Vol.1, No.8, November-2023

APPLIED SCIENCES RESEARCH PERIODICALS

TABLE OF CONTENTS

EDITORIAL ADVISORY BOARD
DISCLAIMER

Prevention and Treatment of Diabetes Mellitus Using Vitality Therapy™ 01
Dorothy M. Murray and Kwasi Donyina

Amino Acid, Antinutritional and Sensory Properties of Ready to Eat Snack from Cassava, Soybean and Cricket Composite Flour 07
Torkuma, S.T., Leke, L and Ayuba, B.

Neutrosophic Structures in Statistical General Mathematical Functions 18
Adebisi Sunday Adesina

Pot Screening of Twenty Tomato Varieties (*Solanum lycopersicum L*.) in Response to Single and Interactive Effects of *Fusarium oxysporum f.sp. lycopersici* (Schlecht) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (Treub) 24
Okocha, A.H., Iheukwumere, C.C., Aguoru, C.U. and Olasan, J.O.

Effectiveness of Combined Anti-Retroviral Therapy [cART] on Viral Load and Cd 4 Count, in Reducing Progression to AIDS, in Improving the Quality of Life of Patients of HIV/AIDS in the Context of the U.N. Sustainable Development Goals (SDGs): A 5-Year Prospective Study in Himachal Pradesh (India) 39
Pankaj Katoch, Anup Kumar, & Jyoti Bala

EDITORIAL BOARD

DISCLAIMER

All the manuscripts are published in good faith and intentions to promote and encourage research around the globe. The contributions are property of their respective authors/owners and Applied Sciences Research Periodicals (ASRP) is not responsible for any content that hurts someone's views or feelings. Authors are responsible for if any plagiarism is found in published material.

Applied Sciences Research Periodicals
November 2023, Vol. 1, No. 8, pp. 01-06

Prevention and Treatment of Diabetes Mellitus Using Vitality Therapy™

Dorothy M. Murray and Kwasi Donyina

1. The Institute of Judo-Christian Studies and Immunology, Wellness and Longevity University, Kumasi and Global Campuses.
2. Wellness and Longevity University, Kumasi and Global Campuses.

Abstract:
Objective: Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use and other factors. The objective of this study was to find a protocol to prevent and treat the deadly disease. Methods: The volunteers were advised to drink about 500 millimeters of vital water™ before each session to enhance resonance and to prevent dehydration. Participants were irradiated with infrared energy with a wavelength of 5 to 20 microns in a sauna for 60 minutes every other day. In addition, the participants were encouraged to ensure that their diets contained multivitamins and minerals. Results and Conclusion: Diabetes affects the rich and the poor and it is a major disease which imposes economic challenge in both the developed and developing nations. This disease can be prevented and treated by Vitality Therapy™. Prevention and treatment of diabetes entails making the body alkaline by drinking Vital Water™ and avoid drinking acidic beverages like Coke and other soft drinks. A good and balanced diet are also essential. Irradiation of the body using infrared with the wavelength of 5 to 20 microns prevents and treats diabetes.

Keywords: Diabetes, Vitality Therapy™, Vital Water™, Infrared Energy, Multivitamins and Minerals.

INTRODUCTION

Diabetes is almost a worldwide epidemic. It is increasing rapidly, and the global prevalence is a significant cause for concern. According to the World Health Organization (WHO), 346 million people worldwide have diabetes (1). Diabetes affects the rich and the poor and it is a major disease which imposes economic challenge in both the developed and developing nations. Diabetes mellitus is a disease in which glucose is not sufficiently metabolized. This results in high glucose levels in blood and glucose in the urine. Cells can starve because glucose is not being metabolized.

Type 1 diabetes is also called "juvenile-onset diabetes" or "insulin-dependent diabetes" because the symptoms usually appear during childhood and insulin injections are necessary to treat it. It usually occurs after a viral infection triggers an immune response that results in the body destroying its own insulin-producing cells. Type 1 diabetes is an autoimmune disease in which the body's immune system damages the cells in the pancreas (Islet cells) that make insulin. Because insulin is necessary to process blood sugar, this leaves people with high blood sugar. The disease is caused by a lack of insulin, and it can be treated with insulin injections.

Type 2 diabetes is more common than type 1. Type 2 diabetes is caused by a deficiency in insulin production or by changes in insulin receptors on the target cells. In either case, blood glucose level may be high because cells do not receive the message to metabolize glucose.

Both types of diabetes can cause dangerous complications, including blindness, heart and blood vessel disease and kidney failure. A decrease in circulation may eventually require the amputation of limbs. In addition, a person with type 2 diabetes can experience life-threatening reactions to low blood sugar or extremely high blood sugar. A temporary form of diabetes can occur when a woman is pregnant. This form is called gestational diabetes and often has no symptoms. Though it usually disappears after the baby is born, more than 50% of women with gestational diabetes eventually develop type 2 diabetes later in life.

REVIEW OF RELEVANT LITERATURE

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use and other factors (2).

According to MAYO Clinic, some of the symptoms of type 1 diabetes and type 2 diabetes are (3):

- Feeling more thirsty than usual.
- Urinating often.
- Losing weight without trying.
- Presence of ketones in the urine. Ketones are a byproduct of the breakdown of muscle and fat that happens when there's not enough available insulin.
- Feeling tired and weak.
- Feeling irritable or having other mood changes.
- Having blurry vision.
- Having slow-healing sores.
- Getting a lot of infections, such as gum, skin and vaginal infections.

In spite of numerous researches over the years, there is no cure for diabetes and its complications. The objectives of our study are how to prevent and find cure for all types of diabetes (4).

Human Agency and Oversight

It includes both the ethical and the legal dimension as it refers to fundamental rights protection aimed at maintaining the balance between human control and technical progress in terms of human agency and oversight. Human beings shall be protected both as individuals and groups, taking into account inclusiveness, fairness, non-discrimination and vulnerabilities protection as paramount interests.

METHODOLOGY

An overweight female Caucasian with blue feet was wheeled to our clinical research facility. She pleaded for help. Her physician had advised her to get prepared for the amputation of both legs otherwise she would die. The type 2 diabetes had advanced to a stage that there was no adequate blood circulation or oxygen supply to the legs. The remaining volunteers were three normal males with type 2 diabetes. The volunteers were advised to drink about 500 millimetres of vital water™ before each session to enhance resonance and to prevent dehydration. Participants were irradiated with infrared with a wavelength of 5 to 20 microns in a sauna for 60 minutes each other

day. Figures 1 and 2 show a typical infrared sauna and a participant. In addition, participants were encouraged to ensure that their diets contained multivitamins and minerals.

DISCUSSION

A decrease in sugar content of the blood and insulin intake was observed after one week of the vital energy irradiation. The feet of the female participant began to change colour from blue to the normal Caucasian colour.

After 3 months of vital energy irradiation, the male volunteers were completely off insulin and after 5 and half months of irradiation, the female volunteer no longer needed insulin. She had also lost 40 lbs. of her weight.

The most important points for this clinical trial are the effect of vital energy irradiation on the endocrine system, blood circulation, oxygen and nutrient supply to the cells, the immune system, body's communication system and the functioning of the nervous system.

The islets of Langerhans are groups of cells within the pancreas that secrete insulin and glucagon. The islets are endocrine glands (ductless); the circulatory system carries their hormones to target cells. Insulin promotes the removal of glucose from the blood for storage as glycogen, fats and protein. It promotes the buildup of fats and proteins and inhibits their use as an energy source.

Glucagon is produced in the islets of Langerhans but by different cells from those that produce insulin. The effects of glucagon are opposite to those of insulin. It raises the level of glucose in the blood. Fig. 3 shows the normalization of glucose in the blood by insulin and glucagon.

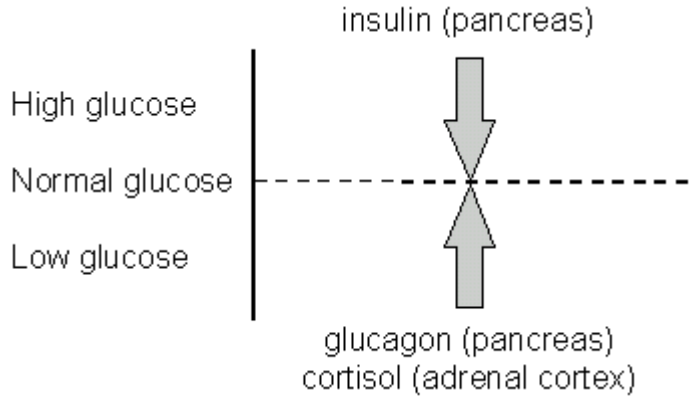

Figure 3: The Normalization of Glucose in The Blood by Glucagon and Insulin

What happens when patients with diabetes are irradiated with vital energy? Firstly, the irradiation of the endocrine system, including the pancreas, improves and restores the functions of the endocrine system. Secondly, the functions of the nervous system are enhanced. These two conditions lead to the restoration of the body's communication system. The autoimmune disease in which the body's immune system damages the cells in the pancreas that make insulin is reversed. Cells then receive the message to metabolize glucose as the circulatory system carrying hormones to target cells is enhanced. Vital energy irradiation lowered the blood sugar level. Blood circulation, oxygen and nutrient supply to the cells and the strengthening of the immune system contribute to the well-being of the diabetes patients. The immune system is enhanced by sleep and rest (5) and is impaired by stress (6). Vital energy enhances the immune system through relaxation, thereby removing stress. The irradiation also raises the body temperature.

The Endocrine System

The endocrine system is one of the body's main systems for communicating, controlling and coordinating the body's work. The major glands of the endocrine system are the hypothalamus, pituitary, thyroid, parathyroids, adrenals, pineal body, pancreas and the reproductive organs (ovaries and testes). Endocrine disorders happen when one or more of the endocrine systems in the body are not working well.

Vitality Therapy™ improves the functions of the endocrine system and enhances the body's immune system. The novel therapy exhibits positive effect on some autoimmune diseases like diabetes and Aids (7).

The Body's Communication System

The endocrine system acts with the nervous system to coordinate the body's activities. Both systems enable cells to communicate (information signal system) with others by using chemical messengers. The endocrine system uses chemical messengers (hormones) that are transported by the blood. They act on target cells that may be anywhere in the body. The endocrine system is slower than the nervous system because hormones must travel through the circulatory system to reach their target.

The chemical messengers used by the nervous system are neurotransmitters. Neurotransmitters travel across the synaptic cleft and bind to receptors on the target cell. The nervous system conducts signals much quicker than the endocrine system.

The nervous and endocrine systems work together to coordinate the actions of all other systems of the body to produce behavior and maintain homeostasis. When these functions, which rely on communication fail, disease sets in. Vital energy penetrates the body's tissues to a depth of 1.5 to 3 inches. It is hereby postulated that the radiation of the CNS and the endocrine system with vital energy restores the body's communication system, leading to a healthy body. The damaged cells are repaired.

CONCLUSION

Diabetes affects the rich and the poor and it is a major disease which imposes economic challenge in both the developed and developing nations. This disease can be prevented and treated by Vitality Therapy™.

Prevention of diabetes entails making the body alkaline by drinking Vital Water and avoiding acidic beverages like Coke and other soft drinks. Good and balanced diet are also essential. At least weekly irradiation of the body using infrared with the wavelength of 5 to 20 microns is recommended.

Treatment of diabetes is similar to the prevention methods described above. In the case of treatment, infrared irradiation is recommended at least every other day until the patient becomes well.

Figure 1: 2-Person Infrared Sauna

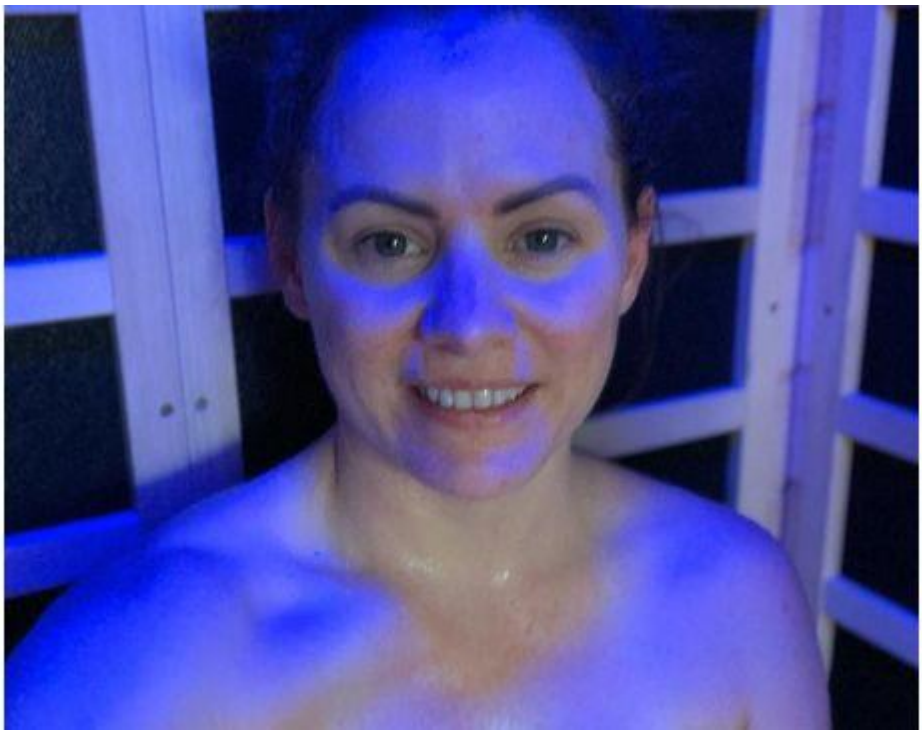

Figure 2: A Diabetic Patient Using Infrared Sauna to Increase Her Energy Level, Including the Energy for the Brain and the Endocrine System.

REFERENCES

1. World Health Organization Fact Sheet #312, August 2011
http://www.who.int/mediacentre/factsheets/fs312/en/index.html

2. National Institutes of Health,
https://www.ncbi.nlm.nih.gov/books/NBK551501/#:~:text=Diabetes%20mellitus%20(DM)%20is%20a%20metabolic%20disease%2C%20involving%20inappropriately,endocrinopathies%2C%20steroid%20use%2C%20etc.

3. https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444

4. Donyina K "Far Infrared Treatment for Diabetes: https://classic.clinicaltrials.gov/ct2/show/NCT00573456

5. Lange, T; Perras B, Fehm HL, Born J (2003). "Sleep Enhances the Human Antibody response to Hepatitis A Vaccination". *Psychosomatic Medicine* **65**: 831–835.

6. Khansari, DN; Murgo AJ, Faith RE (1990). "Effects of stress on the immune system". *Immunology Today* **11**: 170–175

7. Donyina K (2021), "Treatment of HIV/Aids Using Vitality Therapy™" J Clin Trials 11p:456.

Applied Sciences Research Periodicals
November 2023, Vol. 1, No. 8, pp. 07-17

Amino Acid, Antinutritional and Sensory Properties of Ready to Eat Snack from Cassava, Soybean and Cricket Composite Flour

Torkuma, S.T., Leke, L and Ayuba, B.

