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Preface

We would like to present, with great pleasure, the inaugural volume-10, Issue-3, March 2024, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestrial ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



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High Prevalence of Stunting and CKD-1/Obesity in Low- and Middle-nSES Population: A Review Article Support by p53-p21-p16 Axis

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Abstract—

Introduction: Stunting /pygmy or growth impairment parallel with obesity, is in high prevalence in the Aflatoxins exposure population, based on p16 upregulation. Mutation of p16 or epigenetic silencing, gives proliferation of BAT (UCP 132), SMCs (CKD-1), and later cancer cell proliferation and metastasis.

Method: Review articles using my Library, and academic search engines like PubMed, Science Direct, and EBSCOhost. Keywords searching use Stunting-AFB1 exposure (23 Systematic Reviews), and Meta-Analysis (MA) ^ stunting (7), urine AFM1 (82) sub ppb, MA p16 upregulated (35). Acute exposure is excluded. Bayesian Analytical is used to find the reference that supports the p53-p21-p16 axis in AFB1exposure, which causes stunting in low- and middle- neighborhood Socioeconomic Status/nSES populations.

Result: One figure and 3 tables of Senescence barrier, AFB1-stunting, MA p53 axis-stunting, MA p16 upregulation-stunting, and p16 downregulation-cancer.

Discussion: p16 upregulated in stunting then in the older years p16 mutation or epigenetic silencing in proliferation cells due to the senescence barrier.

Conclusion: Detect in the population by epidemiology, laboratory in sub ppb, anthropometric measurement, and anamnesis survey where the refrigerator is full of leftover food, on winning to fight stunting.

Keywords— Stunting, AFB1 exposure, Urine AFM1, Pygmy, p53-p21-p16 axis.

I. INTRODUCTION

There is an urgent need to inform the public and political decision-maker (mainly the legislative) to lead people to mitigate AFB1exposure, and promote the technique in preparing clean food, and the useful of good food stuff management ^{1,2,3,4} in fighting most diseases^{5,6,7,8} especially stunting in low- and middle-neighborhood Socioeconomic Status (nSES) population.⁹ The people should throw away the AFB1 exposure food which is not success to do until now.^{10,11} Moreover, don't give it to animals because Aflatoxin doesn't destruct with high temperatures till 250°C and metabolite to AFM1.¹¹

Stunting/pygmy or growth impairment, associated with the p53-p21-p16 axis has been broadly known. This study focuses on upregulated p16^{9,12,13} as tumor suppressor induce growth impairment mechanism, not the later stage which knock out of p16, induced proliferation.^{14,15,16,17,18,19,20,21} and cancer.²² Post-weaning associated with stunting²³ and paradox downregulation,²⁴ has been reported.

Stern, 2001, has reported p53 mutation codon 249 in HCC patients in China (AFB1 Meta-Analysis).¹⁹

Leong, 2009: downregulated p53 induced upregulated p16 (p53-p21-p16) axis pathway.¹⁴

II. METHOD

A review of articles on the prevalence of stunting and obesity in low- and middle-nSES areas by the p53-p21-p16 axis is built. Systematic Reviews and Meta-Analysis references are preferable when using my library and search engine EBSCOhost, ScienceDirect, and others while chasing academic references. Focus on p16 upregulated-stop the cell cycle growth using Bayesian network and analysis. Keywords searching use Stunting-AFB1 exposure (23 Systematic Reviews), and Meta-Analysis (MA) ^ stunting (7), urine AFM1 (82) sub ppb, MA p16 upregulated (35). Acute exposure is excluded.

III. RESULT

P16 or P16-INK4A also known as CKDI 2A gene upregulated by p53 and p21 mutation due to AFB1 exposure. In this low- and middle- nSES population, epidemiology of stunting and obesity, and later Diabetes Mellitus are in high prevalence.¹¹ Downregulated p16 which gives proliferation at a later age, becomes controversial while upregulated p16, which gives stops the cell cycle (stunting).¹⁵ Epidemiology of both (stunting-obese), parallel happen in the same area.^{12,25} This study reveals the upregulated p16 in the younger age, while in the older, obesity is in high prevalence too. It includes Diabetes Mellitus underlying CVD/CHD as the cause of death, and other complication of diabetes. In another sentence, in the high prevalence of stunting, adult people: the parents, executive, and legislative, are at high prevalence of Obesity, Diabetes, and CVD, similar with reported by Smith.²⁵ By AFB1 induced p53-p21-p16 axis, firstly, all cases of up-regulated p16 inhibit proliferation such as cancer, also specific induce growth impairment are chased.^{26,27,28}

Up-regulated of p16 by miR-877-3p inhibits the proliferation of bladder cancer.²⁹

The p16-dependent upregulation of PD-L1 impairs immunosurveillance of senescence cells.³⁰ Upregulated p16 inhibit cycle's G1/S in cancer, stimulus direct or indirect has been reported.^{20,21,22,31,32}

The p53-p16 axis is a senescence pathway in mouse and human cells.²⁰

Whereas p16INK4a overexpression is a marker for survival/good prognosis/low grade in cancerous patients (Meta-Analysis).³³ Also overexpression was reported in good outcomes of cancer,^{22,32} and mastocytosis (urticarial pigmentosa).³⁴ And Alternative reading frame protein (ARF) is a novel protein in activating p53.^{21,31}

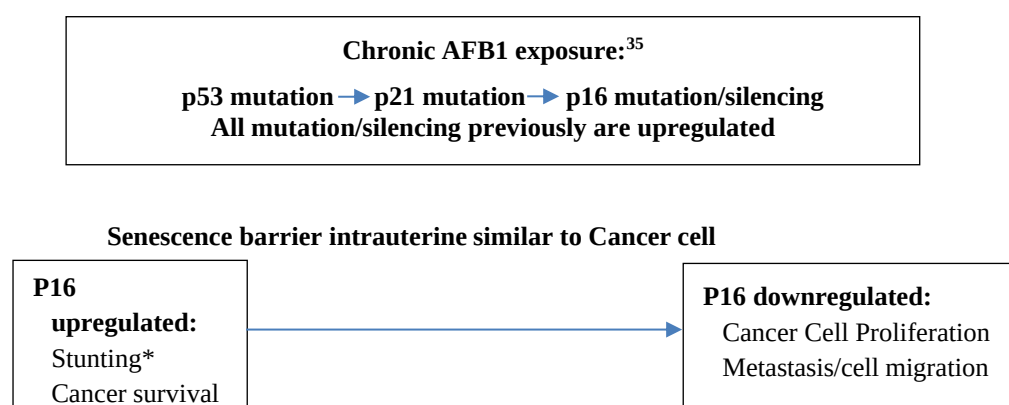


FIGURE 1. Pathogenesis of stunting from molecular pathways mechanism p53-p21-p16 axis

**Upregulated p16 inhibit cycle's G1/S in cancer, stimulus direct or indirect has been reported*

IV. DISCUSSION

Many scientists have revealed that pygmy and small stature is simply a genotype factor geographical area, and with the imported food and feed, developed countries also have prone to a little stature Caucasian.¹¹ In Table 1. Aflatoxin effect on stunting and

since good nutrition and micronutrient fail to fight stunting,³⁷ pay attention change to find AFB1 in food which is metabolite to AFM1 and on urine could be as AFB1 exposure marker.^{47,5,6,7,47,49,50,54,58,61,62,63}

TABLE 1
DESCRIPTION OF IDENTIFIED 18 LITERATURES ON AFLATOXIN EXPOSURE-STUNTING, BUT NEGLECTED AND ABANDONED IN MOST COUNTRIES

Study, year	Mutation p53, p21, p16 upregulation	Stunting population	Aflatoxin exposure	Countries
⁹ Miller, 2016	Stern 2001 Leong 2009	Meta-analysis Stunting and child development	Peni Oct 2018	15 low- and middle-income countries
¹² Sousa, 2016	Stern 2001 Leong 2010	Stunting and overweight	Brazilian	Brazilian
³⁶ Olivero, 2016	Stern 2001 Leong 2011	Pygmies	Tropical rainforest	Central African forest
² Smith 2012	Stern 2001 Leong 2012	Impaired growth/stunting senescence	Food chain mycotoxin	Developing countries
³ Smith LE, 2015	Stern 2001 Leong 2013	Stunting Child undernutrition Likelihood of being overweight, CVD, diabetes	Mycotoxins contributor	SHINE Trial
²⁵ Smith LC, 2015	Stern 2001 Leong 2014	Child undernutrition Likelihood of being overweight, CVD, diabetes	Not mention	116 countries
³⁷ Watson, 2015	Stern 2001 Leong 2015	Undernourished child	May be undermined by dietary Exposure to Aflatoxin	Targeting Child undernutrition in developing Countries
³⁸ Watson 2016	Stern 2001 Leong 2016	Young children Micronutrient Status	Dietary aflatoxin exposure	Guinea
³⁹ Knipstein, 2015	Stern 2001 Leong 2017	Induced stunting and liver injury	Dietary Aflatoxin	Rat model
⁴⁰ Wild CP, 2015	Stern 2001 Leong 2018	Cancer and Stunting	Mycotoxin control	Low- and Middle-income countries
⁴¹ Wild CP, 2007	Stern 2001 Leong 2019	Growth impairment/stunting	Aflatoxin exposure	Developing countries
⁸ Ezekiel, 2014	Stern 2001 Leong 2020	Children, adolescents adult	LC-MS/MS multi biomarker	Rural North Nigeria
⁴² Lombard, 2014	Stern 2001 Leong 2021	Infant and young child growth	Mycotoxin exposure	Africa
⁴³ Khlangwiset, 2011	Stern 2001 Leong 2022	Growth impairment Stunting and underweight	Aflatoxin in utero also	Less developed countries
⁴⁴ Gong, 2012	Stern 2001 Leong 2023	School children	Chronic hepatomegaly	Kenyan School Children
²³ Gong, 2004	Stern 2001 Leong 2024	Impaired child growth	Post weaning exposure to aflatoxin	Benin, West Africa
⁴⁵ Gong, 2003	Stern 2001 Leong 2025	Critical role of weaning in youngchildren	Determinants of aflatoxin exposure	Benin and Togo
⁴⁶ Stevens, 2012	Stern 2001 Leong 2026	Mild, moderate and severe stunting	Peni Oct 2018: AFB1 exposure	141 developing countries

¹⁹Stern 2001: p53 mutation codon 249 in HCC patients in China (AFB1 Meta-Analysis)

¹⁴Leong 2009: downregulated p53 → upregulated p16 (p53-p21-p16 axis pathway)

TABLE 2
DESCRIPTION OF 17 REFERENCES OF URINE AFLATOXIN M1 AS METABOLITE OF AFB1 EXPOSURE, IN P53-P21-P16 AXIS PATHWAYS

Study, year	Patients	Urine AFM1	Countries	P53, p21, p16
¹⁹ Stern, 2001	HCC, Meta- analysis	AFB1 exposure	China	P53 -codon 249 mutation
²⁰ Wadhwa, 2004	Senescence mouse and human cells	P53-p16 signaling	Japan	P53, p16
²¹ Hasan, 2002	Senescence cell of Mouse and human	Activating p53	Japan	P16-p21-p53
⁴⁷ Ali, 2016	AFB1	Urine AFM1	Rural vs. urban Bangladesh	P53 mutation
⁵ Gerding, 2014	AFB1	Urine AFM1	Bangladesh Haiti	P53 mutation
⁷ Jager, 2016	AFB1	Urine AFM1	Brazil	P53 mutation
⁸ Ezekiel, 2014	Cross-sectional communities	Multibiomarker morning urine	Rural Northern Nigeria	P53 mutation
⁴⁸ Warth, 2012	AFB1	Urine AFM1	Cameroon	P53 mutation
⁴⁹ Warth, 2014	AFB1	4 urine biomarkers	Bangkok	P53 mutation
⁶ Mitchel, 2013	AFB1	Urine AFM1	Ghana	P53 mutation
⁵⁰ Solfrizo, 2011	AFB1	Urine AFM1 biomarkers	Human and pig	P53 mutation
⁵¹ Solfrizo, 2014	AFB1	Urine AFM1	Southern Italy	P53 mutation
²⁶ Romero De Cassia, 2009	AFB1	Urine AFM1	Brazilian	P53 mutation
⁵² Jonsyn F, 1995	AFs in cord blood pregnant 58%	Urine AFM1 No	Sierra Leone	P53 mutation
⁵³ Jonsyn FE, 1999	AFB1	Urine sample were 100% contaminated	Infants in Sierra Leone	P53 mutation
⁵⁴ Jonsyn E, 2007	AFB1	Urine AFM1	Boys and Girls Sierra Leone	P53 mutation
⁵⁵ Chen, 2017	AFB1 infant rice cereal	Aptamer AFB1	Detection AFB1	P53 mutation

TABLE 3
DESCRIPTION OF 12 IDENTIFIES LITERATURES ON META-ANALYSIS (MA) OF P16INK4A UPREGULATION, SENESENCE BARRIER, AND DOWNREGULATION

Study, year	MA	Upregulated P16	Stunting	Cancer/Proliferation
²⁰ Wadhwa, 2004	P53 Signaling	The ARF-p53 in mouse and human		Senescence pathway
⁵⁶ Xiao, 2016	MA	Promoter methylation		Ovarian cancer
⁵⁷ Wang, 2016	MA	Overexpressed p16: prognosis		Esophageal squamous cell carcinoma
⁵⁸ Cao, 2016	MA	Overexpressed p16		Vulvar cancer
⁵⁹ Yifan, 2015	MA	Promoter methylation		Lung cancer diagnostic
³³ Bu, 2014	MA	Overexpression p16: survival		Osteosarcoma
³¹ Hasan, 2004	P53 Signaling	CARF-p53		Negative Feedback control
⁹ Miller, 2016	MA	Low- and middle- income countries	Stunting	Multidimensional Child development
¹² Sousa, 2016	SR and MA	Brazilian children	Stunting	Overweight/obesity
⁶⁰ Altare, 2016	MA	Emergency pockets: Ethiopia	Child wasting	
¹³ McDonald, 2013	MA	Developing countries	Multiple anthropometric deficits	Child mortality
¹⁹ Stern, 2001	MA	China: AFB1-p53 codon 249 mutation		HCC patients

Stunting or pygmy is due to high and chronic exposure to AFB1 which metabolites to AFM1, which is parallel to high prevalence in central Africa.³⁶ Mitigation of AFB1 exposure by avoiding wet and warm storage rooms, but is also had thought as others approach to decrease aflatoxin exposure by gamma ray,⁶⁵ culture, and lifestyle.^{36,62,66} WHO guidelines⁶⁷ give the tolerable daily intakes used by governments and international risk managers to establish maximum levels for mycotoxins in food. The maximum levels for mycotoxins in food are very low due to aflatoxin severe toxicity. This study supports WHO by recording the argumentation to fight AFB1 exposure that induces stunting in low- and middle-nSES. It is as follows:

