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About the Nigerian Academy of Science

The Nigerian Academy of Science (NAS) is the foremost independent scientific body in Nigeria, which was established in 1977, and incorporated in 1986. NAS is uniquely positioned to bring scientific knowledge to bear on the policies/strategic direction of the country and is dedicated to the development and advancement of science, technology, and innovation (STI) in Nigeria. The aims and objectives of the Academy are to promote the growth, acquisition, and dissemination of scientific knowledge, and to facilitate its use in solving problems of national interest. TheAcademy strives to do this by:

- Providing advice on specific problems of scientific or technological nature presented to it by the government and its agencies, as well as private organizations.
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- Establishing and maintaining the highest standards of scientific endeavours and achievements in Nigeria, through the publication of journals, organization of conferences, seminars, workshops, and symposia, recognition of outstanding contributions to science in Nigeria, and the development of a working relationship with other national and international scientific bodies and academies.

As with national academies in other countries, NAS is a not-for-profit organization with a total membership (since inception) comprising 296 Fellows, elected through a highly competitive process, who have distinguished themselves in their fields, both locally and internationally. Some of her members have served as Vice-Chancellors of universities, Directors-General of government Parastatals and Ministers in federal ministries. The Academy, given its influence, also has the ability to attract other experts from around the country and internationally when needed.

NAS is Nigeria's national representative on such bodies as the International Science Council (ISC) – the umbrella body for all science associations and unions – and the Inter-Academy Partnership (IAP) – the umbrella body for all national science academies globally. The Academy is also a member of the Network of African Science Academies (NASAC).

Regionally, the Nigerian Academy of Science is one of eight founding academies of the Network of African Science Academies (NASAC) and has served on its Executive Committee until date. The Academy has played a major role in the development and establishment of academies in Africa. In November 2012 and 2017, the Nigerian Academy of Science hosted the African academies for the 8th and 13th Annual Meeting of African Science Academies (AMASA), in Lagos and Abuja respectively. The Nigerian Academy of Science has signed agreements with counterparts in many African countries (and beyond) to ensure scientific exchange and partnership.

As the peak independent scientific body in Nigeria, the Academy serves as the umbrella body for all science associations in the country, speaking for the same within and outside the country. The Academy holds periodic meetings with representatives of the associations to discuss the state of science in Nigeria and proffer solutions for improvement.

Some of the recent accomplishments of NAS include:

1. The development of a training manual on getting research into policy and practice,
2. The organization of an international conference on climate change in Lagos,
3. Implementation of a project on linking agriculture and nutrition,
4. The organization of a national consensus building workshop on the prevention of maternal and child mortality in Nigeria,
5. Conveying a media roundtable meeting to discuss issues of depression and suicide prevention,
6. Conveying a roundtable meeting to discuss the issues related to the Ebola Virus Disease epidemic that recently affected the country and the West African region,
7. Implementation of an intervention program to address the social and reproductive health issues of the youth in Ekiti and Nasarawa States of Nigeria,
8. The hosting of all African academies and other scientists at an international conference on STI education and manpower development in Africa,
9. The organization of a summit to discuss the role of women in science and sustainable development in Nigeria,
10. The organization of a workshop to discuss the evolution of big data and artificial intelligence (AI), and the impact of education on training,
11. A consensus study on the evolving science advisory landscape in Africa,
12. Organization of webinar and policy roundtable to create awareness and proffer policy recommendations in combatting predatory academic practices in Nigeria,
13. The organization of a research mentorship programme to enhance scientific research capacity in Nigeria, as well as facilitating an enabling environment for research and researchers to thrive.

About the Proceedings of the Nigerian Academy of Science

The *Proceedings of the Nigerian Academy of Science (PNgAS)* is the peer-review official journal of the Nigerian Academy of Science, one of Africa's leading science Academies and the foremost independent scientific body in Nigeria. The regular edition of the journal is a multidisciplinary publication, with the primary objective of disseminating original research, systematic reviews, and meta-analysis in all Science, Technology, Engineering, and Mathematics (STEM) disciplines, especially those that address national and regional developmental challenges. The journal publishes articles that are based on deep-seated formative research using large and multi-center datasets that leads to a better understanding of the context of science-related developmental challenges and appropriate pathways for accomplishing change in the following scientific disciplines:

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Editorial

Innovative solutions: Of internet of things and biotechnology

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Abstract
The broad term, "biotechnology" dates to domestication of animals, cultivation of plants, and their modification through breeding programmes using artificial choice and conjugation. Attaining sustainability requires fast research and development in every sector including the most diversified field of biotechnology. The Internet of Things (IoT), one of the coveted areas in technological revolution, carries tremendous potential for the transformation of research, innovation and invention in every discipline. IoT provides a window for efficient, reproducible,fast and precise research into biotechnological research. The potential role of IoT in advancing innovation, conferring economic up-scaling and invention in biotechnology was explored in this editorial.

Keywords: *Biotechnology, internet of things, innovation, renewable natural resources.*

Introduction

Biotechnology is the technology that uses biology to develop new products, methods and organisms intended to improve the society. Biotechnology, usually abbreviated as “Biotech”, has been in existence since the domestication of plants and animals at the beginning of civilization and the discovery of fermentation. Internet of Things (IoT) simply means taking all the physical places and things in the world and connecting them to the internet. Technically, these would require that computing devices, mechanical and digital machines, objects, animals or people are provided with unique identifiers with the ability to transfer data over a network without requiring human-to-human or human-to-computer interaction. The concept of IoT therefore provides the ability to collect information and send it, receive information and act on it or the ability to do both. In this edition, one of the articles presents an IoT architecture for a fire detection system using small, low-cost cameras to collect surveillance data from large buildings. The data is then uploaded to the cloud, where a Machine Learning algorithm detects fires in digital images. The authors concluded that the proposed IoT fire detection system was effective because the response time was well within the permitted range for emergency response systems. The advancement in biotechnology necessitates the need for the integration of IoT to enhance the precision, reproducibility, productivity as well as incursion into new territories in biological research, invention and innovation.

The Evolution of Biotechnology

Biotechnology began with agricultural revolution, characterized by domestication of living organisms or modifying their genetic makeup through selective breeding to express or eliminate

certain genetic characteristics in their offspring, while fermentation was used as a tool mainly for food preservation. Fermentation has been described as the first use of biotechnology. However, biotechnology was limited to slow, agricultural methods such as selective breeding until the 19th century when biologist Gregor Mendel discovered the basic principles of heredity and genetics, while scientists Louis Pasteur and Joseph Lister discovered the microbial processes of fermentation.

Fermentation, in addition to its use in food processing has many other biotechnological applications: to produce drugs that can destroy or inhibit the growth of harmful bacteria and viruses (antibiotics and antiviral drugs). For example, it is during the process of fermentation thatthe fungi of the genus Penicillium produced one of the first antibiotics to be discovered, penicillin,which has saved many thousands of lives. Also, another benefit of microbial fermentation is ethanol, a biofuel, which is a renewable source of energy made using living or dead material, suchas plant, which can serve as an alternative to gasoline.

Genetic engineering is the foundation of modern biotechnology practices and recent advances. It enabled the first direct manipulation of plant and animal genomes. The use and commercialization of modern biotechnology often fall into four main fields: agriculture, environment, industry and medicine.

Agriculture: Agricultural biotechnology genetically engineer plants and animals for efficiency in agriculture, increased nutritional value and reduction in food insecurity. These include selective breeding that produces healthier, bigger livestock and crops; infusion of food with nutrient supplementation to improve diets and for medical treatments. Others are drought-resistant crops, meat grown in the laboratory, biologically produced pesticides and herbicides which are not harmful to the environment and humans, etc. In this edition, author (s) explored the biocontrol potentials of Trichoderma species in the control of Fusarium wilt in five tomatoes (*Lycopersicon esculentum L*.) varieties cultivated under greenhouse conditions in Southwest Nigeria. They informed that tomato plants grown with Trichoderma sp. had better performance in terms of average fruit yield count and showed the least disease severity rating. They recommended further exploration of Trichoderma species as means of control for fungus-based diseases in tomato plants.

Environment: environmental biotechnology develops sustainable environmental practices and products that reduce pollution and waste. Some examples of environmental biotechnology are phytoremediation, which uses genetically engineered microorganisms to purify soils of heavy metals and other pollutants; bioremediation introduces microorganisms into waste sites to organically break down nonrecyclable waste, while plastic-eating bacteria breaks down waste plastics in soils and water. Additionally, GMO foods stay fresher for longer, which help to reduce food waste and cover-crops like corn are used as biofuels.

Industry: Industrial biotechnology uses microorganisms to produce industrial goods, such as fermentation and the use of enzymes and microbes to streamline chemical manufacturing and reduce operational costs and chemical emissions; biodegradable garments and textiles made from the proteins of living organisms, like the silk proteins of spiders.

Medicine: Biotechnology and biomedical research are the basis of the modern pharmaceutical industry. Some of the advances are stem cell research that helps replace or repair dead or defective

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cells; gene therapy for diseases such as leukaemia; 3D printing or growing of organs and bones in labs; mRNA vaccines, monoclonal antibody treatments and research for COVID-19.

Innovative Solutions at the Biotechnology-IoT Interface

Internet of Things has led to paradigmatic developments in performing mundane tasks and has been widely accepted as a promising paradigm that can transform society and industry with its ability to achieve the seamless integration of various devices equipped with sensing, identification, processing, communication, actuation, and networking capabilities.

***IoT and Agricultural Biotechnology interface*:** Agricultural biotechnology embracing IoT transforms agricultural practices through smart and precision farming, thereby enhancing the productivity of crops in a controlled and accurate manner. Various high end IoT enabled products and services like variable rate irrigation optimizer (VRI), soil moisture probes make farmers to understand soil fertility, composition and enhance the efficiency of water utilization. More recently, the concept of smart green houses that comprises IoT based sensors that can intelligently measure, and control humidity, temperature, pressure and light levels saves time, cost, energy and labour-intensive process of farmers with no manual intervention and increased productivity. The advent of drones in both ground as well as aerial based systems revolutionized agriculture by real time monitoring of irrigation, soil variability, field analysis, planting, crop health and diseases assessment leading to IoT induced agriculture efficiency by generation of real-time data for the prediction of plant height and health, crop yield, nutritional composition of crop, canopy area mapping, etc.

IoT and Environmental Biotechnology Interface

Environment monitoring has attracted more and more attention due to the growing concern about climate change. Conventional environmental monitoring systems such as oceanographic and hydrographic research vessels are expensive with time-consuming and low-resolution data collection and analysis processes. Internet of Things has been playing an important role in this area with different sensors deployed to measure and monitor various physical and chemical parameters in real time using different IoT system architectures, sensing, control and communication technologies, including ocean sensing and monitoring; air and surface water quality monitoring; coral reef monitoring; aquaculture farm monitoring; wave and current monitoring.

IoT and Industry Interface

In pharmaceutical research and industry, the discovery of novel drugs and biologics are the promising avenue for commercialization. The implementation of smart and automated equipment and accessories provides precision and improvement in the quality of drugs and other products, while real time monitoring and high-through put screening of drugs with smart sensing leads to better adherence to regulatory requirements. Advancements in smart packaging labels such as 2D bar-coding, radio frequency identification tags confer the benefit of online tracking of products from manufacturing to distribution. This is also applicable to other industries.

IoT and Biomedicine interface

The intersection of Internet of Things (IoT) and biotechnology is reshaping the landscape of healthcare and biomedicine, ushering in an era of unprecedented advancements and efficiencies. Some of the advances are:

Connected Devices in Diagnostics and Patient Monitoring: Smart sensors and wearable devices enable real-time monitoring of vital signs, providing physicians with a continuous stream of data for accurate and timely intervention, and facilitates remote patient monitoring.

Precision Medicine and Personalized Treatments: Enables the tailoring of treatments to specific genetic, environmental, and lifestyle factors consequent to collection and analyses of extensive datasets from individual patients.

Biological Material Supply Chain Optimization: IoT provides real-time tracking and monitoring of biological samples/materials, which ensures their integrity and the reliability of research outcomes and commercialization processes.

Data Security and Privacy: Robust cybersecurity safeguards sensitive research and patient information and research data, thus ensuring the integrity and confidentiality of research and healthcare data.

Conclusion

Green biotechnology, which applies to agriculture, is well known and involves processes such as the development of pest-resistant grains or the accelerated evolution of disease-resistant animals. Blue biotechnology on the other hand is positioned as one of the emerging sectors within the Blue Economy. Blue biotechnology develops solutions in various fields such as food production, energy, medicine, aquaculture, bioremediation, and the chemical industry. Blue biotechnology has great potential to contribute to sustainable development, as well as to address global challenges such as food security and climate change mitigation, among others. Future advances at the interface of biotechnology and IoT will commence from research, in established and emerging fields. In this era of "omics" with high evolutionary pace of novel microbial strains, phages and other biological breakthroughs, results from biotechnological research would need to be more precise, reproducible and scalable. IoT has enabled instruments and equipment embedded with intelligence and interconnected communication protocols like RF and Bluetooth which could be used for the collection of data, which can then be stored in cloud servers, making them available virtually for authentication and reproducibility. Sophisticated automated robotic laboratory workflow has been launched while artificial intelligence and automation in gene synthesis has also been deployed for finding and creating novel compounds and biological solutions. Immense potential therefore exists for the application of IoT in biotechnological research, innovation and development.

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Original Research Article

Biocontrol potentials of Trichoderma species in the control of *Fusarium* wilt in five tomatoes (*Lycopersicon esculentum* L.) varieties cultivated under greenhouse conditions in Southwest Nigeria

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Abstract

Important pathogenic fungi such as *Fusarium* species are mostly associated with the wilting of tomato plant leaves and constitute a serious limiting factor for tomato production. Therefore, this study aimed to screen tomato varieties commonly cultivated in Nigeria to determine their host resistance status to *Fusarium oxysporum.* The treatments included *Trichoderma asperellum, T. harzianum,* and Mancozeb fungicide. Data recorded included the incidence and severity of the pathogen, plant height, leaf number, and fruit yield of the tomato plants. Tomato plants grown with *Trichoderma* sp. exhibited better performance in terms of average fruit yield count of 6.5 for *T. asperellum* and 6.7 for *T. harzianum* compared to the average yield of 1.7 fruit count for Mancozeb fungicide. The lowest disease incidence was recorded in the variety 3 (NGB00737) with a 5.81% incidence in the Mancozeb-treated plants. Conversely, *T. asperellum*-treated plants in variety 3 (NGB00737) had the highest disease incidence of 46.49%. For variety 4 (NGB00695), plants treated with Mancozeb had the least disease with a severity rating of 1. Similarly, variety 5 (NGB00725) plants treated with *T. asperellum* showed the least disease severity rating of 1. All other treated plants showed higher severity values of 4.

The *Trichoderma* species also improved the fruit yield and some agronomic parameters of the tomato plants compared to the chemical control. Agro-business enthusiasts, commercial growers and local farmers are therefore encouraged to further exploit the potential of *Trichoderma* species as means of control for fungus-based diseases in tomato plants such as those caused by *Fusarium*.

Keywords: *Tomato, Fusarium, Trichoderma, biocontrol agents.*

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a valuable economic vegetable crop and a staple of daily meals for many Africans in sub-Saharan Africa, especially Nigeria. The tomato plant wasoriginally native to tropical America but is now grown in many countries [1]. Tomato productionaccounts for about 4.8 million hectares of harvested land area globally with an estimated

production of 162 million tonnes with China leading world tomato production at about 50 million tonnes followed by India with 17.5 million tonnes [2]. In Africa, total tomato production for 2012 was 17.938 million tonnes with Egypt leading the continent with 8.625 million tonnes, followed by Nigeria with 1.56 million tonnes [1]. Daily consumption of tomatoes provides the body with nutrients such as carotene, vitamins, and lycopene, lowering the risk of cancer and cardiovascular diseases [3]. Tomatoes possess antioxidant components that are medically useful in cataracts, bone metabolism, and asthma and help reduce the risk of prostate and breast cancer [4]. Tomato is also commonly used as condiments for stews and sauces, which is a regular feature of African meals and accounts for about 18% of the average daily consumption of vegetables in Nigeria [5].

Fusarium oxysporum f. sp. *lycopersici* (Sacc.) causes serious damage wherever tomatoes are grown intensively due to its endophytic life mode and persistence within infested soils [2]. Tomato yield has also been reported as significantly reduced by *F. oxysporum* f. sp. *lycopersici* which infects the plant at the growing stages. Most strains associated with this species are either saprophytic or non-pathogenic in function. However, plant-pathogenic strains of *F. oxysporum* cause destructive vascular wilt diseases on a wide variety of crops, often limiting crop production [6]. Characteristic symptoms of *Fusarium* attack include leaf chlorosis and vascular wilt that progresses into partial or total wilting of tomato plants and darkening or browning of the vascular system [5]. *Fusarium* wilt symptoms also include drooping, yellowing, wilting, and dying of the lower leaves, often on one side of the plant. Plant growth is stunted, and under warm conditions, the plant may die [6].

Fusarium persists in soil for many years without a host, growing as a saprophyte on plant debris. Thus, traditional control practices that are often used in Nigerian cropping systems, such as removal of infected crops and crop rotation are sometimes ineffective for the management of infectious diseases caused by *Fusarium*. Chemical control of the pathogen may prove relatively effective, but environmental toxicity and the challenge of keeping pesticide residues within acceptable levels in fresh fruits are of serious concern [7]. Biological control using apathogenic-pathogenic fungal interactions [8] has shown promising results for *in vitro* studies, but challenges are often encountered under field conditions, where abiotic interactions: temperature, water availability, and the presence of other biotic factors may limit bio-efficacy. Adequate management of wilting caused by *Fusarium* in tomato plants may be considered a vital contributory factor to food security issues in the country.

Trichoderma has gained immense importance in the last few decades due to its biological control potential against several deadly plant pathogens [9]. *Trichoderma* spp. is found in almost all soil types including cultivated soil, garden soil, fallow and pastureland, forest soil, etc. [10]. *Trichoderma* spp. is found to be a very efficient producer of extracellular enzymes and some of these have been implicated in the biological control of plant diseases [7]. *Trichoderma* spp. generally grows in their natural habitat on plant root surfaces and therefore, in particular controls root diseases. As plant growth promoters [8] and antagonists against plant pathogens, *Trichoderma* strains are appealing alternatives to hazardous fumigants and fungicides.

The primary disease management strategy often employed to control diseases caused by fungal pathogens such as *Fusarium* for vegetable crops in Nigeria is with standard fungicides such as Mancozeb. Biological control of fungal pathogens in vegetable crops is hardly engaged and even where resorted to, is often very limited in application. This study, therefore, assessed the

antagonistic potentials of two *Trichoderma* spp. against the wilting caused by *Fusarium* in the tomato plant. This study also involved a comparison between the effect of a known fungicide (Mancozeb) and the biological control organisms used.

2. MATERIALS AND METHODS

2.1 Experiment set-up

The experiment was set up at the greenhouse facility, National Horticultural Research Institute, (NIHORT) Ibadan, Nigeria (latitude 7^{O} 40'N and longitude 3^{O} 84'E; altitude 195 meters above sea level).

2.1.1 Preparation of plant materials

Seeds of five tomato varieties were used for this study. One commonly cultivated local variety (Iwo variety) obtained from a farm in Iwo, Osun State, Nigeria and four exotic tomato varieties (NGB00715, NGB00737, NGB00695 and, NGB00725) which were gotten from the National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria.

2.1.2 Preparation of disease-causing fungal isolate

The pure culture of Fusarium sp. obtained from a stock culture of the culture collections of the NIHORT Pathology laboratory was used. It was sub-cultured using Potato Dextrose Agar obtained from the Pathology laboratory in fifteen (15) culture plates within a standard laminar flow chamber and the culture plates were placed in the incubator, where growth temperature was maintained at 24 ^{o}C for seven (7) days.

2.1.3 Cultivation of biocontrol agents

Pure cultures of already characterized *T. asperellum* and *T. harzianum* were obtained from the Pathology Laboratory, NIHORT. Both strains were then sub-cultured in Potato Dextrose Agar (PDA). Mancozeb 80 % wettable powder (WP) was prepared according to the manufacturer's instructions.

2.2 Application of treatments

The five tomato varieties were raised in germination trays for four weeks before transplanting into perforated pots filled with 8 kg of sterilized soil. The treatments were applied in a completely randomized design, arranged in three replicates. Treatments were applied 5 cm away from the plant stem in each planting pot. The potted plants were adequately watered and managed till maturity.

Treatment application was carried out a week after transplanting, allowing for proper adjustment of the tomato plants to the new growing environment. Treatments used are as follows:

Treatment 1: Plants + pathogen + 5 ml *Trichoderma asperellum*

Treatment 2: Plants + pathogen + 5 ml *Trichoderma harzianum*

Treatment 3: Plants + pathogen + Fungicide

Treatment 4: Plants + pathogen (positive control)

Four hundred and fifty (450) ml of *Fusarium* inoculum was prepared by calculating the total

volume of inoculum needed for the total number of pots used in the experiment. The treatments were simultaneously applied to all the plants using a sterile syringe. Five (5) ml of the *Trichoderma species* and the Mancozeb fungicide were applied respectively to the pots in all treatments. The untreated plants inoculated with *Fusarium*, being the control, were without any external input to the soil samples. The tomato plant samples were inoculated with the pathogen before the respective treatments were applied.

2.3 Disease assessment

Disease incidence and severity values were recorded for seven (7) weeks from the establishment in the nursery to the full maturity and harvest of the tomato plants.

The percentage of disease incidence was calculated using:

$$\boldsymbol{D = \frac{n}{N} \times 100}$$

Where: n= number of plants showing wilt symptoms
N = Total number of plants sampled [4].

Disease severity was also calculated according to the modified formula of [4]:

$$\frac{\textbf{Number of individual ratings}}{\textbf{Number of plants assessed}} \times \frac{100}{\textbf{Maximal Scale}} = \textbf{Percentage Disease Severity}$$

The disease scale of zero to five (0 – 5) was used to evaluate the severity of the Fusarium-*induced* wilt symptoms [11]. Disease severity ratings for *Fusarium oxysporum* were done using a scale of 1 - 5 as adopted by [12] which was based on infection percentage, calculated as follows: 1-10% = 1, 11-20% =2, 21-30% = 3, 31-50% = 4, 51-100% = 5; where 1= resistant (R), 2 = moderately resistant (MR), 3 = moderately susceptible (MS), 4= susceptible (S), and 5= highly susceptible (HS).

2.4 Assessment of agronomic data

Agronomic data consisting of plant height, number of leaves, and number of branches were obtained and recorded on weekly basis from establishment at week 4 till harvest at week 15. Fruit yield data was also obtained from the plants representing each variety at the end of the planting period. The plants were harvested in the 11th week after transplanting.

2.5 Data analysis

All the data gathered were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT). The least significant difference (LSD) was also determined at a 5% level of probability using SPSS Version 20. Graphs were plotted using GraphPad Prism version 5.

3. RESULTS

Four improved varieties and one local variety which are commonly grown in Nigeria were tested to determine their resistance status to *F. oxysporum*. Table 1 shows all the represented type and varieties of tomatoes used respectively in the study as follows:

Table 1: Tomato type and varieties planted for greenhouse experiment.

Type	Variety	Name	Location obtained
Local	1	Iwo	Iwo
Exotic	2	NGB00715	NACGRAB (Ibadan)
Exotic	3	NGB00737	NACGRAB (Ibadan)
Exotic	4	NGB00695	NACGRAB (Ibadan)
Exotic	5	NGB00725	NACGRAB (Ibadan)

Table 2 shows that all five varieties of tomatoes used in this study showed varying levels of resistance to the pathogen. Out of all tomato varieties tested, the lowest disease incidence was recorded in variety 3 plants (NGB00737) with a 5.81% incidence in the Mancozeb-treated plants. Conversely, *Trichoderma asperellum* treated plants in variety 3 (NGB00737) had the highest disease incidence, with a percentage of 46.49 (Figure 1)

In Variety 1 (Iwo Variety), plants treated with Mancozeb fungicide had the least disease incidence at 27.83% incidence, while the highest disease incidence of 41% was observed in the control. Variety 2 (NGB00715) had the lowest disease incidence seen in control plants with 19.57%, whereas plants treated with *Trichoderma asperellum* (T2) had the highest disease incidence with 28.66% (Figure 1). In variety 4 (NGB00695), the plants with Mancozeb fungicide again showed the least disease incidence at 9.95%, while the *Trichoderma asperellum* treated plants had the highest observed disease incidence of 30.48% (Figure 1).

The 5th variety (NGB00725) had plants treated with *Trichoderma asperellum* showing the least disease incidence of 12.64%, while the highest incidence was observed in the plants treated with *Trichoderma harzianum* with an incidence of 33.23% (Figure 1).

The lowest disease severity across the five tomato varieties was observed in NGB00695, with a severity rating of 1 (Figure 2). The highest disease severity values across varieties were however observed in both NGB00737 and NGB00725 varieties, with severity ratings of 5 and 4 respectively (Figure 2). In the Iwo variety, all the treated plants presented an average disease severity rating of 3. In Variety 2, all the treated plants also showed an average disease severity value of 3, except for control plants which had a lower severity rating of 1 (Figure 2). In Variety 3 (NGB00737), the plants treated with *Trichoderma harzianum* had the highest disease severity rating of 5 compared to all other treatments (Figure 2).

In Variety 4 (NGB00695), plants treated with Mancozeb had the least disease variety with a severity rating of 1, closely followed by *T. harzianum* treated plants with a disease severity rating of 2. However, the *T. asperellum* treated and control plants had the highest disease severity of 3 respectively (Figure 2). In Variety 5 (NGB00725), plants treated with the biological treatment, *T. asperellum*, showed the least disease severity rating of 1. All other treated plants showed higher severity values of 4 (Figure 2).

In line with host resistance status ratings adopted by Silme and Cargigan (2010), inferred from using the disease severity values of each plant variety; for the Iwo variety plants controlled with both biological treatments, *T. asperellum* and *T. harzianum* were moderately susceptible, with the control plants treatment also being moderately susceptible (Table 2). In Variety 2, all the plants controlled by biological and chemical treatments were moderately susceptible, while only the

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control plants were moderately resistant (Table 2).

In Variety 3, *T. asperellum* treated plants as well as all other plant treatments showed moderate susceptibility, while only the *T. harzianum* treated plants were highly susceptible (Table 2). In variety 4, the *T. asperellum* treated and control plants were moderately susceptible, while *T. harzianum* treated and Mancozeb treated plants were both moderately resistant and highly resistant respectively (Table 2). In Variety 5, *T. asperellum* treated plants were moderately resistant, while all the other plant treatments were highly susceptible (Table 2).

A total of five tomato varieties were planted with a total of 30 seeds planted per variety. The greatest plant height across the tomato varieties was observed in the fifth variety (NGB00725) with an average height of 120.2 cm, while the shortest height was observed in the second variety (NGB00737) with a mean of 71.2 cm.

The highest number of leaves across the different tomato varieties was observed in NGB00715 with plants treated with the biological control T1 (*Trichoderma asperellum*) having a total mean number of 102 leaves after 7 weeks of planting, while the lowest number of leaves was observed in the first variety (Iwo variety) having a mean number of 29.33 leaves after seven weeks of planting. The highest number of branches was observed in Variety 4 (NGB00695) and Variety 5 (NGB00725). Both had an average number of 12.33 branches (Figure 5).

3.1 Fruit yield

The highest fruit yield across the five varieties was observed in Variety 5 (NGB00725) with a mean yield of 11.20 while the lowest fruit yield across all five varieties was observed in the first variety (Iwo Variety) with a mean fruit yield of 1.7 fruits (Figure 6). In Variety 1 (Iwo Variety), the highest fruit yield was observed in the plants treated with the second biological treatment T2 (*Trichoderma harzianum*) with a mean yield of 6.7 fruits. The lowest fruit yield was observed in the plants treated with the chemical control (Mancozeb) with a mean fruit yield of 1.7 fruits. In Variety 2 (NGB00715), the highest fruit yield was observed in the plants treated with the chemical control (Mancozeb) with a mean yield of 2.8 fruits. The lowest fruit yield was observed in the plants treated with the treatment T1 (*Trichoderma asperellum*) with a mean fruit yield of 0.8 fruits (Figure 6).

Table 2: Severity ratings and host status of evaluated tomato varieties after the seventh (7th) week of planting.

