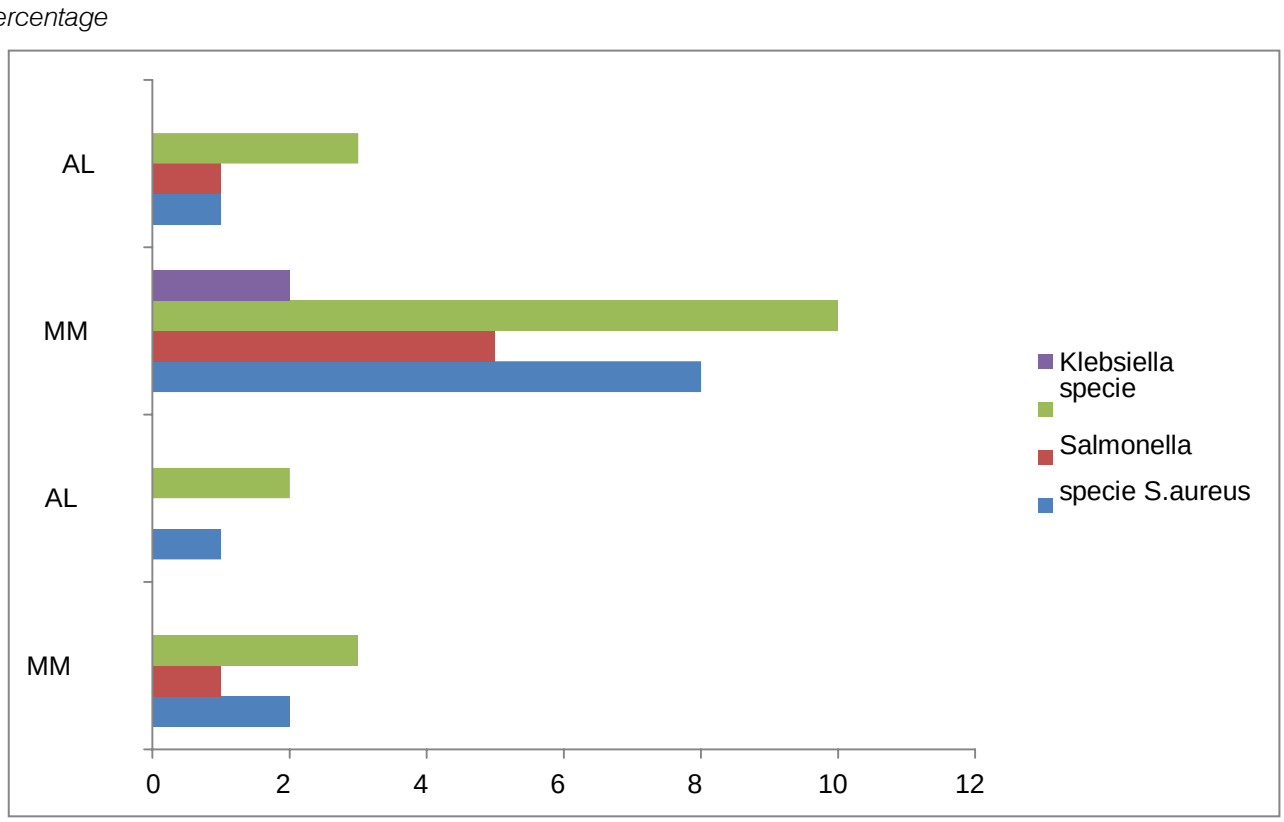

Fig. 6: Percentage occurrence of total bacterial isolates of garri and ogi from markets in Keffi. KEY= AL- Angwan lambo, MM- Main market

Table 5: Antibiotics susceptibility of *Salmonella* species isolated from Garri and Ogi

S/N	Antibiotics	Susceptibility (%)
1	CIP	0 (0.00)
2	S	13 (72.2)
3	C	13 (72.2)
4	CRO	0 (0.00)
5	AMP	0 (0.00)
6	CXM	0 (0.00)
7	CAZ	0 (0.00)
8	AUG	0 (0.00)
9	SXT	7 (38.9)
10	CN	6 (33.3)

Where: CIP- ciprofloxacin, S- streptomycin, C- chloramphenicol, CRO- ceftriaxone, AMP- ampicillin, CXM- cefurixime sodium, CAZ- ceftazidime, AUG- amoxicilin/ clavulanic acid, SXT-sulphamethoxazole, CN- gentamicin.

V. Discussion, Recommendation and Conclusion

a) Discussion

The study has made it evident that ogi had the highest bacterial contamination with *Salmonella* as the highest contaminant, followed by *E.coli, S.aureus* and *Klebsiella* specise, this microbial contamination can be attributed to the fact that Ogi is produced mainly by natives of the area and does not undergo any heat process like garri before final consumption. The presence of these organisms causes food spoilage, food poisoning or food intoxication in the food product this is in agreement with an earlier study by Awada *et al* (2005).

The differences in the level of contamination of garri and ogi can be as a result of the differences in their method of preparation, storage, handling during sales, fermentation. Garri during preparation or processing goes through heat at a temperature capable of distorting the activities of these bacteria, but ogi production does not undergo any of these processes (Ayehu *et al.,* 2014). Use of bare hands, unhygienic practices, use of contaminated utensils for processing and sales, poor personal and environmental hygiene, use of unsanitised or unsterilized packaging bags for sale of Ogi, use of contaminated water, contamination of raw material, and post processing handling are the major sources of contaminations. This tally with a study reported by Ezendianefo and Dimejesi (2014), who listed their risk factors as poor fermentation condition, poor personal and environmental hygiene post processing handling as well as contamination of raw materials.

Processing of the food is carried out by small holders who produce a large quantity for commercial purposes, most times rudimentary or un-standardized equipment are used for its production, Microbes are said to be wide spread and prevalent, this makes the

product prone however to unintentional contamination by microbes found in the environment.

Salmonella and *E.coli* were the predominant organisms at 65% and 45% in this product, this indicate recent fecal contaminations, they get into food through contaminated water, human, animal (rodents), and poor hygienic practices (Adams and Moss 1995). Hence, it can be deduced that the presence of this organisms has resulted from any of the above mentioned sources of contamination. This is in agreement with a study reported by Izah and Ineyougha (2015), they listed a group of microorganism including *Salmonella* and *E.coli* as species commonly found in different portable water sources in Nigeria such as lakes, boreholes, and hand dug wells e.t.c.

Oyelana and Coker (2012) studied the growth of *Klebsiella* specie and other organisms to significantly reduce at the end of fermentation hence, its occurrence according to them could have resulted from the water used for fermentation, it could also be a normal flora of the maize before fermentation.

Staphylococcus aureus was one of the bacteria isolated in this study, they may have found their way into this food product through carriers during sales; this organisms are found around the nose, throats, hands, and clothing's of the carriers (Cheesebrough, 2000). The storage procedures of this food product are mostly carried out in environment devoid quality control. Food gets contaminated by means of storage materials too and this include bagging, fermentation in water after bagging prior to use during which it could get contaminated by this microorganisms. This corroborates with a study reported by Sylvester *et al* (2016), the authors reported this in their study as a source of contamination which put prospective consumers of the product at risk of food borne illnesses.

Control measures are lacking in terms of ogi production by small holders; no laid down step by step procedure for processing such as stating the quantity of water that could be used for a certain weight and quantity of maize during fermentation. Fermentation period also lies on individuals and locality, the only quality control often carried out by producers is the organoleptic characteristics and to a great extent depends on individuals (Sylvester *et al*., 2016). Garri samples were also contaminated and *Salmonella* species was the predominant organism as well. This does not corroborate with a study reported by Almeida (1994) who showed that *Staphylococcus* spp had the highest rate of occurrence in garri. This also agrees with a report by Center for Disease Control, U.S who showed Salmonellosis as the most frequently occurring bacterial food borne infection. It was observed that there was more microbial contamination in white Garri than in yellow Garri, which may be due to the antimicrobial property of the oil added to the yellow Garri. This is in consonance with the work of Orji *et al.* (2014), but on a contrary in this study *Salmonella* contamination was higher in yellow Garri than in white Garri. The variation in these results can be explained by point of sale contamination that could have raised the *Salmonella* burden of the Yellow Garri in this study (Thoha *et al.,* 1890). This food borne pathogen have been marked as one of the organisms which has developed high resistance to antimicrobials. The emergence of antimicrobial-resistant *Salmonella* has been a problem (Dabney, 1997).

Garri contamination maybe associated with inadequate post processing, some post processing handling practices such as spreading on the mat, spreading on the floor after frying, open display in bowls and basins in the market, measurement with bare hands, coughing, sneezing while selling, and use of non-microbiological packaging material. Agboonlahor (1997) listed these factors as a source of contamination in a similar study. Typically it was observed that cassava for gari production is harvested manually in the farm with equipment such as cutlass, hoe and flat iron sheets, which occasionally inflicts injury on the root tuber which are later hauled in their numbers and heaped in the market for sales under humid and warm conditions, this practices predispose the tuber to contamination by organisms prior processing.

