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EKSU JOURNAL OF SCIENCE AND TECHNOLOGY (EJST)

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c. Manuscript should not exceed 15-20 pages.

d. Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution.

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Pelczar J. R. Harley J. P, Klein D. A. (1993) Microbiology: Concept and applications, McGraw-Hill Inc., New York, pp 591-603.

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Table of Contents

ASSESSMENT OF RESOURCE USE EFFICIENCY IN TOMATO PRODUCTION IN AYEDIRE LOCAL GOVERNMENT, OSUN STATE, NIGERIA.

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ABSTRACT

The study examined gender and resource use efficiency in Tomato production in Ayedire Local Government Area, Osun State, Nigeria. Multistage random sampling technique was used toselect 120 tomato farmers from the sampling frames in the Local Government Area. The farmers were interviewed using detailed questionnaire sets. Data collected were analysed using descriptive statistics, gross margin analysis and stochastic frontier model. The main findings showed that tomato farmers in the study area were predominantly Males (63.3%), Married(74.6%) and the mean age of the tomato farmers was (42 Years). Majority (66.4%) of the tomato farmers were educated with a mean household size of (9) persons per household. This study revealed that tomato farmers operated on a large scale of operations as shown by the mean farm size of 3.24 hectares. The mean of farming experience of the farmers was 12 years. There was a very weak institutional framework between tomato farmers and extension agents in the study area, only few (14.2%) of the tomato farmers had access to extension services. The profitability indicators show that tomato production was a profitable enterprise in South Western region of Nigeria. Three (3) profitability indicators estimated were, the Rate of Return on Investment(0.75), Earning per Naira Invested (1.74) and Rate of Return on Fixed Cost (5.96). The result shows that resources such as seeds of tomato, farm size, labour and herbicides were underutilized in the study area. While, fertilizer and fungicides were over utilized. Also, age, education status, access to credit and access to extension services were the factors determining the resource use efficiency of tomato farmers in the study area. Furthermore, the major constraints affecting the tomato farmers in the study area include; pest and diseases, perishability of tomato, unfavourable weather condition, poor storage mechanism and high cost of improved seeds. The study established that tomato production was highly profitable in the study area because the total revenue and total cost has a pristine difference, with high profit ratio and earning per naira invested (EPI). Also, age of the tomato farmers, household size, farm size, farming experience, practice of organic farming, pest infestation and extension services contacts were the factors determining the resource use efficiency. The study therefore recommends among others that female participation in tomato production should be encouraged by Implementing gender-sensitive agricultural policies and programs that specifically target female farmers. Tomato farmers should have access to training, and improved technologies to enhance their productivity and resource use efficiency. Formation of women's farmer groups to facilitate knowledge sharing, resource pooling, and access to markets should be enhanced and encouraged to address gender disparities in land ownership and access to credit to ensure equitable opportunities for female tomato farmers.

Key words: Assessment**,** Resource, Use, Efficiency, Tomato, Production.

1. Introduction

Tomato is an important vegetable crop that is widely cultivated all over the world (FAO, 2015) . In many developing countries, tomato cultivation is a major source of income for small-scale farmers, especially women (FAO, 2015). Women have been reported to play a crucial role in farming, processing, and marketing and it is estimated that over 60 percent of all agricultural production, processing, and marketing activities were carried out by women (Yusuf, 2015). The issue of gender differences in relation to farm productivity in subsistence farming has been of special interest from the standpoint of public policy in developing countries (Kabeer, 2016).

Women are key stakeholders in agriculture, yet they face numerous formidable obstacles (Sisay, 2016). Access to productive resources/inputs is an obstacle to agricultural growth in Africa, thus access to productive resources such as land, modern inputs, technology, education and financial services is a critical determinant of agricultural productivity (FAO, 2015). Resource use efficiency is an important aspect of agricultural productivity that refers to the amount of output obtained per unit of input used. Therefore, the role of rural women in agricultural development draws not only the attention of the academics but also the policy makers. The issue of gender in agriculture has had an increasing interest for many researchers and investigators over the years and across the globe because of the debate on the role of women in economic development, Thus the analysis of women participation in agricultural activities such as irrigated and non - irrigated vegetable production is important and cannot be over emphasized in their contribution to the Gross Domestic Product (GDP) (Karim, *et al.*, 2016).

Across Sub-Saharan Africa, several empirical studies have found that female farmers have lower yields than male farmers (Larson, *et al.*, 2015). Their access to technology, information, and agricultural extension tends to be more limited compared to men (Bravo-Monroy, *et al*., 2016). In growing crops, women are more prone to be constrained in their access to inputs, resulting in lower levels of fertilizer, labour, and other inputs than is optimal (Cadzow, 2016).

Nigeria is an agrarian country with about 70 percent of the population engaged in agricultural production (Yusuf *et al.*, 2016). However, the efficiency of tomato production is often affected by gender disparities in access to resources such as land, credit, inputs, and labour (Oladeebo and Fajuyigbe, 2017). However, a body of empirical evidence from many different countries shows that female farmers are just as efficient as their male counterparts, but they have fewer resources, resulting in

inadequate use of resources, limited alternatives and low income so they produce less (Yusuf, 2015).

Ayinde *et al.*, (2013a) opined that, it is of importance to have strategy to put men and women's concerns and experiences at the centre of research design, implementation, monitoring, and evaluation. Bridging the gap in access to technology between men and women, we could increase productivity; Ayinde *et al.*, (2013b) further affirmed that technological adoption among male and female farmers is crucial to improving the productivity.

The gendered nature of resource access and use can have a significant impact on resource use efficiency in tomato production. Furthermore, there are gender differences in levels of efficiency in resource management in agricultural production (Nwaru, 2013). Invariably, this could affect technology adoption, utilization and outputs of various farmers groups. This study therefore examined the gender-related factors influencing the resource use efficiency in smallholder tomato farms in Ayedire Local Government Area of Osun State, Nigeria.

1.1 Problem statement Agricultural production is the primary source of Nigeria's economic growth (Akinboro, 2014). The UNFAO, (2010) stressed the importance of agriculture to the livelihoods of many millions of poor people. Nigerian agriculture is dominated by small-scale farmers who produce the bulk of food requirements in the country (FAO, 2014).

Despite their unique and pivotal position, the small farmers belong to the poorest segment of the population and therefore, cannot invest much in their farms. The vicious circle of poverty among these farmers has led to the unimpressive performance of the agricultural sector. According to Ajibefun and Daramola, (2013), resources must be used much more efficiently, with more attention paid to an increase in productivity and income. Gender disparities in resource access and use can lead to sub-optimal resource use efficiency in tomato production, which in turn can impact the profitability and sustainability of small-scale farming systems (Oladeebo and Fajuyigbe, 2017). Women farmers often have limited access to productive resources and face greater constraints in terms of credit, land tenure, and labour availability, which can reduce their ability to efficiently utilize inputs and achieve optimal yields (Nwaru, 2013). Additional research has contributed to the debate surrounding gender and agricultural productivity, most existing studies used household headship as a gender indicator, again with mixed findings. Three studies in Ethiopia found female-headed households have persistently lower productivity measures compared to their male-

headed counterparts (Sisay,2016). However, Thapa, (2018) discovered no significant productivity differences by gender of household head in the Gambia and Nepal, after controlling for other inputs.

Oladeebo and Fajuyigbe, (2017), hypothesized that there are differences in the productivity of men and women farmers. Such differences are likely because men and women within the African rural households pursue their own activities both on and off the farm. They also have different endowments, strengths, access, and adoption to technologies, factors of production, etc which enhance the efficiency of production.

There is a dearth of gender-disaggregatedresearch and documentation data in tomato production, especially viz-a-vis accessibility and utilization of resources. It is, therefore, necessary to assess gender accessibility to resources among tomato farmers in this study area to establish the benchmark for developing strategies for promoting gender equity in the accessibility and use of resources, involving tomato farmers in the area. This becomes imperative to conduct this research to evaluate gender differences in resource utilization among tomato farmers in the study area. This study will attempt to answer the following research questions:

i. What are the socio-economic characteristics of the tomato farmers?
ii. What are the gender patterns of resourceuse in tomato production?
iii. What is the cost, return, and profitability of tomato production?
iv. What are the factors influencing resource use efficiency in tomato production?
v. What are the constraints to tomato production in the study area?

1.2 Objectives of the Study

The main objective of this study was to investigate the gendered nature of resource use efficiency in tomato productionin Ayedire Local Government Area of Osun State, Nigeria. The specific objectives of the study include:

i. describe the socio-economic characteristics of the tomato farmers.
ii. assessing the gendered patterns of resource access and use in tomato production.
iii. estimate the profitability of tomato production in the study area.
iv. analyze the factors that influence resource use efficiency in tomato production, with a focus on gender-related factors.
v. identify the constraints to tomato production in the study area.

1.3 Justification

Chukwuji and Oyaide (2005) reported that income per head and technical efficiency were not significantly different for men and women, Ohajianya and Onyenweaku (2011) reported from their profit function analysis that there were no significant differences in economic efficiencies of male and female rice farmers in Ebonyi State of Nigeria. None of these reports considered tomato production which is a major vegetable crop of great importance in the State. The beauty of empirical studies using the stochastic frontier model to estimate production efficiency among male and female tomato farmers in this part of Nigeria gives further justification to this attempt.

2. METHODOLOGY

2.1 The Study Area

The study was conducted in Ayedire Local Government Area of Osun State. Osun State is located in Southwestern Nigeria and its geographic coordinates are approximately 7.5 degrees North latitude and 4.5 degrees East longitude. The state is one of the six states comprising south-western Nigeria and it was created in 1991 from the Eastern part of Oyo State with its capital located in Osogbo. The state has a land area of 9251 km2 (about 0.93 million hectares) and a population of about 4 million people (NPC, 2006). The state is situated entirely within the tropics and is suited to produce permanent crops such as cocoa, coffee, and oil palm and arable crops such as maize, yam, cassava, and rice. The annual rainfall is between 1000 mm and 1500mm with daily temperatures ranging between 280 C and 300 C. The economy of Osun State is largely based on agriculture, with the state being a significant producer of crops such as cocoa, palm oil, cassava, yam, maize, and vegetables. The State also has a growing presence in the mining sector, with significant deposits of gold, tantalite, granite, and talc.

Ayedire Local Government Area is one of the 30 Local Government Areas in Osun State. It is in the northern part of the state and shares borders with Oyo State to the North and Kwara State to the East. Ayedire Local Government Area has an area of 1,077 square kilometers and a population of over 60,000 people. The local government area is divided into several towns and villages, including Ile Ogbo, Akindele, Gbongan, Obaagun, Aba Igbira, Kuta, and Aba Nla. The major occupation of the people is farming, and the area is known for its crop production. Overall, Ayedire Local Government Area is vibrant and culturally rich part of Osun State, with a strong focus on agriculture and a growing infrastructure.

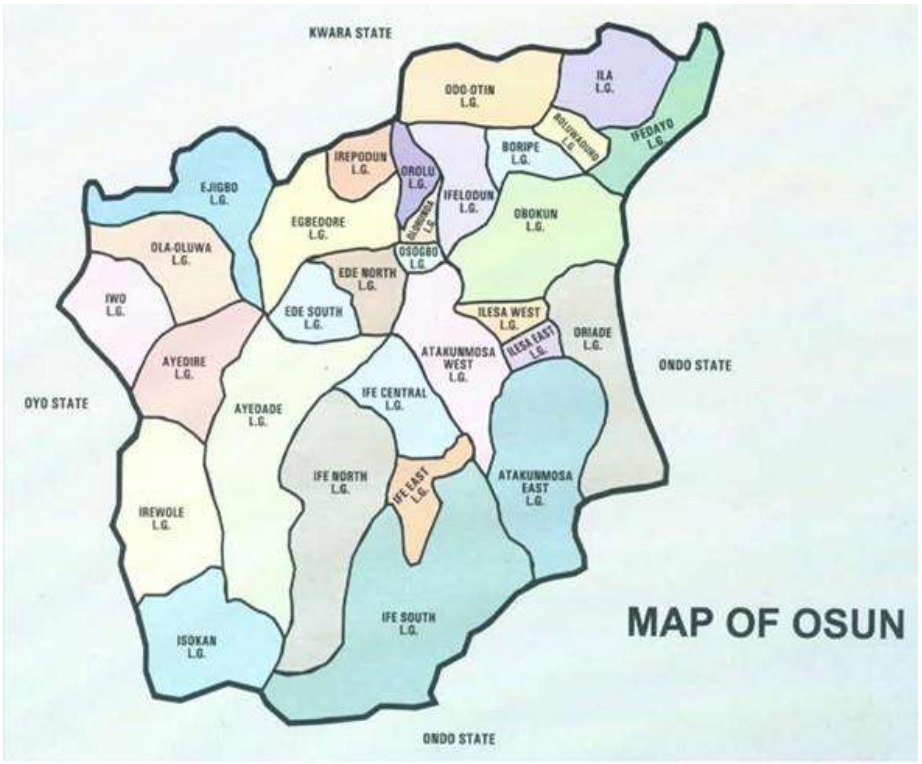

Figure-1. Map showing the location of Ayedire Local Government Area within Osun State.

Source: Wikipedia.org

2.2 Sampling Technique

Three-phase multistage sampling method was used for the study. The first stage involved the purposive selection of Ayedire Local Government area based on the a priori knowledge that the LGAs is producing tomatoes in both the rainforest and savannah agro-ecologies of Osun State and both men and women are actively involved in it. The second stage involved a random selection of Six (6) communities from Ayedire Local Government Areas. The last stage involved the selection of 20 tomato farmers including men and women household heads from each of the Six (6) communities using the snowball technique. This gives a total of 120 tomato farmers.

2.3 Data Analysis

Data for analysis were generated primarily using interview scheduled and s t ructured questionnaires administered to one hundred and twenty (120) respondents selected for the study. Data analysis was achieved through the use of descriptive statistics, budgetary analysis, and Cobb-Douglas stochastic frontier production function

.

2.4 Analytical Technique

Data for the study were analyzed using both descriptive and inferential statistics. Objectives (i) and (ii) were analyzed using descriptive statistics such as mean, percentages and frequency distribution. Objective (iii) was analyzed using Budgetary Analysis Technique. Objective (iv) was analyzed using Cobb-Douglas stochastic frontier production function

2.5 Model Specifications

2.6 Budgetary Analysis Technique

Budgetary Analysis was used to analyze this objective. In order to know the cost implications, returns, and profit on tomato production, this technique was used to compute the cost and returns in tomato production in the study area. The budgetary analysis (Gross Margin Analysis) was used by Henri-Ukoka *et.al.*,(2015) to analyze the Net Farm Income, Gross Margin, and Cost-Benefit Ratio. The budgetary technique which estimates the financial outcome and profitability of farm enterprise was used to determine and analyze the cost and returns to factors of production of the tomato farmers.

$$GM = \sum_{i=1}^{n} P_i Q_i - \sum_{j=1}^{m} C_j X_j \dots\dots\dots\dots\dots\dots (1)$$

Where :

GM = Farm Gross Margin (?)

P_i = Unit farm gate price of output i (?)

Q = Quantity of output for crop; (kg)

C_j = Unit price of variable input (?)

X_j = Quantity of variable input j

i = Crop and n is the total number of cultivated crops

j = Variable input and m is the total number of the variable input used in the farm enterprise

Thus,

Gross Margin (GM) = TR – TVC..... (2)

TR = Price X Quantity

TC = TFC + TVC

GM = TR – TVC

NI = GM – TFC

Where,

TR = Total Revenue

TVC = Total Variable Cost

NI = Net Income

TC = Total Cost

TFC = Total Fixed Cost.

And,

Gross Ratio = TR/TC

2.7 Cobb-Douglas stochastic frontier production function

Several studies from both developing and developed countries have used the Cobb Douglas functional form to analyze farm efficiency (Coelli, 1996). The model is represented as:

$Y = f(X_1, X_2, X_3, X_4 \dots X_6 + Vi - Ui)$ .....(1)

This is defined as follows:

$InY_1 = b_o + b_1InX_1 + b_2InX_2 + b_3InX_3 + b_4InX_4 + b_5InX_5 + b_6InX_6 + Vi-$

Ui… ..... (2)

Where;

In = Logarithm to base e

Yi = Output of Tomato (Kg)

X_1 = Age (Years)

X_2 = Marital Status

X_3 = Household Size (Number)

X_4 = farm size (Ha)

X_5 = Tomato seeds (Kg)

X_6 = labour (Man days)

X_7 = fertilizer (Kg)

X_8 = capital (Naira)

X_9 = other inputs (Kg)

V_i = a symmetric error term which accounts for random variations in output due to factors beyond the control of the farmer.

$b_o, b_1, b_2, b_3, b_4, b_5$ and b_6 are regression

parameters to be estimated

U_i = a non- negative random variable representing inefficiency in production relative to the stochastic frontier. In order to determine the factors contributing to the technical efficiency the following model was formulated and estimate jointly with equation (2) in a single stage by the methods of maximum likelihood using the computer program FRONTIER 4.1 (Coelli, 1996):

$$TE_i = 0 + 1Z_1 + 2Z_2 + 3Z_3 + 4Z_4 + \ldots + 10Z_{10}$$

Where; TE_i = the technical efficiency of the farmer

Z_1 = ownership of land (Ha)

Z_2 = household size (number)

Z_3 = membership of cooperative/farmers' associations (number)

Z_4 = contact with extension agent (number)

Z_5 = age (years)

Z_6 = marital status (dummy variable; 1 for married, 0 otherwise)

Z_7 = Educational status (number of years spent in school)

Z_8 = access to credit (dummy variable; 1 for access, 0 otherwise)

Z_9 = farming experience (years)

Z_{10} = farm size (Ha)

0 = the intercept 1, 2, 3, 4, 5,…..... 10 are parameters to be estimated.

3. Result and Discussion

3.1 Socio-economic characteristics

The socio-economic characteristics of the respondents considered were gender distribution, age , marital status, family type, household size, farm size, educational status, years of experience, primary occupation, access to extension services, source of capital, experience in pigeon pea production, membership of cooperative associations, access to extension services, System of cropping, planting variety, pigeon pea improved variety, and disease resistant.

The study recognizes and gives equal consideration to both genders during the data collection. The tomato farmers were predominantly males in the study area. The result shows that the majority (63.3%) of the tomato farmers were males, while the remaining (36.7%) were females during the period under consideration.

The gender distribution of the tomato farmers indicated that the farming activities are less attractive to females fold due to the energy-demanding nature of manual farm work.

The age of tomato farmers varied between a minimum of 23 years and a maximum of 60 years. The mean age was 42 years, which simply implies that tomato farmers in this region were still in the economically active period of their lives. Significantly, this will improve their productivity, profitability, and efficiency of agricultural labour use. This equally agreed with many other findings that the mean age of farmers in Southwest, Nigeria is between 40 and 50 years. In Sekumade, *et.,al.* (2014) findings, mean ages of 46, 50.5, and 45.8 years were recorded for

farmers in Ekiti, Osun, and Oyo States respectively.

The result showed that 40.1% of the tomato farmers who were predominantly males were aged above the mean age of 42 years while only about 26.3% of them, predominantly males, were aged below the mean age of 42 years in the study area. This generally implies that tomato farmers in this study area were adults but still active physically and economically. If properly harnessed, the age of tomato farmers in this study area will contribute to their efficiency and optimal performance.

The distribution of the tomato farmers by marital status showed that the majority (74.6%) were married and still living with their spouses during the period of this study. The percentage of unmarried or single tomato farmers who were predominantly males was (18.9%) indicating that only an infinitesimal population of the tomato farmers were still unmarried at the time of this study. It was also revealed that very minute populations (0.8%) of the tomato farmers were divorced at the period of this finding, while a small fraction of the farmers (4.1%) were widowed. The study clearly shows the very low level of the divorced situation, as only one (1) farmer constituting (0.8%) of the entire tomato farmers' population was divorced. This higher percentage of married tomato farmers in this study area shows the degree of socio-cultural and religious values that place importance on marriage.

This could enhance efficiency in their tomato production with better economic opportunities. These results agreed with Oyekale *et al.* (2012) whose findings indicate that most rural farmers were married and living together with their spouse.

The distribution of the tomato farmers by family type showed that the majority (63.9%) practiced polygamy with more than one wife in their households. While the remaining (34.1%) were practicing monogamy with only one wife in their households. This implies that tomato farmers in this study area are adherent of the Islamic faith with cultural support for many wives.

The family or household size varied between a maximum of (18) people in one household and a minimum of (4) people with a mean of (9) persons per household. The distribution shows that the majority representing about (49.2%) of the tomato farmers have a family size between 6 and 10 persons. This simply implies that there were more people within the family or household in the study area. This result can be traced to the type of marriage practices and socio-cultural beliefs of the tomato farmers in the study area.

The result revealed that only (22.1%) of the tomato farmers had below (5) persons in their households while only

(28.7%) of them had above Ten (10) persons in their households. This result implies the availability of family labour to the average farming households for their farming and economic activities. Obviously, this will reflect in the size of their farms and their scale of production.

The average farm size in Nigeria according to the FAO, (2013) is 0.53 hectares for smallholders and 3.14 hectares for large farms. The maximum farm size of smallholder farmers is 1.7 hectares, and a large farm is 14.7 hectares. This study shows that tomato farmers operated on a medium scale of operations. This was shown by the mean farm size of 3.38 hectares, implying that the tomato production in the study area was characterized by medium scales of production as the majority (54.1%) of the tomato farmers cultivated around the mean farm size. This large scale of operation will enhance the production capacity in relation to the tomato output coming from this region. Because it is assumed that the higher the farm size, the higher the total outputs from the farm, and the smaller the farm size, the lower the output from the farm. The result also showed that the majority (54.1%) of the tomato farmers cultivated between 1 and 5 hectares of farmland, and about (27.8%) of the tomato farmers operated between 6 and 10 hectares of land. The few remaining (16.4%) of the tomato farmers operated on above 10 hectares of land.

The distribution of the tomato farmers according to their educational status showed that the majority (64.8%) of the tomato farmers were educated as they had one form of education or the other ranging from primary, secondary, and tertiary to vocational education including other forms of professional training. Only very few (33.6%) of the tomato farmers had no formal education at all.

It significantly implies a high rate of literacy among the tomato farmers in this region. Invariably, this contributes positively to their technical and economic efficiency due to their ability to read instructions and accustomed to new information on farming activities. Accordingly, the Majority (39.3%) of the tomato farmers claimed to have secondary education.

It was also observed that about (14.8%) of the tomato farmers claimed to have primary Education. Only (10.7%) of them had tertiary education.

This is in line with *Okorie et al.,* (2016) who reported that education is an important factor that can influence small-scale farmers to adopt new innovations and research findings related to their area of production.

The years of farming experience varied between a minimum of two (2) years of experience and a maximum of thirty-five (35) years of experience with a mean of 18 years. The distribution of the

tomato farmers according to their farming experience shows that only about (24.5%) of the tomato farmers had less than (10) years of farming experience. Only (28.7%) of the tomato farmers had farming experience of more than (20) years and the majority (46.8%) of them had farming experience between (11) and (20) years. This implies that the tomato farmers have many years of farming experience. This may be reflected in their productivities and efficiency of production including the level of output/hectare.

The primary occupation of the respondents is tomato farming as the majority (63.9%) claimed to do this. About (9.8%) of the farmers were artisans involved in one form of work or the other. About (21.3%) of the farmers claimed to have been involved in other primary occupations such as driving. while only (3.3%) of them have civil service as their major primary occupation.

The outcome of this study indicated that about (62.5%) of the tomato farmers had access to extension agent services forming the majority. While only a few of the tomato farmers accounting for (37.5%) had no access to the services of extension agents in this study area. This implies that tomato farmers in the study areas had good access to the services of extension agents.

The majority (53.3%) of the tomato farmers claimed to have been denied access to credit facilities for their tomato farming activities while only (46.7%) of the tomato farmers had access to credit. This implies poor access to credit facilities or probably not reaching the main targeted farmers in therural communities. This unavailability of credit facilities had discouraged majority of the respondents from engaging in agricultural productive activities that could boost production within the study area as opined by Oyinbo and Olaleye,(2016).

Despite all the different intervention programs of the government on Agricultural finance, many farmers were yet to have access to credit facilities for their farming operations.