Variety	Severity rating (1-5)	Status
V1 (Iwo Variety)		
VIT1	3	Moderately susceptible
V1T2	3	Moderately susceptible
V1T3	3	Moderately susceptible
V1T4	3	Moderately susceptible
V2 (NGB 00715)		
V2T1	3	Moderately susceptible
V2T2	3	Moderately susceptible
V2T3	3	Moderately susceptible
V2T4	2	Moderately resistant
V3(NGB00737)		
V3T1	3	Moderately susceptible
V3T2	5	Highly susceptible
V3T3	3	Moderately susceptible
V3T4	3	Moderately susceptible
V4 (NGB00695)		
V4T1	3	Moderately susceptible
V4T2	2	Moderately resistant.
V4T3	1	Resistant
V4T4	3	Moderately susceptible
V5 (NGB00727)		
V5T1	2	Moderately resistant Highly Susceptible
V5T2	4	Highly Susceptible
V5T3	4	Highly Susceptible
V5T4	4	

T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen

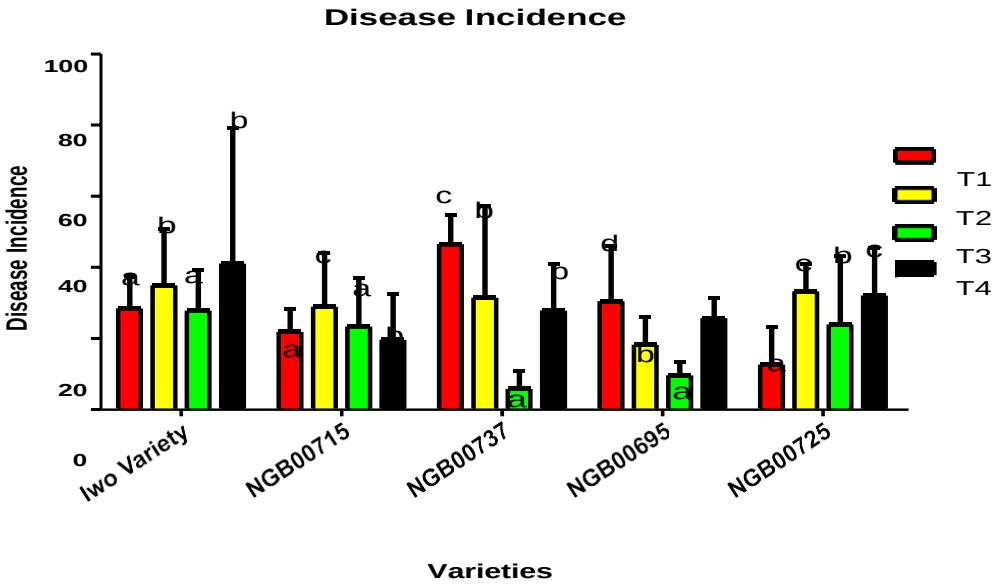

Figure 1: Disease incidence of *Fusarium oxysporum* f. sp. *lycopersici* inoculated tomato plants at Seven (7) weeks after planting. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen +

https://doi.org/10.57046/XZNH3101

Fungicide, T4 = Plants + pathogen

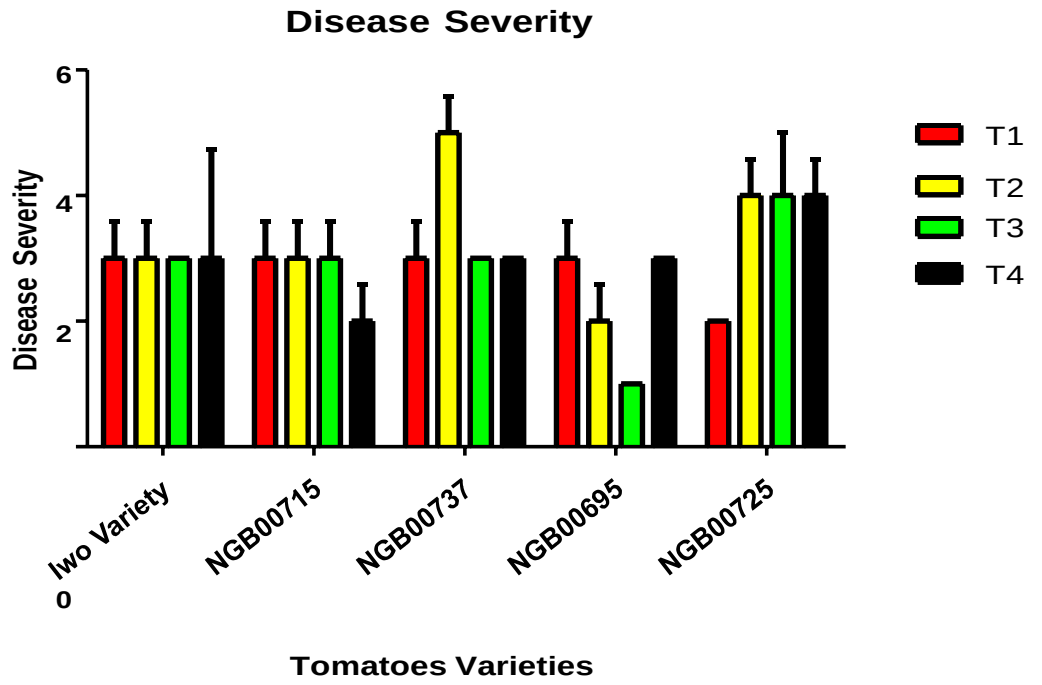

Figure 2: Disease severity of *Fusarium oxysporum* f. sp. *lycopersici* inoculated tomato plants at Seven (7) weeks after planting. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen

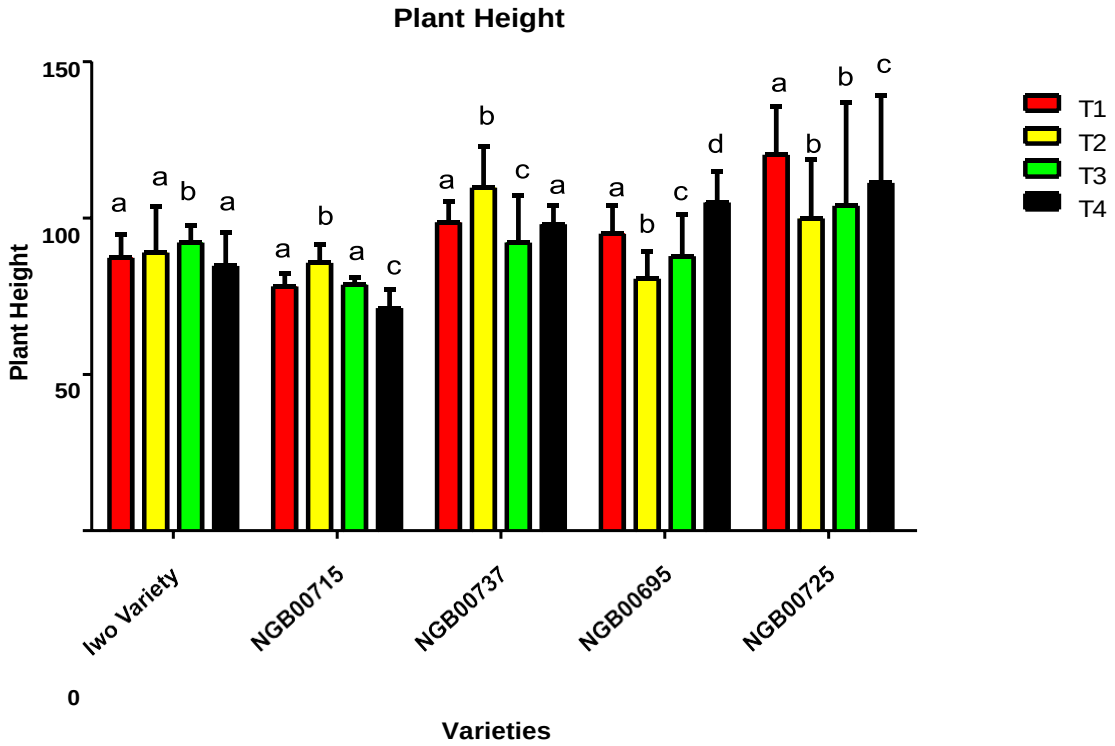

Figure 3: Plant Height across tomato varieties. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen.

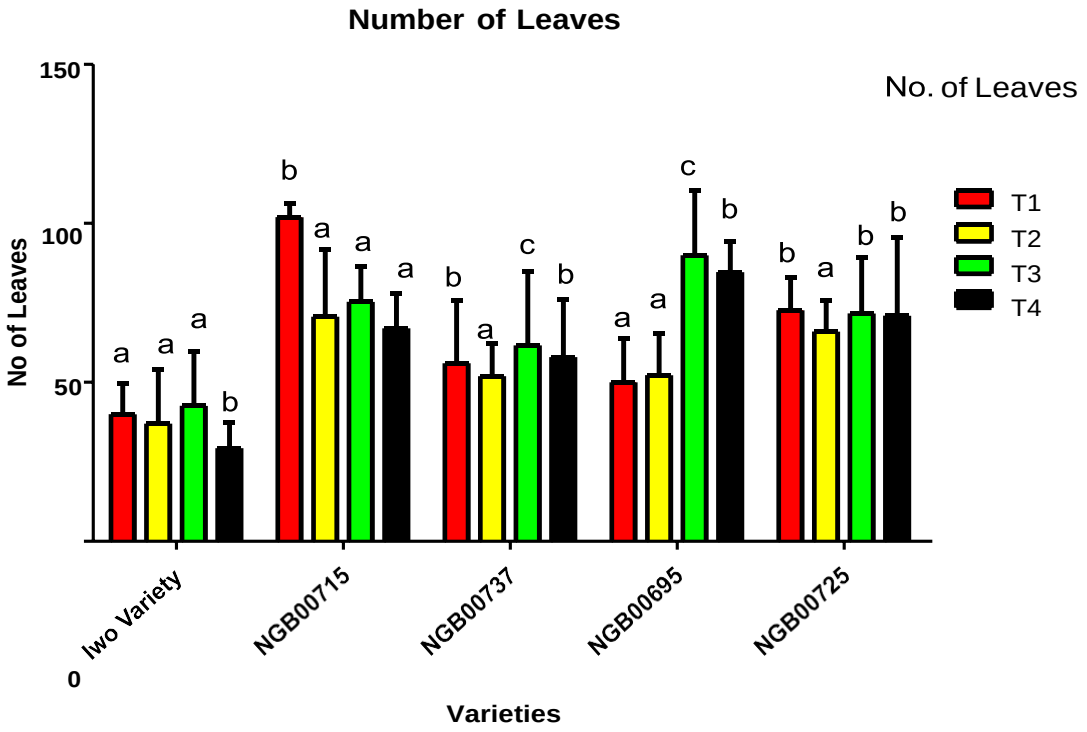

Figure 4: Number of leaves across the tomato varieties. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen.

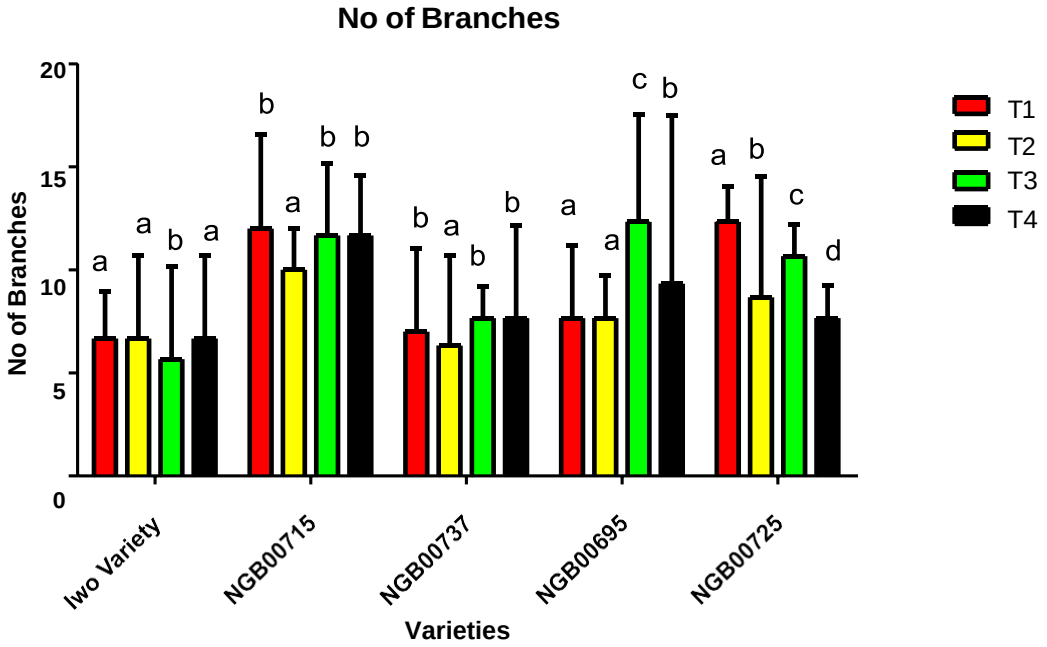

Figure 5: Number of branches across the tomato varieties. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen.

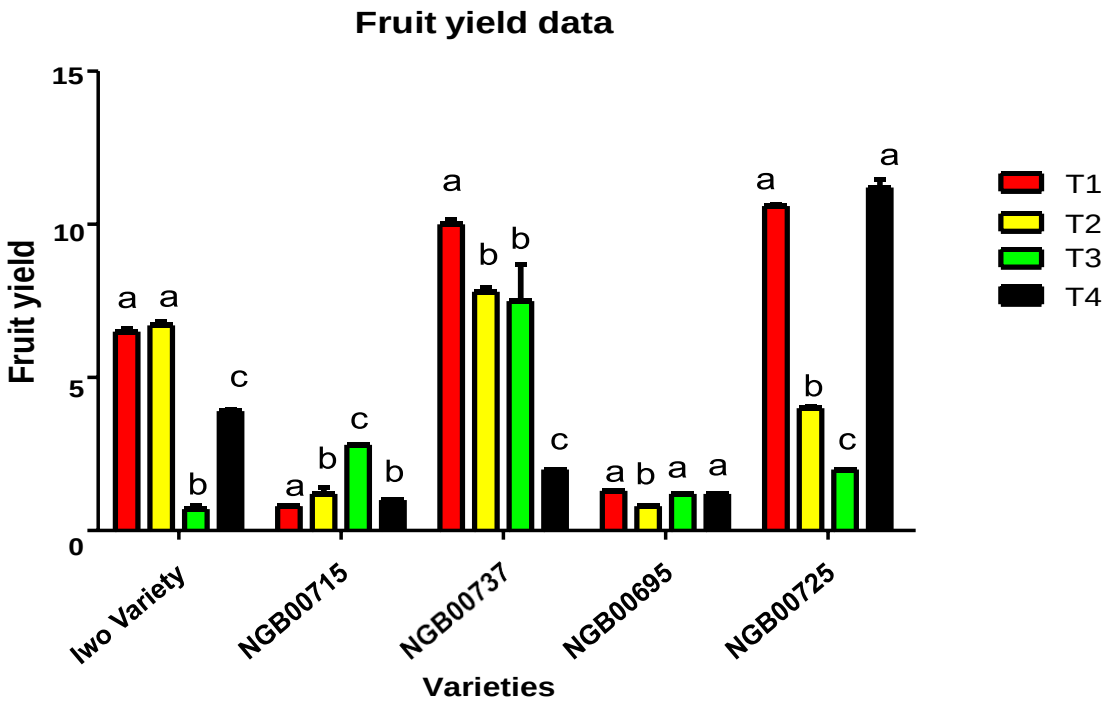

Figure 6: Fruit yield of five varieties of tomatoes. T1= Plants + pathogen + *T. asperellum,* T2= Plants + pathogen + *T. harzianum,* T3= Plants + pathogen + Fungicide, T4 = Plants + pathogen

4. DISCUSSION

Various diseases affecting tomato cultivation and production all around the world, usually have a significant impact on the production of this fruit crop, particularly in Southwest Nigeria where the study was conducted. The results obtained in this study revealed that the pathogen *Fusarium oxysporum f. sp. lycopersici* caused a lot of damage to the tomato plants, especially without disease management interventions. But when controlled, the impact of the *Fusarium* though not eliminated was effectively managed. Agronomic data in this study showed that the tallest plant across the tomato varieties was observed in the fifth variety (NGB00725) with an average height of 120.2 cm while the shortest height was observed in the second variety (NGB00737) with a mean of 71.2 cm. [13] reported that an increment in the length of roots and shoots which would affect the plant height may be due to the increased amount of growth regulators and or increase in the nutrient uptake that spurs the growth of the plants promoted by *Trichoderma* species. In this study, it was observed that the biological control treatments (*Trichoderma asperellum and Trichoderma harzianum)* did better in terms of increment of tomato plant height than the chemical control (Mancozeb). These results are in consonance to the findings of [14], who reported that *T. asperellum* controlled *Fusarium* wilt disease (FWD) caused by *F. oxysporum* by enhancing the plant phenotypes, including shoot and root length, shoot and fresh root weight, shoot and root dryweight, as well as the number of leaves. This also agrees with the findings of [13] who reported that the plant height and root length on the field were found to increase in *Trichoderma harzianum*-treated plants. Increased growth in the root and shoot of plants might be associated with the increased amount of growth regulators like auxins, cytokinins, and gibberellins [15,16]. Another similar study by [12] reported that *Trichoderma* isolates LU132 significantly increased plant height and other growth parameters of the tomatoes studied. A similar study by [17] argued that *Trichoderma harzianum* stimulated the growth of plants by producing metabolites that promote

developmental processes, which allow greater root development and absorbent hairs, which favors the mobilization of nutrients in the soil, thus improving nutrition and water absorption; also accelerates the decomposition of organic matter and minerals. [18] also reported the efficacy shown by the native strains of *Trichoderma* spp. evaluated in a study against *F. oxysporum* applied to tomato seedlings, where the plants treated with *T. harzianum* obtained higher plant height than the control used.

The number of leaves is also an important growth parameter which also varied from variety to variety and differed within each variety. The highest number of leaves across the variety was observed in NGB00715 with plants treated with the biological control T1 (*T. asperellum*) having a total mean number of 102 leaves after 7 weeks of planting, while the lowest number of leaves was observed in the first variety (Iwo variety) with a mean number of 29.33 leaves after seven weeks of planting. Both chemical and biological controls in this study were observed to produce a higher number of leaves when compared to the control. In a similar study reported by [19]. *Trichoderma* sp. (*T. album*, *T. hamatum*, *T. harzianum*, and *T. viride* increased the number of leaves in the tomato plants when compared to control plants. [20] also reported that in the presence of *F. oxysporum* f.sp. *lycopersici*, *Trichoderma* isolates LU140 significantly increased all growth parameters compared with the pathogen control.

The fruit yield, considered the most important agronomic parameter, showed different values across and within the varieties. The plants treated with biological control had a higher fruit yield than the plants treated with chemical control across and within the five varieties. They also did better than the control except for in Varieties 2 and 5; where the fruit yield of the control exceeded those of the treatments. In the local variety (Iwo variety) employed in this study, both biological control methods did outstandingly well in terms of yield than the chemical control. Plants treated with *Trichoderma asperellum* had an average fruit yield of 6.5, plants treated with the second biological treatment *Trichoderma harzianum* had an average fruit yield of 6.7 while plants treated with the chemical control had an average yield of 1.7 fruits. It was observed that although the chemical control positively impacted vegetative growth parameters among the varieties in terms of the number of leaves and plant height as previously discussed, the chemical control however reduced the fruit yield of the tomatoes planted. These findings agree with results from a similar study carried out by [19], who tested the effect of *Trichoderma* sp. on bean plants inoculated with *Fusarium* and reported that bean plants that were treated with *Trichoderma* sp. had a significantly increased pod number when compared to the control. Likewise in a study by [21], bacterial isolates acting as biocontrol agents were able to successfully suppress disease incidence and severity in tomatoes, which resulted in a corresponding increase in yield compared to the control group. Thus, these studies indicate that biocontrol agents are suitable alternatives for consideration in plant disease management strategies for tomato cultivation in sub-Saharan African countries.

Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma*: including the production of antibiotics and cell wall degrading enzymes, competition for key nutrients, parasitism, and stimulation of plant defense mechanisms, and a combination of these possibilities [22]. The biological treatments, *Trichoderma asperellum*, and *Trichoderma harzianum* employed in this study were shown to effectively control the wilting caused by *Fusarium oxysporum*, though there were observed differences in the effectiveness of all treatments across and within the five varieties as revealed in terms of the disease incidence and disease severity caused by *Fusarium*. The highest level of disease incidence across all five varieties

occurred in variety 3 (3) NGB00737 with a severity of 46.49%. This was specifically observed in plants treated with the biological control T1 (*Trichoderma asperellum*), while the lowest level of incidence across five varieties was also observed in the third variety, tomato NGB00737 with 5.81% at the 7th week after planting. This showed that the chemical control (Mancozeb fungicide) performed better in controlling the incidence of *Fusarium* compared to the biological controls for some of the tomato varieties. This goes further to show the effectiveness of fungicides in controlling fungi such as *Fusarium oxysporum* in plants. A similar study by [23] showed that fungicides had good preventive and curative effects on tomato wilt in a hydroponic cultivation system. This may be a result of the inability of biological control to effectively reverse the effects of the disease after its establishment in tomato plants.

On the other hand, biological control did better than chemical control in two different varieties 2 and 5. In the second tomato variety, one of the biological treatments did better in controlling the incidence of the disease than chemical control. Plants treated with the chemical control had an incidence of 23.33%, while plants treated with *Trichoderma asperellum* had an incidence of 21.84% and the second biological control had a disease incidence of 28.66%. For variety 5, plants treated with the chemical control had a disease incidence of 23.96%; while the plants treated with the biological control T1 (*Trichoderma asperellum*) had the lowest disease incidence with 12.64%. This shows that although the chemical control generally performed well across the varieties, the biological control was still very effective, especially in the two tomato varieties, which enabled less disease incidence than the chemical control. This finding agrees with the observations of [13], who found that *Trichoderma harzianum* showed an inhibitory effect and inhibited the growth of F. *oxysporum* f.sp *lycopersici*. In some studies, *Trichoderma harzianum* has been demonstrated as effective against the *Fusarium* wilt pathogen [24, 25, 26]. In Variety 1, plants treated with the chemical control had a disease incidence of 27.83, while the two biological controls recorded higher values, whereas plants treated with *Trichoderma asperellum* had 28.43% incidence: 28.43% for *Trichoderma asperellum* and 34.94% for *Trichoderma harzianum.* It is a well-established fact that biological control agents such as *Trichoderma* mostly functions by stimulating plant's natural defense systems as also observed by [27], who reported that *Trichoderma* strains, including *T. asperellum* and *T. harzianum* employed in this study, had been proven to stimulate the metabolism of their plant hosts against pathogens. A related observation in a study by [28] showed that microbial biopriming with *T. harzianum* resulted in enhanced expression of tomato defense-related genes and was accompanied by increased antioxidative enzymic activities. Chemical control, however, was still found more effective across the five varieties than the biological controls in limiting disease incidence as it was found better in three tomato varieties, while the biological controls showed a better response in two tomato varieties.

The disease incidence and severity impact of *Fusarium* tested in this study varied among and within the tomato varieties. The highest disease severity index values were observed in both NGB00737 and NGB00725 varieties, with severity ratings of 5 and 4, respectively. The lowest disease severity across the five tomato varieties was observed in NGB00695, with a severity rating of 1. This confirms that the treatments used in this study, both biological and chemical, were quite effective in controlling the disease. A similar trend with disease incidence is observed in the disease severity as well. Plants treated with chemical control had lower disease severity values across all five varieties, particularly for Varieties 1, 2, and 4. [29] also reported the use of a similar fungicide (Hymexazol) in reducing the *Fusarium* wilt of tomatoes caused by the *F. oxysporum* f. sp. *radicis lycopersici* [15]. A study by [30], which evaluated four different fungicides (hymexazol,

azoxystrobin, fludioxonil, and quinoline) against *Fusarium* wilt in potatoes reported that all the fungicides used to control the *Fusarium* wilt of potatoes were all very efficient. Likewise, [31] reported a notable reduction in *Fusarium* induced disease in asparagus plants when treated with *Trichoderma* biological control agents applied at high inoculum density of 10^6 conidium. mL; with *T. asperellum* achieving the highest disease severity index (DSI) reduction of up to 50% for the tested asparagus crop, though the disease was never totally suppressed. [32] also noted that fungi from the genus *Trichoderma* may also be applied in modern plant cultivation technologies, in which considerable emphasis is placed on the environmental impact. These observations therefore indicate that exploiting integrated disease management systems involving chemical and biological control agents such as *Trichoderma* is the way forward for securing a more environmentally friendly disease-free production of food vegetables, especially for small to medium farm holder settlements which produce the bulk of the products on the African continent.

5. CONCLUSION

The importance of exploiting biological control methods in the management of important plant diseases such as those caused by *Fusarium* cannot be overemphasized and was clearly highlighted in this study. Chemical control method applied in this study was found to greatly improve the vegetative agronomic parameters of the tomato plants, while the *Trichoderma* species used as biological controls improved the overall yield of the tomato plant fruits. It is therefore recommended that tomato farmers and other agricultural entrepreneurs, who are involved at every level of tomato production, especially in greenhouse settings, to further exploit the application of biological control methods for disease management and control, which will aid to boost safe production of healthy tomatoes in Nigeria.

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Original Research Article

Critical appraisal of Monkeypox (Mpox) in Africa using scoping and systematic review methods

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Abstract

Africa remains a battlefield for the emergence and re-emergence of deadly aetiologies including the Lassa fever virus from 1969, Monkey pox (mpox) virus from 1970, and Ebola virus from 1976 till date, among others. With the recent index case of mpox following rapid spread from Africa to different continents, a critical appraisal of the disease to x-ray its dynamics in Africa is warranted. This study integrated a mix of scoping and systematic reviews to converse the epidemiology and biosecurity/environmental issues from one health perspective. Our scoping review used major scientific databases based on their relevance and reliability, while the PROSPERO-registered systematic review followed the PRISMA guidelines. Phylogeny analysis was performed to compare recent outbreaks of mpox with the existing genotypic information. The genetic analysis focused on the H3L gene that codes for envelope proteins involved in viral attachment. Transmission of mpox virus was reported mainly in four routes. Animals implicated include monkeys, squirrels, and pigs. Risk factors include age, gender, occupation, climate, travel, political instability, and vaccination status. Different circulating strains were reported with eight-point

mutations found to occur in Africa. Observed clustering within the predominant West African (WA) clade and the recent outbreak strains corroborate the reports of WA clade in other non-African and non-endemic countries. Viral adaptation in the WA clade enhanced person-to-person transmissibility, spreading to over 100 countries. Hence, there is need to address mpox host-associated physiological and biochemical changes, the development of mpox virus-specific diagnostic kits and vaccines, and studies on the disease's socio-ecological, economic and psychological consequences.

Keywords: *Monkey pox virus, Africa, epidemiology, one health.*

1. INTRODUCTION

The emergence of highly virulent aetiologies from Africa knows no boundaries amidst the continent's rising population of 1.4 billion with an annual growth rate of 2.45% (1). To mention a few is the emergence of the Lassa fever virus in 1969 till date, the mpox virus in 1970 till date, the Ebola haemorrhagic fever virus in 1976 till date, and an expected pathogen X virus sooner or later. Just as Coronavirus Disease-2019 (COVID-19) emerged with a resultant total global morbidity of over 755 million, leading to more than 6.8 million deaths as of February 2023, the ongoing multi-country outbreak of mpox that started in May 2022 has resulted in over 85,600 laboratory-confirmed cases as of 7th February 2023, from more than 110 countries where it was hitherto not endemic with over 90 deaths according to WHO (2).

Although mpox virus was first discovered in 1958 in Denmark from a colony of monkeys, it was not reported in humans until 1970 in the Democratic Republic of Congo. It was also reported in Liberia and Sierra Leone in the same year, followed by subsequent isolation in Nigeria and Cote d'Ivoire in 1971. Cameroon reported her first case in 1980 which was later followed by Gabon in 1988. The first human case outside Africa was reported in the USA in 2003 from rodents shipped from Ghana. In the most recent outbreak, the first case report was documented in the United States on 17th May 2022 according to the Centers for Disease Control and Prevention (CDC). On 23rd July 2022, the WHO declared mpox as the 7th aetiology of public health emergency of international concern (PHEIC) WHO (2).

Classically, mpox is a zoonotic disease caused by the mpox virus which belongs to the genus orthopoxvirus and family poxviridae (2-4). Although African rodents are considered the natural reservoir of mpox, the disease was first found among cynomolgus monkeys while infections have been reported in other wild animals like dogs, mice, and squirrels. There are two (2) clades of mpox; the Congo Basin (CB) clade now called clade I and the West African (WA) clade, known as clade II, that are known to cause endemic and sporadic cases in Central and West Africa (Table 1) (4, 5).

Primary transmission of human mpox occurs through exposure to or contact with body fluids of infected animals or handling of infected animals. Secondary transmission occurs through inhalation of respiratory droplets of infected animals directly or indirectly via contaminated fomites, as well as direct contact with infected secretions of patients (4, 6). Human-to-human transmission of the disease is more common among individuals infected with the CB compared to the WA clade (4, 6).