Garri may be contaminated during processing and production and contaminants may be killed when frying locally with a temperature ranging from 22-60^{0}c (Aseidu and Wieneke 1989) and using a modernized oven for 8hrs at 110^{0}c, Garri still gets contaminated after cooling during packaging and unhygienic practices during sales.

Bacteria load was high in samples collected in main market than in Angwan lambo market, this can be attributed to the fact that the main market is more populated and activities that will make these food products prone to contamination are high. The distribution of the organisms varied from both markets. The main market also harbors many waste dumps, muddy environment with stagnant water hence recorded more contamination than Angwan Lambo market which is a mini market with lesser population and activities. This is in line with findings made by Almeida (1994) who attributed variation in distribution of organisms to environmental condition and practice of the food handlers. Insects such as flies may play an important role in spread of these bacteria especially from contaminated fecal matters to food. (Center for Disease Control 1978).

The isolates showed resistance to cephalosporin's, quinolones and penicillin class of antibiotics, and susceptible to aminoglycoside class of antibiotics. This is not in agreement with a similar study reported by Bacon *et al* (2002) who reported that the *Salmonella* isolates were susceptible to the cephalosporin and quinolones class of antibiotics. This

could be as a result of the fact that this test organism developed resistant pattern over the years.

All isolates studied were found to be multi-drug resistant (MDR) as all of them were resistant to 60% of the antibiotics tested; this tally's with a report by Gordana *et al* (2012) who reported that multidrug-resistant (MDR) strains of *Salmonella* are now frequently occurring worldwide and the rates of multidrug-resistance have increased considerably in recent years.

Drug resistance is one of the nature ending processes, organisms develop tolerance for new environment which may be due to pre-existing factors in the organism or acquired factors. This bacterium is capable of inflicting economic losses such as cost of treatment, cost of disease investigation and control. Mead *et al.,* (1999) estimated *Salmonella* strains to be responsible for over 1.4million illnesses and subsequently accounted for 9.7% of total food borne illnesses and many cases of death.

b) Conclusion

This work on Garri and Ogi sold in Keffi markets showed that these food products are mostly unfit for consumption and present significant risk of food poisoning to consumers. This work should serve as a pointer for relevant agencies of government to carry out frequent checks and ensure compliance with best hygiene practices for the safety of food sold in the markets of Keffi. The enforcement of these measures could help reduce diseases related to contaminated food sold in the market. A strict application and implementation of quality control (QC), good manufacturing practices (GMP), quality assurance (QA) and hazard analysis critical control point principles (HACCP) will ensure the safety of Garri and Ogi consumed by the people in Nigeria. The emergence of antibiotics resistance suggests excessive use of antibiotics in human which has resulted to an increased risk to human health. Controlling the use of antibiotics wisely is needed to reduce spread of antibiotic resistant strains of microorganisms.

c) Recommendations

The following recommendations should be taken into consideration:

1. To protect against bacterial infection, heating food for at least 10mins to an internal temperature of 75^0c is recommended.
2. Sellers of Garri and Ogi and other food products should stop exposing their products but cover with a transparent poly-ethane bag to protect against contamination.
3. Sanitized containers should be used during production and sales.
4. Adequate inspections should also be carried out by public health sectors or services to ensure clean environment and hygienic practices during the sales of these food products.
5. The state and federal government should establish standard Garri and especially Ogi processing industries as Ogi is a common weaning food for children and as such should be prevented from contamination.
6. Sellers should also use gloves instead of their bare hands in sales of these products.
7. Good water sources should be made priority during production.
8. Sensitivity test for food infection should be conducted often in order to hinder administration of wrong drugs to affected persons.
9. The government through the ministries of water resources, health and education should collaborate with various public health organization and lunch a campaign in order to create awareness to smallholders, market women and even the public on the need for hygienic practices during production and sales of food products.
10. A hand wash station or site should be made set up and made compulsory in markets especially large markets in the country, with the availability of disinfectants and clean water for use often.

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Appendix I

1. Nutrient agar (oxoid CM3)

	gms/ltr
Agar	15.00
Peptone	5.00
Sodium chloride	5.00
Yeast extracts	2.00

Dissolve 28g in 1000ml of water completely and sterilize by autoclaving at 121^{0}c for 15 minutes

2. Nutrient broth (oxoid CM1)

	gms/ltr
Yeast extract	2.00
Lab-lemco powder	1.09
Peptone	5.00
Sodium chloride	5.0

Dissolve completely 13g in 1000ml 0f water and sterilize by autoclaving at 121^{0}c for 15 minutes.

3. MaCConkey agar (TM MEDIA)

	gms/lrt
Peptic digest animal tissue	20.00
Agar	12.00
Lactose	10.00
Bile salts	5.00
Neutral red	0.075
pH	7.4 at 25^{0}c

dissolve 47g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes.

4. EMB (L:S BIOTECH)

	gms/ltr
Peptic digest animal tissue	10.00
Dipotassium phosphate	2.00
Lactose	5.00
Sucrose	5.00
Eosin-Y	6.40
Methylene-blue	0.065
Agar	13.50
pH	7.2 at 25^{0}c

Dissolve 36g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes.

5. Simon citrate agar

	gms/ltr
Agar	15.00
Sodium chloride	5.00
Sodium sitrate	2.00
Dipotassium phosphate	1.00
Ammonium dyhydrogen	1.00
Magnesium sulphate	0.20
Bromothymol-blue	0.80
pH	6.8 at 25^{0}c

Dissolve 24g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes.

6. Indole test medium (HI-MEDIA)

	gms/ltr
peptone	25.00
sodium chloride	5.00

Dissolve 15g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes.

7. SSA (HI-MEDIA)

	gms/ltr
Proteose peptone	5.00
Lactose	10.00
Bile salts mixture	8.50
sodiumcitrate	8.50
sodium thiosulphate	8.50
ferric citrate	1.00
brilliant green	0.003
neutral red	0.25
agar	13.50
pH	7.0 at 25^{0}c

Dissolve 60g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes

8. Mueller Hinton Agar

	gms/ltr
Beef extract	2.00
Acid hydrolysate	17.00
Starch	1.50
Agar	17.00
pH	7.3 at 25^{0}c

Dissolve 38g in 1000ml of water and sterilize by autoclaving at 121^{0}c for 15 minutes.

Urea agar	gms/ltr
Peptone	1.00
Glucose	1.00
Sodium chloride	5.00
Disodium phosphate	1.20
Potassium dihydrogen phosphate	0.80
Phenol red	0.012
Agar	15.00

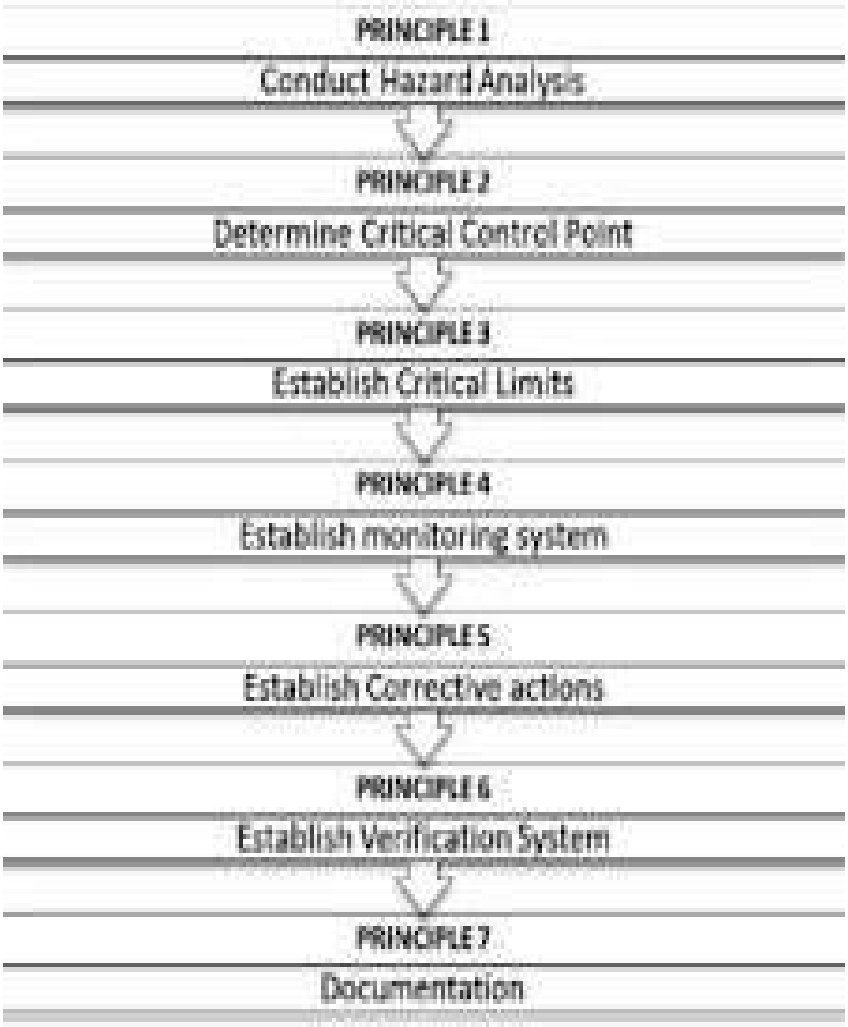
Appendix II

Table 6: Total bacteria isolate of Ogi and Garri from markets in Keffi (figure 6)