4.1 Reducing P16 increase proliferation, upregulated p16 induced stunting:

Upregulated in younger age, then downregulated in older age after experiencing of Senescence barrier phase. The senescence barrier could be intrauterine due to maternal mycotoxin exposure and adverse pregnancy outcomes,^{52,68} pregnant women,^{42,51,52,69} which have associated with growth impairment.^{3,8,42,43,70} emphasize that AFB1, especially in utero, is associated with DNA methylation in white blood cells of infants in the Gambia.⁷⁰ Table 3. Review the sequence of upregulated p16 which induced stunting, through the senescence barrier phase,²⁰ with the silencing/reducing p16 (downregulated p16) induced proliferation such as overweight and obesity,¹² and cancer.^{14,19}

4.2 Urine AFM1 sub ppb could be useful for global fighting on stunting^{5,7,47,50}:

Also anthropometric measurement of child overweight/obesity and anamnesis survey.⁴ Table 2. said many patients, urine AFM1, p53-p21-p16, mainly of developing countries and low-middle-nSES with senescence,^{2,20,21} growth impairment,^{1,9,43,46} obese,^{11,12} and cancer^{1,40} with high urine AFM1 as metabolite of AFB1 in Asia, Africa and Latin America.^{4,11}

4.3 Brazilian overweight has also been associated with stunting:

Geographic wet and warm climate, a good condition for *Aspergillus* sporulation, produce AFB1 exposure, and induce stunting Table 1, by upregulated p16 gene which the p53-p21-p16 axis could be described in Fig. 1. Imported food goes nSES globally.¹¹ So reported overweight,¹² obese,¹¹ and Diabetes.²⁵ A systematic review on the prevalence and factors associated with overweight and obesity in Brazilian children and adolescents is about 20-30%, and it was observed that the socioeconomic factors were associated with the outcome, and are subject to change from the adoption of a healthy lifestyle.⁶⁴

4.4 Mitigation AFB1 on food contamination using gamma-ray to fight stunting, overweight, obesity, and cancer:

Inactivation of aflatoxin B1 by using the synergistic effect of hydrogen peroxide and gamma-ray, has been reported since 1989.⁶⁵ Reducing aflatoxin exposure in agriculture is an issue for the prevention of aflatoxin-related health problems.^{40,41}

4.5 Urine AFM1 in pg/ml (sub ppb)^{5,7,47,50,51}:

Anthropology measurements (BMI, WC) and keeping the Refrigerator empty, are good lifestyle to prevention from AFs exposure to various cooking spices, especially small red onions and candlenut which are everyday spices in Indonesian cuisine, and should be stored in open-air ventilation air to prevent from AFs exposure.⁴

4.6 Expression of p16 induces transcriptional downregulation of the RB gene Oncogene.⁶³:

In another way, Viral E6/E7 in HPV, HBV, EBV represent the oncogenic activity parallel with inducing senescence barrier of downregulated p16 gene-activate the RB gene.^{17,71,72,73}

Decreased prevalence of stunting and increased obese children, confused as successes the project as given of good nutrition for the undernourished and supplement and probiotic. It was never considered that both stunting and obesity took place successively following in order in sequence obese after stunting, through the senescence barrier (methylation promoter of p16 gene) period. An independent cross-sectional household survey for 12 years, of children from Alagos, Northeast Brazil was reported has the prevalence of stunting and overweight, both were very close in 2005 and if the trends were maintained, at this time, the childhood overweight prevalence has already exceeded that of the stunting.⁷⁴

V. LIMITATION

It is reported that p16(INK4a) in p53-p21-p16 axis, upregulated (growth impairment) than down regulation (proliferation) after the senescence barrier, but many studies do it partly in period of age,⁷⁴ associated with nSES.⁷⁴ Our study joint both the whole life how p53 mutation, upregulated p21 then p21 downregulated, which upregulated p16 (growth impairment) in stunting and precancerous or good survival stage per individual. By the downregulated p16 genetic or epigenetic hypermethylation, proliferation of cancer cells and migration take place. The aflatoxin exposure could be also intrauterine since pregnancy,^{68,69,42,52,70} which the exist of senescence barrier (DNA methylation) reported in infant.⁷⁰

Epidemiology of high prevalence of stunting, obesity, and CKD1 in low- middle-nSES/ income populations in developing and developed countries (AFB1 exposure),¹⁸ Fighting for AFB1 exposure should become a political concept led by the legislative. The molecular pathway based on p53-p21-p16 axis biology molecular signaling in proven sub-ppb technology aspect in nSES population should be known by the people. Till now, it has been considered, an effort to target fighting stunting could be genetic,⁷⁵ deficient of zinc^{76,77} and another supplement, meta-analysis probiotic,⁷⁸ and undernourished due to gut health.^{2,79} Agriculture & Health setting to address this aflatoxin problem, has called us to participate.

VI. CONCLUSION

Evidence-Based Diagnosis of AFB1 exposure by urine AFM1 sub-ppb marker for public services, could decrease the prevalence of stunting which should be paralleled with Indonesian heritage management from A to Z to get zero-level food free of AFB1 exposure. Various molecular pathophysiological effect was already known as p53-p21-p16 axis pathways, that upregulation of p16INK4a slow down the G1→S cell cycle and support the cause of stunting.

Urine AFM1 level represents aflatoxin B1 exposure is needed for public services simply to convince people, policymakers, legislation is urgent to fight stunting. We propose that legislation should address the role of AFB1 exposure in the pathogenesis of stunting and evaluate interventions to limit AFB1 exposure directly (UPLC) or indirectly (BMI/WC/SsST/refrigerator fullness) to reduce childhood stunting. Stunting and obesity are markers especially vulnerable to mycotoxin exposure.

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CONFLICT OF INTEREST

The author declares the possession to fight AFB1 exposure with no vested interest in it.

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Improving Poultry Waste Management for Energy Production in Nigeria: A Case Study of Poultry Management Systems in Selected Local Government Areas of Anambra State, Nigeria

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Abstract— The aim of this study was to conduct a survey of poultry production systems and waste management in some local government areas of Anambra State of Nigeria. The basics of poultry farming were discussed including various types of chicken kept by farmers, production systems and scales. A proposal for improved poultry waste management through anaerobic digestion for biogas production was also discussed. The work highlighted anaerobic digestion process of poultry waste in biogas production. The research methodology adopted in the work is primary data obtained by use of questionnaires distributed to respondents, also secondary data obtained from journals and newspapers. The study revealed that medium scale poultry farms are predominant in the study area where majority of the farms still operate deep litter system. Only few of the farms operate battery cage system. It was found out that mechanized poultry farming is still at its lowest ebb in the state. It was found out that the poultry wastes generated by farmers are widely used for fertilizer. It is recommended that some level of mechanization is introduced in the industry to minimize the drudgery associated with poultry farming; technical training programs should be organized on regular basis to familiarize farmers with modern technology in poultry farming. Extension services are also recommended to educate farmers on recent best practices in the industry.

Keywords— Poultry Farm Mechanization, Poultry Waste, Waste Management, Anaerobic Digestion, Biogas Production.

I. INTRODUCTION

Agriculture is the mainstay of Nigeria's economy, employing more than two-thirds of her total active labour force and has been contributing more than 42.2% of her gross domestic product (GDP). Also in 2007, agriculture provided about 88% of the country's non-oil earning. However, despite all these achievements, animal protein especially meat is expensive, in short supply and out of reach of the majority of the population. Thus, most of the people get far too little of these nutritious and protective foods such as meat and eggs that are required for normal growth, energy and resistance to various diseases (Chukwuji *et al*, 2006). The effect of this inadequate meat intake is felt more by a large proportion of the population especially in rural areas, where inhabitants constitute over 70% of Nigerians who constitute about 85% of the extreme poor in the country (Anosike *et al*, 2015).

The term poultry generally used in agriculture refers to all the domesticated birds kept for eggs or meat production. These include chickens or domesticated fowls, turkeys, ducks and geese. However, at times the term poultry is seen as being synonymous with chickens. The rising standard of living in the country has resulted to rising demand for eggs and meat. Meanwhile, the fact that people cannot raise their own poultry has called for more establishments of poultry farms in the country. Under this system, birds are kept in runs made of wire-netting where they can move about in the runs during the day but are kept in poultry house at night.

Poultry meat and eggs play a very useful role in bridging the protein gap in Nigeria (Nwagu, 2002). The total poultry bird population in Nigeria reached a total of 169 million heads in 2021 (Doris, 2023; Ajala *et al*, 2007), same as the count of the preceding year, however, the count observed that within the period, the highest stock of live poultry birds in the country was registered in 2018 which stood at 184 million birds. The Nigerian poultry industry has been contributing approximately 25%

to her agricultural GDP; and since 2008, there has been a deliberate national drive to promote agriculture as business. But Adedotun (2002), noted that despite the expansion of the poultry industry in recent years, poultry farming in the country only caters for about 30% of chicken eggs and meat needs of Nigerians, which amounts to about 300metric tons and 600metric tons of eggs respectively per year. The poultry industry in Nigeria is worth about N1.6tr, making it the most commercialized sub-sector of all Nigeria's agricultural sub-sector (Emefiele, 2019; FAO, 2019; Udo *et al.* 2006; PAN, 2017; Adene and Oguntade, 2006; World Bank, 2005). There is consensus that about 90% of the figures derived from the local poultry stocks are composed of chickens (91%), guinea fowl (4%), duck (3%), turkeys and others (2%).

1.1 Poultry Management:

Depending on the purpose for which the birds are reared, and available capital, there are various methods of poultry management:

1.1.1 Intensive Systems:

In this system, the birds are kept indoors all the year round. More fowls can be kept in a small area of land than under the semi-intensive or free range systems. There are different methods or intensive management systems.

1.1.2 The Fold System:

Under fold system, the birds are kept in moveable houses which allow the birds certain amount of freedom. Thus, this method is successful where land is clear and well-drained, with good pasture, however, the system is very expensive and demands much attention.

1.1.3 Deep Litter System:

In this system, birds are kept in as completely closed but well-ventilated house where they are fed. The birds are kept indoors all the time on a layer of litter made of absorbent materials, such as straw, saw dust, or wood shavings, which absorbs the liquid droppings of the poultry. It is essential that the house is airy and cool, providing about 1 sq. m space per bird for light breeds and about 1.3 sq.m space for birds for heavy birds.

Inside the deep litter house, feeding and drinking troughs are necessary, as well as suitable length of perch on which birds can rest at night. Both feeders and drinkers should be moveable, and should be kept not less than 46 cm high such that birds cannot perch on them or defecate into them this is to avoid feed wastage or spread of disease.

1.1.4 Battery Cage System:

Birds especially layers can be kept in battery cages made of galvanized wire. The birds may be kept single in each cage or in groups of two to four. Some battery cage systems permit automatic supply of both feed and water and egg collection while other operations can be carried out manually. This system allows poultry droppings to fall through the wire at the bottom of the cage from where they can be cleared away.

1.1.5 Free Range System:

In free range system, birds are allowed to roam freely about under natural conditions and feed themselves. However, this system exposes the birds to the dangers of wild animals and theft.

1.1.6 Semi-intensive System:

1.2 Types of Chicken:

Although there are many different breeds of chickens used in poultry farming, they all can be divided into three types:

1.2.1 Layers:

These breeds primarily are for egg production. These birds usually weigh about 1-2 kilograms. They are lighter than chicken bred used for meat production, and they need less feed to maintain their body weight while laying as many or more eggs than the big birds.

1.2.2 Meat Chickens:

These types of chicken grow very rapidly and reach marketable size after two to three months, and are commonly called broilers. However, their size and age determine whether they are called a fryer or a rooster.

1.2.3 Dual-Purpose Chickens:

These birds are raised for both their eggs and meat. Females of the new, improved breeds are kept to lay eggs while the males are separated and sold for meat as soon as they reach about 15 weeks of age.

1.3 Basic Requirement for Poultry Housing:

There are some important basic requirements for poultry housing such as: space, ventilation, light, and protection.

1.3.1 Space:

Space is most important basic requirement for poultry housing as it determines the number of poultry that can be kept (Chukwuji *et al.* 2006). For instance, a deep litter size of 6m x 11m can hold 200 laying hens at stock density of 3 birds/m². (3.6 ft²/bird), while the recommended requirement for chickens for floor and perch space is presented in Table 1. Less space creates stressed social behaviour which encourages disease vulnerability and cannibalization, weaker birds deprived of feed or perch space (Sonaiya, 2000; Ugwu, 1990; Adeyemo and Onikoyi, 2012).

TABLE 1
REQUIREMENT OF CHICKEN FOR FLOOR AND PERCH SPACE.

Chicken Types	Floor Space (Birds/m ²)	Floor Space (ft ² /Bird)	Perch Space (per Bird)
Layer	3	3.6	25 cm (10 in)
Dual purpose	4	2.7	20 cm (10 in)
Meat	4 – 5	2.1 – 2.7	15 – 20 cm (6 – 8in)

1.3.2 Ventilation:

A building with open sides is ideal, otherwise cross-ventilation at bird-level should be allowed for in the form of floor level inlets, open in a direction to allow the prevailing wind to blow across the width of the building. Heat stress can lead to death of the birds. Birds can withstand several degrees below freezing point, but cannot tolerate temperature above 40°C (Feddes *et al.* 1992; Kocaman *et al.* 2005; Salum *et al.* 2002). Building materials such as tin or other metal should be avoided for this reason. Heat stress affects the birds in several ways:

- i. Reduction in feed intake as ambient temperature rises.
- ii. An increase in water consumption in an attempt to lower temperature.
- iii. A progressive reduction in growth rate.
- iv. Reduction in rate of laying eggs

1.3.3 Light (Duration and Intensity):

A well-lit house is essential for birds as a dark house leads to lethargic, inactive and unproductive (Kenneth and Larry, 1981). Light is important for feeding, increased egg production, thus regular and reliable electricity supply is required.

1.3.4 Protection:

There are many factors that affect the type of houses. Birds need to be properly housed to protect them from adverse effect of weather or predators. These factors include local climate, space, size and number of the flock, and management system (Onwualu *et al.* 2006). In extensive systems, birds must be protected from disease and predators such as snake, kites, rats, theft, and other vermin (Conroy *et al.* 2005; Sonaiya *et al.* 2004).

1.4 Poultry Waste:

Poultry raised for commercial purposes produce large amount of wastes which contain valuable plant nutrients and other chemicals that if properly managed, can be returned to the land or processed for other uses.

1.5 Production of Biogas from Poultry Waste:

1.5.1 Definition of biogas:

Biogas is a flammable gas produced when organic materials are fermented under anaerobic condition. It originates from biogenic material and it is a type of bio-fuel (Ghosh, 1997; Jenner, 2006). Biogas has globally remained a renewable energy source derived from plants that use solar energy during the process of photosynthesis. Being is source of renewable natural gas; it has been adopted as one of the alternatives to fossil fuels after 1970's world energy crisis. Biogas is a product of the metabolism of methane bacteria and is created when bacteria decomposes a mass of organic materials. It is smokeless, hygienic and more convenient to use than other solid fuels. To produce biogas, water is added to animal/plant waste in a certain ratio to form slurry and digestion takes place in the process of anaerobic digestion. Anaerobic digestion (AD) is a microbial process in which micro-organisms breakdown and organic waste because it provides volume and mass reduction of the input material. Anaerobic digestion is also a biological process in which organic material is decomposed in the absence of oxygen to produce biogas. The organic matter can be degraded by the sequential action of hydrolytic, acetogenic and methanogenic bacterial to produce biogas.