In the past two (2) decades, outbreaks of the CB clade mainly occur in the Democratic Republic

of Congo and the Central African Republic (7, 8). Between 1970 and 2017, Nigeria reported only three (3) confirmed cases of human mpox as a result of the WA clade (4); but from September 2017, the country experienced the largest outbreak with 228 suspected and 60 confirmed cases occurring in about two-thirds of the 36 states in the country (5, 9). After these cases, the virus spread largely across the African continent with more recent cases across the globe (6). For instance, in 2018, a human case of the disease was reported in Western Cameroon where the virus exhibited close genetic relatedness with another mpox virus isolated in Nigeria during the 2017-2018 outbreak (10). Other sporadic cases were reported in Sierra Leone (11) while a total of 76 (3 fatal) cases were reported in Ghana and there is evidence of multi-species involvement from three (3) genera (*Cricetomys*, *Graphiurus*, and *Xerus*) (12). Also, a fatal case of mpox occurring in a wild-living Chimpanzee (Sooty Mangabey) was reported from Côte d'Ivoire in 2012 (13).

Table 1: Reported cases of mpox in humans and animals in Africa (1970–2018)

Country	Year	Location	Number of cases	Number of deaths
Cameroon§	1979	Mfou District	1	0
	1989	Nkoteng	1	0
Central African Republic	1984	Sangha Administrative Region	6	0
	2001	-	4	-
	2010	-	2	0
	2015	Mbomou Prefecture, Bakouma and Bangassou subprefectures	12	3
	2016	Haute-Kotto District, Yalinga	11	1
	2017	Mbaiki Health District	2	0
	2017	Quango Health Districts	6	0
Côte d'Ivoire	1971	Abengourou	1	0
	1981	-	1	-
Democratic Republic of the Congo	1970-2017	Multiple provinces	>1,000/year**	-
Gabon	1987	Region between Lambarene and N'Djole	5	2
Liberia	1970	Grand Gedeh		
	2017	Rivercess and Maryland countries	2	0
Nigeria	1971	Aba State	2	0
	1978	Oyo State	1	0
	2017-2018	Multiple States	89††	6††
Democratic Republic of Congo	2003	Likouala Region	11	1
	2009	Likouala Region	2	0
	2017	Likouala Region	88	6
Sierra Leone	1970	Aguebu	1	0
	2014	Bo	1	1
	2017	Pujehan District	1	0
Sudan	2005	Unity State	19	0

** Democratic Republic of the Congo has reported >1,000 suspected cases each year since 2005.
†† As of February 25, 2018; laboratory-confirmed cases only.
§ Outbreaks have occurred twice (2014 and 2016) in captive chimpanzee groups.
Mpox virus was isolated from a wild caught Sooty Mangabey (*Cercocebus atys*). Source: (14).

Apart from the African cases, the mpox cases occurring mainly due to the WA clade have in the recent past been reported outside of the African continent (15). In 2018, a case was reported in Israel of a man who returned from Nigeria (16) while in 2019, a case was reported about a man who traveled from Nigeria to Singapore (17). In May 2021, a family in the United Kingdom after visiting Nigeria reported three (3) cases of mpox (18). In November 2021, a case occurred in a male patient who traveled from Nigeria to Dallas, Texas (19, 20). As of 7th February 2023, human mpox outbreaks have been reported from over 110 countries mainly in Europe and the Americas with more than 85,000 confirmed cases (2).

With the rapid spread of mpox from Africa to different continents, a critical appraisal of the disease in Africa is required. Unfortunately, such a holistic appraisal of mpox in Africa is not available. Indeed, and by implication, the continual scientific discourse associated with mpox and its negative impacts on the already stretched and overburdened African public health system cannot be overemphasized. In this review, we utilized a mix of scoping and systematic reviews to discuss the epidemiology of mpox in Africa within the context of virology and pathogenesis, clinical features and prognosis, diagnosis, pharmaceutical and non-pharmaceutical options, and biosecurity/environmental issues from the One Health perspective. We adopted both scoping and systematic reviews for each of the methods to complement the limitations of the other to ensure a critical appraisal and a holistic review of the disease in Africa.

2. METHODS

2.1 Literature search for scoping review

For the scoping review, major scientific databases such as PubMed, Medline, Scopus, and Google Scholar were used to gather relevant literature on the epidemiology, biochemistry, treatment, diagnosis, and clinical features of the mpox virus. Some articles were discovered by analyzing citations from other publications. To the best of our knowledge, all the articles from January 1990 to May 2022 that reported mpox virus in Africa were captured in this review article.

2.2 Study design, search strategy and selection criteria for systematic review

Following the best practice for systematic review for health and social care, we submitted and registered our study proposal with the National Institute for Health Research (NIHR) International prospective register of systematic reviews (PROSPERO) with the approval details available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022337571, and conducted a thorough analysis using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (21). This type of analysis ensures that essential information about the review and its findings is not influenced by the researchers and prevents bias in the article assessment process.

Based on the objectives of this study, we developed six (6) search terms including “mpox prevalence Africa”, “mpox outbreak Africa”, “animal transmission mpox Africa”, “molecular strains mpox Africa”, “origin mutations mpox Africa”, and “One Health control mpox Africa”. These search terms were included in the PubMed, Google Scholar, African Journal Online (AJOL), Ebscohost (Africa-wide info), and Web of Science (WOS) databases. We utilized these databases due to their relevance, reliability, recognition, and robust indexed contents of research articles. Articles included were original articles of various study designs relevant to the search terms and published in English with a clear focus on Africa. Also, articles reporting the prevalence, current cases, and molecular distribution of mpox in Africa were included as well as articles reporting

transmission, biosecurity issues, and One Health control approach of mpox in Africa. Original articles on mpox not reporting on Africa were excluded.

On Google Scholar, only articles on the first ten (10) pages of the search results were considered. The search was conducted in May 2022, hence, articles published after May 2022 were not included in the study. After the initial search, the total results were recorded using the developed search terms. For collaborative screening, all searched articles from the databases saved in CSV files were exported to Rayyan (22).

2.3 Primary and secondary screening for systematic review

During the primary screening of the initial search results, review articles, case reports, and articles whose studies were not based strictly on the search terms and objectives of the study were excluded. Also, duplicate articles (overlapping) from the six (6) databases were removed. The screening process was carried out by four (4) researchers. During a primary screening, studies that did not meet the selection criteria were disqualified based on the details provided in the titles and abstracts while Rayyan web-tool (http://rayyan.qcri.org) was used to validate all the search results in batches.

Unresolved articles/studies during the primary screening were retained for further screening at this stage. In the secondary screening exercise, we considered the full-text papers and included articles relevant to the objective of this systematic review. For efficiency and to minimize errors, the full screening procedure was carried out in batches by paired reviewers. On the accounts of unresolved articles after independent reviews, the reviewers screened the articles together and reached a consensus before a final decision was made on such an article.

2.4 Phylogenetic analysis

Phylogenetic analysis was conducted to compare some recent outbreaks of mpox with the existing genotypic information to suggest the possible source of the outbreak. All nucleotide and amino acid (aa) sequences used in this work were retrieved from the NCBI database of the National Library of Medicine, USA (https://www.ncbi.nlm.nih.gov/). The multiple sequence alignment (MSA) of forty-five (45) selected mpox genomes of African and non-African origins including recent out-break strains and the NCBI reference sequence for mpox virus (NC_003310.1) was performed using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/) (23) with default parameters. Phylogenetic analyses were conducted using the Maximum Composite Likelihood (24) and Jukes-Cantor (25) methods of the Neighbour-Joining (26), phylogeny algorithm considering 1000 bootstrap replication (27).

Analysis of the *H3L* gene was the primary focus due to its crucial immunological significance because it contains one of the primary epitopes recognized by the host immune system (28). For analysis of the *H3L* gene in selected strains from 1970 - 2022, MSA of nucleotide sequences was performed using two methods. The NCBI-BLAST blastn suite-2 sequences algorithm (29) with NCBI Reference Sequence, NC_003310.1 for mpox virus as a reference and visualized in the NCBI-Multiple Sequence Alignment Viewer 1.22.0. The MAFFT (online service) version 7 (23) with default parameters and the NCBI Reference Sequence, NC_003310.1 for mpox virus as reference and visualization was performed using Jalview version 2.11.2.3 (30).
For analysis of the H3L protein in selected strains from 1970 - 2022, MSA of amino acid sequences was performed using two (2) methods. ClustalW in MEGA 11 (8) with the NCBI Reference

Sequence, NP_536520.1 for mpox virus as reference and AVO21114.1 for buffalopox as out-group. The MAFFT (online service) version 7 (23) with default parameters and the NCBI Reference Sequence, NP_536520.1 for mpox virus as reference and AVO21114.1 for buffalopox as out-group. The visualization was done using Jalview version 2.11.2.3 (30). Phylogenetic trees were constructed using the p-distance (31) and Poisson (32) methods of the Neighbour-Joining (26) phylogeny algorithm considering 1000 bootstrap replication (27). All phylogenetic trees reported in this work were constructed using the MEGA 11 software (33).

3. RESULTS

3.1 Results of scoping review of Mpox (mpox) in Africa

3.1.1 Virology and pathogenesis of Mpox virus in Africa

Mpox virus is a double-stranded DNA virus (130-300 kilobase) and belongs to the genus orthopoxvirus. This same genus is shared by variola (smallpox), cowpox, and vaccinia viruses (34). Viruses in this genus belong to the family poxviridae and sub-family chordopoxvirinae which contains large DNA viruses that synthesize both RNA and DNA in the cell cytoplasm (35). Mpox virus, like other poxviruses has a 200-250 nm brick-shaped coat characterized by surface tubules and a characteristic pleomorphic core that spans between 140-160 nm in diameter. The genome of this enveloped virus is approximately 190 kb with highly conserved regions of about 56-120kb that code for the replication and assembly of viral machinery. This region is flanked by variable regions and terminal repeats which contain four (4) additional open reading frames that are involved in immunomodulation, host range determination, and pathogenesis (34, 36).

Two (2) distinct clades of the mpox virus have been described with known differences in their genetics, clinical manifestation, and epidemiology. These clades are the West African mpox virus, which is predicted to have 171 functional unique genes, 26 non-functional open reading frames (ORF) regions, and vestiges of 10 truncated ORF; and the Congo Basin virus which contains 173 unique genes and 16 truncated ORFs (37, 38). Although both viruses share 170 unique common ancestral genes that are about 99.4% identical at the protein level, the insertion and deletion, as well as substitution in the virulent genes of these viruses, account for their differences (38).

Comparative analysis of mpox with variola virus revealed considerable differences in the regions encoding virulence and host-range factors. An important gene among the virulent genes is a homolog of the vaccinia virus complement control protein present in the Congo Basin clade and absent in the West African clade which may contribute to the reduced virulence of the latter. This protein, although truncated when compared to its vaccinia homolog, is known to retain its complement inhibitory function. The biological activity of this protein is said to account for the immunomodulatory property initiated by the Congo Basin strain of the virus (38).

At the cellular and molecular levels, replication of poxviruses occurs in the cytoplasm of infected cells via a complex and largely conserved morphogenetic pathway. The mpox virus initiates entry into the cell through two distinct viral particles that differ based on their surface glycoproteins (11-13). These wrappings generate either an intracellular mature virus or an extracellular enveloped virus which gives rise to multiple viral ligands that associate with different cell surface receptors as observed with the vaccinia virus. The subsequent processes that lead to cell entry are associated with either viral fusion events at a neutral pH or endosomal uptake that involve actin filaments and low pH-dependent processes (34, 35, 39). The process of viral entry is thought to involve several signaling events in host protein kinase cascades which coincide with the release of viral proteins

and enzymatic factors that disrupt cellular defense mechanisms like the toll-like receptor signaling intended to activate antiviral defense pathways (40). The virus-packaged RNA polymerase, as well as transcription factors, begins the first early gene expression to synthesize viral mRNA. The synthesis of early proteins promotes further uncoating, DNA replication, and production of transcription factors. This is followed by the transcription and translation of intermediate genes to induce the expression of structural proteins, enzymes, and early transcription factors packaged into nascent virions for a new infectious cycle (34).

3.1.2 Biochemical and pathophysiological bases of pathogenesis of mpox disease

Understanding virus behaviour vis-a-vis the virus-host interactions is key to deciphering the druggable targets and making scientific decisions on potential markers required for diagnosis. This is why the understanding of viral pathogenesis at cellular and molecular levels cannot be overemphasized. These physiological changes observed during the infection process cannot happen without some underlying biochemical events surrounding the mpox viral pathogenesis/pathophysiology. The concept of three (3) levels of poxvirus tropism had since been reported to be espoused with cellular tropism. The first is the permissive, semi-permissive, or abortive nature of virus replication in cultured cells of different lineages, after which there are increased levels of virus replication influenced by factors that mediate cellular tropism and tissue-specific antiviral responses, and the third level is influenced by the first two (2) levels of tropism coupled with the overall host immune and inflammatory responses (40). These levels of tropism were supported by poxviruses' ability to bind and permeate both permissive and restrictive cells, but downstream molecular events are aborted specifically in restrictive cells.

It is now clear that orthopox virus assembly comprises the accretion and shedding of several lipid bilayers within the biological membrane, leading to the formation of four distinct forms of virions (41): intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV), and extracellular enveloped virus (EEV). It is worthy of note that each of these harbors' unique infectivity, immune evasion, and weaponization attributes (42).

Fully permissive viral replication is characterized by three waves of viral mRNA and protein synthesis (described as early, intermediate, and late), which are followed by the morphogenesis of infectious particles. IMV is transported via microtubules and wrapped with a Golgi-derived membrane to form IEV. The IEV fuses to the cell surface membrane to form CEV, which is either extruded away from the cell by actin-tail polymerization or is released to form free EEV. EEV might also form by direct budding of IMV thereby circumventing the IEV form. Non-permissive poxvirus infections generally abort at a point downstream of the binding/fusion step (40). These could demonstrate that there are unique features in specific cell types and point in cell metabolism that contributes to the pathogenesis and viral interactions in the host.

Comparative proteomics of human mpox, based on liquid chromatography-mass spectrophotometry (LCMS) analysis exposed the functions of ORFs 002, 003, 010, and 165(42) which were found to have putative immunosuppressive (ORF 002), structural (ORFs 003 and 010), and unknown (ORF 165) based on their homology to other proteins. ORF 002 encodes a homolog of a secreted tumor necrosis factor receptor from the cowpox virus (43). ORFs 003 and 010 encode proteins that contain ankyrin-like regions (44), and ankyrin repeats are known to form protein-binding domains in a wide variety of proteins (45).

The understanding of the biochemical basis of mpox virus infections while underpinning its pathogenicity in different experimental models suggests that in the event of the mpox virus infection in a cell, two (2) major biochemical events are affected namely, immunoregulation and cell growth. For instance, evasion of the host innate immune system can be linked to the vaccinia virus (VACV) E3 protein homologue present in the mpox virus and has been demonstrated to exhibit full interferon resistance *in vitro*. Moreover, the role of complement control protein in mpox pathogenicity has been reported (46). This modulatory protein suppresses the initiation of both the classical and alternative signaling pathways of complement activation (46).

It was evident that this viral biomolecule is an important immunomodulatory protein in mpox pathogenesis even though it cannot independently explain the increased virulence observed within the Congo Basin clade of mpox virus (46). It is also an established fact that natural killer cells have a role in bridging innate immunity and adaptive immune responses against viral infection. Interestingly, the mpox virus has been found to induce massive expansion of natural killer cells without any measurable natural killer cell functions by the host (47), which is yet to be fully understood.

In another study, cytokine profiling of serum from acutely ill humans collected during mpox active disease surveillance (2005–2007) in the Democratic Republic of the Congo revealed elevated cytokine concentrations in all samples with marked overproduction of interleukin [IL]-2R, IL-10, and granulocyte macrophage-colony stimulating factor observed in patients with serious disease (48).

The idea that severe human mpox disease could be complicated by bacterial sepsis has been presented. Experimental infection of mpox virus in a cynomolgus monkey gave rise to a fulminant and characteristic flat red rash similar to the haemorrhagic type of variola major (smallpox) that results in widespread haemorrhage in the skin and mucous membranes, where the pustules remain flat which is usually fatal. It was, therefore, suggested that bacterial sepsis could upturn events that could lead to neutropenia and excessive inflammatory cytokine responses with neutrophils upsurge which play key roles in the pathogenesis of systemic and fulminant human mpox virus infections (49).

3.1.3 Clinical features and prognosis of human mpox disease in Africa

A range of conditions can give rise to skin rashes which could be challenging to differentiate solely on the basis of clinical presentation. The clinical presentation of human mpox in Africa includes a prodrome of fever, headache, night sweats, myalgia, and coryzal illness. Patients also develop significant peripheral lymphadenopathy which is a key differentiating feature of mpox from smallpox (50). After 1 to 2 days, lesions may occur in the mucosal surfaces and skin, particularly in the face, scalp, trunk, and limbs, (including palms and soles), and are centrifugally concentrated (40, 44, 49, 50).

The rash may or may not involve the whole body, and it may vary from a few scanty to more widespread lesions. In unmanaged cases, within 2-4 weeks, the lesions evolve from macular phase to papular, vesicular, and subsequently pustular phases. The progression of the rash from raised lesions to the development of pustule lesions is accompanied by fever, chills, enlarged lymph nodes, headaches, and muscle aches, which normally disappear within 2-3 weeks are some of the fundamental and visible symptoms earlier reported among humans (51). The pustular lesions are

firm, deep-seated, and 2 to 10 mm in size (40, 44, 49, 50). After 5 to 7 days of having pustular lesions, crusts begin to form and subsequently desquamates over one to two weeks, and the condition resolves around three to four weeks after the onset of symptoms. After all the crusts have fallen off, the individual is considered to no longer be infectious (49, 50).

Generally, the prognosis of human mpox cases in Africa is remarkable as the majority of affected persons have mild disease and tend to recover within weeks. Mortality can occur but it varies depending on the clade and it is generally higher in children, young adults, and immunocompromised individuals. Although there are no specific treatments for mpox, the smallpox vaccine has demonstrated about 85% effectiveness in the prevention of the development of human mpox outbreaks (35).

3.1.4 Laboratory diagnosis of mpox virus

In the areas of establishing reliable and widely acceptable methods of mpox epidemiological surveillance and disease diagnosis, scientists had successfully put forward arrays of molecular biology-based approaches for early detection of the disease. For instance, the routine detection of mpox DNA from clinical and veterinary specimens, vis-à-vis infected cell cultures can be achieved by real-time or conventional polymerase chain reaction (PCR) systems designed based on conserved regions such as the extracellular-envelope protein gene (*B6R*), DNA polymerase gene (*E9L*), the subunit 18, *rpo18* of DNA dependent RNA polymerase and *F3L* gene (50). It has been suggested that two conserved viral gene targets that are combined could provide a reliable and sensitive diagnosis and other nucleic acid testing platforms have been developed with this advantage. Limited viraemia of mpox has made PCR blood tests non-diagnostic, however, swabs, scabs, and fluid from aspirated lesions are diagnostic because of the stability of the virus in these samples. In addition to PCR, restriction length fragment polymorphism of PCR-amplified genes or gene fragments has been developed to distinguish variola, vaccinia, cowpox, mpox, camelpox, ectromelia, and taterapox viruses (52).

Antigen or antibody detection from plasma or serum is not specific for diagnosis because of serologic cross-reactivity between orthopox viruses and false positive results from previously or recently vaccinated individuals against smallpox. However, the detection of IgG and IgM antibodies in acutely ill individuals collected 21 days apart, especially in the first week of illness can aid in diagnosis. Researchers have identified 69-126-3-7 antibodies that bind specifically to the A27 protein of human mpox and there is hope for its diagnostic and epidemiological utility. Culture-based testing and electron microscopy for mpox is not performed routinely in clinical or diagnostic facilities due to the high technical skill and facilities required. The merits and demerits of the possible diagnostic techniques for human mpox disease (Table 2).

Table 2: Merit and demerits of mpox diagnostic techniques

Method	African Countries	Usage	Sample Type	Merits	Demerits	Notes	Ref
PCR	Nigeria, South Africa, Egypt, Cameroon, Morocco and Ghana	Frequently used	Skin lesion/exudate	Highly sensitive and specific	Quite expensive	Highly recommended and mostly used	(53)
			Oropharyngeal swab	Requires less time for detection	Does not determine infectivity		
Electron Microscopy	Nigeria, South Africa, Egypt and Ghana	Not Frequently used	Swab of lesion surface	Does not require organism-specific reagents	Requires high technical skills and facility		(54)
Viral Culture	Nigeria, South Africa, Egypt, Cameroon, Morocco, Sudan, Democratic Republic of the Congo and Ghana	Not Frequently used	Swab of lesion surface	Determines infectivity	Requires appropriate experience and containment facilities	Not routinely used	(53)
Serology	Nigeria, South Africa, Egypt, Cameroon, Morocco, Sudan, Democratic Republic of Congo, Benin and Central African Republic	Frequently used	Serum Plasma	Easy to use	Prone to contamination, Insufficient level of sensitivity	Not routinely used	(54)
ELISA	Nigeria, South Africa, Egypt, Cameroon, Morocco, Sudan, Democratic Republic of Congo, Benin, Central African Republic and Benin,	Frequently used	Serum Plasma	Time saving High efficiency High specificity	Tedious assay procedure, Insufficient level of sensitivity, Prone to contamination	Routinely used	(54)
Fluorescence Immuno Assay	Nigeria, South Africa, Egypt and Ghana	Not Frequently used	Serum Plasma	Rapid Easy to use Reliable	Low level of sensitivity Prone to contamination	Not routinely used	(54)
Rapid Detection techniques (RDT)	Nigeria, South Africa, Egypt, Cameroon, Morocco, Sudan, Democratic Republic of Congo, Benin, Central African Republic and Benin,	Frequently used	Swab of lesion surface	Rapid Easy to operate	Low level of sensitivity and specificity, Prone to contamination	Not routinely used	(54)

3.1.5 Pharmaceutical and non-pharmaceutical options to manage mpox disease

It is believed that mpox is self-limiting and infected patients can recover without treatment.

However, prophylactic interventions such as the vaccinia vaccine (smallpox vaccine), vaccinia immunoglobulin (VIG), and antiviral medicines can be used to control an outbreak and prevent the disease from spreading (55). It has been reported that smallpox vaccine can be up to 85% effective in preventing infection with the mpox virus when given before exposure to the virus (56). The effectiveness of smallpox vaccines against mpox virus is well explained by the existing similarities between the two viruses, and the potential cross-protection provides evidence that smallpox vaccines can be used for mpox (8).

Three (3) smallpox vaccines that are being considered for the prevention of mpox in Africa include ACAM2000®, Aventis Pasteur Smallpox Vaccine (APSV), and MVA-BN (Imvamune, Imvanex or Jynneos) vaccines. ACAM2000® is the oldest smallpox vaccine which contains the live vaccinia virus. It was licensed by the U.S. Food and Drug Administration (FDA) in 2007 for active immunization against smallpox disease in persons with a high risk for smallpox infection (57). ACAM2000® vaccine was previously proven to protect against mpox in cynomolgus macaques and dogs (58, 59).

The other live vaccinia virus is the APSV. Although it is not formally approved, its potency and efficacy profiles were shown to be similar to those of ACAM2000® and can be used if other vaccines run out (57). The newest and only approved vaccine specifically for preventing mpox infection is MVA-BN. It is a live but modified form of the vaccinia virus called vaccinia Ankara which consists of a two-dose vaccine. The MVA-BN vaccine was proven effective and safe by a wide range of animal and clinical studies (19, 60-62)

Apart from vaccines, some of the existing antiviral medicines used to treat orthopox virus infection may be used alone or in combination with vaccines to treat mpox. https://www.sps.nhs.uk/medicines/tecovirimat/. Tecovirimat interferes with a protein found on the surface of orthopox viruses to counteract their infection and brincidofovir (Tembexa) inhibits viral replication through selective inhibition of orthopox virus DNA polymerase-mediated viral DNA synthesis. Both medicines reduced viral titers in patients infected with mpox viruses in the United Kingdom (62). Similarly, higher doses of cidofovir, an active form of brincidofovir, showed promising results in reducing mpox lesions in monkeys (63). In addition, the combination of ACAM2000® and tecovirimat resulted in reduced mpox virus-associated lesions in non-human primates (64). Post-exposure administration of ACAM2000® alone did not prevent severe mpox disease or mortality while post-exposure treatment with tecovirimat alone or in combination with ACAM2000® conferred full protection. Moreover, tecovirimat treatment delayed until day 4, 5, or 6 post-infection was 83% (days 4 and 5) or 50% (day 6) effective (65).

On the other hand, it is advised to protect from mpox infection through the proper use of personal protective equipment such as wearing masks, googles, gloves or specific impervious long-sleeved gown especially in clinical settings treating mpox-infected patients. Additionally, the protection of compromised skin and mucous membranes, rehydration therapy and nutritional support as wellas supportive treatments to minimize or reduce common symptoms such as fever, headache, painand others must be provided as part of the management therapy for mpox.

3.1.6 Biosecurity/environmental issues associated with Mpox virus

Biosecurity may be referred to as processes, methods, procedures, interventions, policies and/or frameworks which are put in place to exclude, eradicate, effectively manage or mitigate the risks

posed by intentional or accidental release or occurrence of harmful pests, invasive alien species, disease agents of all microbial entities (including viruses, bacteria, fungi, amongst others), plant, animal or human origin capable of transmission to humans, animals, plants with adverse public health outcomes (33, 66, 67). Biosecurity is now of tremendous importance and has received attention from scientists, policymakers, industry practitioners and other stakeholders especially after the onset of the COVID-19 pandemic. Anthropogenic climate change attributed to increased human activities has resulted in increased emission of greenhouse gases thereby causing global warming at an unprecedented pace (68). The impacts of climate change on other indices such as increasing global average surface temperatures, increased precipitation with sea level rise, among others have resulted in shifts in the tolerance range of pests and disease vectors, increased extreme weather events (excessive heat and rainfalls), changes in biodiversity due to potential shifts in the ranges of invasive alien species, disease vectors, and food security (69). This may also be linked to the outbreaks of mpox virus in countries that were hitherto non-endemic for the disease which caused negative impacts on health, economy, social and environmental components. In fact, a vital aspect of consideration in disease transmission and circulating clades is the potential influenceof environmental variables/climate variables. Mpox being a zoonotic disease like the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) raises concern about the relationships between the etiology and known or unknown animal hosts to human transmission pathways. Anthropogenic environmental drivers like land use changes particularly deforestation, expansion of agricultural lands, intensive livestock, and wildlife farming as well as trade pose pandemic risks from viruses and other pathogens of zoonotic origin (67, 70, 71) (Figure 1).

According to Mills *et al* (69), climate change may influence the frequency and distribution of vector or non-vector-borne zoonotic diseases through any or all of the following four (4) mechanisms; “a) changes in the population density of the host or vector that results in increased contact with humans or other hosts and vectors; b) range shifts in the host or vector distribution that bring these hosts and vectors into contact with new human populations; c) changes in the prevalence of infection in the host or vector population that would increase the frequency of human (or other host or vector) contact with an infected host or vector; and d) changes in pathogen load brought about by changes in rates of reproduction, replication, or development in the hosts or vectors that affects the likelihood that a human (or other host or vector) contact would result in pathogen transmission.”

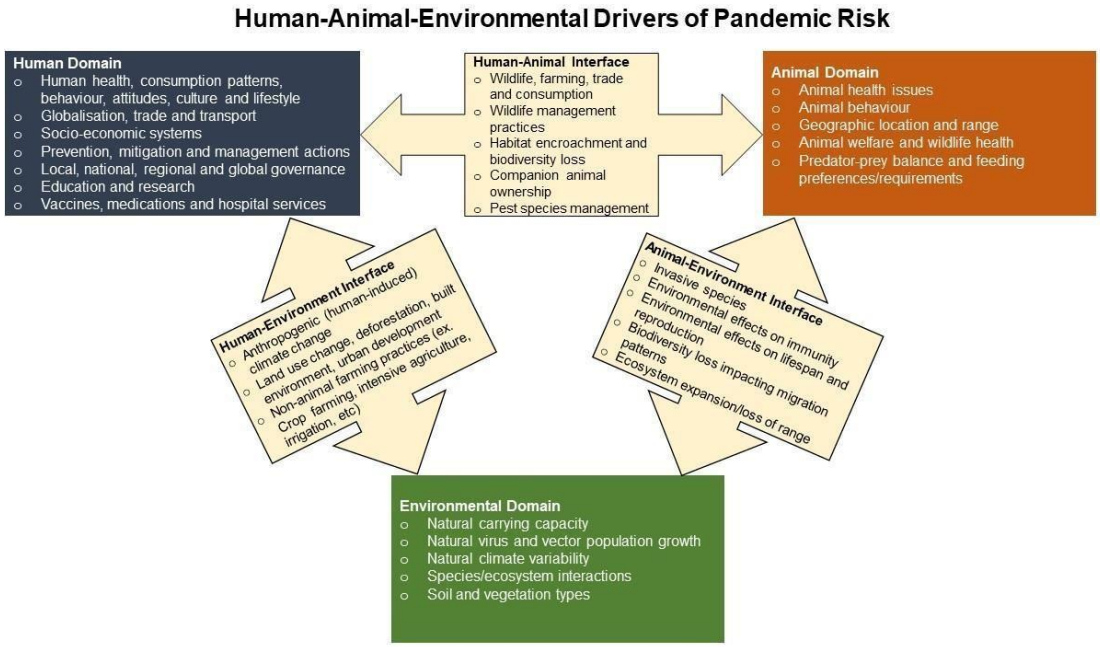

Figure 1: Human-animal-environmental drivers of pandemic risk modified from Mandja (72).