Markets	No. of samples	E.coli (%)	S.aureus (%)	Salmonella specie(%)	Klebsiella specie(%)
MM garri	10	2(20.00)	1(10.00)	3(30.00	0(0.00)
AL garri	10	1(10.00)	0(0.00)	2(20.00)	0(0.00)
MM ogi	10	8(80.00)	5(50.00)	10(100.00)	2(20.00)
AL ogi	10	1(10.00)	1(10.00)	3(30.00)	0(0.00)
Total	40	12(30.00)	7(17.50)	18(45.00)	2(5.00)

Keys: MM= main market, AL= angwan lambo market

Hazard Analysis Critical Control Point Principle



Appemdix III

Antibiotics Used

Antibiotics used	Abbreviation	Concentration(μg)	Company name
Chloramphenicol	C	30	Oxoid Ltd
Sulphamethoxazole	SXT	25	Oxoid Ltd
Gentamicin	CN	30	Oxoid Ltd
Ciprofloxacin	CIP	5	Oxoid Ltd
Ceftazidime	CAZ	30	Oxoid Ltd
Amoxicillin	AUG	30	Liofilchem roseto italy
Ampicillin	AMP	10	Oxoid Ltd
Cefurixime	CXM	30	Oxoid Ltd
Ceftriaxone	CRO	30	Oxoid Ltd
Streptomycin	S	30	Liofilchem roseto italy
Antibiotics Break Points			
Antibiotics	Break points (mm)		
Ciprofloxacin	≥ 31		
Streptomycin	≥15		
Chloramphenicol	≥18		
Ceftriaxone	≥23		
Ampicilin	≥22		
Cefurixime	≥18		
Ceftazidime	≥21		
Amoxicillin	≥18		
Sulphamethoxazole	≥16		
Gentamicin	≥15		

Antibiotic Susceptibillity Test

Isolates	CIP	S	C	CRO	AMP	CXM	CAZ	AUG	SXT	CN
1	R	S	R	R	R	R	R	R	R	R
2	R	S	S	R	R	R	R	R	R	R
3	R	S	R	R	R	R	R	R	S	S
4	R	S	S	R	R	R	R	R	R	R
5	R	R	S	R	R	R	R	R	R	R
6	R	S	S	R	R	R	R	R	R	R
7	R	S	S	R	R	R	R	R	S	R
8	R	S	S	R	R	R	R	R	R	S
9	R	R	R	R	R	R	R	R	R	R
10	R	R	S	R	R	R	R	R	R	S
11	R	S	R	R	R	R	R	R	R	R
12	R	R	S	R	R	R	R	R	R	R
13	R	S	S	R	R	R	R	R	S	S
14	R	S	S	R	R	R	R	R	S	S
15	R	R	S	R	R	R	R	R	S	R
16	R	S	S	R	R	R	R	R	S	R
17	R	S	S	R	R	R	R	R	S	S
18	R	S	R	R	R	R	R	R	R	R

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Effect of Rust (*Uromyces Phaseoli* Var. *Vignae*) Infection on Photosynthetic Efficiency, Growth and Yield Potentials of Cowpea (*Vigna Unguiculata* L. Walp) in an Open Field System

By S. M. A. Tagoe, T. A. Mensah & A. T. Asare

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Abstract- Cowpea rust fungus affects photosynthetic rate and physiological performance of the host plant through the induction of structural changes in the host cell. The study assessed the effects of rust fungus on net photosynthetic efficiency and yield performance of cowpea genotypes under field conditions. The experiment was conducted in the minor cropping season following a randomized complete block design outlay with three replications. Chlorophyll fluorescence of healthy leaves and rust-infected leaves of each cowpea genotype was determined by a non-destructive method. Rust disease severities of the infected leaves were assessed based on a diagrammatic scale. The effect of rust on the net photosynthetic rate was quantified by the model $Px / Po = (1 - x)^{\beta}$. Growth and yield traits of the cowpea genotypes and correlation between seed yield and photosynthetic efficiency were determined. The cowpea genotypes responded differently to rust infection as expressed in net photosynthetic rates, growth, and yield. The pathogen impaired photosynthetic efficiency of leaf tissue beyond the observed diseased leaf area. Seed yield was more closely related to the β estimate than that observed for the relative net photosynthetic rate.

Keywords: *cowpea; rust pathogen; disease severity; photosynthetic rate; growth; yield.*

GJSFR-C Classification: *FOR Code: 069999*

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Keywords: cowpea; rust pathogen; disease severity; photosynthetic rate; growth; yield.

I. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important leguminous food crop and plays a critical role in sustenance of about 194 million people in Africa (Abate et al., 2012; Dugje et al., 2009). In Ghana, cowpea is both food security and a cash crop (Rusike et al., 2013). More than 95% of the dry matter content of cowpea is obtained from photosynthesis (Bate and Canvin, 1971; Chapin III and Eviner, 2003; Ho, 1976). Cowpea is typically a C3 plant and therefore follows the C3 photosynthesis pathway (Kassman et al., 1991). Cowpea belongs to the crop stability group II of C3 plants and operates under warm conditions with a potential rate of photosynthesis (40–50 mg CO_2 $dm^{-2}h^{-2}$) greater than group I C3 crops (20–30 mg CO_2 $dm^{-2}h^{-2}$) such as common bean, barley, wheat, and oat (Kassman et al., 1991). C3 photosynthesis is a multi-step process and occurs in virtually all leaf mesophyll cells (Ehleringer and Cerling, 2002). The photosynthetic process is largely dependent on stomatal regulation and the amount of chlorophyll present (Brito et al., 2012; Evans, 2013). However, the concentration of chlorophyll may be influenced by responses to biotic and abiotic stresses (Hailemichael et al., 2016; Lobato et al., 2010; Sheikh et al., 2017). Variation in chlorophyll content of healthy and stressed plants provide valuable information about the physiological condition of the plant (Kamble et al., 2015; Sims and Gamon, 2002; Steele et al., 2008).

Cowpea rust fungus (*Uromyces phaseoli* var. *vignae*) is the most important biotic constraint to high yield and production of cowpea (Deshpand et al., 2010; Honnur et al., 2016; Uma et al., 2016). The fungus infects host leaves and is disseminated through the airborne urediniospores (Uma et al., 2016). The pathogen reduces foliar area and photosynthetic activity considerably in cowpea through destruction and reduction of most of the photosynthetic surface area, and interference with the photosynthetic activity of the healthy green parts of the host plant (Honnur et al., 2016; Newcombe, 2004; Singh, 2011). Toxins such as tentoxin and tabtoxin produced by rust fungus inhibit the activities of some enzymes involved directly or indirectly in photosynthesis in cowpea (Agrios, 2004). Cowpea rust fungus consumes photosynthetic products of host leaves and causes premature defoliation and mortality of young and mature plants, leading to reduced seed size and severe yield loss of 60–80% (Deshpand et al., 2010; Leonard and Szabo, 2005; Uma et al., 2016; Voegele and Mendgen, 2003). The effects of rust disease on cowpea is more severe in the tropical and subtropical regions of the world, particularly Africa, due to the dry, warm, and high humid conditions that are

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conducive for rust disease development and spore dissemination (Kumar and Narain, 2005).

Despite these, knowledge about the photosynthetic metabolism of cowpea cultivars infected with cowpea rust fungus is limited. Determination of the amount of chlorophyll is often employed by scientists to assess photosynthetic performance in vegetative plants. Destructive techniques have been conventionally used to determine leaf chlorophyll content in crops (Brito et al., 2012). The chlorophyll content of various plant species have been determined using 80% acetone (C_3H_6O) and magnesite ($MgCO_3$) powder followed by spectrophotometric analysis (Kamble et al., 2015; Sheikh et al., 2017). Makeen et al. (2007) estimated chlorophyll content in Black gram (*Vigna mungo* L.) using methanol and dimethyl sulfoxide (DMSO). Diethyl ether (DEE) and dimethylformamide (DMF) are highly recognized as best solvents for chlorophyll extraction (Etemadian et al., 2017; Stiegler et al., 2005; Sumanta et al., 2014). However, these techniques are very laborious, expensive, time-consuming, and involve destructive sampling, various procedural steps, and a high volume of solvent which leads to dilute or poor yield of chlorophyll (Kamble et al., 2015; Makeen et al., 2007). Complete extraction of chlorophyll is difficult to achieve in etiolated plants due to the presence of a low concentration of chlorophyll (Makeen et al., 2007). Besides, the use of solvents such as methanol, DMF, and DMSO is not recommended for chlorophyll extraction as the solvents are toxic to humans and easily absorbed by the skin (de Abreu Costa et al., 2017; Kadam et al., 2018; Long et al., 2001).