Biogas is a colourless, flammable gas produced through anaerobic digestion of animal, plant, human, industrial and municipal waste amongst others. It is composed of methane (50-70%), carbon dioxide (20-40%), water vapour (2-7%), and traces of other gases such as ammonia, nitrogen, hydrogen, hydrogen sulphide as shown in Table 2 below.

TABLE 2
COMPOSITION OF BIOGAS

Component	Concentration by volume (%)
Methane (CH ₄)	50 – 70
Carbon Dioxide (CO ₂)	20 – 40
Water (H ₂ O)	2 – 7
Hydrogen Sulphide (H ₂ S)	2
Ammonia (NH ₃)	0 - 0.55
Nitrogen (N)	0 – 2
Oxygen (O ₂)	0 – 2
Hydrogen (H)	0 – 1

Source: Mattocks, (1980)

Biogas technology is a biochemical conversion technology of bio-energy conversion where decomposition or degradation of organic matter occurs in the absence of oxygen by microorganisms (Legett, 2006). Biogas technology is based on the phenomenon that when organic matter containing cellulose is fermented in the absence of air (anaerobically), combustible gases (chiefly methane) are emitted. Biogas technologies commonly apply consortia of microbes. These communities form an intricate microbiological food chain.

1.5.2 Bases for Biogas Technology:

Biogas is produced by the biological breakdown of organic matter in the absence of oxygen. It originates from biogenic material and is a type of bio-fuel. One type of biogas is produced by anaerobic digestion or fermentation of biodegradable materials such as biomass, manure or sewage, municipal waste, green waste and energy crops. This type of biogas comprises primarily of methane and carbon dioxide. The other principal type of biogas is wood gas which is created by gasification of wood or other biomass. This type of biogas is comprised primarily of nitrogen, hydrogen, and carbon monoxide, with trace amounts of methane.

Biogas generators or digesters yield two products: The biogas itself and a semi-solid by-product called effluent or sludge. Biogas systems are most popular for their ability to produce fuel from products that might otherwise be wasted crop residues or manures. The fuel is a flammable gas suitable for cooking, lighting, and fuelling combustion engines.

1.5.3 Formation of Ammonium Fertilizer from Poultry Waste:

The digested waste or sludge is a high-quality fertilizer. The digestion process converts the nitrogen in the organic materials to ammonium, the form in which it becomes more stable when ploughed into the soil. Ammonium is readily fixed or bonded in the soil so that it can be absorbed by plants. Moreover, biogas systems offer a need to sanitize wastes. Thus, the systems are capable of destroying most bacteria and parasitic eggs in human and animal wastes, enabling the digested sludge to be applied safely to crops.

1.5.4 Biogas models:

The development of biogas plant that co-digests agricultural waste with other organic wastes, energy crops or industrial wastes has been aggressive over the past two decades. This is as the result of economic, social and environmental pressure. The Kyoto Protocol, which requires countries to meet 1990 levels of greenhouse gas (GHGs), is a very significant driver (Energy Commission of Nigeria, 1998). In Europe, Denmark has been the world leading country in anaerobic digestion development and implementation, especially for generating manure for fertilizer and for electricity production. One of the driving forces in Denmark is their goal of achieving 33% of their total energy requirement to be derived from renewable energy sources by the year 2030. Biogas generators or digesters operate throughout Asia, for example, more than 100,000 biogas generators or digesters have been reported to be in use in India, about 30,000 in Korea, and several millions in China. Ancient Chinese experimented burning the gas given off when vegetables and manures were left to rot in a closed vessel (Nwoye *et al.* 2014; Chukwu *et al.* 2006).

Presently, China has successfully promoted the use of biogas as a source of household energy since 1980s, especially in the rural areas where wood for fuel was in short supply and rural electricity was not available. Each household builds its own plant to channel waste from the domestic toilet and nearby shelters for animals, usually pigs, into a sealed tank. The waste ferments and is naturally converted into gas and compost. In addition, the project has resulted in better sanitary conditions in the home. In Nigeria, biogas technology can serve as a means to overcome energy poverty, which poses a constant barrier to economic development in Africa, (Chukwuma *et al.*, 2021). Biogas production from energy crops, agricultural wastes, industrial wastes, municipal water, crop residues etc., does not compete for land, water and fertilizers with food crops like is in the case with bio-ethanol and biodiesel production.

1.5.5 Biogas technology in Nigeria:

Anaerobic digestion has been deemed one of the most useful decentralized sources of energy supply by the United Nations Development Programme. In United States of America, Europe and Asia, there has been considerable interest in the process of anaerobic digestion as an approach to generating a safe clear fuel as well as source of fertilizer (Chukwuma *et al.* 2013; Umeghalu *et al.* 2015). In the past decades, the consumption of poultry in Nigeria and in many other countries has been on the increase. Growing demand for poultry product has resulted to corresponding increase in the poultry industry and consequently increased amount of organic solid by-products and wastes. It was reported that annually about 724.8 tons of poultry droppings and 184,128 tons of paunch are produced from poultry farms and from cows slaughtered in major abattoirs respectively in Anambra State of Nigeria (Umeghalu *et al.* 2012). Only a small proportion of the poultry droppings generated in major farms in the state is utilized for manure application (majorly during planting season) and fish farming. Poultry droppings can be considered as a sustainable biomass. The rapid growth of poultry industry in the country has been causing increasing concern about the disposal of poultry wastes with respect to non-point source pollution. The management of poultry waste constitutes a major problem in poultry industry (Umeghalu *et al.* 2012; Chukwuma *et al.* 2012).

II. MATERIALS AND METHODS

2.1 The Study Area

The study was carried out in seven out of the twenty one local government areas that make up Anambra State of Nigeria. Anambra State is located in the South-East Geopolitical Zone of Nigeria between Latitude 5°37'60N and Longitude 7°10'0E (NPC, 2006). Fig. 2.1 shows the map of the Anambra State which is bounded in the East by Enugu State, in the North by Kogi State, in the South by Rivers and Imo States and in the west by Delta State. The state comprises of 21 LGAs with a population of 4.06 million (NPC, 2006) people and a population density of 1,500 to 2,000 persons living within every square kilometer. Anambra State occupies a land mass of about 4,844 km² (1,870.3 square miles).

The poultry farms studied were randomly selected and are located in the following local government areas of State: Awka North, Awka South, Oyi, Anaocha, Ekwusigo, Nnewi North, and Idemili North Local Government Areas.

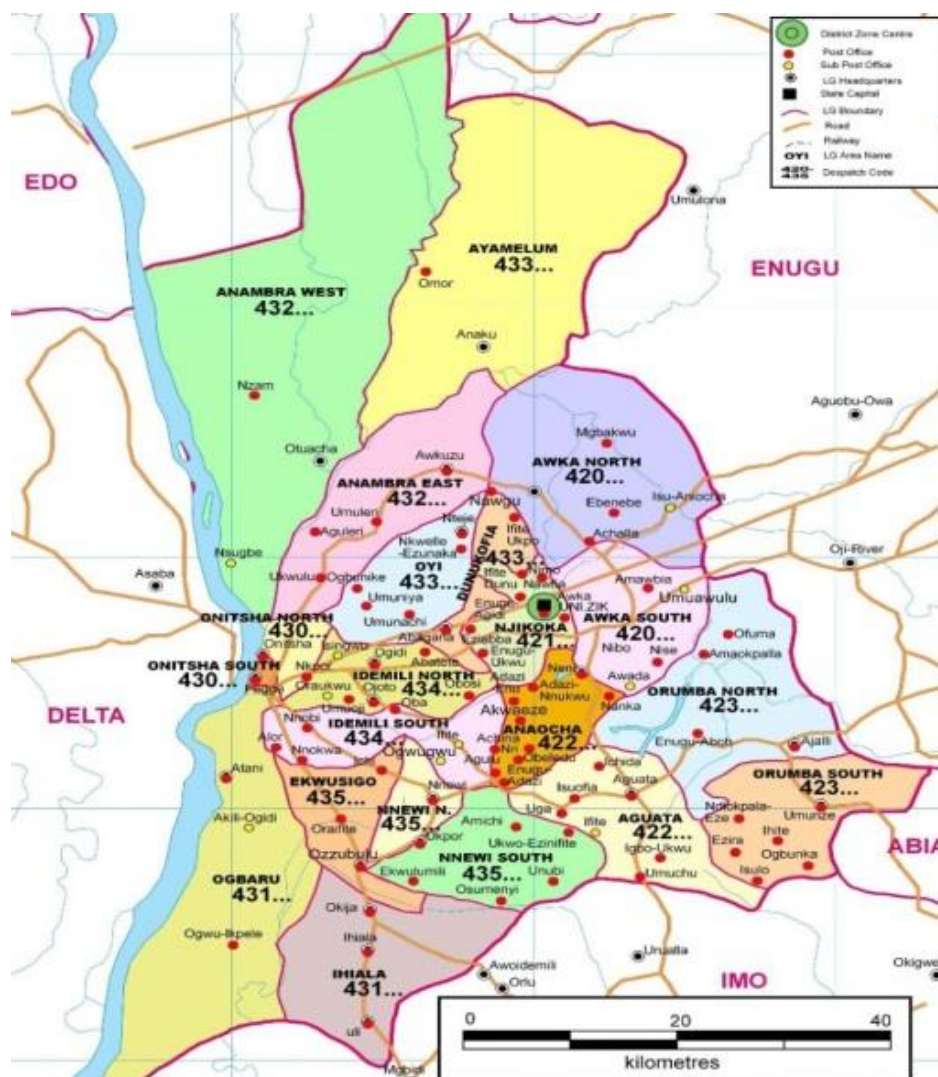


FIGURE 1: Map of Anambra State of Nigeria.

The criteria for the selected nine poultry farms were based on their categorization as being small or medium scale in addition to size of the farms, number of years of experience in poultry farming and membership of Poultry Association of Nigeria (PAN). The primary data used for this research were obtained from questionnaires designed for nine (9) respondents. All the nine (9) questionnaires were filled properly and returned.

III. RESULTS AND DISCUSSIONS

Analytical tools such as graphs and simple descriptive statistics were used to characterize and analyze the data generated from the study. Also, in the course of data analysis in this work, the percentage distribution of data were first obtained for easy and proper analysis of the data as shown in the tables below.

3.1 Percentage Distribution of the poultry types.

The data presented are shown in the tables and analyzed in percentages as shown. Table 3 below represents the percentage distribution of each poultry type (layers, broilers and day-old chicks) and number housed respectively by the farms. The estimated total production of poultry in the study area is 234,035 birds comprising 41,750 (17.84%) layers, 65,985 (28.19%) broilers and 126,300 (53.97%) day-old chicks.

The numbers of birds housed by the farms differ markedly. Out of the 41,750 layers in the study area, Ausco Farms has 47.90%, Chika Ebele Farms has 11.98%, Labour Farms has 14.37%, Ozubulu Monastery Farms has 1.8%, Aroma Farms has 9.58%, Chidera and Ifeukwu Farms both have 2.4% while Michael and F.C. Muonwem Farms both have 4.79%.

Out of the 65,985 broilers, in the study area, Ausco Farms has the largest percentage of 90.93% followed by Aroma Farms with 4.85%, Chika Ebele Farms has no broiler, Labour Farms has 2.27%, Ozubulu Monastery Farms has 0.13%, Chidera Farms 0.61% and Ifeukwu Farms have no broiler. Michael Farms has 0.76% and F.C. Muonwem Farms has 0.45%.

Ausco Farms has 95.10% of the total number of 126, 300 day-old-chicks housed in the farms studied, Chidera Farms has 1.19%, Aroma Farms has 1.58%, Michael Farms 0.63%, F.C. Muonwem and Labour Farms both have 0.79%, while Chika Ebele, Ifeukwu and Ozubulu Monastery Farms have no day-old chicks.

TABLE 3
PERCENTAGE DISTRIBUTION OF POULTRY TYPE AND NUMBER IN THE FARMS STUDIED

S/N	Name of Farms	Distribution of Layers (%).	Distribution of Broilers (%).	Distribution of Day-old Chicks (%).
1.	Ausco Farms	47.90	90.93	95.01
2.	Chika Ebele Farms	11.98	-	-
3.	Labour Farms	14.37	2.27	0.79
4.	Ozubulu Monastery Farms	1.80	0.13	-
5.	Chidera Farms	2.40	0.61	1.19
6.	Ifeukwu Farms	2.40	-	-
7.	Aroma Farms	9.58	4.85	1.58
8.	Michael Farms	4.79	0.76	-0.63
9.	F.C. Muonwem Farms	4.79	0.45	0.79
	Total	100	100	100

3.2 Percentage scale of poultry production:

Table 4, below presents the percentage distribution of production scale of the poultry farms studied. Out of the nine farms studied, two farms can be categorized as small scale farms with (22.22%) each, six of the farms are medium scale farms which made up (66.66%) of the total poultry production and only one is categorized as large-scale farm with (11.11%) of the total poultry production.

This shows that there are more of the medium scale farms in the study area. The research indicates that the level of mechanization in poultry farming in Anambra State is at its low ebb. The methods of feeding, brooding of day-old chicks, egg and waste collection and disposal predominantly are carried out manually by the employees of the farms.

TABLE 4
PERCENTAGE SCALE OF POULTRY PRODUCTION

S/No	No. of Farms	Production Scale (No. of Chickens)	Percentage distribution (%)
1.	2	Small Scale (less than 2,000)	22.22
2.	6	Medium Scale (2,000-10,000)	66.67
3.	1	Large Scale (10,000 and above)	11.11
		Total	100

3.3 Distribution of daily egg production in crates of 30 eggs each:

The estimated total egg production is 871 crates daily of 30 eggs each with Table 5 below showing the distribution respectively by the farms studied. From the table, Ausco Farms produces about 500 crates of eggs (57.41%) followed by Aroma Farms with 135 crates (15.50%), while Ifeukwu Farms and Ozubulu Monastery Farms produce 26 crates (2.99%) and 11 crates (1.26%) respectively representing the least daily egg production among the farms studied indicating those farms with the highest number of layers produce more eggs.

TABLE 5
SHOWING DISTRIBUTION OF DAILY EGG PRODUCTION IN CRATES OF 30 EGGS EACH.

S/N	Name of Farms	Daily egg production in crates of 30 eggs each.	Percentage Distribution (%)
1.	Ausco Farms	500	57.41
2.	Chika Ebele Farms	40	4.59
3.	Labour Farms	60	6.89
4.	Ozubulu Monastery Farms	11	1.26
5.	Chidera Farms	30	3.44
6.	Ifeukwu Farms	26	2.99
7.	Aroma Farms	135	15.50
8.	Michael Farms	35	4.02
9.	F.C. Muonwem Farms	34	3.90
	Total	871	100

3.4 Brooding size of poultry farms studied:

Brooding stage is the first and most delicate stages of poultry husbandry when the strength foundation of the birds are laid with respect to future performance as regard to egg laying, body weight, growth rate, feed consumption, conversion efficiency and resistance to diseases. Table 6 shows the percentage distribution of brooding size of the respective farms. Ausco Farms broods 20,000-day-old chicks (62.02%), Chika Ebele, Labour, Aroma and Michael Farms brood 2,000 chicks (6.2%) each, while Chidera and F.C. Muonwem Farms brood 1,500 chicks (4.65%) each. Ifeukwu and Ozubulu Monastery Farms brood 900 chicks (2.79%) and 350 chicks (1.09%) respectively. Factors affecting brooding of day-old-chicks are temperature, light, relative humidity, ventilation, floor space, nutritional requirement and ammonia concentration. There are optimum temperatures for chicks of different ages. Too high or too low temperatures will slow down their growth rate. Extreme temperatures may cause death. The most important condition for brooding is to keep the chicks comfortable and to avoid extreme temperature.