1. Department of Chemistry Benue State University, Makurdi

Abstract:
The amino acid, antinutritional and sensory properties of formulated ready to eat snacks prepared from yellow cassava, soybeans and cricket (protein source) composite flour was obtained using simultaneous equation and material mass balance method, it was determined and compared statistically with two proprietary snacks sold in the market. Essential amino acid profile showed that the formulated snack was significantly lower than the two proprietary snacks while the antinutritional properties were significantly higher in the formulated snack than the proprietary snack, but fell under the 0.50 safe permissible unit for antinutrient in formulated snacks, making bio-unavailability of calcium and magnesium needed for bone development minimal. Sensory evaluation showed that the formulated snacks did not differ significantly ($p = 0.05$) from the two proprietary snacks in colour flavour, taste smell, texture and overall acceptability. Sensory panellists had a high preference for the formulated snacks than the two proprietary snacks because the egg and soybeans component were mildly perceivable. The formulated snacks mostly complied with Emergency Food Product (EFP) recommendation for snacks. It therefore recommended that substitution of cassava flour, soybean flour and cricket flour using simultaneous equation and material mass balance equation in snacks formulation should be embarked upon by snacks industries as this will help in conserving national foreign exchange and improving the national value of snacks

INTRODUCTION

The formulation and deployment of Emergency food product (EFP) may hold potential in tackling protein energy malnutrition (PEM) among children, refugees and internally displaced persons in war torn and disaster-prone areas. This is because emergency food products are intended to provide a compact self-contained, high-energy, nutrient-dense emergency food for school children, refugees and internally displaced persons (IDP) and victims of disasters for a short duration at the initial stages of an emergency (Burgess 2005). Protein energy malnutrition (PEM), which may cause disease such as kwashiorkor, marasmus, and immune deficiencies, can be explained by several factors, the main one being the lack of quantitative and qualitative protein intake (Amal et al 2012). For children from 4 - 6 month, these conditions are usually due to the fact that breast milk no longer meets the needs for energy, protein and micronutrients including calcium, iron, Zinc, and vitamin A (Zoumas et al 2002). An EFP should contain 10-20 % of protein, 5-15 % of fat and 50-70 % of carbohydrates, 5-10 % of ash and 1-29 % moisture (Amal et al 2012). Furthermore, the EFP must be safe, palatable, and easy to dispense, easy to use and nutritionally complete (Zoumas et al 2002). There are many alternative forms of EFP one among them is bakery food product, snacks (Vanlaanen 2010).

snacks are an excellent food for the incorporation of different nutritionally rich ingredients, thus making it a useful tool in meeting the nutritional requirements of increasing global population (Adegoke *et al* 2017). Snacks have become very popular in Nigeria among all age groups particularly children and babies both in rural as well as urban areas owing to their sweet taste and this is perhaps because it comes ready to eat, low cost among other processed foods, varied taste, easy availability, good eating quality and relatively long shelf life (Adegoke *et al* 2017). Snacks are nutritive snacks made from unpalatable dough that has been transformed into appetizing product through the application of heat in the oven (Olaoye *et al*, 2007). In Nigeria, ready-to-eat baked products consumption is continually growing and there has been increasing reliance on imported wheat to sustain this trend (Akpapunam and Darbe 1999). Nigeria, moreover, grows staple crops other than wheat such as cassava, yam, sweet potatoes and cereals that can be used in baked foods (Chinma and Gernah 2007). It would therefore be economically advantageous if imported wheat could be reduced or even eliminated and the demand of baked foods such as snacks could be met by the use of domestically grown alternatives to wheat (Chinma and Gernah 2007). Snacks made from staple crops are high in carbohydrates, fats and calorie but low in protein, fiber and vitamin and mineral which make it unhealthy for daily use (Serrem et al, 2011). Moreover, snacks have only about 6-7 % space protein (Agarwal *et al*, 1990). This may be enhanced through incorporation of protein-rich food source such as soy bean, and cricket flours.

Cassava (*Manihot esculenta*), is a perennial woody shrub with edible root (Omolara and John, 2017). It grows in tropical and sub-tropical regions and is known by different names in different parts of the world, such as yuca, manioc and mandioca (Omolara and John 2017). Cassava is highly drought tolerant with the ability to grow on marginal lands where cereals and other crops do not grow well; it can also grow in soils where the nutrient levels are low. Because cassava roots can be stored on the ground for a long time (from 24 - 36 months in some varieties), the harvest is usually delayed until market processing, or other conditions are favorable (Fauquet and Fargette *1990*). It is a major staple food in Nigeria, consumed daily by more than 100 million people, it is known to be highly perishable and thus often processed immediately after harvest into gari, akpu, achicha and furaka (Olatunde *et al*, 2016). It is also an important source of calories to millions of people particularly in the tropics (Lasekan *et al*, 2016). The major limitations of cassava include low protein, low mineral and vitamin contents together with cyanide toxicity (Ihekoronye *et al*, 1985). The cassava amino acid such as methionine, lysine and tryptophan are also low in quality (Badifu *et al,* 2000). The commonly available white cassava can provide most of the body's daily energy requirements; it lacks micronutrients, such as vitamin A, that are essential for a healthy and productive life (Ayankunbi *et al*, 1991). Populations which eat a lot of white cassava do not receive adequate intake of good quality protein and such populations are prone to protein malnutrition, which may cause diseases such as kwashiorkor, marasmus, immune deficiencies and eye damage that can lead to blindness and even death (Ayankunbi *et al.*, 1991). Recently, new varieties of cassava have been produced and pro-vitamin A cassava which is rich in β-carotene is one of such varieties. Pro-vitamin A cassava is currently been used as an aid in reducing the prevalence of dietary vitamin A deficiency due to its high β-carotene content (Ihekoronye *et al*, 1985). According to (Omodamiro *et al*., 2019), pro-vitamin A cassava have the potential of providing up to 40 % of the vitamin A recommended daily requirements of children and women. Therefore, incorporation of soya bean and cricket flour into cassava flour for the production of snacks may hold potential for increasing the protein caloric value, amino acid, mineral and vitamin content of the resulting product (Badifu *et al.,* 2000).

Soybean (*Glycine max*) is among the major industrial and food crops grown in every continent and have long been recognized as a plant food that when compared with other plants, is relatively high in protein (40 %), lipid (20 %), minerals (5 %) and B vitamins for human nutrition (Lee *et al.*,2007). Moreover, most of the oilseeds contain 40-50 % oil, whereas soybean contains 18 % of oil (Badifu *et al,* 2000). The amino acid profile of soy protein is excellent among plant proteins (Tasnim *et al*., 2015). Hence, it is superior to other plant proteins as it contains most of the essential amino acids except methionine (Tasnim and Suman 2015). Soy protein directly lowers serum cholesterol levels (Mirrahimi *et al*., 2010). Soybeans also contain biologically active proteins such as enzymes, trypsin inhibitor hemagglutinins, and cysteine proteases very similar to papain (Tasnim *et al*., 2015). Soy food is quite important to us as they reduce the risk of heart disease improve mental health and physical abilities, memory power and hemoglobin level of children (Tasnim and Suman 2015).

Crickets (*Gryllus assimilis*) are large insects that live underground where they feed on the roots of plants in the soil (Oibiokpa *et al*., 2017). They are rich in essential nutrients; Cricket protein is considered complete proteins because it contains all the essential amino acids such as leucine, isoleucine, valine, methionine, tryptophan, threonine, lysine, histidine and phenylalanine. These are considered "essential" because it must be through diet (Ayieko and Millicent 2010). Cricket is a source of branched chain amino acid (BCAAS) crucial for muscle growth (Belluco *et al.,* 2013). It provides the following essential minerals; zinc, copper, iodine and manganese that are required by the body (Belluco *et al.,* 2013).it is also a good source of vitamin B_2 and B_7 rich in chitin, a probiotic fiber that may support gut health. It is a seasonal insect but can be reared in other state like Kano, Niger and Gombe States (Belluco *et al.,* 2013).

Malnutrition and hunger have become serious problems in Nigeria (Zoumas *et al*., 2002). The country's population which depends on staple foods such as cassava is prone to malnutrition which causes diseases such as kwashiorkor, marasmus and immune deficiencies. It has also been a problem because both men, young and old are suffering from insufficient and qualitative protein intake (Zoumas *et al*., 2002). For children and babies from 4 - 6 months, these conditions are usually due to the fact that breast milk no longer meets the needs for energy, protein and micronutrients including calcium, iron, zinc and vitamin A (Zoumas *et al*., 2002). Also there appear to be a growing need for an indigenous nutrient dense emergency food product (EFP) that can help curtail the problem of malnutrition experienced by affected population seeing as there is a growing number of internally displaced persons (IDP) and refugees arising from herders/farmer clashes, natural disasters, communal clashes e. t. c. The aim of this research was to produce and evaluate the amino acid, antinutritional and sensory properties of ready to eat snacks from cassava, soybean and cricket composite flours.

MATERIALS AND METHODS

Sources of Materials and Equipment

Soybean seeds, freshly harvested yellow cassava cultivar root and edible cricket was procured from the local farmer and cricket sellers, all in Gboko town, in Benue State, and was taken to the Department of Biological Science, Benue State University, for identification by the botanist and zoologist respectively.

Processing of Cassava Roots into Flour:

The method of international institute of tropical agriculture IITA (Messinger-Rapport *et al.,* 2009) was adopted. Three kilogram of cassava roots was washed manually, peeled with a knife, washed

again and cut into chips. The chips were soaked for 9 h in tap water at ambient temperature. The water was changed at intervals of 3 h after which the chips was rinsed and dried in an air. It was milled into flour using hammer mill and the resultant flour was sieved into a particle size of 80 µm. The flour was packaged in low density polyethene bags and stored for further use (Chinma *et al.*, 2007).

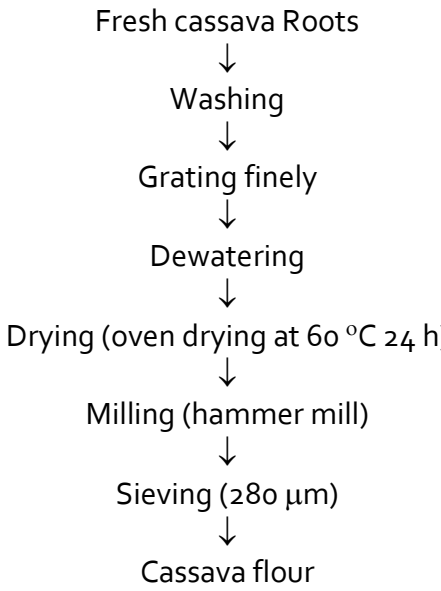

Figure 1: Flow chart for the production of cassava flour (**Chinma *et al.*, 2007**)

Processing of Soy Bean Flour:

The soya bean was processed into flour as outlined in the flow chart in Figure 2. The process ensures effective removal of most of the anti-nutritional factors.

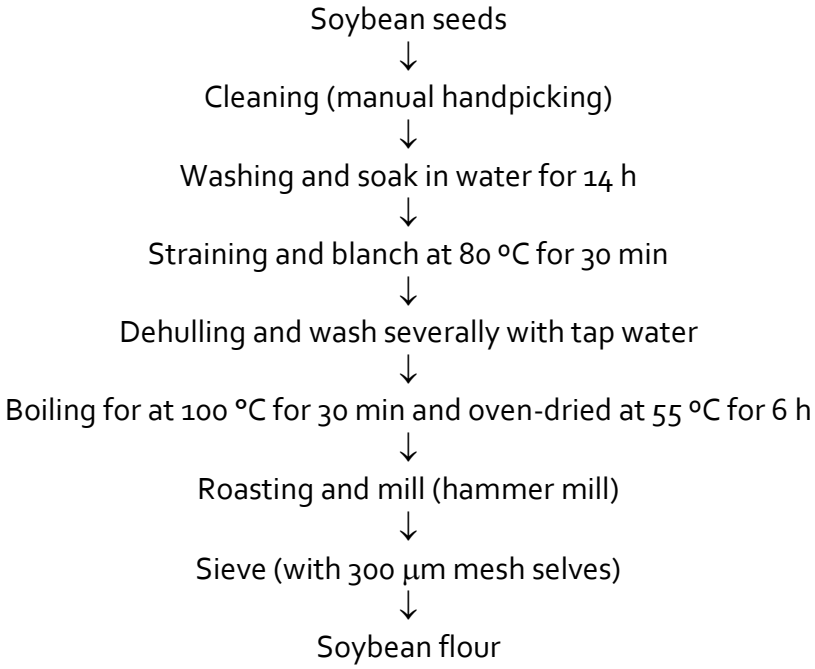

Figure 2: A flow chart for the Preparation of soybean flour

Processing of Cricket into Flour:

The cricket was processed into flour using the procedure shown in the flow chart below.

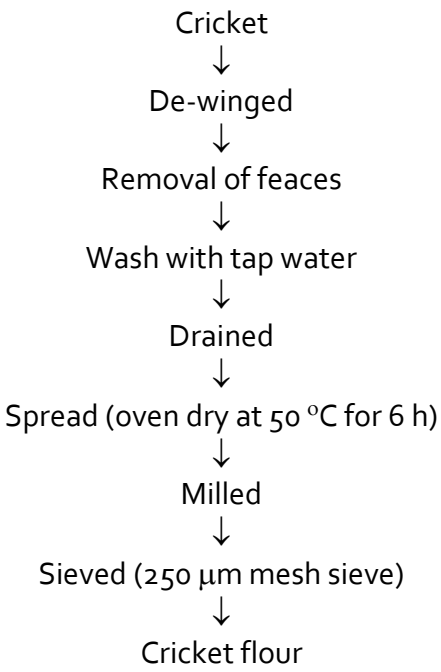

Figure 3: Flow chart for the production of cricket flour (Shittu *et al.*, 2007)

Determination of Amino Acid Profile of the Formulated and Two Proprietary Snacks

About 30 g of the sample was dissolved in 10 mL of 6 N hydrochloric acid containing 0.1 % of phenol. The sample was hydrolyzed under nitrogen at 110 °C for 24 h. After cooling and adjusting pH to 2.2 using NaOH, 0.5 mL of leucine was added as an internal standard. The sample was filtered through a 0.2 µm filter and 20 µl of the filtrate was analyzed by high performance liquid chromatography equipped with sodium oxidized column, cation exchange resin followed by post-column derivatisation of the amino acids to ninhydrin and spectrometric detection at 570 mm. The amino acids to be determined are essential amino acids such as; Valine, Tyrosine, Tryptophan, Cystine, Leucine, Iso-leucine, Lysine, Methionine, Phenylalanine, and threonine, and non-essential amino acids such as; Arginine, Histidine, Alanine, Aspartic, Glutamic, Glycine, Proline and Serine.

Evaluation of Anti- Nutrient Factors of The Formulated and Two Proprietary Snacks

Determination of Cyanogenic Glycoside:

The alkaline pictrate method described by Oke was adopted. Five grams (5.0 g) of samples was weighed and dissolved in 50 mL distilled water in corked conical flasks. The mixtures were allowed to stay overnight and then filtered. The extracts (filtrates) were collected and labeled. Different concentration of hydrogen cyanic acid (HCN) was prepared. The absorbance was taking in a spectrophotometer at 490 nm and the cyanide standard curve was plotted. One milliliter (1 mL) of sample filtrate and standard cyanide solution was measured into three (3) test tubes respectively and 4 mL of alkaline pictrate solution was added and incubated in a water bath for 15 min. After color development (reddish brown), the absorbance of the sample in the test tubes was taken in a spectrophotometer at 490 nm against a blank containing only 1 mL distilled water and 4 mL alkaline pictrate solution (1 g of pictrate and 5 g of sodium carbonate (Na_2CO_3) was dissolved

in a warm water in 200 mL flasks and made up to 200 mL with distilled water). The cyanide content for the sample was extrapolated from the cyanide curve.