Chlorophyll fluorescence meters have been extensively used in research and agricultural applications to determine chlorophyll content of leaves because they are non-destructive to leaf tissue, portable, quick, and easy to use. They also have high accuracy and do not require specially trained personnel (Cate and Perkins, 2003; Chapman and Barreto, 1997; Ling et al., 2011). The plant photosynthesis meter (mini PPM-300, EARS) was used to determine leaf photosynthesis in this work. The meter uses a light emitting diode to elicit and recognize the fluorescence of chlorophyll (EARS, 2009; Fernandez-Jaramillo et al., 2012). The mini PPM-300 instantaneously measures fluorescence yield, photosynthesis yield, photosynthetically active radiation and photosynthetic rate of plant leaves based on quantification of light intensity wavelengths between 300 nm and 800 nm. The data generated is stored in the meter and can easily be assessed.

Although crop productivity is mainly determined by the efficiency of photosynthesis under a specific environmental condition and available resources, measurement of leaf photosynthesis does not necessarily predict improvement in crop growth and yield since the individual leaves used are not representative of the photosynthetic behavior of the entire crop canopy (Dutton et al., 1988; Elmore, 1980; Singh, 2011). A positive correlation between photosynthesis and productivity in crops, therefore, require a considerable sampling and assessment of various plant parts (Ambavaram et al., 2014; Jiang et al., 2017; Long et al., 2015; Silva et al., 2017). Components for determining cowpea growth and yields such as plant height, number of branches per plant, number of seeds per pod, pod length, and 100-seed weight have been adjudged as important selection traits for genetic improvement of the crop since they are moderate to highly heritable (Adigun et al., 2014; Nwofia et al., 2014).

Several studies have reported variability in cowpea growth and yield components, but the relationship of these parameters with photosynthetic performance in rust-infected leaves is limited (Afutu et al., 2016; Agyeman et al., 2014; Sharma et al., 2017; Shweta and Singh, 2018). The study showed the effect of cowpea rust infection on the efficiency of the net photosynthetic rate of cowpea genotypes based on the virtual lesion concept by Bastiaans (1991) and described the relationship between net photosynthetic efficiency and seed yield. The identification of promising cowpea genotypes with high yield potentials amidst rust infection is necessary to establish the appropriate agronomic manipulations for obtaining high yields in cowpea production, particularly in endemic rust areas in producing countries.

II. Materials and Methods

a) Study area

The study was conducted at the University of Cape Coast Teaching and Research Farm during the minor cropping season of 2017 because rust disease development in the area is generally higher in the minor season compared with the major season. The study area falls within the coastal savannah agro-ecological zone of Ghana and coordinated on latitude 05°08' N and longitude 01°18' W. The area was characterized by a mean temperature of 28.5 °C and rainfall of 545.50 mm during the study period.

b) Sowing of cowpea seeds

Twenty-four (24) cowpea genotypes comprising two local lines from the Savanna Agriculture Research Institute (SARI), six accessions from the International Institute of Tropical Agriculture (IITA), and 16 recombinant inbred lines (RILs) from the University of Cape Coast (UCC) were obtained from the Department of Molecular Biology and Biotechnology, UCC for the study (Table 1). The selected cowpea genotypes have different responses to rust infection (Mensah et al., 2019), maturity periods (55–75 days), and usage (grown for seeds and leaves). The genotypes have flower color of either violet, cream, violet-pink, or white. The seed coat color ranges from white, red, brown, cream,

mottled red, rough brown to speckled white. The experimental plot was cleared, demarcated, and divided into blocks at 1 m intervals. The blocks were further divided into subplots of 3 m x 3 m at 1 m interval between two subplots. The plot was sprayed with a 2% solution of Roundup weedicide (Monsanto Europe N.V.) after two weeks of weed emergence. One cowpea genotype was sowed in a subplot. The cowpea seeds were sowed at two seeds per hole at a spacing of 40 cm within rows and 60 cm between rows. The experiment was set up in a randomized complete block design outlay with three replications and was manually weeded at 3 and 6 weeks after seed germination.

Table 1: Cowpea genotypes used for evaluation of rust disease

Sl. No.	Genotype	Source	Response to rust	Sl. No.	Genotype	Source	Response to rust
1	UCC-11	UCC	Moderately resistant	13	UCC-490	UCC	Moderately resistant
2	UCC-24	UCC	Moderately resistant	14	UCC-513	UCC	Resistant
3	UCC-32	UCC	Moderately resistant	15	UCC-523	UCC	Resistant
4	UCC-153	UCC	Resistant	16	UCC-Early	UCC	Resistant
5	UCC-221	UCC	Resistant	17	Padi-Tuya	SARI	Moderately resistant
6	UCC-241	UCC	Moderately resistant	18	Apagbaala	SARI	Resistant
7	UCC-328	UCC	Resistant	19	IT08K-125-107	IITA	Resistant
8	UCC-366	UCC	Resistant	20	IT08K-193-14	IITA	Resistant
9	UCC-445	UCC	Moderately resistant	21	IT10K-817-3	IITA	Moderately susceptible
10	UCC-466	UCC	Resistant	22	IT10K-819-4	IITA	Moderately resistant
11	UCC-473	UCC	Resistant	23	IT10K-832-3	IITA	Resistant
12	UCC-484	UCC	Resistant	24	IT97K-499-35	IITA	Resistant

c) *Assessment of leaf photosynthetic rate in cowpea genotypes*

Net photosynthetic rates of cowpea genotypes at six weeks germination were determined with the miniPPM-300 photosynthesis system at mean light intensity of 400 μmol photons $m^{-2}s^{-1}$, photosynthetically active radiation (PAR) of 400–700 nm, temperature of 31 °C and relative humidity of 53%. Rust-infected and uninfected intact leaves in the uppermost layers of five cowpea plants excluding border plants were randomly selected per subplot. Rust-infected leaves showed the presence of pustules with different levels of severities while uninfected leaves showed absence of pustules on both abaxial and adaxial leaf surfaces. Three photosynthetic measurements were taken at 5 min intervals on each leaflet with an area of 7 cm^2. The net photosynthetic rate was calculated according to the model: $(1 - (F / Fm)) \times PAR$, where F is fluorescence yield, Fm is maximum fluorescence yield, and PAR is photosynthetically active radiation (Genty et al., 1989; van der Tol et al., 2014).

d) *Assessment of rust disease severity in cowpea genotypes*

The severity of rust disease on the infected leaves were measured to determine the effect of rust infection on photosynthetic rates of the genotypes. Rust severities were assessed by direct estimation using a diagrammatic scale by Godoy et al. (1997), where 0 = 0%, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% of the leaf area covered by pustules on the adaxial and abaxial leaf surfaces.

e) *Measurement of growth and yield parameters in cowpea genotypes*

The relationship between growth and yield parameters and mean β value of cowpea genotypes were assessed during the cropping season. The growth parameters were determined by measuring plant height and canopy diameter at six weeks germination. The number of branches per plant was obtained by a numerical count of individual branches emerging from the main stem of each sampled plant.

The yield parameters measured were the number of days to 50% flowering, number of pods per peduncle, pod length, number of seeds per pod, 100-seed weight, and seed yield. The number of days to 50% flowering for each cowpea genotype was determined by counting the number of days from seed sowing to 50% flowering of the plants. A numerical count of the number of pods per peduncle was done for each sampled plant at eight weeks germination. The dried cowpea pods on each subplot (excluding border plants) were harvested and air-dried. Fifteen pods were randomly selected from each subplot, and the lengths and number of seeds per pod were determined. All the pods were threshed, and 100 seeds of each cowpea genotype were randomly counted and weighed. The total seed yield of each cowpea genotype (kg ha^{-1}) was estimated.

f) *Data analysis*

The relationships between rust disease severities and photosynthetic rates were determined by Bastiaans (1991) model: $P_x / P_o = (1 - x)^{\beta}$, where P_x is net photosynthetic rate of a leaf with disease severity x, P_o is net photosynthetic rate of a healthy leaf, and β (Beta) is a ratio between virtual lesion area and visual lesion area. The value of β was determined using nonlinear regression in GenStat v16.1.0 (VSN International Ltd, UK). Two-sided *t*-test was used to compare β values with 1, and significant differences in β values were determined among cowpea genotypes.

Analyses of variance (ANOVA) were conducted to test variation in growth and yield components of cowpea genotypes, and significant differences in seed yield were separated with the Tukey test. The relationships between the relative net photosynthetic rates and seed yield of cowpea genotypes were performed by linear regression. Principal component analysis (PCA) biplot of the estimated β values, growth and yield traits was constructed to assess relatedness among the variables and distribution of cowpea genotypes under rust infection.