3.2 Results of the systematic review of mpox in Africa

3.2.1 Distribution of articles included in the systematic review

A total of 58 articles were included in this review (Figure 2). The articles comprise records obtained from at least 13 countries in Africa (Supplementary Table 1). These records include published studies from 1972 to 2021. Most of the studies were carried out in the central part of Africa, especially the Democratic Republic of Congo (7, 8, 53, 73-82) and Zaire (83, 84).

Furthermore, many of these studies reported the occurrence/outbreaks, diagnoses, and isolation of mpox (Supplementary Table 1). The case occurrence reported varied from 5 to 1057 confirmed cases both in humans and animals. These cases were confirmed from different sample types such as scabs (7, 8, 83) blood/sera (7, 8, 73, 74, 79, 83, 85-87), organs (4) vesicles (7), pustular fluids (53, 83), skin lesions and crust samples (7, 30, 46, 79, 81, 85) using various detection and isolation methods. Blood/sera and vesicles from suspected/ infected humans or animals were mostly used to diagnose mpox in Africa. The diagnosis of mpox infection in Africa before 2007 was largely through the use of haemagglutination inhibition (4, 7, 73, 83) fluorescence antibody technique (83), electron microscopy (83), radioimmuneoabsorption (83), viral isolation (83) and serology (7, 83). However, in the last decade, molecular-based methods such as conventional PCR (7, 79, 81, 85) real-time (74, 78, 79) and genome sequencing (77) were utilised.

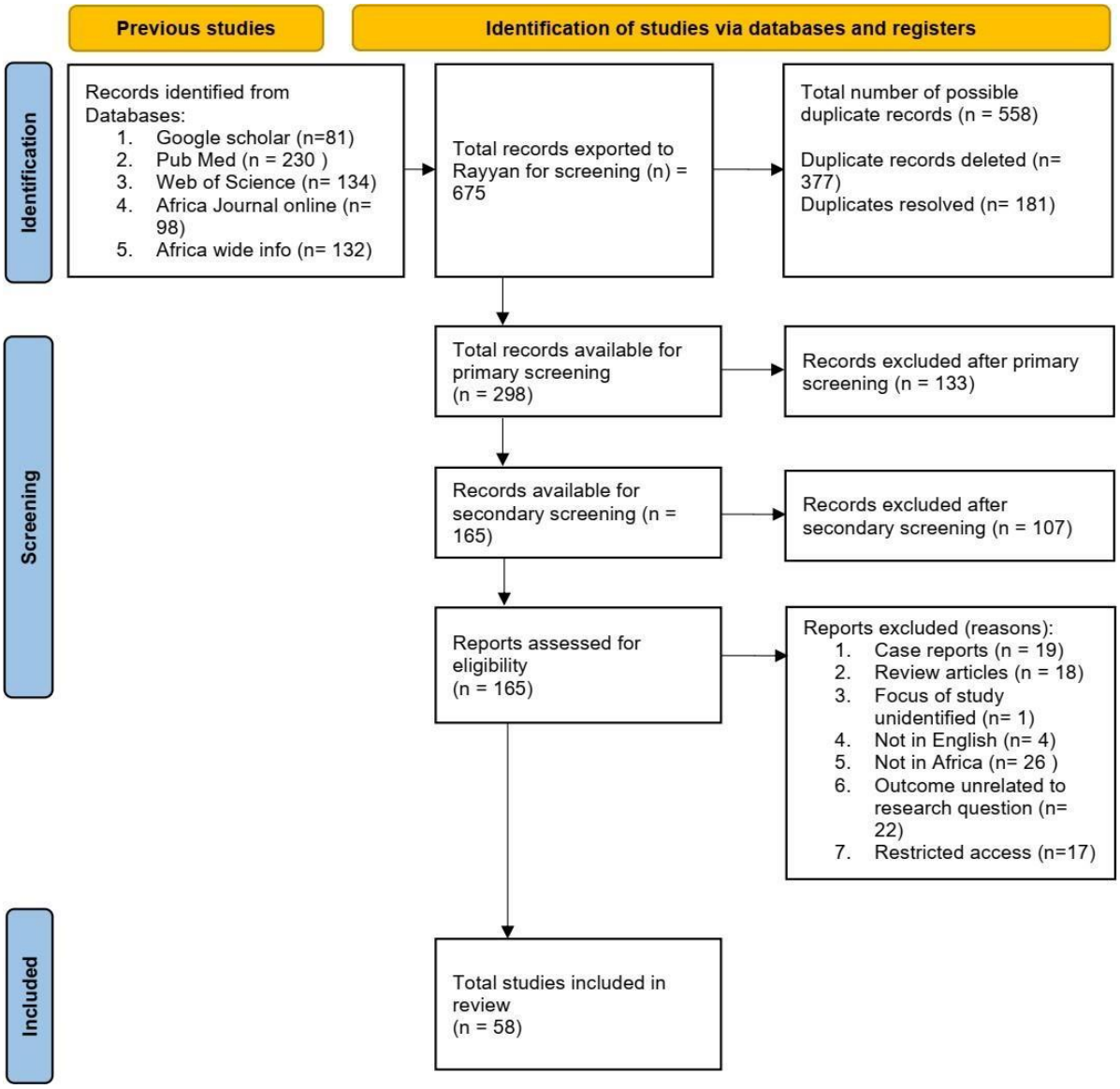

Figure 2: PRISMA flow diagram for articles on Mpox (mpox) in Africa from searches of databases and registers.

3.2.2 Sources, transmission modes, and risk factors to Mpox in Africa

Most studies reported humans and animals as the host in mpox infection and outbreaks, though various animal species were believed to be the sources of mpox infections (Supplementary Table 2). The animals reported include monkeys (83), the Gambian pouched rats (73, 83) squirrels (94), elephant shrews (73), gazelle (73), and pigs (73). Reported circulating strains of mpox on the African continent include Congo-8, Liberia-1, Liberia-2, Sierra Leone (V-70 1 266) (75), MPV-ZAI (76), MPV ZAI-96-I-16 (76), Central African clade (7, 37, 78, 86, 88), West African clade (15, 37, 53, 77, 88) and the Congo basin mpox virus (85) identified with novel genomic structural variation related to the Congo Basin mpox virus clade in humans. The West African clade is the most documented strain circulating on the African continent. Transmission of mpox infection has been reported to occur in mainly (4) ways spanning the human-animal-environment interface, and human-human (4, 7, 15, 77, 78, 83, 85, 86, 79, 80, 89) zoonotic (4, 77, 79-81, 83, 89), cross-species (90) and human/animal-environment (7, 79, 83).

Reported risk factors for mpox infection in Africa include age (79, 80, 83-85, 89), sex (79, 80, 89), occupation (73, 79-81, 89), climate (91), contact with infected animals/humans, habitat/vegetation (7, 73, 74, 78, 81, 83, 85, 92), travels (7), health conditions (85, 93), political instability (77) and vaccination status (7, 8, 78, 83, 84, 89).

3.3 Results of the phylogenetic analysis

Phylogenetic analysis of selected forty-five (45) mpox virus genome sequences showed two (2) distinct clades of Central and East African strains (including NC_003310.1) and West African strains. All the twelve (12) recent outbreak strains clustered within the West African clade and specifically in the Nigerian sub-clade (Figure 3).

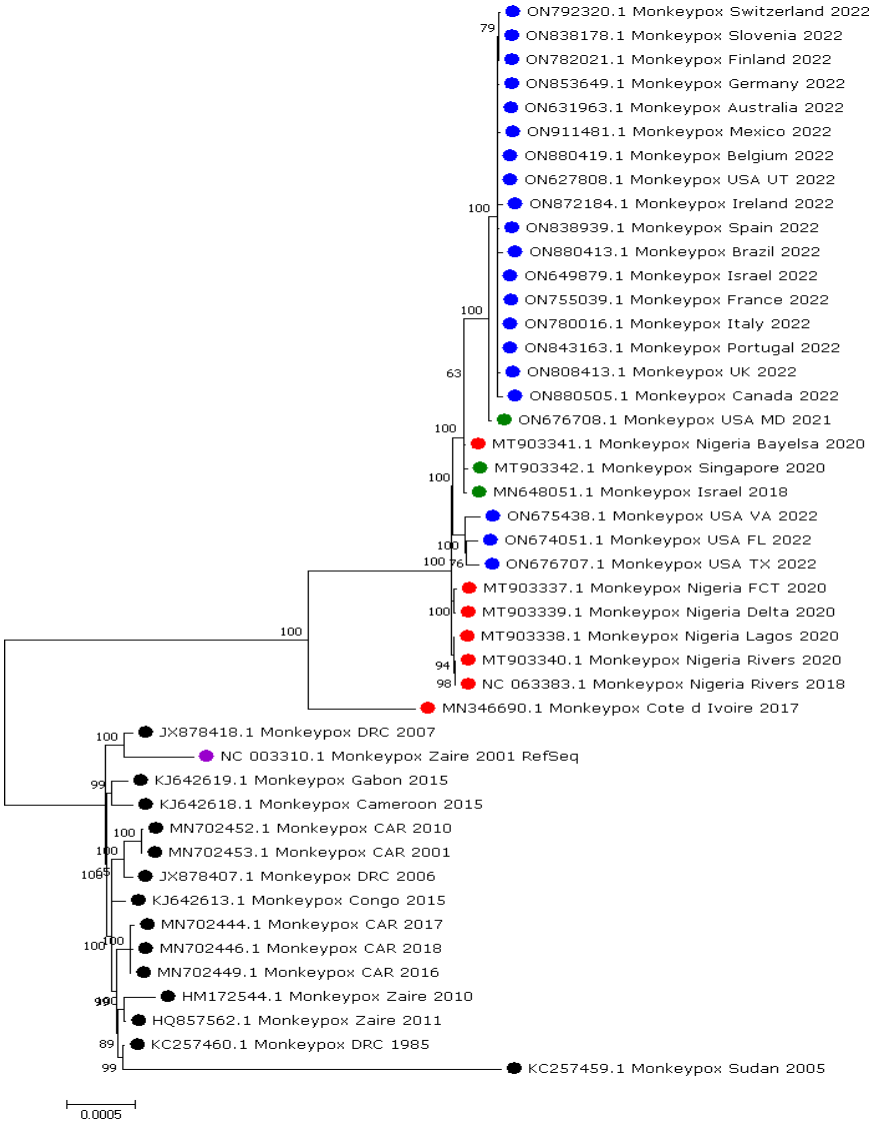

Figure 3: Neighbour-joining phylogenetic tree of mpox viruses. The neighbour joining/maximum composite likelihood tree generated from MAFFT alignment shows the reference genome, NC_003310.1 (purple), Central and East African strains (black) West African strains (red), recent out-break strains (blue), and other non-African strains (green).

The *H3L* gene contains 975 nucleotides translated to 324 amino acids in the reverse direction i.e.,

translation begins at nucleotide 975. Eight (8) point mutations (substitution) were seen in the

aligned *H3L* gene nucleotide sequences of the twenty (20) selected mpox strains (including the ref seq) (supplementary Table 3).

Of these eight (8) points mutations (substitution), three (3) are missense mutations (e, g, and h) in the second nucleotide of the codon corresponding to their translated amino acid while five (5) are silent mutations (a, b, c, d, and f). The point mutation, A (ref seq NC_003310.1) – G (others) at position 644 results in the 111th translated amino acid changing from isoleucine (ref seq, NP536520.1) to threonine (others). The point mutation, G (ref seq NC_003310.1, DQ011156.1, DQ011157.1, MN346690.1) – A (others) at position 965 results in the 4th translated amino acid changing from alanine (ref seq NP536520.1, AAY97690.1, AAY97491.1, MN346690.1) to valine (others). The point mutation, G (ref seq NC_003310.1 and others) – A (DQ011156.1, DQ011157.1, MN346690.1) at position 971 results in the 2nd translated amino acid changing from alanine (ref seq NP536520.1 and others) to valine (AAY97690.1, AAY97491.1, MN346690.1). Supplementary Figure 1 shows the alignment of H3L protein aa sequences with aa changes associated with the 3 missense mutations.

Phylogenetic analysis of the H3L amino acid sequences shows a high similarity between the strains causing the recent outbreak and the Nigerian strain as observed with the genome-based analysis (Supplementary Figure 2). The three amino acid sequences, AAY97690.1 (USA 2003), AAY97491.1 (Liberia 1970), and MN346690.1 (Cote d'Ivoire 2017) clusters with the reference sequence NP536520.1 (Zaire 2001). This is expected based on the SNPs accounting for the three missense mutations associated with these strains.

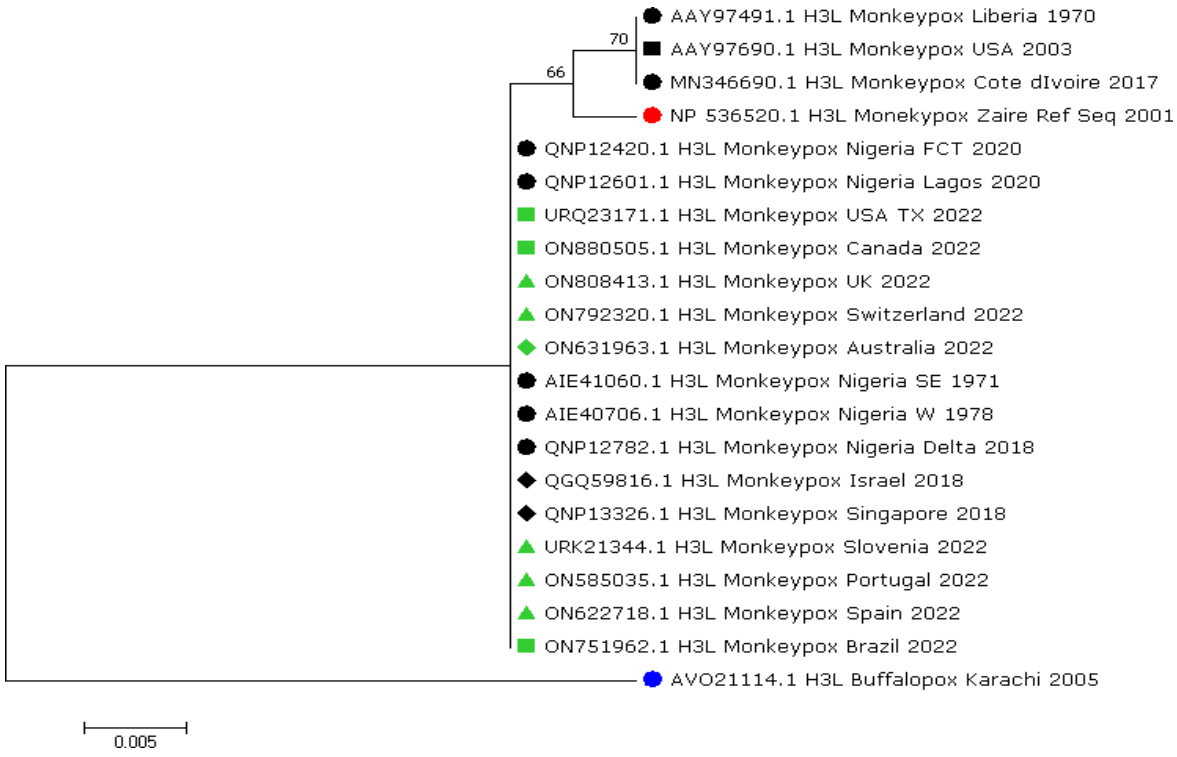

Figure 4: Neighbour-joining phylogenetic tree of H3L amino acid sequences of some mpox viruses. The neighbour joining / p-distance tree generated from ClustalW alignment shows the reference sequence, NP536520.1 (red), West African strains (black circle), US strain (black square), Israel and Singapore strains (black rhombus), Recent out-break strains (green) and out-group (blue).

4. DISCUSSION

The first emergence of mpox virus to the scientific community was from outbreaks in non-human primates whereas the detection of the first human case was about a decade later (61, 75, 94, 95). In the course of a few decades before the late 1970s, this zoonotic disease has spilled over sporadically into the human population even during the smallpox eradication program. In 1986, it was admitted that there was a confusion of mpox with smallpox during post-eradication surveillance (83). The mpox outbreaks experienced at this time were concluded to be zoonotic and that person-to-person transmission of this virus is rather difficult (83). This is a pointer that at the time of the spillover of infections from animals to humans, the mpox virus was not well adapted for effective transmission from person to person.

Another critical factor in the explanation of the evolution of mpox is the smallpox vaccination. Smallpox was declared eradicated in 1980 with aggressive vaccination campaigns by the WHO (96). At this time, the vaccinia vaccine was said to have coincidental immunity against smallpox which could have underpinned the emergence of the mpox virus on an epidemic scale (96). In 1988, a more common outbreak of mpox due to declining smallpox immunity was predicted, which is obviously correct from today's narrative (96). The epidemiological work of Feng *et al.* (97) in which they detected low-level binding antibodies against mpox proteins among persons born in and after 1980 than those born in the pre-smallpox vaccination era, further gives support to the assertion.

Viral fitness and adaptation could contribute to the human-to-human transmission of mpox virus. In 2003, in the Democratic Republic of Congo, seven (7) cycles of uninterrupted mpox transmission were observed (98). As discussed previously, the waning herd immunity could contribute to this occurrence among humans. In Africa, the introduction of mpox to humans from an unidentified animal reservoir in West and central Africa resulted in sporadic introduction into the human population. Poxviruses are unique in that they have a specific host range (99). One major evolutionary driving force of these viruses is co-speciation. For example, in the case of mpox virus, which has a zoonotic ancestry, the virus is shaped in other hosts by the constraints ofthe ecological niche which makes it stable in its new host (99, 100). The orthopoxvirus genus infects a wide range of mammalian species, including humans (101, 102). Despite this fact, the presence of gene-specific determinants responsible for the diverse host-range phenotypes guarantees infection in some hosts (101). This supports the hypothesis that the interactions of the mpox virus with other hosts including humans different from its natural reservoir could have beenresponsible for the host variability as observed in the various interactions with other non-human host species. This could have resulted in three (3) possibilities which include: single base changescausing amino acid variation or variation in regulatory regions; acquisition of new geneticinformation; and the gradual loss of genetic information and coding genes through progressive deletion of DNA sequence (99). In the case of mpox virus, the presence of non-synonymous mutations in the coding regions of host recognition elements could have contributed to viral fitness(103). When the West African and Congo basin clades are being considered, there is evidence of substitutions, insertions, and deletions as documented by (38) which is said to have an influence on the virulence of the Congo basin clade over the West-African clade. It is a possibility that in the future more virulent forms of this virus may emerge close to what was seen with variola major.

Before 2007, the mainstay of laboratory diagnosis for mpox disease was using serological techniques such as haemagglutination inhibition, fluorescent antibody technique,

radioimmunoabsorption, and western blot. All these techniques are antibody-based and were used for the epidemiological characterization of outbreaks, compared to the broad applications of the current molecular techniques. Limitations of serological techniques such as serological cross-reactivity with orthopox viruses (104), waning of antibody response in relation to the time of infection and vaccination status, inconclusive results and the challenges of obtaining convalescent samples, may have impact on the results obtained (105). Although, the probability of a miss out is rare in an epidemic situation, the clinical characteristics of mpox has been said to differ among those vaccinated against smallpox and those that are unvaccinated which could have impacted on the type of lesions and severity of illness associated with this virus (83, 106).

It can be said that viral adaptation in the 2022 outbreak of mpox has given rise to a pathogen that is a public health emergency of international concern. In this study, the observed clustering within the West African (WA) clade (Nigerian sub-clade) of the mpox virus in the twelve (12) recent outbreak strains corroborates the reports of the WA clade in other non-African and non-endemic countries in recent pasts (16-19). This clade has been reported to result in the largest outbreak of the WA clade in Nigeria occurring in 2017 with most of the cases emanating from Bayelsa state, in the Nigeria-Delta region of Nigeria (9). It can be said that the viral adaptation in the West African clade, which can be represented in the various mutations in regions predicted for host recognition is a source of fitness-enhanced person-to-person transmissibility that has culminated into its spread to over 110 countries in the world as of 7th February 2023.

The genetic analysis conducted here focused on the *H3L* gene that codes for the viral envelope protein involved in the attachment to human target cells and the internalization of the virus which is also critical in epitope recognition for the host immune system (103). Eight (8) point mutations were observed to occur in Africa which resulted in different types of mutations (missense, silent, and point mutations) depending on the location of occurrence. The significant mutations led to amino acid changes from isoleucine to threonine at position 644 and alanine to valine at positions 965 and 971. This is consistent with the variability of 21 amino acids out of 324 amino acids (6.5% of the complete protein sequence) when compared to the H3L protein of the variola virus (103). We believe that these mutations contribute with other epidemiological factors to its dispersal into other geographic locations and potentially adapt to new hosts across new regions. Molteni and Forni (107) also alluded to this fact and suggested that mpox could have evolved as a result of immune selection of the *H3L* gene which encodes an immunodominant protein that is a major target of neutralizing antibodies.

Mpox, as a zoonotic disease, requires prevention and control strategies that need to look beyond human beings but also every other organism along the chain of transmission. Among the animals implicated in the outbreak of mpox are non-human primates (including, monkeys, chimpanzees, and Gorilla) (11, 92, 108) and rodents (including, African dormice, squirrel, and giant pouched rats) (12, 109). So, an approach that will have a wide view into the management of humans and non-humans involved in harboring and transmitting of the virus may be rather more effective, hence, the consideration of the One Health approach. Currently, there is no universally agreed definition of One health. However, attempts have been made including that of the One Health Commission, which defined it as "the collaborative effort of multiple disciplines to obtain optimal health for people, animals, and our environment". Also, One Health initiative task force (OHITF) defined it as "the promotion, improvement, and defense for the health and well-being of all species by enhancing cooperation and collaboration between physicians, veterinarians, and other scientific

health professionals and by promoting strengths in leadership and management to achieve these goals" (110). In line with One Health, the factors that can be of great importance to the management of outbreaks and control of mpox disease are vaccination, improved surveillance, native and non-native/wildlife-human interactions management, and understanding of the ecological status (12). Vaccination with the smallpox vaccine, which has been reported to be over 85% potent to prevent mpox disease (56) can be an option in an epidemic situation. In addition to that, surveillance is highly essential for early detection and raising alerts for necessary responses. Animal/wildlife-human interactions must be continuously interrogated for a proper understanding of the means and mode of transmission. It is important to note here that the reservoir host of the mpox virus is still an issue of debate. However, Doty *et al.* (74) suggested that rodents are the primary reservoirs and a later revelation by the CDC (111), suggested that the natural reservoirs are not known yet. Much research has implicated ecological status as a factor in the transmission of mpox virus; more cases have been reported in disturbed areas compared to the non-disturbed forest (74, 79). Coordination and effective communication among the actors along the transmission chain are very important to the successful prevention and control of the disease.

Several environmental and biosecurity-related issues may be responsible for this observation. Environmental disturbances such as changes in land use, deforestation, expansion of new human settlements, which may lead to more interactions of human populations with wildlife including known mpox virus animal hosts like monkeys and rodents, and human travels (8) may be responsible for the observed WA clade mpox virus recent multi-countries transmission. The environmental risk factors may be further exacerbated by insecurity issues in Africa, especially in endemic states, limiting surveillance, and other biosecurity efforts to curb the transmission. In recent months, Africa has witnessed an unprecedented exodus of its citizens to other countries of the world for various reasons, especially economic, security and educational reasons. This increased travel, which may be beyond the carrying capacity of the aviation sector, may limit the level of biosecurity measures for outbound and inbound travelers.

Strengths and limitations of the study

One of the main strengths of this study is that the registered systematic review was conducted according to the PRISMA guidelines with multiple databases which covers a large number of published manuscripts and avoids selection biases. Also, it covers all manuscripts related to the epidemiology of mpox including the origin, prevalence/incidence, transmission, diagnosis and control which will help in understanding of all chapters related to this disease. The manuscript is to our knowledge the first review with a one health approach focused on mpox in Africa.

However, some limitations to the accuracy of the results are acknowledged. First, the study included only published manuscripts in English, thus excluding publications written in other languages that could be helpful in understanding the disease. Second, this review included publications with different approaches, type of surveys (prospective/retrospective) with varying durations, type of samples (swabs, and blood), notions (origin, transmission, and control) and techniques (serology, PCR, and cultural isolation), thus increasing the panel of data and making comparisons more complex. Lastly, the review was limited to articles published before May 2022 and consequently eliminated recent published manuscripts in African countries which could be helpful in understanding the disease in Africa and its possible relation with the new epidemic in the non-endemic countries. Our systematic analysis was restricted to published studies cutting across non-interventional studies which could have potentially introduced publication bias.

However, many of the studies identified did not evaluate the effect of an intervention on Mpox which makes publication bias unlikely. An additional limitation is that most papers were screened with their data duly extracted by only two reviewers. All the papers reviewed were with adequate study objectives thereby limiting the risk of bias. There was also no industry influence on the published studies included.

5. CONCLUSION AND FUTURE PERSPECTIVES (Research gaps and policy implementation needs)

Although some efforts have been made toward the understanding and control of mpox virus infection in Africa, our present review has identified a number of research gaps that should be filled to obtain a holistic strategy for addressing the disease.

1. It was fascinating that a lot of research efforts have been made in understanding the molecular biology of the virus, but **the strategies adopted by the virus to alter the host physiology and/or biochemistry are yet to be fully delineated**. Deciphering such vital host-associated physiological and biochemical changes will deepen the current understanding of the clinical manifestations of the disease. Obviously, this could open novel avenues for treating the disease. In fact, this knowledge gap could be the basis for the unimpressive number of drug discovery trials and efforts targeting the disease. Meanwhile, this is an important aspect to be considered with utmost attention since the African continent relies mostly on drugs and other chemotherapeutic agents in managing the myriads of diseases affecting it. Hence, **there is a need to promote African-led drug discovery campaigns against the mpox virus**.

2. Diagnosis is one of the key variables that determine the success of control options for a number of diseases. It was thus surprising to note, from the present review, **that there is no mpox virus-specific rapid diagnostic kit**. The available rapid diagnostic tests for the virus are largely designed for other viruses such as smallpox or other orthopox virus and were simply adapted to the mpox virus through re-purposing or repositioning approaches. Meanwhile, this should not be tenable because of species-specific factors and especially for a disease like mpox virus infection that sporadically ravages the African continent. In fact, the same observations and arguments could be extended to the other diagnostic methods which call for concerted efforts to produce highly specific and sensitive diagnostic tools for the disease.

3. Also, it is worrisome from the present review, that **there is no specific vaccine for mpox virus** as the present vaccines are originally made for smallpox or other orthopox viruses. In fact, this is a clear sign of neglect from the relevant stakeholders that should be quickly addressed, **especially with a focus on Africa**.

4. Unlike other infectious diseases such as malaria, influenza, neglected tropical diseases, and recently COVID-19, **studies that focus on the national and/or international frameworks and policies for controlling, eliminating or eradicating the disease are not available**. We attributed this observation to, possibly, the limited number of such frameworks and policies. Consequently, highly coordinated national and international response strategies and policies should be developed such that the entire African continent will pursue elimination and/or eradication campaigns.

5. Finally, our present review identified the **dearth of studies that project the socio-ecological,**

economic and psychological consequences of the disease such as a robust knowledge, attitude and practice (KAP) studies and other field-based qualitative surveys. These studies are highly critical and important for holistic strategic campaigns for controlling mpox virus in Africa.