The few (46.7%) of the tomato farmers that had access to credit facilities claimed to have sourced them from money lenders (9.0%), Commercial Banks (9.0%), Microfinance Bank (11.5%), Cooperative Society (18.2%) and Family/Friends (1.8%). This result shows that the tomato farmers depend largely on cooperative societies for their credit facilities in the study area

This implies that cooperative societies are playing a critical role by contributing to agricultural finance in the study area.

Table 1: Socio-Economic Characteristics of the Respondents

Characteristics	Frequency	Percentage	Mean
Gender:			
Male	76	68.3	
Female	44	36.7	
Age (Years):			
< 20	0	0	
Between 21 – 30	16	13.3	
Between 31 – 40	41	34.2	
Above 40	63	52.5	42
Marital Status:			
Married	93	74.6	
Single	23	18.9	
Divorced	1	0.8	
Widowed	5	4.1	
Family Type:			
Monogamy	42	34.1	
Polygamy	78	63.9	
Household Size:			
Less than 5 Persons	27	22.1	
Between 6 -10	59	49.2	9
Above 10 Persons	34	28.7	
Farm Size:			
Less than 5 hectares	65	54.1	3.38
Between 6 – 10 hectares	33	27.8	
Above 10 hectares	22	18.1	
Education Status:			
No formal Education	41	33.6	
Primary	18	14.8	
Secondary	48	39.3	
Tertiary	13	10.7	
Years of Farming Experience:			
Less than 10 Years	29	24.5	
Between 11 – 20	54	45	
Above 20 Years	37	30.5	
Primary Occupation:			
Tomato Farming	78	63.9	

Civil servants	4	3.3
Artisans	12	9.8
Others	26	21.3
Access to Credit:		
No	64	53.3
Yes	56	46.7
Sources of Credit:		
Money Lender	11	19.6
Commercial Bank	6	10.7
Micro finance Bank	12	21.4
Cooperative Society	20	35.7
Family and friends	7	12.5

Source: Field survey, 2023.

3.2. Gender patterns of resource access and use in tomato production in the study area.

The gender analysis was used to describe the gender pattern of resource access and use in tomato production. The majority (50.8%) of the tomato farmers claimed that tomato production is a masculine task and as such is the sole business of men. About (49.2%) claimed that tomato production is for both males and females. While the remaining (1.7%) of the respondents perceived tomato production as a feminine task. This implies that tomato production is perceived to be a male occupation rather than female work because of the rigor and risk involved in its production.

The result of resource access and use by the tomato farmers were reported in the study area. Very few women have access to large hectares of land as the majority (19.2%) of the women in tomato production operated on less than 5 hectares in the study area. Only (8.3%) of female tomato farmers have access to and operate on above 10 hectares of land in the study area. On access to credit, only (16.7%) female tomato farmers have access to credit. This implies poor access to credit by most female tomato farmers. On access to Extension Agents, male tomato farmers which constituted the majority (40.0%) have access to extension agents compared to female tomato farmers with only (22.5%) of them having access to extension agents. On access to the market, the result shows that the majority (56.7%) of the male tomato farmers have access to the market while the minority (34.2%) of female tomato farmers have access to the market in the study area. There is poor access to other inputs such as fertilizer among both male and female tomato farmers in the study area. Only 7.5% and 4.2% of the male and female farmers respectively have access to other inputs.

Table 2: Gender distribution of Tomato Production

	Frequency	Percentage (%)
Masculine	61	50.8
Feminine	2	1.7
Both	59	49.2

Source: Field Survey, 2024.

Table 3: Gender Distribution of Resource Access and use in tomato production.

Variables	Frequency		Percentage (%)	
	Male	Female	Male	Female
Farm Size) = < 5 ha	44	23	36.7	19.2
Betw (6-10) ha	23	11	19.2	9.2
> 10 ha	20	10	16.7	8.3
Access to Credit				
Yes	36	20	30.0	16.7
No	40	24	33.3	20.0
Access to Extension Agents				
Yes	48	27	40.0	22.5
No	28	17	23.3	14.2
Access to Market				
Yes	68	41	56.7	34.2
No	8	3	6.7	2.5
Access to other inputs				
Yes	9	5	7.5	4.2
No	67	39	55.8	32.5

Source: Field Survey, 2024.

3.3. Estimate of Costs, Returns, and Profitability of Tomato Production

The budgetary technique was explored to estimate the profitability of tomato production. The result showed that an average tomato farmer realized about N523,600.00 per hectare as total revenue per hectare of tomato farm while the average yield of tomato was estimated at about 2,380kg/ha. (2.4 tons). Tomatoes are measured with a basket and one (1) basket is equivalent to 10kg.

An average farmer spent N192, 856.00 per hectare as the total variable cost (TVC). These costs include the cost of land rent, land clearing, cost of purchase of seeds, planting, herbicides, pesticides, fertilizer, organic manure, weeding, and labour inputs. The total variable cost constituted 85% of all total cost incurred in tomato production.

The estimated result showed that, about 70% of all the total variable cost was spent on labour inputs which include planting, weeding, spraying of pesticides, fertilizer applications and harvesting. The average fixed cost incurred by tomato farmer (N129,447.09) constituted about 15% of the total cost (TC). It implies that, tomato production in the area required little capital investment on fixed cost. These fixed costs were majorly on cutlasses, hoes, knapsack sprayers etc. The result further revealed that, the gross margin of an average tomato farmer was N287,050.00 which imply that tomato production had desirable returns. Three business indicators were estimated, earning per Naira Invested (1.74), the Profit Ratio (0.74) and Rate of Return on Fixed Cost (3.07). A gross return per naira invested of N1.74 obtained showed that, for every one naira (N1) invested in tomato farming, the farmer would earn N1.74. The profitability indicators show that tomato farming is a profitable enterprisein Ayedire Local Government Area of Osun State, Nigeria.

Table 4: Cost, Return and Profitability of Tomato Production per Hectare

INCOME				₦
Revenue from 2,380 kilogram of Fresh Tomato (Per ha)				523, 600.00
Total Revenue				**523, 600.00**
Variable Cost	**Unit**	**Price/Unit (₦)**	**Days**	**Cost (₦)**
Land Clearing (Man/day)	5 Man	3,500	1	17,500.00
Cost of Seeds (Kg/ha)	4kg	4,000		20,060.00
Planting (Man/day)	5 Man	3,450	1	17,250.00.
Weeding (Man/day)	5 Man	3,200	1	15,966.00
NPK	2 Bags	10,500		27,066.00
Urea	2 Bags	9,500		23,062.00
Herbicides	10 Litres	3,600		15,972.00
Insecticides	7 Litres	1,800		12,690.00
Fertilizer Application (Man/day)	4 Man	2,700	1	10,831.00
Cost of spraying Herbicides (Man/day)	2 Man	3,750	1	7,529.00
Cost of Spraying Insecticides (Man/day)	2 Man	3,750	1	7,360.00
Harvesting (Man/day)	3 Man	2,500	2	17,570.00
Total Variable Cost (TVC)				**192,856.00**
Fixed Cost				
Land lease				40,699.00
Depreciation on Cutlass				15,513.00
Depreciation on Knapsack Sprayer				29,010.00
Depreciation on Wheel Barrow				9,621.91
Depreciation on Hoe				12,603.00,
Total Fixed Cost				**107,446.00**
Total Cost				**300, 302.00**
Gross Margin				**330,744.00**
Profit				**223,298.00**
Profitability Indicators:				
EPI				1.74
PR				0.74
RRFC				3.07

Source: **Field Data, 2024.**

Note: EPI = Total revenue/Total Cost
PR = Profit/Total Cost
RRFC = Gross Margin/Total Fixed Cost.

3.4. Factors Influencing resource use efficiency in Tomato production

The result of Maximum Likelihood Estimates (MLE) for the production frontier is presented in Table 5. The result showed that the estimated parameters of sigma-squared ($ó^2$) are 0.642 and 0.507 for male and female tomato farmers respectively. The values of Sigma-squared show a significant difference from zero at a 1% level of significance, which hypothesized the perfect goodness of fit of data with the Cobb- Douglas stochastic frontier model, and also the assumption of the composite error term was properly specified.

The generalized likelihood ratio statistics of 3.7 was obtained for male farmers while 6.4 was obtained for female tomato farmers. These ratios exceed the critical chi-square values at a 1% level of significance. The log-likelihood ratio value represents the value that maximizes the joint densities in the estimated model. Thus, the functional form that is, Cobb-Douglas used in this estimation is an adequate representation of the data. It was further revealed that the values of the gamma statistics were 0.52 and 0.73 for male and female farmers respectively. These indicate that 52% and 73% of the changes in the output of both male and female tomato farmers respectively are attributable to farmers' inefficiency factors. The result revealed that technical inefficiency effects were present in tomato production in the study area.

The stochastic frontier model estimates Cobb-Douglas tomato production function based on six (6) basic input variables: labour, land (farm size), seed, herbicides, pesticides, and fertilizer with the help of maximum likelihood estimation techniques. The results show that area under tomato cultivation (land size), herbicide quantity applied, and labour quantity used were important in determining tomato production in the study area. Acreage under tomato cultivation (land size), the quantity of labour used, and herbicides quantity had positive coefficients that were significant at a 1% level of significance for both male and female tomato farmers. Thus, increasing hectares under tomato production by 1% would increase tomato output by 0.837 and 0.651% for male and female tomato farmers respectively. Also, increasing the quantity of labour used by 1% would increase tomato output by 0.624 and 0.703% for male and female tomato farmers respectively and a 1% increase in herbicide usage will significantly increase the output of tomato by 0.708 and 0.873% for male and female tomato farmers respectively.

Table 5a: Maximum likelihood estimates of stochastic frontier production for Tomato production.

Variable Coefficients						
Variables	**Male**			**Female**		
	Coefficient	**Std. Error**	**t-value**	**Coefficient**	**Std. Error**	**t-value**
Constant ($\hat{a}_0$)	7.305	0.6362	4.38***	4.621	0.6224	2.76**
Seed (X_1)	0.0611	0.014	1.18	0.374	0.013	1.02
Labour (X_2)	0.624	0.042	3.03***	0.703	0.086	3.42***
Fertilizer (X_3)	-0.086	0.049	-1.024	-0.127	0.046	-1.07
Farm size (X_4)	0.837	0.069	3.98***	0.651	0.082	4.01***
Herbicides (X_5)	0.708	0.087	3.63***	0.873	0.076	3.18***
Fungicides (X_6)	-0.251	0.096	-1.55	-0.008	0.098	-0.029
Variance parameters						
Sigma squared (ó2)	0.642	0.201	4.72***	0.507	0.072	5.81***
Gamma (ã)	0.520	0.088	4.54***	0.730	0.420	3.00**
LR test	3.70			6.40		
log likelihood function	-383.43			-465.81		

$ó^2 = óv^2 + óu^2$, $ã = óu^2 / ó^2$, Std. - Standard *** Significant at 1%, ** Significant at 5%.

3.4.1. Result of Resources Use Efficiency of Tomato Production

The result of the resource use efficiency is presented in Table 5b. The result shows that the ratio of marginal value product (MVP) to marginal factor product (MFP) for fertilizer and fungicides is less than 1 for both male and female tomato farmers; this implies that the quantities of fertilizers and fungicides were over-utilized. This may be because of a lack of technical know-how or knowledge of the best practices in fertilizer and fungicide application. Any reduction in the usage of these inputs will lead to an increase in the output of tomatoes in the study area. Seeds of tomato, land, labour, and herbicide were under-utilized in tomato production activities of both male and female farmers in the study area. This may be a result of scarcity and high prices of improved seeds variety, labour, and herbicides especially during production periods, meaning that to increase the profitability of tomato production in the area, the level of such inputs utilized should be increased.

Table 5b: Determination of resource use efficiency in tomato production

Variables	Coefficients	MVP	MFC	MVP/MFC	Decision
Seeds (kg)	0.0611	1896.2	400	4.7405	under utilized
Labour (man/day)	0.624	6380.67	300	21.269	under utilized
Fertilizer (kg)	-0.086	- 5439.65	900	- 6.0441	over utilized
Farm size (Land) ha	0.837	4218.98	12.0	6.4321	under utilized
Herbicides (Litres)	0.708	1746.54	340	5.1369	under utilized
Fungicides (Litres)	-0.251	- 1832.60	250	- 7.3304	over utilized

Source: Field Survey, 2024

3.4.2. Determinants of technical efficiency:

After estimating technical inefficiency variables by using the single-stage estimation approach of the stochastic frontier model, identified the following significant determinant factors of technical efficiency of tomato producers.

Age: The age of the tomato farmers had a positive and significant (at a 5% significance level) effect on the technical efficiency of tomato production. It implies that as the age of the tomato farmers increases so does the technical efficiency increased.

Education Status: It is measured by the level of school education attained by the respondents, in this study education was a positive and statistically significant (at a 1% significance level) effect on technical efficiency. This might be due to, educated farmers were eager to disseminate technology that they were able to receive, interpret, and disseminate new information and improved technologies such as improved seeds, fertilizer, and pesticides. Education also improved the unobserved labour quality and management capability of farmers, this, in turn, increased technical efficiency.

Access to extension Service: It is a dummy variable consisting of 1 and 0, It had a statistically significant and positive relationship with the technical efficiency of tomato production at a 5% level of significance. This implies that more access to extension services will increase the productivity of tomato production.

Access to Credit. It is a dummy variable indicating 1 if a farmer gets credit, and 0 otherwise. Table 5 revealed that access to credit has a favorable or positive effect on the technical efficiency of tomato producers at a 1% level of significance. Cash requirements for purchasing inputs on time (improved tomato seed, pesticides, additional labour force, and fertilizer) will result in the farmer being more efficient than the counterpart.

Table 5c: Determinants of Technical Efficiency

Variable	parameter	Coefficient	Std Error	z	p -value
Constants	$\hat{a}_0$	5.3289	0.67323	5.212	0.0001
Gender	$\hat{a}_1$	-0.6217	0.4328	0.532	0.1041
Age	$\hat{a}_2$	0.4974**	0.3290	3.032	0.0010
Education Level	$\hat{a}_3$	0.3219***	0.8320	4.091	0.0036
Farm Size	$\hat{a}_4$	0.7145	0.5931	1.042	0.8532
Household Size	$\hat{a}_5$	0.6219	0.4209	0.5432	0.3021
Access to Extension	$\hat{a}_6$	0.9521**	0.5197	1.910	0.0042

Source: Field Survey, 2024.

Note: ** (1%), ** (5%)**

4.0 Conclusion and recommendation

The study established that tomato farmers were mostly male, married, physically and economically active in age. They were experienced, educated, and efficient in their resource utilization for tomato production. Tomato production was highly profitable in the study area because the total revenue and total cost have a pristine difference, with a high profit ratio and earnings per naira invested (EPI). Age of the tomato farmers, household size, farm size, farming experience, practice of organic farming, pest infestation, and extension services contacts were the factors determining the resource use efficiency. Since the study revealed that most of the farmers in the study area were predominantly male. It is therefore recommended that female participation in tomato production should be encouraged by Implementing gender-sensitive agricultural policies and programs that specifically target female farmers. The study showed that there is a very weak institutional framework between tomato farmers and extension agents in the study area. Tomato farmers should have access to training, and improved technologies to enhance their productivity and resource use efficiency. Formation of women's farmer groups to facilitate knowledge sharing, resource pooling, and access to markets should be enhanced and encouraged to address gender disparities in land ownership and access to credit to ensure equitable opportunities for female farmers. Agricultural input supply centers should be established in Ayedire Local Government to ensure timely and affordable access to fertilizers, pesticides, and other essential inputs for tomato production. Use of climate-smart and sustainable agricultural practices to reduce input costs and enhance resource use efficiency should be promoted and practiced amongst Tomatoes Farmers in the study area. Training and support the farmers on processing and value addition of

tomatoes, such as producing tomato paste, juice, or dried tomatoes, to increase income generation and reduce post- harvest losses should be encouraged in the study area. Access to electricity and energy-efficient technologies to support irrigation, processing, and storage of tomatoes should be provided and enhanced in the study area.

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PREVALENCE OF ANTIFUNGAL RESISTANCE AND VIRULENCE FACTORS IN CANDIDA ALBICANS ISOLATED FROM DIABETIC PATIENTS IN A TERTIARY HEALTCARE FACILITY

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ABSTRACT

Owing to its immunosuppressive effects on patients, diabetes mellitus predisposes individuals to candidal infections. Therefore, this study aimed to determine the antifungal resistance of Candida albicans in diabetic mellitus patients attending a tertiary health care facility in Ekiti State. A structured questionnaire was used to obtain demographic data from the subjects. Fifty-three (53) mid-stream urine samples were collected and the urine samples were cultured on Sabouraud dextrose agar and identified using cultural and molecular methods. Virulence factors (phospholipase, haemolysis, protease, and biofilm formation) were also determined using standardized methods. Two virulence (ALS 1 and SAP 5) genes were also detected in the isolates. Antifungal susceptibility was determined using a standard method. Of the 53 urine samples analyzed, 19 (35.8%) yielded C. albicans, with the highest isolation rate of 57.9%, while non-albicans Candida had an isolation rate of 42.1%. The age group of 41-50 years had the highest C. albicans isolation rate of 8 (42.1%), followed by the age group of 31-40 years with a prevalence rate of 6 (31.5%). The urine of female diabetic subjects yielded more candidal growth than their male counterparts. All the C. albicans strains tested in this study possessed virulence factors. The antifungal resistance among the C. albicans isolates ranged from 27.3% to 54.5%. Additionally, SAP5 was present among Candida albicans isolated from diabetic subject. Nevertheless, ketoconazole and fluconazole, with the least antifungal resistance, should therefore be included in the regimen of diabetic patients with candiduria.

Key words: Diabetes Mellitus, Candida albicans, antifungals, virulent factors, candiduria, fungi

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INTRODUCTION

The opportunistic pathogenic yeast *Candida albicans* is a common constituent of the human microbiome. It is often a commensal organism, but in immunocompromised people with several diseases such as diabetes mellitus, HIV, and cancer, it can become pathogenic (Gow, 2017; David *et al.*, 2020). One of the most prevalent *Candida* species commonly implicated in nosocomial infections is albicans. This species has also been reported to be associated with different diseases in critically ill or otherwise vulnerable patients. *Candida albicans* causes a variety of infections, from superficial skin infections to severe systemic infections, depending on the circumstances. The skin, genitals, throat, mouth, and blood are mainly infected by this fungus (Vazquez, 2016).

Candida albicans can adapt quite quickly to protect themselves and thrive, even in unfavourable environments. *Candida* species can exist in at least two different forms: the yeast form, which survives well in acidic conditions, and the hyphal form, which thrives in a neutral or alkaline environment. As the conditions in the intestine change, *Candida* can switch between these two forms and survive dramatic alterations in its environment. The organism also modifies the pH of its immediate environment by releasing metabolites that actively alter the pH of its surroundings, enabling *Candida albicans* to switch from its yeast form to its hyphal form (Ksiezopolska and Toni, 2018).

In recent times, populations of susceptible immunocompromised or diabetic patients and even the elderly have resulted in increasing cases of resistant *Candida albicans* (Ksiezopolska and Toni, 2018). These groups of individuals develop invasive fungal infections that damage the blood, heart, brain, eyes, or other organs. Treatment-resistant bloodstream fungal infections can result in major health complications (CDC, 2019). Antifungal resistance is a problem associated with Candida infections. Although *Candida albicans* is the most common cause of severe Candida infections, resistance is most common in other species, particularly *Candida glabrata* and *Candida parapsilosis* (Toda *et al.*, 2019). Therefore, this study aimed to isolate and identify antifungal-resistant *Candida albicans* from the urine of diabetic subjects attending a tertiary institution in Ekiti State, Nigeria. The presence of virulent factors in the isolates were also determined. Molecular method was also used to determine the presence of two virulence (*ALS* 1 and *SAP* 5) genes in the isolates.

MATERIALS AND METHODS

Sample Collection and Identification

Fifty-three (53) mid-stream urine samples were collected from diabetic patients into a sterile universal bottle. The samples were collected only from subjects who consented to participate in

the study. The study population includes patients attending the Outpatient Department (OPD) unit of the healthcare facility who have been diagnosed with type 2 diabetes. A diagnosis which was confirmed after a patient meets specific clinical criteria. These criteria include a fasting blood glucose level of 126 mg/dL (7.0 mmol/L) or higher after an overnight fast, a postprandial blood glucose level of 200 mg/dL (11.1 mmol/L) or higher two hours after a meal, or a hemoglobin A1c (HbA1c) level of 6.5% or higher, indicating chronic hyperglycemia. Additionally, a random blood glucose level of 200 mg/dL (11.1 mmol/L) or higher also qualifies a person as diabetic.

The subjects were instructed to allow the first voided urine to flow, after which a sterile universal bottle was placed, and midstream urine was collected. The midstream urine sample was examined visually, and the colour and transparency were recorded accordingly. A semi-quantitative urine culture technique was employed using the standard loop method (Cheesbrough, 2000). The samples were incubated on Sabouraud dextrose agar (SDA) incorporated with 0.05% chloramphenicol at 37°C for 48 h. Twenty-four-hour-old cultures of the isolates were prepared on a clean grease-free slide, the Grams stain test was performed on each of the isolates, and the results were recorded as described by Ochei and Kolhaktar (2000).

Cultural Identification of the isolates

A cream-coloured, raised, entire, smooth, and butyrous colony on SDA was inoculated into an SDA slant. The carbohydrate assimilation test was performed by singly incorporating different carbohydrates (glucose, lactose, galactose, and sucrose) into Yeast Nitrogen Base Agar (YNBA) and observing for growth after incubation at 25°C for 24 to 48h. Signs of growth on the medium and changes in the colour of the indicator were recorded, as described by Chakrabarti *et al*. (2016).

Molecular Identification of the isolates

The DNA of the isolates tentatively identified as *C. albicans* was extracted as described by Kanbe *et al*. (2002). The sequence of the primers was as follows: forward 5'CGCCTCTGATGGTG ATGAT3' and reverse 3'TCCGG TATCACCTGGCTC5' as reported by Jordan (1994). The PCR was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µL of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM MgCl2, 200 µM of each deoxynucleotide triphosphates (dNTP) (Solis Biodyne), 25 pmol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne); however, additional Taq DNA polymerase was

incorporated into the reaction mixture to make a final concentration of 2.5 units of Taq DNA polymerase, Proofreading Enzyme, 2 µL of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. A total of 36 cycles were performed during gene amplification, and denaturation was carried out at 95°C for 1 min. Annealing and extension were performed at 52 and 72°C, respectively, for 1 min. The amplification product was separated on a 1.5% agarose gel, and electrophoresis was performed for 1 h 30 min at 80 V. DNA bands were visualized using ethidium bromide staining after electrophoresis. A 100 bp DNA ladder was used as the DNA molecular weight standard. The sequence of the primers used for the detection of the *ALSI* and *SAP5*-resistant sequence condition genes are shown in Table 1.

Antifungal Susceptibility Test

The susceptibility of the isolates to different antifungals was determined using the method described by CLSI (2015). Colonies of each isolate were randomly selected and inoculated in 2 mL of sterile Sabouraud broth in bottles. The mixture was incubated at 37°C for 4 to 8 h. The turbidity of the culture was adjusted by serial dilution in phosphate buffer saline (pH 7.2) to match an opacity tube containing 0.5 mL of 1% barium chloride in 1% sulfuric acid (0.5 McFarland's turbidity standard equivalent to 10^6 CFU/mL of the inoculum). The inoculum was seeded on Sabouraud dextrose agar medium and allowed to dry. Five antifungals: ketoconazole, itraconazole, fluconazole, nystatin and micafungin at standard concentrations stipulated by CLSI (2015) were tested against the isolates in this study. The antifungals were impregnated paper disc and were were gently placed on the surface of the SDA Agar previously seeded with different isolates. The plates were then incubated at 37°C for 18-24 h. The diameter of the zone of inhibition on the isolates on SDA plate was measured in millimetres using a ruler and then interpreted according to Clinical Laboratory Standard Institute (CLSI) (2015) guidelines.

Determination of the virulence factors

Detection of germ tube

Isolated colonies of *Candida* spp. were inoculated into glass tubes with 1 mL of human serum and incubated at 37°C for 2 h. A drop of the inoculum was then placed on a clean microscope slide and a coverslip was placed on the slide. Wet mount preparations were examined under the objective lens of a microscope (×40 magnification) and tests were performed in duplicate.

Detection of phospholipase production

Potato Dextrose Agar was prepared aseptically, allowed to cool to around 50°C followed by the aseptic addition of 10% sterile egg yolk. Gelling was allowed and the plug of organisms at the growing edge was placed at the centre of the plate and incubated at 37°C for 48 h.