III. Results

a) Relationships between rust disease severity and leaf photosynthesis

Rust disease severity differed significantly ($P < 0.001$) with an average of 21.50 ± 7.33% among the cowpea genotypes. The highest disease severity (75.40 ± 3.97%) was observed in IT08K-817-3 (Table 2). In contrast, UCC-11 showed the lowest severity of 10.20 ± 3.34%. The net photosynthetic rate of healthy leaves and rust-infected leaves did not differ significantly ($P = 0.91$). In healthy leaves, the net photosynthetic rate ranged from 29.53 μmol $m^{-2}s^{-1}$ (IT08K-193-14) to 98.12 μmol $m^{-2}s^{-1}$ (Apagbaala). UCC-Early recorded the highest net photosynthetic rate of 78.80 μmol $m^{-2}s^{-1}$, whereas IT08K-193-14 recorded the lowest net photosynthetic rate of 28.74 μmol $m^{-2}s^{-1}$ in rust-infected leaves. Eleven cowpea genotypes (IT97K-499-35, IT10K-819-4, IT10K-832-3, UCC-221, UCC-241, UCC-366, UCC-473, UCC-490, UCC-513, UCC-523, and UCC-Early) showed higher net photosynthetic rates in rust-infected leaves compared with healthy leaves.

The model by Bastiaans (1991) effectively characterized the effect of rust disease on photosynthesis of the cowpea genotypes (Table 2). The estimated β values differed significantly ($P < 0.001$) from 1 with coefficient of determination (r^2) ranging between 0.88 and 0.98 (average of 0.94). Rust infection considerably reduced relative net photosynthetic rate ($\beta > 1$) than expected in all the cowpea genotypes except IT08K-817-3 ($\beta = 0.93 \pm 0.23$, $r^2 = 0.95$). The reduction was severest in IT97K-499-35 ($\beta = 9.94 \pm 1.74$, $r^2 = 0.93$). Similar observations were made in UCC-490 ($\beta = 9.53 \pm 1.08$, $r^2 = 0.97$), UCC-366 ($\beta = 9.52 \pm 1.56$, $r^2 = 0.92$), and UCC-473 ($\beta = 9.31 \pm 1.34$, $r^2 = 0.96$). High values of β ranging from 5.49 to 7.50 (average r^2 of 0.96) were found in six cowpea genotypes (Apagbaala, UCC-11, UCC-24, UCC-241, IT08K-193-14, and IT10K-832-3). Besides, regression analysis of relative net photosynthetic rates and rust severities for the 24 cowpea genotypes gave a significant β value of 2.98 ± 0.98 (Fig. 1).

Table 2: Effect of rust infection on net photosynthetic rates of cowpea genotypes on-field

Genotype	Severity (%)	Net photosynthetic rate		β	Standard error of β	r^2
		Healthy leaves	Rust-infected leaves			
UCC-11	10.20 ± 3.34	60.31 ± 13.86	49.27 ± 10.32	7.50	1.35	0.97
UCC-24	17.00 ± 4.65	52.39 ± 12.94	33.32 ± 9.34	6.09	0.94	0.96
UCC-32	20.10 ± 4.09	62.26 ± 9.84	55.15 ± 10.52	4.11	0.74	0.97
UCC-153	11.10 ± 4.83	58.02 ± 9.81	53.17 ± 10.59	2.27	0.57	0.96
UCC-221	17.30 ± 5.71	45.47 ± 10.42	63.88 ± 15.18	2.92	0.89	0.91
UCC-241	15.90 ± 5.75	37.21 ± 9.52	44.57 ± 10.36	5.49	0.96	0.94
UCC-328	53.00 ± 8.83	63.45 ± 15.71	38.97 ± 18.41	1.88	0.71	0.80
UCC-366	14.50 ± 4.82	31.07 ± 5.62	36.77 ± 10.74	9.52	1.56	0.92
UCC-445	11.10 ± 6.37	71.86 ± 15.83	46.85 ± 10.78	3.66	0.85	0.93
UCC-466	14.40 ± 4.80	53.38 ± 13.18	39.43 ± 8.93	3.19	0.81	0.92
UCC-484	19.70 ± 4.48	52.66 ± 11.17	51.80 ± 9.58	4.92	0.77	0.95
UCC-473	17.70 ± 2.75	33.25 ± 4.11	63.86 ± 16.56	9.31	1.34	0.96
UCC-490	16.30 ± 4.08	45.13 ± 7.90	52.56 ±10.93	9.53	1.08	0.97
UCC-513	16.30 ± 4.02	46.77 ± 10.09	68.86 ± 13.10	3.69	0.94	0.97
UCC-523	19.40 ± 7.28	46.00 ± 8.96	54.90 ± 7.54	2.52	0.78	0.93
UCC-Early	16.90 ± 5.34	55.47 ± 8.71	78.80 ± 13.26	3.33	0.74	0.91
Padi-Tuya	23.40 ± 5.71	65.54 ± 10.84	59.69 ± 9.06	4.48	0.71	0.97
Apagbaala	21.70 ± 5.73	98.12 ±20.06	58.68 ± 10.88	7.37	1.28	0.95
IT10K-125-107	15.00 ± 4.06	51.38 ± 6.78	32.52 ± 5.98	4.15	0.49	0.98
IT08K-193-14	16.40 ± 5.37	29.53 ± 7.14	28.74 ± 7.69	7.19	0.87	0.97
IT97K-499-35	14.20 ± 4.81	42.36 ± 8.03	64.95 ± 18.41	9.94	1.74	0.93
IT08K-817-3	75.40 ± 3.97	67.43 ± 9.90	58.56 ± 14.05	0.93	0.23	0.95
IT10K-819-4	46.00 ± 6.11	39.95 ± 7.91	71.29 ± 13.47	2.49	0.97	0.88
IT10K-832-3	13.10 ± 3.61	44.26 ± 10.70	55.37 ±6.94	5.85	0.99	0.97

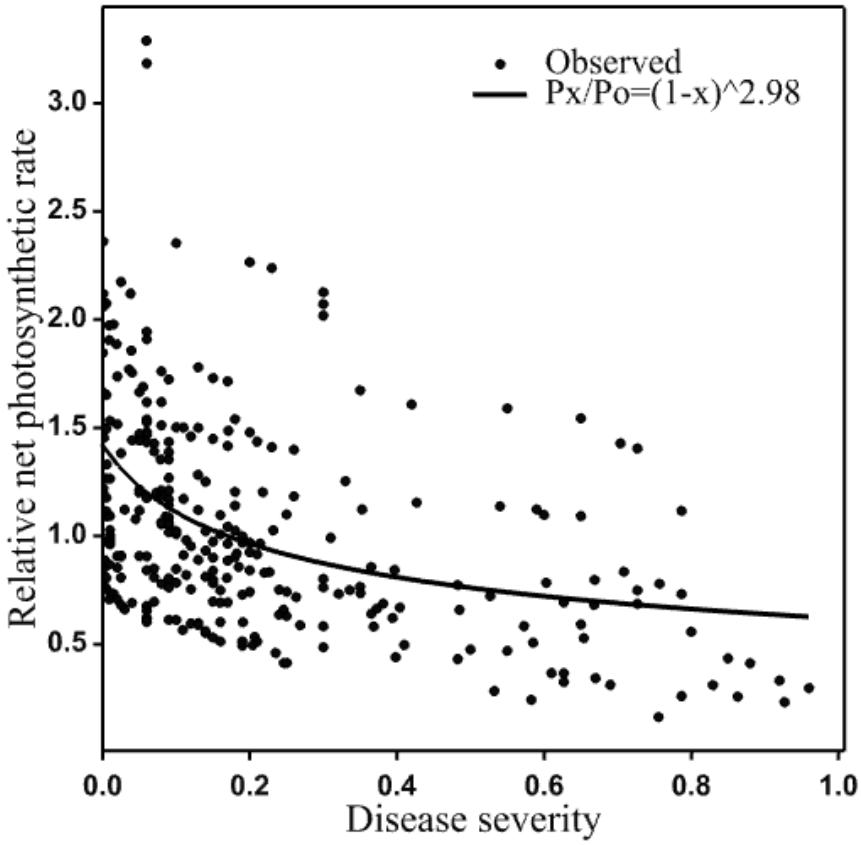

Fig. 1: Relative net photosynthetic rate of cowpea leaves in relation to rust disease severity

b) Effect of rust infection on growth and yield performance of cowpea genotypes under field conditions

Growth and yield parameters significantly differed ($P < 0.001$) among cowpea genotypes. Cowpea genotypes showed significant differences ($P \leq 0.017$) in plant height (30.85 ± 1.42 cm, $P \leq 0.001$), number of branches (3.79 ± 0.15 cm, $P \leq 0.001$), canopy diameter (95.14 ± 6.35 cm, $P = 0.017$), days to 50% flowering (41.12 ± 0.63 cm, $P \leq 0.001$), pod length (16.16 ± 0.34 cm, $P \leq 0.001$), number of seeds per pod (13.39 ± 0.23 cm, $P = 0.003$), 100-seed weight (17.04 ± 0.56 g, $P < 0.001$), and seed yield (850.14± 51.02 kg ha^{-1}, $P = 0.002$) (Table 3). However, there was no difference in number of pods per peduncle (2.06 ± 0.03, $P = 0.566$) among the cowpeas. UCC-Early recorded the highest plant height (46.10 ± 6.12 cm), whereas Apagbaala showed the lowest plant height (18.64 ± 1.46 cm). The highest number of branches (5.25 ± 0.73), canopy diameter (183.91 ±43.30 cm), and pod length (21.00 ± 1.70 cm) was found in UCC-466, UCC-366, and IT10K-819-4 respectively.