Determination of Phytate:

The phytic acid was determined using the procedure described by Markkar (Olaoye *et al.*, 2005). Two grammes (2.0 g) of sample was weighed into 250 mL conical flask. 100 mL of 2 % concentrated HCL acid will be used to soak sample in the conical flask for 3 h and then filtered through a double layer of hardened filter papers. 50 mL of filtrate was placed in 250 mL beaker and 100 mL of distilled water was added to give proper acidity. 10 mL of 0.3 % ammonium thiocyanate solution was added into solution as indicator. The solution was titrated with standard iron chloride solution. The end point color was slightly brownish - yellow which persisted for 5 min. The percentage phytic acid was calculated.

Determination of Oxalate:

Oxalate was determined by using the method described by Oke (Olaoye *et al.*, 2005). One gram (1.0 g) of sample was placed in a 250 mL volumetric flask, 190 mL of distilled water and 10 mL of 6 M HCL was added. The mixture was warmed on a water bath at 90°C for 4 h and the digested samples were centrifuged at a speed of 2,000 rpm for 5 min. The supernatant was diluted to 250 mL. Three (3) 50 mL aliquots of supernatant were evaporated to 25 mL, and then the brown precipitate was filtered off and washed. The combined solution and washings were titrated with concentrated ammonia solution in drops until Salmon pink color of methyl orange changed to faint yellow. The solution was heated on a water bath to 90 °C and the oxalate was precipitated with 10 mL of 5% calcium chloride ($CaCl_2$) solution. The solution was allowed to stand overnight then centrifuged. The precipitate was washed into a beaker with hot 25 % H_2SO_4, diluted to 125 mL with distilled water and after warming to 90 °C it was titrated against 0.05 m $KMnO_4$. The oxalate content is given by the relationship that 1 Ml of 0.05 M $KMnO_4$ solution $_{= 0.00225}$ g oxalate (Omolara *et al.*, 2017).

$$\text{Oxalates content (mg/100 g)} = \frac{\text{T}\times[\text{Vme}]\ [\text{DF}]\times 2.4\times10^2}{MEXMf}$$

$$\text{Saponin content} = \frac{\text{weight of dry residue}}{weightofsample}\ x\ 100$$

$$\text{Xannine content} = \frac{\text{weight of dry residue}}{weightofsample}\ x\ 100$$

$$\text{Cyanogenic glycoside} = \frac{\text{weight of dry residue}}{weightofsample}\ x\ 100$$

$$\%\ \text{phytate} = \frac{100\text{xAu xcxvt}}{wxAsx1000xva}$$

Where

w	=	Weight of sample
au	=	Absorbance of sample
as	=	Absorbance of standard phytate solution
c	=	Concentration of standard phytate (mg / ml)
vt	=	Total extract volume
va	=	Volume of extract used

Where: T = titer of $KMnO_4$, Vme = Volume-mass equivalent (i.e 1 mL of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), DF = Dilution factor, VT/A VT = Total volume of filtrate (75 mL), A = Aliquot used (25 mL), ME = molar equivalent of $KMnO_4$, Mf = Weight of sample use

Determination of Trypsin Inhibitor:

The method outlined by Kakade (Olaoye *et al*., 2005) was employed. Two grams (2 g) of sample was weighed into a screw cap centrifuge Tube. Ten milliliter (10 mL) of 0.1 M phosphate buffer was added and the contents was shaken at room temperature for 1 h on a shaker. Each suspension obtained was centrifuged at 5000 rpm for 5 min and filtered through Whatman No. 42 filter paper. The volume of the filtrate was adjusted to 2 mL with phosphate buffer in test tubes. The test tubes were transferred to a water bath, maintained at 37 ºC. 6 mL of 5 % TCA solution was poured into one test tube to serve as a blank. 2 mL of casein solution was added to the test tubes, which was previously kept at 37 ºC, then incubated for 20 min. The reaction was stopped after 20 min by adding 6 mL of TCA solution to the experimental tubes and was shaken. The reaction was allowed to proceed for 1 h at a room temperature. The mixture was filtered through Whatman No 42-filter paper. Absorbance of filtrates from sample and trypsin standard solutions was read in a spectrophotometer at 280 nm.

Determination of Tannins:

The method described by Markkar (Olaoye *et al*., 2005) was adopted. Briefly, 400 mg of sample was placed into two conical flasks and 40 mL diethyl ether containing 1% acetic acid (v/v) was added, then the mixtures was properly mixed to remove the pigment materials. the supernatant was carefully discarded after 5 min and 20 mL of 70 % aqueous acetone was added and the flasks was sealed with cotton plug covered with aluminum foil, then kept in electrical shaker for 2 h for extraction. Each content in the flasks was filtered through Whatman filter paper and samples (filtrates) were used for analyzing. 50 mL of tannins extract from sample was taken into test tubes and the volume was made up to 1.0 mL with distilled water. 0.5 mL Folic ciocalteu reagent was added and mixed properly. Then 2.5 mL of 20% sodium carbonate solution was added and mixed. The mixture was kept for 40 min at room temperature, after which absorbance was taken using spectrophotometer and concentration was estimated from the tannic acid standard curve.

Determination of Saponins*:*

Five grams (5 g) of samples was weighed and mixed with 100 mL of 20 % ethanol. The suspension was heated and stirred continuously on a water bath for 4 h at about 55 °C. The mixture was filtered and the residue was re-extracted with 100 mL of 20 % ethanol. The combined extract was concentrated on a water bath to a volume of about 40 mL. The concentrate was washed with diethyl ether and extracted with n-butanol and the n-butanol extract was washed with 5 % aqueous sodium chloride. The residual solution was first heated in a water bath and then dried in the oven to constant weight. The saponin content was calculated in percentage (Olaoye *et al*., 2005).

Sensory Evaluation of the Formulated and Two Proprietary Snacks

The formulated snacks and 2 other proprietary Snacks brands were subjected to sensory evaluation. A total of twenty (20) untrained panelist drawn from the Benue State University, Center for Food Technology and Research (CEFTER) at the Benue State University, Makurdi, Benue State. Based on their familiarity with the product. The panelist was used for the evaluation. The parameter that was evaluated include taste, colour, flavor, texture and general acceptability.

The coded sample was served in clean white plastic plates at room temperature, in individual booths with adequate florescent lights. Sample was presented to the panelists at random and one at a time. They eat the samples and check how much they like or disliked each one and rate them as such. The panelist was given enough water to rinse their mouth between each sample. The nine-point hedonic scale (Chinma *et al*., 2007) was used for the evaluation.

Statistical Analysis

The result of the analysis was expressed as mean ± standard deviation and SPSS Statistical Package version 22.0 was used to analyze the variances using one-way analysis variance (ANOVA) post-hoc test was used to determine the differences between and within the different biscuit formulations and to compare with two (2) proprietary biscuits brands obtained from the market. The analysis was done at 95 % confidence level

RESULTS AND DISCUSION

The Derived Blend Proportions for Raw Flour.

Table 1 shows a detailed solution to the simultaneously equation and mass balance equation from which the blend proportions for the legume's tubers insect blend (cassava, soya-bean and cricket blend) and the final formulated food.

Table 1: Derived blend proportions for raw flour from simultaneous equation and material mass balance

Feed materials	Blend proportions (%)
Soybean	50.95
Cricket	25.11
Cassava	23.94

Blend Proportions of Components in Formulated Snacks

Table 2. shows that if the legumes tubers insect composite must meet emergency food product recommendation of 7 % fat and 20 % protein, then it must comprise 50.95 % soy-bean, 25.11 % cricket and 23.94 % cassava. The proportion of soybean flow is required in the composite is highest (50.95 %) followed by cricket because soybean flour is highest in protein and cricket is higher in fat and as such, both must complement tubers flour with the same nutrients

Table 2: Recipe for ready to eat food making

Ingredient	Blend proportions (%)
Cassava, soybean and cricket (CSC)	86.5
Sugar	2.5
Vegetable oil	3.0
Butter	2.5
Baking powder	3.0
Egg	2.5

Comparative Anti-Nutrient Analysis of Snacks

Anti- nutrient content of the formulated food in table 3 Shows that the anti - nutrient content in the formulated Snacks is higher than that of the proprietary snacks sold in the market except tennis which is lesser, but all fall within range of 0.50 safe permissible limits for anti-nutrients in formulated snacks. The implication is that the chances of anti-nutrient chelation of divalent

mineral nutrients such as ca and mg that are needed for bone development leading to their bio-unavailability will be minimal.

Table 3: Antinutritional Contents of Ready to eat Samples

S/N	Parameter	Formulated (%)	Control "A" (%)	Control "B" (%)
1.	Tannins	$0.10^{ab} \pm 0.01$	$0.21^{ac} \pm 0.02$	$0.39^{bc} \pm 0.04$
2	Phenol	$0.45^{ab} \pm 0.04$	$0.69^{ac} \pm 0.03$	$0.92^{bc} \pm 0.04$
3	Oxalate	$0.49^{a} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.10^{ab} \pm 0.01$
4	Flavonoids	$0.91^{ab} \pm 0.03$	$0.61^{ac} \pm 0.03$	$0.50^{bc} \pm 0.03$
5	Alkaloids	$0.47^{ab} \pm 0.03$	$0.24^{ac} \pm 0.01$	$0.39^{bc} \pm 0.03$

All values are triplicates means ± standard deviation. Different superscript along row depicts significant difference ($P \geq 0.05$).

Comparative Assessment of Amino Acid Profile of Snacks

Table 4 shows the result of the essential amino acid for the formulated snack is less than that of the proprietary snacks. The essential amino acid help to breakdown saturated fatty acid which cause cancer, obesity and type 2 diabetes

Table 4: Amino acid Profile of Ready to eat Samples

S/N	Parameter	Formulated (mg/100g)	Control "A" (mg/100g)	Control "B" (mg/100g)
1.	Leucine	$4.95^{ab} \pm 0.08$	$5.18^{ac} \pm 0.06$	$6.82^{ab} \pm 0.04$
2	Isoleucine	$3.16^{a} \pm 0.03$	$4.68^{ab} \pm 0.04$	$4.17^{ab} \pm 0.04$
3	Lysine	$2.05^{a} \pm 0.08$	$3.96^{ab} \pm 0.09$	$4.91^{ab} \pm 0.06$
4	Phenylalanine	$2.99^{ab} \pm 0.06$	$3.52^{ac} \pm 0.06$	$3.21^{bc} \pm 0.02$
5	Tryptophan	$0.21^{ab} \pm 0.02$	$0.84^{ac} \pm 0.04$	$0.61^{bc} \pm 0.04$
6.	Valine	$1.26^{ab} \pm 0.01$	$2.15^{ac} \pm 0.06$	$1.06^{bc} \pm 0.02$
7.	Methionine	$0.92^{ac} \pm 0.04$	$0.74^{ac} \pm 0.02$	$1.90^{bc} \pm 0.02$
8.	Histidine	$2.81^{a} \pm 0.04$	$2.17^{ac} \pm 0.02$	$2.42^{bc} \pm 0.04$
9.	Proline	$4.02^{ac} \pm 0.09$	$3.18^{ac} \pm 0.03$	$2.60^{bc} \pm 0.05$
10	Arginine	$5.61^{a} \pm 0.04$	$8.11^{a} \pm 0.05$	$7.26^{a} \pm 0.06$
11.	Tyrosine	$2.99^{a} \pm 0.02$	$4.25^{ab} \pm 0.04$	$3.92^{ab} \pm 0.05$
12	Cysteine	$1.06^{a} \pm 0.02$	$0.66^{ab} \pm 0.01$	$0.80^{ab} \pm 0.02$
13.	Alanine	$1.97^{a} \pm 0.06$	$2.46^{ab} \pm 0.02$	$2.03^{b} \pm 0.06$
14.	Glycine	$4.11^{a} \pm 0.04$	$3.60^{ab} \pm 0.03$	$4.62^{ab} \pm 0.06$
15	Glutamic Acid	$10.22^{a} \pm 0.33$	$8.90^{ac} \pm 0.04$	$10.45^{bc} \pm 0.09$
16	Threonine	$1.90^{ab} \pm 0.04$	$0.99^{ac} \pm 0.04$	$1.54^{bc} \pm 0.04$
17.	Serine	$2.31^{a} \pm 0.03$	$4.05^{ab} \pm 0.09$	$4.60^{ab} \pm 0.03$
18.	Aspartic Acid	$6.51^{ac} \pm 0.08$	$6.01^{ac} \pm 0.04$	$5.62^{ba} \pm 0.04$

All values are triplicates means ± standard deviation. Different superscript along row depicts significant difference ($P \geq 0.05$).

Sensory Evaluation of Snacks

Table 5 showed the sensory evaluation of formulated snacks and two proprietary snacks sold in the market the taste, colour texture, overall acceptability of the formulated snacks does not differ significantly ($P = 0.05$) from those of the proprietary snacks sold in the market. Most judges observed that the formulated snacks could be improved by further reducing its particle size to give it a much fine texture.

Table 5: Mean Sensory Scores of Ready to Eat Samples

S/N	Parameter	Control "A"	Control "B"	Formulated
1.	Colour	$7.98^{a} \pm 0.99$	$4.88^{ab} \pm 1.96$	$8.00^{b} \pm 1.07$
2	Texture	$7.75^{a} \pm 0.89$	$5.13^{ab} \pm 1.64$	$7.13^{b} \pm 0.99$
3	Smell	$7.88^{a} \pm 1.25$	$5.88^{b} \pm 2.42$	$7.50^{a} \pm 2.07$
4	Taste	$8.00^{a} \pm 1.41$	$6.13^{b} \pm 2.62$	$7.50^{c} \pm 1.41$
5	Flavour	$7.87^{a} \pm 1.25$	$6.50^{b} \pm 2.78$	$7.00^{a} \pm 1.41$
6	Total Acceptability	$7.75^{a} \pm 0.71$	$6.50^{b} \pm 2.51$	$8.00^{c} \pm 1.41$

All values are triplicates means ± standard deviation. Different superscript along row depicts significant difference ($P \geq 0.05$).

CONCLUSION AND RECOMMENDATION

The study successfully formulated a highly nutritious snacks from yellow cassava, soybean and cricket obtained from Gboko Local Government, Benue state, Nigeria. The formulated snacks mostly complied with Emergency Food Product (EFP) recommendation for snacks. The studied made remarkable contribution to knowledge as it has developed an efficient new formula with which locally grown feed materials can be blend into a potential nutritious low-cost emergency food product. Sensory assessment showed that the formulated snacks did not differ significantly ($p = 0.05$) from the two proprietary snacks sold in the market. But judges had a higher preference for the formulated snacks because of the residue flavor of the egg and soybean component were still slightly perceived. This study indicates that biscuit with higher protein content can be produced composite flour of cassava, soybean and cricket flours.

REFERENCES

Adegoke, G.O., Oyekunle, A.O, Afolabi, and M.O (2017) Functional Snacks from wheat, soya bean and turmeric (curcuma longa): optimization of ingredients levels using Response surface methodology. Research journal for food and nutrition vol, 1, issue 1, 2017, PP 13-22.

Agarwal, S.R. (1990). Prospects for small-scale biscuit industry in the nineties.Ind. food indust. 24:19-21.

Akpapunam, M.A, Darbe, J.W (1999). Chemical composition and functional properties of blend of maize, Barbara, Groundnuts flours for cookie production. Plants food for human Nutri, 46:147-155.

Ayankunbi, M.A, Kesninro O.O, Egele P (1991) Effect of methods of preparation on the nutrient composition of some cassava products. Gari (Eba), cafun and fufu. Food chem. 41:316-322.

Ayieko, M.A, and Millicent F.N, (2010). Climate change and the abundance of edible insects in the Lake Victoria Region. J. Cell Animal Biology 4; 112-118.