TABLE 6
BROODING SIZE OF POULTRY FARMS STUDIED.

S/N	Name of Farms	No. of day-old-chicks	Percentage
1.	Ausco Farms	20000	62.02
2.	Chika Ebele Farms Name	2000	6.2
3.	Labour Farms	2000	6.2
4.	Ozubulu Monastery Farms	350	1.09
5.	Chidera Farms	1500	4.65
6.	Ifeukwu Farms	900	2.79
7.	Aroma Farms	2000	6.2
8.	Michael Farms	2000	6.2
9.	F.C. Muonwem Farms	1500	4.65
	Total	32250	100

3.5 Monthly poultry waste generation by the farms studied:

Table 7 below shows the distribution of monthly poultry waste generated by the farms. Out of the 1,244 bags (25 kg each) of poultry wastes generated, Ausco Farms generates the highest quantity of waste generated with 600 bags representing about (48.23%) of the total quantity of the poultry wastes, Aroma Farms generates about 200 bags (16.08%), F.C. Muonwem Farms generates 112 bags (9%), Michael farms 150 bags (12.06%), Ifeukwu Farms generates 85 bags (6.83%), Labour Farms produces about 40 bags representing 3.22%, while Ozubulu Monastery Farms and Chidera Farms each generates 20 bags representing 1.61% each and Chika Ebele Farms generates about 17 bags (1.37%). With these data, if the wastes are effectively managed as source of bio-energy production, it will result to having cleaner environment, sustainable source of raw material for bio-energy production and fertilizer for farmers.

TABLE 7
MONTHLY POULTRY WASTE GENERATION BY THE FARMS STUDIED IN 25KG BAGS.

S/N	Name of Farms	Monthly poultry waste ((25kg bag)	Percentage
1.	Ausco Farms	600	48.23
2.	Chika Ebele Farms Name	17	1.37
3.	Labour Farms	40	3.22
4.	Ozubulu Monastery Farms	20	1.61
5.	Chidera Farms	20	1.61
6.	Ifeukwu Farms	85	6.83
7.	Aroma Farms	200	16.08
8.	Michael Farms	150	12.06
9.	F.C. Muonwem Farms	112	9
	Total	1,244	100

3.6 General overview of poultry production system of the farms studied:

TABLE 8
RESULT OF THE SURVEY NOT SHOWN IN THE TABLES.

S/N	Farms	Production purpose	Production system	Method of waste management	Price of day-old chick (N)	Constrains	Common diseases
1.	Ausco Farms	Day old chicks, eggs & meat production	Battery cage & Deep litter	Fertilizer, Feedstock & Bio-digester	-	Finance	Newcastle
2.	Chika Ebele Farms	Meat and egg production	Deep litter	Fertilizer	200 – Layers	Finance	Gomboro, Newcastle & Coccidiosis
3.	Labour Farms	Day old chicks, eggs & meat production	Deep litter	Fertilizer & Feed	230 - Layers; 180 – Broilers	Bad road network	Newcastle
4.	Ozubulu Monastery Farms	Meat and egg production	Deep litter	Fertilizer	210 - Layers; 205 – Broilers	Finance	Green & white
5.	Chidera Farms	Meat and egg production	Deep litter	Fertilizer	180 – Layers	Disease	Coccidiosis
6.	Ifeukwu Farms	Egg production	Deep litter	Fertilizer & Feed	150 – Layers	Lack of mobility, capital	Gomboro, Newcastles, Almonella & Coccidiosis
7.	Aroma Farms	Egg production	Deep litter	Fertilizer	230 - Layers; 180 – Broilers	Unavailability & high cost of day-old chicks	Newcastle, & Coccidiosis
8.	Michael Farms	Meat and egg production	Deep litter	Fertilizer	230 - Layers; 180 – Broilers	High cost of feeding	Cough
9.	F.C. Muonwem Farms	Meat and egg production	Battery cage & Deep litter	Fertilizer	220 - Layers; 120 – Broilers	Slow market	Newcastle,& Coccidiosis

IV. CONCLUSION

Brooding stage is the first and the most delicate stage in poultry husbandry. It is at this stage that the strength of performance of the birds are laid in respect to egg laying, body weight and good growth rate in broilers, feed consumption and conversion efficiency and resistance to diseases.

Efficient use of the poultry wastes generated by these poultry farms for biogas energy production will contribute meaningfully in solving the energy crisis in the country as are found in many countries of the world such as in Asia, Europe and America.

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Mosquito Repellent Activity of Various Formulations of Scent Leaf Essential Oil

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Abstract— Mosquitoes are the most deadly vectors of parasites that causes diseases such as malaria, yellow fever, dengue fever and phillieriasis, in view of the recent interest of developing plant based mosquito repellent on a replacement to the synthetic repellence. Hence, this study aimed at evaluating the mosquito repellent activity of *O.gratissimum* with the objective of accessing its incense, spray and cream formulation. The plant material used in this study was sourced from Akanu Ibiam Federal Polytechnic Unwana and processed into crude extracts for incense and spray and also extraction of oil for cream formulation. The mosquitoes used in this study was cultured from stagnant water kept in the lab. The evaluation of incense burning and spray of the crude extract was done using 5 different household for 3 consecutive days. The landing time and percentage repellency was done using mosquito cages containing 30 female blood starved mosquito. ODOMOS was used as standard, petroleum jelly and essential oil as the test and petroleum jelly alone as the control. Each evaluation was done for 10mins and all in triplicate. The results obtained showed 4hours and 2hours for incense burning and spray respectively. The landing time for DEET is 6 minutes, 1 minutes and 3minutes for standard, control and test and total bite of mosquito is 1, 26 and 12 for standard, control and test and repellency of 96.6% 13.3% and 60% respectively. The result showed that *Ocimum gratissimum* has significant mosquito repellency. Therefore, more research attention is being invoked towards harnessing the potential of using this plants extracts as a replacement for synthetic repellent.

Keywords— cream, incense, mosquito repellent and spray.

I. INTRODUCTION

1.1 Background of the Study

Female mosquitoes are one of the most disturbing blood sucking insects that afflicts human beings. They need blood meal in other to produce viable eggs that will hatch. Mosquito belongs to the family culcidea and order disptera (Ralph, 2008; Molavi; 2013). Mosquitoes are important primary host in the spread of malaria, yellow fever, and severe arboviral infections.

Malaria which is caused by plasmodium parasites transmitted through the bites of female Anopheles mosquitoes continue to impact a major disease burden on infants and young children in endermic region (WHO 2014; and Chaiyakunapruk; 2011) in 2012, there were about 207 million cases of malaria and an estimated 627,100 deaths all around the globe. (WHO; 2014). Malaria is among the biggest public health issues globally, especially in tropical Africa, in which Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths reported annually (approximately 30%of total malaria burden in Africa). While 97% of the total population (Approximately, 173 million) is at risk of infection (WHO; 2014). Therefore, the control of mosquitoes and prevention of their bites are important public health concern around the world. One

of the approach for control of these mosquito-borne diseases is the interruption of diseases transmission by either killing the mosquitoes or preventing them from biting individuals (Adeogun *et al.*, 2012).

Mosquito repellents generally function by hindering the capacity of the female mosquito to recognize the external stimuli (for example, carbon-dioxide water vapor, and heat) that she utilizes to spot a host (Pears; and Granshaw 2000). The mosquito and other insect repellent properties of N,N-diethyl-3-toluamide also known as DEET, were discovered as the first DEET product was introduced in 1956. Since it became commercially available, it has generally been regarded as safe. However, toxic effects have been recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate. An alternative to repel mosquitoes could be plant-based natural materials like plant oils to prevent the adverse effects of synthetic repellents. In comparison with synthetic repellents, they are deemed safe and good for the environment (Azeem *et al.*, 2019).

Additionally, the use of chemical insecticide has been greatly impeded due to development of physiological resistance in the insects intermediary, environmental pollution resulting in bio-application of food chain contamination and harmful effects on beneficial non-target animals. However, people may be ignorant of the facts that overuse and injudicious application of such synthetic insecticides may result in resistance and unwanted toxic or lethal effects on a non-target organism as well as human and other environmental health challenges (WHO;2018).

This study therefore, aimed at determining the mosquitoes repellent activity of formations of scent leaf (*ocimum gratissimum*) essential oil in its various formulations.

II. MATERIALS AND METHODS

2.1 Collection of Plants:

Source: The scent leaf (*Ocimum gratissimum*) was obtained from the department of Science Laboratory Technology Herbarium in Akanu Ibiam Federal Polytechnic Unwana, Afikpo Ebonyi State, into a clean sack bag. The leaves were science and the unwanted leaves were removed. The leaves were taken to a plant taxonomist in the department of science laboratory technology to be authenticated.

2.2 Sample processing:

Crude Extraction (spray and incense burning): Under running tap water and was left to dry under room temperature and was grounded into a fine powder. The grounded leaves were divided into 2 portions, one portion was soaked in ethanol for 48 hours and was filtered with a Whatman No 1 filter paper. The filtrate was concentrated to a constant dry weight.

2.2.1 Essential oil extraction (cream):

The other portion was soaked in N-hexane for 36 hours and then it was filtered. Ethanol was added and it was shaken which forms two layers (N-hexane at the bottom and ethanol at the top).

2.2.2 Formulation of incense, spray (aerosol) and cream

Incense: A known quantity of the plant leaf *ocimum gratissimum* was allowed to dry in room temperature for about 2 - 3 days and it was burnt to generate the incense.

- **Spray (aerosol) :** A 40ml of the oil extracts was combined with a 40ml of the ethanol to form the spray.
- **Cream :** A 10% of the essential oil which was dissolved in acetone was mixed with a 10% quantity of the petroleum jelly to form a cream (Sofowara, 2018, and Ojo *et al.*, 2010).

2.3 Experimental Design

The mosquito was gotten from a cultured larva from stagnant water, using physical characteristics of the larva for identification. The mosquito was maintained with 10% sucrose for 4 days at normal room temperature at 12 hours day light and 12 hours darkness before use. (Karunamoorthy *et al.*, 2010).

2.4 Determination of Landing Time:

Landing time is the average time required for the first mosquito to land on the exposed area and attempt to take a blood meal. A untreated hand was exposed to the mosquitoes and the time of landing was recorded to determine the readiness of the mosquito to take blood. This procedure was repeated for about 3 times in each cage and the average landing time was calculated.

2.5 Percentage Repellency:

The repellency of the essential oil was evaluated by using arm-in-cage test. The student of Akanu Ibiam Federal Polytechnic Unwana Afikpo North Ebonyi State who volunteered themselves for this study was recruited. One of the creams was moderately rubbed on the dorsal part of the hand and the other hand un-rubbed serves as control.

$$\text{Therefore \% repellency} = \frac{C-T}{C} = \frac{100}{1}$$

Where c = control

T = test

Oshagi, 2003 and Carrol *et al.*,2006)

2.6 Protection Time:

Five household was used for the incense, and spray for about 3 consecutive days. The response from them was obtained using structural questionnaires.

2.7 Statistical Analysis

The data obtained was analyzed and expressed as mean $\pm$ standard deviation. Comparism of mean was done by ANOVA Using SPSS 20.0 version.

III. RESULTS

TABLE 1

A TABLE SHOWING THE PROTECTION TIME OF SPRAY (AEROSOL) FORMULATION

HOUSE HOLD	DAY 1	DAY 2	DAY 3
1	2hrs 30 minutes	2hrs 30 minutes	1hrs 30 minutes
2	1hrs 30 minutes	1hrs 45 minutes	2hrs 50 minutes
3	2hrs 30 minutes	2hrs 30 minutes	1hrs 45 minutes
4	1hrs 45 minutes	1hrs 45 minutes	2hrs 30 minutes
5	1hrs 45 minutes	1hrs 30 minutes	1hrs 45 minutes

TABLE 2

A TABLE SHOWING THE PROTECTION TIME FOR INCENSE FORMULATION

HOUSE HOLD	DAY 1	DAY 2	DAY 3
1	4hours	3hrs 20 minutes	4hrs 30 minutes
2	3hrs 20 minutes	4 hours	4hrs 50 minutes
3	3hrs 25 minutes	3hrs 25 minutes	3hrs 20 minutes
4	4hrs 30 minutes	4hrs 15 minutes	4 hours
5	4hrs 15 minutes	4hrs 30 minutes	3hrs 25 minutes

TABLE 3

A TABLE SHOWING LANDING TIME, NUMBER OF BITES AND REPELLENCY % FOR CREAM FORMULATION.

	Landing time (minutes)	No of Bites	% Repellency (%)	No of mosquitoes
Standard	6 minutes	1	99.6	30
Control	1 minutes	26	13.3	30
Test	3 minutes	12	60	30

- **Key:** DEET formulation repellent is "Odomos" mosquito repellent control.
- Negative contains petroleum Jelly
- Test contains petroleum and essential oil from *Ocimum gratissimum*.

IV. DISCUSSION

Many mosquito-borne diseases, such as malaria, dengue fever, and yellow fever, are serious public health problems in tropical regions, especially in Nigerians. These diseases are transmitted to human beings through mosquito bite only. Prevention of mosquito bites is one of the main strategies to control or minimize the incidences of these diseases. The use of insect repellents can provide practical and economical means of preventing mosquito-borne diseases and death. It is important not only for local people in disease risk areas in Nigeria but also for travelers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from home countries. Many mosquitoes repellent formulations have been made containing plant materials (Andeniran *et al* 2012, WHO 2009). Efforts are been made to ensure safety and effectiveness of such repellants (Dickens *et al* 2013).

DEET (N,N- diethyl-3-methylbenzamytes) is grown to be the best chemical insects repellent over the years and at such as they widely applied in the control of insects vectors such as mosquito. Most of the modern mosquito repellants coming in diverse forms including repellent creams is made of DEET as most widely used. Through regarded as safe but toxic effects has been recorded including encephalophathy in children, utilized heart beat (Petersen, 2001).

Many mosquito formulation has been made containing oil of plants *O. gratissimum* (Esmonye *et al* 2011, Aderi and Fabiyi 2012, Dickens and Bohhot 2013). The limitation of these products is that they have short time of effectiveness because of their rapid volatility (Apywatt *et al* 2001).