Days to 50% flowering was relatively low in UCC-490 (36.43 ± 0.08), UCC-221 (36.50 ± 0.00), UCC-Early (36.62 ± 2.19), UCC-32 (37.80 ± 0.00), and Apagbaala (37.00 ± 0.00) compared with IT08K-817-3(46.33 ± 0.33) and IT10K-819-4 (46.86 ± 3.48) (Table 3). Five cowpea genotypes (UCC-328, UCC-473, UCC-484, UCC-490, and UCC-513) gave the highest mean number of pods per peduncle (2.33 ± 0.33). The number of seeds per pod (15.00) was highest in UCC-11, UCC-Early, and IT97K-499-35. IT10K-819-4 showed the highest 100-seed weight (24.50 ± 1.19 g) followed by Padi-Tuya (23.50 ± 0.65 g). Padi-Tuya, however, recorded the highest seed yield (1,389.17 kg ha^{-1}). UCC-153, UCC-523, and UCC-32 similarly had high seed yield values of 1,330.00 kg ha^{-1}, 1,162.50 kg ha^{-1}, and 1,155.00 kg ha^{-1} respectively, whereas UCC-366, IT08K-193-14, and UCC-Early recorded low seed yield of 490.83 kg ha^{-1}, 504.17 kg ha^{-1}, and 539.17 kg ha^{-1} respectively.

Table 3: Comparison of growth and yield performance of cowpea genotypes infected with rust fungus on-field

Genotype	Plant Height (cm)	No. of Branching	Canopy Diameter (cm)	Days to 50% Flowering	No. of Pods/ peduncle	Pod Length (cm)	No. of Seeds/ pod	100-Seed Weight (g)	Seed Yield (kg ha^{-1})
UCC-11	34.13± 3.32	3.89± 0.59	111.13± 14.83	41.00± 0.00	2.00 ± 0.00	16.83 ± 0.78	15.00 ± 0.58	18.00 ± 0.41	1005.83^{abc}
UCC-24	32.11 ± 3.73	4.22± 0.43	55.12± 5.09	39.80± 0.00	2.00 ± 0.00	17.00 ± 0.32	12.67 ± 0.33	18.67 ± 0.33	801.25^{abc}
UCC-32	25.19 ± 1.56	4.22 ± 0.47	108.32± 27.31	37.80± 0.00	2.00 ± 0.00	15.97 ± 0.57	13.00 ± 0.00	20.00 ± 0.58	1155.00^{abc}
UCC-153	35.81 ± 2.31	2.67± 0.37	124.94± 36.64	38.30± 0.00	2.33 ± 0.33	16.63 ± 1.22	13.67 ± 1.33	17.00 ± 0.41	1330.00^{ab}
UCC-221	26.90 ± 2.05	3.78± 0.47	78.50± 7.50	36.50± 0.00	2.00 ± 0.00	16.23 ± 0.15	14.67 ± 0.33	16.50 ± 0.29	912.50^{abc}
UCC-241	28.42 ± 2.44	4.44± 0.44	85.10± 12.82	42.80± 0.00	2.00 ± 0.00	15.10 ± 0.58	12.67 ± 0.88	16.50 ± 0.87	682.50^{abc}
UCC-328	23.34 ± 1.97	4.89± 0.39	140.73± 45.39	41.80± 0.00	2.33 ± 0.33	15.50 ± 0.89	12.67 ± 1.20	16.75 ± 0.63	846.67^{abc}
UCC-366	30.47 ±2.85	4.56± 0.50	183.91± 43.30	41.80± 0.50	2.00 ± 0.00	14.33 ± 0.03	14.00 ± 0.58	15.00 ± 0.41	490.83^{c}
UCC-445	28.11 ± 3.27	3.67± 0.62	104.90± 23.17	40.80± 0.00	2.00 ± 0.00	14.57 ± 0.37	13.00 ± 0.58	17.75 ± 0.48	958.33^{abc}
UCC-466	26.75 ± 3.92	5.25± 0.73	135.36± 19.20	42.30± 0.00	1.67 ± 0.33	13.13 ± 0.09	11.00 ± 0.58	17.00 ± 0.41	649.17^{abc}
UCC-484	24.92 ± 0.83	4.44± 0.34	72.67± 18.47	41.36± 0.06	2.33 ± 0.33	14.90 ± 0.27	13.00 ± 0.58	14.25 ± 0.63	784.17^{abc}
UCC-473	28.46 ± 2.32	3.00± 0.50	65.13± 10.46	43.17 ± 0.17	2.33 ± 0.33	15.20 ± 0.87	11.00 ± 1.16	14.67 ± 0.88	728.75^{abc}
UCC-490	25.01 ± 2.44	3.11± 0.42	59.34± 4.44	36.43± 0.08	2.33 ± 0.33	15.17 ± 0.43	11.67 ± 0.89	16.25 ± 1.84	633.33^{abc}
UCC-513	28.46 ± 3.56	3.44± 0.48	79.19± 25.19	38.80± 0.00	2.00 ± 0.00	16.13 ± 0.92	13.00 ± 1.00	16.75 ± 0.25	1031.67^{abc}
UCC-523	31.24 ± 4.05	5.11± 0.56	105.04± 14.60	40.25± 0.25	2.00 ± 0.00	16.60 ± 0.67	14.00 ± 0.00	15.25 ± 0.48	1162.50^{abc}
UCC-Early	46.10 ± 6.12	2.78± 0.36	74.72± 15.01	36.62± 2.19	2.00 ± 0.00	18.47 ± 1.13	15.00 ± 0.58	13.25 ± 0.48	539.17^{bc}
Padi-Tuya	36.20 ± 3.39	3.33± 0.17	113.46± 29.74	44.26± 0.00	2.00 ± 0.00	18.77 ± 0.39	14.33 ± 0.33	23.50 ± 0.65	1389.17^{a}
Apagbaala	18.64 ± 1.46	4.33 ± 0.33	77.06 ± 16.35	37.00± 0.00	2.00 ± 0.00	14.60 ± 0.21	13.00 ± 0.00	14.00 ± 1.08	829.17^{abc}
IT10K-125-107	29.04 ± 1.86	3.44± 0.24	109.13± 32.74	42.85± 1.48	2.00 ± 0.00	17.40 ± 0.67	13.33 ± 0.33	19.25 ± 0.25	1065.83^{abc}
IT08K-193-14	25.31 ± 2.02	2.89± 0.42	109.56 ± 31.30	45.31 ± 2.03	2.00 ± 0.00	15.90 ± 0.55	13.33 ± 0.67	18.00 ± 1.00	504.17^{bc}
IT97K-499-35	29.09 ± 1.70	3.56± 0.69	94.46± 23.13	40.80± 0.00	2.00 ± 0.00	17.47 ± 0.89	15.00 ± 1.16	15.75 ± 0.63	632.50^{abc}
IT08K-817-3	43.16 ± 3.30	3.67± 0.24	62.83± 3.91	46.33± 0.33	2.00 ± 0.00	15.90 ± 0.61	14.00 ± 0.00	16.50 ± 0.50	895.00^{abc}
IT10K-819-4	45.06 ± 3.92	3.56± 0.41	70.78± 4.14	46.86± 3.48	2.00 ± 0.00	21.00 ± 1.70	13.67 ± 0.89	24.50 ± 1.19	790.83^{abc}
IT10K-832-3	38.51 ± 2.56	2.78± 0.62	61.97± 6.45	44.02± 2.32	2.00 ± 0.00	14.93 ± 0.64	14.67 ± 0.33	13.75 ± 0.63	585.00^{abc}
Mean	30.85	3.79	95.14	41.12	2.06	16.16	13.39	17.04	850.14
S.e. of mean ±	1.42	0.15	6.35	0.63	0.03	0.34	0.23	0.56	51.02
P value	<0.001	<0.001	0.017	<0.001	0.566	<0.001	0.003	<0.001	0.002

c) Relationship between relative net photosynthetic rate and seed yield of cowpea genotypes