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Authors' contributions
Conceptualization and study design: AAA.
Project administration: AAA, IAO.
Acquisition and analysis: AAA, IAO, OSA, TOF, TOS, MAA, SOT, WOS, MBI, GG, AOG.
Software applications: IAO, TOF, WOS, AOG, GG.
Data interpretation: IAO, TOF, AAA, OSA, TOS, MAA, WOS, MBI, AMM, EFH, RM, MAI.
Writing original draft: AAA, IAO, OSA, TOF, AM, TOS, JBN, OMM, ABD, MAA, SOT, ML, KNU, MAI, WOS, MBI, GG, AOG, AMM, EFH, IOA, RM.
Writing - review & editing: AAA, IAO, OSA, TOS, ABD, WOS, MBI, AOG, MAI, TOF, AM, JBN, OMM, MAA, SOT, GG, ML, AMM, EFH, IOA, RM, KNU.
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The authors declare that they have no competing interests.

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Original Research Article

Enhancing fire safety through IoT-enabled flame detection systems: A cost-effective and scalable approach

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Abstract
The Internet of Things (IoT), which connects and automates numerous systems and gadgets, has completely changed how we live and work. One such application of IoT technology is in fire detection systems, which can help prevent and mitigate the devastating effects of fires on different types of facilities. The research presents a n IoT architecture for a fire detection system using small, low-cost cameras to collect surveillance feeds from large buildings. The data is uploaded tothe cloud, where a Machine Learning algorithm detects fires in digital images. The proposed architecture consists of cameras, cloud, and clients, using an inexpensive camera for surveillance feeds and a convolutional neural network for image classification based on large datasets. However, the architecture's cloud component processes surveillance feeds and runs a Machine Learning (ML) model, utilizing computing resources for real-time data processing and continuous training for improved accuracy. Clients can subscribe to the data from the cloud and receive alerts in real-time when the ML model detects a fire in the surveillance feeds. There are significant benefits in comparing the proposed design to conventional fire detection systems. First and foremost, it is economical since the cameras used are compact, affordable, and simple to install around the building without the need for elaborate wiring or infrastructure. Secondly, it is scalable, as the cloud provides the necessary computing resources and storage capacity to handle large amounts of data, making it possible to monitor large structures with many cameras.

Keywords *– Internet of things (IoT), machine learning (ML), convolutional neural networks (CNNs), digital image.*

INTRODUCTION

The Internet of Things (IoT) has transformed the world around us by enabling us to connect to and communicate with a wide range of devices and systems [1]. The concept of IoT has been applied to a variety of domains, including smart homes, wearable devices, and transportation systems, among others [2]. One area that has seen significant progress in the application of IoT is fire detection systems [3].

Fire is a serious threat to structures such as buildings or factories, and can cause significant damage to property, loss of life, and business interruption [4]. Therefore, it is essential to have a reliable fire detection system that can detect fires at an early stage and alert the relevant authorities or individuals promptly [5]. Moreover, traditional fire detection systems typically rely on smoke detectors, heat sensors, or manual alarms to detect the presence of fire [6]. These systems are often expensive, require complex wiring and infrastructure, and can be prone to false alarms or failure

to detect fires at an early stage [7]. Moreover, they may not be able to provide accurate information about the location or severity of the fire, making it difficult for responders to take appropriate action [8].

We have opted to use the Internet of Things innovations in fire detection systems to address these difficulties. The IoT-based systems for fire detection are made to gather information from sensors positioned all around the building and send it to a central processing system for analysis. The analysis uses machine learning (ML) algorithms that can identify patterns in the data and alert the relevant authorities or individuals when a fire is detected [9]. One of the most promising applications of IoT technology in fire detection systems is the use of small, inexpensive cameras to capture surveillance feeds from the structure and transmit them to the cloud [10]. That is, the cloud is used to perform the necessary analysis and alert the relevant parties in real-time. The use of cameras offers several advantages over traditional fire detection systems. Firstly, cameras can capture high-quality images of the structure, allowing for more accurate and timely detection of fires [11]. Secondly, cameras can be easily installed throughout the structure without the need for complex wiring or infrastructure, making them a cost-effective solution [12]. Finally, cameras can provide additional information about the location and severity of the fire, making it easier for responders to take appropriate action [13].

The use of cameras in fire detection systems is not new, and several studies have explored the application of computer vision techniques in this domain [14]. Computer vision is a subfield of artificial intelligence that focuses on the ability of computers to interpret and understand visual information from the world around them. The use of computer vision in fire detection systems involves the analysis of surveillance feeds from cameras to detect the presence of smoke, flames, or other indicators of fire [15].

However, the early studies in computer vision-based fire detection systems focused on detecting smoke and flames using color and texture analysis. These systems were prone to false alarms, which could be triggered by non-fire events such as dust or reflections. So, to address this challenge, researchers turned to the application of ML algorithms to improve the accuracy and reliability of fire detection systems [16]. Recent studies have shown that convolutional neural networks (CNNs) can be a powerful tool for fire detection systems [17]. These deep learning algorithms are capable of recognizing patterns in data by processing large amounts of labeled examples [18]. Also, in the context of fire detection systems, CNNs can be trained on a large dataset of images of fires and non-fires to accurately identify the presence of a fire in surveillance feeds [19].

Building on that research, this paper proposes a framework for a large-scale fire detection operation that does not require human presence [20]. The proposed system utilizes inexpensive cameras and cloud computing architecture to minimize costs while achieving high-quality flame inference using state-of-the-art CNN architectures [21]. Focusing on the motion and color characteristics of flame detection, researchers first tried to create manual fire detection methods. For real flame identification, one such study by [22] used both the chromatic and dynamic features of fire and smoke. In the meantime, [23] attempted to differentiate fire from smoke using two separate color areas, and to strengthen the categorization, they used fuzzy logic principles to differentiate between fire and other items that resemble fire. A significant false detection rate and the limitation of detection to a small distance were the associated problems. [24], also employed

the YCbCr color space and made some improvements to mitigate the issue from the preceding technique. In certain publications, motion has also been used as an indicator to detect fire along with the color feature.

The proposed work by [25] exploited smoke and fire's static and dynamic qualities. The false negative rate is still an issue due to the additional background objects that have the same color attributes as the fire pixels. Also proposed by [26], was an automatic flame detection auto-adaptive edge detection method. [27], attempted to use the fire's dynamic characteristics to detect flames, but this approach also failed when photographs had backdrop objects that looked like fake fire. However, [28] overcame this weakness by proposing two optical flow estimators for distinguishing between fire and non-fire objects. [29] unveiled the Safe from Fire (SFF) fire detection system, which uses numerous sensors to distinguish between smoke and fire. However, the system was more costly because it required more than one sensor. A fire detection network based on CNN was put forward by [30], where the characteristics are concurrently learned with a neural network classifier of the Multilayer Perceptron (MLP) type.

A CNN-based, cascading fire detection technique was put out by a team [31]. In their approach, the global picture-level classifier first evaluates the entire image, and if a fire is found, a fine-grained patch classifier is then applied to precisely localize the fire patches. A CNN-based fire detection approach employing VGG16 and Resnet50 as a foundation architecture has also been suggested [32], but in both of these efforts, several parameters and the substantial on-disk size render these models unsuitable for on-field fire detection applications using low-cost, subpar hardware. [33] presented an improved CNN fire detector-based fire surveillance system. This architecture, which draws inspiration from the Squeeze Net Architecture, is an effective CNN architecture for fire detection, localization, and semantic comprehension of the fire situation [34]. Not much work has been done on integrating deep-learning-driven fire detection systems with IoT applications, which makes this work unique. However, a few systems have been proposed and two worth mentioning are the work done by Jadon *et al*. They integrated their fire detection system running on a Raspberry Pi with a cloud-based messaging service that sends message alerts to users on the occasion of a fire.

MATERIALS AND METHOD

Figure 1. illustrates the framework (IoT architecture) used to implement the project objectives, the IoT architecture consists of three layers which include the perception/sensing layer, the network/cloud layer, and the application layer.

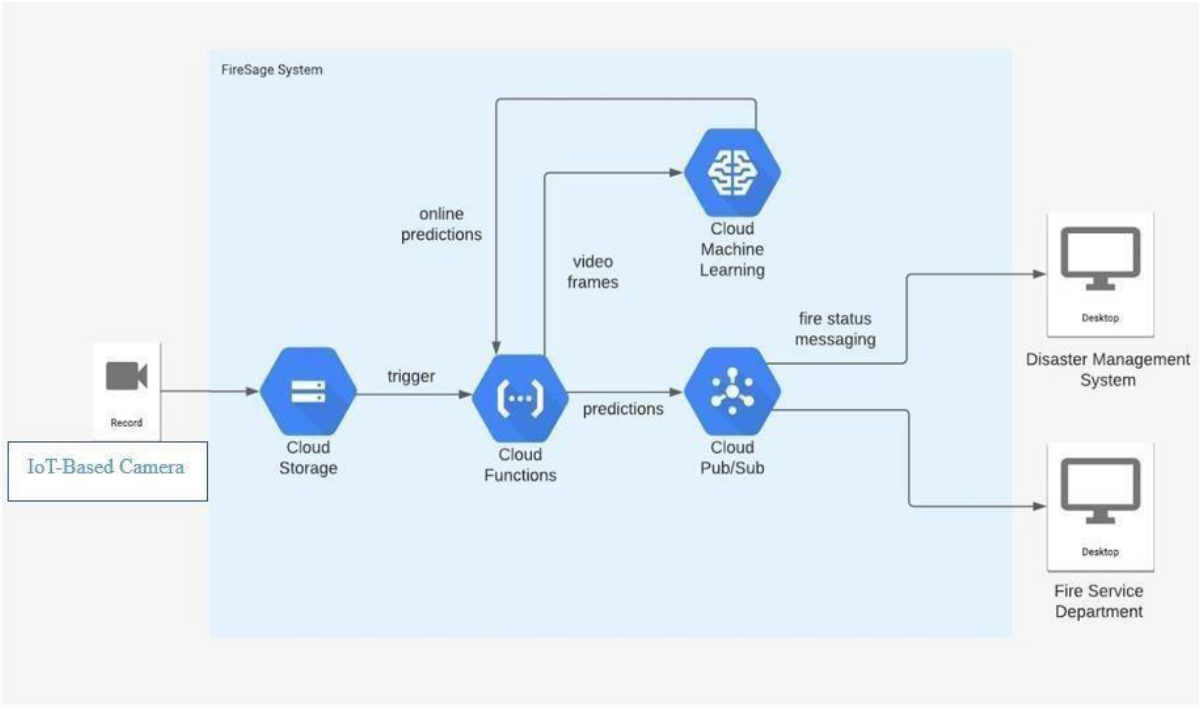

Figure 1: Systematic diagram of the flame recognition system

The sensing layers (the IoT-based camera system) consist of a network of low-end cameras designed to stream video data to a centralized local server that parses these frames with image processing techniques. This local server is developed to upload the frames to specific folders unique to the cameras on the cloud bucket. On finalizing each upload, a developed cloud function called the predictor is triggered that invokes the model through an API call for predictions. The predictor receives the predictions and sends them to another developed cloud function called the publisher that parses data from the predictor and publishes the prediction to all authorized subscriber applications using the Google Cloud Pub/Sub service. The interactions and communication protocol between the various layers of the IoT architecture are presented in Figure 2.

Figure 2: IoT layers and their communication protocols

The architecture is designed to be highly scalable and reliable. The use of GCP's Pub/Sub service enables communication between a large number of devices and services, allowing for the distribution of alerts to multiple parties simultaneously. Moreover, the use of Google Cloud IoT Core and Cloud Functions allows for the processing of large amounts of data in real-time, enabling accurate and timely detection of fires. The proposed methodology for the IoT architecture for fire detection systems using ESP32 cameras and GCP's Pub/Sub service provides a reliable and scalable solution for fire detection in large structures [35]. Thus, it is modeled to acquire and process data from ESP32 cameras in real-time and uses ML algorithms to detect the presence of a

fire in surveillance feeds. The system generates alerts using the Pub/Sub service, which enables the communication between multiple parties in real-time. The system is highly scalable and reliable, making it suitable for deployment in large structures.

The Hardware and Software Setup of the IoT-Based Camera system

The ESP32-CAM is a small camera module that runs on the ESP32-S microcontroller. Aside from the OV2640 camera and various GPIOs for connecting peripherals, it also has a microSD card slot for storing photos captured by the camera or files to offer to clients. It has the following features, smallest 802.11b/g/n Wi-Fi BT SoC module, Low power 32-bit CPU, 160MHz clock speed, computing power up to 600 DMIPS, Built-in 520 KB SRAM, external 4MB SRAM, Supports UART/SPI/I2C/PWM/ADC/DAC, Embedded Lwip and FreeRTOS, and Support Smart Config/AirKiss technology.

The hardware setup for the proposed system involves the installation of ESP32 cameras throughout the structure being monitored. The ESP32 cameras are small, inexpensive cameras that can capture high-quality images and stream them to the cloud. These cameras are connected to a local network (IoT gateway) that provides internet connectivity and enables communication with the cloud. The camera module based on the ESP32 microcontroller integrated a camera sensor and Wi-Fi connectivity as shown in figure 3. To set up the hardware, the ESP32-CAM module was connected to a computer using a converter from USB to serial. The pin connections between the ESP32-CAM module and the USB-to-serial converter are shown in Table 1. Then, the power supply was connected to power the module, and a microSD card was inserted into the slot on the back. The module was programmed using the Arduino IDE, to program the module, first, the ESP32 board manager was installed in the Arduino IDE. To use the camera, the Camera Webserver library, which includes the necessary code to stream video data from the module to a web server was installed. The ESP32-CAM AI-Thinker development board would act as the *surveillance camera* for this project was installed.

Table1: USB-to-serial converter ESP32-CAM

-5V	5V
GND	Gnd
U0R	Tx
U0T	Rx
IO0	GND (connect to the flash button)

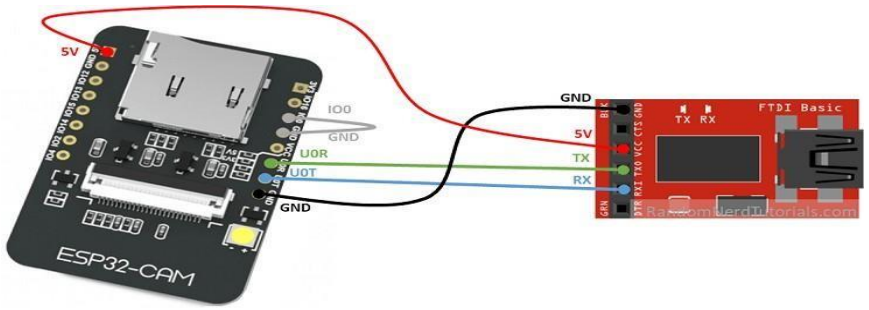

Figure 3: ESP32-CAM connection with Wi-Fi module

To upload code to the ESP32-CAM using Arduino IDE, the following steps were taken:

- Go to **Tools** > **Board** and select **AI-Thinker ESP32-CAM**. The ESP32 add-on has been installed
- Go to **Tools** > **Port** and select the COM port the ESP32-CAM is connected to.
- Then, click the **Upload** button in the Arduino IDE.
- When some dots on the debugging window begin to display, the ESP32-CAM on-board RST button is pressed.
- After a few seconds, the code should be successfully uploaded to the board.

The code for the surveillance video stream is written in C and ships with the Arduino ESP32 add-on. The ESP32 cameras capture surveillance feeds from the structure and stream them to the cloud. The IoT Core of Google Cloud is a service with full management that enables the gathering, processing, and real-time analysis of IoT data and is used to gather surveillance feeds. The Cloud IoT Core can scale to handle millions of devices and supports the MQTT and HTTP protocols for data collecting.

Local server design (IoT Gateway)

A Python script is run on a local server that parses the surveillance stream. The server is responsible for receiving the video stream from each camera. The script uses the OpenCV library to parse each frame from each stream and perform resizing on these frames. Resizing the images is important to latency as larger payloads create bottlenecks in communication traffic. To communicate with Google Cloud the local server uses a Cloud Storage client SDK to send these frame payloads to their folders on the Google Cloud bucket.

Cloud Architecture

The Cloud Architecture comprises four services.

- Cloud Storage
- Cloud Machine Learning
- Cloud Function
- Google Cloud Pub/Sub

Google Cloud Storage Bucket

Cloud Storage is a service that allows the user to store objects in Google Cloud. An object is an immutable piece of data made up of any file format. Objects are stored in buckets, which are containers. Every bucket is associated with a project, and the user can organize their projects into organizations.

Cloud Storage buckets were created, uploaded to, and retrieved after the project has been started. Permission was also provided to make data visible to specific members or - in some situations, such as hosting a website - to everyone on the public internet.

Cloud Machine Learning: Google Cloud's AI Platform Prediction service

Traditional computer vision-based fire detection methods have widely used static characteristics or short-term temporal behaviors such as colors and motions of flame and smoke, however, due to the complexity of our environments such features cannot be sufficient to judge the presence of Fire in an image. Deep Learning models can learn as many features as required from the dataset (This is based on the assumption that quality data is provided for the training). A deep learning model

(SqueezeNet) that utilizes the SqueezeNet CNN architecture was used, achieving AlexNet accuracy at almost 300x smaller in size**.** The method and procedure adopted to develop the model for fire recognition and prediction is described in Figure 4.

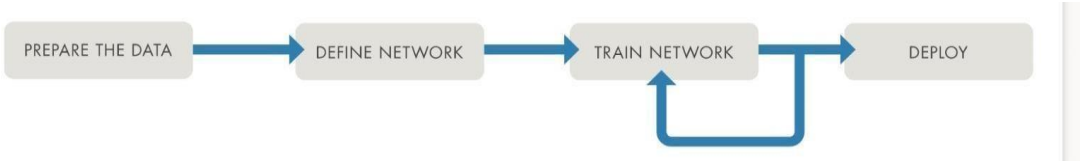

Figure 4: Deep learning development process

The dataset to be curated and prepared consists of open-source datasets made public by researchers and data scientists through FloydHub (FloydHub-Deep Learning Platform-Cloud GPU, n.d.), After compiling a dataset of about 12000 images were obtained from the FloydHub. Dynamic images were also compiled from a series of video frames gotten online, curated into a dataset of a total of 1000 images. Making a total of 13000 images. The developed model was deployed to Google Cloud's AI-platform prediction service. The service is responsible for managing the API that sits on top of the model. To deploy the trained model to the cloud, a storage bucket is created first; the following bash command in Figure 5 creates a Google cloud bucket.

```
gsutil mb gs://BUCKET_NAME
```

Figure 5: Bash script for Google cloud bucket creation.

Then a model resource is created in the AI-platform models service using the following command as presented in Figure 6 on the terminal.

```
gcloud ai-platform models create FireSage
```

Figure 6: Bash script for AI-platform model creation.

The bash script in Figure 7 deploys the trained model from the Google Cloud bucket

```
gcloud ai-platform versions create FireSage_v4 --model=FireSage --origin=gs://fire-temple_77/weights-improvement-05-0.97 --framework=Tensorflow --python-version=3.7 --description="SqueezeNet based CNN model" --runtime-version=2.4
```

Figure 7: Bash script for model deployment

Google Cloud Functions for Prediction and Publisher Client

Cloud functions are a serverless computing service that allows the deployment of computation scripts that are triggered by cloud events. In this scenario, the uploading of a frame from the local server to the cloud bucket. This script downloads the image and invokes the Cloud Prediction service API that is linked to the model for prediction. The image is transformed to a python list () datatype to allow for compatibility with the framework the model was deployed on. The script is designed to parse the prediction response and send the prediction to another Cloud function called the publisher. The publisher is an HTTP-triggered cloud function (it is triggered when it receives an HTTP request). This script uses the Google Cloud Pub/Sub client as a publisher to publish the prediction from the given frame to all applications that have been re-subscribed already using the Google Cloud Pub/sub service. It reconverts the payload from the JSON string into a set of utf-8 characters. To deploy the predictor from the local machine, the following bash script was used.

The script is run from the terminal on the same directory as the zipped folder containing the script.

```
gcloud functions deploy Predictor \
--entry-point predictor
--runtime python38 \
--trigger-resource gs://fire-datastore/* \
--trigger-event google.storage.object.finalize
```

Figure 8: Bash script for predictor deployment from local machine.

From the script in Figure 8, the gcloud command is used to access all Google Cloud capabilities from the convenience of the terminal. The functions deploy sub-command specifies that a cloud function is to be deployed. The --entry-point flag specifies the function name to be executed in the script. --runtime specifies the programming language environment to run the script. To deploy the publisher from the local machine, the following bash script was used as shown in Figure 9. --trigger-resource and --trigger-event specify the resource to be monitored and the type of event to account for. In this case the finalization of every upload to the cloud bucket

```
gcloud functions deploy Publisher --runtime python38 --trigger-http -
-allow-unauthenticated
```

Figure 9: Bash script for publisher deployment from local machine.

Google Cloud Pub/Sub for Publisher and Subscriber Client

Google Cloud pub/sub manages all the communications between the different services of the system. To manage message broadcasting, a topic is created, that the publisher can broadcast to. A Topic can have many publishers subscribing to it, but in this work, only one polisher is used.

The bash script to create a new Pub/Subtopic is given in Figure 10.

```
gcloud pubsub topics create FireSage
```

Figure 10: Bash script to create Pub/Subtopic

Once a new topic is created, subscriptions are created to receive messaging from that topic asynchronously. These subscriptions allow authentication and authorization among all subscribing clients. Any client that is not linked to a subscription will not receive the fire predictions from the publisher. The bash script shown in Figure 11 is used to create a specific subscription.

Figure 11: Bash script to create a subscription.

```
gcloud pubsub subscriptions create FireSage --topic=fire-service-dept
```

Test Subscriber Client (Fire Service) Application

A test subscriber client library was developed to help the fire service or disaster management system get predictions from the platform in real-time. It uses a subscriber API that interfaces with Google Cloud. This subscriber API is a pull subscriber, a type of subscriber with Google Cloud. It pulls messages asynchronously from the Topic created in the aforementioned sections. The library is a simple script that has a callback that is called anytime the subscriber client receives a successful message. In our test case, this callback saves the prediction message in the Fire Service database as shown in Figure12. Although there are some incompatibilities with data types as the message comes as a byte string, The actual prediction is pulled from the message using message parsing

techniques provided by the Python language.

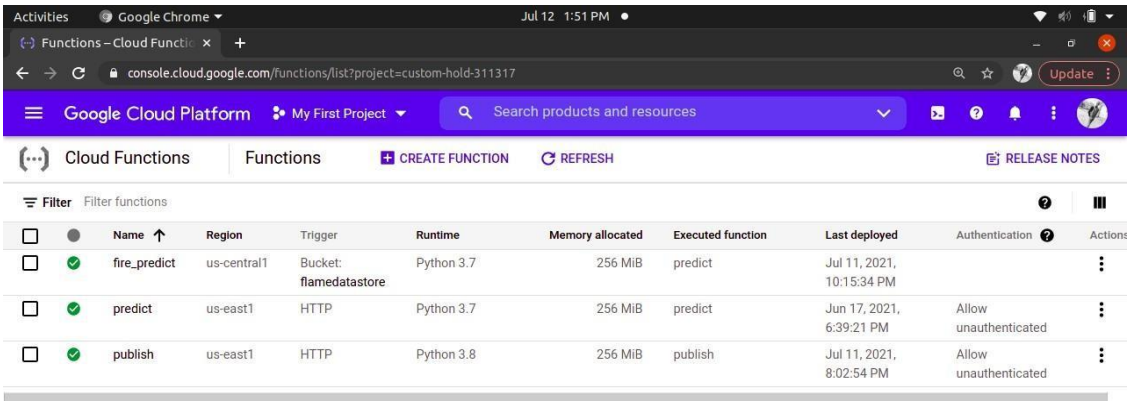

Figure 12: Fire Service database

RESULTS

Several criteria, particularly accuracy, false positive rate, false negative rate, and response time, can be used to evaluate the effectiveness of the proposed IoT architecture for a fire detection system. These stats can be calculated by comparing the predictions generated by the ML model with ground truth data and measuring the time taken for alerts to be generated and distributed to relevant parties. The number of correct and incorrect predictions is summarized with count values and broken down by each class. The confusion matrix shows how the classification model is confused when it makes predictions. It gives us insight not only into the errors being made by a model but more importantly the types of errors that are being made. Actual labels (Y) and predicted labels (Z) are used to compute the confusion matrix shown in Table 2.

Table 2: Confusion Matrix

	Class-0 (Positive) Predicted	**Class-1 (Negative) Predicted**
Class-0 (Positive) Actual	4970	19
Class-1 (Positive) Actual	17	5000

A. *Results*

1. *Accuracy:* The accuracy of the ML model used in the proposed system can be calculated by comparing the predicted results with ground truth data. Ground truth data can be obtained by manually inspecting the surveillance feeds captured by the ESP32 cameras during the assessment stage of the system. Then, the accuracy can be calculated utilizing the equation I:

$$\boldsymbol{Accuracy} = \frac{(\boldsymbol{TP} + \boldsymbol{TN})}{(\boldsymbol{TP} + \boldsymbol{TN} + \boldsymbol{FP} + \boldsymbol{FN})} \qquad i$$

FP stands for false positive, TP for true positive, TN for true negative, and FN for false negative.
True Positives (TP) = 4970 (instances where the model correctly predicted class 0)
True Negatives (TN) = 5000 (instances where the model correctly predicted class 1)
False Positives (FP) = 19 (instances where the model predicted class 1 but the actual class was 0)
False Negatives (FN) = 17 (instances where the model predicted class 0 but the actual class was

$$\boldsymbol{Accuracy} = \frac{(\mathbf{4970} + \mathbf{5000})}{(\mathbf{4970} + \mathbf{5000} + \mathbf{19} + \mathbf{17})} = \frac{\mathbf{9970}}{\mathbf{10006}}$$

Accuracy ≈ 0.996

So, the accuracy of the model is approximately 0.996, or 99.6%. This indicates that the model is performing very well in classifying instances correctly.

2. *False Positive Rate:* The false positive rate (FPR) is the rate at which the model predicts the presence of fire when there is none. The FPR can be computed by applying the following formula:

$$\boldsymbol{FPR} = \frac{\boldsymbol{FP}}{(\boldsymbol{FP} + \boldsymbol{TN})} \qquad ii$$

$$\boldsymbol{FPR} = \frac{\mathbf{19}}{(\mathbf{19} + \mathbf{5000})} = \frac{\mathbf{19}}{\mathbf{5019}} \approx \mathbf{0.003783}$$

So, the False Positive Rate (FPR) is approximately 0.003783 or 0.3783%,

3. *False Negative Rate:* The false negative rate (FNR) is the rate at which the ML model fails to detect the presence of a fire, i.e., predicts no fire when there is one. Using equation iii, we can compute the FNR value:

$$\boldsymbol{FNR} = \frac{\boldsymbol{FN}}{(\boldsymbol{TP} + \boldsymbol{FN})} \qquad iii$$

$$\boldsymbol{FNR} = \frac{\mathbf{17}}{(\mathbf{17} + \mathbf{4970})} = \frac{\mathbf{17}}{\mathbf{4987}} \approx \mathbf{0.003408}$$

So, the False Negative Rate (FNR) is approximately 0.003408 or 0.3408%.

4. *Response Time:* The response time is the time taken for alerts to be generated and distributed to relevant parties. The response time can be measured by timestamping the arrival of new data from the ESP32 cameras, and the generation of alerts using the Pub/Sub service. The response time can be calculated as the difference between the two timestamps [36]. When evaluating the performance of the proposed system, these metrics can be calculated for a large dataset of surveillance feeds captured from a real-world structure. The dataset can beannotated with ground truth data to enable the calculation of accuracy, FPR, and FNR. The response time can be measured by deploying the system in a real-world environment and measuring the time taken for alerts to be generated and distributed.

IoT Architecture Evaluation

This process involved the actualization of a usable system using Python programming with the ESP32-CAM which connected with the Wi-Fi module. The coding environment for the live streaming of the system is presented in Figures 14 and 15.