In this study, the repellency of the *O. gratissimum* essential oil was tested in a various formulations of spray, incense burning and cream. For the incense burning and spray (aerosol), the repellency was measured in terms of protection time. This is the maximum length of time it take for mosquito presence and bite is observed after their application. The spray protection time of two hours was observed suggesting it takes about two hours for the active repellent ingredients in scent leaf essential oil to degrade upon exposure to air. In similar manner, the burnt product of essential oil of scent leaf takes protection time of four hours to lose mosquito repellent activity. The loss of this activity may be due to oxidation on exposure to oxygen producing a non or less repellent products.

A similar studies done by Shankar *et al* 2013, Awosola *et al* 2018, shows that *O.gratissimum* has mosquito repellency by incense burning and spray. From the study also, the cream formulation presented with delay landing time. Protection from mosquito bite and consequently high repellency. The repellency of *O.gratissimum* is significant when compared with negative control but lower than that of the DEET standard. This implies that the essential oil *O.gratissimum* could to a great extent serve as a better competitive alternative to the synthetic DEET.

Similar study done by (Obeta *et al* 2021) on Moringas, scent leaf menthe, spicata, Agbalaka *et al* 2021 on dry scent leaf, and Shankar *et al* 2013 on Neem, lemon, curry leaf showed similar effect on repellency to the current study. Through the mechanism of action of the formulated repellent in this study are yet to be established, several lines of the evidences suggest that incense repellent molecules reduces mosquito host contact by interacting with odourance and odourant receptors, thereby ultimately affected olfactory- driven behavior (Chemo-attraction) (Bohbot *et al.*, 2011). The first detailed mode of action of attraction was summarized by Davis 1985. Techniques were available for single cell recordings for olfactory Neurons from antenna of mosquito and number of repellent tested for activity on these cells Based mostly on these electrophysiological studies. Davis hypothesized that repellent had their effect by modified or blocking responses of olfactory receptor neurons. Normally sensitive to attractants. The same point was made by Dickens and Bohbot 2013. In this study , one or more compound should be responsible for modifying or blocking responses by olfactory , receptors neurons, normally sensitive to attractive.

In humans, some of this attractants include (TMAO) in the sweat, odour of haemoglobin in RBC and heat emission from the living body. In conclusion, the essential oil of scent leaf in the various formulations of aerosol, incense burnig and cream exhibited significant observable mosquito repellent activity with highest effect associated with the cream. However, the would be side effect of it application on the skin is unknown and the oil components responsible for repellency unelucidated. To these, we finally recommend further studies in order to tap from this potential in scent leaf in providing organic based mosquito repellent.

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Biochemical Profiling of *Bombyx mori* L. Droppings: Insights into Protein and Carbohydrate Composition for Agricultural Implications

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Abstract— This study meticulously examined the protein and carbohydrate contents in aqueous and chloroform extracts of *Bombyx mori* L. (silkworm) droppings from six distinct silkworm breeds (CSR2, CSR4, M43, M46, SK4 and Sanish 8) using standardized protocols. The findings consistently indicated heightened protein and carbohydrate levels in aqueous extracts across both 4th and 5th instar droppings. Particularly noteworthy was the CSR4 breed, displaying the highest protein content at 10.76% and 8.09% in 4th and 5th instar, respectively, while the M46 breed showcased the lowest protein content. Conversely, carbohydrate content peaked in the droppings of the M46 breed. This investigation underscores the indispensable role played by these constituents in soil health and agricultural practices, emphasizing their potential as organic fertilizers. It advocates for further comprehensive exploration of these elements to unlock their manifold applications beyond silk production. Recognizing their significance in sustainable agriculture and environmental management, this study prompts further research endeavors to harness their potential across various sectors.

Keywords— *Bombyx mori* L., Silkworm droppings, Carbohydrate, Protein.

I. INTRODUCTION

Bombyx mori L., commonly known as the mulberry silkworm, has garnered enduring scientific interest over centuries, owing to its multifaceted economic, ecological, and scientific implications (Goldsmith *et al.*, 2005; Hardy *et al.*, 2008). Beyond its iconic role in silk production, the silkworm serves as an invaluable model organism for unravelling diverse biological processes. A facet often overshadowed in silkworm biology pertains to the composition of its excretory byproduct, colloquially known as droppings, which offers insights into dietary habits, metabolism, and potential applications. Employing biochemical profiling, a potent analytical approach, allows for an in-depth exploration of the intricate molecular makeup of biological samples (Smith and Fieldsend, 2021).

Silkworm excrement, a consequential byproduct of sericulture, emerges as a subject of interest due to its intricate composition. Despite consuming mulberry leaves at a rate approximately ten times its body mass, only 40% of the leaves undergo digestion, with the remaining 60% expelled as droppings. Investigation reveals that some compounds in the excreta originate from mulberry leaves, while others undergo bio-transformation in the silkworm intestine (Katayama *et al.*, 2007). The reported chemical constituents of silkworm excreta encompass chlorophyll and its derivatives, xanthophyll, carotenoids, and flavonoids (Park *et al.*, 2003; Park *et al.*, 2011). Complementary examinations have probed the lipid profile of silkworm excreta (Uzakova *et al.*, 1987), while Chen (2003) comprehensively outlined the nutrient composition, including moisture, crude protein, crude fat, crude fiber, non-nitrogen extracts, and minerals.

In the broader context of soil nutrition and plant growth, the pivotal roles played by proteins and carbohydrates cannot be overstated (Fernandez-Carazo *et al.*, 2020). Proteins, acting as nitrogen sources, enrich soil fertility by facilitating nutrient availability, supporting microbial activity, and influencing biochemical processes vital for nutrient cycling (Gupta *et al.*, 2018). Simultaneously, carbohydrates function as organic matter, enhancing soil structure, aeration, water retention, and serving as a carbon source for beneficial soil microorganisms (Garcia *et al.*, 2018).

This synergy between proteins and carbohydrates creates a dynamic soil environment that fosters plant growth. The ensuing interplay of nutrient availability, improved soil structure, and microbial interactions collectively contributes to enhanced plant health, development, and overall agricultural productivity. Silkworm droppings, often underestimated yet rich in proteins and carbohydrates, emerge as a valuable resource with profound implications for agriculture. Research indicates the presence of essential proteins vital for plant growth and development in silkworm excrement (Seo *et al.*, 1985). Nitrogen-rich proteins act as potent fertilizers, augmenting soil fertility and promoting microbial activity for enhanced nutrient cycling (Park *et al.*, 2011). Concurrently, carbohydrates in silkworm droppings act as organic matter, enhancing soil structure and providing a carbon source for beneficial soil microorganisms (Chen, 2003). This microbial activity further supports nutrient availability for plants, fostering sustainable nutrient recycling practices in agriculture.

The amalgamation of proteins and carbohydrates from silkworm droppings not only nurtures soil health but also provides a promising avenue for organic fertilization and nutrient management in crop production. This aligns seamlessly with the principles of sustainable and eco-friendly agriculture. The present study seeks to quantify protein and carbohydrate levels in the droppings of diverse mulberry silkworm breeds, contributing to the expanding understanding of the agronomic potential inherent in this often-overlooked byproduct.

II. MATERIALS AND METHODS

2.1 Collection of Material:

As shown in Table 1, 100 grams of excreta on 2nd day of 4th instar and 3rd day of 5th instar of 06 different breeds of silkworm (*Bombyx mori*. L) was collected separately during the rearing conducted at College of Temperate Sericulture, Mirgund. The samples were dried in tray dryer at 60°C until constant weight was obtained. Then the samples were crushed into fine powder before analysis.

TABLE 1
COLLECTION OF DROPPINGS FROM SILKWORM *BOMBYX MORI* L. BREEDS

Breed	Name of Breed
B1	CSR2
B2	CSR4
B3	M43
B4	M46
B5	SK4
B6	Sanish-8

2.2 Preparation of extract:

Extraction of the samples was carried out using two solvents viz., water and chloroform by mixing 1.25 gram of excreta in 25ml of solvent filtered through Whatman filter paper 42 followed by centrifugation at 5000 rpm for 15 minutes. The extracts were pooled and collected up to a final volume of 25 ml and stored in cryo vials at -5°C till further analysis.

2.3 Bioactive compound analysis:

2.3.1 Estimation of Total Protein content

Protein content in aqueous and chloroform dropping extract of 06 different breeds of *Bombyx mori*. L, was estimated by Lowry's method (Lowry *et al.*, 1951). 500mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. This solution was centrifuged and the supernatant was used for protein estimation. 0.2,0.4,0.6,0.8 and 1ml of the working standards were pipetted into a series of test tubes. The aqueous and chloroform sample extracts were pipetted in other test tubes. The volume was made to 1 ml in all the test tubes. The tube with 1ml of water served as the blank. 5ml of Alkaline copper reagent was added to each tube including the blank. This was mixed well and allowed to stand for 10 min. Then 0.5ml of Folin-Ciocalteu reagent was added, mixed and incubated at room temperature in the dark for 30 min and blue colour was developed. The readings were recorded at 660 nm. Standard graph was drawn and the amount of protein in the sample was calculated. Protein percentage was calculated in relation to fresh weight and dry weight basis. The amount of protein mg/g or 100g sample was expressed.

2.3.2 Estimation of Total Carbohydrate

The total carbohydrate content in aqueous and chloroform dropping extract of 06 different breeds of *Bombyx mori*. L, was estimated by anthrone method (Hodge and Hofreiter, 1962). 100mg of the sample was weighed into a boiling tube. This was hydrolyzed by keeping it in a boiling water bath for three hours with 5ml of 2.5 N – HCl and cool to room temperature. Then, this solution was neutralized with solid sodium carbonate until the effervescence ceases and made to 100ml and centrifuged. The supernatant was collected and 0.5, 1ml aliquots were taken for analysis. Standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard 'O' served as blank. The volume is made to 1ml in all the tubes including the sample tubes by adding distilled water. Then 4ml of anthrone reagent was added and this was heated for eight minutes in a boiling water bath and made to cool. Green to dark green colour was recorded at 630nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present in the tube was calculated from the graph. Carbohydrate percentage was calculated relation to fresh weight and dry weight basis. The Calculation is done by using this formula:

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100 \quad (1)$$

III. RESULTS AND DISCUSSION

3.1 Total Protein (%)

The comprehensive analysis of total protein content in the 4th and 5th instars of silkworm droppings across six distinct breeds and varied extracts is elucidated in Table 2 and Fig. 1. The aqueous extract exhibited significantly higher total protein content, closely followed by the chloroform extract in both instars. In the aqueous extract, the total protein content in silkworm droppings ranged from 7.32% to 10.76% in the 4th instar and 5.63% to 9.55% in the 5th instar. Notably, the highest total protein content in the 4th instar droppings was observed in the CSR4 breed (10.76%), succeeded by the CSR2 breed (10.01%), while the M46 breed exhibited the lowest total protein content at 7.32%. Similarly, in the 5th instar droppings, CSR4 recorded the highest total protein content (8.09%), followed by CSR2 (7.72%), with the lowest total protein content at 5.31% observed in the M46 breed.

The total protein content in the chloroform extract of silkworm droppings ranged from 5.31% to 8.09% in the 4th instar and 4.15% to 7.04% in the 5th instar. In the 4th instar droppings, CSR4 breed exhibited the highest total protein content (8.09%), followed by CSR2 (7.72%), while M46 breed displayed the lowest at 5.31%. In the 5th instar droppings, CSR4 again recorded the maximum total protein content (7.04%), followed by CSR2 (6.85%), with M46 breed showing the lowest total protein content of 4.15%.

The observed trends in the present study align with the findings of Murthy (2015), who reported a decrease in protein content with each passing instar, thereby highlighting the developmental dynamics of protein synthesis in silkworms. This conformity not only underscores the robustness of the methodology applied in the present study but also adds to the body of knowledge concerning the physiological changes in silkworm droppings across different breeds and developmental stages (Patil *et al.*, 2013).

TABLE 2
TOTAL PROTEIN CONTENT IN 4th AND 5th INSTAR AQUEOUS AND CHLOROFORM EXTRACT OF SILKWORM DROPPINGS OF SIX BREEDS (%)

Breed \ Extraction Method	4 th instar			5 th instar		
	Aqueous	Chloroform	Mean	Aqueous	Chloroform	Mean
B1: CSR2	10.01	7.72	8.86	9.11	6.85	7.98
B2: CSR4	10.76	8.09	9.42	9.55	7.04	8.30
B3: M43	7.54	5.51	6.52	5.81	4.45	5.13
B4: M46	7.32	5.31	6.31	5.63	4.15	4.89
B5: SK4	8.17	6.17	7.17	8.55	5.47	7.01
B6: Sanish-8	7.55	5.79	6.67	8.30	5.01	6.65
Mean	8.56	6.43		7.82	5.49	

C.D(p≤0.05)

Extraction Method (M) : 0.149

Breed (B) : 0.258

MxB : 0.365

C.D(p≤0.05)

Extraction Method (M) : 0.054

Breed (B) : 0.094

MxB : 0.133

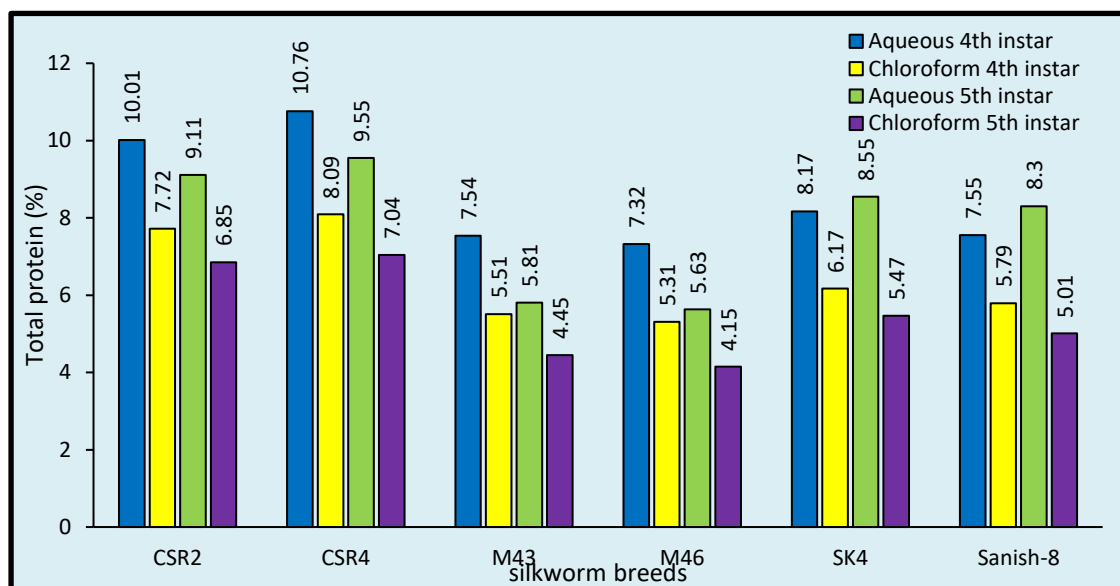


FIGURE 1: Total protein content in aqueous and chloroform extract of 4th and 5th instar silkworm droppings of 06 different breeds (%)

3.2 Total Carbohydrate (%)

The investigation into the total carbohydrate content of silkworm droppings in the 4th and 5th instars, encompassing six different breeds and various extracts, is summarized in Table 3 and Fig. 2. Notably, the aqueous extract consistently exhibited significantly higher total carbohydrate content compared to the chloroform extract in both instars. In the aqueous extract, the total carbohydrate content in silkworm droppings ranged from 4.31% to 7.15% in the 4th instar and 4.03% to 7.03% in the 5th instar. The M46 breed demonstrated the highest total carbohydrate content in the 4th instar (7.15%), followed by M43 (6.63%), while CSR4 exhibited the lowest at 4.31%. Similarly, in the 5th instar, M46 recorded the maximum total carbohydrate content (7.03%), followed by M43 (6.49%), with CSR4 presenting the lowest content at 4.03%. The chloroform extract of silkworm

droppings exhibited total carbohydrate content ranging from 2.82% to 4.10% in the 4th instar and 2.12% to 3.54% in the 5th instar. In the 4th instar, M46 breed displayed the highest total carbohydrate content (4.10%), followed by M43 (3.58%), with CSR4 exhibiting the lowest at 2.82%. For the 5th instar, M46 again recorded the maximum total carbohydrate content (3.54%), followed by M43 (3.50%), while CSR4 displayed the lowest at 2.12%.