The linear regression analysis revealed variations in the relationship between the relative net photosynthetic rate and seed yield of cowpea genotypes as shown by the coefficient of linear estimation (γ) (Table 4). A unit increase in the relative net photosynthetic rate decreased seed yield (-0.790 ± 1.260 <γ< -0.006 ± 0.139) in 11 cowpea genotypes. The highest effect was observed in IT08K-193-14 followed by UCC-24 (-0.726 ± 0.428). In contrast, an increase in the relative net photosynthetic rate increased seed yield in 13 cowpeas. The linear coefficient estimates were high in UCC-490 (γ = 0.554 ± 0.121), UCC-Early (γ = 0.533 ± 0.713), and UCC-241 (γ = 0.386 ± 0.193). Padi-Tuya (γ = 0.002 ± 0.086) and Apagbaala (γ = -0.006 ± 0.139) recorded the lowest γ estimates for reduction and increase in seed yield among the cowpea genotypes, respectively. The relative net photosynthetic rates in UCC-221 (γ = -0.077 ± 0.001) and UCC-473 (γ = -0.172 ± 0.012) accounted for the most variation (r^2 = 0.99) in seed yield with significant differences (P = 0.011 and 0.045). However, the relative net photosynthetic rates of seven cowpea genotypes (UCC-153, UCC-Early, Apagbaala, Padi-Tuya, IT10K-125-107, IT08K-193-14, and IT10K-819-4) were inconclusive (r^2 = 0.00) (Table 4).

Table 4: Linear regression analysis of relative net photosynthetic rate and seed yield of cowpea genotypes

Genotype	Y-intercept	γ	r^2
UCC-11	0.302 ± 0.089	0.059 ± 0.047	0.24
UCC-24	1.445 ± 0.701	-0.726 ± 0.428	0.48
UCC-32	0.612 ± 0.069	-0.119 ± 0.047	0.71
UCC-153	0.821 ± 0.333	-0.232 ± 0.257	0.00
UCC-221	0.428 ± 0.001	-0.077 ± 0.001*	0.99
UCC-241	-0.087 ± 0.187	0.386 ± 0.193	0.60
UCC-328	-0.026 ± 0.045	0.222 ± 0.025	0.97
UCC-366	0.374 ± 0.153	-0.175 ± 0.144	0.19
UCC-445	0.323 ± 0.017	0.032 ± 0.007	0.91
UCC-466	0.316 ± 0.013	-0.042 ± 0.009	0.91
UCC-484	0.081 ± 0.129	0.232 ± 0.123	0.56
UCC-473	0.379 ± 0.015	-0.172 ± 0.012*	0.99
UCC-490	-0.264 ± 0.116	0.554 ± 0.121	0.91
UCC-513	0.334 ± 0.009	0.114 ± 0.014	0.97
UCC-523	0.365 ± 0.031	0.117 ± 0.036	0.83

UCC-Early	-0.160 ± 0.512	0.533 ± 0.713	0.00
Padi-Tuya	0.553 ± 0.109	0.002 ± 0.086	0.00
Apagbaala	0.341 ± 0.219	-0.006 ± 0.139	0.00
IT10K-125-107	0.487 ± 0.382	-0.037 ± 0.235	0.00
IT08K-193-14	0.990 ± 1.250	-0.790 ± 1.260	0.00
IT97K-499-35	0.559 ± 0.059	-0.357 ± 0.065	0.94
IT08K-817-3	0.269 ± 0.041	0.044 ± 0.015	0.79
IT10K-819-4	0.133 ± 0.253	0.282 ± 0.365	0.00
IT10K-832-3	0.123 ± 0.098	0.140 ± 0.115	0.19

d) Principal component analysis (PCA) biplot of estimated β values, and growth and yield traits

Principal component analysis (PCA) biplot demarked cowpea genotypes with variations in traits explained by principal components 1 (PC1) and 2 (PC2) (Fig. 2). PC1 accounted for 29.21% of the variation observed in the cowpea genotypes. Pod length (PL) and plant height (PH) correlated most to this variation with values of 0.510 and 0.469, respectively (Data not shown). PC2 similarly explained 18.49% of the variation. This observation was mainly spanned by the number of branches (NB) and canopy diameter (CD) with values of -0.537 and -0.463, respectively (Data not shown). The β estimate contributed high values to the variations in PC1 (-0.279) and PC2 (0.341) and showed a significant ($P = 0.016$) negative linear relationship to seed yield. UCC-513 revealed average performance in each trait as well as β value with close relatedness to UCC-24 and UCC-221. Padi-Tuya and IT10K-819-4 genotypes showed similar performance but were most dissimilar to UCC-466, UCC-490, UCC-Early, and Apagbaala.

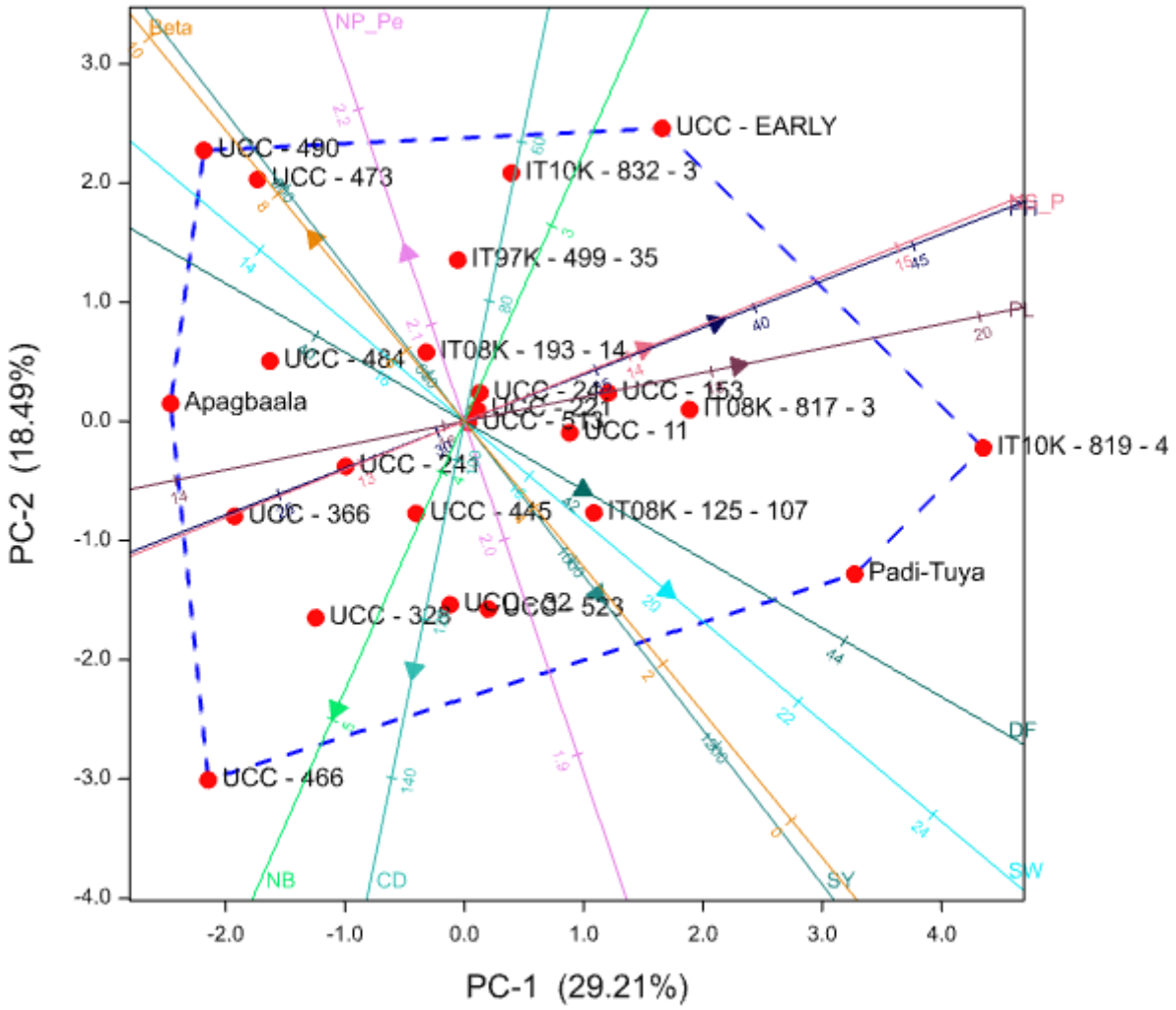

Fig. 2: Principal component analysis (PCA) biplot of estimated β values, growth and yield parameters of cowpea genotypes to open field rust infection

IV. Discussion

The extent of rust disease damage to cowpea depends on the level of resistance of the cowpea genotype to the pathogen in the face of favorable environmental conditions for the pathogen's development. Cowpea genotypes that were studied in this work showed varied responses to rust severity. The variations observed suggest that the cowpea genotypes have different levels of resistance and recovery rates to cowpea rust infection. This finding conforms to the study by Mensah et al. (2019), who reported that different cowpea genotypes respond differently to rust infection. Similar observations in rust disease severities of cowpeas have been reported (Edema and Adipala, 1995; Uma et al., 2015).