The whitish colouration observed around the organism was recorded as positive(i.e. phospholipase production).

Detection of haemolysin production

The haemolysin assay for *Candida albicans* was performed according to a protocol previously validated by Luo *et al.* (2001). Briefly, Sabouraud dextrose agar supplemented with 6% human blood and 3% glucose (pH 5.6) was used to determine haemolysin production. A suspension of yeast (10^6 cells/mL) was prepared in saline solution, and 10 ìL was spot inoculated on human blood agar plates and incubated at 37°C in 5% CO_2 for 5 days. Haemolytic activity was calculated by dividing the diameters of the colonies by the translucent zones of haemolysis.

Detection of gelatinase production

The gelatinase assay was performed on SDA plates prepared with 1% gelatin (Ramesh *et al*., 2011). The single diffusion technique was applied in triplicate. The plate was incubated for 5 days at 37°C. The appearance of the inhibition zone was visualized by the addition of 0.1% mercuric chloride. The zone diameters were measured and recorded.

Detection of proteinase test

Protease production was determined on agar containing bovine serum albumin as previously described by Mohan and Ballal (2008). A solution was prepared by dissolving 11.7 g of yeast carbon base, 0.1 g of yeast extract, and 2 g of bovine serum albumin in 200 mL of distilled water. The solution was sterilized by filtration and added to a previously sterilized stock solution of 16 g agar-agar in 800 mL of distilled water. After inoculation, the plates were incubated at 37°C, and the results were recorded after six days of incubation. The enzyme activity was measured as the diameter of the lytic area surrounding the growth area of the serum medium.

Detection of biofilm production

The Congo Red Agar Method (CRA) was used to detect biofilm formation (Mathur *et al*., 2006). The CRA medium was prepared by supplementing the Brain Heart Infusion medium with 5% sucrose and Congo red. Medium: BHI (37 g/L), sucrose (50 g/L), agar (10 g/L), and Congo Red (0.8 g/L). Congo Red was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 min, separately from other medium constituents, and added when the agar had cooled to about 55°C. The plates were inoculated and incubated aerobically for 24–48 hours at 37°C. Positive results were shown as black colonies with dry crystalline consistency (Pallavi *et al*., 2014).

Determination of the virulent genes

DNA extraction

The method described by Kathrin *et al*. (2019) was used for the DNA extraction. The oligonucleotide primer pair (50' -CGGAGATTTTCT

CAATAAGGACCAC, and 50' - AGTCAATCTCTGTCTCCCCTTGC) designed by Galan *et al.* (2006). Mechanical lysis was carried out using 1 mm silica spheres with the addition of the detergents sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), and proteinase K (Qiagen, Venlo, Netherlands). After adding chloroform-isoamyl alcohol (24:1), the water-soluble polar layer was transferred to a new tube, followed by precipitation with ammonium acetate and isopropanol, and was subsequently washed using ethanol. Air-dried DNA was resuspended in 10 mM Tris-EDTA buffer. After extraction, the amount of DNA was determined using Qubit 2.0, the dsDNA HS kit (Life Technologies, Carlsbad, California, USA), and a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, Massachusetts). Additionally, the $A_{260/280}$ and $A_{260/230}$ were used to estimate DNA purity. Sequencing was performed using a targeted resequencing design on the MiSeq platform (Illumina, San Diego, California USA).

RESULTS

Table 2 shows the isolation rate of *Candida* spp. among diabetic patients attending a tertiary health facility in Ekiti. Of 53 urine samples from diabetic patients, only 19 (35.8%) were positive for *Candida* species, while the fungus was not recovered from the remaining 34 (64.2%). The isolation rate of *Candida* spp. among diabetic subjects revealed that out of the 19 Candida isolates, only 11 (57.9%) were *Candida albicans* and eight (42.1%) were non-*albicans Candida*. The prevalence of *Candida* spp. among patients with diabetes and their age is shown in Table 2. The 41-50 year age bracket exhibited the highest isolation frequency, with 8 cases representing 42.1% of the total. The 31-40 year age group followed, showing a prevalence of 6 cases, or 31.5%. The older age groups, 51-60 years and 61-70 years, showed lower prevalence rates, with 3 cases (15.8%) and 2 cases (10.5%) respectively.

The prevalence of *Candida* spp. among diabetic patients about sex showed that female subjects had the highest prevalence rate of 11 (57.89%) compared to their male counterparts, with a prevalence rate of 8 (42.11%). Table 3 shows the Gram reaction, biochemical, and pathogenicity tests of *Candida albicans* isolated from diabetic subjects. The fungus showed a Gram-positive reaction, oval-shaped cells, that ferment glucose, galactose, and sucrose, but not lactose. The detection of virulence factors in *Candida albicans* showed that 10 out of the 11 isolates produced germ tube and phospholipase, Virulence factor analysis reveals that *C. albicans* exhibits higher biofilm formation (100 %), germ- tube production (90.91%), and phospholipase activity (90.91%) compared to *non-albicans Candida* , which shows lower percentages except for protease production (87.50%).as shown in Table 2.

Table 4 shows the antifungal susceptibility of *Candida albicans* isolated from diabetic patients. Three (27.3%), 5(45.4%), 3(27.3%), 6(54.5%), and 4(36.4%) *Candida albicans* isolates were resistant to ketoconazole, itraconazole, fluconazole, nystatin, and micafungin, respectively. Concurrently, two (25.0%), six (75.0%), three (37.5%), five (75.0%), and three (75.0%) *Candida tropicalis* isolates were resistant to ketoconazole, itraconazole, fluconazole, nystatin, and micafungin, respectively. Three (27.3%), 5 (45.4%) and 3 (27.3%) were resistant to ketoconazole, itraconazole and fluconazole. The percentages of isolates that were resistant to nystatin and micafungin were 54.5% (n=6) and 36.4% (n=4), respectively.

Plate 1 shows agarose gel showing positive amplification of specific primers for the identification of *Candida albicans*. Lane M is the ladder (marker), lane 3 is the gene from the control organism (*C. albicans* ATCC 10231), with 46, 22, 9, 34, 21, 4, 8, and 5 being the test isolates, while lane 31 is a negative control. Plate 2 shows agarose gel of *Candida* spp. amplification using ALS1 specific primers. Only the sample in lane 5 had an amplification of approximately 240 bp which indicates positive amplification using primer *ALS* 1. Plate 3 shows the agarose gel electrophoresis of *Candida albicans* isolates using *SAP* 5-specific primers. Only *C. albicans* isolates in lanes 3, 46, 22, 34, 21, and 4 showed positive amplification of approximately 280 bp genes

DISCUSSION

In this study, the prevalence rate of *C. albicans* among the isolates recovered from diabetic subjects was 19 (35.8%). This prevalence is similar to that reported by Khaled *et al*. (2006), who reported a prevalence of 22.5%. This might be because opportunistic candiduria remains an emerging health condition and its prevalence might be increasing if not properly checked (Armstrong-James *et al*., 2014). This is because the immune system of diabetic patients coupled with the high level of glucose remains a good medium for fungus. Also, Kotran (2007) reported that diabetic patients are prone to high blood sugar concentration which weakens the immune system and causes several health complications including systemic Candida infection.

In this study, we encountered more *Candida albicans* 11 (57.9%) than non-*albicans Candida* which accounted for only 42.1% (n=8). According to Adebiyi *et al*. (2016), *C. albicans* remains the most common opportunistic pathogen among patients with diabetes mellitus. Molecular characterization of the isolates further confirmed the identity of the isolates. The presence of genes with a molecular weight of 122 bp further confirms that the isolates were *C. albicans* and is in agreement with the reports of Trindade *et al.* (2007), who reported a similar base pair in *C.*

albicans isolates btained in their study.

The prevalence of *C. albicans* among diabetic subjects and the age of the subjects is shown in Table 3. The table showed that subjects within the age group 41–50 years had the highest prevalence rate of *C. albicans* [8 (42.1 %)] followed by the age group 31-40 years with the prevalence rate of 6(31.5%), while subjects within the age group 51 – 60 yr. had a prevalence rate of 3(15.8%). This is in agreement with a study conducted by Khaled *et al.* (2006), who reported that age differences among patients with diabetes remain a factor in systemic fungal infections, which may be due to the interaction between aging and suppressed immunity due to diabetes (Khaled *et al*., 2006). The prevalence of *C. albicans* among diabetic patients (Table 5) showed that female subjects had the highest burden of candiduria compared to their male counterparts at a ratio of 11:8.

Candida species are frequent and common factor responsible for urinary tract infections (approximately 10–30%) as reported by David *et al.* (2020). This was due to the proximity of the urethra and anus. The vulva vestibule region may be periodically colonized by microbes from the alimentary tract, including yeast-like fungi. The ascending pathway may be a route for tract infections. Urinary Tract Infections (UTIs) are more often diagnosed in women than in men because of their anatomical structures (a short urethra and the closeness of the vagina and anus). As many studies have shown, 3 %– 8 % of the female population develops a UTI at least once in their lifetime (Behzadi *et al*., 2010). At varying degrees, *C. albicans* isolated from diabetic subjects possessed virulent factors. All the isolates were positive for biofilm formation, while 90.91% of the isolates produced both gelatinase and germ-tube. The number of the isolates that produce gelatinase and protenase were 81.81 and 72.73% respectively. Non-*albicans Candida* species recorded lower occurrence of pathogenic factors compare to *C. albicans.* This finding is in agreement with the results reported by Adebiyi *et al*. (2016). This further confirmed that *Candida albicans* are highly pathogenic and often possess more pathogenic factors as reported by David *et al.* (2022). The presence of these virulence factors contributes to the pathogenicity of *Candida albicans.* Protease and phospholipase enhance its attachment to the human epithelium, which plays an important role by i ncreasing physiological changes, and the increase in glucose level in diabetic patients provides an adequate supply of utilizable sugars that favours *Candida albicans* growth (Nwadioha *et al*., 2010).

Antifungal susceptibility of *Candida* spp. isolated from diabetic patients showed that the resistance of *Candida albicans* to the selected antifungal

agents ranged from 54.5% for nystatin to 27.3% for ketoconazole and fluconazole. In addition, the resistance of *Candida tropicalis* ranges from 75.0% to 25.0%. In this study, azole groups of antifungal agents appeared to be very effective in the treatment of *Candida albicans* infection. This was also supported by Sheehan *et al*. (1999), who reported that these drugs act by targeting the cytochrome P450 enzyme lanosterol 14á-demethylase, which converts lanosterol to ergosterol. As a result of the action of azoles, the candidal cell membrane is depleted of ergosterol and accumulates toxic 14á-methylated sterols. This causes a decrease in membrane fluidity and, in most cases, inhibits cell growth.

The presence of the two virulence genes were detected in the isolates. The genes were *ALS* 1 and *SAP* 5; only one strain (*Candida albicans* in lane 5) was positive for *ALS* 1 gene, as shown in Plate 2. The gene (*ALS* 1) was not frequently present among *Candida albicans* in this study. However, *SAP* 5 was present in all the six *C. albicans* isolates. This confirms that the isolates have the gene responsible for the virulence of *Candida albicans*. This gene has been reported to be associated with the ability of isolates to form biofilms. The burden of candidal infections is linked to an increasing number of at-risk populations. Subjects with metabolic disorders due to diabetes mellitus have a weakened immune system and several health complications (Kotran, 2007). *Candida albicans* remains a major threat to this group of patients owing to its pathogenicity, which was also detected in this study.

In summary, the *C. albicans* strains isolated in this study exhibited resistance to first-line antifungal agents and possessed various virulence factors. The SAP 5 was the most prevalent out of the two virulent genes screened for in the isolates. The presence of the virulence factors might have contributed to the severity of the infection caused by the organism in people living with diabetes. Therefore, proper hygiene and control of high blood glucose remain great factors in controlling the spread of infections caused by the fungus.

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Table 1: The primers sequence and condition of reaction for the detection of the *ALSI* and *SAP5*

Genes	Primer name	Primers	Condition for the reaction
ALS1	ALS-F	CCATCACTGAAGATATCACCACA	94°C for 5 min; followed by a 30 cycles of 94°C for 30 s and the 72°C for 30 s and a termination at 72°C for 10 mins. and cooling at 4°C.
	ALS-R	TGGAGCTTCTGTAGGACTGGTT	
SAP5	SAP-F	AGAATTTCCCGTCGATGAGACTGG	
	SAP-R	CAAATTTTGGGAAGTGCGGGAAGA	

Table 2: Prevalence of *Candida* spp. among diabetic patients in relation to age.

Years	Number screened	Number positive
21-30	2	0
31-40	23	6 (31.5)
41-50	18	8 (42.1)
51-60	6	3 (15.8)
61-70	4	2 (10.5)

Table 3: Gram reaction, biochemical and pathogenicity tests of *Candida* spp. isolated from diabetic patients

Test	Reaction	*Candida* species	
		Candida albicans	**Non-*albicans* *Candida***
Sugar utilization	Glucose	+	±
	Galactose	+	±
	Lactose	-	-
	Sucrose	-	±
Virulence factors [n (% +ve)]	Biofilm	11 (100.00)	1 (12.50)
	Gelatinase	9 (81.81)	2 (25.00)
	Germ-tube	10 (90.91)	1 (12.50)
	Haemolysin	7 (63.63)	3 (37.50)
	Phospholipase	10 (90.91)	5 (62.50)
	Protease	8 (72.73)	7 (87.50)

Table 4: Antifungal susceptibility of *Candida* spp. isolated from diabetic subjects

Reaction	***Candida* species**
	Candida albicans
Ketoconazole	3(27.3)
Itraconazole	5(45.5)
Fluconazole	3(27.3)
Nystatin	6(54.5)
Micafungin	5(45.5)

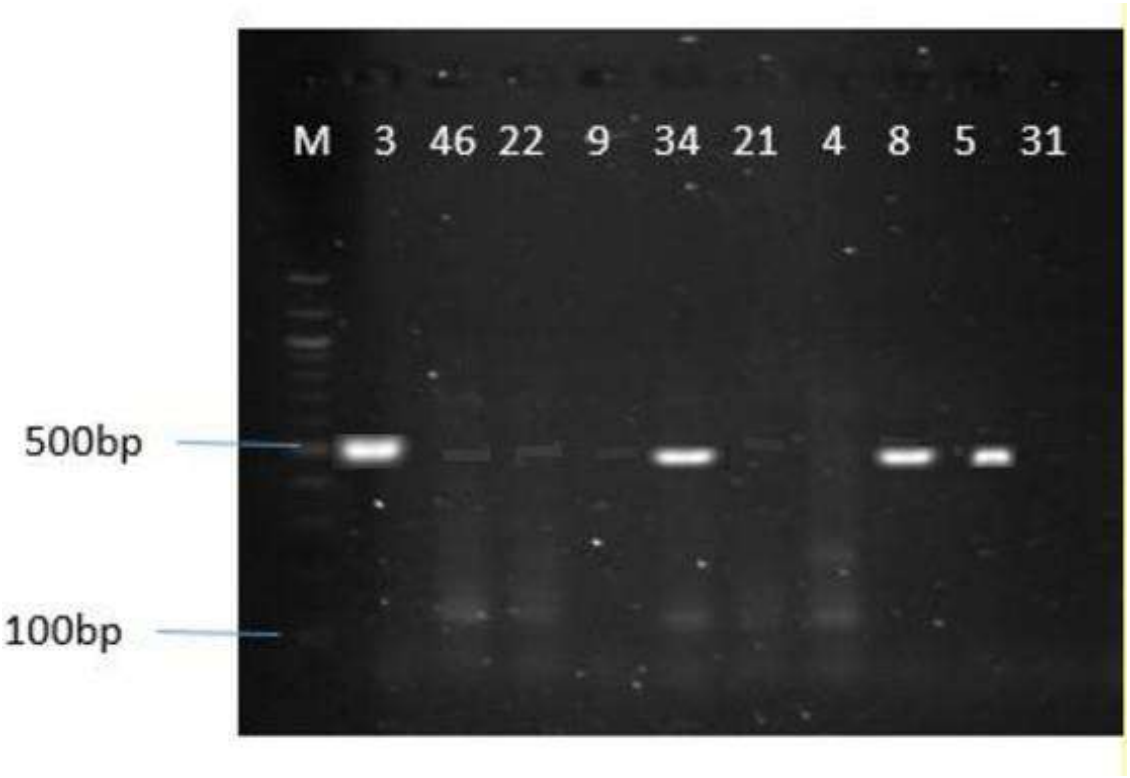

Plate 1: Agarose gel electrophoresis showing the positive amplification of Candida albicans using specific primer for the identification of Candida albicans. Lane M is the ladder (marker), lane 3 is the gene from the control organism (C. albicans ATCC 10231), while lanes 46, 22, 9, 34, 21, 4, 8 and 5 are the test isolates while lane 31 is a negative control.

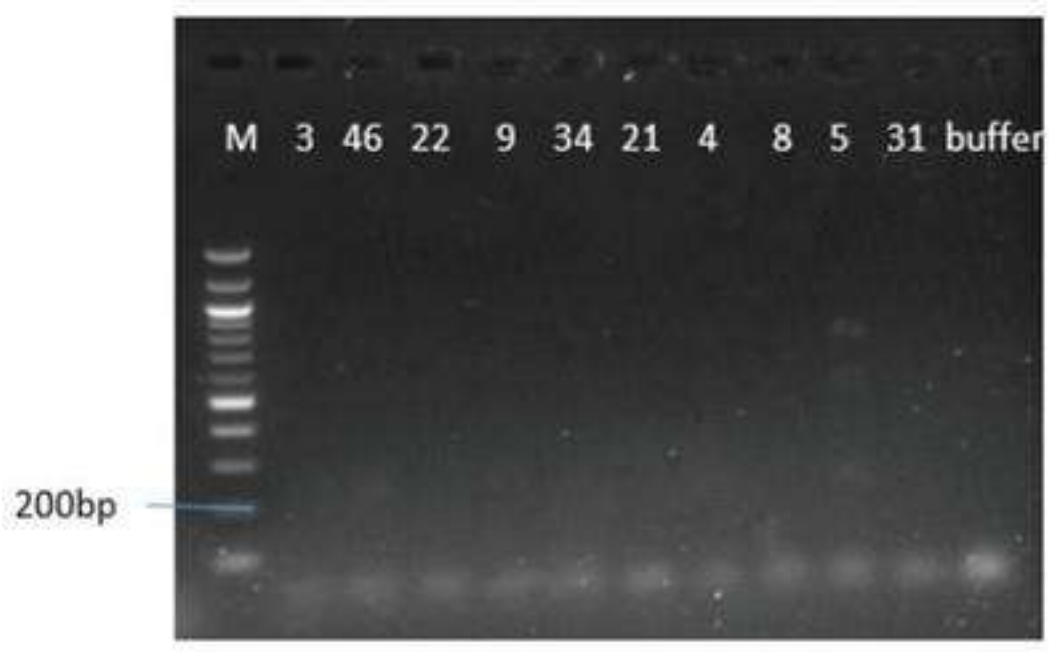

Plate 2: Agarose gel electrophoresis showing the positive amplification of Candida albicans using ALS1 primers. Lane M is the marker.

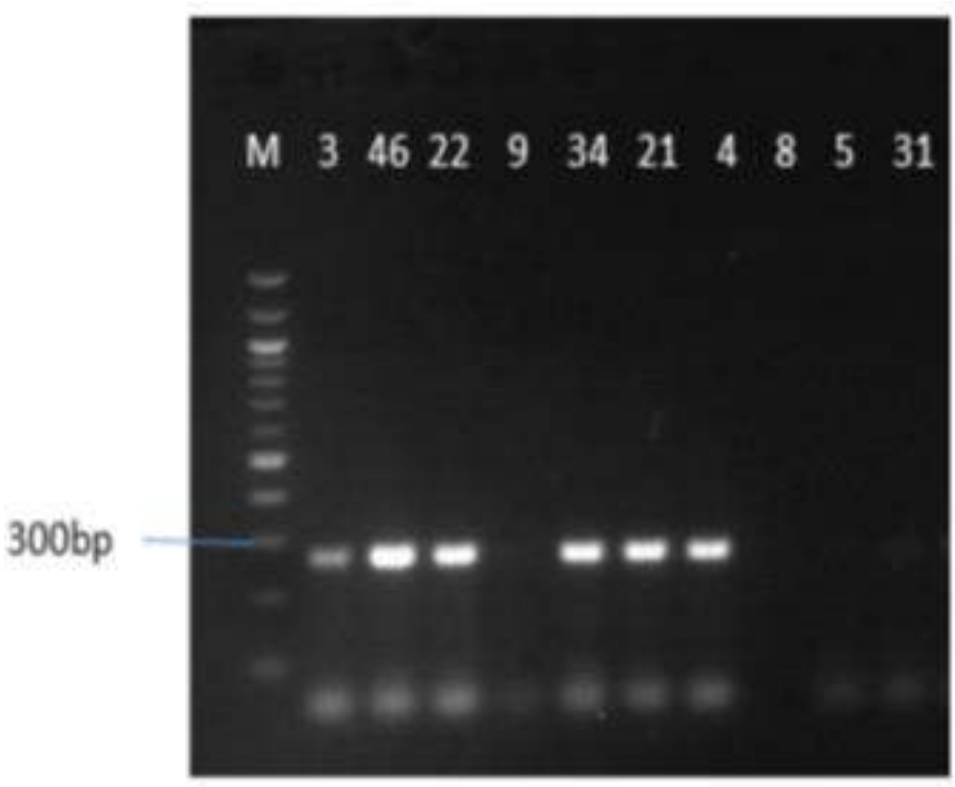

Plate 3: Agarose gel electrophoresis of Candida albicans using SAP5 primers. Lane M=molecular marker, lane 31 was the negative lane, while other lanes were the test isolates

MARKOV CHAIN ANALYSIS OF INFECTIOUS DISEASES USING NON-PHARMACEUTICAL INTERVENTION AS CONTROL STRATEGY (A CASE OF COVID-19 DISEASE IN NIGERIA)

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ABSTRACT

Various mathematical and standard epidemiological models have been proposed in studying infectious disease dynamics which assist to comprehend the spread of disease infections. However, various diseases like, Ebola, chickenpox (Varicella), chikungunya, dengue, diphtheria and corona virus disease 2019 (COVID-19) are global health challenge. In this work, discrete time Markov chain analysis of infectious diseases using non-pharmaceutical intervention as control strategy with particular attention to the case COVID-19 disease in Nigeria. A deterministic analysis is performed and the estimated value of the basic reproduction number

1. INTRODUCTION

The use of drugs and vaccines are largely required for the treatment of many infectious diseases. But in the cases where such drugs and vaccines are limited or not available, non-pharmaceutical interventions (NPIs) are needed to control the outbreaks of such diseases. Diseases like, Ebola, chickenpox (Varicella), chikungunya, dengue, diphtheria and corona virus disease 2019 (COVID-19) are global health challenge. Non Pharmaceutical Interventions (NPIs) like social and physical distancing, covering nose when coughing, washing of hands etc., are to be strictly implemented to curtail the spread of the disease (Xu *et al.*, 2021; keeling *et al.*, 2008).

Access to health care facilities in Nigeria and many other African countries is very low hence NPIs could be effective strategies. For example, Nigeria was one of the epicenters of COVID-19 in Africa with an estimates population of over 200 million (Nigeria population 1950 – 2024). Since the index case, which was from an Italian immigrant on 27th of February 2020, Nigeria experienced a daily surge in the number of confirmed cases of the infection. By September 30, 2020 Nigeria has recorded 58,848 confirmed cases, 11,730 of active 7,378 cases, 50,358 discharge cases and 1,112 deaths respectively (NCDC; 2020).

Several mathematical models have been developed to depict COVID-19 transmission in human and individual host as well as many nations including Nigeria (Adegboye *et al*., 2020; Gumel *et al.*, 2021 and Iboi *et al*., 2020, Mmaduakor, 2022). Also, many quantitative and qualitative methods have been employed to analyze infectious models models (Ogunlade *et al*., 2020, Ogunmiloro *et al.*, 2021). An epidemic threshold called the basic reproduction number is a key quantity used in describing the elimination or persistence of a disease when an infection arises through an individual on human vulnerable host community (Zhou *et al.*, 2018).