These findings are consistent with the results reported by Patil *et al.* (2013), where a similar methodology revealed a 4% carbohydrate content in silkworm droppings. The observed agreement reinforces the reliability of the methodology employed in the present study. The significant variations in total carbohydrate content across breeds and instars underscore the dynamic nature of silkworm droppings and their potential significance in agriculture. Further research could delve into the underlying factors influencing these variations and explore the implications for soil health and plant growth.

TABLE 3
TOTAL CARBOHYDRATE CONTENT IN 4th AND 5th INSTAR AQUEOUS AND CHLOROFORM EXTRACT OF
SILKWORM DROPPINGS OF SIX BREEDS (%)

Breed \ Extraction Method	4 th instar			5 th instar		
	Aqueous	Chloroform	Mean	Aqueous	Chloroform	Mean
B1: CSR2	4.77	3.10	3.93	4.37	2.92	3.65
B2: CSR4	4.31	2.82	3.56	4.03	2.12	3.08
B3: M43	6.63	3.58	5.11	6.49	3.50	4.99
B4: M46	7.15	4.10	5.62	7.03	3.54	5.29
B5: SK4	6.30	3.51	4.91	5.60	2.33	3.97
B6: Sanish-8	6.35	3.52	4.93	6.49	3.01	4.75
Mean	5.92	3.44		5.67	2.90	

C.D(p≤0.05)

Extraction Method (M) : 0.033
Breed (B) : 0.057
MxB : 0.081

C.D(p≤0.05)

Extraction Method (M) : 0.010
Breed (B) : 0.017
MxB : 0.024

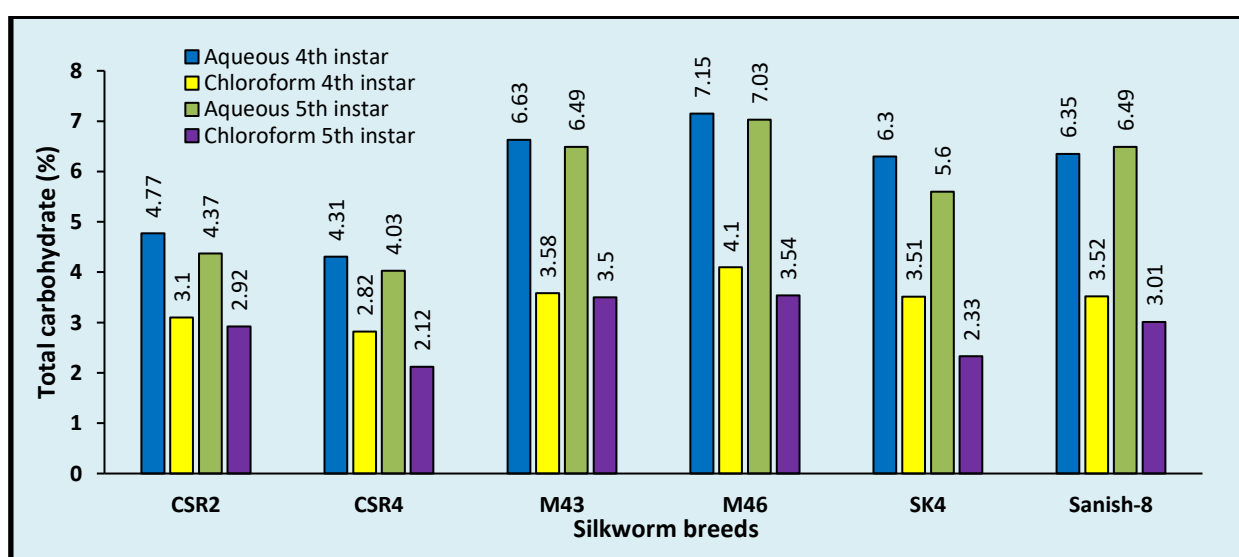


FIGURE 2: Total carbohydrate content in aqueous and chloroform extract of 4th and 5th instar silkworm droppings of 06 different breeds (%).

Understanding the factors influencing protein dynamics in silkworm droppings can provide valuable insights into soil fertility and plant growth, presenting a rich avenue for further exploration in sustainable agriculture (Gupta *et al.*, 2018; Fernandez-Carazo *et al.*, 2020). The intricate interplay between protein content, soil health, and plant productivity invites a nuanced investigation, with potential implications for optimizing agricultural practices. Future research endeavors in this direction may contribute to the development of eco-friendly and sustainable strategies in agriculture.

The observed variations in protein and carbohydrate content within silkworm droppings hold significant implications for their utilization in agriculture, offering potential benefits for soil health and overall productivity.

Silkworm droppings, enriched with proteins, can serve as valuable organic fertilizers due to their nitrogen-rich composition. Proteins play a crucial role in soil nutrition by acting as potent nitrogen sources. Nitrogen is an essential component for plant growth, and its availability in the soil directly influences crop productivity (Gyaneshwar *et al.*, 2002). The proteins in silkworm droppings can enhance soil fertility by facilitating the availability of nitrogen to plants, promoting robust growth and development. Moreover, proteins support microbial activity in the soil, contributing to the decomposition of organic matter. This decomposition process releases essential nutrients, making them available for plant uptake. Proteins also serve as building blocks for soil enzymes, influencing biochemical processes vital for nutrient cycling (Marschner, 2011). The microbial activity supported by proteins in silkworm droppings enhances the overall nutrient cycling in the soil, fostering a nutrient-rich environment for plants.

Carbohydrates present in silkworm droppings, especially in the aqueous extract, contribute to improving soil structure and fostering better aeration and water retention (Lehmann *et al.*, 2015). Carbohydrates serve as organic matter in the soil, enhancing soil fertility and creating a conducive environment for plant growth. The carbon source provided by carbohydrates supports the growth and activity of beneficial soil microorganisms. The microbial activity fueled by carbohydrates in silkworm droppings further contributes to nutrient transformation and availability for plants (Bardgett and van der Putten, 2014). Microorganisms break down complex organic matter into simpler forms, releasing nutrients that are vital for plant nutrition. This symbiotic relationship between carbohydrates, microorganisms, and nutrient availability creates a dynamic soil environment that supports plant growth.

The combination of proteins and carbohydrates in silkworm droppings offers a holistic approach to enhancing soil health and, consequently, agricultural productivity. The availability of essential nutrients, improved soil structure, and microbial interactions influenced by these biomolecules collectively contribute to enhanced plant health, development, and overall agricultural productivity.

By utilizing silkworm droppings as organic fertilizers, agricultural practices can align with principles of sustainable and eco-friendly farming. The recycling of nutrients through the incorporation of silkworm droppings into soil contributes to the development of nutrient-rich soils, reducing the dependency on chemical fertilizers and promoting environmentally sustainable agriculture.

IV. CONCLUSION AND FUTURE SCOPE

In the current study, a meticulous examination of the biochemical composition of silkworm droppings, specifically focusing on the aqueous and chloroform extracts of the 4th instar droppings of CSR4 and M46 breeds, revealed noteworthy variations in total protein and carbohydrate content.

The aqueous extract of the 4th instar droppings from the CSR4 breed exhibited the highest total protein content at 10.76%, surpassing the chloroform extract with a content of 8.09%. Similarly, concerning total carbohydrate content, the aqueous extract from the 4th instar droppings of the M46 breed displayed the highest concentration at 7.15%, outperforming the chloroform extract with a content of 4.10%. In conclusion, this comprehensive analysis provides valuable insights into the nutritional dynamics of silkworm droppings, shedding light on breed-specific variations in protein and carbohydrate content. The higher protein content in the CSR4 breed suggests potential genetic influences on protein synthesis, while the elevated carbohydrate content in the M46 breed implies variations in dietary habits or metabolic processes. The unraveling of the intricate biochemical profile of silkworm droppings not only contributes to our understanding of silkworm biology but also

presents potential applications in various fields. These applications could range from the development of organic fertilizers enriched with specific nutrients to biotechnological processes leveraging silkworm-derived biomolecules.

As we delve deeper into the biochemical nuances of silkworm droppings, the avenues for future research become increasingly promising. Exploring the genetic and environmental factors influencing the observed variations, optimizing the extraction processes, and assessing the practical applications of these findings in agriculture are directions that merit further exploration. In essence, this study marks a crucial step in decoding the biochemical intricacies of silkworm droppings, laying the groundwork for future research endeavors and practical applications that could contribute to advancements in agriculture and related fields.

Overall, the protein and carbohydrate content in silkworm droppings represents a reservoir of untapped potential with implications spanning agriculture, biotechnology, medicine and environmental sustainability. Continued research efforts in this area promise to unlock further insights and innovations for the benefit of society and the ecosystem alike.

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The Impact and Future Prospects of Mutation Breeding in Indian Agriculture

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Abstract— The scientific breeding method that creates mutations, mutation breeding, revolutionizes agriculture by cultivating a higher yield of the crop and making them more resilient. Challenges such as mutation unpredictability and ethical aspects exist. Nevertheless, mutation breeding should be considered as a sustainable practice of agriculture that ensures climate adaptation and food security. Coordinating technological developments, suiting it with other methods of breeding and dealing with socio-economic challenges are essential in this regard for the future of gene editing in the different agro-climatic regions of India.

Keywords— Mutation breeding, Agriculture, India, Sustainability.

I. INTRODUCTION

Mutagenesis or mutational breeding, which is a scientific method, has tremendously changed many aspects of agriculture. The technique involves the deliberate induction of mutations in the genetic information of an organism, thus creating a wide array of variants that can be selectively utilized to improve crops. The functions of mutation breeding in agronomy are multifaceted but significant which had lead to the increasing crop productivity. The world's population is getting bigger and thus the demand for food is growing as well. To meet the higher consumer demand, it is thus essential to enhance crop varieties that yield more, irrespective of the favourable conditions. Mutation breeding has been able, therefore, to overcome this problem, and has played a key part, along with other methods, in the development of high-yielding crop varieties (Ahloowalia *et al.*, 2004). Particularly, mutation breeding is one of the indispensable means to increase the nutritional value of crops. It has been possible to achieve enhanced nutritional qualities in crop varieties through the use of induced mutation. Likewise, a variety of rice with higher protein content as well as maize with increased essential amino acid level has been developed. This aspect is especially true in areas where malnutrition is the norm that the improved varieties of crops can also bring about better health outcomes. (Shu *et al.*, 2012). The other important issue of mutation breeding is that it can be used to create cultivars resistant to pests and diseases. It not only limits the dependency on the synthetic pesticides, hence lowering the risk on the environment and agriculture, but also helps in the sustainability of crop production. Breeding pest and disease resistant crop varieties can virtually eliminate or dramatically reduce crop losses, thus maintaining food security and supply (Jankowicz-Cieslak *et al.*, 2017). The emergence of mutation breeding as the crucial means for generating climate resilient crops is a response to the threats of climate change. It is used, in the development of varieties, which are tolerant to different types of abiotic stresses for instance, drought, salinity and extreme temperatures. Hence, this is particularly important for regions like India, where agriculture is a key economic sector but remains sensitive to climate change impacts. The development of climate-resistant crop varieties will make sure that farming remains sustainable in the face of so many challenges (Parry & Hawkesford, 2010). On the other hand, it needs to be pointed out that mutation breeding also comes with several difficulties. Altering the genetic material by means of inducing mutations is random and unpredictable, and not all induced mutation results in beneficial traits. On the other hand, there also exists a set of ethical issues related to the alteration of hereditary material. Hence, a detailed research must be conducted to observe ethical standards whenever this technique is use, mutation breeding potentially resonates on the scene of contemporary agriculture. It provides a multi-purpose tool to combat these problems such as food security,

malnutrition and climate change. Nevertheless, this technology needs to be approached with caution owing to its problems that it poses.

II. HISTORY OF MUTATION BREEDING

Mutation breeding is a method that involves the intentional creation of mutations through induction, with the purpose of introducing variations in the plant species. This has been one of the main elements driving agricultural development in India. This technique, which edits an organism's DNA to produce a permanent mutation, has been a vital factor in the breeding process and in enhancing crop varieties during the early 20th century. Varies de Vries, a Dutch botanist, was the first one who brought the theory of mutation in the early 20th century. De Vries, without selecting for any particular trait, recorded mutations that occur in the evening primrose and posited that new species may emerge suddenly in this manner. This pivotal discovery has become the foundation for the innovation of mutation breeding. Within the 1930s and 1940s, the experts discovered that they could set the mutations using irradiation and chemicals. This resulted in the rise of accelerated breeding using mutations as an alternative for developing crops. This was seen as a novel strategy of increasing yields and disease resistance (Larkin & Scofcroft, 1981). In India the first varieties of crop which were evolved through mutagenesis were released in the 50s and early 60s. To start with, the varieties of peanuts, barley, and rice that were resistant to diseases, or had increased yield, were also introduced. The successes of some of these early attempts identified the potential of mutation breeding as an improvement tool for agriculture. Form the 1970s; mutation breeding has been exploited to establish different varieties of many crops. In India, rice, pulses, and oilseeds are species that have benefited greatly from hybrid breeding. This technology has been used to increase the production, biofortification (nutritional value) and disease resistance in these crops, and thus has helped immensely in ensuring food security in the country (IAEA, 2000).

Bhabha Atomic Research Centre (BARC) is the leading institute that conducts mutation breeding in India. They have built new varieties of crops such as groundnut, mustard and chickpea that help to produce more and have resistance to diseases. The intensity of these varieties has been extensively accepted by farmers hence, increasing the agricultural productivity (BARC, 2000). Alongside BARC, other research centres such as IARI and agricultural universities in different states of the country have also made notable contributions to mutation breeding in India. These institutions have implemented a multitude of research projects on the mutation breeding and have bred numerous mutant crop varieties. Although the approach has gained some traction, it is also limited or, perhaps, risky. These are the cases of the evil nature of mutations and the challenge in controlling the mutation process. So, it is significant to take this approach in a responsible way and together with other methods of breeding. It is imperative for the scientists to do more and more research and development to eliminate flaws of this technique and control its adverse consequences (Larkin & Scowcroft, 1981).