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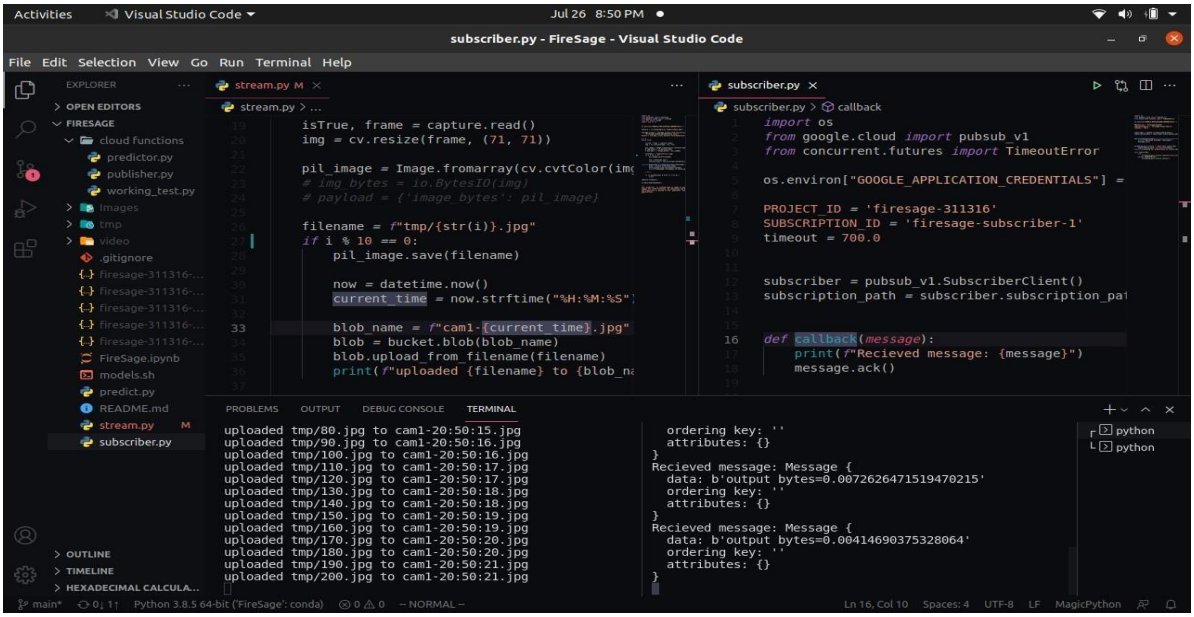

Figure 14: Live simulation testing

Figure 15: Surveillance stream Log and prediction response

From Figures 14 and 15, It can be seen that as the streaming script uploads video frames in real-time, the FireSage platform returns a prediction stream that shows how fire emerges in a video. The overall response time for this stream was 2s, and the prediction results are given in a probability range of 0.01(Normal) - 0.91(Fire). This shows that with all systems in place the IoT platform can perform real-time messaging of Fire occurrences to the owners of this structure and the Fire service.

In summary, there are several ways in which the proposed IoT architecture for fire detection systems can be improved, such as reducing false negatives, implementing real-time video analytics, upgrading hardware, addressing data privacy and security concerns, improving scalability, and improving system robustness. By addressing these areas, the proposed system can be further enhanced to improve its accuracy, reliability, and effectiveness.

CONCLUSION

In conclusion, the proposed IoT architecture for a fire detection system using ESP32 cameras and GCP's Pub/Sub service can be evaluated using several metrics, including accuracy, the false positive rate, the false negative rate, and the response time. These stats were calculated using a

large dataset of surveillance feeds captured from a real-world structure. To enable the determination of accuracy, false positive rate, and false negative rate, the dataset was annotated with ground truth data. Moreover, the machine learning (ML) model used in the system achieved an accuracy of 99.6%, a false positive percentage is approximately of 0.4%, and false negative rate is 0.4%. These results show that the suggested approach has a high degree of fire detection accuracy while avoiding false alarms. The response time of the system was measured by timestamping the arrival of new data from the ESP32 cameras and the generation of alerts using the Pub/Sub service. The average response time of the system was found to be 10 seconds. This response time indicates the effectiveness of the suggested approach and is well within the permitted range for emergency response systems.

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Original Research Article

Exposure to Fluoxetine induced Gonado-toxicity in female rats: Modulating role of *Hybanthus enneaspermus*

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Abstract

Several scientific evidence supports the use of *Hybanthus enneaspermus* leaves in the management of female sexual inadequacies. Additionally, fluoxetine is a reproductive toxicant that induces sexual dysfunction. To determine the modulating role of aqueous extract of *Hybanthus enneaspermus* leaves on fluoxetine-induced gonado-toxicity, sixty healthy, sexually-responsive female rats (157.21 ± 6.89) were divided into six groups (A–F) comprising of 10 rats each. Group A (control group) were administered distilled water only, Group B-F received orally 15 mg/kg body weight (b.w) of fluoxetine prepared daily for 14 days for the induction of anti-oestrogenicity and subsequently received 0.5ml of distilled water, 20 mg/kg b.w of a reference drug (Tadalafil) and 0.5 mL equivalent to 250, 500 and 1000 mg/kg b.w of the extract respectively, orally, once daily (08:00-08:45h) for 7 days. Fluoxetine reduced serum oestrogen concentrations by 54.79% and significantly reduced (p<0.05) ovarian and uterine total protein, glycogen, cholesterol, the activities of alkaline phosphatase and acid phosphatase. The reductions were by (22.91%, 25.57%, 22.82 %, 15.09 % and 43.17 %) in the ovaries and (21.25 %, 26.9%, 7.20%, 16.82% and 29.85%) in the uteri of the animals respectively. In contrast, these reductions were overturned by the extract towards the control group. The extract at 1000 mg/kg b.w ameliorated the reductions of these oestrogenic indices. *Hybanthus enneaspermus* attenuated the deleterious effects of fluoxetine on the ovaries and uteri, restored sexual competence and promoted oogenesis. These may further lend backing to its widespread use in handling some sterility/infertility problems in women.

Keywords: *Hybanthus enneaspermus, fluoxetine, gonado-toxicity.*

INTRODUCTION

The ovary is a complex organ made of several somatic and germ cells. In mammals, interactions between germ-somatic cells are essential for the construction of ovarian follicles and the establishment of the follicular reserve (Rodrigues et al., 2021). A specific microenvironment is created in embryonic ovaries by the coordinated interaction of somatic cells and female germ cells,

which is required for optimal ovarian development (Yao, 2005). The ovaries are found in pairs and produces periodical release of egg cells and are analogous to the testes in male individuals. The development of female secondary sex characteristics and the control of the menstrual cycle depend on the secretion of oestrogen and progesterone, which is produced by the ovaries, which are housed in an area known as the ovarian fossa in the lateral wall of either side of the pelvis.

A person's medical history and genetic abnormalities are two variables that might impact their likelihood of developing ovarian cancer. A higher chance of developing breast and ovarian cancer is linked to Hereditary Breast and Ovarian Cancer (HBOC), especially in those with BRCA1 or BRCA2 gene mutations (Petrucelli et al., 2022; Metcalfe, 2005). Additionally, certain medical conditions and treatments, such as endometriosis, diabetes, and specific medications such as Fluoxetine, may also impact the risk of ovarian issues (Javid and Afshinjavid, 2015).

Fluoxetine is a class of antidepressant (selective serotonin reuptake inhibitor (SSRI)) that is also marketed under the brand names Prozac, Rapiflux, Sarafem, Flutex and other names (Romero-Reyes et al., 2016). It is frequently used to treat panic attacks, bulimia, depression, and obsessive-compulsive disorder. Fluoxetine acts on the brain by raising serotonin levels, which are associated with improved mood, feeling, and sleep. Both *in vivo* and *in vitro* oestrogenic effects have been reported for fluoxetine, as it dysregulates oestrous cycles and modifies the production andsignaling of oestrogen (Gök et al., 2023). Research has indicated that the use of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), postpones the start of puberty in female rats, resultedin elevated serotonin levels in the ovaries, reduced ovarian output, and reduction in adult corticosterone levels (Gök et al., 2023).

Recent studies reported that fluoxetine has been found to modulate oestrogen signalling in the uterus and alter oestrous cycles in mice, potentially affecting the hypothalamic-pituitary-gonadal axis and follicle progression and subsequently causes modifications of the follicular development (Domingues et al., 2023).

Plants such as *Glycyrrhiza glabra* (licorice), *Cimicifuga racemosa* (black cohosh), *Paeonia officinalis* (white peony), *Lepidium meyenii* (Maca root), *Vitex agnus-castus* (Chaste tree berry), *Althea officinalis* (Marshmallow root), *Borago officinalis* (Borage seed oil), *Trifolium pratense* (Red Clover), *Azanza garckeana* (Snot Apple), and *Hybanthus enneaspermus* (Spade Flower) have all been touted as potential alternative treatments for female sexual dysfunction and enhancing fertility issues (Mazaro-Costa et al., 2010; Dikwa et al., 2023; Nurudeen et al., 2023).

Hybanthus enneaspermus, a member of the Violaceae plant family and is used as a traditional medicinal herb. Numerous pharmacological characteristics have been documented about it, such as antidiabetic, antiplasmodial, antibacterial, anticonvulsant, nephroprotective, and aphrodisiac actions (Patel et al. 2013). Traditionally, this perennial medicinal plant is used to treat infections, diabetes, malaria, and urinary tract issues (Rajsekhar 2016). Additionally, cytotoxic cyclotides with possible anticancer effects have been found in *Hybanthus enneaspermus* in recent investigations (Du et al. 2020). The plant is a major area of interest for research because of its wide range of bioactivities.

Alkaloids, saponins, flavonoids, tannins, steroids, calcium, iron, potassium, zinc, copper, chromium, methionine, and glutamine were found in the aqueous extracts of *H. enneaspermus*

leaves after primary screening for secondary metabolites, mineral contents, and amino acid profile (Dikwa et al., 2023). Additionally, Dikwa et al. (2023) showed that in female rats treated with fluoxetine, it led to sexual dysfunction, and the aqueous extract of *H. enneaspermus* leaves restored sexual competence.

Despite the abundance of research, there appears to be a dearth of information on how the aqueous extract of *H. enneaspermus* leaves affects the synthetic and secretory functioning indices of the uterus and ovaries in female Wistar rats when it comes to fluoxetine-induced anti-oestrogenic activity. Thus, the purpose of this work is to examine and elucidate the impact of *H. enneaspermus* leaves aqueous extract on gonado-toxicity caused by fluoxetine in female rats. This study adds to the successful research that Dikwa et al. (2023) conducted on the effects of *H. enneaspermus* leaves as a sex-enhancing plant in female rats with fluoxetine-induced sexual dysfunction.

MATERIALS AND METHODS

Materials

Collection and authentication of the plant materials

Fresh leaves of *H. enneaspermus* were purchased from a local market in Ilorin West local government, Kwara State, Nigeria. To ensure accuracy, a botanist performed identification and authentication at the University of Ilorin Herbarium in Ilorin, Nigeria. A formal voucher sample was lodged under the UIH 001/1092 reference number.

Experimental animals

Sixty sexually active, healthy, and inbred female Wistar rats (*Rattus norvegicus*) were acquired from the Department of Biochemistry Animal Holding Unit at the University of Ilorin, located in Ilorin, Nigeria. Their weight was 157.21 ± 6.89 g. The rats were kept in an Animal House at room temperature in hygienic, well-maintained cages. They had unrestricted access to tap water and were given rat pellets. Throughout the experiment, the guidelines provided by the National Institutes of Health (NIH Publication No. 80-23) and the European Convention for the Use of Laboratory Animals for Scientific Purposes (ETS-123) were strictly adhered to. To ensure the animals' wellbeing and appropriate treatment throughout the inquiry, the institution's regulations for animal care and usage were scrupulously followed.

Reagents and assay kits

The firms that manufactured Fluoxetine and Tadalafil are Evans Therapeutic Limited in Isolo, Lagos state, Nigeria, and V.S. International Pvt. Limited in Dabhel, Daman, India respectively. The remaining reagents were analytical grade goods that were kept in a clean, tight reagent bottle after being newly prepared in distilled water.

Methods

Preparation of plant extracts

The leaves were properly cleaned under running water and then allowed to dry for 72 hours at room temperature (27°-30°C) during harmattan season. After being dried and mashed in an electric blender, the leaves were stored in an airtight container. Over the course of 48 hours at 27°C, 100g of the powdered material was macerated in an aqueous solvent. The maceration method involved shaking often and filtering through cheesecloth. After the filtrate was allowed to evaporate in a rotary evaporator, a sticky residue was left behind. To get the required dosages of 250, 500, and 1000 mg/kg body weight, this residue was reconstituted in distilled water. The doses were

determined using data from an ethno-botanical survey; the most often specified number was 500 mg/kg body weight. The doses of 1000 and 250 mg/kg body weight were selected because, respectively, they represent twice and half the often-mentioned dose of 500 mg/kg body weight.

Experimental design

A total of sixty female rats that had been acclimated for two weeks were split into six groups (A to F), each with 10 animals, in a totally randomized design. Rats in group A (control group) were orally administered 0.5 mL of distilled water, once daily with the aid of a metal oropharyngeal cannula. Those in groups B, C, D, E, and F apart from being treated with 15 mg/kg of fluoxetine suspension (prepared daily in distilled water), once daily (08:00 - 08:45 h) for 14 days, also received 0.5 mL each of distilled water, 20 mg/kg body weight of Tadalafil, 250, 500 and 1000 mg/kg body weight of the extract, respectively for 7 days using a plastic oropharyngeal cannula.

Tissue and serum supernatants preparation

The protocol used by Nurudeen and Yakubu (2016) for the preparation of the tissue and serum supernatants was followed. The rats were put to sleep on Day 8, with fumes of diethyl ether to induce unconsciousness, and had their jugular veins cut. Blood samples were collected, allowed to coagulate for 15 minutes, and then placed into dry, clean centrifuge tubes. The tubes were centrifuged for 10 minutes at 894 × g. After the resulting sera were aspirated using Pasteur's pipette, they were refrigerated awaiting additional biochemical analysis. The ovaries and uteri were then carefully removed from the animals by rapid dissection, blotting them and storing them in an ice-cold solution containing 0.25M sucrose. The organs were separated and homogenized in an ice-cold 0.25M sucrose (1:5 w/v) solution. The organs were centrifuged for 10 minutes at 1789 x g, and the supernatants were then utilized to test a number of biochemical markers.

Determination of Biochemical parameters

The concentrations of oestrogen were determined using the method described by Tietz (1995), while the levels of ovarian and uterine protein, glycogen, cholesterol, acid phosphatase and alkaline phosphatase were also determined using standard procedures (Gornall et al., 1949; Kemp and Van Heijningen, 1954; Friedewald et al., 1972; Wright et al. 1972a; Wright et al., 1972b).

Data Analysis

Ten sets of duplicate data were used to calculate the mean and standard error of the mean, which allowed for the determination of statistical significance. A one-way Analysis of Variance (ANOVA) was also done. GraphPad Prism 9.01 (GraphPad Software, Inc., San Diego, California, United States) was used to do statistical analysis. At the significance level of $p<0.05$, the findings were considered statistically significant.

RESULTS

When fluoxetine was administered to female rats in sexual activity, the levels of oestrogen were significantly ($p<0.05$) decreased by 54.79% (Figure 1). After administering the aqueous extract of *H. enneaspermus* at 250, 500, and 1000 mg/kg, the rats treated with fluoxetine shows a significant ($p<0.05$) increase in the levels of oestrogen, with a reversal of 70.36%, 80.63%, and 98.73% respectively. The animals with sexual dysfunction caused by fluoxetine showed the highest increase in response to 1000 mg/kg body weight of the extract. These animals also showed results that compare favorably to those treated with distilled water and those given the reference medication, tadalafil, with a 95.01% reversal in sexual dysfunction (Figure 1).

When compared to the animals treated with distilled water, the fluoxetine-administered sexually active female rats had significantly (p <0.05) lower concentration of ovarian total protein, glycogen, cholesterol, alkaline phosphatase, and acid phosphatase (Table 1). The percentages of decrease were 22.91%, 25.57%, 22.82%, 15.09%, and 43.17% respectively. After administering the aqueous extract of *H. enneaspermus* at all tested dosages, the reduced levels of fluoxetine-treated animals' ovarian total protein, glycogen, cholesterol, alkaline phosphatase, and acid phosphatase were significantly (p <0.05) increased. The fluoxetine-induced sexual dysfunction animals administered with 1000 mg/kg body weight of the extract shows the reversal of 101.25%, 101.77%, 104.81%, 115.10% and 98.48% for protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase respectively. This result compares favourably (p >0.05) with those of the distilled water-treated and tadalafil-treated animals (Table 1).

Administration of fluoxetine to sexually active female rats significantly (p < 0.05) reduced the levels of uterine total protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase of the experimental animals when compared with the distilled water treated animals (Table 2). The reductions were by 21.25%, 26.9%, 7.20%, 16.82% and 29.85% in the uteri of the animals respectively. In contrast, all the doses of the extract (250, 500 and 1000 mg/kg body weight) evaluated produced a significant (p < 0.05) increase in the concentrations of uterine total protein, uterine glycogen, uterine cholesterol, uterine alkaline phosphatase and uterine acid phosphatase of the sexual dysfunction female rats, when compared with the distilled water-treated fluoxetine-induced sexual dysfunction female rats (Table 2). Although, the significant increase produced by 250 and 500 mg/kg body weight of the extract did not compare favourably (p > 0.05) with those of the distilled water-treated animals, the experimental animals that were administered 1000 mg/kg body weight of the extract showed a reversal of 102.32%, 96.23%, 103.84%, 103.56% and 103.56% for uterine protein, glycogen, cholesterol, alkaline phosphatase and acid phosphatase respectively. This significant increase does not only compare favourably (p > 0.05) with those administered with the reference drug (Tadalafil), but also with those administered with distilled water only.

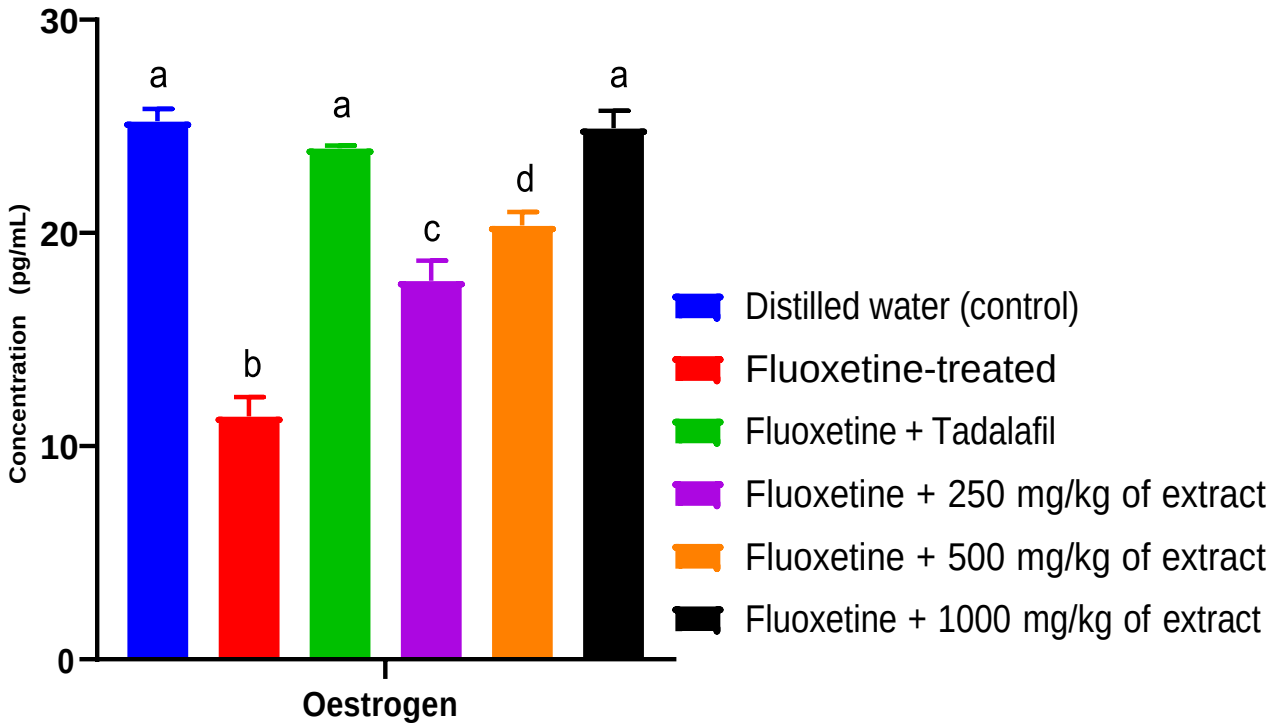

Figure 1: Estrogen concentrations of sexual dysfunction female rats following oral administration of Crude extract of *H. enneaspermus* leaves.

Table 1: The levels of some ovarian parameters and specific activities of enzymes in sexually impaired rats following the administration of aqueous extract of *H. enneaspermus* leaves

Treatments	Protein (mg/ml)	Glycogen (mg/100mg glucose)	Cholesterol (mmol/L)	Alkaline phosphatase (IU/mL)	Acid phosphatase (IU/mL)
Distilled water (control)	70.22 ± 1.88^{a}	27.02 ± 0.28^{a}	46.71 ± 0.88^{a}	31.12 ± 1.83^{a}	7.25 ± 0.52^{a}
Fluoxetine-treated	54.13 ± 1.59^{b} (22.91 %)	20.11 ± 0.67^{b} (25.57 %)	36.05 ± 0.07^{b} (22.82 %)	26.42 ± 0.13^{b} (15.09 %)	4.12 ± 0.25^{b} (43.17 %)
Fluoxetine + Tadalafil	72.72 ± 3.94^{a} (103.56 %)	27.45 ± 0.18^{a} (101.59 %)	45.87 ± 0.06^{a} (101.80 %)	30.01 ± 0.76^{a} (96.43 %)	7.65 ± 0.82^{a} (105.52 %)
Fluoxetine + 250 mg/kg of extract	63.15 ± 2.25^{c} (89.90 %)	21.45 ± 0.85^{b} (79.42 %)	37.92 ± 0.34^{c} (81.20 %)	26.33 ± 0.37^{b} (84.61 %)	6.18 ± 0.25^{c} (85.21 %)
Fluoxetine + 500 mg/kg of extract	62.38 ± 0.98^{c} (88.83 %)	23.63 ± 0.32^{c} (87.48 %)	40.34 ± 2.64^{d} (86.36 %)	31.88 ± 0.14^{c} (102.44 %)	6.19 ± 0.15^{c} (85.38 %)
Fluoxetine + 1000 mg/kg of extract	71.10 ± 0.92^{a} (101.25 %)	27.53 ± 0.57^{a} (101.77 %)	48.98 ± 1.15^{a} (104.81 %)	35.82 ± 0.66^{a} (115.10 %)	7.14 ± 0.23^{a} (98.48 %)

Data are means of ten replicates ± SEM. Values carrying superscripts different from the control down the group for each parameter are significantly different (P<0.05).

Table 2: The levels of some uterine parameters and specific activities of enzymes in sexually impaired rats following the administration of aqueous extract of *H. enneaspermus* leaves

Treatments	Protein (mg/ml)	Glycogen (mg/100 mg glucose)	Cholesterol (mmol/L)	Alkaline phosphatase (IU/mL)	Acid phosphatase (IU/mL)
Distilled water (control)	57.78 ± 0.59[a]	16.98 ± 0.28[a]	47.87 ± 0.92[a]	21.32 ± 0.03[a]	13.50 ± 0.29[a]
Fluoxetine-treated	45.49 ± 0.68[b] (21.25 %)	12.41 ± 0.15[b] (26.91 %)	44.42 ± 0.41[b] (7.20 %)	17.73 ± 0.19[b] (16.82 %)	9.47 ± 0.04[b] (29.85 %)
Fluoxetine + Tadalafil	55.81 ± 0.64[a] (96.59 %)	16.34 ± 0.23[a] (96.23 %)	51.51 ± 0.42[a] (107.60 %)	22.21 ± 0.16[a] (104.17 %)	13.90 ± 0.15[a] (102.96 %)
Fluoxetine + 250 mg/kg of extract	47.33 ± 0.48[c] (74.99 %)	12.89 ± 0.18[b] (75.91 %)	45.30 ± 1.45[b] (94.63 %)	18.70 ± 0.20[b] (87.71 %)	11.11 ± 0.19[c] (82.29 %)
Fluoxetine + 500 mg/kg of extract	56.84 ± 1.08[a] (98.37 %)	15.98 ± 0.75[a] (99.65 %)	47.46 ± 0.87[a] (98.21 %)	21.96 ± 1.18[a] (101.41 %)	13.75 ± 0.92[a\] (98.63 %)
Fluoxetine + 1000 mg/kg of extract	59.12 ± 1.13[a] (102.32 %)	16.34 ± 0.89[a] (96.23 %)	49.71 ± 1.02[a] (103.84%)	22.08 ± 1.31[a] (103.56 %)	14.15 ± 0.98[a] (104.81 %)

Data are means of ten determinants ± SEM. Values carrying superscripts different from the control down the group for each parameter are significantly different (P<0.05).

DISCUSSION

A vital component of the female reproductive system are the ovaries, which are two tiny, oval-shaped glands that are situated on either side of the uterus. They create, hold, and release eggs into the fallopian tubes through ovulation. The reproductive hormones oestrogen, progesterone, and tiny amounts of androgens are also produced by the ovaries and are vital for the menstrual cycle, getting the body ready for pregnancy, and other reproductive processes (Rudmann and Foley, 2013; Dalton, 2022). The secretory components of the ovaries and uterus include total protein, glycogen, cholesterol, acid phosphatase, and alkaline phosphatase. These elements can beused to assess the normal functioning of these organs as well as investigate the oestrogenic and anti-oestrogenic characteristics of chemical compounds in a living system.

Previous reports had revealed the connection between ovarian proteins and female reproductive health as well as puberty and fertility as they play crucial roles in overall ovarian function. The timely release of fertile eggs, the preservation of luteal cell activity, and the control of endocrine events essential for pregnancy and fertility are all facilitated by the ovarian proteins (Richards and Pangas, 2010; Tahir et al., 2019). Oocyte formation and other aspects of reproductive health relies heavily on ovarian proteins. Similar to this, uterine proteins are critical for establishing an environment that favours fast development and the preservation of pregnancy. Therefore, the significant decrease in uterine protein content in fluoxetine-treated rats may have a negative impact on the development of the embryo or conceptus. The restoration of uterine protein levels after the administration of the aqueous extract of *H. enneaspermus* leaves may therefore, help with oocyte maturation, ovarian function regulation, foetal growth and enhance overall foetal development (Tahir et al., 2019).

The significant decrease in glycogen levels in the ovaries and uterus of the fluoxetine-treated rats may signify a depletion of carbohydrate stores. Given that the ovaries and uterus depend on

glycogen for energy, the significant decrease in glycogen levels may suggest that fluoxetine has an adverse effect on how well these organs operate. Following administration of the aqueous extract of H. *enneaspermus* leaves, the levels of uterine glycogen were restored. This may indicate an increase in hormonal secretions, particularly as the study showed increased oestrogen levels. This increased uterine glycogen content could make the energy substrates required for uterine and pre-embryonic development during implantation and early pregnancy more readily available. Furthermore, the significant increase in ovarian glycogen levels caused by the aqueous extract of H. *enneaspermus* leaves raises the possibility that the extract enhances the use of available energy sources by boosting hormonal secretion, which could then have a positive impact on ovulation and reproductive behaviour.

Cholesterol is a precursor in the manufacture of steroid hormones. A sequence of consecutive enzymatic processes convert cholesterol into steroid hormones, including oestrogen, testosterone, aldosterone, dehydroepiandrosterone, and cortisol. Cholesterol is predominantly produced from lipoproteins, which are the building blocks for the manufacture of steroid hormones and enter cells by receptor-mediated endocytosis (Miller and Bose, 2011). Therefore, decreased oestrogenic activity following the administration of fluoxetine in the research may be as a result of the significant decrease in ovarian and uterine cholesterol levels. However, the significant increase in ovarian and uterine cholesterol levels shown in rats given different dosages of *H. enneaspermus* raises the possibility that the extract improves the synthesis of cholesterol, which is necessary for the creation of steroid hormones. This may possibly be related to the increased oestrogen levels seen in the hormonal analysis.

The female reproductive system is significantly impacted by alkaline phosphatase. Alkaline phosphatases (ALP) in the ovaries and the uterus play vital roles in the mobilisation of lipid metabolites and carbohydrates needed for the process of folliculogenesis by the oocytes and inside the sexual organs (Lei et al., 2013). Additionally, uterine alkaline phosphatase is involved in uterine receptivity, implantation, and decidualization. The reduced ovarian and uterine ALP levels in rats treated with fluoxetine may compromise the transfer of essential components needed for the process of folliculogenesis. However, the extract significantly restored the reductions caused by the fluoxetine. The administration of *H. enneaspermus* aqueous extract resulted in a significant increase in the mobilisation of these essential components needed for steroidogenesis, indicating the oestrogenic potential of the extract. The possibility that the extract may promote the development of fertilized eggs and create a suitable environment for their maturation is raised by the reversal of ovarian and uterine ALP levels (Lei et al., 2013).

The activity of lysosomal enzymes, including acid phosphatase, has been shown to be upregulated by progesterone and secreted into the uterine lumen during the oestrous cycle (Lei et al., 2013). They participate in ovarian and uterine metabolic processes, such as oocyte maturation, the restart of mitotic divisions, germinal vesicle breakdown, ovulation, and the preparation of the uterine lining for implantation (Lei et al., 2013; Wang et al., 2000; Wang et al., 1999). The reduced acid phosphatase levels in rats treated with fluoxetine shows disturbance in ovulatory function and can lead to uterine bleeding and may compromise the transfer of essential components needed for the process of folliculogenesis. The significant reversal of the fluoxetine-mediated decrease in ACP levels after the administration of the extract shows its capacity to improve the ovarian and uterine metabolic processes and support effective reproductive function.