Badifu, G.O, J.C Hochi, J.V Dutse and M.A. Akpapunam, 2002. Use of mango mesocard flour to enrich the provitaminA content of a complementary food blend of maize and soya bean flour for porridge. Food Nutr. Bull., 21:316-322.

Belluco, S.C; Lassasom, Maggioletti C.C, Alonzi, M.G, Paoletti and Ricci A. (2013). Edible insects in food safety and Nutritional Perspective.A critical review.compr. Rev. Food Sci. Food safety 12: 296-313.

Burgess A. (2005) Guide de nutrition familial. Rome: organization des Nations unis pour l'alimentation et l'agriculture. P 121.

Chinma, C.E, Gernah D.I (2007) phy. and sensory properties of cookies produced from cassava/soy/mango composite flours. Journal of food technology 5 (3): 256-260.

Chinma,C.E and Gernah, D.I (2007). Physico-chemical and sensory properties of cookies produced from cassava soyabean/mango composite flours. Journal of food technology 5(3):256-260, 2007. (c) medwell journals, 2007.

Fauquet Claude; Fargette Denis (1990). "African Cassava Mosaic Virus: Etiology, Epidemiology, and Control" (PDF). Plant Disease. **74** (6): 404–11. doi:10.1094/pd-74-0404.

Ihekoronye, A.I and P.O Ngoddy, 1985. Intergrated food science and technology. Macmillan publishers, New York, PP: 296-301.

Lasekan, O.O, J.M. Babajide and O.A. Tuwase, 2004. Nutrient composition and acceptability of soy pururu flour and dough. Nig. food J.,21-7-10.

Lee, G.J, Wu, X., Shannon, G.J Sheper, A.D, Nguyen T.H. Soybean, chapter 1 in genome mapping and molecular breeding in plants; oilseeds Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany. 2007. Volume 2, PP. 1-53

Makkar HP, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. *Feed Sci Technol*. (2014) 197:1–33. doi: 10.1016/j.anifeedsci.2014.07.008

Messinger-Rapport B, Thomas D, Gammack J, Morley J (2009) Clinical update on nursing home medicine. J of the American Medical Directors Association 10: 530-53.

Mirrahimi, A.K. Strichaikul, C.E. Berryman, L.Wang, A. Carleton, S. Abdulnour, et al. 2010. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. J. Nutr. 140: 23025-23115.

Oibiokpa, F.I., Akanya, H.D., Jigam, A.A, Saidu, A.N (2017). Nutrient and Anti-nutrient compositions of some edible insect species in Northern Nigeria.A publication of college of Natural and applied sciences, Fountain University, Osogbo, Nigeria. ISSN: 2354-337X(online) 2350-1863(print).

Olaoye, O.A, Onilude, A.A, and Oladoye, C.O (2007), Breadfruit flour in biscuit making: Afr.J. Food sci., PP 20-23.

Olatunde, S.J, Adetola, R.O, Oyeyinka, A.T, Oyeyinka, S.A (2016). Production and quality evaluation of tapioca substituted with fermented bambara flour. Ukrainian food journal 5:36- 43.

Omolara, O.O, John O.O, (2017). Comparative studies on the phytochemicals nutrients contents and Anti-nutrients content of cassava varieties. Journal of the chemical Turkish chemical society chemistry.

Omolara, O.O, John O.O, (2017). Comparative studies on the phytochemicals nutrients contents and Anti-nutrients content of cassava varieties. Journal of the chemical Turkish chemical society chemistry.

Serrem, C.H kock, and J. Taylor. 2011. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuit fortified with defatted soy flour. Int. J. food sci. Technol. 46: 74-83.

Shittu, T., Raji, A. O. and Sanni, L. O. (2007). Effect of baking time and temperature on some physical properties of bread loaf.*Food Research International* 40: 280–290.

Tasnim, F. Suman, M., (2015). Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuit fortified with mushroom. Food Sci. and Nutr. 2015; 3(5): 363-369 doi: 10.1002/fsn 3. 228.

Vanlaanen, P 2010 Emergency food and Water supplies (Texas A&M: Agrilife Extension ELL 599).

Zoumas, BL., Armstrong L.E., Backstrand, J.R, Chemoweth, W.L., Chinachoti, P, Klein, B.P., Lane, H.W, Marsh, K.S, and Tolvanen, M 2002 High-Energy Nutrient-Dense Emergency Relief food production (Washington D.C: National Academy press)

Applied Sciences Research Periodicals
November 2023, Vol. 1, No. 8, pp. 18-23

Neutrosophic Structures in Statistical General Mathematical Functions

Adebisi Sunday Adesina

ORCID: 0000-0003-3766-0910

1. Faculty of Science, Department of Mathematics, University of Lagos, Akoka, Yaba, Nigeria, West Africa

Abstract:
The neutrosophic structures are very much relevant, vey essential and of course highly applicable to statistical and general mathematical concepts and structures. In practice. Sets in neutrosophic statistics are used, instead of crisp numbers in classical statistics. In addition, the neutrosophic concepts are undoubtedly very much applicable to a host of important mathematical ideals and concepts such as the transcendental functions and identities. In Classical Statistics all data are determined and this makes a very clear and vivid distinctions between neutrosophic statistics and classical statistics. In many cases, when indeterminacy is zero, neutrosophic statistics coincides with classical statistics. In many cases the neutrosophic can be used as a means for measuring the indeterminate data. Neutrosophic Data is the data that contains some forms of indeterminacy in this paper, efforts are intensified as much as possible to examine to some extent, the usefulness as well as the applicability of the concepts of neutrosophism in general mathematical functions and most especially in the area of problem solving.

Keywords: Neutrosophic Statistics, Classical Statistics, crisp numbers discrete neutrosophic data, continuous neutrosophic data, transcendental functions

INTRODUCTION

An extension of the classical statistics is the modern neutrosophic Statistics. In classical statistics the data is known and formed by crisp numbers while in neutrosophic statistics the data may have some forms of indeterminacies. Multiple problems such as the attributes in decision making processes are often being solved using the hesitant fuzzy linguistic information. In the case of the neutrosophic statistics, the data may be so direct. It may seem to be vague, ambiguous, incomplete, imprecise or even unknown. (Please, see [1]). In practice. Sets in neutrosophic statistics are used, instead of crisp numbers in classical statistics. In addition, the neutrosophic concepts are undoubtedly very much applicable to a host of important mathematical ideals and concepts such as the transcendental functions and identities.

ON NEUTROSOPHIC STATISTICS

In the neutrosophic statistics, the data may be ambiguous, vague, imprecise, incomplete, even unknown. Instead of crisp numbers used in classical statistics, one uses sets (that respectively approximate these crisp numbers) in neutrosophic statistics. (See [1])

Also, in neutrosophic statistics the sample size may not be exactly known (for example the sample size could be between 90 and 100; this may happen because, for example, the statistician is not sure about 10 sample individuals if they belong or not to the population of interest; or because

the 10 sample individuals only partially belong to the population of interest, while partially they don't belong).

In this example, the neutrosophic sample size is taken as an interval n = [90, 100], instead of a crisp number n = 90 (or n = 100) as in classical statistics.

Neutrosophic Statistics refers to a set of data, such that the data or a part of it are indeterminate in some degree, and to methods used to analyze the data. (See [1])

In Classical Statistics all data are determined; this is the distinction between neutrosophic statistics and classical statistics.

In many cases, when indeterminacy is zero, neutrosophic statistics coincides with classical statistics.

We can use the neutrosophic measure for measuring the indeterminate data.

Neutrosophic Data is the data that contains some indeterminacy.

Similarly, to the classical statistics it can be classified as:

- discrete neutrosophic data, if the values are isolated points;for example: $6+i1$, where $i1\in[0,1]$,7,26+$i2$, where $i2\in[3,5]$;
- and continuous neutrosophic data, if the values form one or more intervals, for example: [0,0.8] or [0.1,1.0] (i.e., not sure which one).

Another classification:

- quantitative (numerical) neutrosophic data; for example: a number in the interval [2, 5] (we do not know exactly), 47, 52, 67 or 69 (we do not know exactly);
- and qualitative (categorical) neutrosophic data; for example: blue or red (we don't know exactly), white, black or green or yellow (not knowing exactly) (see [1]).

Also, we may have:

- univariate neutrosophic data, i.e., neutro-sophic data that consists of observations on a neutrosophic single attribute;
- and multivariable neutrosophic data, i.e., neutrosophic data that consists of observations on two or more attributes.

As a particular case we mention the bivariate neutrosophic data, and trivariateneutrosophic data.

A Neutrosopical Statistical Number *N* has the form: $N=d+i$,

where *d* is the determinate (sure) part of *N*, and *i*is the indeterminate (unsure) part of *N*.

For example, $a=5+i$, where $i\in$ [0,0.4], is equivalent to$a\in$ [5,5.4], so for sure $a\geq5$ (meaning that the determinate part of a is 5), while the indeterminate part $i\in$ [0,0.4] means the possibility for number „a" to be a little bigger than 5.

While the Classical Statistics deals with determinate data and determinate inference methods only, the Neutrosophic Statistics deals with indeterminate data, i.e., data that has some degree of indeterminacy (unclear, vague, partially unknown, contradictory, incomplete, etc.), and indeterminate inference methods that containdegrees of indeterminacy as well (for example, instead of crisparguments and values for the probability distributions, charts, diagrams, algorithms, functions etc.

Neutrosophic Numbers of the form N = a+bI have been defined by W.B. Vasantha Kandasamy and F. Smarandache in 2003 [see 3], and theywere interpreted as "a" is the determinate part of the number N, and "bI"as the indeterminate In Imprecise Probability: the probability of an event is a subset T in [0,1], not a number p in [0, 1], what's left is supposed to be the opposite,subset F (also from the unit interval [0, 1]); there is no indeterminatesubset I in imprecise probability [see 4].

The function that models the Neutrosophic Probability of a random variable x is called *Neutrosophic distribution*: NP(x) = (T(x), I(x), F(x)), where T(x) represents the probability that value x occurs, F(x) represents the probability that value x does not occur, and I(x) represents the indeterminate / unknown probability of value x

I can deduce that the Neutrosophic idea is continuous within the interval while the crisp idea is discrete. And so, combining the cases we have as required and indicated: NP(x) = (T(x), I(x), F(x)) A true neutrosophic number contains the indeterminacy I with a non-zero coefficient.

Neutrosophic Real or Complex Polynomial.

A polynomial whose coefficients (at least one of them containing I) are neutrosophic numbers is called Neutrosophic Polynomials.

Similarly, we may have Neutrosophic Real Polynomials if its coefficients are neutrosophic real numbers, and Neutrosophic Complex Polynomials if its coefficients are neutrosophic complex numbers.

Proposition 1:
Let P(x) be a polynomial of degree 2. Then, thee exists two district solutions for P(x).

Proof: From [1] Let $P(x)$= (A + B.I) x^2 + (C + D.I) x + (E + F.I) = 0, and assume that the two solutions are given by $x_1 = a_1 + b_1I$ and $x_2 = a_2 + b_2I$ be the two neutrosophic real solutions of $P(x)$=0.

Then, set P(x) = (A+ B.I) [x – ($a_1 + b_1I$)]. [x- ($a_2 + b_2I$)] ≡(A + B.I) x^2 + (C + D.I) x + (E + F.I)

By equating the components, after the expansion of the. LHS, we have from the coefficients of x that C + D.I = - [A ($a_1 + a_2$) + (A ($b_1 + b_2$) + B ($a_1 + a_2$) + B ($b_1 + b_2$)) I], from where we would have that A ($a_1 + a_2$) = - C ..... (1) (A + B) ($b_1 + b_2$) + B ($a_1 + a_2$) = - D..... (2)

And from the constant term, we have, E + F.I = a_1a_2 + ($a_1b_2 + a_2b_1 + b_1b_2$) I, from where we have: a_1a_2 = E ..... (3), and $a_1b_2 + a_2b_1 + b_1b_2$ = F ..... (4).

We have a_1 = E/a_2, $a_1 + a_2$ = - C/A, a_2 + E/a_2 + C/A = 0.

Hence, $a_1 = \frac{-\frac{C}{A} \pm \sqrt{\frac{C^2}{A^2} - 4E}}{2} = K_1 \in \mathbb{C}$ and $a_2 = E/a_1 = \frac{2E}{-\frac{C}{A} \pm \sqrt{\frac{C^2}{A^2} - 4E}} = K_2 \in \mathbb{C}$.

Also, for b_1 and b_2 we have that $b_1 + b_2 = \frac{BC - AD}{A(A*B)} = K_3 \in \mathbb{C}$. So, $b_2 = k_3 - b_1$.

Substituting this into (4), we have $k_1k_3 - k_1b_1 + b_1k_2 + k_3b_1 - b_1^2 = F$, so that $b_1^2 + b_1(k_1 - K_2 - k_3) - k_1k_3 - F = o$ from where $b_1 = \frac{K_2 + K_3 - K_1 \pm \sqrt{K_1 - K_2 - K_3{}^2 + 4(K_1K_3 + F)}}{2} = K_4 \in \mathbb{C}$, And $b_2 = K_3 - b_1 = K_3 - K_4 = K_5 \in \mathbb{C}$

By this, the following proposition is immediate:

Proposition 2:
A real polynomial equation of degree $n$possesses an n number of neutrosophic solutions in a neutrosophic.

Transcendental Functions
Transcendental function, in mathematics, a function not expressible as a finite combination of the algebraic operations of addition, subtraction, multiplication, division, raising to a power, and extracting a root. The transcendental functions are: Exponential functions, Trigonometric functions, Logarithmic functions, Inverse trigonometric functions. Examples include the functions log x, sin x, cos x, e^x and any functions containing them. (See [2])

For this aspect, we begin as follows:

The Exponential Function
Let $f(x) = e^x$. Here, we set x = a + bI

Addition

$$e^{(a_1+b_1I)} + e^{(a_2+b_2I)} = e^{(a_1+b_1I)} + e^{(a_2+b_2I)}$$

Multiplication

$$e^{(a_1+b_1I)}.e^{(a_2+b_2I)} = e^{(a_1+b_1I)+(a_2+b_2I)} = e^{(a_1+a_2)+(b_1+b_2)I}$$

Division

$$\frac{e^{(a_1+b_1I)}}{e^{(a_2+b_2I)}} = e^{(a_1+b_1I)-(a_2+b_2I)} = e^{(a_1-a_2)+(b_1-b_2)I}$$

Trigonometric Functions
Let A = $(a_1 + b_1I)$and B = $(a_2 + b_2I)$

Then, Sin (A $\pm$ B) = SinACosB$\pm$ CosASinB = Sin$(a_1 + b_1I)$Cos$(a_2 + b_2I) \pm$ Cos$(a_1 + b_1I)$Sin$(a_2 + b_2I)$ and Cos(A $\pm$B) = CosACosB$\mp$SinASinB = Cos$(a_1 + b_1I)$ Cos$(a_2 + b_2I) \mp$Sin$(a_2 + b_2I)$Sin$(a_2 + b_2I)$

Moreover, let P = A + B = $(a_1 + b_1I)+(a_2 + b_2I)$ = $((a_1 + a_2) + (b_1 + b_2)I)$and Q = A – B = $((a_1 - a_2) + (b_1 - b_2)I)$

Then, sinP + sinQ = 2sin$\frac{1}{2}$(P+Q) cos$\frac{1}{2}$(P-Q), sinP-sinQ = 2cos$\frac{1}{2}$(P+Q) sin$\frac{1}{2}$(P-Q).