According to Ross (2006) and Kobayashi, 2012, the Markov chain is a stochastic process. Markov Chain is being commonly applied to solve and analyse problems in manpower planning, whether forecast, stock exchange, etc., that can be seen in various studies (Fernando, 2012; Choji, 2013; Chung, 2011; Fernado,2012; Masseran, 2015; Saad *et al*., 2014; Güler *et al*., 2016; Ogunlade , 2020; Ogunlade *et al.*, 2024**)**. In this work, discrete time Markov chain analysis of infectious diseases using non-pharmaceutical intervention as control strategy with particular attention on the case COVID-19 disease in Nigeria. A deterministic COVID-19 model with cases of isolation and non-isolation of infected human with mass action and saturated incidence function is formulated. The basic analysis and the computation of basic reproduction number is carried out and the Markov chain method is further on applied as a predictive tool to determine the future state of the disease prevalence. The article is placed into sections. Section 2 involves the mathematical model derivation, parameter estimation and data fitting and basic analysis of the model. Also, Section 3 deals with the extension of the model into a Markov chain process as well as Markov chain estimations of the probability values and finally, Section 4 deals with the discussion of results.

1. THEMATICAL MODEL DERIVATION

Consider a system of nonlinear first ordinary differential equations. Let the total human host population be denoted by $N_h(t)$ at time $t > 0$, vulnerable individuals $S_c(t)$, infected isolated individual $I_1(t)$, infected non - isolated individuals $I_2(t)$ and recovered individuals R_c. So that $N_h(t) = S_c(t) + I_1(t) + I_2(t) + R_c(t)$. Also, a compartment depicting an infested environmental sources I_3 is considered, given by

$$\begin{aligned}
\frac{dS_c}{dt} &= A - \left(\beta_1 I_1 + \frac{\omega_0 \beta_2 I_2}{1 + m_0 I_2} + \omega_1 \beta_3 I_3\right) S_c - \mu S_c \\
\frac{dI_1}{dt} &= \beta_1 I_1 S_c - (\mu + \alpha\phi_1 + c_1 + \sigma_0) I_1 \\
\frac{dI_2}{dt} &= \frac{\omega_0 \beta_2 I_2 S_c}{1 + m_0 I_2} - (\mu + \phi_2 + c_2 + \xi\sigma_1) I_2 \\
\frac{dI_3}{dt} &= \sigma_0 I_1 + \xi\sigma_1 I_2 - v_0 I_3 \\
\frac{dR_c}{dt} &= \alpha\phi_1 I_1 + \phi_2 I_2 - \mu R_c
\end{aligned} \qquad (1)$$

together with the initial condition $S_c(0) \geq 0, I_1(0) \geq 0, I_2(0) \geq 0, I_3(0) \geq 0, R_c(0) \geq$

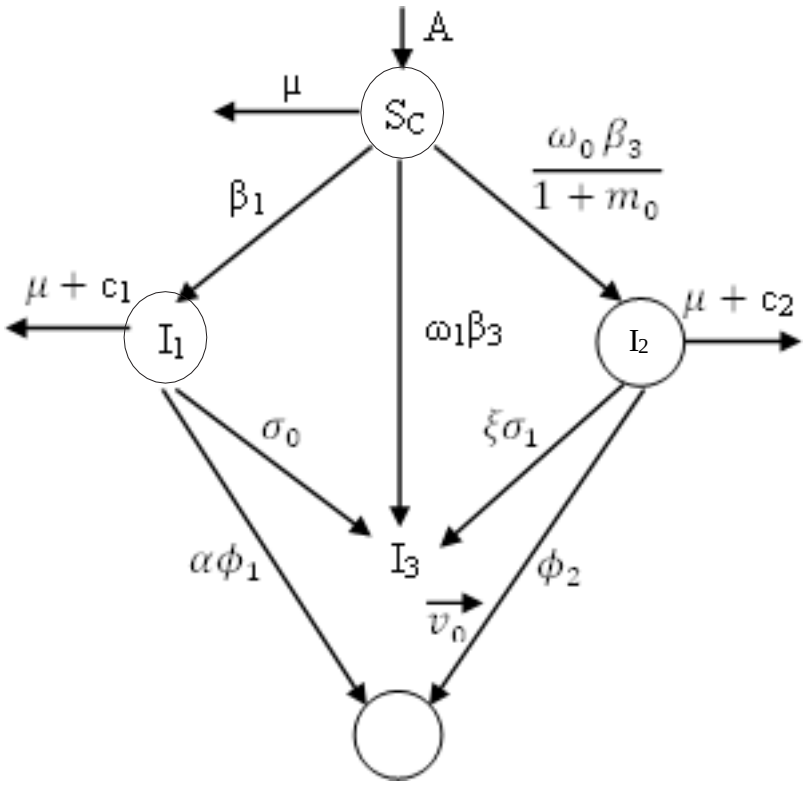

Fig 1. COVID-19 transmission flow diagram in human and environment host community

Fig. 1 is the depiction of the COVID-19 transmission in the human and environment host population. The steady influx of vulnerable individuals into the vulnerable sub-population of humans is denoted A, while a decrease of vulnerable human sub-population is denoted by the quantities

$$\beta_1 I_1 S_c \text{ and } \frac{\omega_0 \beta_2 S_c}{1 + m_0 I_2}$$

which represent the mass action and nonlinear saturated incidence function,

where β_1 and β_2

represent the transmission rate between vulnerable, isolated and non-isolated infected humans

. $\omega_0 > 0$

denote the modification parameter that considers the higher tendency of non-isolated infected human to pass on infection relative to isolated infected humans.

$$\text{If } \omega_0 = 1,$$

then they exhibit equal abilities to pass onCOVID-19 infections, while

$$\beta_1 I_3$$

denote indirect transmission rate between vulnerable individuals and infected environmental sources. The quantity $\alpha\phi_1$

leads to increase of the recovered human sub-population,
where ϕ_1

denote recovery rate of isolated infected humans and α

is the modification parameter that considered the higher ability of isolated infected humans to recover quickly relative to non-isolated infected humans. The quantity

$$\phi_2 I_2$$

also increases the recovered class, where ϕ_2 denote the recovery rate of non-isolated infected humans. The natural death rate μ is associated to all sub-population of humans and the COVID-19 infected related mortality denoted

$$c_1 \text{ and } c_2$$

reduces the isolated and non-isolated infected human sub-population. The quantities

$$\sigma_0 I_1 \text{ and } \xi\sigma_1 I_2$$

increases the environment sub-population through the infection contribution of isolated and non-isolated infected humans at the rate of

$$\sigma_0 \text{ and } \sigma_1,$$

where ξ

denote the modification parameter that account for the relative ability of non-isolated infected individuals to contribute infection higher than isolated infected humans, while v_0 represent the net death rate of the virus. The model cannot encompass on the complexities involving COVID-19 dynamics. Hence assumptions guiding the model implementation are presented.

- A non-constant population is considered.
- Trial drugs and vaccine are still being considered.
- Strong immunity, natural therapy, trial drugs leads to recovery.
- There is natural and COVID-19 infection related deaths.
- Virus disintegrate with time in the environment.
- Infectious humans contributes to the environment.

2.1.Estimation of Parameters and Fitting of Data

The model system (1) parameters are estimated from literature and cumulative cases of COVID-19 prevalence published by Nigeria Center for Disease Control (NCDC, 2020) as at August 31, 2020. The nonlinear least square method, which deals with minimizing the sum of squares of cumulative cases predicted by the model and cumulative cases in Nigeria. The demographic birth rate for Nigeria is denoted by

$$\frac{\Lambda}{\mu} = 3.2192\text{year}^{-1} \text{ where } \mu$$

is the demographic natural death rate taken to be 11.577, so that

$$\frac{1}{\mu} = 0.0863\text{year}^{-}$$

Mortality rate associated to COVID-19 infected as at August 31, 2020 was 1013, so that

$$\frac{1}{c_1} = 0.000987\text{day}^{-1} \text{ and } c_2 = 0.00137\text{day}^{-1} \therefore$$

Also, the rate of recovery of the two groups of infected individuals is given by $\frac{1}{7}\text{day}^{-1}$.

The variables and parameters are tabulated below.

Table 1: Interpretations of Variables of a model

Variables	Interpretations
S_c	Vulnerable human individuals
I_1	Isolated infected human individuals
I_2	Non-isolated infected human individuals
I_3	Infected environmental sources
R_c	Recovered individuals

Table 2: Interpretations of Parameters of the model

Estimated parameters	Interpretations	Values	Sources
A	Birth rate	3.2192 year^{-1}	Estimated NCIA (2020)
μ	Natural mortality rate	0.0863 year^{-1}	Estimated NCIA (2020)
α	Modification parameter	0.001139 day^{-1}	Gumel *et al* (2021), Iboi *et al*. (2020)
ϕ_1	Recovery rate of isolated individuals	$\frac{1}{7}\text{day}^{-1}$	Gumel *et al* (2021), Iboi *et al*. (2020)
ϕ_2	Recovers infected rate of individuals	$\frac{1}{7}\text{day}^{-1}$	Gumel *et al* (2021), Iboi *et al*. (2020)
c_1	Mortality due to COVID-19 in isolated individuals	0.1 day^{-1}	NCDC (2020)
c_2	Mortality due to COVID-19 in non-isolated individuals	0.001137 day^{-1}	Estimated NCDC(2020)

Table 3: Interpretation of the Fitted Parameters of the model

Fitted parameter	Interpretations	Values	Sources
ω_0	Modification parameter	0.7532	Fitted
β_1	Direct transmission rate	0.9213	Fitted
β_2	Direct transmission rate	0.8043	Fitted
β_3	Indirect transmission rate	0.2100	Fitted
m_0	Emergence of SARS-COV-2 virus	0.3110	Fitted
σ_0	Infectious contribution of isolated humans	0.2330	Fitted
σ_1	Infectious contribution of non-isolated humans	0.0032	Fitted
ξ	Modification parameter	0.4310	Fitted
v_0	Net death rate of virus	0.2020	Fitted

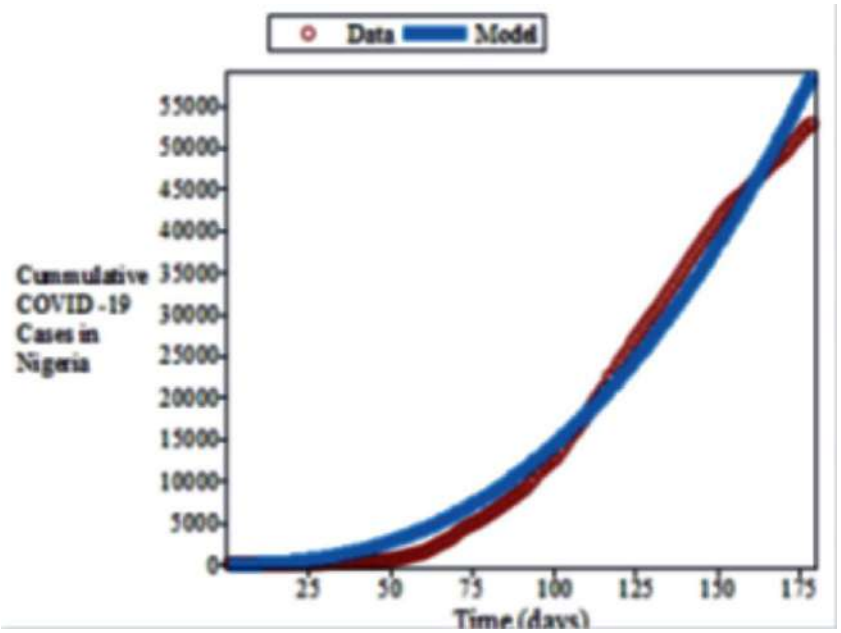

Figure 2: Data fit of cumulative cases of COVID - 19 as at August 25, 2020

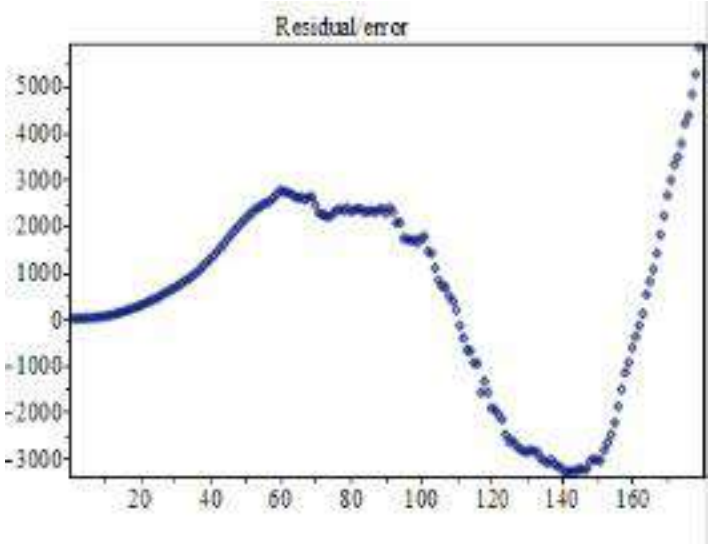

Figure 3: Errors of the fit in Nigeria using model system (1)

2.2. BASIC ANALYSIS OF THE MODEL

Here, the model is analyzed in a feasible region where COVID – 19 transmission is relevant. That is, if the whole equation (1) is summed up, we obtain

$$\dot{N}_c = A - \mu(S_c + I_1 + I_2 + R_c) - c_1 I_1 - c_2 I_2 \quad (2)$$

In the absence of COVID – 19 infection related mortality after simple simplification, becomes

$$\dot{N}_c = A - \mu N_h \quad (3)$$

In turn, solving (3) yields

$$N_c(t) \leq N_c(0)e^{-\mu t} + \frac{A}{\mu}(1 - e^{\mu t}) \quad (4)$$

It is observed in (4), that $N_c(t) \leq \frac{A}{\mu}$ if $N_c(0) \leq \frac{A}{\mu}$ and $N_c(0) > \frac{A}{\mu}$ when $N_c(t) \to \frac{A}{\mu}$ as $t \to \infty$. Then, the model is said to be positively invariant in the sense of COVID-19 transmission as shown in (1). Furthermore, model system (1) possess a continuum of COVID-19 absent equilibria given by

$$C_v^*(S_c^*, I_1^*, I_2^*, I_3^*, R_c^*) = (S_c(0), 0, 0, 0, 0) \quad (5)$$

Where $S_c(0)$ is the initial total size of vulnerable individual population.

A key threshold called the basic reproduction number (R_{cv}) is obtained using the next generation matrix method [25, 28]. The R_{cv} for model system (1) is given by

$$R_{cv} = \frac{\omega_0 A \beta_1 \beta_2}{\mu^2(\mu + \alpha\phi_1 + c_1 + \sigma_0)(\mu + \phi_2 + c_2 + \xi\sigma_1)} \quad (6)$$

The quantity in (6) measures the average number of new COVID-19 cases arising through the introduction of a COVID-19 typical infected individual into a population of vulnerable humans. When $R_{cv} < 1$, COVID-19 infection dies out, when $R_{cv} > 1$,

$$R_{cv} \approx 7.51,$$

which indicate that 7 persons are infected on the average through the presence of an infected individual in the human and environment host population. With the estimation of

$$R_{cv}$$

in this work, COVID-19 remains in the endemic state in Nigeria. Hence, Non-pharmaceutical interventions are to be forestalled to curtail the pandemic and prevent a likely second deadly wave .

2. OF THE MODEL INTO A MARKOV CHAIN PROCESS

Here, the interest is changing the parameters in model system (1) into a Markov chain process. Consider five discrete states: susceptible (state 0), isolated infected (state1). Non - isolated infected(state 2), infected environmental sources (state 3) and recovered (state 4) states. If

$$P(H_n = i_n | H_{n-1} = i_{n-1}, \ldots, H_1 = i_1, H_0 = i_0) = P(H_n = i_n | H_{n-1} = i_{n-1}) \quad (7)$$

Then the transition matrix, N, which indicates the observed frequency of transition or jump from one state to another state. Thus,

$$N = \begin{bmatrix} A & \beta_1 & \frac{\omega_0\beta_2}{1+m_0} & \omega_1\beta_3 & \mu \\ \beta_1 & \alpha\phi_1 & c_1 & \sigma_0 & \mu \\ \frac{\omega_0\beta_2}{1+m_0} & \phi_2 & c_2 & \zeta\sigma_1 & \mu \\ \sigma_0 & \zeta\sigma_1 & v_0 & 0 & 0 \\ \alpha\phi_1 & \phi_2 & \mu & 0 & 0 \end{bmatrix} \quad (8)$$

Then the transition probability

(P_{ij}) for $i, j = 0.1.2.3.4$ is denoted in matrix form as:

$$P_{ij} = \begin{bmatrix} p_{00} & p_{01} & p_{02} & p_{03} & p_{04} \\ p_{10} & p_{11} & p_{12} & p_{13} & p_{14} \\ p_{20} & p_{21} & p_{22} & p_{23} & p_{24} \\ p_{30} & p_{31} & p_{32} & p_{33} & p_{34} \\ p_{40} & p_{41} & p_{42} & p_{43} & p_{44} \end{bmatrix} \quad (9)$$

Where $\sum_{j=0}^{4} P_{ij} = 1, \quad i = 0,1,2,3,4.$ (10)

3.1. ESTIMATING TRANSITION PROBABILITIES

The transition events are independent of one another; the likelihood of the transition probability P_{ij}, follows a binomial model:

$$L(P_{ij}|N,x) = \binom{N_i}{x_{ij}} P_{ij}^{x_{ij}} (1-P_{ij})^{N_i - x_{ij}} \quad (11)$$

Where N_{ij} is the number of observed transition that starts from state i to j and

$$\Sigma_j P_{ij} = 1 \quad (12)$$

From (9), the transition probability matrix is estimated as a multinomial distribution given as

$$\hat{P}_{ij} = \frac{x_{ij}}{\Sigma_j x_{ij}} = \frac{x_{ij}}{N_i} \quad (13)$$

For $i, j = 0,1$ with standard errors $(\hat{s}.e)$ from the sampling distribution of the ME estimate given as

$$\hat{s}.e(P_{ij}) = \sqrt{\frac{\hat{P}_{ij}(1-\hat{P}_{ij})}{N_i}} \quad (14)$$

3.2. MARKOV PROCESS PROBABILITY VALUES

Stationary probability distribution and mean return time can be obtained for Markov process probability values which will describe the behavior of COVID-19 in long term forecasting where the chain is enough for a long period of time with steady-state probabilities that are independent from initial conditions (Ibe, 2013) . The stationary probability distribution is given as

$$\rho_j = \lim_{n\to\infty} P(X_n = j | X_0 = i) \quad (15)$$

The probability of finding the process in state j is irrespective of starting state for a long duration in the process (Pinsky & Karlin, 2011). The value of ρ_j will be high if the probability occurrences of state j is high (Grinstead *et al.*, 2006).

The mean return time can be calculated to identify the average time for specific states to return back to itself, h_{ij}. This is given as

$$h_{ij} = \frac{1}{\rho_j} \quad (16)$$

3.3. ESTIMATING P^n TRANSITION MATRIX

To estimate the nth step probability matrix $\mathbf{P}^n$ That (1984) uses Eigen value and Eigen vector. The

$$P_{ij}^n . i, j = 0.1.2.3.4$$

transition probability matrix was estimated for COVID-19 infection using a decomposition method that requires eigenvalues and their corresponding eigenvectors. Hence, it can be estimated using the decomposition below:

$$P^n = Q \wedge^n Q^{-1} \quad (17)$$

where Q is a 5×5 nonsingular matrix $(X_0, X_1, X_2, X_3, X_4)$ and $X_j, (j = 0,1,2,3,4)$ is the right eigenvectors corresponding to the eigenvalues $\lambda_j (j = 0,1,2,3,4)$. Thus,

$$PX_j = \lambda_j X_j \quad (18)$$

$$\wedge^n = \begin{bmatrix} \lambda_0^n & 0 & 0 & 0 & 0 \\ 0 & \lambda_1^n & 0 & 0 & 0 \\ 0 & 0 & \lambda_2^n & 0 & 0 \\ 0 & 0 & 0 & \lambda_3^n & 0 \\ 0 & 0 & 0 & 0 & \lambda_4^n \end{bmatrix} \quad (19)$$

3. CHAIN ESTIMATION

We obtain the estimate of the transition probability for the COVID-19 model system (1). Using the maximum likelihood estimate, we get the transition probability matrix given by (20)

$$P_{covid} = \begin{bmatrix} 0.6199 & 0.1450 & 0.0636 & 0.1549 & 0.0166 \\ 0.6550 & 0.0002 & 0.0870 & 0.1827 & 0.0751 \\ 0.2990 & 0.1294 & 0.0010 & 0.4925 & 0.0781 \\ 0.2773 & 0.7184 & 0.0043 & 0 & 0 \\ 0.0009 & 0.6229 & 0.3762 & 0 & 0 \end{bmatrix} \quad (20)$$

Fig 4 describes the transition probablity of the model of system given by (1)

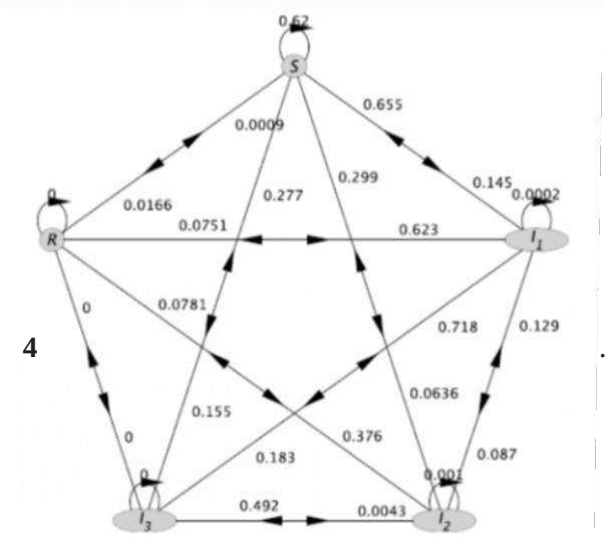

Figure 4: Chain of transition probability for COVID-19 model

Also, the mean stochastic matrix of (20) yields

$$P_{covid}(mean) = \begin{bmatrix} 0.6204 & 0.1453 & 0.0634 & 0.1541 & 0.0168 \\ 0.6542 & 0.0002 & 0.0872 & 0.1837 & 0.0747 \\ 0.2978 & 0.1311 & 0.0007 & 0.4925 & 0.0784 \\ 0.2787 & 0.7170 & 0.0042 & 0 & 0 \\ 0.0009 & 0.6245 & 0.3747 & 0 & 0 \end{bmatrix} \quad (21)$$

and the standard deviation of the stochastic matrix (20) yields

$$P_{covid}(S.D) = \begin{bmatrix} 0.0473 & 0.0360 & 0.0246 & 0.0356 & 0.0134 \\ 0.0485 & 0.0016 & 0.0288 & 0.0395 & 0.0263 \\ 0.0455 & 0.0354 & 0.0024 & 0.0491 & 0.0278 \\ 0.0447 & 0.0446 & 0.0042 & 0 & 0 \\ 0.0034 & 0.0484 & 0.0489 & 0 & 0 \end{bmatrix} \quad (22)$$

While the 1% and 99% confidence intervals (C.I) are respectively given by

$$P_{covid}(1\%\,(C.I)) = \begin{bmatrix} 0.5212 & 0.0793 & 0.0234 & 0.0855 & 0.0012 \\ 0.5545 & 1.0535\times10^{-05} & 0.0366 & 0.1098 & 0.0282 \\ 0.2072 & 0.0655 & 2.9410\times10^{-19} & 0.3906 & 0.0355 \\ 0.1918 & 0.6222 & 0.0000 & 0.0000 & 0.0000 \\ 4.4446\times10^{-32} & 0.5207 & 0.2795 & 0.0000 & 0.0000 \end{bmatrix} \quad (23)$$

$$P_{covid}(99\%\,(C.I)) = \begin{bmatrix} 0.7283 & 0.2358 & 0.1346 & 0.2442 & 0.0637 \\ 0.7594 & 0.0072 & 0.1622 & 0.2847 & 0.1463 \\ 0.4195 & 0.2144 & 0.0092 & 0.0028 & 0.1601 \\ 0.4031 & 0.8135 & 0.0300 & 0.0000 & 0.0000 \\ 0.0152 & 0.7353 & 0.4919 & 0.0000 & 0.0000 \end{bmatrix} \quad (24)$$

4.1 STATIONARY PROBABILITIES

In the long run, the value below derived the steady state probability of COVID-19 infection in the future by using equation (9)

$$p_j = [0.535156546280454 \quad 0.215609042590436 \quad 0.0648612328168834 \quad 0.154231678262430 \quad 0.0301418000497959] \quad (27)$$

The mean return time for every state of COVID-19 as shown in the matrix below.

$$b_{ij} = \begin{bmatrix} 1.8686121041597 & 4.63802439816 76 & 15.417529956965 \\ 6.4837523086435 & 33.176518932112 \end{bmatrix} \quad (28)$$

4.2. ESTIMATING THE P^n TRANSITION PROBABILITY

The P^n transition probability matrix which predicts the transition probability for COVID-19 at any time step was estimated from equation (17) and it is given by

$$P^n_{covid} = \begin{bmatrix} 0.2787 & 0.7170 & 0.0042 & 0 & 0 \\ 0.0009 & 0.6245 & 0.3747 & 0 & 0 \end{bmatrix} \quad (29)$$

Clearly, n = 1 from the estimated P^n Transition matrix fives the actual first transition matrix P_{covid}. Therefore, the transition matrix at any time step $(n \geq 1)$ can be generated from the P^n matrix.