III. TECHNIQUES IN MUTATION BREEDING

Mutation breeding, known alternatively as induced mutation, is a scientific process that entails the intentional induction of mutations to give rise to modifications in plant species. This technique was highly instrumental in crop varieties improvement and in enhancing productivity hence, agricultural development in India. The mutation-breeding process consists of several stages. The first stage consists in mutation induction, which can be done by the physical mutagens (e.g. radiation) or chemical mutagens (e.g. EMS). The type of mutagens determines the choice of mutagen and the species of plant which is the key factor. For example, radiation is a common technique of applying large-scale chromosomal variations, while chemical mutagens are a technique used for inducing point mutations (Shu *et al.*, 2012). After the induction of mutations, the mutated plants are cultivated on the field and the same are selected on the basis of favourable characteristics. This process is referred as phenotypic screening which involves watching out the plants for varieties of changes in their characteristics such as yield, resistance against diseases or nutritional content. This step is the most crucial one since it allows breeders to select the most favoured mutant plants which will later be subjected to the next round of breeding. Having a plant with the traits you want is the first step for producing seeds of this plant which will be able to produce stable lines of plants with the same traits. Likewise, there is a process called backcrossing in which the mutant plants are crossed with the parents so as to keep the desirable traits stable and transferred to the next generation. The development of the trait is the last but not the least important step for the market introduction of the mutant varieties (Bado *et al.*, 2015).

India has seen good progress in mutation breeding in terms of improving the varieties of rice, pulses and oilseeds. The technology has helped to increase the yield, nutritive value and resistance to diseases of these crops, and thus, the country can now achieve food security. The Bhabha Atomic Research Centre (BARC) is one of the top research institutes in India that have contributed to mutation breeding research through their human resource development, technical know-how, and genetic stock importation program. A number of these crops have been made into multifarious varieties, e.g. groundnut, mustard, and

chickpea, which are high in yield, and resistant to diseases. These hybrid varieties are widely adopted by farmers; they have resulted in the increase in agricultural productivity. However, mutation breeding is not faultless since it has limits also. These include the fact that the process of mutation desirability of the mutation process itself and the difficulty of controlling the mutations. Due to this, we should be careful about the use of this kind of technology and it should be combined with other breeding methods. The necessity for continuous research and development is crucial to improve the technique and to reduce its potential risks (Larkin & Scowcroft, 1981).

IV. CASE STUDY: THE IMPACT OF MUTATION BREEDING

Mutation breeding, or induced mutation, is a very popular method applied in the agricultural science as a very means of improving plants of a particular species. This technology has played the very decisive role in the enhancing crop yields and in the developing cultivars especially in India. The process involves inserting mutations in the plant's genetic material to create the variations that can increase the crop yield, also boost disease resistance or increase nutrient content. The first phase of the mutation breeding is the creation of the mutations. It is often accomplished using mutagens, the physical ones like radiation and also chemical ones like ethyl methanesulfonate (EMS). The choice of mutagen is determined by the type of mutation for which the best options and also the species of the plant. Such as, the large-scale chromosomal changes are mostly induced by the radiation, while chemical mutagens for the point mutation (Shu *et al.*, 2012). The mutated plants are then grown and the desirable characteristics are identified after many mutations have been introduced. This process is known as the phenotypic screening which is a set of the observations of the plants for any changes in characteristics like yields, disease resistance, and also nutritional content. This step is a pivotal point where the backcrossing and also other methods are used to choose the best of the mutant plants (Bado *et al.*, 2015). The next stage is to select a plant that has the desired characteristics. Then, breeding is done to produce the lines of plants that have the same traits. This procedure is named as backcrossing and it involves the crossing of the mutated plant with its parent to make the desirable traits stable and to be able to pass them on to the future generations. The introduction of these mutant varieties is thus a prerequisite to a more extensive commercialization of the many varieties. (Bado *et al.*, 2015).

In India, mutation breeding has thus far had greater success in improving variants of rice, pulses and oilseed crops. This technique has been applied to improve crops yield, nutritive content, and disease resistance and hence the country food security is influenced in a great way. Bhabha Atomic Research Centre (BARC) is one of the major stakes in mutation research in India. They have transgenic ones too, such as Bt groundnut, Bt mustard, and Bt chickpea, which have high yield and disease-tolerance characteristics. These varieties were widely accepted by farmers causing increase in agricultural yield. Likewise, mutation is also limited. There are two prime defects associated with this method which include bringing in harmful mutations in the body and the difficulty in controlling mutations process. Hence, this approach has to be handled appropriately and with other breeding strategies. It will be important to continue research and development in order to further improve and minimize the risks involved (Larkin and Scowcroft, 1981). The application of mutation breeding has resulted in a considerable rise in agricultural productivity in India, leading to the construction of varieties resistant to yield and diseases. The interactive case study will deal with the question of mutation breeding in India, showing the application in rice, pulses and oilseeds, as well as the organizations which participate in this project.

The BARC (Bhabha Atomic Research Centre) has been a pioneering institute in the arena of mutation breeding research in India. Many types of these crops have been developed containing genes of the various wild species which are resistant to disease and capable of producing good yield. This type of crop varieties is widely accepted by farmers which resulted in an increased agricultural productivity (Bhabha Atomic Research Centre, 2018). One of the most well known cases of mutant breeding in India is by BARC where the Trombay groundnut, a variant of groundnut, was developed. Unlike the other types, this variety is rich in oil, has high yield, and is resilient to diseases (like leaf spot). The Trombay Groundnut is widely adopted by farmers in many states and which also helped increasing oilseed production in India (Bhabha Atomic Research Centre, 2018). Mutation breeding has also been applied to create more improved varieties of rice in India. 'Trombay Basmati' is a mutant variety that was developed by BARC and is known to be of more yield and quality when cooked than the traditional basmati rice. This strand, which has been embraced by farmers in many states, has contributed to higher production and exports as well (Bhabha Atomic Research Centre, 2018). Besides all its achievements, mutation breeding also has disadvantages. These include the fact that they might produce dangerous mutations and the troubles associated with monitoring mutation processes. Therefore it is highly important to apply this technique in a responsible way, combined with other breeding techniques. The pursuit of perfection through constant research and development may eventually enable to remedy the possible effects (Larkin and Scowcroft, 1981). Despite some concerns which exist, mutation breeding has undoubtedly made a noticeable contribution to agriculture in India by introducing high yielding and disease resistant crops varieties. Institution like

BARC has contributed to this research greatly, creating types of crops mutant that have been dominated for decades by farmers all over the world.

V. CHALLENGES AND LIMITATIONS OF MUTATION BREEDING

This method has been employed in India to develop improved varieties of crops such as rice, wheat, and pulses. However, there are several challenges and limitations associated with mutation breeding.

Firstly, the disagreement and randomness of mutations are a challenge. Mutations can occur in regulatory regions, protein-coding regions, and often, they are detrimental. In that way, the random nature of mutations makes it hard to obtain the highly preferred trait and thus the large number of plants has to be screened to find good mutation. (Bhatia *et al.*, 2016).

One of the major problems associated with mutation breeding is that it is a time and resource consuming technology. Stabilizing mutations translates too many generations of plants, a process which is usually very long, sometimes even exceeding a decade. Besides that, screening and selection of mutants pose high resource demand, and thus infrastructure may be a limiting factor in low-resource settings (Shu Q. Y., 2012 & Forster B. P., 2012).

In IPCC context, the major challenges in India are agriculture-climate conditions and economic and social status of farmers. Indian farmers are mostly smallholders having limited resources so they find it hard to afford with new technologies and practices. Additionally, India's native agro-climatic diversity necessitates development of crop varieties adapted to different regions, which increases the complexity of utilizing mutation breeding tool (Sikka V. K. & Saini R. G., 2014).

To overcome these constraints, mutant breeding is a way for the crop improvement and contribute to food security in India. This approach must be complemented by various varieties of plant breeding and be accompanied by suitable policies and investments (Bhatia, S., Bhatia, N., and Sharma, K., 2016)

VI. FUTURE PROSPECTS: MUTATION BREEDING - TOOL FOR SUSTAINABLE AGRICULTURE

Mutation breeding, or mutagenesis, has a lot of prospect as a tool of a sustainable agriculture in India due to its numerous agro-climatic zones and the requirement of improved crop varieties. This tactic helps to obtain enhanced resistance against diseases and pests, abiotic stresses such as drought, salinity and extreme temperatures in crop-varieties. Climate change and the growing pressure on agricultural resources are the highlights in which determining these traits are important.

But, the effective use of the mutation breeding to achieve the sustainable agriculture in the Indian context needs to take into account multiple factors. For the start, it involves improving knowledge in the field of plant genetics and the theory about mutation. This information supports predicting and restraining the implications of mutations, so that the application of mutation breeding works well. Gene mapping and bioinformatics facilitate this process. Another is that mutation breeding needs to be harmonized with the other plant breeding methods, such as hybridization and marker assisted selection. This integrated approach is possible by combining various methods together and enhancing the success for designing new improved varieties of crops. For example, mutation breeding is responsible for inducing genetic variation across species and marker-assisted supporting in identifying and keeping only beneficial mutations.

Thirdly, the utilization of mutation breeding demands remarkable resources, irrespective of time, human labor, and infrastructural support. Therefore, the right policies and resources need to be in place. Here we refer for instance, to the funding of research and development, capacity strengthening of researchers and farmers, and the creation of infrastructure for mutation breeding and screening of mutants. At last, the socio-economic situation about farmers needs to be taken into account. The majority of farmers in India are the smallholders with less resource and they find it difficult to accept the new technologies and the practices. This implies that mechanics need to be put in place to ensure that benefits of mutation breeding are readily available for these farmers.

VII. THE PATH FORWARD FOR MUTATION BREEDING IN INDIA

The future of mutation breeding in India crucially goes along with different important factors and strategies.

On the first hand, the issue of uncertainty and randomness of the mutations might show to be solve by means of technological progress and deep plant genetics understanding. Using such approaches as, the application of molecular markers and genomic selection may be relevant to detecting positive mutations and facilitating the breeding process.

Moreover, the resource-dependent character of mutation breeding can be dealt with through engineering efficient screening and selection standards and the application of biotechnology to speed up the breeding. Thus, the agro-climatic conditions

specific to the farming system in India and the socially and economically defined predictions of the agriculture in India must be taken into consideration. This calls for developing novel crop types that are climate-smart, locally tailored and easy to accept by small-scale farmers. In fact, farmers too should be given training and support as and when required for their ability to adapt new technologies and techniques. Besides the mutation breeding, plant breeding is one of the main techniques that can be used in combination with other breeding techniques. This could mean the application of the traditional breeding methods as well as the advanced ones such as genetic engineering and genome editing. Finally, effective policies and investments will also help in increasing security. This could cover such areas as research and development funding as well as acceptance policies of new and improved cropping patterns.

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Nutritional Assessment and Microbial Safety of Croaker (*Micropogonias Undulatus*) Fish from Three Frozen Food Centers in Afikpo, Ebonyi State Nigeria

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Abstract— Microbial activity on food leads to its spoilage. This is usually by enzymatic processes that bring about loss of nutrients experienced on decaying food. Food decay, affect virtually all classes of nutrients in food especially, the organic aspect with proteins and lipids being the most. This study aimed at determining the nutritional assessment and microbial safety of croaker fish from different frozen food centers. The nutritional assessment was determined through proximate analysis by standard method of AOAC and microbial safety by aerobic count, proteolytic and lipolytic count method using appropriate media. The results for proximate analysis showed moisture (62.38, 62.58 & 64.03), protein (23.09, 23.40 & 22.98), lipid (10.21, 10.31 & 10.25), ash (1.11, 1.12 & 1.13) and carbohydrate (2.51, 2.65 & 1.61) for center A, B & C respectively. The mean values for aerobic plate count, proteolytic, and lipolytic counts were 7.30 ± 0.18 , 4.00 ± 0.03 , and 2.00 ± 0.06 , respectively, for center A, 6.31 ± 0.29 , 3.85 ± 0.07 , 2.61 ± 0.01 for center B, and 7.70 ± 0.82 , 4.20 ± 0.29 , 2.82 ± 0.13 for center C all in $\times 10^3$ CFU/ml. The presence of salmonella sp and straphylococcus were confirmed. These finding however, suggest contamination with aerobes with both proteolytic and lipolytic activities and particularly with pathogenic salmonella and straphylococcus which could endanger the health of people upon their consumption.

Keywords— frozen food, lipolytic, microbes, proteolytic, safety, spoilage.

I. INTRODUCTION

Fish has been considered an indisputable source of animal protein available in the tropics and is widely accepted as a good quality source of protein and other nutrients for the maintenance of good health (Andrew, 2001). The developing countries capture 50% of the world's harvest, with a large proportion of the catch consumed internally. In many Asian countries, over 50% of animal protein intake comes from fish. Methods of preservation and storage are the major factors affecting the rate of loss of quality and shelf life of fish. However, its effects bordered on the nutrient quality and safeness upon consumption (Ewo 2019).

World consumption of fish per capita increased in developing regions from 5.2kg in 1961 to 18.8kg in 2013, while in the least developed countries with deficits increased from 3.5kg to 7.6kg in the same period. Hence, fish account for about 17% of the intake of animal protein by the world population (FAO, 2016). Fish is known for its high nutritional value, and the chemical composition of fish regarding other food is unique (Simopolous, 2002). Fish tissue is the main source of long-chain polyunsaturated fatty acids, especially omega-3 and omega-6. These fatty acids have particular importance in fish since their

consumption contributes to the reduction of the appearance of cardiovascular diseases (Turkmen, Aro, Nurimi, and Kailio, 2005) and the improvement of learning ability. Fish also contains a high-quality protein with all essential amino acids, being a source of dietary minerals such as calcium, iodine, or selenium and providing an important amount of polyunsaturated fatty acid (Araujo, Soares, and Gois, 2010). Pelagic species, usually smaller ones such as sardines, are generally rich in omega-3 fatty acids, mainly eicosapentaenoic acid and docosahexaenoic acid (Pestana, 2007).

Fish is a very perishable food, being highly susceptible to oxidation and microbiological deterioration. Therefore, efficient storage strategies need to be employed in order to increase its shelf-life and guarantee its safety and quality from catch to consumption. This shelf life of fish is dependent on several factors, such as storage time, temperature, fish species, the stress suffered during the catch, and the amount of ice (Mahmud, Abraha, Samuel, Mohammedidris, and Abraham, 2018). Therefore, these preservation methods need to be optimized to increase fish shelf life to guarantee its quality and safety, with consequent satisfaction of consumer requirements, reduction of economic losses from fishing industries, and food waste. This optimization can involve the effect of freeze/chill temperature and time, thawing, fish preparation, and bleeding condition (Rong, Ruchuan, Huihui, and Qi, 2020; Nguyen and Phan, 2018)

Fish contaminants are of great concern for export earnings because of their high nutritive value, such as high protein content with little or no carbohydrate and fat value. However, fish may be contaminated at various stages of transport, handling, and processing. This contamination may be related to the raw materials, personnel, and processing tools, such as forklifts, through leakage, insects, and pest harbourage. Additionally, seafood can become contaminated during storage and processing. Contamination may be caused by foodborne pathogens that are naturally present in aquatic environments, such as vibrio spp, or derived from sewage-contaminated water, such as salmonella spp (Gnanambal and Patterson, 2005). Consumption of these contaminated fish may cause infection or intoxication to the consumer.