Also, cosP + cosQ = 2cos$\frac{1}{2}$(P+Q) cos$\frac{1}{2}$(P-Q), and cosP- cosQ = - 2sin$\frac{1}{2}$(P+Q) sin$\frac{1}{2}$(P-Q). For the tangent, tanA = sinA/cosB. So, let tan$\frac{1}{2}$A = t. Then, tanA = $\frac{2t}{1-t^2}$ = $\frac{2\tan\frac{1}{2}A}{1-\tan^2 A}$ = $\frac{2\tan\frac{1}{2}(a_1+b_1I)}{1-\tan^2(a_1+b_1I)}$ and tan(A$\pm$ B) = $\frac{tanA \pm tanB}{1\mp tanAtanB}$ = $\frac{tan(a_1+b_1I) \pm(a_2+b_2I)}{1\mp tan(a_1+b_1I)tan(a_2+b_2I)}$

Logarithmic Functions

By the rule of logarithms, difine A and B as stated above, then, we would have that: logA + logB = logAB = log$(a_1 + b_1I)(a_2 + b_2I)$= log$(a_1a_2+ (a_1b_2 + a_2b_1 +. b_1b_2)I)$ and logA - logB = logA/B = log$\frac{(a_1+b_1I)}{(a_2+b_2I)}$ = log($\frac{a_1}{a_2}$ + $\frac{a_2b_1 - a_1b_2}{a_2(a_2+ b_2)}$ $.I$)(see [1])

Change of Base:

Let A = $(a_1 + b_1I)$, B = $(a_2 + b_2I)$and C = $(a_3 + b_3I)$.

Then,

$$\log_B A = \frac{log_C A}{log_C B} = \frac{log_{(a_3+b_3I).(a_1+b_1I)}}{log_{(a_3+b_3I).(a_2+b_2I)}}.$$

APPLICATIONS

The neutrosophic ideas could be seen as having a great deal of importance and applications in analyzing the states, status and nature of any given mathematical structures and the way to make use of them in reality and general practical sense.

CONCLUSION

Neutrosophic and fuzzy science has been viewed from statistical as well as mathematical dimensions such as the transcendental functions and the like.

FUNDING

This research received no external funding

ACKNOWLEDGMENTS

The author is grateful to the anonymous reviewers for their helpful comments and corrections which has improved the overall quality of the work.

CONFLICTS OF INTEREST

The author declares that there is no competing of interests.

REFERENCES

[1] FLORENTIN SMARANDACHE (2014) Introduction to Neutrosophic StatisticsSitech& Education Publishing

[2] https://en.wikipedia.org/wiki/Transcendental_function

[3] W. B. Vasantha Kandasamy, FlorentinSmarandache, *Fuzzy Cognitive Maps andNeutrosophic Cognitive Maps*, Xiquan, Phoenix, 211 p., 2003, http://fs.unm.edu/NCMs.pdf

[4] F. Smarandache(2013) Introduction to Neutrosophic Measure, Neutrosophic Integral,and Neutrosophic Probability, Sitech Publishing House, Craiova, http://fs.unm.edu/NeutrosophicMeasureIntegralProbability.pdf

Applied Sciences Research Periodicals
November 2023, Vol. 1, No. 8, pp. 24-32

Pot Screening of Twenty Tomato Varieties (*Solanum lycopersicum L.*) in Response to Single and Interactive Effects of *Fusarium oxysporum f.sp. lycopersici* (*Schlecht*) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (*Treub*)

Okocha, A.H., Iheukwumere, C.C., Aguoru, C.U. and Olasan, J.O.

1. Plant Biotechnology and Molecular Genetics Unit, Department of Botany, Federal University of Agriculture Makurdi, Makurdi 970212, Benue State, Nigeria

Abstract:
Pot screening of twenty (20) tomato varieties was carried out with a view to determining wilting and root gall responses in terms of resistance or susceptibility to single and interactive effects of *Fusarium oxysporum f.sp. lycopersici* (Schlecht) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (*Treub*). The experiment was carried out in the screen house of the Department of Crop Protection, University of Abuja. Seeds of twenty varieties of tomato were sourced from the National Centre for Genetic Resources and Biotechnology (NACGRAB) and National Institute for Horticultural Research (NIHORT) in Ibadan. The test pathogens (*F. oxysporum* and *M. javanica*) were characterized conventionally and subsequently identified using molecular method through Polymerase Chain Reaction and gene sequencing techniques carried out carried out in the Bioscience/Molecular /Virology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The Completely Randomized Design (CRD) was used with 4 treatments and 5 replications per treatment. The disease symptomology of the interaction was documented. As a result, all the varieties recorded an average wilt severity of 15.00 ±1.58 and root gall of 37.20 ± 3.71. The red cherry tomato had the highest wilt severity (40.0%) caused by *F. oxysporum* while *Tropimech* (75.6) and Alausa (75.5) had the highest number of root galls caused by M. javanica. Simultaneous inoculation of *F. oxysporum* and *M. javanica* in pot screening experiment resulted in high Fusarium wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry. However, Onitrye variety showed tendency for resistance to both pathogens. On the basis of their responses and performances, four varieties were selecte: Onityre, Zumoured, Roma savanna and Riogrande. These varieties should be subjected to further pot and field evaluations to determine the effects of the pathogens on their agronomic characteristics including growth and yield parameters, and make an informed decision to growers and breeders.

Keywords: Fusarium oxysporum, Meloidogyne javanica, Screening, Tomato, Responses

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide. Tomato has its origin in the South American Andes (Joy *et al*., 2015). It is an annual plant grown in many parts of Nigeria both as wet and dry season crops. World production of tomato was estimated at 162 million tons of which Nigeria produces about 555.630 tones (Adenuga *et al*., 2013; FAOSTAT, 2014). Tomato and tomato-based foods provide a wide variety of nutrients and many health-

related benefits to the body. In regions where it is being cultivated and consumed, it constitutes a very essential part of people's diet (FAOSTAT, 2014). It can be consumed fresh or processed into paste. It is also rich in minerals, vitamins, essential amino acids, sugars and dietary fiber (Aneta *et al.*, 2015). The fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes.

Tomato production is very important to the food security of Nigeria where different varieties are grown as a result of breeding efforts (Joy *et al*., 2015). However, tomato production is hampered several biotic stresses including pathogenic fungi, bacteria, viruses and nematodes (Adenuga *et al*., 2013). Interactive effects of *Fusarium* and *Meloidogyne* spp have been implicated in the poor yield of tomato (FAOSTAT, 2014; Waheed *et al*., 2014). This accounts for about 90-100% yield loss of the crop as a result of interaction of the pathogens in diseased crops which cause augmentation of wilt disease incidence and severity (FAOSTAT, 2014; Waheed *et al*., 2014). The use of resistant varieties of crops against *Meloidogyne* spp and *Fusarium* spp are cheap and also an alternate method of managing *Fusarium* wilt disease and root-knot nematodes population in the vegetable crops as compared to fungicides and nematicides which most often leave toxic residues in the soil (Muhammad *et al*., 2015). Therefore, the use of tomato varieties that are resistant to the myriads of pathogens that attack tomato plants are of significant importance (Bawa *et al*., 2014). Furthermore, identification and selection of more tomato varieties with resistant factors to *Fusarium* wilt diseases and root- knot nematode will reduce losses and increase yield as well as fruit quality. This will eventually enhance the economic power of rural peasant farmers that are engaged in the production of the crop. The emergence of these resistant varieties will no doubt, provide the much-needed alternative methods for effective management of nematode-*Fusarium wilt* disease complexes in tomato. The aim of this study was to screen twenty (20) tomato varieties and determine their responses to single and interactive effects of *Fusarium oxysporum* f.sp. *lycopersici* (Schlecht) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (Treub).

MATERIALS AND METHODS

Sources of Materials and Study Area

Seeds of twenty tomato varieties were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan and National Institute for Horticultural Research (NIHORT) Ibadan Oyo state. The varieties were: Uc82, Roma VF, Beske JM4, Ibadan Local Cobra F1, Alausa, Beske Jm3, Hanit, Assila, Tropimech, Plantnium, Onitrye, Tolin 1 ks, Roma Savanna, Chibli, Riogrande, Ife 1 tomato, Gamad , Zumoured and Red Cherry. Extensively galled roots of tomato were collected from an infested farm land in Bounguo-Kwali village in Kwali Area Council of the Federal Capital Territory (FCT) in Nigeria. A screening pot experiment was carried out in the screen house of the Department of Crop Protection, University of Abuja.

Sterilization of Materials

Soil samples from fertile land were collected at the back of Science Faculty block of University of Abuja. Sterilization of soil and seeds was done following the procedure outlined by Nikki (2015) and Ganpati and Judy (2014). Isolation and culturing were conducted under aseptic conditions in the laminar flow cabinet.

Morphological Identification of Nematode

Infected roots were carefully teased out in the tray using a pair of forceps and a scapel to remove the adult female nematodes for the perineal patterns (Aneta *et al*., 2015; Bello *et al*., 2015) and

confirmed with the pictorial key described by Eisenback *et al*. (1981) to determine the identity of the nematode.

Morphological Identification of Fungus

A diseased tomato stem that was suspected to be infected by the fungus *Fusarium oxysporum* (Schlecht) Snyder and Hansen f.sp. *lycopersici* was cut into bits and sterilized in 1.05% sodium hypochlorite solution for 5 minutes. They were then rinsed in 6 changes of sterile distilled water and dried in sterile blotter paper (Iheukwumere *et al*., 2009; Bonger *et al*., 2016). The cut tissues where aseptically placed on each plate containing water agar. The plates were incubated at room temperature at 27 ± 3°C for 3 days before being sub cultured on Potato Dextrose Agar plates. The plates were then incubated for 5-7 days and sub cultured repeatedly on a clean PDA until pure cultures of the isolate was obtained (Iheukwumere *et al*., 2008; Mohit *et al*., 2014). The pure culture of the fungus was observed for colony and spore characteristics.

Molecular Characterization of Pathogens

Molecular characterization was carried out in the Bioscience /Virology Molecular Laboratory of the International Institute of Tropical Agriculture (IITA). Standard methods were employed in DNA extraction (Mwangi *et al*., 2019; Zijlstra *et al*., 2021) and Polymerase Chain Reaction. Sequence Characterized Amplified Region (SCAR) markers (F-GGTGCGCGATTGAACTGAGC and R–AGGCCCTTCAGTGGAACTATAC) were used as primers for the amplification of nematode DNA. Internal transcribed spacer molecular markers (ITS4: TCCTCCGCTTATTGATATGC and ITS5: GGAAGTAAAAGTCGTAACAAGG) were used for the amplification of the fungus DNA. Amplicons were separated on the agarose gel electrophoresis chamber and viewed under UV transilluminator and photographed. The PCR products were sequenced at GATC Biotech AG, Pretoria South Africa using the Sanger's methods.

Seed Preparation and Planting

Seeds were sterilized for 5 minutes in 1.05% sodium hypochlorite and rinsed for 5 minutes in 6 changes of sterile distilled water prior to planting (Koenning and McClure, 1981; Ganpati and Judy, 2014). Four hundred perforated polythene bags of 15-centimeter diameter and 20cm height were filled with 1kg sterilized sandy-loamy soil. They were used as experimental pots. The twenty tomato varieties were with five replicates each of the experimental pots. A plant was maintained per pot at one week (seven days) after planting and I ml of the suspension containing 500 juveniles (J_2) of *M. javanica* was inoculated in the soil around the root system of the plant.

Inoculation of Potted Test Plants

Plants were not watered the day before inoculation. Seven–day old seedling was inoculated with 500 juveniles of the nematode and 1.0×10^6 / ml spore suspension of the fungus per plant (Waheed *et al*., 2014; Nasrin *et al*., 2015). The experiment was arranged in a completely randomized design with 4 treatments and 5 replications per treatment on a cemented platform in a screen house at temperature of 32 ± 3°C. Treatments given to the 7-day-old seedlings are gigen as follows:

N= Inoculation of test plant with 500 juveniles (J_2) of nematodes only
F= Inoculation of test plant with 1.0×10^6/ ml spore suspension of the fungus only
N+F= Simultaneous inoculation of test plants with 500 juveniles of nematodes and 1.0×10^6/ ml spore suspension of the fungus)
C= the uninoculated control plants

Harvesting of Tomato Plants

The test plants were harvested eight weeks after inoculation. To ensure easy removal of the plants from the soil, the sides of the polythene sleeves were pressed to loosen the soil. The soil was then removed from the roots by gently shaking the plants. The roots of the harvested tomato were each washed separately and dabbed dry with tissue paper (Kankam and Adomako, 2014).

Disease Assessment

Plants were observed weekly for the appearance of symptoms such as wilts and leaf yellowing. The plants were also watered on alternate days till the end of the experiment at eight weeks after inoculation.

Root gall index were measured on a scale as 0 = No galls (Highly Resistant, HR); 1 =1-2 galls (Resistant, R); 2 =3-10 galls (Moderately Resistant, MR); 3 =11-30 galls (Moderately Susceptible, MS); 4 =31-100 galls (Susceptible, S); 5 = More than 100 galls (Highly Susceptible, HS (Muhammed *et al.*, 2014). Disease severity by the fungus were scored on a scale of 0-5 as follows: 0 = No wilt (Highly Resistant), 1 = 1-10% wilted (R: Resistance); 2 = 11-20% wilted (Moderately Resistant, MR); 3 = 21-30% wilted (MS: Moderately Susceptible); 4 = 31-50% wilted (S: Susceptible) and 5 = 51-100% wilted (HS: Highly Susceptible) (Muhammed *et al.*, 2014). Wilt severity was assessed by visually counting the number of infected plants (wilted plants) after inoculation and dividing it by the total number of both healthy and infected plant in each variety and then multiplying by 100 (Mohit *et al.*, 2014). Plant showing wilting and dropping was then quantified on the basis of scale, of percentage wilt severity and was calculated with this formula:

$$\text{Percentage wilt severity} = \frac{\text{Number of plants wilted}}{\text{Total plant population}} \times 100$$

Data Analysis

The Genstat 17 application package was used to analyse the data set using the one-way ANOVA (Analysis of variance) tools. Mean separation was done using the LSD method at P≤0.05.

RESULTS

Table 1 showed that there were significant differences at (P ≤ 0.05) in percentage wilt severity among the tomato varieties when *Fusarium oxysporum* was inoculated singly. Red cherry tomato plant had the highest percentage wilt severity of (40.0%) and it was susceptible to the fungus. Tropimech (20.0%), Riogrrande (20.0%) and Ife (20.0%) varieties were moderately resistant to the fungus. Some varieties including Gamad, Ibadan local, Chibili, Plantinum, Assilia, Alausa, Haint, Zumoured and Roma Savanna varieties were resistant to the fungus. Four varieties (Roma Savanna, Cobra, Onitrye and Roma VF had no cases of wilting (0.0%) and were highly resistant to the fungus. All the varieties had an average wilt severity of 15.00 ±1.58.

Table 2 showed the effects of single inoculation of *M. javanica* on the number of root galls of twenty tomato plant varieties. There were significant differences in the number of galls among the tomato varieties (P ≤ 0.05). Tropimech (75.6) and Alausa (75.5) had the highest number of galls and were susceptible to the root-knot nematode, followed by Gamad and Regrade (60.4). The lowest number of galls were observed among Plantimum (1.0), Onityre (0 .90) and Haint (0.80^{k} varieties resistance to the nematode. There was no variety found to be highly resistant or highly susceptible to the nematode. All the varieties produced an average of 37.20 ± 3.71 galls

Table 3 shows the effects of simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment. There were significant differences among the tomato varieties (P ≤ 0.05). The highest wilt severity recorded was 50.0% as observed in Beske 11 Jm4, Beske 1 Jm3 and Red cherry varieties. This made susceptible to the fungus *F. oxysporum*. Tropimech and Ife 1 recorded 30.0% wilt severity and they were moderately susceptible to the fungus. Chibili (10.0%), Roma Savanna (10.0%), Cobra F1 (10. 0%), Onitrye (10.0%) and Roma VF (10.0%) had the lowest wilt severity and were resistant to the fungus. No variety was found to be highly resistant or highly susceptible to the nematode. Variety mean wilt severity was 27.00 ±2.38. Significant differences (P ≤ 0.05) were recorded in the number of root galls among the tomato varieties. Beske 11 Jm4, (10.30) and Beske 1 Jm3 (29.70) had the highest number of root galls being susceptible to nematode while Plantimum (0.80), Onitrye (0.60) and Haint (0.50) had the lowest value being resistant to the nematode. None of the varieties was found to be highly resistant or highly susceptible to the nematode. Variety mean number of galls was 24.63 ± 2.60.