DISCUSSION

We formulated a model depicting the transmission of COVID – 19 disease in human and environment host population and fit the model using data on COVID – 19 cumulative cases recorded as at August 31 2020 in Nigeria published by NCDC (2020). Figures 2 and 3 display the model fit using the data and the errors of the fit. It is observed that Nigeria still experiences daily surge of cases of infection in humans. Analytically the model is well behaved and the basic reproduction number

$$R_{cv} \text{ is estimated to be } R_{cv} \approx 7.51,$$

which indicated that the disease was still endemic in the nation at that time. The results of this study also revealed that Markov chain forecasting model is suitable for the data and it is able to predict future COVID-19 behavior.

CONCLUSION

In this paper, a model representing COVID-19 transmission amidst isolated and non-isolated humans is considered. The transition estimates of the disease outcomes at discrete time steps for future predictions is obtained through a developed discrete-time Markov chain model. The developed model surmises that the transmission of COVID-19 among humans is utterly influenced by the stochastic factors. We fit the data published by NCDC using the model understudy, which provide an effective fit and low residuals. The reproduction number R_{cv} was obtained

to be approximately 7.51 by using the estimated parameters, which indicates that an average COVID-19 infected human is efficient of infecting at least 7 persons in the absence of strict implementation of NPIs in the community host. The Markov chain model is appropriate and it's a suitable technique in estimating epidemiological quantities of infectious disease.

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ASSESSMENT OF THE EFFECTS OF HEPATITIS B INFECTION ON SOME HEPATIC BIOMAKERS IN INFECTED PREGNANT WOMEN AT WESLEY GUIDE HOSPITAL, ILESA, OSUN STATE, NIGERIA.

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Abstract

Hepatitis B virus (HBV) is a blood borne pathogen transmitted through parenteral (intravenous drug use) and mucosal (sexual contact), with mother-to-infant transmission being an important source of endemic in the absence of maternal screening and infant vaccination. HBV causes severe liver disease, leading to liver cirrhosis and hepato-cellular carcinoma. This study was carried out to access the impact of hepatitis B infection on some hepatic biomarkers in infected pregnant women attending Wesley Guild Hospital Ilesha, Osun state. The subjects used for this study were made up of 50 pregnant women infected with Hepatitis B as the test and 50 Hepatitis B negative pregnant women as the control. They were tested and confirmed to be Hepatitis B positive and negative respectively.Venous blood sample was collected from each participant into lithium heparinised anticoagulant bottles using sterile syringe after issuing them the consent form. The blood samples collected were subjected to centrifugation for 5 minutes at 1000rpm to obtain the plasma which was stored at room temperature prior analysis.Plasma activity of aspartate aminotransferase (AST), alanine transaminase(ALT) and alkaline phosphatase (ALP) and levels of both total and conjugated bilirubin were determined in both test and control using standard methods, The result of the study shows a significant increase ($p<0.05$) in the activity of ALT (37.80 ± 0.48 IU/L)and ALP (364.10 ± 11.82 IU/L)in the plasma of hepatitis B infected pregnant women when compared with the control. However, there is no significant difference in the activity of AST (23.52 ± 0.81 IU/L)(24.94 ± 0.64 IU/L) in both test and control respectively. The plasma level of total bilirubin (29.32 ± 1.90Umol/L) was significantly raised in pregnant women infected with hepatitis B when compared with the control group (18.84 ± 0.69 Umol/L). The elevated plasma activity of these enzymes and the raised level of total bilirubin in these patients indicate liver damage which could be as a result of hepatitis B infection. This could be however a potent prediction of an acute liver infection which can be used in diagnosis of HBV infection.

Introduction

Hepatitis B is an infection of the liver caused by the hepatitis B virus. It causes acute or chronic infection and puts human at high risk of death from cirrhosis and liver cancer. Its mode of transmission is through contact with infected body fluids like blood, saliva, vaginal fluids and semen. It can also be passed from a mother to her baby. It is estimated that over 254 million people are chronically infected with hepatitis B virus (HBV) worldwide (WHO, 2024)

and over 880,000 annual deaths resulting from HBV-related liver diseasesuch as hepatocellular carcinoma(HCC) and liver cirrhosis(Tan et al., 2021). Although childhood vaccination programs have been operational since the 1990s, yet a significant number of individuals worldwide live with this life-threatening disease that require monitoring of treatment response, viral activity, and disease progression to minimize the imminent mortality and morbidity as a result of the disease. (Lampertico*et al*.,2017)

HBV binds via its surface antigen to the sodium taurocholate co-transporting polypeptide (NTCP) on the surface of the hepatocytes to enter and infect cells (Afraieetal., 2023). After internalization of HBV, the viral relaxed circular DNA (rcDNA) genome is transported to the nucleus where it is completed into cccDNA by host enzymes. The cccDNA is later transcribed in the nucleus into mRNA transcript from which the viral protein is translated.

Hepatitis B vaccine is safe for pregnant women in each trimester and those who are seronegative can be vaccinated during pregnancy. Serum level protection in pregnant women is only 45%, this is lower than 55% mentioned for non-pregnant women as well as for women during the postpartum period that had received three doses of the vaccine (Wiseman *et al*., 2009). Vaccination during pregnancy, in addition to being beneficial for the mother, also provides partial immunity for the infant.Most infections of pregnant women are chronic and asymptomatic and are diagnosed in prenatal screening.

Acute hepatitis in pregnancy has been implicated to cause jaundice and induce premature labor and premature complications. Also, chronic hepatitis in pregnancy or cirrhosis poses a higher risk of fetal loss during pregnancy. Developed countriesroutinely screened pregnant women for HBV infection. However, in developing countries, the case is different, as many women are not screened during pregnancy because of less awareness. The HBV status of the mothers should be known before parturition in order to prevent HBV transmission from mother to child and to mitigate associated risks (Iklaki et al., 2015)

Biomarkers are equally useful in the evaluation and assessment of hepatic function and disease severity because HBV infection may alter the serum levels of certain hepatic enzymes (Sharif et al., 2016).Therefore, this study is set to assess the effect of HBV infection on the hepatic enzymes in pregnant women and its predictive role in diagnosis of the infectionin order to reduce or mitigate inherent danger it poses on the liver and the foetus

Materials

Subjects

The subjects for this study were 50 pregnant women infected with Hepatitis B and 50 Hepatitis B negative pregnant women at Wesley Guild Hospital, Ilesha, Osun state. They were tested and confirmed to be Hepatitis B positive and negative respectively.

Methods

Collection of Blood Samples

5mls of venous blood was collected from each participant into lithium heparinised anticoagulant bottles using sterile syringe after issuing them the consent form. The blood samples collectedwere subjected to centrifugation for 5 minutes at 1000rpm to obtain the plasma which were later stored at room temperature until analysis.

Assay of Plasma Aspartate Aminotransferase (AST) Activity

This wascarried out spectrophotometrically using enzymatic method of Reitman and Frankel (1957).

Assay of Plasma Alanine Aminotransferase (ALT) Activity

This assay was done spectrophotometricallyusing enzymatic method as reported by Reitman and Frankel (1957).

Determination of Plasma Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase activity wasassayed spectrophotometrically according to enzymatic method of [*]Henry (1964).

Estimation of Plasma Levels of Bilirubin (Total and Conjugated)

Plasma bilirubin concentration was estimated spectrophotometrically according to Jendrassik's method (1938).

Statistical analysis

Statistical analysis will be carried out by One Way Analysis of Variance (ANOVA) using the Package for the Social Sciences (SPSS) version 22.0, while t-test will be used for comparison within the groups. The test of variability will be carried out at 5% level of significance ($p<0.05$).

Results

Table 1:Plasma Concentration of Total Bilirubin and Conjugated Bilirubin in Pregnant Women Infected with Hepatitis B and Control Subjects.

Biochemical indices	Test Subjects (A) (n=50)	Positive control (B) (n=50)	Negative control (C) (n=50)
Total bilirubin(Umol/L)	29.32 ± 1.90*[a]	18.84 ± 0.69	13.14 ± 0.55
Conjugated bilirubin(Umol/L)	6.29 ± 0.07	8.20 ± 0.44	7.72 ± 0.27

Values were expressed as mean ± standard error of mean of three (3) determinants. Values with different superscript are significantly. *= compared with negative control, a= compared with the positive control, Test Subjects A = Hepatitis B Positive pregnant subjects, Positive control B = Hepatitis B negative pregnant individual, Negative Control C =Hepatitis B Negative non-pregnant subjects

Table 2:Plasma Concentration of Hepatic Enzymes in Pregnant Women Infected with Hepatitis B and Control Subjects.

Biochemical indices	Test Subjects (A) (n=50)	Positive control (B) (n=50)	Negative control (C) (n=50)
AST (IU/L)	.....	24.94 ± 0.64	22.06 ± 1.22
ALT (IU/L)	37.80 ± 0.48*	31.29 ± 0.12	20.42 ± 0.90
ALP (IU/L)	364.10 ± 11.82*[a]	232.30 ± 15.51*	77.30 ± 1.70

Values were expressed as mean ± standard error of mean of three (3) determinants. Values with different superscript are significantly. *= compared with negative control, a= compared with the positive control, Test Subjects A = Hepatitis B Positive pregnant subjects, Positive control B = Hepatitis B negative pregnant individual, Negative Control C =Hepatitis B Negative non-pregnant subjects

Table 3: Pearson's correlation coefficients of hepatic function indices in pregnant women infected with Hepatitis B

Parameter		Total bilirubin(Umol/L)	Conjugated bilirubin(Umol/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Total bilirubin(Umol/L)	R	1	0.781**	0.603**	0.406*	0.160
	p		0.000	0.000	0.029	0.518
Conjugated bilirubin (Umol/L)	R		1	0.420**	0.540**	0.199
	p			0.000	0.002	0.310
AST (IU/L)	R			1	0.744**	0.218
	p				0.000	0.255
ALT (IU/L)	R				1	0.350*
	p					0.032
(IU/L)	R					1
	p					

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

AST = Aspartate Aminotransferase, ALT=Alanine Aminotransferase, ALP= Alkaline Phosphatase, R = Pearson correlation value, p = p-value.

Discussion

HBV infection causes severe liver disease, leading to liver cirrhosis and hepato-cellular carcinoma (WHO, 2024). When there is liver inflammation or damage, enzymes located in the liver are often found in the bloodstream. The result of this study showed a significant increase ($p<0.05$) in the plasma activity of ALT while there was no significant difference in the plasma activity of AST in pregnant women infected with hepatitis B virus when compared with the positive control subjects. However, the activity of Alkaline Phosphatase (ALP) in these patients showed a significant increase ($p<0.05$) when compared with both negative and positive control subjects. There was also a significance difference in the plasma activity of ALP in positive control subjects when compare to negative control subjects. Similar outcome was also observed by (Abulude *et al*., 2017) .

The increased activity of ALT reported in this study may be as a result of damage to the hepatocytes due to HBV infection. In a similar study conducted by Koki *et al*. (2015) to show the activity of liver enzymes among HBV-positive patients, elevated level of ALT was observed among tested groups. In another work reported by Taura *et al*. (2013), on the analysis of the liver enzyme activity in 200 hepatitis B surface antigen (HBsAg)-seropositive patients, 17.0% were seropositive for HBeAg with elevated serum level of ALT and 44.8% of the HBeAg-infected subjects had ongoing liver damage.

The result of this study also shows that the plasma concentration of total bilirubin was raised ($p<0.05$) in pregnant women infected with hepatitis B virus when compared with the control subjects and no significant difference in the plasma concentration of conjugated bilirubin was observed among the three groups. Prolong increase in the concentration of bilirubin could mean severe liver disease and decrease hepatic clearance due to hepatitis B infection. This outcome agrees with the findings of Min *et al*., (2016), who reported significant increase in the concentration of total bilirubin in (HBs Ag) positive group when compared with those without HBsAg infection.

Conclusion

The alteration of the hepatic enzymes and compounds observed in this study might be as a result of inflammation of the liver caused by the HBV infection. However, the abnormal levels of ALP observed in the test subject and positive control group may be due to pregnancy. Therefore hepatic enzymes can be used in predicting and diagnosis of HBV infection.

Limitation of the study

This study is not without limitations, as it did not consider trimester of pregnancy, medications used currently or previously, herbal or alternative remedies, or occupational exposure to toxins by subjects prior to this study, which may have effects on liver enzymes.

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CRUDE PROTEIN AND AMINO ACIDS PROFILES OF THREE UNDEREXPLOITED LEAFY VEGETABLES CONSUMED IN EKITI STATE, NIGERIA

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ABSTRACT

The study was conducted to determine the protein and amino acids content in three underutilized semi-wild leafy vegetables (Celosia argentea, Asystasia gangetica, Launaea taraxacifolia). The crude protein (CP) content was determined using the methods prescribed by AOAC and amino acids (AA) composition was determined using Technicon Sequential Multisample Amino Acid Analyzer (TSM) (Technicon Instruments Corporation, New York). The results obtained showed that CP content in the leafy vegetables ranged between 14.3 and 17.2 g/100g and a CV % value of 9.54 with C. argentea having the highest concentration. The AA composition showed the following ranges in individual samples: 0.475 – 13.7 g/100 g with a total of 88.2 g/100g in C. argentea, 0.419 – 11.0 g/100 g with a total AA of 88.0 g/100g in A. gangetica and 0.496 – 12.0 g/100 g and total AA of 94.6 g/100g in L. taraxacifolia. The quality parameters of the AA respectively in the three vegetables C. argentea, A. gangetica, L. taraxacifolia were as follows: TEAA with histidine (43.7 – 49.7 g/100g, 49.6 – 52.8 %); TNEAA (41.5 – 44.9 g/100g, 47.2 – 50.4 %), predicted protein efficiency ratios (P-PER$_1$ and P-PER$_2$): 1.53 – 2.15 and 1.69 – 1.83; isoelectric point pI (5.15 – 5.77); biological value (BV) and Essential amino acid index (EAAI): 80.8 -85.6 and 84.9 -89.3. The three scoring patterns of the amino acids showed tryptophan as the first limiting AA across the three vegetables analysed. The statistical analysis showed that significant differences existed among the results of methionine, proline and tyrosine. This study has shown that the three vegetables would serve as cheap sources of protein and amino acids in human nutrition especially L. taraxacifolia.

Keywords: *Esesential;Non-essential;Amino acids; Crude protein;Vegetables*

1. Introduction

According to reports, leafy vegetables—especially the ones that are typically consumed in many Nigerian households—are an essential component of the majority of culinary preparations in many families, particularly in the western region (Arowora et al., 2017). Although each location has a certain variety of

vegetable that is typically grown due to environmental needs and the relative importance of such vegetables, they are generally available throughout the nation. Nigeria alone is thought to consume more than 60 different types of green leafy vegetables (Okaka et al., 1985). Nigeria and other developing nations frequently consume wild and semi-wild food resources as a primary source of leafy vegetables due to food shortages and the high cost of cultivatedgreen vegetables (Kubmarawa et al., 2008). It is often recognized that vegetables are a good supply of minerals, vitamins, and proteins, which people need on a daily basis for healthy metabolic and biochemical processes (Arowora et al., 2017). Additionally, veggies are a good source of essential nutrients.

The development of goods enriched in crude plant components, particularly those with a high-quality protein content, may be the answer to the problem of improving the quality of food (Sokolova et al., 2021). Many vegetables have been reported to produce these ingredients in the literature, such as Amaranth (Gins et al., 2017); Telfalria occidentalis and Sesamum Indicum and Balanites aegyptical (Kubmarawa et al., 2008); Talinium triangulare, Vernonia amygdolia, and Solanum macrocarpon (Arowora et al., 2017; Adesina et al., 2020).

According to reports, Asystasia gangetica, Launaea taraxacifolia, and Celocia argentae are commonly consumed in Southwest Nigeria (Adesina et al., 2020). However, little is known about their main nutritional benefits, particularly their vitamin, protein digestibility, and amino acid compositions. Protein's amino acid composition—more especially, the amount of essential amino acids—is recognized as the primary determinant of its quality. The crude protein and amino acid profiles of the semi-wild leafy vegetables mentioned above need to be examined in order to promote their broader use and exploitation.

2. Materials and methods

2.1. Materials

2.2.1. Samples collection

The Department of Plant Science and Biotechnology's herbarium at Ekiti State University in Ado Ekiti, Nigeria, authenticated the three leafy vegetables that were analyzed. They were sourced from nearby farms in Ire-Ekiti, along the Ilupeju-Ekiti road in the Oye Local Government Area of Ekiti State.

2.2.2. Samples treatment

The three veggies' fresh leaves were properly cleaned with distilled water, then individually shred and allowed to air dry for around a week. After the leaves were ground into a powder, 10 g of each sample were chosen at random and kept in an airtight plastic container at 4^0C while the assays were being conducted.

2.2.3. Determination of amino acids

Defatting: Samples weighing between 0.137 and 0.169 g were placed in an extraction thimble, and fat was extracted using a Soxhlet extraction device and a chloroform/methanol mixture (AOAC, 2006) . The evacuation took five to six hours.

Hydrolysis of the samples: Glass ampoules were filled with 30–35 mg of the defatted samples. To prevent the oxidation of some amino acids during hydrolysis, seven milliliters of 6 M HCl were added, and nitrogen gas was passed into the ampoule to expel oxygen. After that, each glass ampoule was sealed using a Bunsen flame and baked for 22 hours at 105°C ±5°C. After cooling, the ampoule was cracked open at the tip, and the contents were filtered to get rid of the humins.

After that, the filtrate was vacuum-evaporated in a rotary evaporator until it was completely dry at 40°C. Five milliliters of acetate buffer were then used to dissolve each residue, which was then placed in a plastic specimen container and kept in the deep freezer.

Ion exchange chromatography (IEC) was used as the sample analysis technique (FAO/WHO, 1991). For every sample, 5–10 µl of material were loaded. These were poured into the analyzer's cartridge. The study was performed using the Technicon Sequential Multisample Amino Acid Analyzer (TSM) (Technicon Instruments Corporation, New York). The hydrolysate's free acidic, neutral, and basic acids can be separated and analyzed using the TSM analyzer. For every sample, the analytical time lasted 76 minutes. At 60 degrees Celsius, the column flow rate was 0.50 milliliters per minute, and the repeatability was within ±3%. Every peak created by the TSM chart record—each of which represents an amino acid—had its net height measured and computed. Two determinations were averaged to produce the reported values. Cost prevented tryptophan from being determined. The micro-Kjeldahl method (Pearson, 1976) was used to determine the nitrogen, using N x 6.25 for crude protein. An artificial amino acid called norleucine (PubChem, CID 21236) is utilized in experiments to investigate the structure and function of proteins. In the analyses for the composition of amino acids, it served as the internal standard.

Some calculations were made from the analytical results:

I. **Estimation of isoelectric point(*pI*)**: The formula of the form (Olaofe and Akintayo, 2000) can be used to estimate the isoelectric point (pI) for a mixture of amino acid

$$IP_m = \sum_{i=1}^{n} IP_i X_i \quad (1)$$

where IP_m is the isoelectric point of the mixture of amino acids, IP_i is the isoelectric point of the i^{th}

amino acid in the mixture and Xi is the mass or mole fraction of the i^{th} amino acid in the mixture

ii. **Estimation of predicted protein efficiency ratio (P-PER):** Computation of protein efficiency ratio (C- PER or P-PER) was done using the equationssuggested by Alsmeyer et al. (1974).

$P\text{-}PER_1 = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr)$ (2)

$P\text{-}PER_2 = -0.684 + 0.456\ (Leu) - 0.047\ (Pro)$ (3)

iii. *Leucine/isoleucine ratio:* The leucine/isoleucine ratios, their differences and their percentage differences were calculated**.**

iv. **Estimation of essential amino acid index (EAAI):** The method of EAAI calculation according to Oser (1959) using the egg protein amino acid as the standard was used.

v. **Estimation of biological value (BV):** Computation of biological value (BV) was calculated following the equation of Oser (1959):

Biological value =1.09 (EAAI) – 11.73 (4)

Computation of amino acid scores: Three methods were used to calculate the amino acid scores: essential amino acid scoring pattern (FAO/WHO, 1973), essential amino acid values compared to the amino acid profile of a whole hen's egg (Paul et al., 1978), and essential amino acid suggested pattern of requirements for preschoolers (FAO/WHO/UNU, 1985).***2.2.4***.

Statistical evaluation

The grand mean, standard deviation (SD), and coefficient of variation (CV%) were computed using the data results from the tables. Additionally, the IBM was used to statistically analyze the amino acid contents (linear correlation).

3.Results and Discussions

Table 1 displays the crude protein content and amino acid composition of the chosen vegetables. Since it includes all kinds of nitrogen (nucleic acids and nitrogen) that are digested as protein, the protein content achieved is referred to as "crude protein" (Arowora *et al.*, 2017). The veggies' crude protein contents (g/100g) varied from 14.3 to 17.2, with an average of 15 and a CV% of 9.54. These values were comparable to those reported for *S. aethiopicum* (12.57g/100g), but they were within the range of crude protein results reported by Arowora et al. (2017) for *T. occidentalis, A. hybridus, and T. triangulare* (11.17-25.03 g/100g) and *Abelmoschus esculentus, Corchorus olitorius, Ipomea batatas, Solanum melongena,* and *Vigna unguculeta* leaves (10.58-19.15 g/100g) (Athanase *et al.,* 2018). (Aja *et al.,* 2021) and *Hibiscus canabinus* and *H. barteri* (12.4-13.75 g/100g) (Kubmarawa *et al*., 2009). According to reports, vegetables are a primary source of plant protein for both human and animal

nutrition, with the body absorbing around 80% of plant protein from vegetable sources (Arowora et al., 2017). NAS (2005) states that adults synthesize and breakdown over 250g of protein per day, while the typical daily consumption is between 55 and 100g. This suggests that protein has to be replaced, and leafy vegetables may be the best way to provide the additional protein that this age group needs (Arowora *et al*., 2017). The Institute of Medicine's Food and Nutrition Board study suggests that adult men and women should consume 800 mg of high-quality protein per kilogram of body weight daily (Waterlow, 1984; FAO/WHO, 1991). This suggests that while Celocia argentae had the greatest protein content of the three vegetables examined, all three vegetables had good protein amounts that may be used to satisfy daily human needs. The three veggies showed the following trend: CA>WL>AG.