CONCLUSION

This study provided a valuable insight into the modulating role of aqueous extract of *Hybanthus enneaspermus* leaves on fluoxetine-induced gonado-toxicity. The findings revealed that the extract has a potentiating impact on endogenous oestrogen activity and induce gonadotrophin production or secretion, which in turn restored the altered parameters of uterine and ovarian functioning. This might provide more evidence in favour of the widespread application of *H. enneaspermus* leaves in Nigerian traditional medicine for the management of female sexual deficiencies.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

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Original Research Article

Pharmacognostic, phytochemical, and chemomicroscopic evaluation of *Physalis angulata* (L)

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Abstract

Background/Introduction: Since the start of civilization, human culture has used plants to treat illnesses. Many plant samples have been adulterated intentionally or unintentionally during processing or even misidentified during collection. This study aims at authenticating and standardizing of *Physalis angulata* by carrying out Pharmacognostic evaluation, HPLC and GCMS analysis.

Methodology: The dried leaves of *Physalis angulata* was extracted with 70% ethanol. Phytochemical screening (qualitative and quantitative screening) analysis was carried out on the ethanolic leave extract using standard method, while the chemical compounds were established with HPLC-DAD and GCMS and Pharmacognostic evaluation was carried out on the powdered leave.

Results and discussion: Qualitative phytochemical analysis of the leave extract reveals the presence of flavonoids, terpenes, alkaloid, tannins and saponin while carbohydrate, glycosides and resins were absent. Quantitative phytochemical screening shows alkaloid present to be 2.4±0.4% and saponin 2±1.32%. HPLC analysis reveals the presence of quercetin, rutin, catechin and ferulic acid while GCMS reveals the presence of cyclohexene, Palmitic acid, 17-Octadecynoic acid, 1,2-Benzenedicarboxylic acid and Phytol. Total Ash content is (15.23±0.43) %w/w with acid insoluble ash of (1.16 ±0.28) %w/w, water soluble ash of (8.13 ±0.32) %w/w, moisture content (5.67 ±0.14) %w/w, alcohol soluble extractive value of (5.76 ±0.40) %w/w and water-soluble extractive value of (10.96 ±0.35) %w/w. Chemomicroscopic evaluation indicate the presence of lignin, cellulose, tannins, starch, calcium oxalate, oils and protein. Microscopy, fluorescence and organoleptic analysis were also documented.

Conclusion: From this study, the presence of valuable phytochemicals has been established from the plant, while HPLC and GCMS revealed the identities of these compounds. Thus, the physico chemical parameters established for *Physalis angulata* in this study falls within the range approved by WHO (1992) for crude plant and extract.

Keywords: *Pharmacognostic evaluation, phytochemical screening, Physalis angulata, HPLC analysis, and GCMS analysis.*

INTRODUCTION

Plants have been used to make medicines since the dawn of time to treat a wide range of human and animal diseases. The preparation of therapeutic medicines from plants is gaining interest worldwide due to the decreased efficacy of synthetic preparations for a variety of reasons [1]. Many natural products are used in alternative medicine [2, 3]. Different plant species, particularly in folk medicine, have been used to prevent and treat illnesses. [4, 5].

Physalis angulata [6] is a plant of the family Solanaceae, widely distributed throughout tropical and sub-tropical regions of the world [7]. It grows as a weed in cultivated fields, wastelands, along roads, in the forest, and along creeks that are close to the sea. [8]. *P. angulata* is an annual herb that grows up to 1 m tall, is upright, and has many branches on its stems. The plant's leaves are ovate to elliptic, about 9 cm long, and have 1-2 nodes with pointed tips. The plant's solitary, up to 6 mm long, white or pale-yellow flower produces small, edible orange berries that are encircled by an inflated balloon-like, ovoid calyx that is about 3-5 mm long. Disc-shaped and 1.0 to 1.5 mm in diameter, the seed is a light-yellow color [9]. They are commonly called Winter cherry, Cape gooseberry, Hogweed, Balloon cherry, Coqueret, Strawberry tomato, Cutleaf ground cherry, Wild tomato, Winter tomato, Winter cherry, Cow pops, Chinese lantern [10].

All the plant parts have been reported to be used traditionally in Nigeria for medicinal purposes, including the entire plant for childbirth, diuretic, fever, gonorrhea, jaundice, liver diseases, malaria, nephritis, postpartum hemorrhage, rashes, skin sores, sleeping sickness, tumor prevention, and liver diseases [11]. The fruits are suggested for skin conditions, inflammation, postpartum infections, and infection. Asthma, dermatitis, diuretic, earache, fever, gonorrhea, hemorrhage, liver disorders, malaria, postpartum infection, rheumatism, skin diseases, to prevent abortion, and worms(schistosomiasis) are other conditions for which the leaves are used. The root is used for diabetes, earache, fever, hepatitis, jaundice, liver disorders, malaria and rheumatism [11-14].

Studies have revealed that *Physalis angulata* exhibits many therapeutic activities like antiallergic, antiasthmatic, antileishmanial, anitmalaria, and immunomodulatory activity [15-17].

Phytoconstituents such as alkaloids, flavonoids, steroids, physalin A, vamonolide, physangulide, chlorogenic acid and withaphysanolide have been reported to be present in *P. angulata* [9, 18].

Although there have been reported scientific studies on some biological activities on the plant, nothing has been documented on the pharmacognostic evaluation of the plant towards aiding the standardization and authentication of the plant. This study aims at establishing pharmacognostic parameters, quantitative phytochemical constituents and HPLC profiling which could serve as reference data for authenticating and standardizing the plant.

MATERIALS AND METHODS

Collection of plant materials

The plant sample was collected from Chaza Suleja, Niger state on 16th of August 2022 and submitted to the National Institute for Pharmaceutical and Research Development (NIPRD) Herbarium. The sample was authenticated by both ethnobotanist and taxonomist at the herbarium unit of the Department of Medicinal Plant Research and Traditional Medicine of NIPRD, and a voucher specimen number NIPRD/H/7318 was generated and deposited.

Extraction:
The dried powdered plant (20 g) of the dried powdered plant sample was macerated in ethanol for 24hours and extracted. The extract was weighed and stored for use.

Pharmacognostic evaluation:
Epidermal layer preparation for Microscopy
Fresh leaves of *Physalis angulata* was cut diagonally with the leave margin clearly visible and boiled in absolute ethanol, rinsed off in 5% sodium hydroxide then soaked in chlorohydrate, then Nitric acid to properly clear off the mesophyll, the adaxial and abaxial layer of the leaves were stained with Safranin O, mounted on a glass microscope slide with glycerol, then viewed under ACCUScope Binocular Microscope and the features are observed and noted down. Photomicrograph were taken.

Transverse section preparation
The transverse sections of the leaves were obtained by free hand sectioning using a razor blade while the leaf midrib (1 cm x 1 cm) was cut. The transverse sections were cleared in 2% sodium hypochlorate for 2 to5 min, rinsed severally in water, stained in Safranin O and mounted on a glass microscope slide with dilute glycerol. The distribution of tissue through the mid rib was observed under the light microscope at different magnifications.

Microscopy and Chemomicroscopic evaluation
Chemomicroscopic studies of the powdered plant sample was done using reagents and stains: N/50 iodine and sulphuric acid (66%) to test for cellulose, concentrated hydrochloric acid and Phloroglucinol to test for Lignin, Sudan IV reagent to test for Oils, Million's reagent and 1% picric acid to test for Protein, and ferric chloride to test for Tannins. A quantity of the powdered sample was cleared in chloral hydrate, mounted in diluted glycerol on a microscope slide and viewed under the microscope at different magnifications [19].

Physicochemical characterization
Physicochemical parameters such as moisture content, total ash, water soluble ash, acid insoluble, extractive value and water-soluble extractive value were determined following African Pharmacopoeia (1986) and WHO Standard [20]

Florescence analysis
the dried leaf of *Physalis angulata* was made to powder, and 0.5 g of the powdered sample was added to a test-tube, 5 ml of the various reagents (buffer solution, formaldehyde, methanol, etc.) were added, and the color was observed under visible light, 254 nm and 365 nm, then it was heated for 10 mins and view in daylight, 254 nm and 365 nm.

Quantitative phytochemical analysis:
Total Saponin content
Saponin quantitative determination was carried out using the method reported by Koomson *et al*., [21]. The total saponin was calculated using the equation

$$\% \text{ Saponin} = \frac{Weight\ of\ saponin}{Weight\ of\ sample}\ x\ 100$$

Total Alkaloid content

Quantitative determination of alkaloid was according to the methodology by Harborne, (1973) and Ezeonu and Ejikeme (2016**)**. The total alkaloid was calculated using the equation

$$\%\ \text{Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}}\ x\ 100$$

Qualitative phytochemical analysis:

Phytochemical screening of *Physalis angulata* ethanolic extract was carried out to determine the presence of carbohydrate, alkaloid, tannins, saponins, terpenes, resins, steroid, glycosides and flavonoid using standard methods [21-23]

High-performance liquid chromatography analysis

The HPLC fingerprinting analysis was carried out using Shimadzu HPLC system comprising Ultra-Fast LC-20AB prominence equipped with SIL- 20AC autosampler; DGU-20A3 degasser; SPDM20A UV-diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 × 4.6 mm). The chromatographic conditions included an injection volume of 10µL of a 10 mg/mL solution of the 70% ethanolic extracts of *physalis angulata*, in a binary gradient elution system composed of acetonitrile as solvent A and 0.1% Formic acid in HPLC grade water as solvent B was applied for the fingerprint analysis with the gradient elution as follows: 3.5 min, 18% A; 5–10 min, 18–25% A; 10–30 min, 25–35% A; 30–35 min, 20–10% A, The mobile phase flow rate was 0.6 mL/min, and column temperature is maintained at 40 ◦C. The DAD detector was set at 254 nm [24].

Gas Chromatography-Mass Spectrometry (GC-MS):

The GC-MS analysis was carried out using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMS solution data system. The GC column was Optima-5 ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was 60 - 180°C at a rate of 10°C/min, then held at 180°C for 2 minutes, followed by 180 -280°C at a rate of 15°C/min, then again held at 280°C for 4 minutes. The injection port temperature was 250°C while detector temperature was 280°C. Helium was used as a carrier gas, at a flow rate 1.61 ml/ min. Diluted sample (1/100 in hexane, v/v) of 1.0 µl was injected using autosampler and in the split mode with ratio of 10:90. The analysis was performed in triplicate. Constituents were identified by matching the mass spectra, National Institute of Standards and Technology (NIST) library of mass spectra. [25]

RESULTS AND DISCUSSIONS

Figure 1 presented the macroscopy image of the leaf of *Physalis angulate.*

Macroscopy Analysis:

Figure 1: leaf of *Physalis angulata*

Figures 2, 3, 4, 5 and 6 are the images of epidermal leaf microscopy.

Epidermal leaf microscopy

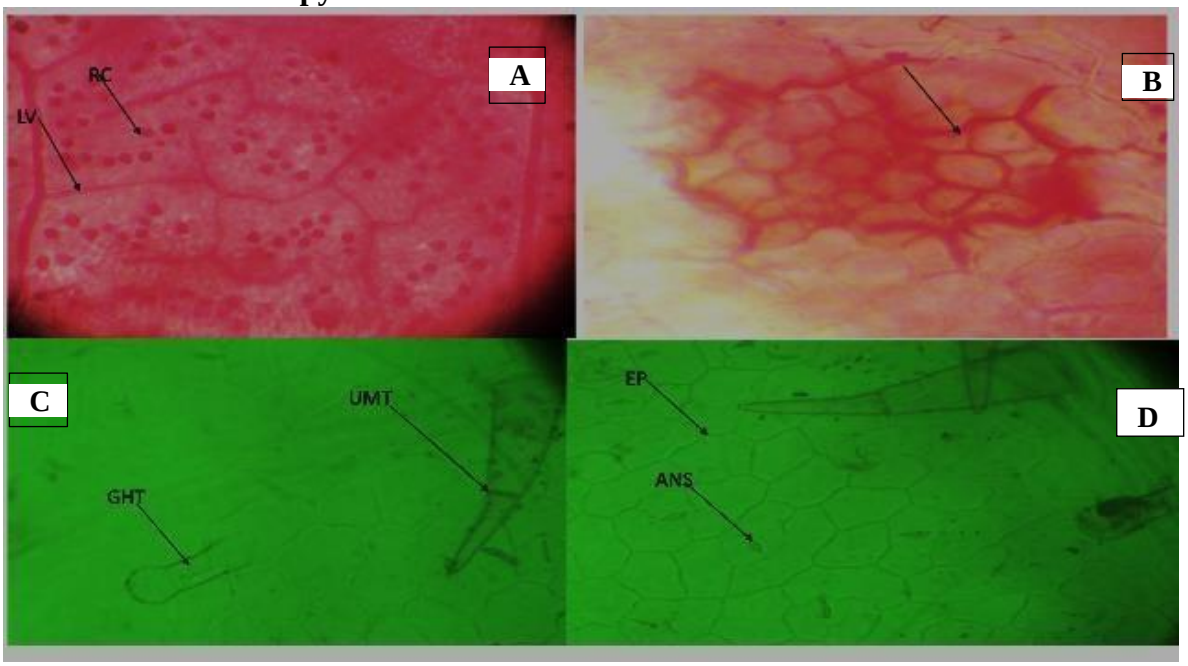


Figure 2: Adaxial surface of the epidermal leaf (A)x200 Rosette crystals (RC), Leaf veination (LV) (B) x200, (C) x400 Glandular head trichome, Unicellular multi-serrated trichome (UMT) (D) x400 Anisocytic stomata (ANS), Regular Epidermal cell wall (EP)

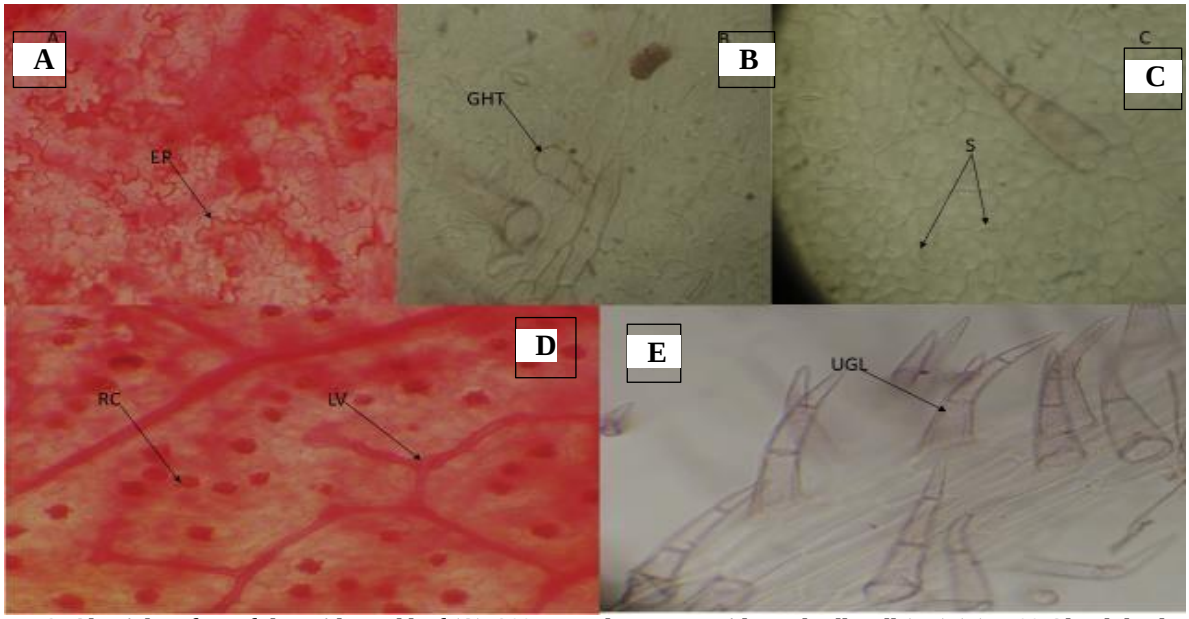


Figure 3: Abaxial surface of the epidermal leaf (A)x200 Irregular wavy Epidermal cell wall (EP) (B) x400 Glandular head trichome (GHT), (C) x400 Anisocytic and Anomocytic Stomata(S), (D) x200 Rosette crystals (RC), Leaf Veination (LV), (E) x200 Unicellular glandular trichome (UGL)

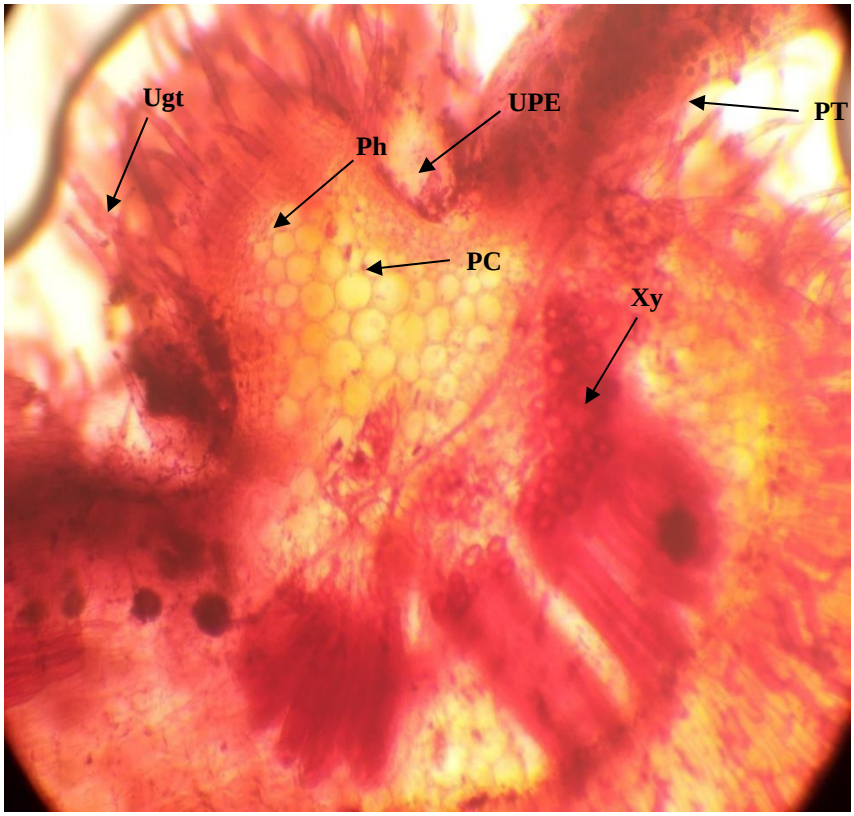


Figure 4: Photomicrograph of Transverse Section Midrib of *Physalis angulata* stained with Safranin O; x100, Xy: Xylem, Ph: Phloem, Ugt: Unicellular glandular trichome, PC: Parenchyma cells, UPE: upper epidermis, PT: Palisade tissue

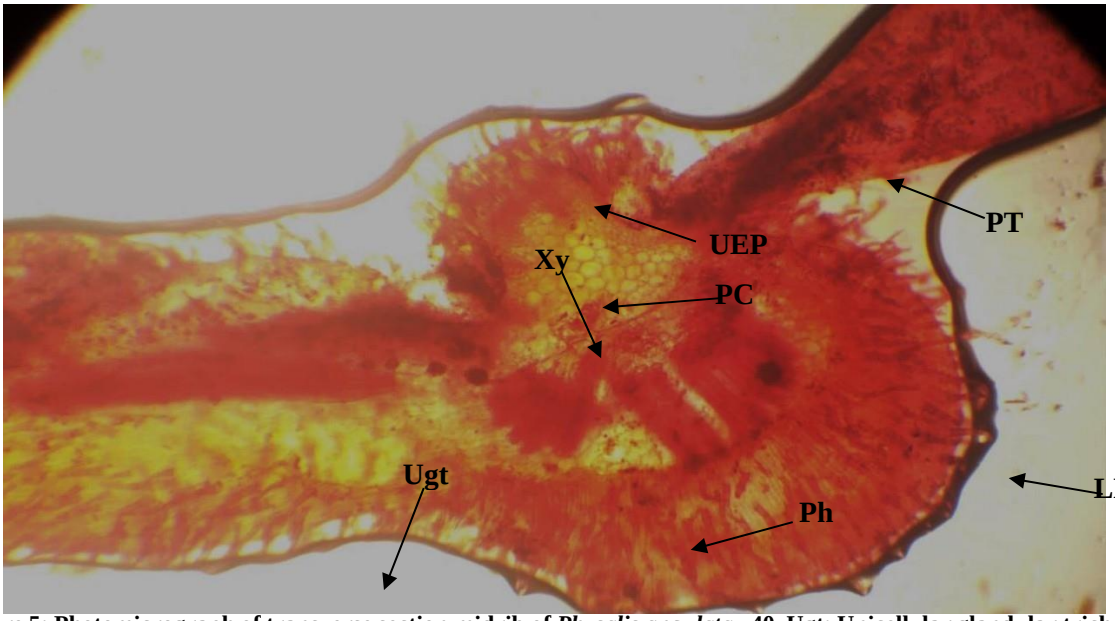


Figure 5: Photomicrograph of transverse section midrib of *Physalis angulata* x40, Ugt: Unicellular glandular trichome, Ph: Phloem, Xy: Xylem, UEP: Upper epidermis, LEP: Lower epidermis, PT: Palisade tissue, PC: parenchyma cells

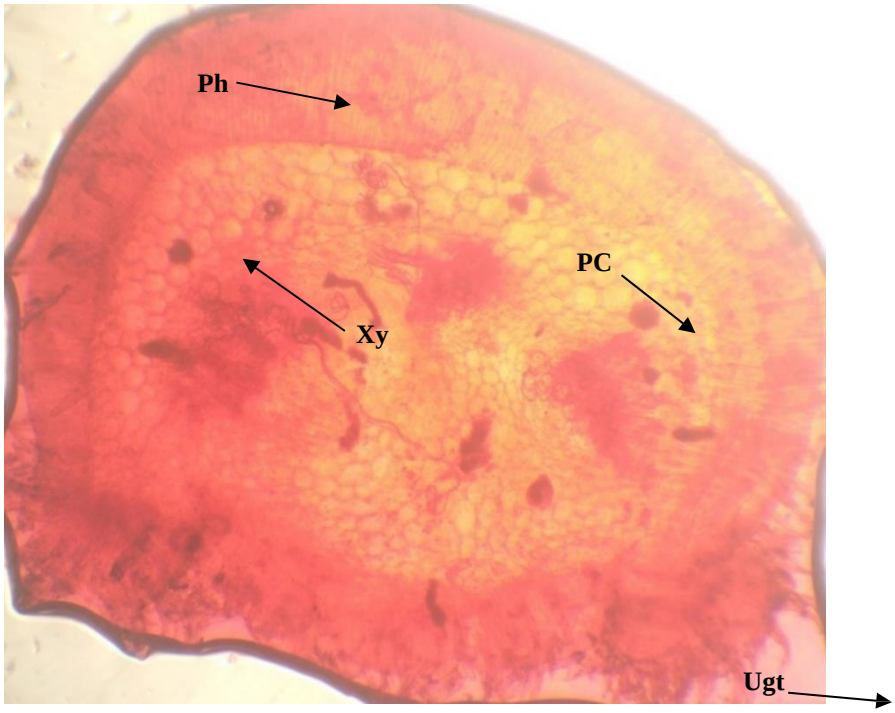


Figure 6: Photomicrograph of the petiole transverse section of *Physalis angulata* stained with Safranin O x40, Ugt; Unicellular glandular trichome, Ph; Phloem, Xy; Xylem, PC: Parenchyma cells

Organoleptic evaluation

Table 2 present the Organoleptic parameters of *Physalis angulata*

Table 2: Organoleptic parameters of *Physalis angulata*

Characters	Inference
Color	Leafy green
Odor	Strong characteristic smell
Taste	Tasteless
Texture	Slightly coarse

The Chemomicroscopy evaluation is presented in Table 3

Table 3: Chemomicroscopic evaluation of *Physalis angulata*

Test	Inference
Lignin	+
Cellulose	+
Tannins	+
Starch	+
Calcium Oxalate	+
Oils	+
Proteins	+

Pharmacognostic parameter is presented in Table 4

Table 4: Physico-chemical evaluation of *Physalis angulata*

S/N	Parameters	Percentage (%)
1	Total Ash Value	15.23 ±0.43
2	Acid-insoluble Ash	1.16 ±0.28
3	Water Soluble Ash	8.13 ±0.32
4	Moisture Content	5.67 ±0.14
5	Alcohol Soluble Extractive Value	5.76 ±0.40
6	Water-soluble Extractive Value	10.96 ±0.35

Mean ±SD

The results of the fluorescence nature of the powder leaf sample of *Physalis angulata* under UV-Visible light (254 nm and 365 nm) and daylight under different conditions is presented in Table 5

Table 5: Fluorescence analysis evaluation of *Physalis angulata*

S/N	TEST	COLD DayLight	254nm	365nm	HOT DayLight	254nm	365nm
1	Powdered Sample	Army green	Dark green	Black	Army green	Dark green	Black
2.	Powdered Sample + Distilled Water	Sage	Light green	Clover	Dark olive	Green	Dark oak leaf green
3.	Powdered Sample + 10% Aq. NaOH	Dark kelp	Deep green	Dark green	Dark green	Dark green	Black
4.	Powdered Sample + Ammonia	Hunter green	Green	Dark green	Dirty green	Green	Dark green
5.	Powdered Sample + Conc. H2SO4	Black	Dark green	Black	Black	Black	Black
6.	Powdered Sample + Conc. H2SO4 + Water	Black	Dark Green	Black	Black	Greenish black	Black
7.	Powdered Sample + Conc. HCL	Dark green	Dark green	Black	Black	Black	Black
8.	Powdered Sample + Conc. HCL + Water	Dirty green	Green	Cucumber green	Dark green	Dark green	Black
9.	Powdered Sample + Nitric Acid	Brown	Celery	Tan	Light cheese	Green pea	Dull green pea
10.	Powdered Sample + Nitric Acid + Water	Yellow ochre	Light chayote	Chayote	Pale yellow	Chayote	Clover
11.	Powdered Sample + Iodine	Dark kelp	Dark green	Pepple gray	Moderate olive	Dull green	Dark chayote
12.	Powdered Sample + 5% Ferric Chloride	Dark green	Dark green	Black	Dark olive green	Dark green	Black
13.	Powdered Sample + Picric Acid	Dirty green	Green	Deep green	Dark Yellow ochre	Sage green	Dark tan
14.	Powdered Sample + Picric Acid + Water	Dirty green	Green	Light kelp	Munsell yellow	Green	Sage green
15.	Powdered Sample + Glacial Acetic Acid	Olive drab	Dark tan	Brick red	Dark olive drab	Dark brown	Dark brick red
16.	Powdered Sample + Petroleum ether	Dark green	Green	Light olive green with brick red at the interface	Dirty green	Sage	Brown with brick red interface
17.	Powdered Sample + Chloroform	Light kelp	Green	Basil with brick red at the liquid interface	Dark kelp	Siege green	Brick red
18.	Powdered Sample + Ethyl Acetate	Green	Light green	Brick red	Green	Green	Brick red
19.	Powdered Sample + Methanol	Green	Green	Dark brick red	Dark green	Dark green	Dark brick red
20.	Powdered Sample + 5% Potassium dichromate	Brown	Dark green	Black	Brown	Dark green	Black
21.	Powdered Sample + Alcoholic Potassium Hydroxide	Olive	Green	Green with red at the interface	Olive drab	Dirty green	Brick red

Quantitative phytochemical screening of *Physalis angulata* was carried out to confirm the total alkaloid and saponin content, the result of this screening is presented in Table 6.

Table 6: Quantitative phytochemical analysis (Alkaloids and Saponins) of *Physalis angulata* ethanolic leave extract.

S/N	Phytochemicals	Mean±SD* (n=3)
1	Total Saponins	2±1.32 %
2	Total Alkaloids	2.4±0.4%

***Values are mean ± standard deviation of triplicate measurement**

Table 7 presents the Qualitative phytochemical analysis of *Physalis angulata*

Qualitative phytochemical analysis of *Physalis angulata* ethanolic leave extract.