Table 1: Effect of single infection of *F. oxysporum* on percentage wilt severity and responses on twenty tomato varieties in pot screening experiment

Tomato Varieties	% Wilt Severity	Wilt responses
Red Cherry	40.00 [a]	S
Beske Jm3	30.00 [b]	MS
UC82B	30.00 [b]	MS
Tolin I ks	30.0 0[b]	MS
Beske Jm4	30.00 [b]	MS
Tromipech	20.00 [c]	MR
Riogrande	20.00 [c]	MR
Ife 1	20.00[c]	MR
Gamad	10.00[d]	R
Ibadan Local	10.00[d]	R
Chibili	10.00[d]	R
Plantimum F1	10.00[d]	R
Assisla	10.00[d]	R
Alausa	10.00[d]	R
Haint	10.00[d]	R
Zumoured	10.00[d]	R
Roma Savanna	0.00[e]	HR
Cobra F1	0.00[e]	HR
Onitrye	0 00[e]	HR
Roma VF	0.00[e]	HR
Variety means	15.00 ±1.58	
LSD at P ≤ 0.05	0.42	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ Significantly y (P ≤ 0.05) according to Duncan's multiple range test. Gall indices Percentage wilt severity on a scale of 0-5 where 0= No wilt (Highly resistant), 1 = 1-10% wilted (Resistant) 2 = 11-20% wilted (Moderately resistant), 3 = 21-30% (Moderately susceptible), 4 = 31-50% wilted (Susceptible) and 5 = 51-100% (Highly susceptible

Table 2: Effect of single infection of *M. javanica* on number of root galls and responses on twenty tomato varieties in pot experiment

Tomato varieties	Number of galls	Responses
Tropimech	75.60 [a]	S
Alausa	75.50 [a]	S
Gamad	60.40[b]	S
Riogrande	60.40[b]	S
Tolin I Ks	59.70[b]	S
Roma Savanna	56.10[b]	S
Ife 1	53.50[c]	S
Ibadan local	51.10[d]	S
Beske Jm4	50.80[d]	S
Red cherry	44.10[d]	S
Roma VF	40.70[f]	S
Cobra F1	38.80[h]	S
UC82B	36.90[h]	S
Assila	15.00[i]	MS
Beske Jm3	13.80[i]	MS
Zumoured	5.00[j]	MR
Chibili	3.90[j]	MR
Plantimum	1.00[k]	R
Onityre	0.90[k]	R
Haint	0.80[k]	R
Variety means	37.20 ± 3.71	
LSD at P ≤ 0.05	1.53	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ significantly (P ≤ 0.05) according to Duncan's multiple range test. Gall indices on the scale of 0-5 where 0= No galls (Highly resistant), 1 = 1- 2galls (Resistant), 2 = 3-10 galls (Moderately resistant), 4= 11-30 galls (Moderately susceptible), 5= More than 100 galls (Highly susceptible)

Table 3: Effects of simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment

Tomato varieties	(%) Wilt severity	Responses	Number of galls	Responses
Beske 11 Jm4	50.00[a]	S	10.30[f]	MS
Beske 1 Jm3	50.00[a]	S	29.70[d]	MS
Red cherry	50.00[a]	S	26.60[ed]	MS
Riogrande	40.00[b]	S	43.60[bc]	S
UC82B	40.00[b]	S	24.60[ed]	MS
Zunoured	40.00[b]	S	0.90[g]	R
Tolin 1 KS	40.00$_b$	S	28.60[ed]	MS
Tropimech	30.00[c]	MS	43.30[c]	S
Ife 1	30.00[c]	MS	51.10[a]	S
Ibadan local	20.00[d]	MR	43.70[bc]	S
Assilia	20.00[d]	MR	2.90[g]	R
Gamad	20.00[d]	MR	50.00[ba]	S
Haint	20.00[d]	MR	0.50[g]	R
Alausa	20.00[d]	MR	50.20[ba]	S
Plantimum	20.00[d]	MR	0.80[g]	R
Chibili	10.0[e]	R	2.80[g]	MR
Roma Savanna	10.0[e]	R	49.80[ba]	S

Cobra F1	10.0^{e}	R	10.2^{f}	MS
Onitrye	10.0^{e}	R	0.60^{g}	R
Roma VF	10.0^{e}	R	22.30^{e}	MS
Variety means	27.00 ±2.38		24.63 ± 2.60	
LSD at P≤ 0.05	2.35		3.02	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ significantly (P ≤ 0.05) according to Duncan's multiple range test. Gall indices Percentage wilt severity on a scale of 0-5 where 0= No wilt (Highly resistant), 1 = 1-10% wilted (Resistant) 2 = 11-20% wilted (Moderately resistant), 3 = 21-30% (Moderately susceptible), 4 = 31-50% wilted (Susceptible) and 5 = 51-100% (Highly susceptible

DISCUSSION

In single infection with pathogens during pot screening experiment, it was found that red cherry tomato had the highest wilt severity and high susceptibility to *F. oxysporum* whereas Tropimech and Alausa varieties were predisposed to gall formation as induced by *M. javanica*. Siddiques *et al*. (2022) recently reported that the disease wilt severity on tomato was an indication of the virulence of the pathogens on the crop tested. Bonger *et al*. (2016) reported that resistant /moderately resistant varieties have gene resistance to *M. javanica* and that root of resistant varieties reacted to root-knot nematodes attack by reducing its catalase activity, thus conferring resistance to the host plant. Resistant plants may possess active principles that inhibit candidate enzymes involved in pathogenicity. This mechanism might explain the resistance pattern to nematode and fungus as observed in few tomato varieties tested. The above observation is in agreement with Zahid *et al.* (2021) who reported that resistance and susceptibility to root-knot nematode reflects the effect of the plant product on the nematode's ability to reproduce in the host plant. The comparable responses of all the twenty tomato varieties tested for resistance to nematode infection showed that some of the tomato varieties had resistance genes that directed the synthesis of inhibitory proteins which in turn conferred to the host plant the ability to stop the penetration, development and reproduction of nematode juveniles.

Simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment resulted in high *Fusarium* wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry as a result of their susceptibility to *F. oxysporum* and *M. javanica* respectively. In spite of this, some varieties were screened and selected for their resistance to the pathogens. For instance, Roma Savanna, Cobra, Onitrye and Roma VF had no cases of wilting and were highly resistant to *F. oxysporum*. Onitrye tomato variety showed tendency for resistance to both *F. oxysporum* and *M. javanica* while other varieties showed tendency for resistance to only one of the two pathogens.

The differential responses of the twenty screened varieties to fungus and nematode infection could possibly be due to genetic factors, depending on the depending on the susceptibility or resistance pattern of the varieties to infections. The above observation was supported by Kumar *et al.* (2017) who found that all genotypes of the tested crops showed variation in responses to root-knot nematode from resistance to susceptibility. This implies that some varieties were more tolerant/resistance to disease than others. This view agrees with the findings of Aslam *et al*. (2016) who similarly noted the influence of genetic makeup of different cultivars on the *Fusarium* wilt severity. The present study agreed with the views of Karssen and Moenens (2021) who reported that susceptible tomato plant varieties supported greatest/ maximum number of juveniles penetrated and completed their development to maturity as shown by highest number of galls present in the roots. On the other hand, resistant and moderately resistant tomato varieties

allowed only a limited number of juveniles of *M. javanica* to penetrate the roots, leading to maturity as an evident by number of galls on their roots. This was the position of other authors who reported that resistant cultivars contained fewer developed nematodes than susceptible plants (Ansari *et al.,* 2014). Tomato crop, being an important vegetable food crop has received attention among breeders to meet set objectives. Some varieties have been improved for disease resistance and yield among other agronomic qualities.

CONCLUSION

Simultaneous inoculation of *F. oxysporum* and *M. javanica* in pot screening experiment resulted in high *Fusarium* wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry. However, Onitrye variety showed tendency for resistance to both pathogens. On the basis of their responses and performances, four varieties were selecte: Onityre, Zumoured, Roma savanna and Riogrande. These varieties should be subjected to further pot and field evaluations to determine the effects of the pathogens on their agronomic characteristics including growth and yield parameters, and make an informed decision to growers and breeders.

REFERENCES

Adenuga, B., Anwar, S A. and Khan, S.A. (2013). Evaluation of Tomato Genotypes against Meloidogyne *incognita* Infection. *Pakistan Journal of Phytopathol*ogy, 23(1): 31-34

Aneta, T. G., Kandrzey, H. and Kononowicz, K. (2015). Tomato (*Solanum lycoperiscum* L.) in the Service of Biotechnology. *Plant Cell Tissue and Organ Culture*, 120: 881- 902.

Ansari, S., Shahab, S., Mazid, M. and Ahmed, D. (2014) Comparative Study of *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita* Race-2 on Plant Growth Parameters of Tomato. *Agricultural Sciences,* 5: 844-847.

Aslam, M., Maqbool, M.A., Akhtar, S. and Faisal, W. (2016). Estimation of genetic variability and association among different Physiological traits related to biotic stress (*Fusarium Oxysporum*) in Chickpea. *The Journal of Animal and Plant Sciences,* 23:1679-1685.

Bawa, J. A., Bashir, K. A. and Sani, Z. R. (2014). Pathogenicity Study of Southern Root-Knot Nematode (*Meloidogyne incognita* Chitwood) on Roma King Tomato Cultivar. *International Research Journal of Biological Sciences*, 3(11):5-9.

Bello, T.T., Fawole, B. and Abiodun, C. (2015). Susceptibility of Seven Varieties of Pepper and Tomato to Root – Knot Nematodes (*Meloidogyne* Species) in Ibadan, Nigeria. *IOSR* Journals *of Agriculture and Veterinary Science*, 8:79-82.

Bonger, C.W., Kariuki, G.M., Elashry, A., Mishra, B., Thines, M., Grunder, F.M.W. and Schouten, S. (2016). Fungal root endophytes of tomato from Kenya and the nematode biocontrol potential. *Mycological Progress,* 15:30.

Eisenback, J. D., Hirschmann, H., Sasser, J.N. and Triantaphyllou, A.C. (1981). A guide to the four most common species of root-knot nematodes (*Meloidogyne* spp.) with pictorial key. North Carolina State University Graphics, Raleigh, NC, USA, 48 pp.

FAO (Food and Agriculture Organization) (2016) Crop Production: Food and Agricultural Organization of United Nations (www.fao.org)

FAOSTAT (2014). "Production - Crop, 2014 data". Food and Agriculture Organization of the United Nation. Pp166 (www.fao.org)

Ganpati, J. B. and Juddy, C. (2014). Sampling for Plant-Parasitic Nematodes, Identification and Diagnosis. CBS Publication. Delhi, India.6-7.

Iheukwumere, C.C., Iheukwumere, C.I., and Aguoru, C. U. (2009). Host-parasite relationship of okra and single and combined infection of *Meloidogyne javanica* and *Fusarium oxysporum*. *Nematologia Meditarenean*, 37: 31-37.

Iheukwumere, C.C., Mutsaers H. J. W. and Dashiell, K.E. (2008). Population and pathogenicity of root -knot nematode *Meloidogyne incognita* on soyabeans in Nigeria. *International Journal of Nematology*, 8(1): 54-60

Joy, A., Elsie, I. H. and Mohammed, K. (2015). Germination and vegetative growth of selected hybrid tomato (*Solanum lycoperiscum* L) cultivars under hot and wet enivromental conditions in Rivers State Nigeria. *Journal of Plant Science,* 3(2): 99-105.

Kankam, F. and Adomako, J. (2014). Influence of inoculum level of root-knot nematode (*Meloidogyne* spp) on tomato (*Solanum lycoperiscum*). *Asian Journal of Agriculture and Food Science*, 02.

Karssen, G. and Monens, M. (2021). Root–knot nematodes in plant nematology. *Turkish Journals of Nematology*, 5(3) :124-126

Koenning, S.R. and McClure M. A. (1981). Interaction of two potyviruses and *Meloidogyne incognita* in Chili pepper. *Phytopathology,* 71: 404-408.

Kumar, N., Bhatt, J. and Sharma, R.L. (2017) Interaction between Meloidogyne incognita with *Fusarium oxysporum* f.sp. *lycopersici* on Tomato. *International Journal of Current Microbiology and Applied Sciences*, 6:1770-1776.

Mohit, K., Tripathi, U.K., Ajay, T., Pankaj, K. and Anchal, S. (2014). Screening of Linseed Germplasm for Resistance/Tolerance against *Fusarium oxysporum* f.sp. Lini (Bolley) Disease. *Journal of Plant Pathology and Microbiology,* 5: 235

Muhammad, A.H., Tariq, M., and Muhammad, Z. (2014). Characterization of Susceptibility and resistance responses to root- knot nematode (*Meloidogyne incognita*) infection in okra germplasm. *Pakistan Journal of Agricultural Science*, 51(2): 319-324.

Muhammad, U.N., Sarfran, H. and SaqibJabbar, H. (2015). Tomato processing, lycopene and health benefit: A review. *Open Access Journal,* 3 (1).

Mwangi, M.W., Muiru, W.M., Narla, R.D., Kimenju, J.W. and Kariuki, G.M. (2019). Management of *Fusarium oxysporum* f. sp. *lycopersici* and root-knot nematode disease complex in tomato by use of antagonistic fungi, plant resistance and neem. *Science and Technol*ogy *of Biocontrol,* 29: 229-238.

Nasrin, K., Abrahim, P. and Narazollah, S. (2015). Elicitation of Defense Responses in Tomato against *Meloidogyne javanica* and *Fusarium oxysporum* f.sp *lycoperisci* wilt complex. *Jerop Publication,* 4(1): 29-38.