Individual levels (g/100g) of the amino acid content of the three vegetables under examination were displayed by the amino acid analysis (Table 1). The findings demonstrated that the amounts of essential and non-essential amino acids in the plants varied. The essential amino acids in the three vegetables were as follows: WL (0.585-7.36 g/100g), CA (0.475-5.79 g/100g), and AG (0.419-6.60 g/100g). Tryptophan was the least concentrated amino acid in all three vegetables, while valine (5.79 g/100g), lysine (6.60 g/100g), and arginine (7.36 g/100g) were the most concentrated essential amino acids in the vegetables, respectively. With values of 0.682 (CA), 0.496 (AG), and 0.496 (WL), lysine was the least concentrated non-essential amino acid among the three vegetables. Glutamic acid, on the other hand, had the highest concentration of non-essential amino acids, with values (g/100g) of 13.7 (CA), 11.0 (AG), and 12.0 (WL). According to reports, free amino acids dissolved in plasma and tissue (some of which are generated from vegetables) make up a relatively small percentage of the body's overall mass of amino acids, despite being extremely important for the biochemical processes of the body's proteins (Arowora *et al*., 2017). In contrast to total body protein, the amounts of particular free amino acids in bodily fluids can vary significantly in response to dietary changes or medical circumstances (Furst, 1989). It is evident from the findings that the vegetables under investigation may supply the particular amino acids that the human body requires. According to reports, the human body's capacity to conserve individual amino acids varies greatly at very low intakes. As a result, the pattern of amino acids needed in the diet to match their individual catabolic rates does not directly correlate with the protein composition of the body (Arowora *et al.*, 2017). In this results, essential amino acids of major concentrations (g/100g) were: CA [Valine (5.79), Leucine (5.53), Lysine (5.59), Phenylalanine (5.47), Hulidine

(5.45) and Arginine (5.48)], AG [lysine (6.60), valine (6.48), arginine (6.37), leucine (6.08) and histidine (5.34)] and WL [arginine (7.36), valine (6.69), leucine (6.08). lysine (6.60), hisidine (6.69) and phenylalanine (5.40)]. Gold (2009) asserts that these amino acids are essential for human nutrition for at least one of the following healing processes: muscle tissue repair, dressing wounds, growth hormone production, blood sugar control, brain disease treatment, normal central nervous system function, depression symptom management, and chronic pain management. USDA (2016) states that tryptophan plays a key role in the synthesis of the neurotransmitter serotonin, which regulates mood and sleep patterns and is used to treat depression, binge eating, and jet lag. Additionally, it is essential for the management of retinoid arthritis, panic episodes, vascular migraines, and the regular operation of the central nervous system (Adesina, 2013; Aja *et al*., 2021). Tryptophan levels in the current study varied from 0.419 to 0.585 g/100g. These values were comparable to those reported by Adesina (2013) and ten commonly consumed indigenous vegetables (Omoyeni et al., 2015), but they were lower than those reported for similar leafy vegetables: *T. occidentales, A. hybridus, V. amygdalina, and T. triangulare* (3.13-5.35 g/100g) (Arowora *et al*., 2017), and *S. aethiopicum* (2.21 g/100g) (Aja *et al*., 2021).

Glutamic acid was the most abundant of the non-essential amino acids found in the three vegetables under analysis (11.0–13.7 g/100g), with Celosia argenteahavingthe greatest concentration (13.7 g/100g). Numerous publications from the literature that indicated glutamic acid to have the highest content (Arowora *et al*., 2017; Omoyeni *et al.*, 2015; Adesina, 2013; Athanase *et al*., 2018; Kubmarawa *et al*., 2008, 2009) corroborated this observation.

Table 2 displayed the quality parameters of the amino acid composition of the chosen vegetables that were analyzed. The highest value was found in WL (wild lettuce), where the total amino acid content (TAA) was 88.0-94.6 g/100g cp with a CV% value of 4.18. The histidine-containing total essential amino acids (TEAA) ranged between 43.7 and 49.7 g/100g with corresponding percentage values (49.6-52.8), the histidine-free total essential amino acids ranged between 38.3-42.8 g/100g cp (47.2-50.4%), and the total non-essential amino acid in the samples ranged between 41.5-44.9 g/100g (47.2-50.4%). According to FAO/WHO (1973), the percentage of TEAA found in this study was all above the 39% threshold for an optimal protein diet for newborns, 26% for children, and 11% for adults. Additionally, the values were comparable to those found for raw and heat-treated Treculia africana seed flour (Adesina *et al.*, 2016), pigeon peas (452 g/100 g cp) (Nwokolo, 1987), and other

vegetables consumed in South West Nigeria (Omoyeni et al., 2015). The TNEAA values in this study were within the ranges reported for raw and processed flour made from Treculia africana seeds (Adesina *et al.*, 2016), roasted cocoa, and cocoa nibs and shells (Adeyeye *et al*., 2014).

Only roughly 50–55% of the 5.80 g/100 g cp advised for babies (FAO/WHO, 1973) could be satisfied by the TSAA values of 2.05-2.99 g/100 g cp (2.16-3.39%) in the current report. For babies, the recommended TArAA intake ranges from 6.8 to 11.8 g/100g Cp. The TArAA values from this report, which ranged from 6.41 to 10.9 g/100g cp (7.28 to 12.3%), showed that the examined vegetables could easily provide the recommended amount of protein for infants. FAO/WHO/UNU (1985) does not specify the percentage of total sulfur amino acid that can be satisfied by cystine, despite the fact that it is recognized that cystine can partially substitute for methionine. For example, the percentage is roughly 50% for rats, chicks, and pigs (FAO/WHO, 1991). Although many vegetable proteins, particularly those from legumes, contain significantly more cystine than methionine, it has also been noted in the literature that the majority of animal proteins are low in cystine. Nevertheless, it is evident from this report that the levels of cystine in the three vegetables were comparable to those of methionine. The present report's Cys in TSAA percentages were generally low (22.8-24.2), indicating that if these vegetables are consumed as the primary source of protein in a person's diet, cystine can only make a little contribution to total sulfur amino acids.

According to Muller and Tobin (1980), the computed protein efficiency ratio (PER) typically ranged from 0.0 for a mediocre protein to a maximum of just over 4.0 for a very good protein. The current reagent revealed that the veggies had PER (P-PER, 8.2) averages of 1.78 and 1.92, suggesting that the protein's efficiency in the samples is only roughly 50% of what is needed for a very good protein. Since the isoelectric point (PI) is the lowest pH at which a protein becomes soluble, it is crucial to prepare the protein isolate of any given food sample. In the current report, the PI ranged from 5.15 to 5.77, indicating a relatively acidic pH for the protein's solubility (Adesina et al., 2016). Although it does not take into consideration variations in protein quality brought on by different processing techniques or specific chemical reactions, the essential amino acid index (EAAI) provides a quick tool to assess food formulation for protein quality (Adesina *et al*., 2016). EAAI is also necessary for determining a protein's biological values (BV), according to Albanese (1959). The equivalent BV values for the EAAI in this study were WL (84.9, 80.8), AG (88.5, 84.7), and CA (89.3, 85.6). These

results demonstrated that the protein in the examined vegetables was of extremely good quality and compared favorably with those found in diets derived from both animal plant sources: raw and treated *T. africana* seed flour (Adesina *et al*., 2016), and Pandalus borealis (Adeyeye, 2015).

Tables 3, 4, and 5 displayed the amino acid scores based on various scoring systems. Table 3 showed scores based on the entire hen's egg amino acid scoring pattern; Table 4 showed the FAO/WHO provisional amino acid scoring pattern; and Table 5 showed scores based on the amino acid scores of preschool-aged children (2–5 years). Only five amino acids—tyrosine, histidine, phenylalanine, glycine, and glutamic acid—had scores higher than 1.0 in the entire hen's egg amino acid scoring pattern. In AG, six amino acids—glycine, alanine, proline, lysine, histidine, and arginine—had scores higher than 1.0, and in WL, eight amino acids—glycine, alanine, proline, lysine, glutamic acid, phenylalanine, histidine, and arginine—had scores above 1.0. According to this scoring pattern, tryptophan, cystine, and serine were the three main limiting amino acids in all three vegetables, with average scores of 0.274, 0.310, and 0.529, respectively. Vegetables with AA scores above 1.0 were classified as CA (valine, isoleucine, lysine, and phenylalanine + tyrosine), AG (valine, threonine, isoleucine, lysine, and phenylalanine + tyrosine), and WL (valine, threonine, lysine, and phenylalanine + tyrosine) according to the FAO/WHO provisional amino acid scoring in Table 4. Nevertheless, tryptophan received the lowest score among the three vegetable samples examined, making it a limiting amino acid that requires adjustment.

With an average score of 0.448, tryptophan likewise had the lowest score among the pre-school child (2–5 years) pattern scores. This suggests that tryptophan would need to be adjusted if these veggies were the only source of protein in the diet.

4. Conclusions

According to the study, the green vegetables under investigation had respectable amounts of protein and amino acids. They are also a good source of amino acids and high-quality protein. These vegetables, particularly Celosia argentea and Launaea taraxacifolia, which have high protein levels, high-quality amino acids, and high biological values, may be helpful as inexpensive and quick sources of protein and amino acid replenishment for undernourished children. Therefore, substituting these vegetables for some or all of the animal or human food could improve the nutritional status of amino acids in the diets of people in impoverished nations.

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Table 1. Amino acids profile (g/100g) of *Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL)

Amino acid	CA	AG	WL	Mean	SD	CV%
Glycine	4.70	5.55	6.54	5.60	0.921	16.5
Alanine	4.56	5.52	5.72	5.27	0.620	11.8
Serine	3.91	4.12	4.51	4.18	0.304	7.28
Proline	3.05	5.97	5.58	4.87	1.59	32.6
Valine	5.79	6.48	6.69	6.32	0.471	7.45
Threonine	3.32	4.51	4.69	4.17	0.744	17.8
Isoleucine	4.32	4.49	3.87	4.23	0.320	7.58
Leucine	5.53	6.08	6.08	5.90	0.318	5.39
Aspatic acid	8.93	7.55	8.11	8.20	0.694	8.47
Lysine	5.59	6.60	6.60	6.26	0.583	9.31
Methionine	2.31	1.55	1.55	1.80	0.439	24.3
Glutamic acid	13.7	11.0	12.0	12.2	1.38	11.3
Phenylalanine	5.47	4.63	5.40	5.17	0.466	9.02
Histidine	5.45	5.34	6.89	5.89	0.865	14.7
Arginine	5.48	6.37	7.36	6.40	0.940	14.7
Tyrosine	4.91	1.36	1.98	2.75	1.90	69.0
Tryptophan	0.475	0.419	0.585	0.49	0.084	17.1
Cystine	0.682	0.496	0.496	0.56	0.107	19.2
Crude protein	17.2	14.3	15.2	15.6	1.48	9.54
Total	88.2	88.0	94.6	90.26	3.77	4.20

SD=standard deviation, CV = coefficient of variation

Table 2. Quality parameters of amino acids profile (g/100g) of *Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL)

Parameters	CA	AG	WL	Mean	SD	CV%
TAA	88.2	88.0	94.6	90.3	3.77	4.18
TEAA with His	43.7	46.5	49.7	46.6	2.99	6.42
TEAA without His	38.3	41.1	42.8	40.7	2.29	5.63
TNEAA	44.4	41.5	44.9	43.6	1.82	4.18
TArAA	10.9	6.41	7.97	8.41	2.26	26.8
TAAA	22.6	18.5	20.1	20.4	2.07	10.1
TBAA	16.5	18.3	20.9	18.6	2.18	11.7
TNAA	7.23	8.63	9.20	8.35	1.01	12.1
TSAA	2.99	2.05	2.05	2.36	0.550	23.1
%TEAA with His	49.6	52.8	52.5	51.6	1.78	3.44
%TEAA without His	43.4	46.7	45.3	45.1	1.66	3.68
%TNEAA	50.4	47.2	47.5	48.4	1.78	3.67
%TArAA	12.3	7.28	8.42	9.34	2.64	28.2
%TAAA	25.7	21.0	21.2	22.6	2.61	11.5
%TBAA	18.7	20.8	22.0	20.5	1.67	8.12
%TNAA	8.20	9.81	9.72	9.24	0.900	9.78
%TSAA	3.39	2.32	2.16	2.63	0.670	25.5
Cys in TSAA	0.682	0.496	0.496	0.560	0.110	19.2
% Cys in TSAA	22.8	24.2	24.2	23.8	0.840	3.52
P-PER$_1$	1.53	2.15	2.08	1.92	0.340	17.8
P-PER$_2$	1.69	1.81	1.83	1.78	0.070	4.02
Leu/Ile	1.28	1.35	1.57	1.40	0.150	10.8
Leu-Ile	1.21	1.59	2.21	1.67	0.500	30.2
%Leu-Ile	21.9	26.2	36.3	28.1	7.43	26.4
P*i*	5.15	5.35	5.77	5.42	0.320	5.83
BV	85.6	84.7	80.8	83.7	2.57	3.07
Lys/Trp	11.8	15.8	11.3	12.9	2.45	19.0
Met/Trp	4.86	3.70	2.65	3.74	1.11	29.6
EAAI	89.3	88.5	84.9	87.58	2.35	2.69

TAA= total amino acids, TEAA= total essential amino acids, TNEAA= total non-essential amino acids, TAAA= total acidic amino acids, TBAA= total basic amino acids, TNAA= total neutral amino acids, TSAA= total sulphur amino acids, P-PER= predicted protein efficiency ratio, p*I*= isoelectric point, BV= biological value, EAAI= essential amino acids index

Table 3. Amino acids scores based on the whole hen's egg amino acids scoring pattern of *Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL)

Amino acid	CA	AG	WL	Mean	SD	CV%
Glycine	1.57	1.85	2.18	1.87	0.307	16.5
Alanine	0.844	1.02	1.06	0.975	0.115	11.8
Serine	0.495	0.52	0.571	0.529	0.039	7.28
Proline	0.803	1.57	1.47	1.28	0.417	32.6
Valine	0.772	0.864	0.892	0.843	0.063	7.45
Threonine	0.651	0.884	0.920	0.818	0.146	17.8
Isoleucine	0.771	0.802	0.691	0.755	0.057	7.58
Leucine	0.666	0.733	0.733	0.710	0.038	5.39
Aspatic acid	0.835	0.706	0.758	0.766	0.065	8.47
Lysine	0.902	1.06	1.06	1.01	0.094	9.31
Methionine	0.722	0.484	0.484	0.564	0.137	24.3
Glutamic acid	1.14	0.914	1.00	1.02	0.115	11.3
Phenylalanine	1.07	0.908	1.06	1.01	0.091	9.02
Histidine	2.27	2.23	2.87	2.46	0.360	14.7
Arginine	0.898	1.04	1.21	1.05	0.154	14.7
Tyrosine	1.23	0.340	0.495	0.688	0.474	69.0
Tryptophan	0.264	0.233	0.325	0.274	0.047	17.1
Cystine	0.379	0.276	0.276	0.310	0.060	19.2
Total	0.883	0.881	0.947	0.904	0.038	4.18

Table 4. Amino acids scores based on FAO/WHO Provisional scoring pattern of *Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL)

Amino acid	CA	AG	WL	Mean	SD	CV%
Valine	1.16	1.30	1.34	1.26	0.094	7.45
Threonine	0.830	1.13	1.17	1.04	0.186	17.8
Isoleucine	1.08	1.12	0.968	1.06	0.080	7.58
Leucine	0.790	0.869	0.869	0.842	0.045	5.39
Lysine	1.02	1.20	1.20	1.14	0.106	9.31
Tryptophan	0.475	0.419	0.585	0.493	0.084	17.1
Methionine +Cystine	0.855	0.585	0.585	0.675	0.156	23.1
Phenylalanine+Tyrosine	1.73	1.00	1.23	1.32	0.374	28.3
Total	1.07	1.02	1.05	1.05	0.026	2.46

Table 5. Amino acids scores based on Pre-school child (2-5 years) amino acids requirement scoring pattern of *Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL)

Amino acid	CA	AG	WL	Mean	SD	CV %
Valine	1.65	1.85	1.91	1.81	0.135	7.45
Threonine	0.976	1.33	1.38	1.23	0.219	17.8
Isoleucine	1.54	1.60	1.38	1.51	0.114	7.58
Leucine	0.838	0.921	0.921	0.893	0.048	5.39
Lysine	0.964	1.14	1.14	1.08	0.101	9.31
Tryptophan	0.432	0.381	0.532	0.448	0.077	17.1
Methionine +Cystine	1.20	0.818	0.818	0.945	0.218	23.1
Phenylalanine+Tyrosine	1.65	0.951	1.17	1.26	0.356	28.3
Histidine	2.87	2.81	3.63	3.10	0.455	14.7
Total	1.29	1.24	1.32	1.28	0.043	3.36

Table 6. Statistical analysis results showing the correlation between amino acids compositions the analysed vegetables (*Celosia argentea* (CA), *Asystasia gangetica* (AG), *Launaea taraxacifolia* (WL))

AA	Gly	Ala	Ser	Pro	Val	Thr	Ile	Leu	Asp	Lys	Met	Glu	Phe	His	Arg	Tyr	Trp	Cys	Cp
Gly	1																		
Ala	.919	1																	
Ser	.992	.861	1																
Pro	.771	.960	.683	1															
Val	.942	.998*	.891	.940	1														
Thr	.902	.999*	.840	.970	.995	1													
Ile	-.733	-.405	-.814	-.131	-.462	-.367	1												
Leu	.843	.987	.768	.992	.975	.993	-.252	1											
Asp	-.555	-.838	-.444	-.958	-.802	-.860	-.160	-.915	1										
Lys	.843	.987	.768	.992	.975	.993	-.252	1.00**	-.915	1									
Met	-.843	-.987	-.768	-.992	.975	-.993	.252	-1.00**	.915	-1.00**	1								
Glu	-.588	-.859	-.480	-.968	-.825	-.879	-.120	-.931	.999*	-.931	.931	1							
Phe	-.031	-.423	.096	-.661	-.365	-.460	-.657	-.564	.849	-.564	.564	.827	1						
His	.856	.583	.915	.330	.633	.549	-.979	.444	-.045	.444	-.444	-.085	.490	1					
Arg	1.00**	.924	.990	.779	.946	.908	-.724	.850	-.566	.850	-.850	-.598	-.044	.849	1				
Tyr	-.744	-.947	-.653	-.999*	-.925	-.960	.091	-.987	.969	-.987	.987	.978	.691	-.291	-.753	1			
Trp	.684	.341	.771	.062	.399	.302	-.998*	.185	.228	.185	-.185	.188	.708	.963	.674	-.021	1		
Cys	-.843	-.987	-.768	-.992	-.975	-.993	.252	-1.00**	.915	-1.00**	1.00**	.931	.564	-.444	-.850	.987	-.185	1	
Cp	-.641	-.892	-.538	-.983	-.861	-.909	-.053	-.953	.994	-.953	.953	.998*	.788	-.151	-.651	.990	.122	.953	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

User's Perception of Smart Technologies in Buildings in Lagos State.

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Abstract

The integration of smart technology into buildings has become a major trend in urban development. As a major urban developing area in Nigeria, Lagos State presents a dynamic environment for the adoption and perception of smart technology in buildings. The study encompasses a comprehensive analysis of the benefits, challenges, and implications of smart technologies in buildings, as well as the potential impact on user experience. The objectives of this research are to investigate users awareness and knowledge of smart technologies, examine users' experiences and satisfaction levels with existing smart building solutions as well as identify challenges and concerns influencing users perceptions of smart technologies in buildings in the state. This research aims to investigate the user's perception of smart technology in buildings within Lagos State through the use of surveys. The findings from this research showed that though there has been a bit of lack of awareness about it, majority of the Lagos residents embraced the idea of smart buildings and it was recommended that more awareness should be done.

Keywords: *Lagos State, Smart Technology, Smart Design, Sustainability, User Perception, Urban Development.*

Introduction

Therapid urbanization and technological development in Lagos State have led to increased interest in smart building solutions. This urban development is due to some major factors such as population growth, rural-urban migration, and economic opportunities which has led to increased demand for infrastructure, including buildings, to accommodate the rising population. (Boadi et al. 2017). The combination of the urban development and technological evolution has heightened the interest in smart buildings in Lagos State. Stakeholders, including developers, policymakers, and building users, are increasingly exploring the potential benefits of implementing smart technologies to improve building efficiency, sustainability and user experience (Oluwatayo, 2020). The Smart technology offers these

numerous benefits in terms of energy efficiency, security, comfort, convenience, and sustainability which are major needed factors in the ever-growing metropolis of Lagos.

As buildings become more intelligent and interconnected, it is important to understand the perception of users towardsthese technologies. Understanding users perception involves studying how individuals, communities and organizations in Lagos State perceive, accept, and interact with smart building technologies. It is very crucial due to the fact that different cultural norms, values and social structures shape how each individual perceives and embraces technological innovations, including smart building technologies. It also involves identifying the needs, preferences and expectations regarding smart building technologies as well as the economic and environmental factors such as energy savings and cost efficiency. Studying the usability, accessibility and user interface of the buildings also matter as well as identifying any barriers or challenges users encounter when engaging with these technologies in the environment.

This research aims to address the user's perception of smart technology integration in buildings within Lagos State, shedding l ight on the opportunities and challenges associated with the adoption of these innovative solutions.

Literature Review

Smart Technology in Buildings

(Ismail et al. 2019) Intelligent building concepts are not recent developments from an architectural perspective. Even inprimitive and indigenous architectural forms, there were advanced instances of clever design. These designs showcased intelligence through the symbiotic relationship between occupants and the technologies embedded in the built environment. This close connection was intentionally integrated into the structure, enabling users to engage with various systems. Users possessed the capability to modify, adjust, and customize the building in response to changing circumstances, be they social, functional, or related to natural phenomena. The achievement of comfort, utility, and satisfaction depended on the user's ability to manipulate the architectural form. In this context, the occupant played a crucial role in both the design and utilization of the building. The absence of the occupant rendered the building non-functional and devoid of any intelligent features. The ongoing interaction between the user and the building, including its technologies, was dynamic and responsive, representing a form of intelligent architecture as outlined by Walter M. Kroner in 1989. This perspective underscores the historical and fundamental connection between occupants and the inherent intelligent

design in architectural structures. The evolving relationship between users and the technologies integrated into contemporary buildings demonstrates a more complex and dynamic integration compared to the intelligence observed in primitive smart buildings.

A smart building is a structure that incorporates various systems, including lighting, HVAC (heating, ventilation, and air conditioning), voice and data communications, and other building functions. These systems are integrated to efficiently manage resources in a coordinated manner. The objective is to enhance tenant performance, optimize investments, achieve cost savings, and improve overall flexibility within the building. Smart buildings utilize technology and automation to create a seamless and efficient environment that responds to the occupants' needs and contributes to sustainable and resource-efficient operations (The National Research Council, Washington DC).

According to Ismail's 2019 definition, a smart building is identified as an "electronically enhanced building." The description goes on to elaborate that a smart building is fully equipped with controllers and systems, complemented by a robust infrastructure, to facilitate the utilization of cutting-edge communication, data processing, and control technologies by both its inhabitants and operating staff. This type of building includes essential infrastructure elements such as wires, cables, conduits, power supply, heating, ventilation, cooling, lighting, sound insulation, and security systems. The primary goal is to support the performance requirements of modern office environments. Essentially, a smart building incorporates advanced technologies to improve communication, data processing, and control, benefiting both occupants and operational staff.

The incorporation of smart technologies into buildings signifies a significant transformation in their design, operation, and the way users experience the built environment. From sensor systems driven by the Internet of Things (IoT) to sophisticated data analytics and automationplatforms, smart technologies are reshaping how buildings operate and how occupants engage with their surroundings. This comprehensive integration of technology holds the potential not only to optimize building efficiency but also to significantly enhance the user experiencewithin the built environment.

Technologies involved in the integration of a Smart Building (Features)

- **HVAC Systems:** Smart HVAC systems utilize a variety of sensors to oversee and manage their performance. Such systems

use software to analyze information from different sensors to enhance the system's efficiency and the comfort level of those within the building. Advanced HVAC controls have the capability to lower energy use in areas that are not in use, detect and troubleshoot issues, and decrease the operation of HVAC systems, especially during times of high energy demand.

- **Lighting:** Intelligent lighting systems come with enhanced features, including natural light utilization and progressive controls for occupancy detection and dimming, to prevent excessively bright areas. Lighting fixtures with adjustable brightness settings are evolving quickly, with demand-response initiatives promoting both incremental and smooth dimming options. These setups offer wireless management and can be integrated into lighting control platforms, allowing users to operate them via online dashboards.
- **Plug Loads:** Intelligent plug load controls help manage the energy use of various portable and ad-hoc devices in buildings. In established structures, these systems consist of smart receptacles and power strips that can shut off electricity to inactive equipment based on timers, motion detectors, or usage monitoring. Some smart strips can detect a main device, like a desktop computer, and control connected ancillary gadgets accordingly. These load schedules can also be integrated with lighting and building management systems for centralized governance, enhancing energy economy and streamlining building operations.
- **Window Shading:** Smart window systems are designed to regulate solar heat and daylight entering the building. These systems include both passive and active window glazing and films responsive to changes in sunlight or temperature. Additionally, auto-controlled shades operate on specific schedules to manage light levels and solar heat gain. In retrofitting, smart shading technologies prove particularly effective in buildings with untinted, single-pane windows.
- **Automated System Optimization (ASO):** Adaptive Systems Optimization (ASO) challenges traditional Building Automation Systems (BAS) by harnessing real-time feedback rather than static schedules. ASO utilizes Information and Communication Technology (ICT) to gather and scrutinize data on energy use and system function within a building. It

preemptively adjusts settings in response to changing variables like occupancy trends, weather predictions, and fluctuating utility costs. Cloud-powered remote observation of buildings is becoming commonplace in ASO application, facilitated by web-based energy management interfaces, which allow for more dynamic and responsive building management..