In the present study, cowpea genotypes showed varied responses in net photosynthetic rates of rust-infected and healthy leaves. The differences in net photosynthetic rates of the cowpea genotypes may be due to differences in anatomical, biochemical and physiological characteristics of the cowpea genotypes because photosynthetic efficiency of cowpea remain the same after development of rust pustules regardless of the stage of disease development, incubation temperature, phenological stage of the host, and cultivar-type (Bassanezi et al., 2001; Rivas et al., 2016; Santos et al., 2009; Wentworth et al., 2006). Despite these variations, there was a lack of significant difference in the average net photosynthetic rates recorded for rust-infected and healthy cowpea leaves. Plants are considered to accumulate dry matter in healthy areas of diseased leaves proportional to the amount of photosynthetically active radiation (PAR) that the healthy tissue intercepts (Robert et al., 2004). A ring of enhanced photosynthesis has been detected surrounding an area with decreased photosynthesis at the infection sites of *Albugo candida* and *Botrytis cinerea* in *Arabidopsis* and tomato leaves, respectively (Berger et al., 2007). Besides, rust infection may be latent within healthy leaves of cowpea genotypes. Livne (1964) observed no appreciable difference in photosynthetic rate between a heavily rust-infected and healthy bean leaves using radioactivity before a visible symptom of rust infection. Currently, it is not clear if this photosynthetic response in disease infected plants is due to the defense strategy of the plants (Berger et al., 2007).

Bastiaans' model, however, revealed that rust infection generally reduced the net photosynthetic rate ($\beta = 2.98$) of the asymptomatic leaf area of cowpea genotypes. The β value was similar to that reported for leaf rust of bean ($\beta = 2.17$) and wheat ($\beta = 2.20$) cultivars (Bassanezi et al., 2001; Robert et al., 2004). Whereas the reduction in photosynthesis to rust infection was higher ($\beta > 1$) in 23 cowpea genotypes, the photosynthetic efficiency in rust diseased leaf areas in IT08K-817-3 was functionally active ($\beta < 1$). The differential responses of the cowpea genotypes to the pathogen may account for the differences in estimated β values for the genotypes. Erickson et al. (2004) similarly observed larger disparity in β values (1.49 and 6.94) of two clones of hybrid poplar plants in response to the leaf spot pathogen, *Marssonina brunnea*. Pathogenic infection in plants generally leads to a reduction in photosynthetic efficiency (Kocal et al., 2008) as observed in this work. Similar observations were made by Bauer et al. (2000) and Zhao et al. (2011) on the infection of needle rust fungus, *Chrysomyxa rhododendri* in Norway spruce and orange rust fungus, *Puccinia kuehnii* in sugarcane plants. In contrast, Robert et al. (2005) showed that wheat leaf rust fungus (*Puccinia triticina*) did not affect the photosynthetic rate of symptomless parts of rust-infected wheat leaves.

The significant variations in plant height, number of branching, canopy diameter, days to 50% flowering, pod length, number of seeds per pod, 100-seed weight, and seed yield indicate existence of sufficient genetic variability in the cowpea germplasm and potential for improvement in cowpea production. Several studies have similarly observed significant differences in growth and yield components of cowpea genotypes, as observed in this study (Futuless and Bake, 2010; Kwaga, 2014; Manggoel and Uguru, 2011; Nwofia et al., 2014; Pethe et al., 2017). An earlier study has contrarily reported a significant difference in the number of pods per peduncle (Aliyu and Makinde, 2016).

These variations in growth and yield parameters may be due to differences in environmental conditions and inherent genetic characteristics of the cowpea genotypes (Badr et al., 2014; Basaran et al., 2011; Mafakheri et al., 2017; Sarath and Reshma, 2017). Xu et al. (2017) established genomic regions, cellular components, and gene regulatory basis as factors responsible for variations in pod length of cowpea cultivars. Several studies have shown that the magnitude of genotypic correlation coefficients was higher than their corresponding phenotypic and environmental correlation coefficients on growth and yield components of cowpea genotypes (Nwosu et al., 2013; Shweta and Singh, 2018; Umar et al., 2010).

All the cowpea genotypes were early flowering (< 45 days) except IT08K-817-3 and IT10K-819-4, which were late flowering (> 45 days). Earlier studies in cowpea have reported early flowering and late flowering genotypes (Manggoel and Uguru, 2011; Singh, 1993). The seed yields of the cowpea genotypes were low compared with the seed yields reported by Tettey et al. (2018) during the major cropping season in the coastal savannah agro-ecological zone. This variation may be due to the high mean temperature of 28.5 °C and rainfall of 545.5 mm observed in the present study compared with low mean temperature of 27.3 °C and rainfall of

171.0 mm reported during the study (Tettey, 2017), which influenced rust development and disease severity. A study on rust infection in cowpea genotypes in the coastal savannah agro-ecology reported higher rust disease incidence and severity in the minor season compared with the major season and concluded that temperature and rainfall are positively associated with cowpea rust disease incidence, severity, and area under disease progress curve (Mensah et al., 2019). Robert et al. (2004) indicated a significant reduction in seed yields of rust-infected wheat cultivars compared with healthy (control) wheat cultivars under field conditions.

Even though the effect of rust disease on photosynthesis was higher ($\beta > 1$) than the visual lesion in Padi-Tuya compared with IT08K-817-3 ($\beta < 1$), the genotype had the highest seed yield. Similar variations in the seed yield were recorded among the cowpea genotypes amidst the estimated β values. These differences may be due to genetic, morphological, and phenotypic variations among the cowpea genotypes. Moreover, stem photosynthesis may be important for biomass accumulation in rust-infected plants (Robert et al., 2004). Studies have identified quantitative trait loci underlying these physiological changes in photosynthetic efficiency, and growth and yield traits of cowpea genotypes to rust infection (Lo et al., 2018; Wu et al., 2018a; Wu et al., 2018b).

Rust infection in cowpea leads to significant losses in biomass yields due to a reduction in relative net photosynthetic rates (Deshpand et al., 2010; Staples, 2001; Voegele and Mendgen, 2003). Quantification of responses of cowpea genotypes to rust infection is, therefore, important to predict potential yield losses in the genotypes. Linear regression analysis of relative net photosynthetic rates and seed yields of cowpea genotypes showed that an increase in the relative net photosynthetic rates of UCC-473 and UCC-221 genotypes reduced seed yields by 7.7% and 17.2%, respectively. However, the modeling tool could not establish a relationship between variations in relative net photosynthetic rates and variations in seed yields of the remaining cowpea genotypes. This suggests that a relationship between relative net photosynthetic rate and seed yield in cowpea genotypes to rust infection may not be a direct effect but an interaction of factors. This lack of direct correlation has been noted by earlier studies and attributed to the single leaf measurement of photosynthetic efficiency at only one stage in the host's development other than photosynthetic efficiency of the whole crop over time (Evans, 1993; Long et al., 2006).

The distribution of the cowpea genotypes, as observed in the PCA biplot indicates a wide phenotypic variability, which was accounted for largely by UCC-466, UCC-490, UCC-Early, Apagbaala, and IT10K-819-4. UCC-513, UCC-221, and UCC-24 were the most stable cowpea genotypes with regards to the effect of rust infection on net photosynthetic rates (β value), and growth and yield parameters. The β value correlated to seed yield with a significant difference and revealed that an increase in β value decreases seed yield in cowpea genotypes. This observation, coupled with the regression analysis, confirms that variation in seed yield is stimulated by photosynthetic efficiency and an interaction variable in the face of rust infection. This interaction variable to photosynthetic efficiency has been noted by earlier studies as the available light energy, efficiency of light interception, and harvest index which collectively describe the physiological and structural properties of the host (Long et al., 2006; Simkin et al., 2019; Zhu et al., 2010).

V. Conclusions

Cowpea rust infection altered net photosynthetic rates, and growth and yield parameters in cowpea genotypes. The pathogen generally reduced net photosynthetic efficiency beyond the visible lesion areas on host leaves. The PCA biplot analysis using estimated β values revealed a significant negative relationship between rust disease severity and seed yields among the genotypes. Rust-resistant cowpea genotypes should, therefore, be used to control cowpea rust fungus. The β value could be used as an effective index to select for potential high-yielding genotypes, which could reduce the time frame and cost of breeding. UCC-24, UCC-241, and UCC-513 showed higher stability to rust infection and can be recommended to farmers for cultivation within the coastal savannah agro-ecological zone of Ghana. The high phenotypic variability associated with UCC-466, UCC-490, UCC-Early, Padi-Tuya, Apagbaala, and IT10K-819-4 can be explored in cowpea breeding programs to improve the crop.

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The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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

General style:

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

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

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

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

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

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

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

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

Results:

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

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

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

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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

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

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

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