Nikki, P. (2015). Tips to sterilize potting soil, garden soil and soil for seeds. Laboratory procedures. Pp 13: 5-8. New Jersey*: Prentice Hall.*

Siddiques, S., Coomer, A., Baum, T. and Wuliama, S.V.A. (2022). Recognition and responses in Plant-nematode interactions. *Annual revision of Phytopathology,* 60(7):1-7

Waheed, A., Tehmina, A. and Aqcel, A. (2014). Basal suspectibility of tomato varieties against different isolates *Fusarium oxysporum* f.sp *lycoperisci*. *International Journal of Agriculture and Biology,* 5 (2)

Zahid, M.I., Guour, G.M., Nikandrow, A., Hodda, M., Fukerson, W.J., Niwi, H.I. (2021). Effects of root and stobo-infecting fungi on root colonization nematodes of white clovers. *Plant Pathology,* SI:242-250

Zijlstra, C, Donkers-Venne, D.T.H.M. and Fargette, M. (2021). Identification of Meloidogyne incognita, M. javanica and M. arenaria using Sequence Characterised Amplified Region (SCAR) based PCR assays. Nematology, 2(8): 847-8

Applied Sciences Research Periodicals
November 2023, Vol. 1, No. 8, pp. 33-42

Effectiveness of Combined Anti-Retroviral Therapy [cART] on Viral Load and Cd 4 Count, in Reducing Progression to AIDS, in Improving the Quality of Life of Patients of HIV/AIDS in the Context of the U.N. Sustainable Development Goals (SDGs): A 5-Year Prospective Study in Himachal Pradesh (India)

Pankaj Katoch[1], Anup Kumar[2], & Jyoti Bala[3]

1. Department of Microbiology, IGMC Shimla
2. Department of Pharmacology Dr. RKGMC Hamirpur Himachal Pradesh India
3. Department of Pathology DrRKGMC Hamirpur Himachal Pradesh India

Abstract:
Background: The increasing prevalence of the Human Immunodeficiency Virus (HIV) every forthcoming year continues to pose a serious threat to mankind. This global problem and its sequelae cause significant loss of precious lives every year despite advances in Antiretroviral (ARV) therapy. CD4 cell count is an essential component in monitoring HIV treatment outcomes but it's monitoring sometimes is unable to estimate the virological failure leading to a switch of treatment lines that leads to drug resistance and limiting of options for patients. CD4 count still is the best measurement of an HIV patient's immune and clinical status, the threat of opportunistic infections, and diagnostic decision-making, especially for patients with advanced HIV disease. Aims and Objectives: This study is an attempt to assess the effectiveness of cART on Cd 4 count and Viral load both as predictors of HIV/AIDS by monitoring the progression of the disease by determining the CD4 level and HIV RNA Viral load and reducing the progression of AIDS improving the quality of life of patients of HIV/AIDS in the context of the U.N. sustainable development goals. Material and Methods: 492 HIV patients were analyzed over five years. Models were selected, for various parameters like CD4 cell count monitoring and HIV load RNA monitoring. Effects of various covariates; gender, age, CD4 baseline count, HIV RNA baseline count, and patient's adherence to treatment were assessed for each of the fitted models. To assess the extent International or U.N. policies are being implemented concerning Viral load and CD4 testing in practice. We also draw on data from key informant interviews to contextualize the policy content and national implementation strategies, and from qualitative interviews with service providers and users to explore how tests are being conducted and interpreted in everyday clinical practice. Results: For people living with HIV/AIDS Antiretroviral therapy must be consumed throughout life to inhibit the levels of the HIV/AIDS virus as it is a chronic disabling disease and improving the quality of life depends on cART adherence and psychosocial support. Results from this analysis revealed that viral load monitoring is a better predictor of HIV/AIDS progression and deaths in HIV/AIDS patients in comparison to CD4 cell count monitoring. Conclusions: Our study concludes that both CD4 and HIV viral load should be performed on patients alternately to cover both aspects of patient management.

Keywords: Combined antiretroviral therapy (cART), CD4+ cell count, HIV RNA Viral load, Life expectancy

INTRODUCTION

This study assesses the effectiveness of cART on Cd 4 count and HIV Viral RNA load both as predictors of HIV/AIDS by monitoring the progression of the disease by determining the CD_4 level and viral RNA load before initiation of cART and viral loads after 6 months of cART initiation and difference in their levels 5 years after. When diagnosed with HIV, the patient's CD 4 count parallel viral load is done. The CD 4 count allows the attending physician to determine the state of the immune system and plan the further course of treatment. If the CD 4 count on presentation is below 200 units, the diagnosis of acquired immunodeficiency syndrome (AIDS) is done. The viral load will indicate the speeds of viral replication. Current medications used nowadays to treat HIV infection attack different phases of viral replication and hinder the process of new viruses being produced. Therefore, a fall in viral load after starting cART is an indicator that the medication is doing its job as per expectation.

The role of the CD_4 cell count to decide the treatment efficacy is changing day by day. However, CD_4 count remains the best measurement of a patient's immune and clinical status, the threat of opportunistic infections, and diagnostic decision-making, especially for patients with advanced HIV disease. For patients who are stable on ART, CD_4 cell counts are no longer needed to monitor the response to treatment where HIV viral load testing is available.

MATERIALS AND METHODS

Two milliliters of sterile blood samples were collected in a diamine tetraacetic acid vacutainer and transported to the microbiology laboratory within 1 hour. The sample was processed within one hour according to the National AIDS Control Society guidelines based on the Clinical laboratory standard institute (CLSI). CD_4 count was estimated by BD FACS machine using flow cytometry with single platform technology as the principle. The 5-year prior CD_4 counts and CD_4 counts after ART intervention of all the 492 patients were analyzed by calculating the standard error of the mean, standard deviation, and paired *t*-tests. For each visit time, blood samples were obtained for each patient's viral load determination and stored frozen until assayed. Plasma HIV RNA was measured using an HIV-1 monitor assay kit which has a lower limit of sensitivity of 50 copies/mL.

DATA ANALYSIS

In this study, the patients suffering from HIV infection were analyzed for various parameters. Out of 492 patients, there were 307 males, 169 females, and 16 children. See Figure 1 Annexure. Out of 492 patients, 390 reported a Heterosexual mode of transmission, Unknown 27. Mother to child 13, MSM 3, IDU 2. See Figure 2 in the Annexure for modes of HIV transmission. Over 5 years 26 patients died, transfer out 31, Left outs were 8, opted out 3. Out of 492 patients, 470 belonged to Himachal Pradesh while 22 were from different states working in Himachal Pradesh.

The CD 4 count was less than 150 in 157 patients, in 164 patients it was below 350, and above 350 in 171 patients. See Table 3 in Annexure. The mean CD_4 count in 492 patients before ART was 315, with a standard deviation ± 141 of the mean value. After 5 years of ART, the mean value of CD_4 count increased to 550 with ± 148 standard deviations of the mean value.

This study of 492 patients as a group after ART intervention for 5 years shows that their mean CD_4 count increased from 315 to 550, an increase of 235 a clear indication that nearly all benefited from the cART. See Table 4 in Annexure. Figure 4

The mean difference in the CD_4 count increase in patients obtained was 145 and is statistically significant ($P < 0.01$) by the paired *t*-test. The confidence limit in 95% of the patients having ART treatment for 5 years will have a mean increase of CD_4 count in between 125 and 175. See Table 5.

RESULTS

The mean difference in the CD_4 count increase in patients obtained was 145 and is statistically significant ($P < 0.01$) by the paired *t*-test. The confidence limit in 95% of the patients having ART treatment for 5 years will have a mean increase of CD_4 count in between 125 and 175.

It was observed that if we consider all patients together, it appears that the CD_4 count of the cohort has increased, but this should not be considered in totality. We separately evaluated the patients who showed a rise and fall in CD_4 count after ART to get the true picture. Patients showing a decrease in CD_4 count should be assessed for viral load and drug resistance studies. Reasons for lowering the count may be non-adherence, lack of support from families and society, social stigma, shortage, and toxicity of drugs. Viral load monitoring is a better predictor of HIV/AIDS progression compared to CD_4 cell count and viral load estimation is considered to be superior.

In our study of 492 patients baseline viral load after six months of cART was performed and the mean value in whom significant viral load above 1000 copies/ml was taken into account and others were classified into Target Not Detected (TND). 61 patients were falling in the zone of consideration above 1000 copies/ ml. They were again tested for viral load monitoring after 6 months and then the count of patients above significant viral load came down to 31. The basic viral load which was 16997 copies/ ml decreased to 11067 copies/ ml after 6 months of cART adherence. See Table 6 in Annexure. Viral load decreased by 5930 copies on average in the selected group. Therefore, we witnessed a significant decline in viral load estimation, a confirmation of the efficacy of cART in decreasing the viral load. See Figure 5 in the Annexure.

DISCUSSION

This study tries to analyze the effectiveness of Combined Anti-Retroviral Therapy on Viral load and CD 4 count, in reducing the progression of AIDS and improving the quality of life of patients with HIV/AIDS in the context of the U.N. sustainable development goals (SDGs). The CD_4 count is the best parameter for guiding general care physicians to initiate cART in the prophylaxis of HIV patients. CD_4 count can also guide general care physicians in managing late presenting patients, or patients on cART if there is suspicion of poor clinical outcome or immunological failure. In monitoring the patient's response to ART, the most reliable test continues to be viral load detection.[1] The Immune system comprises a large network of cells that work mutually in combating infections. Helper T Lymphocytes are a specific type of immune cell that expresses a molecule called Cluster Determinant 4 (CD_4). Helper T lymphocytes are generally referred to as CD_4 cells. The Human Immunodeficiency Virus (HIV) precisely targets CD_4 host cells for viral replication. As the virus replicates in the cells, it destroys its host cell and releases new progenies. Hence, as the viral load (the amount of virus present in the body) increases the host CD 4 cell count decreases. During the early stages of a person getting HIV infection, viral replication occurs at a very fast rate at the expense of CD 4 host cells. A chain of circumstances occurs, where an increase in HIV viral load occurs leading to more CD_4 cells getting infected and leading to an increase of hosts to further increase the viral load. Within the first few weeks of an HIV infection, the CD 4 count falls steeply. The significant fall in CD_4 cells is pathogenic to HIV infection.

When diagnosed with HIV, the patient's CD 4 count parallel viral load is done. The CD 4 count allows the attending physician to determine the state of the immune system and plan the further course of treatment. If the CD 4 count on presentation is below 200 units, the diagnosis of acquired immunodeficiency syndrome (AIDS) is done. The viral load will indicate the speeds of viral replication. Current medications used nowadays to treat HIV infection attack different phases of viral replication and hinder the process of new viruses being produced. Therefore, a fall in viral load after starting cART is an indicator that the medication is doing its job as per expectation.

The role of the CD4 cell count to decide the treatment efficacy is changing day by day. However, CD4 count remains the best measurement of a patient's immune and clinical status, the threat of opportunistic infections, and diagnostic decision-making, especially for patients with advanced HIV disease. For patients who are stable on ART, CD4 cell counts are no longer needed to monitor the response to treatment where HIV viral load testing is available.

Knowledge is a key factor which supports the level of adherence to ARV therapy. Human Immunodeficiency Virus (HIV) infection is a lifelong disabling chronic condition affecting the patient and their families financially and socially. Preventing the progression of the disease and its subsequent disability is a crucial factor in adherence to therapy. Early diagnosis and cART initiation impart better HIV management for the patient ultimately leading to a reduction in mortality and improvement in the quality of life.[2] In the majority of people affected the sequence of HIV infections is not clearly defined. Still, it starts from the time of acquiring of the virus till the eclipse phase through seroconversion (primary HIV infection) which is followed by a period of clinical latency due to antibody evolution. During clinical latency, there is a continuous decline of peripheral CD4 T cells, immune system amplification, and inflammation.[3,4] In HIV infection, the CD4 count decreases to differing levels as per the immune status of the patient. Current CD4 count is a good predictor of the immediate risk of acquired immunodeficiency syndrome (AIDS) or death than HIV RNA level. Antiretroviral therapy (ART) has significantly decreased the morbidity and mortality connected with HIV infection and boosted the prognosis for people living with HIV AIDS.

As per the latest guidelines cART should be started in all children, pregnant and breastfeeding women, and adolescents, adults infected with HIV, notwithstanding WHO clinical staging and CD4 cell count. The cART should be started in all children, adolescents, pregnant or breastfeeding women, adults on a priority basis with severe or advanced HIV clinical disease, and adults with a CD4 count of fewer than 300 cells/mm3 as well as children aged less than 5 years of age with WHO clinical stage 3 or 4 or CD4 count less than 700 cells/mm3. If viral load testing is not routinely available, CD4 count and clinical monitoring should be used to diagnose the treatment failure, with selective viral load testing for confirming viral failure if possible. For children above five years of age, adolescents, and adults, advanced HIV disease is defined as the presence of a CD4 cell count < 200 cells/mm3 or a WHO clinical stage 3 or 4 events. A patient is considered stable on ART based on the following criteria: on ART for at least 1 year, no current illnesses, a good understanding of lifelong adherence, and evidence of treatment success if we classify the patient as Nondetectable viral load [NTD] which means two consecutive viral load measurements below 1,000 copies/ml). Various factors influenced the adoption and implementation of WHO guidance, including historical policies on CD4 counts, governance issues, supply chain challenges, and funding mechanisms. Facility-level practices relating to the use of these tests have often diverged from International and national policies. In addition to continued support for scaling-up viral load

testing, the renewed focus should be placed on the ongoing value of CD4 testing in the era, including its important role in the assessment of disease progression and alarming the clinical management for revision of treatment if needed of cases to reduce HIV-related mortality. In 2015, the World Health Organization (WHO) recommended that patients diagnosed with HIV should be initiated on ART at any CD4 cell count (World Health Organisation, 2015), which means starting treatment on diagnosis a strategy known worldwide as the 'universal test and treat (UTT). The widespread adoption of UTT in India has been recognized as a critical step in moving towards targets to eliminate AIDS by 2030 .[5] While CD4 cell counts indicate the strength of the immune system, it does not report viral activity, which is best measured through a viral load test. However, compared with CD4 testing, viral load testing is expensive and more technically complex, reasons which were widely cited as inhibiting its wide-scale use in most South Asian countries during the first decade of ART scale-up. As evidence is upcoming on the benefits of viral load monitoring for increasing the lifespan of first-line regimens and improving patient outcomes has become established, the WHO recommended viral load (VL) monitoring as part of routine care for monitoring adherence and treatment failure among people taking ART in all settings (World Health Organization, 2017; World Health Organization, 2016). Frontline access for testing CD4 count remains critical for assessing disease progression (World Health Organization, 2017), identifying people diagnosed late with HIV (CD4 < 350 cells/μL),[6] informing differentiated care models (World Health Organization, 2017) and, more broadly, providing healthcare workers with an objective measure of a patient's health.[7] However, several factors have undermined their use including the growing availability of Viral load testing and recent guidance on its use, adoption of UTT, and a decrease in financial donor support for CD4 testing. [8] As a result, several South Asian and African countries have drastically reduced CD4 monitoring in favor of increased Viral Load testing (United States Agency International Development, 2016), and CD4 tests performed in some low- or middle-income countries are not being fully utilized to inform clinical management for revising treatment options.[9] These concerns are intensified with the suggestions that coverage of Viral Load monitoring remains less in sub-Saharan African settings [10] and even where the scale-up of Viral Load testing is under process, the test results are used sub-optimally for clinical decisions about revision.[11] Appreciating the ongoing value of conducting both Viral Load and CD4 testing, we analyzed the extent to which national policies on the use of each biological marker reflect WHO guidance and the degree to which national directives on their use are implemented at the facility level in rural India. We also tried to find out the difficulties faced in their implementation from the viewpoint of in-country stakeholders, and the experiences with the use of both tests among patients and providers. WHO recommends countries should retain their capacity to conduct CD4 testing at diagnosis and up to ART initiation, and CD4 counts remain useful in guiding the clinical management of patients failing treatment or re-engaging in care. [12] Our finding supports the recent expression of anxiety that guidelines promoting Universal tests and treatment may be misinterpreted.[13] Specifically, the importance of CD4 monitoring in identifying and treating people at risk of advanced disease has been put aside due to the attention given to increasing Viral load testing. Our study found the sub-optimal implementation of tests, such as inadequate scheduling, long turnaround times (mainly with VL), and procedures not being followed (mainly concerning CD4) compromised the utility of the markers. Challenges leading to sub-optimal implementation in these settings included a lack of facility space, a lack of refrigeration, and suboptimal laboratory capacity. Developments in the use of point-of-care testing are positive with evidence to suggest such testing is feasible, acceptable, and can increase coverage and effectively identify treatment failures for viral load testing.[14,15] Our study supports their recommendations of the need for continued efforts to train staff regularly, monitor the program, and promote demand for tests. Additionally, our study highlighted various challenges

in health workers' capacity to communicate the meaning of test results to patients and populations.One study concluded the greatest mean increase in CD_4 count of 100 cells/mL after the first year of ART. [16] Cascade Collaboration has reported the median CD_4 count increase at 6 months on ART was 119 cells/mL.[4] Other studies have opined that the negative CD_4 count slope in patients on ART was associated with virological failure. They have concluded that the CD_4 count changes correlated significantly with viral load at a group level.[17] But, this has limited utility in identifying virological failure in individual patients.[5] Some studies also reported poor CD_4 cell recovery or no increase in CD_4 count in a few patients on ART in their studies.[6]

CONCLUSIONS

Based on our study, we can conclude that CD_4 cell count to reach the risk-free zone adherence to cART therapy plays a key role and takes a longer time than for undetectable viral load because if CD_4 cell count is normal, mortality risks are reduced substantially. Significant decisions will be needed in the future regarding the use of CD_4 cell count and its use for clinical disease management. Hence, both viral RNA load monitoring and CD_4 count monitoring are essential to correlate various aspects of disease progression for improving the quality of life of patients living with HIV.