- **Human Operation:** Smart buildings enable operators to interact with systems via intuitive computer dashboards. These interfaces present clear views into building functions and energy consumption, centralizing data analysis and offering alerts to issues flagged by Automated System Optimization (ASO). Staff, including IT experts, use training in network management, data analytics, and advanced smart tech. Occupants have mobile apps to personalize workspace settings like lighting. These apps also show individual energy use and suggest efficiency tips. This tech blend boosts building efficiency and augments occupant comfort.
- **Distributed Energy Resources (DER):** Distributed Energy Resources primarily include energy generation and storage systems strategically placed at or near the point of use, providing power independently of the grid. Examples of DER encompass combined heat and power, solar photovoltaics, other renewable sources, as well as battery and thermal storage. DER relies on communication and control devices for efficient energy dispatch, and integrating a smart inverter to the DER adds smart functionality. Smart inverters are software-controlled devices that enhance the management of onsite energy generation and storage.They facilitate continuous two-way communication between the DER and the electric grid, allowingfor immediate responses to load signals, electricity rates, demand response events, and power outages. The incorporation of smart inverters enhances the responsiveness and adaptability of DER systems, contributing to a more efficient and resilient energy infrastructure.

Benefits of Smart Technology in Buildings

The incorporation of smart building technology in the construction industry of developing countries brings numerous benefits to professionals, clients, and the nation as a whole. Vattano (2014) emphasizes that the use of smart buildings in sustainable construction contributes to minimizing waste, efficiently utilizing resources,

and reducing environmental harm. According to (Honeywell et al., 2015), the economic advantages of smart buildings over traditional structures include a swift return on investment, avoidance of appliance and equipment failures, prevention of fire outbreaks, and addressing energy challenges. (Balta-Ozkan,et al., 2014) point out the social benefits of smart buildings, including concerns related to safety, healthcare management, and security. (Sherif, et al., 2018) identify general benefits, such as reducing energy costs, increasing s taff productivity, improving building operations, providing web-based security, and enhancing the safety and security of occupants. Additional advantages encompass health and safety, data infrastructure connectivity, fault detection in the system, and cost savings (Honeywell et al., 2015). Overall, the adoption of smart building technology yields a range of advantages across economic, social, and environmental dimensions.

(Ejidike, et al., 2023) have distilled these benefits into five major categories;

- **Energy Saving:** Smart building technologies play a crucial role in enhancing energy efficiency and maximizing savings throughout the building' s operational lifespan. As per a study by (Iwuagwu U et al., 2014), smart buildings ensure real-time control and monitoring of energy consumption within the building, contributing to its overall environmentally friendly attributes. Noteworthy contributions to energy savings have been reported, with smart buildings reducing power consumption significantly in documented cases, such as a 34.78% reduction from 765,228.16 to 499,067.01 kWh (Omar O, 2018). This evidence underscores the positive impact of smart building technologies in optimizing energy usage and promoting sustainability.
- **Safety and Security:** As noted by (Honeywell et al., 2015), safety and security systems within smart buildings cover diverse aspects, including responses to threats, access management, protection of lives and assets, and ensuring occupant comfort and productivity. These systems encompassfactors like illumination, thermal comfort, air quality, connectivity, and energy availability. Whether residential or commercial, the prioritization of safety and security in smart building technologies is essential. Designing and implementing these technologies must consider ensuring comprehensive safety and security measures for occupants.

- **Maintenance Cost-saving:** Extending beyond initial construction costs, smart buildings, as highlighted by (Iwuagwu, et al., 2014) and (Ejidike, et al., 2023), consider operating and maintenance costs over the building's entire lifecycle. Automated control, communication, and management systems improvement can lead to cost savings by allowing equipment to be shared among multiple users. By integrating safety, sustainability, and productivity, smart buildings create more interconnected, dynamic, and functionalsystems that contribute to cost savings beyond maintenance alone.
- **Improve Building Comfort:** According to (Buckman, et al., 2014), smart buildings are significant in enhancing occupant comfort through systems that interpret data from previous usage, adapting to occupants' preferences. Optimization of lighting, utilities, and Heating, Ventilation, and Air Conditioning (HVAC) systems aligns with occupancy patterns and desired comfort levels. Real-time monitoring of building systems in smart buildings optimizes energy usage, leading to minimized asset loss and increased comfort for occupants.
- **Productivity and Collaboration:** Lighting plays an important role in smart building technology, influencing occupants'well-being, motivation, and productivity, as noted by (Buckman, et al., 2014). Integrated systems not only improve the quality of life for occupants but also contribute to increased sustainability, safety, and overall productivity in smart buildings.

Integration of Smart Technology in Buildings

The incorporation of smart technology into buildings signifies a groundbreaking shift in our approach to conceptualizing, designing, and engaging with the built environment. Smart buildings, featuring a multitude of interconnected devices and systems, are strategically designed to elevate efficiency, comfort, and sustainability. The integration of smart technology in buildings offers a fresh perspective on how structures are constructed, utilized, and maintained.

Methodology

Research Method and Instrument adopted for this study

The research employed a quantitative methodology, utilizing a random sampling technique to select residents from various age groups in Lagos State, focusing on six specific Local Government Areas (LGAs). Lagos State was chosen for its technological

exposure and recent innovations, situated in Nigeria's south-western region. Geographically, it is located between latitudes 6° 20'00" N and 6° 40'0" N, and longitudes 3° 20'0" E and 4° 20'0" E, covering an approximate area of 3,496 km2 (Kaoje et al., 2017). With a population of around 14 million people and 20 Local Government Areas, Lagos State experiences an annual population growth of 5.7%, making it one of the world's fastest-growing megacities (Dano et al., 2020).

The study's sampling frame involved randomly selecting residents from six specific local government areas out of the total twenty in Lagos State. This selection was based on the prominence of technology-oriented buildings in these areas. Equal representation from each local government area aimed to ensure a statistically balanced sample of the population. This selection strategy is crucial to the study, as it will use questionnaires to gather diverse perspectives from different individuals on the integration of smart technology in buildings.

Data Collection Procedure

A total of 593 questionnaire forms were distributed to selected respondents within the six local government areas in Lagos State, both at their homes and offices. Out of these, 439 completed forms were retrieved, resulting in an overall response rate of 88%. Upon review, 400 of the retrieved questionnaires were deemed relevant and suitable for analysis. These local government areas were selected based on their association with a significant presence of technology-oriented buildings, contributing to the study's focus on the integration of smart technology in the built environment.

Local Government Area	Population	Sample Size
Surulere	504,409	100
Lagos Mainland	317,980	98
Ikeja	313,333	99
Eti-Osa	287,958	99
Lagos Island	209,665	99
Ibeju-Lekki	117, 542	99

Yamane Formula was applied to each LGA to determine the sample size used to distribute the questionnaire

Chapter Four

Findings and Discussion

In this section, the analysis results and a discussion of the findings pertaining to the research questions on awareness, residents' findings, and preferences regarding current smart buildings in Lagos State are presented.

Awareness

A simplified definition of a smart building was provided and the awareness question followed, inquiring if the respondent had ever stepped into a smart building. 66.7% gave a negative response, 20 % gave a positive response that they have been in one while 13.3% said they think they have been to a smart building. The second question was linked to the first which buttressed the point of if the respondent will be willing to step into a smart technology building. The response was a 100%

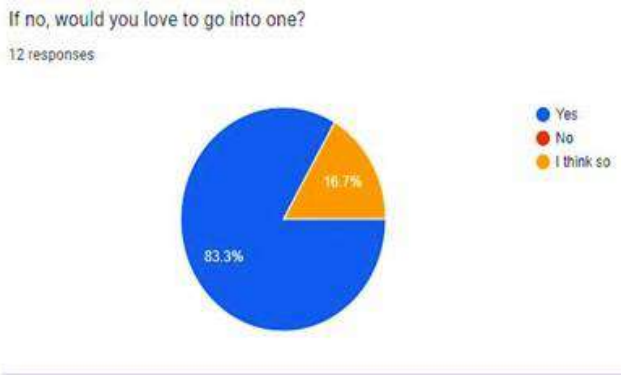


Figure (1) *Figure (2)*

Piechart showing the data respondents

Section II

The second section of the question was directed to the respondents who responded positively about stepping into a smart technology building. A checkbox was created to tick any of the features they noticed when they visited the smart building. A table would be shown of the percentage that noticed each feature listed in the questionnaire. A question was also asked inquiring about how well they enjoyed the experience.

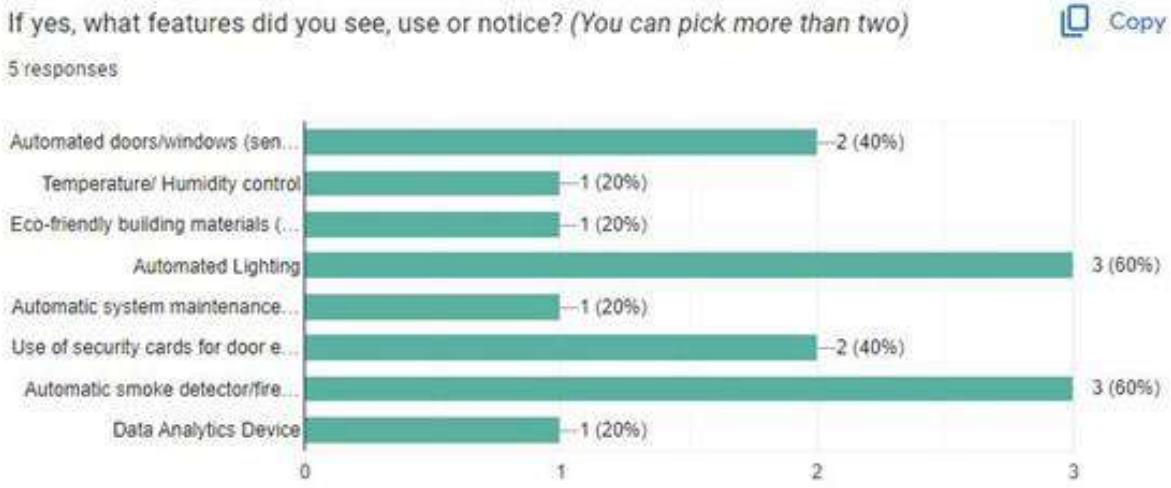
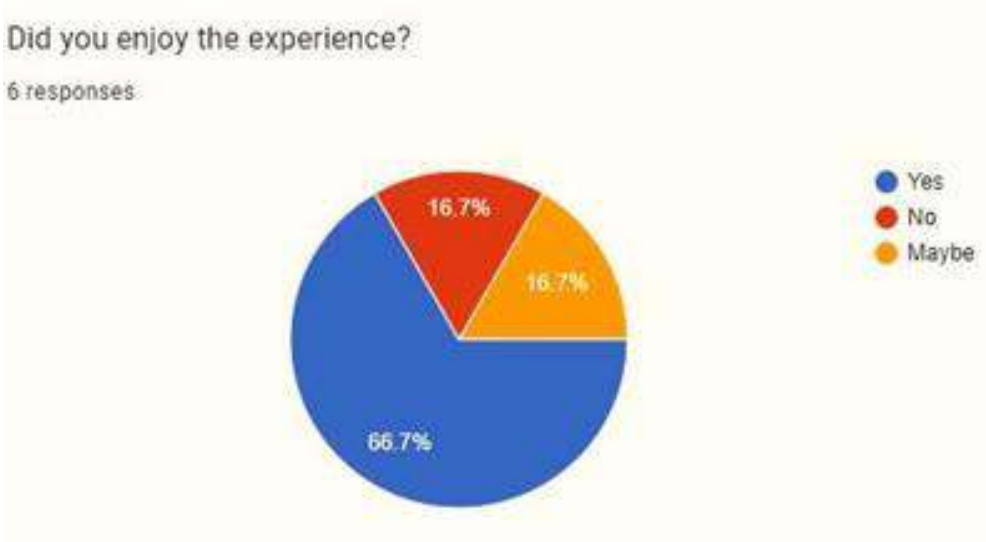


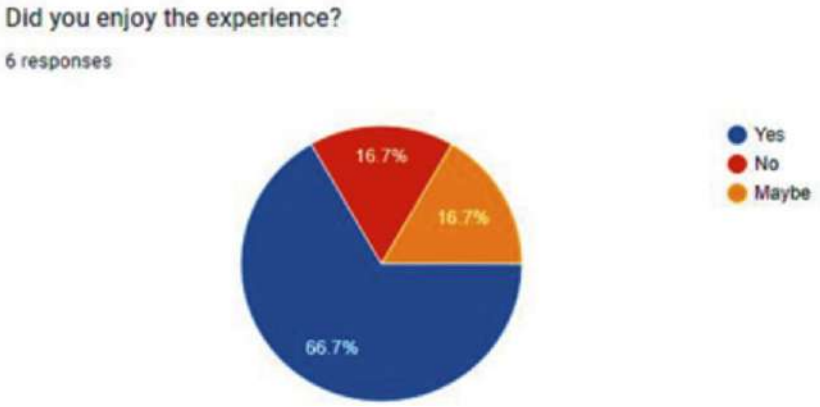
Fig 3.
A Bar Chart showing the features the respondents noticed in the smart building



Pie Chart showing the data of the respondents from section II (Fig. 4)

Section III

This section asked the respondent if the experience was enjoyable and if they would love to see more buildings like it in their Local Government Area. It was a very positive response as every respondent gave a Yes.



Pie Chart (Figure 5)

Conclusion and Anticipated Findings

The study delved into the perceptions of Lagos residents regarding smart technology in buildings, shedding light on the awareness levels among the population. It uncovered that a significant portion of residents lacks awareness of smart buildings, with many not having experienced one. The hypothesis results indicated that those who have encountered smart buildings generally enjoyed the experience and expressed a desire for more such developments in Lagos State.

In conclusion, the limited presence of smart buildings in Lagos has contributed to low awareness levels among residents. To address this, the study recommends organizing seminars and awareness programs, especially targeting the building industry, to educate residents about the benefits of smart buildings and how they can enhance living standards. Increased awareness could lead to improved safety, energy efficiency, cost savings, and environmental protection by reducing greenhouse gas emissions. Furthermore, the building industry plays a crucial role in promoting smart buildings. The study suggests that the industry should offer smart building solutions to clients and the government, thereby reducing the construction of traditional buildings in the state. This can be achieved through training sessions, workshops, conferences, and seminars, creating a knowledge base for the development of smart buildings.

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SYSTEMATIC COMPARATIVE ANALYSIS OF MOBILE ADHOC NETWORK ROUTING PROTOCOLS

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ABSTRACT

Mobile Ad-Hoc Network (MANET) is a decentralized system that enables direct communication between mobile nodes without relying on fixed infrastructure. The study focused on five widely used MANET routing protocols: Destination-Sequenced Distance-Vector (DSDV), Ad hoc On-Demand Distance Vector (AODV), Optimized Link State Routing (OLSR), Dynamic Source Routing (DSR), and Temporally Ordered Routing Algorithm (TORA). This research employed Simulation-based experiments, which were conducted using the NS-2 simulator to evaluate the performance of each protocol. The simulation scenarios include varying network (node) size, mobility pattern, and traffic pattern. The research also offered a detailed evaluation of the routing protocols. It also carried out a comparative analysis based on three key performance metrics: Packet Delivery Ratio (PDR), Throughput, and Average Delay, which are essential in assessing the reliability, efficiency, and timeliness of data transmission in MANET environments. The result of the research showed that Proactive protocols like DSDV and OLSR have proven effective in networks with low mobility and stable conditions, offering high PDR and minimal delays. Reactive protocols such as AODV and DSR excel in dynamic environments, providing greater throughput and better adaptability to frequent changes in network topology. TORA, designed for highly dynamic settings, employs a link reversal algorithm to manage rapid topology changes, although its complex route maintenance can lead to increased delays. This work presented a systematic comparative analysis of prominent MANET routing protocols. The results of this study can be used to inform decision-making for protocol selection and optimization in various MANET scenarios.

Keywords: Average Delay, Throughput, Proactive protocol, PDR, Reactive protocol.

1. Introduction

Wireless communication enables remote access to information and services. Wireless local area networks, based on the IEEE 802.11 standard, are one approach to meet these expectations. However, there is an increasing demand for connectivity in situations/locations where no base station/infrastructure exists (Quy *et al*., 2024). This is where the ad hoc network came into being. Wireless networks are characterized as infrastructure networks, infrastructure-less networks, or mobile ad hoc networks (MANETs) (Mogaji *et al*., 2021).

Mobile Ad Hoc Networks (MANETs) are self-organizing and configuring networks that do not require infrastructure assistance. Because of the high node mobility in such networks, topological changes may occur frequently and unexpectedly. MANETs are ideal for time-critical applications due to their mobility and lack of a stable infrastructure. Ad hoc network applications include students using laptops for interactive lectures, business associates sharing information during conferences, and search and rescue operations (Ramphull *et al*., 2021). They are also a modification of ad hoc networks, characterized by the availability of mobile devices such as phones or laptops that can be moved and connected with other nodes on the network (Rahman *et al*., 2023). The routing protocol in such a network is an authority to determine the routes and offer communication among end points via intermediate nodes (Mogaji *et. al*., 2018). This dynamic aspect poses challenges for routing and communication protocols (Abdan and Seno, 2022).

Applications of MANETs encompass military communications, emergency response systems, collaborative environments, and Internet of Things (IoT) deployments. Nonetheless, the decentralized and dynamic nature of MANETs introduces security challenges, necessitating the addressing of issues such as secure routing, authentication, and data confidentiality within these networks (Kalime and Sagar, 2021, Saravanan and Saravanakumar, 2023).

MANETs are often utilized in scenarios where privacy is paramount, making it imperative to ensure data confidentiality and protect user privacy. The rationale behind conducting a comparative analysis of security attacks within Mobile Ad Hoc Networks (MANETs) is rooted in several key drivers (Tahboush and Agoyi, 2021, Rahman *et al*., 2023). In the sphere of wireless communication, a thorough grasp of Ad-Hoc Networks and the DSR protocol is essential for researching cache optimization solutions (Nurftriana *et al*., 2023)

This research presents a systematic comparative analysis of prominent

MANET routing protocols, including Destination-Sequenced Distance-Vector (DSDV), Ad hoc On-Demand Distance Vector (AODV), Optimized Link State Routing (OLSR), Dynamic Source Routing (DSR), and Temporally Ordered Routing Algorithm (TORA). A comprehensive review of existing literature is conducted to identify the strengths and weaknesses of each protocol.

Multiple routing techniques are usually used in Mobile Ad Hoc Networks (MANETs), which include:

i. *Proactive Routing*: This approach, also known as table-driven routing, entails keeping a complete routing table for each node in the network (Shukla *et al*., 2022). It ensures that routes always available but incurs substantial overhead on route discovery and maintenance. Optimized Link State Routing (OLSR) and Destination-Sequenced Distance Vector (DSDV) are examples of proactive routing protocols.

ii. *Reactive Routing*: Reactive routing also referred to as on-demand routing only finds routes when required. When a node wants to forward its packet to its destination, it starts a search process to find the path towards the target (Srilakshmi *et al*., 2022). This method reduces overhead but may cause delays in packet delivery. Examples of reactive routing protocols include Ad-hoc On-demand Distance Vector (AODV) and Dynamic Source Routing (DSR) (Affandi *et al*., 2023).

iii. *Hybrid Routing*: Hybrid routing is a combination of proactive and reactive routing. For frequently communicated destinations, nodes maintain partial routing tables while other routes are discovered as the need arises. This helps to balance between overhead and delays (Shajin and Rajesh, 2022). In Zone Routing Protocol (ZRP), if the routing destination is within the peripheral zone, the source node can take the shortest path as can be provided by any proactive routing protocol (Al Ajrawi and Tran, 2024). Examples of hybrid routing protocols include Zone Routing Protocol (ZRP) and Temporally Ordered Routing Algorithm (TORA).

iv. *Geographic Routing*: Also referred to as position-based routing, this method uses physical location information in its decision-making process for the routers. Nodes forward packets to the nearest neighbor closer to the destination. Although efficient and scalable it requires that all nodes within the network have accurate location information about themselves. Examples of geographic routing protocols include Greedy Perimeter Stateless Routing (GPSR) and Geographic Distance Routing (GEDIR).

The type of routing protocol that is chosen depends on specific application

requirements as well as network characteristics. Proactive routing is better suited for networks with high traffic loads, whereas reactive routing is more preferred for those with low traffic loads. Hybrid routing works best for networks with mixed traffic loadings, while geographic-based based would be applicable in networks with relatively stable topologies.

2.0 Review of Related Works

This section provides a succinct summary of related research works that have attempted to comparative analysis of routing protocols in Mobile Adhoc Networks.

Sharma and Gupta (2019) compared the performance of reactive (like AODV, DSR) and proactive (like OLSR) routing in MANETs under diverse network conditions. Their findings indicated that proactive protocols have lower route discovery latency but higher control overhead in comparison with reactive alternatives. Al-Turjman (2017) also highlighted the emergency communication system concerning disaster management scenarios using various routing protocols for this purpose through MANETs. It asserts that during periods like emergencies and natural disasters, such technology plays a vital role in maintaining resilient communication infrastructure, which is highly needed.

Hasan (2021) conducted a comparative study on energy-efficient routing protocols in MANETs aimed at reducing energy consumption and extending network lifetime. The paper also considered node mobility and transmission range as some of the mentioned factors in evaluating these protocols, such as LEACH, AODV, and DSR. A study by Johnson *et al.* (2019) compared three different routing protocols, namely AODV, DSR, and OLSR, concerning packet delivery ratio, end-to-end delay, and throughput. In their research report they explained how each protocol had strengths and weaknesses under various network conditions. Smith and Jones (2020) examined the security vulnerabilities affecting MANETs together with performance comparison for encryption algorithms, intrusion detection systems, and authentication protocols. The work established that strong security configurations were necessary to protect data transmitted over the Internet. Wang et al. (2018) did a comparison of energy-efficient routing protocols in MANETs which included energy consumed, network lifetime and packet delivery ratio among others. The findings revealed much about the energy efficiency measures adopted by various routing protocols upon applying them to certain special applications.

Li and Zhang (2021) reviewed the applications of MANETs in disaster management scenarios and compared different routing protocols for

emergency communication. Their research highlighted the critical role of MANETs in providing resilient and adaptive communication infrastructure during natural disasters and emergencies. It also justified the need to compare mobile ad hoc network routing protocols due to their varying efficiency, scalability, and adaptability in dynamic environments. Understanding these differences helps in selecting the most suitable protocol for specific applications, enhancing network performance, and optimizing resource utilization in real-world scenarios.

Khan *et al*. (2024) compared simulation settings for AODV, DSR, and MP-OLSR protocols to investigate QoS limits associated with various routing technologies. The study was primarily concerned with examining several quality measures for service enhancement and measuring procedure performance. Simulation results show that the DSR protocol had an 80% throughput advantage over AODV and MP-OLSR. However, in terms of delay and packet delivery ratio, the hybrid approach beats the AODV and DSR protocols. These findings provided a unique perspective for evaluating MANET compliance services.

Obaid *et.al*. (2024) aimed to consolidate existing research findings, identify best practices, and highlight gaps in the literature that warrant further investigation. The methodology for this review involved a comprehensive search across multiple academic databases, employing specific keywords related to routing protocols such as Ad-Hoc Networks, caching, and Ad-Hoc Networks. Studies were selected based on predetermined inclusion and exclusion criteria, ensuring relevance and quality. The research emphasized the necessity for more detailed research into the balance of cache efficiency, resource management, and security in Ad-Hoc Networks. The author only based their findings on a review of existing works of literature, but did not perform any experiments for more concrete evidence.

3.0 Materials and Methods

3.1 Research Design

In this research, a quantitative approach was adopted. The primary rationale behind selecting this methodology lies in the fact that the objective of this research extends beyond the definition and categorization of collaborative security attacks in MANET. Simulation-based experiments were conducted using the NS-2 simulator to evaluate the performance of each protocol.