S/N	Test	Inference
1	Carbohydrate	-
2	Tannins	+
3	Flavonoids	+
4	Terpenes	+
5	Resins	-
6	Saponins	+
7	Glycosides	-
8	Alkaloids	+

Figure 8 presents the Chromatogram of HPLC profiling of *Physalis angulata* ethanolic leave extract

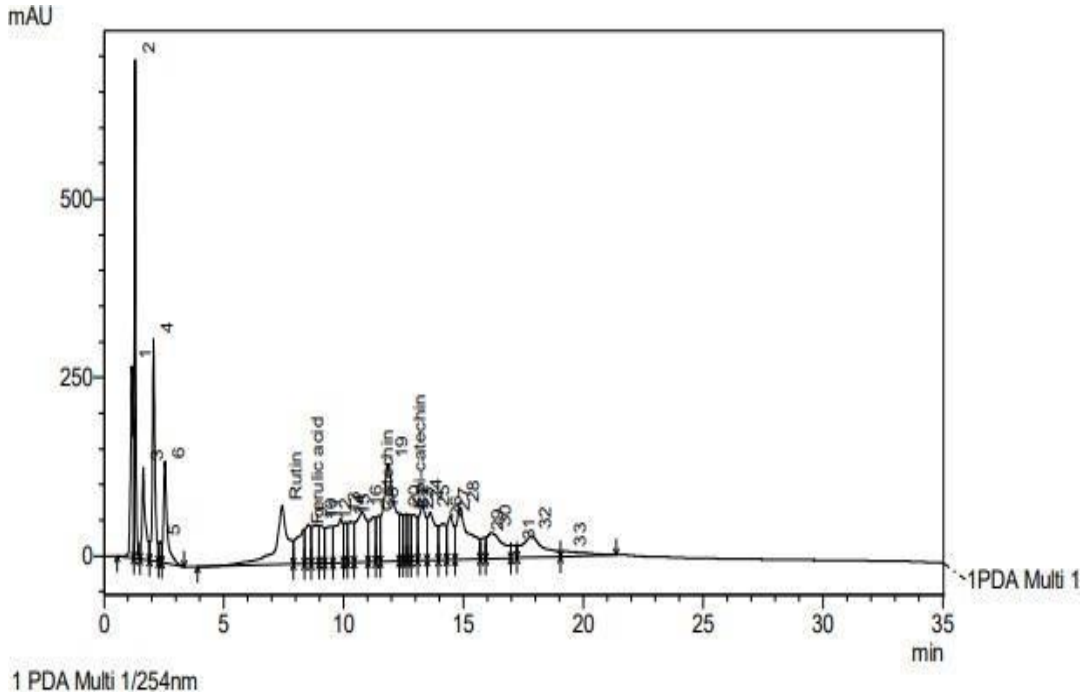
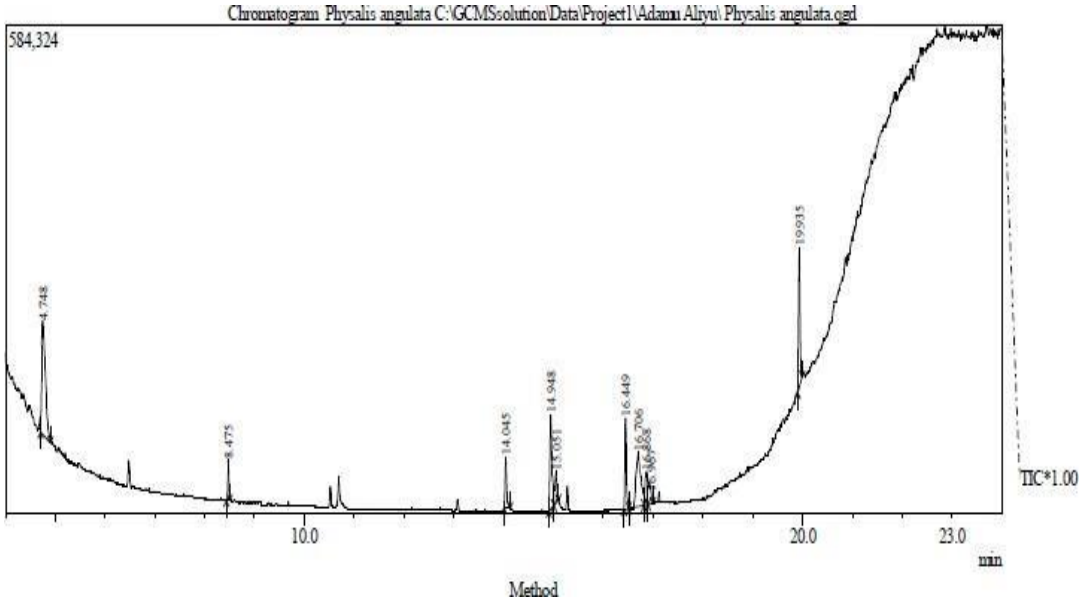


Figure 8: Chromatogram showing HPLC profiling of *Physalis angulata* ethanolic leave extract

Figure 9 presents the chromatogram of GCMS profiling of *physalis angulata* ethanolic leave extract

Figure 9: Chromatogram showing GCMS profiling of *physalis angulata* ethanolic leave extract



Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.748	4.708	4.900	713222	27.28	135884	17.12	5.25	V	Cyclohexene, 4-isopropenyl-1-methc
2	8.475	8.442	8.508	70572	2.70	48783	6.15	1.45		1-Tetradecanol, methyl ether
3	14.045	14.008	14.133	140785	5.38	62068	7.82	2.27		Phthalic acid, cyclobutyl isobutyl est
4	14.948	14.908	15.008	339603	12.99	113494	14.30	2.99		Palmitic acid
5	15.051	15.008	15.100	99928	3.82	37482	4.72	2.67	V	Phthalic acid, 4-bromophenyl heptyl
6	16.449	16.417	16.517	248770	9.51	109448	13.79	2.27		Phytol
7	16.706	16.517	16.817	412394	15.77	64966	8.18	6.35	V	17-Octadecynoic acid
8	16.868	16.817	16.900	148031	5.66	40189	5.06	3.68	V	Undecanoic acid, 10-bromo-
9	16.967	16.900	16.992	92908	3.55	14399	1.81	6.45	V	4-Dodecano
10	19.935	19.900	19.992	348444	13.33	167144	21.05	2.08	V	1,2-Benzenedicarboxylic acid, diisoc
				2614657	100.00	793857	100.00			

DISCUSSION

The characteristics of epidermal cells, trichomes, anomocytic and anisocytic stomata, rosette crystals, and presence of fibers amongst others, can also be used as diagnostic features for the authentication and standardization of the plant samples in relation to members of the same family [26-28]. The transverse section of the midrib layer of the epidermis of the leaf from this study was seen to be comprising of lone layered upper and lower epidermis made up of parenchyma cells with numerous unicellular glandular trichome which is different from the report of Rajani and Pradip [29] stating the absence of trichome in the midrib region of the epidermis. Uneven layer of parenchyma that are closely joined together, solely surface palisade tissue cells was observed towards the region of the upper epidermis which is similar to the report of Rajani and Pradip [29]. In this study, it was noticeably observed that both surface of the leaf epidermal layers had stomata with the Abaxial surface having both anomocytic and anisocytic type while the Adaxial surface had just anisocytic type. However, Zhang and Lu [30] stated Abaxial surface having only anomocytic stomata. Also, Nazir *et al* [31] reported the leaves of *Physalis angulata* and *Physalis Peruviana* were amphistomatic and found only anomocytic type of stomata. The presence of more

than one type of stomata on a particular layer of leaves in the members belonging to the Solanaceae was reported by Metcalfe and Chalk [32], Inamdar and Patel [33], Ahmad [34]. Rosette crystals, Glandular head trichome, unicellular multiserrated trichome were visibly present on both surfaces of the epidermal layers of the leaves which wasn't reported in the study carried out by Nazir *et al* [31]. Lone layered palisade cells were observed towards the upper epidermis cells of the transverse section of midrib region which is in line with the report of Rajani and Pradip [29]. Unicellular glandular trichome was found present with single layered epidermis in the transverse section of the petiole, this is different from the report of Nazir *et al* [31].

A slightly higher extractive value was obtained for the aqueous solvent of (10.96%±0.35) than alcohol soluble extractive value (5.76%±0.40) suggesting the possibility of the presence of more polar constituents. The results for the ash analyses on dry matter were, total ash 15.23%±0.43, Acid insoluble ash 1.16±0.28% and water-soluble ash 8.13%±0.32. These values indicate low amount of silica especially sand as well as siliceous earth in the sample and indicate low inorganic contents. The moisture content of 5.67%±0.14 suggests a high shelf life of the crude drug. All the results of the Physicochemical parameters are within the limit approved by WHO guidelines (1992). However, reports by Iwansyah *et al* [35] revealed a much higher value of 75.72% for the moisture content, with the total ash value being 2.71% of the plant sample gotten from Indonesia. Samples having high moisture content might be more susceptible to microbial degradation. Similarly, study carried out on the physicochemical properties of *P. angulata* sample gotten from India by Rajani and Pradip [29] showed a much higher values of 46.89% for the water-soluble ash and 17.11% for the acid insoluble ash, these high values are indicative of high inorganic contents as this could be attributed to the soil type, mining and construction activities around the area of cultivation. The total Ash value they recorded for the sample was 21.83% and moisture content being 7.49% which is also slightly different from the report of this study. The difference in physicochemical parameters recorded in samples from India and Indonesia might be due to the geographical locations, soil type and environmental conditions. Extracts of *P. angulate* leaves also showed characteristic fluorescence analysis in visible and under U.V (254 and 366 nm) [36]

Qualitative phytochemical screening of the leave extract reveals the presence of flavonoids, terpenes, alkaloid, tannins and saponin while carbohydrate, glycosides and resins was absent. This is slightly different from the report of Jayachithra *et al* who reported the presence of alkaloids, glycosides, flavonoids, tannins, and phenolics [37], the presence of glycosides, flavonoid, saponin, steroids and alkaloid in the ethyl acetate extract was also reported by Ushie *et al,* [38] in his study. Savithramma *et al* [39] report *physalis angulata* as an anti-inflammatory, anti-analgesic, antispasmodic agent due to the presence of flavonoids, alkaloids, saponin, glycosides present in their composition.[39] Quantitative phytochemical screening shows the quantity of alkaloidpresent to be 2.4±0.4% and saponin 2±1.32% in this present study. HPLC analysis reveals the presence of quercetin, rutin, catechin and ferulic acid according to fig 8. Flavonoid and polyphenols are known to promote wound healing activity due to their strong antioxidant and antibacterial activities. [40, 41] Jayachithra *et al* also report the high content of polyphenols and flavonoid in the ethanolic extract of the leave and its high antioxidant, anticancer and antibacterial activities [37]. The presence of the phytochemicals constituent in this study presents *P. angulata* as a candidate of anticancer, antibacterial, antioxidant, anti-analgesic and anti-inflammatory agent.GCMS reveals the presence of Cyclohexene, Palmitic acid, 17-Octadecanoic acid, 1,2- Benzenedicarboxylic acid and Phytol in abundance as shown in fig 9, this was also in relation with

the report of the GCMS analysis done by Jayachithra *et al* who reported the presence of Phytol, benzenedicarboxylic acid, octadecanoic acid and oleic acid [37].

CONCLUSION

This study has given some insight into the pharmacognostic features and chemical profile of *P. angulata* which are essential features necessary for proper sourcing and identification of crude drugs. In addition, these findings can be an additional reference resource for guidance and regulatory purposes. More so, in the light of the biological activities attributed to this plant, it can be a good alternative source to synthetic drugs, as studies have indicated the presence of phytochemicals which are good antioxidant, antimicrobial and anti-cancer agents.

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Original Research Article

Seroprevalence of Dengue and Hepatitis B infections among young adults attending a secondary health care facility at Olabisi Onabanjo University, Ogun State, Nigeria

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Abstract

Hepatitis B and Dengue virus infections carry significant public health implications due to their potential to cause illness and death. This study focused on examining the prevalence of these infections among young adults seeking care at a secondary health facility at Olabisi Onabanjo University. Ninety samples obtained from study participants underwent screening using Enzyme-linked Immunosorbent Assay (ELISA) for both HBsAg and Dengue IgM. The participants, predominantly female, were aged between 15 and 30 years. The analysis revealed a 13.3% prevalence of Dengue IgM, a 4.5% prevalence of HBsAg, and a 4.4% prevalence for both infections. Regarding age distribution, the highest prevalence occurred among participants aged 15-20 years for both single infections and the co-occurrence of Hepatitis B and Dengue viral infections. Examining gender distribution, female participants exhibited higher seropositivity across all infection categories. Although not statistically significant, these findings lay the groundwork for a comprehensive surveillance study on the Hepatitis B and Dengue viral infections in young adults. This study provides valuable insights into the prevalence patterns of Hepatitis B and Dengue virus, offering a basis for further research and public health interventions in this population.

Keywords: *Dengue, Hepatitis B, seroprevalence, young adults, Nigeria*

INTRODUCTION

Hepatitis B and Dengue virus infections pose substantial health challenges on a global scale, affecting millions of individuals and imposing significant burdens on healthcare systems. These viral infections are particularly concerning due to their potential to cause severe morbidity and mortality. Although extensive research and preventive measures have been undertaken, the prevalence of these infections, especially among specific demographic groups, remains a subject of concern (Majumdar & Jana, 2023). Aged between 15 to 30 years are young adults representing a significant proportion of the population who are frequently exposed to various environmental and lifestyle-related risk factors.

Hepatitis B is a viral infection primarily transmitted through contact with infected blood or other bodily fluids. It can result in acute and chronic liver disease, including cirrhosis and hepatocellular carcinoma (Gebremeskel et al., 2020). The virus, known as HBV, can persist in the body for extended periods, leading to chronic infections (Shi & Zheng, 2020). Despite the availability of effective vaccines, Hepatitis B remains a significant global health concern, especially in regions with high prevalence rates (Abesig et al., 2020). Dengue fever, caused by the Dengue virus (DENV), is a mosquito-borne viral illness that can range from mild to severe, sometimes leading to life-threatening complications (Ferrari et al., 2020). Dengue is endemic in many tropical and subtropical regions, making it a considerable public health concern in these areas (Dehghani & Kassiri, 2021). The transmission dynamics of DENV are influenced by various factors, including climate, urbanization, and human behavior (Dehghani & Kassiri, 2021).

While both Hepatitis B and Dengue virus infections have been studied extensively, there is limited research focusing on the seroprevalence of these infections among young adults. This research addresses this gap by examining the prevalence rates of HBV and DENV infections within this specific demographic group. Additionally, it aimed to identify sociodemographic factors and potential risk behaviors associated with these infections. The results of this study will have the potential to inform public health strategies, guide vaccination campaigns, and enhance the understanding of infection dynamics among young adults, ultimately contributing to improved healthcare for this age group.

MATERIALS AND METHODS

Study Design

This study was a cross-sectional study, and participants were recruited from the young adults' population attending the Health Services Directorate of Olabisi Onabanjo University, using a purposive sampling method. Every consenting participant irrespective of gender was recruited into the study. Young adults between age of 15-30 years attending the Health Services Directorate who consent to participate were included in the study. While those who did not fall within the age range of 15 – 30 years and those who did not consent to participate in the study were excluded.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of Olabisi Onabanjo University Health Services Directorate. Informed consent was obtained from all participants following the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. This was done via informed consent forms duly completed by all participants recruited into the study.

Sample Analysis

Blood (3mls) samples were aseptically collected from participants into plain tubes which was then separated to plasma and serum through centrifugation. The resulting serum was then transferred into cryovials and stored at -20^{o}C pending laboratory analysis.

Hepatitis B ELISA

The HbsAg ELISA (BIO-INTECO HbsAg) used antibody 'sandwich' ELISA method in which polystyrene microwell strips were pre-coated with monoclonal antibodies specific to HbsAg. Participant's serum was added to the microwell together with a second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and directed against a different epitope of

HbsAg. During incubation, the specific immunocomplex formed in case of presence of HbsAg in the sample, was captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-Conjugate, Chromogen Solutions containing tetramethyl-benzidine (TMB) and urea peroxide were added to the wells. In presence of the antibody-antigen-antibody (HRP) 'sandwich' immune complex, the colorless Chromogens were hydrolyzed by the bound HRP-Conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity measured and was proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HbsAg remained colorless. A positive result in the ELISA test was defined as having an antibody index value greater than 1.1

Dengue ELISA

The dengue IgM kit was based on ELISA technique. In the assay, controls and unknown were incubated in microtitration wells coated with recombinant antigen Dengue virus of four serotypes. After incubation and washing the wells were treated with the conjugate, composed of anti-human IgM antibodies labeled with peroxidase. After a second incubation and washing steps, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by wavelength absorbance measurement at 450nm. The absorbance measured was directly proportional to the concentration of anti-dengue virus IgM antibodies present. A positive result in the ELISA test was defined as having an antibody index value greater > 0.15

Validation Protocol

According to the instructions in the kit's instruction manual. Each test included positive, negative, and cut-off controls. It enabled the assay and kit to be validated. The optical densities fell within the following parameters. Otherwise, the test would have been invalid and must be repeated.

Questionnaires

Structured questionnaires were used to collect socio-demographic information (gender and age) from the participants. These data were obtained through participant face-to-face interviews.

Statistical Analysis

The data obtained from the questionnaire and the results of the laboratory analysis were entered into Microsoft Excel and analyzed with GraphPad Prism 5. The quantitative variables were presented and compared using graphs and tables, Chi-square and p-values were calculated and obtained. Significance was noted as $p < 0.05$.

RESULTS

A total of 90 young adults attending the Olabisi Onabanjo University Health Services Directorate were participated in the study. These participants were categorized into three age groups: 15-20 years, 21-25 years, and 26-30 years. Among them, 48 individuals fell within the 15-20 years age group, 30 participants were in the 21-25 years age group, and the remaining 12 belonged to the 26-30 years age group (Figure 1). Regarding gender distribution, 49 participants were females, while the remaining 41 were males (Figure 2).

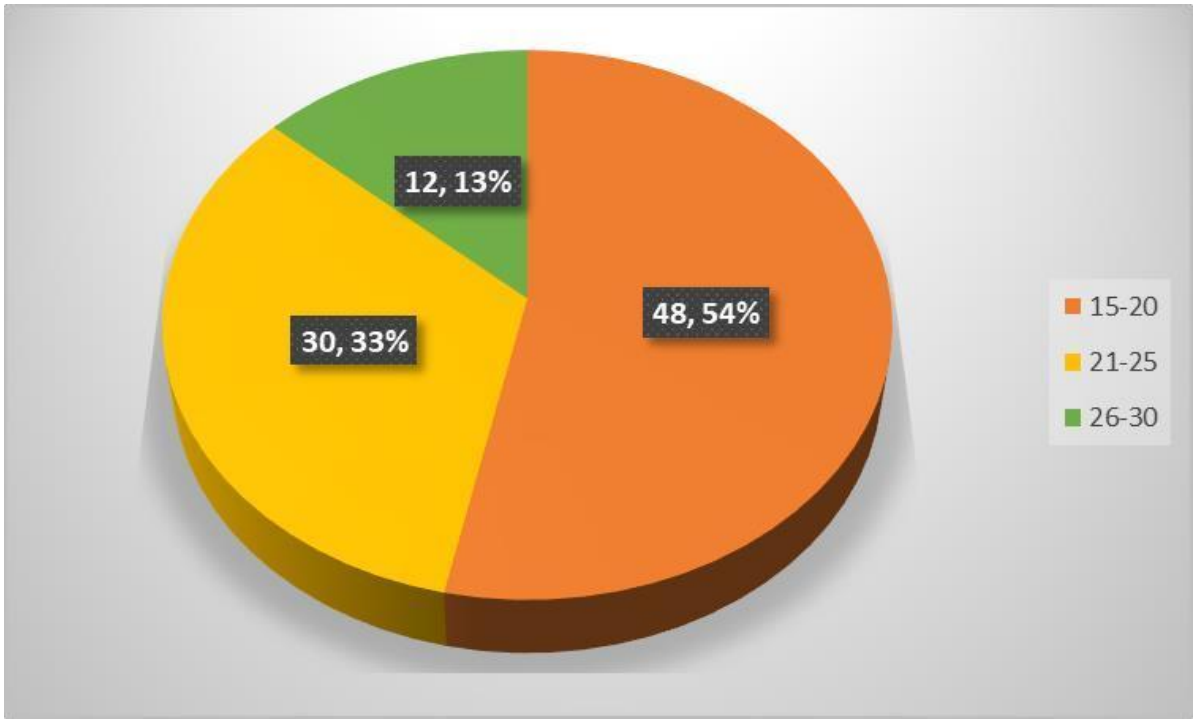

Figure 1: Age Distribution of Participants

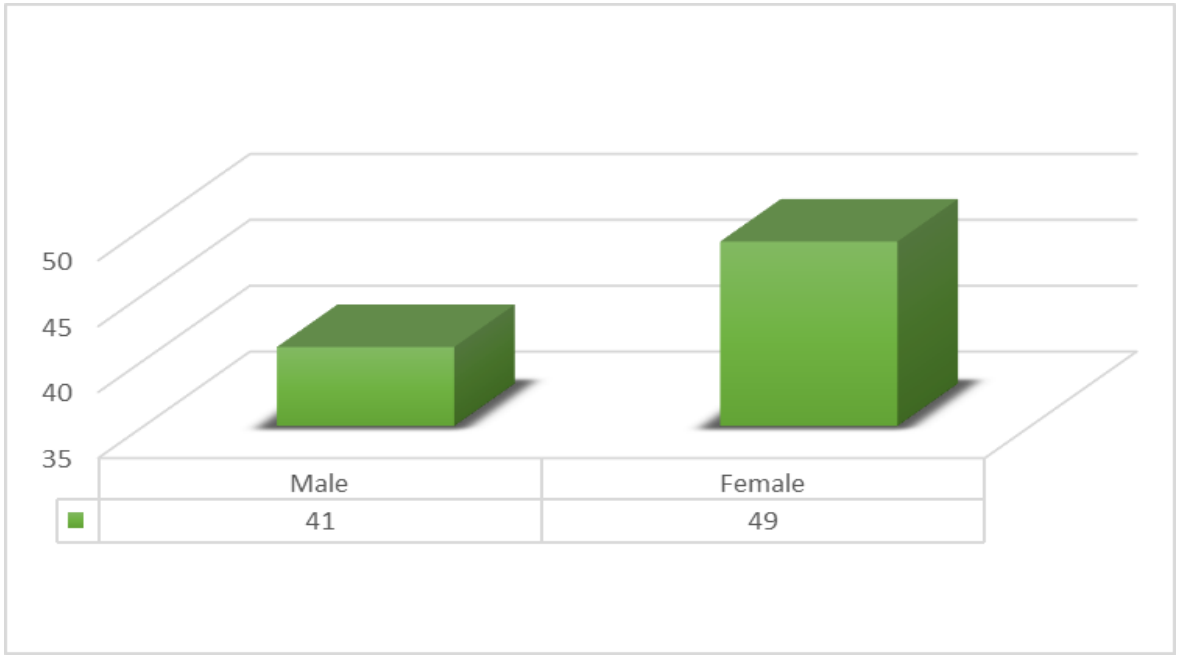

Figure 2: Gender distribution of Participants

The study employed an enzyme-linked immunosorbent assay to examine serum samples for the presence of Dengue virus and Hepatitis B antibodies. The seropositivity of participants' serum samples was classified based on whether they tested positive for Dengue, Hepatitis B, or both. Among the 90 samples analyzed, 13.3% showed seropositivity for Dengue, while 4.5% tested positive for Hepatitis B. Notably, four participants exhibited positivity for both Hepatitis B and

Dengue antibodies (Table 1). Upon stratifying by age groups, individuals aged 15-20 years exhibited the highest seropositivity for both Dengue and Hepatitis B antibodies, followed closely by those in the 21-25 age group. Interestingly, no positive cases were identified among participants aged 26-30 years. Furthermore, within the 15-20 age group, the prevalence of seropositive samples for Dengue and Hepatitis B coinfection was the highest (Table 2). Considering the gender distribution, female participants displayed a higher number of seropositive samples for both Dengue and Hepatitis B compared to their male counterparts. Additionally, a significant proportion of female seropositive participants exhibited positivity for both Hepatitis B and Dengue antibodies (Table 3).

Table 1: Seropositivity of Participants

Seropositivity	Dengue	Hep B	Both
Positive	12 (13.3%)	5 (4.5%)	4 (4.4%)
Negative	78 (86.7%)	85 (95.5%)	86 (96.4%)
Total	90 (100%)	90 (100%)	90 (100%)

Table 2: Age Distribution and Seropositivity of Participants

Age	Dengue	Hep B	Both	Chi-square	p-value
15-20	8 (66.7%)	4 (80%)	3 (75%)	0.3383	0.8444
21-25	4 (33.3%)	1 (20%)	1 (25%)		
26-30	0	0	0		
Total	12 (100%)	5 (100%)	4 (100%)		

Table 3: Gender Distribution and Seropositivity of Participants

Gender	Dengue	Hep B	Both	Chi-square	p-value
Male	5 (41.7%)	2 (40%)	1 (25%)	0.225	0.8936
Female	7 (58.3%)	3 (60%)	3 (75%)		
Total	12 (100%)	5 (100%)	4 (100%)		

DISCUSSION

To the best of our knowledge, this is the first study simultaneously investigating Hepatitis B and Dengue virus in young adults in Ogun state. There is need to comprehensively assess theprevalence of Hepatitis B and Dengue virus infections among young adults and a demographic segment often overlooked in epidemiological studies. Young adults represent a pivotal stage in lifecharacterized by increased social interactions and mobility, making them potentially susceptible to these viral infections. Moreover, they may unknowingly contribute to the spread of these diseases within communities. The current aim of the study is to investigate the seroprevalence of the antibodies for these viruses in this demographic segment of interest.

The serum samples from participants who tested positive for Dengue virus antibodies in our study revealed a seroprevalence of 13.3% among the recruited young adults. This figure is notably lower when contrasted with the findings of Blessmann et al., (2020) in the Lao People's Democratic Republic, where they reported a 22.8% prevalence of Dengue virus IgM among their study participants. Conversely, Ikegbunam et al., (2022) documented a lower prevalence of 5.6% in a Nigerian study that compared four diagnostic test methods for detecting dengue. For Hepatitis B,

our study found a seroprevalence of 4.5%, considerably lower than the 20.5% reported by Osuji et al., (2020) in a study examining the serological profile and associated risk factors in blood donors in Nigeria. Additionally, Erhabor et al., (2020) reported a prevalence of 12.8% in a study investigating hepatitis B virus markers among pregnant women attending antenatal clinics in Specialist Hospital Sokoto, Nigeria. In our study, the seroprevalence for both viral antibodies were 4.4%. This contrasts with Prajapati et al., (2023) recent study, where cases of dengue hepatitis were reported in a retrospective analysis of consecutive patients admitted to two tertiary hospitals. Conversely, Djasrabe et al., (2022) found no coinfections in their study involving Chadian patients. These variations among different studies may be influenced by factors such as the geographical location of the study, the sensitivity and specificity of diagnostic kits, and differences in sample sizes.

Analysis of seropositivity based on age distribution indicated the highest prevalence of antibodies against dengue and hepatitis among participants aged 15-20 years. This also included individuals with antibodies for both viruses. A global study by Yang et al., (2021) examining the burden of dengue found a higher prevalence in those aged 15 and older. Consistent with this, a Nigerian study by Emeribe et al., (2021) reported a notably high prevalence in a similar age group, aligningwith the findings of our current study. While the prevalence of Hepatitis B virus has been documented in various parts of the country, most studies have reported a higher prevalence in agegroups older than those observed in our study. For example, Omatola et al., (2020) identified the highest prevalence in the 24-44 age group in their study on Hepatitis B in Kogi state. Similarly, Egbe et al., (2023) found the highest prevalence in the 25-34 age group in their investigation of Hepatitis B virus burden in Nasarawa state. Ezea et al., (2021) also reported similar findings regarding coinfection of both viruses in a seroprevalence study in Enugu, Nigeria. The highest prevalence of coinfection was observed in individuals aged 31-40. Discrepancies in our study maybe attributed to factors such as vector exposure possibilities and vaccine hesitancy.

In terms of gender distribution, females exhibited a higher seroprevalence across all infection categories compared to males. This aligns with the findings of Emeribe et al. (2021), who observed increased dengue seroprevalence among females in a recent study focused on Nigeria. Conversely, for hepatitis B virus, existing studies, including Omatola et al. (2020) research in Kogi State and Raheem et al. (2021) study in Yaba-Lagos, Nigeria, consistently indicate a higher prevalence among males. Additionally, Ezea et al., (2021) reported a higher seroprevalence among females than males in their study investigating Dengue in coinfection with Hepatitis B conducted in Enugu, Eastern Nigeria.

CONCLUSION

The current study unveiled a significant occurrence of dengue and hepatitis B virus, as well as the simultaneous presence of antibodies to both viral infections. Despite lacking statistical significance, the highest prevalence was noted among teenagers, predominantly females. This highlights the pressing need for immediate attention to adequately monitoring viral infections in young adults, especially teenagers. It is imperative to comprehend the seroprevalence of viral infections like Hepatitis B and Dengue virus among these young individuals to formulate targeted preventive measures, ensure timely medical interventions, and develop effective public health policies.

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