REFERENCES

1. Moorhouse M, Conradie F, Venter F. What is the role of CD_4 count in a large public health antiretroviral program? South Afr J HIV Med. 2016 Feb 12;17(1):446. doi: 10.4102/sajhivmed. v17i1.446. PMID: 29568607; PMCID: PMC5843041.

2. Lee K, Park, et al. (2013) Reduction of the HIV Seroconversion Window Period and False Positive Rate by Using ADVIA Centaur HIV Antigen/Antibody Combo Assay. Ann Lab Med 33: 420-425.

4. Cohen MS et al. The detection of acute HIV infection. J Infect Dis 202: S270-S277.

5. Persistent.immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 17: 1881-1888. Antiretroviral Therapy Guidelines for HIV-infected Adults and Adolescents 2013.

5. N Dravid Mrudula et al. Indian J Community Med. 2012 Oct-Dec; 37(4): 266–267. Statistical Analysis and Evaluation of CD_4 Count after 6 Months on ART.

6. Antiretroviral Therapy Guidelines for HIV-infected Adults and Adolescents Including Postexposure Prophylaxis. New Delhi: Ministry of Health and Family Welfare, Govt. of India; 2007. Aug 29, National AIDS Control Organization.

7. Mocroft A et al. Normalisation of CD_4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. Lancet. 2007; 370:407–13.

8. Babiker A et al. Short-term CD_4 cell response after highly active antiretroviral therapy initiated at different times from seroconversion in 1,500 seroconverters. J Acquir Immune Defic Syndr. 2003; 32:303–10

9. Badri M et al. Utility of CD_4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting. BMC Infect Dis. 2008; 8:89.

10. Highleyman L. Poor CD_4 cell recovery on antiretroviral therapy is linked to late treatment and leads to adverse outcomes. In: HIV and Hepatitis. Com Coverage of the 17th Conference on Retroviruses and Opportunistic Infections

11. Claris Shoko et al. A superiority of viral load over CD4 cell count when predicting mortality in HIV patients on therapy.BMC Infect Dis 2019 Feb 15;19(1):169.

12. Patrick Dakum et al. Effect of community antiretroviral therapy on treatment outcomes among stable antiretroviral therapy patients in Nigeria: A quasi-experimental study.PLoS One. 2021 Apr 26;16(4): 0250345.

13. Jenny Renju et al. Influence of evolving HIV treatment guidance on CD4 counts and viral load monitoring: A mixed-methods study in three African countries.Glob Public Health. 2021 Feb;16(2):288-304.

14. Nicholas Sey et al. Point-of-care viral load monitoring: outcomes from a decentralized HIV program in Malawi. J Int AIDS Soc. 2019 Aug;22(8): e25387. DOI: 10.1002/jia2.25387. PMID: 31441242; PMCID: PMC6706700.

15. Kohler SL, Pham MN et al. Germinal Center T Follicular Helper Cells Are Highly Permissive to HIV-1 and Alter Their Phenotype during Virus Replication. J Immunol. 2016 Mar 15;196(6):2711-22. DOI: 10.4049/jimmunol.1502174. Epub 2016 Feb 12. PMID: 26873986; PMCID: PMC4779697.

16. Mocroft et al. The decline in the AIDS and death rates in the EuroSIDA study: an observational study, The Lancet, Volume 362, Issue 9377,2003, Pages 22-29, ISSN 0140-6736, https://doi.org/10.1016/S0140-6736(03)13802-0.

17. Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. Lancet. 2002 Jun 15;359(9323):2059-64. DOI: 10.1016/S0140-6736(02)08904-3. PMID: 12086758.

Annexure For HIV Manuscript

Table 1: Shows the Sex Distribution

	Sex	
SNO 1	Men	307
SNO 2	Women	169
SNO.3	Children	16

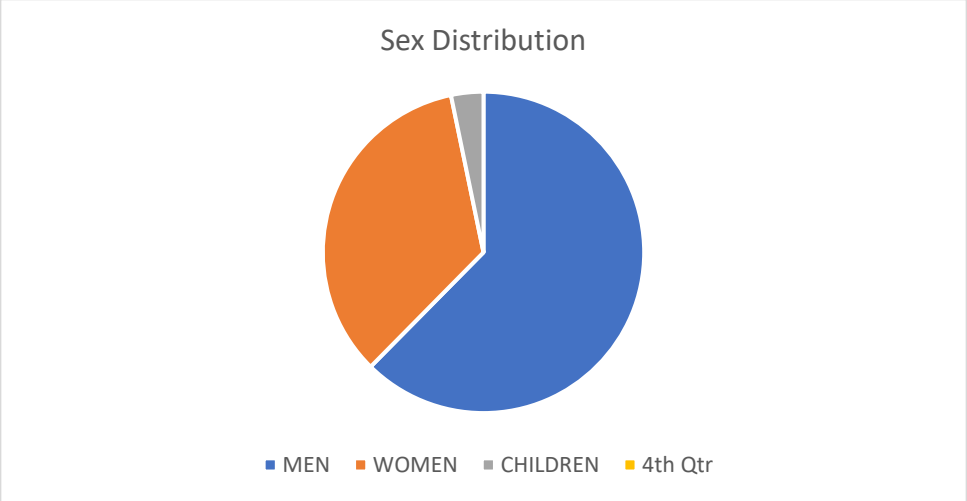

Figure 1

Table 2: Shows the Modes of Transmission

SNO	MODES OF TRANSMISSION	
1	HETEROSEXUAL	390
2	UNKNOWN	27
3	MOTHER TO CHILD	13
4	MSM	3
5	IDU	2

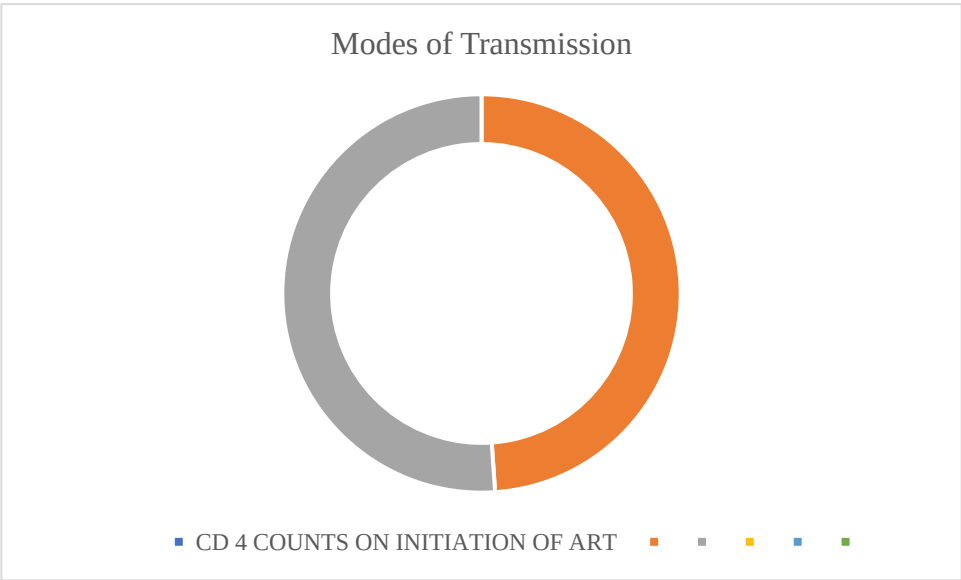

Figure 2: Shows the Modes of Transmission

Table 3: Shows Cd4 Count at Diagnosis

SNO 1	CD 4 COUNT	
1	BELOW 150	157
2	BELOW 350	164
3	ABOVE 350	171

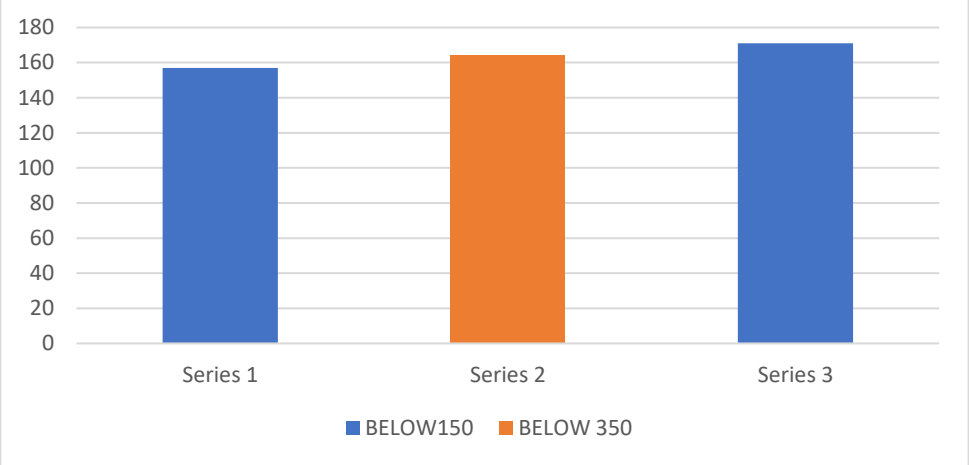

Figure 3: Shows the CD 4 Count at Diagnosis

Table 4: Shows an Increase in CD4 Count After 5 Years

SNO 1	Cd 4 Counts on The Initiation of Art	Total 492	Cd 4 Count After 5 Years of Adherence to Art	Total 424
1	Below 150	157	Below 150	77
2	Below 350	164	Below 350	199
3	Above 350	171	Above 350	148

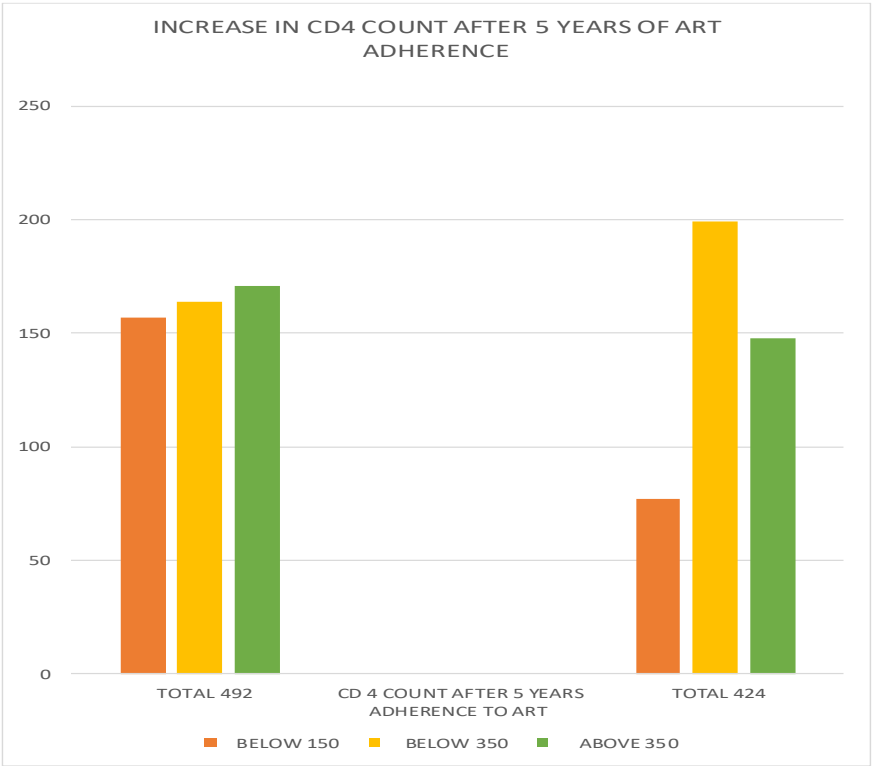

Figure 4: Shows an Increase in CD4 Count After 5 Years of Art Adherence

Table 5

CD4 Count	Baseline	After 5 years	**p-value**
BELOW 150	157	77	<0.01
BELOW 350	164	199	
ABOVE 350	171	148	

Over 5 years 26 patients died, transfer out 31, Left outs were 8, opted out 3.

Table 6: Viral Load After 6 Months Initiation of Art and 6 Months Later

	Viral Load Above 10000 Copies / ML	Viral Load Below 10000 Copies / ML(NTD)
After 6 Months of Art	431	61
After 12 Months of Art	31	461

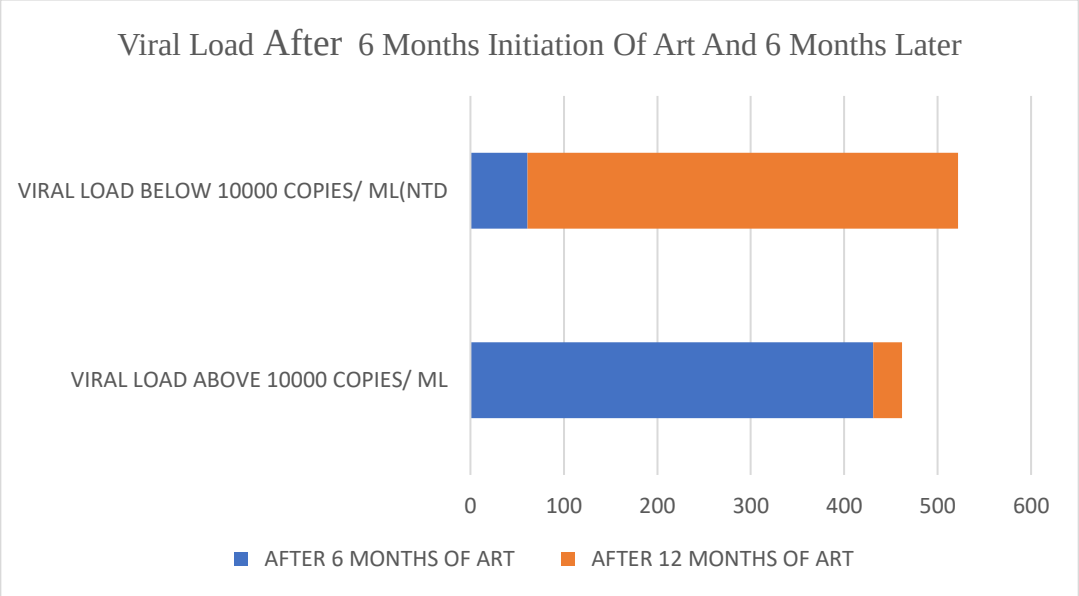

Figure 5: Viral Load After 6 Months Initiation of Art and 6 Months Later