The simulation scenarios include:

i. Network size: Varying the number of nodes in the network.
ii. Mobility: Varying the mobility pattern of nodes.

iii. Traffic: Varying the traffic pattern and intensity.

The performance of each protocol is evaluated using the following metrics:

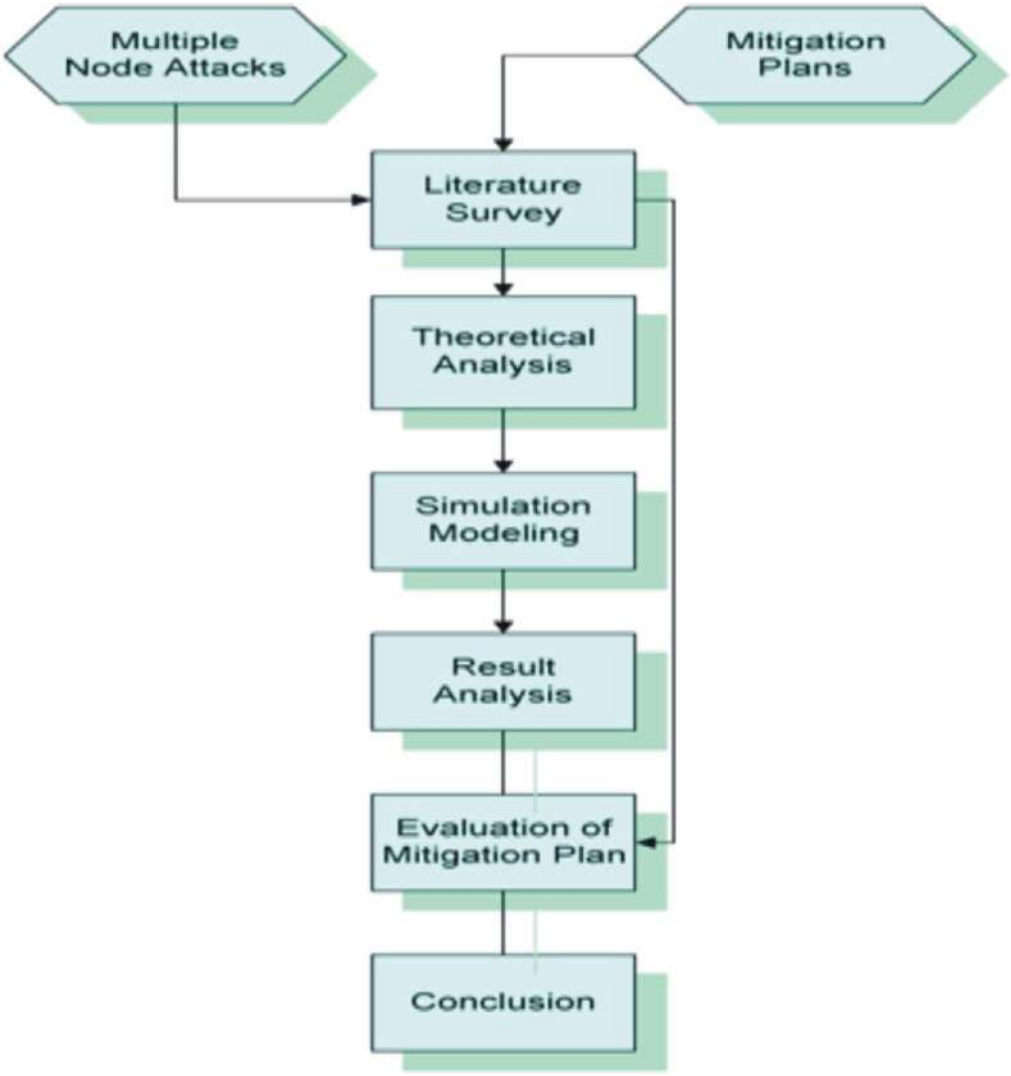
i. Packet delivery ratio: The ratio of successfully delivered packets to the total number of packets sent.

ii. Throughput: Throughput is the amount of data that can be transmitted or processed by a system, network, or communication channel within a given period. It is typically measured in bits per second (bps), bytes per second (B/s), or packets per second (pps)

iii. Average end-to-end delay: The average time taken for packets to travel from the source to the destination.

The design architecture of this work is shown in Figure 1.

Figure 1: The Research Design Architecture



3.2 Routing Protocols

3.2.1 Destination Sequence Distance Vector Protocol (DSDV)

The Destination Sequence Distance Vector (DSDV) protocol is a proactive routing protocol that adds a sequence number as an extra attribute to each route table entry in every node. This information is stored in nodes' routing tables, which makes it possible for packets to be transmitted from one node to another within the network. The protocol was prepared in such a way that it enables data transfer across dynamic and non-predefined paths of interconnection, which may be at any distance from any base station. During broadcast, every node attaches its current sequence number with the rest of this information for each new route: destination address, hops to destination, and destination's original sequence number.

3.2.2 Optimized Link State Routing Protocol (OLSR)

In the proactive link state protocol, OLSR is used as a link-state routing protocol. It has three routing mechanisms: firstly, it detects neighbors with Hello messages; secondly, it uses control packets with multi-point relay (MPR); and thirdly, it picks the route which is optimally computed by using the shortest path first algorithm. Each node selects the MPRs of its two-hop neighbors for access to all two-hop neighbors. The network then deploys these nodes as relay points for forwarding traffic across the network. Essentially, analyses are carried out using a hello message in order to determine if there is any neighbor within one or two hops as stated by Lee and Gerla (2000). However, since this protocol has been optimized using MPRs, it is best suited for larger networks with high density of nodes such MANETs. Such optimizations are more pronounced in dense and large networks than in traditional link state algorithms because of the greater amount of optimization occurring.

3.2.3 AdHoc On-Demand Distance Vector Routing (AODV)

The Ad Hoc On-Demand Distance Vector (AODV) routing protocol is based on the principles of the DSDV algorithm. In a nutshell, AODV acts as an improvement to DSDV by usually reducing the number of broadcasts necessary for establishing routes which are formed 'on demand'. For instance, DSDV algorithm keeps a full list of routes while in contrast, AODV is more likely to make it less in general and per broadcast. Consequently, AODV is defined as a pure on-demand route acquisition system because non-selected path nodes do not remember any information about routing tables and do not participate in their exchange. If a source node wants to send some message to a specific destination node but has no valid route leading towards this destination, it initiates a path discovery procedure aimed at finding that destination node. It incorporates new routes into routing tables and

packets, establishing new rules for the route discovery phase and generating link-disjoint and loop-free multiple paths (Luo *et al*., 2024).

3.2.4 Dynamic Source Routing (DSR) The Dynamic Source Routing (DSR) protocol is an on-demand routing protocol that uses source routing as a fundamental idea. Mobile nodes ought to keep route caches that have source routes they know, and update entries in the cache whenever new routes are obtained. There are two main phases of this protocol: Route discovery and Route maintenance. Before transmitting a packet to a destination, a mobile node checks its route cache to see if it already has a route to the destination. In case there is still an unexpired path, it makes use of this and forwards the packet through the same path. Otherwise, the node initiates route discovery by broadcasting a route request packet.

3.2.5 Temporally Ordered Routing Algorithm

A dynamic routing algorithm, Temporally Ordered Routing Algorithm (TORA), is the mechanism for distributing goods that are flexible and loop-free because it reverses the principle of the link. TORA is custom built to work well in highly mobile networks. It works on a source-initiated basis and provides multiple paths for any given source/destination pair. Localizing control messages around topological changes to a small fraction of nodes close by is the main design principle used in TORA. To guarantee this, the nodes must have routing information about their neighbor one-hop nodes. The three primary functions carried out by the protocol include: Route establishment, route maintenance; and route removal

4.0 System Implementation and Tools

This work was simulated using Network Simulator 2 (NS-2) implemented in C++. The simulation configuration table is depicted in Table 1

Table.1: Simulation Configuration Table

Parameter	Value
Number of Node	100
Speed	5-25 m/s
Routing Protocols	DSDV, OLSR, AODV, DSR, TORA
Source-Destination Pair	Random (varies with mobility)
Transmission Capacity	2 Mbps
Simulation Time	900 seconds
Number of Iteration	10

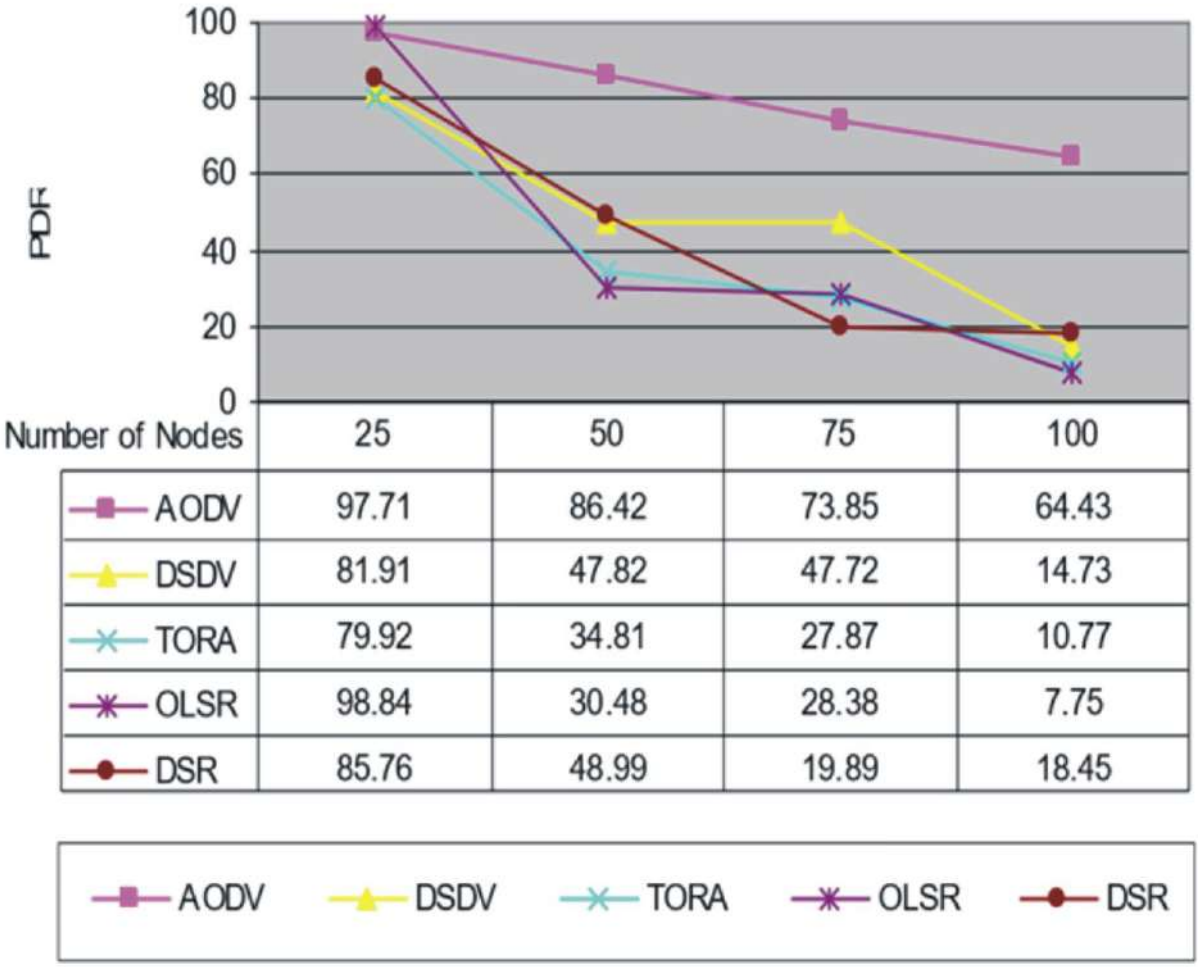
5.0 Results and Discussion

The implementation and results section outlines the deployment of the selected MANET routing protocols and their evaluation against specified metrics. It describes the experimental setup, details the performance and security assessments conducted, and presents a thorough analysis of the results, including key findings and their broader implications.

5.1. Results and Discussion

5.1.1 The Delivery Ratio of the Packet

The packet delivery proportion (PDR) as shown in Figure 2 is the ratio of packets that have been successfully delivered to their intended destination, compared to the total number of packets sent by sources. It also indicates packet loss rate which affects maximum throughput capacity that can be achieved by the network.

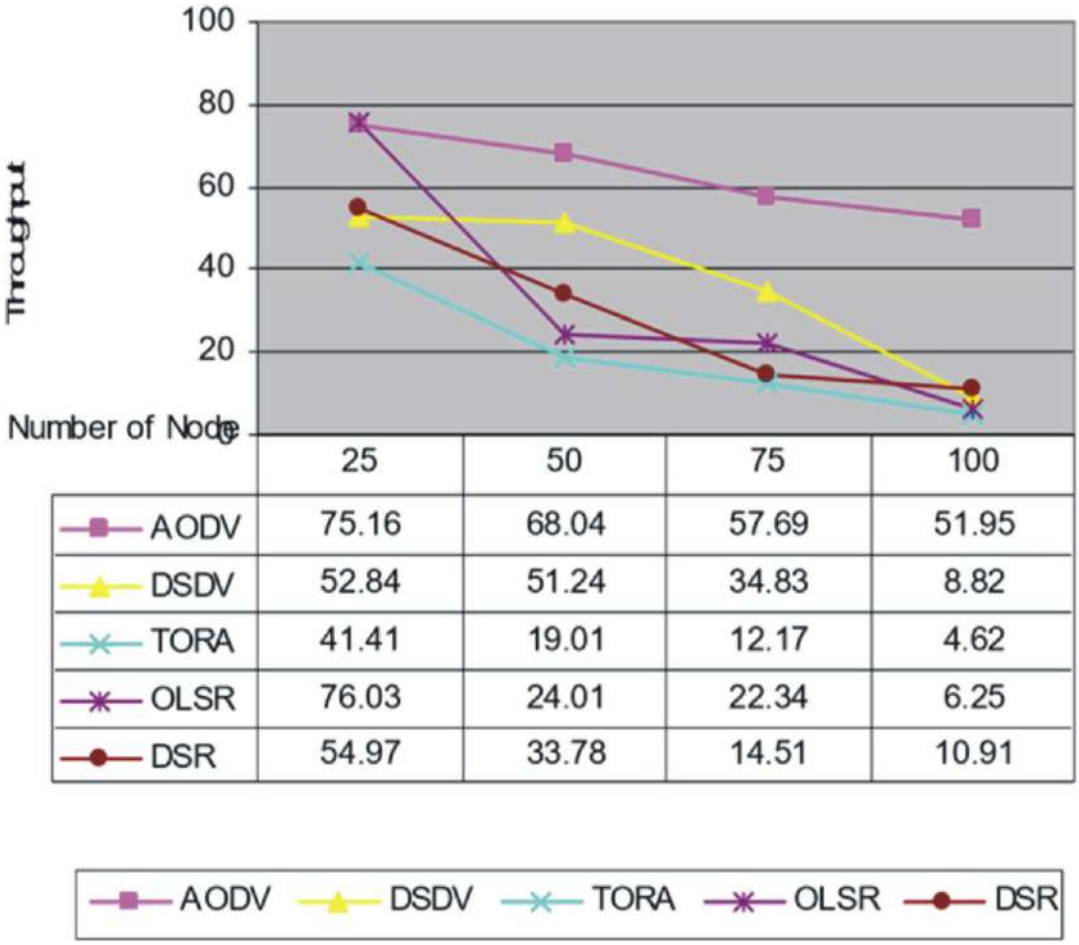


Number of Nodes	25	50	75	100
AODV	97.71	86.42	73.85	64.43
DSDV	81.91	47.82	47.72	14.73
TORA	79.92	34.81	27.87	10.77
OLSR	98.84	30.48	28.38	7.75
DSR	85.76	48.99	19.89	18.45

Figure 2: The Packet Delivery Ratio

5.1.2 The Throughput

The Throughput depicted in Figure 3 defines the average rate at which data packets are successfully transmitted from one node to another across a communication network. This rate is typically measured in kilobytes per second (KB/sec).

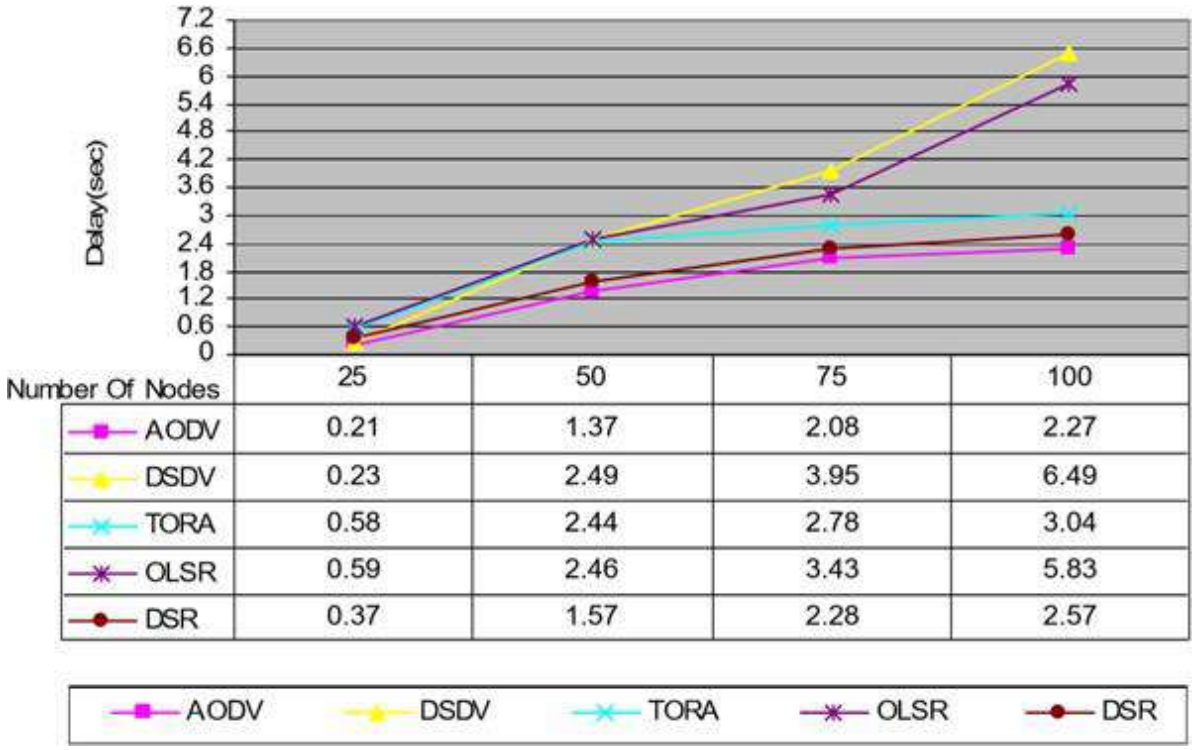


	25	50	75	100
AODV	75.16	68.04	57.69	51.95
DSDV	52.84	51.24	34.83	8.82
TORA	41.41	19.01	12.17	4.62
OLSR	76.03	24.01	22.34	6.25
DSR	54.97	33.78	14.51	10.91

Figure 3: Throughput

5.1.3 The Average End-to-End Delay

The End-to-end delay depicted in Figure 4 refers to the time required for a packet to travel from the source node to the destination node within a network.



Number Of Nodes	25	50	75	100
AODV	0.21	1.37	2.08	2.27
DSDV	0.23	2.49	3.95	6.49
TORA	0.58	2.44	2.78	3.04
OLSR	0.59	2.46	3.43	5.83
DSR	0.37	1.57	2.28	2.57

Figure 4: Average Delay Values

5.2 Discussion

The results from the simulation-based experiments are presented. The performance of each protocol was examined and compared, revealing its strengths and flaws. This analysis considers various routing protocols for Mobile Ad-Hoc Networks (MANETs). The examined protocols are: DSDV, OLSR, AODV, DSR, and TORA. To gauge the effectiveness of these protocols, three metrics are used: Packet Delivery Ratio (PDR), Throughput, and Average Delay.

Packet Delivery Ratio is thus a key metric that measures how reliable the routing protocol is for it shows the number of data packets that have been successfully transmitted to their destination compared to the total number of packets sent by the source. In this study, DSDV, a table-driven protocol, maintains consistent routing tables with up-to-date routes, resulting in a relatively high PDR under stable network conditions. However, its performance can decline in highly dynamic environments due to the overhead of continuously updating the

tables. OLSR, another proactive protocol, uses multipoint relays to reduce control message overhead, leading to improved PDR compared to traditional table-driven protocols like DSDV. Its efficient control of traffic management helps sustain a higher PDR, particularly in networks with moderate mobility. AODV and DSR, both on-demand routing protocols, generally show higher PDR in highly dynamic environments. AODV's route discovery process ensures routes are established only when needed, reducing the likelihood of stale routes. Similarly, DSR's source routing mechanism allows for rapid adaptation to changing network topologies, maintaining a high PDR. (Jothi-Lakshmi and Karishma, 2024). TORA, designed for highly dynamic environments, uses a link reversal algorithm to quickly respond to topology changes, ensuring competitive PDR, especially in scenarios with frequent node mobility.

Throughput, the average rate at which data packets are successfully delivered across the network is essential for evaluating the efficiency of routing protocols. DSDV, with its proactive nature, ensures steady throughput in stable networks. However, frequent table updates can cause congestion, negatively impacting throughput in dynamic scenarios. OLSR's optimization techniques, such as using multipoint relays, help maintain higher throughput by reducing redundant retransmissions. Its proactive approach ensures that data packets can be forwarded quickly, contributing to better throughput. AODV's on-demand nature allows it to achieve high throughput in networks with varying mobility, as routes are established only when necessary, reducing unnecessary overhead. DSR, with its aggressive caching and source routing, also achieves commendable throughput by efficiently managing route information and reducing the need for frequent route discoveries. TORA, while designed for dynamic environments, may suffer from lower throughput due to the complexity of its link reversal process. However, its adaptability to topology changes can sometimes offset this disadvantage, providing reasonable throughput under specific conditions.

Average delay measures the time taken for data packets to travel from the source to the destination. DSDV and OLSR, being proactive protocols, generally exhibit lower delay as routes are pre-established, allowing for immediate packet forwarding. OLSR's efficient control message management further reduces delay compared to DSDV.

Due to their on-demand nature, AODV and DSR experience higher initial delays during route discovery. However, once routes are established, their performance in terms of delay improves significantly. AODV's periodic route maintenance helps keep delays manageable, while DSR's route

caching minimizes the need for frequent discoveries, reducing overall delay. TORA, with its complex route maintenance mechanism, can introduce higher delays, especially during link reversal operations. However, its ability to quickly adapt to network changes helps mitigate excessive delays in highly dynamic environments.

The comparative study highlights that the performance of MANET routing protocols varies significantly based on network conditions and evaluation metrics. Proactive protocols like DSDV and OLSR perform well in stable environments with low mobility, ensuring high PDR, throughput, and low delay. Conversely, on-demand protocols like AODV and DSR excel in dynamic scenarios, offering higher PDR and throughput with acceptable delay. TORA, while specialized for dynamic networks, strikes a balance with moderate performance across all metrics.

6.0 Conclusion

The study underscores that the performance of MANET routing protocols varies significantly based on network conditions and evaluation metrics. Proactive protocols like DSDV and OLSR are well-suited for stable environments with low mobility, ensuring high PDR, throughput, and low delay. DSDV's table-driven approach provides consistent routing information, which is beneficial in static or slowly changing networks. OLSR's use of multipoint relays enhances its efficiency, making it a robust choice for moderately dynamic networks where control message overhead needs to be minimized.

Conversely, on-demand protocols like AODV and DSR excel in dynamic scenarios, offering higher PDR and throughput with acceptable delays. AODV's route discovery process and periodic route maintenance help manage the challenges of frequent topology changes effectively. DSR's aggressive caching and source routing mechanisms allow it to maintain high performance by efficiently managing route information and reducing the need for frequent route discoveries. These characteristics make AODV and DSR ideal for environments with high mobility and rapid changes in network topology. TORA, while specialized for highly dynamic networks, strikes a balance with moderate performance across all metrics. Its link reversal algorithm and adaptability to topology changes ensure it remains a competitive choice in highly variable network conditions. However, the complexity of its route maintenance mechanism can introduce delays, which need to be carefully managed. This research provided a systematic comparative analysis of prominent MANET routing protocols. The results of this study can be used to inform decision-making for protocol selection and optimization in various MANET scenarios.

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