The contaminant of fish is one of the leading causes of foodborne diseases or gastroenteritis, characterized by diarrhea, abdominal cramps, vomiting, nausea, and fever. According to the Centers for Disease Control and Prevention, salmonella is the leading cause of bacterial foodborne illness, causing approximately 1.4 million non-typhoidal illnesses, 15000 hospitalizations, and 400 deaths in the USA annually (Center for Disease Control and Prevention (CDC), 2011).

Water and ice quality is also an important factor for quality fish because water and ice used for fish processing may contaminate the whole processing plant. So, it is important to find out the quality of fish we consume as well as the frozen fish that are exported (Andrew and Hammack, 2001).

This study, therefore, aimed to assess the nutritional value and microbial safety of frozen croaker fish from three different frozen centers in Afikpo Ebonyi State, Nigeria, in order to assess their microbial wholesomeness. The specific objective of the study includes the determination of the nutritional value and microbial safety and comparing the various outcomes of frozen croaker fish obtained from three different frozen centers.

II. MATERIALS AND METHODS

2.1 Sample:

Frozen croaker fish (*Micropogonias undulatus*)

2.1.1 Sampling and Sample Preparation:

A freshly frozen croaker fish of about 1kg size was randomly sourced from three different frozen centers. The sample was put in sterile plastic bags and immersed immediately in an ice-containing flask. The samples were divided into two portions, one for chemical (proximate) evaluation and the other for microbiological analysis.

2.2 Nutritional Analysis:

The moisture content estimation was done following the method AOAC (AOAC, 1995). The crude protein content of the samples was determined by estimating total nitrogen by the Kjeldhal method AOAC (AOAC, 1996). The crude fat of the sample was determined by the Soxhlet extraction method AOAC (AOAC, 1996). The Ash content of the samples was determined by the method described in AOAC (AOAC, 1996). **Carbohydrate Content;** this was done by calculating the percent remaining after all the other components had been measured. Carbohydrate (%) = 100 - (%moisture + %protein + %lipid + % ash).

2.3 Microbiological Analysis:

2.3.1 Sample Preparation.

Ten grams of the sample was aseptically cut and transferred into sterile polyethylene and blended with 90ml of sterile normal saline, then 1ml of homogenate was aseptically transferred to 9ml of sterile normal saline in a test tube. Further decimal serial dilution is required before inoculation.

A. Enumeration of Aerobic Plate Count.

The plating was done by adding a loopful from each dilution on plate count algae medium using the pour plate method. The colonies formed after incubation at 35°C for two days under aerobic conditions were counted.

B. For *Staphylococcus* species.

It was cultured in mannitol salt algae, and purified colonies were confirmed using a coagulase test.

C. For *Salmonella* species.

It was cultured using a tetrathionate broth supplement with iodine.

2.3.2 Determination of Total Proteolytic Count:

It was done as recommended by APHA (APHA, 1996) as follows: 1ml of previous serial dilution was inoculated in a skim milk algae medium aseptically and incubated at 37°C for 48 hours and examined for a clear zone around the growth.

2.3.3 Determination of Total Lipolytic Count:

One ml of each dilution was mixed with tributyrin nutrient media and incubated at 37°C for 48 hours; lipolytic activity was determined by measuring the clear zone.

2.4 Statistical Analysis:

The data from different center samples were presented as mean and standard deviation. Analysis of variance (one-way ANOVA) was performed in order to compare the differences in croaker fish obtained from different frozen centers. The significance of the difference was defined at $p < 0.05$.

III. RESULTS

Food safety is of principal importance to the meat industry. Chemical and microbial contamination of fish meat is a critical global problem (Farg, 2002).

TABLE 1

A TABLE SHOWING THE CHEMICAL COMPOSITION OF ESTIMATES FOR FROZEN CROAKER FISH IN THREE DIFFERENT CENTERS WITHIN AFIKPO

Parameters	Center A (%)	Center B (%)	Center C (%)
Moisture	63.38	62.52	64.03
Protein	23.09	23.4	22.98
Lipids	10.21	10.31	10.25
Ash	1.11	1.12	1.13
Carbohydrate	2.51	2.65	1.61

The moisture, protein, lipid, ash, and carbohydrates content for frozen croaker fish obtained from center A were 63.38, 23.09, 10.21, 1.11, and 2.51, respectively, 62.52, 23.40, 10.31, 1.12, and 2.65, respectively for center B, and 64.03, 22.98, 10.25, 1.13 and 1.61 respectively for center C.

TABLE 2

SUMMARY OF MICROBIAL SAFETY SHOWING AEROBIC PLATE COUNT (APC), PROTEOLYTIC COUNT, AND LIPOLYTIC COUNT OF FROZEN CROAKER FISH OBTAINED FROM THREE DIFFERENT FROZEN CENTERS WITHIN AFIKPO AND ITS ENVIRONS

Parameters	Center A (%) ($\times 10^3$ CFU/ml)	Center B (%) ($\times 10^3$ CFU/ml)	Center C (%) ($\times 10^3$ CFU/ml)
Aerobic plate count	7.30 $\pm$ 0.18	6.31 $\pm$ 0.29	7.70 $\pm$ 0.82
Proteolytic count	4.00 $\pm$ 0.03	3.85 $\pm$ 0.07	4.20 $\pm$ 0.19
Lipolytic count	2.00 $\pm$ 0.06	2.61 $\pm$ 0.01	2.85 $\pm$ 0.13

No significant difference ($p < 0.05$) among the centers under study was revealed by the student t-test.

Microbiological quality: Aerobic plate count is a commonly recommended microbiological method for estimating the shelf-life of fish meat and others. The bacteriological content of frozen croaker fish obtained from different frozen centers is revealed in Table 2. The mean values for aerobic plate count, proteolytic, and lipolytic counts were 7.30 $\pm$ 0.18, 4.00 $\pm$ 0.03, and 2.00 $\pm$ 0.06, respectively, for center A, 6.31 $\pm$ 0.29, 3.85 $\pm$ 0.07, 2.61 $\pm$ 0.01 for center B, and 7.70 $\pm$ 0.82, 4.20 $\pm$ 0.29, 2.82 $\pm$ 0.13 for center C. All in $\times 10^3$ CFU/ml. No significant difference ($p < 0.05$) among the centers under study using the student t-test.

TABLE 3

SUMMARY OF MICROBES PRESENT IN THE ANALYZED SAMPLES FROM DIFFERENT FROZEN CENTERS.

Parameters	Present
Staphylococcus	+
Salmonella	-

Staphylococcus and *Salmonella* were present in all samples obtained from the three different frozen centers under study.

IV. DISCUSSION

Frozen fish are popularly consumed processed food in many countries of the world. They are generally produced by refrigeration methods, which play important roles in the physiochemical and sensory properties of fish products (Rasul et al., 2018). The moisture, protein, lipids, ash, and Carbohydrate values reveal the nutritional quality of every food. It also gives the impression of shelf life and safety. The moisture content of frozen foods is always high, which is consistent with the findings of frozen croaker fish obtained from three different frozen centers within Afikpo and its environs. This is a further indication that outside refrigeration temperature, spoilage, and safety of frozen fish are not guaranteed. The protein content was found to be high in the three centers, suggesting croaker fish is a good source of animal protein, which is substantial enough to supply the protein needs of the body. The lipid composition is also significant, with important essential fatty acids such as omega-3 and others needed for normal physiological conditions. It is also rich in retinol and vitamin E. These are also important for good health. The ash content also points to the mineral composition of croaker fish. As seafood, it has been revealed to be a source of selenium and iodine.

The findings on nutritional assessment are nearly similar to those obtained by (Steffens, 2006, Tawfik, 2009, Nisa and Asadullah, 2011, Nail and Raju, 2015) for some tested parameters. Similar studies with different results were reported by Topper, Albrektsen, Hope, and Aksnes (2007) and Ondo-Azi, Kumulungui, Meworo, Mbina-Kounmba and Ella-Missang (2013). However, some parameters were higher in some centers, which may be attributed to the quality of feed the fish were fed with and the processing procedure used in handling the from catch to refrigeration.

The proteolytic and lipolytic microorganisms grow well in fish meat, leading to loss of fish meat quality and reduction in its shelf-life due to protein and lipid hydrolysis, which may lead to deterioration in the color, flavor, and texture of displayed fish meat. The presence of *salmonella* and *staphylococcus* spp indicates food poisoning and potent health hazards(2017). In conclusion, the croaker fish from the three centers are contaminated but not above the recommended limits, with moderately

low lipolytic and proteolytic activities. However, worrisomely is the presence of pathogenic microbes with potent health hazards.

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Molecular Detection of Plasmodium Falciparum from Malaria Diagnosed Patients attending Mater Hospital

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Abstract— This research was aimed at evaluating the prevalence and molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending Mater Hospital. Venous blood samples (5ml) of 75 patients attending Mater Hospital were collected for preparation of thick blood films for parasite screening and Nested PCR (nPCR) for *Plasmodium falciparum* (*P. falciparum*) gene detection. While thick film was prepared on microscopic slid, air dried, stained in field stain A and B., and viewed under the light microscope with x100 objective lens, 10µl each of whole blood sample used for PCR *Plasmodium falciparum* gene detection using the nested Polymerase Chain Reaction (nPCR) as a diagnostic tool. The PCR products were analyzed in ethidium bromide stained 2% agarose gel. Microscopic examination of the stained blood film showed the presence of ring form trophozoites, schizonts and gametocytes of *P. falciparum* confirming high prevalence of malaria in Afikpo within the sampled population as 50 persons out of the 75 collected blood samples showed positive for malaria parasite. Age distribution of the samples shows that the most affected were those within the age brackets of 10 – 15 years with a prevalence rate of 26 (52%) followed by those within the age bracket of 16 – 21 years 11(22%). However, the nPCR analysis showed the presence of *Plasmodium falciparum* gene that resolved at 250bp and 270bp in all the 50 samples. Thereby indicating that *Plasmodium falciparum* was the prevalent specie that responsible for malaria in Afikpo. Following this result, it is recommended that PCR be included as part of the diagnostic tools for screening of the causative specie of malaria as this will go a long way in ensuring effective treatment to prevent drug resistance.

Keywords— malaria, molecular detection, PCR, plasmodium, prevalence.

I. INTRODUCTION

In this part of the world, malaria has constituted itself the most fearful ailments all year round. Its prevalence has been such that no single day passes without a reference to it as a main cause of an illness. Its devastating health impact has been recognized to be transmitted by species of female anopheles mosquitoes. In 2003, Mbogo *et al.* opined that of the over 400 Anopheles species, only 30 – 40 can transmit malaria with Anopheles gambiae being the principal vector. Malaria is the world's most deadly parasitic disease and is caused by infection with single-celled parasites of the genus *Plasmodium* belonging to the apicomplexan phylum. Anopheles mosquitoes transmit these parasites from one person to another in their bites (Microsoft Encarta, 2009).

In Nigeria and rest of endemic Africa, the bulk of malaria episodes are attributable to *P. falciparum*. With an estimated 28 million cases and 38 000 deaths in 2011, malaria remains a significant public health problem in Sub-Saharan Africa (Olawole *et al.*, 2014). The parasite destroys the red blood cells, leading to the clinical signs and symptoms such as fever, flu-like, chills, headache, muscle aches, tiredness, nausea, vomiting, diarrhea, and anemia and jaundice due to loss of red blood cells unless treated quickly the disease can kill within 24 hours: children under the age of five are particularly at risk (Wells *et al.*, 2009).

Malaria treatment has defied many known antimalarial drugs and so there is need to actually ascertain the exact parasite responsible for malaria transmission in Afikpo, Ebonyi State Niger. It is based on this that this study is on molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending mater hospital was conceived.

II. METHOD

2.1 Study Area

The study was conducted in Afikpo Local Government Area in Ebonyi State of Nigeria. Afikpo Local Government Area is about 140KM South of Ebonyi State and is host to Akanu Ibiam Federal Polytechnic, Mater Misericordiae Hospital and other private health institutions.

2.2 Sample Collection

Blood (5ml) samples of 71 patients attending Mater Hospital were collected. From the samples collected, thick blood films were prepared for malaria parasite screening.

2.2.1 Parasitological preparation and Examination for Malaria Parasite

Thick blood films were made by using the end of a pipette to apply a large drop of blood on the slide to produce a thick smear. An area of about 15 mm × 15 mm was covered by the film. The blood films were air-dried and the slide placed on a horizontal position.

2.2.2 Thick film staining

Field stains A and B were used for staining. The slides were placed face downwards on a slide rack to air dry.

2.2.3 Microscopic Examination of Thick Film

Immersion oil was added by the edge and it spread to cover an area of about that equivalent to the diameter of the film. The blood films were examined under ×100 objective and malaria parasites recorded. The trophozoites, schizonts and gametocytes were looked for.

A smear will be considered negative for malaria parasites if no parasites was seen after examining at least 100 microscopic fields (Cheesbrough, 2005).

2.3 Result Interpretation

The presence of ring forms and Trophozoites of *Plasmodium* indicate positive results. The following plus sign scheme was used to report parasite numbers as described by Cheesbrough, (2005):

- 1 – 10 parasites per 100 high power fields +
- 11 – 100 parasites per 100 high power fields ++
- 1 – 10 parasites in every high power field +++
- More than 10 parasites in every high power field ++++

2.4 nPCR screening for *P. falciparum*

Blood samples of 50 microscopically malaria diagnosed and confirmed positive patients were collected and screened for the presence of *P. falciparum* gen using the nested PCR (nPCR) technique.

2.5 PCR Amplification

The two set of primer sequences used for the *Plasmodium* detection are; forward primer (*Pf1*) 5'-*agc gtg atg aga ttg aag tca g*-3' and the reverse primer (*Pf2*) 5'-*ccc taa acc ctc taa tca ttg tc*-3'. The primers was designed from NCBI sequence data base and synthesized at Inqaba Biotec West Africa. A commercially prepared Master mix (Solis master mix) was purchased from Reddint Scientific, Lagos Nigeria. (The Solis master mix contains 2.5mM dNTPs, 5mM of MgCl₂, DNA polymerase enzyme, and 5X Go Taq buffer). The master mix Mixture for PCR comprises of 4µl of the master mix, 0.6µl each of the primers (*Pf1* and *Pf2*) 5µl of the extracted DNA template, nuclease free water was used to make up the volume to 20µl in a 0.2ml PCR tube.

The following amplification conditions were adopted during amplification process; initial denaturation @95°C for 5min, denaturation @95°C for 30 sec, annealing @56°C for 30 sec, elongation @72°C for 1min, final elongation @72°C for 5 min and final hold @4°C for for 7min. The protocol was adopted and modified from Mohanty *et al.*, 2009.

2.6 Gel Electrophoresis

A 2% agarose gel was prepared by measuring 2g of the Agarose powder into 100ml of TAE buffer and microwaved until the powder dissolves completely. It was allowed to cool to cheek temperature before 10µl of ethidium bromide (DNA stain) was added into the liquid agarose gel. The gel was poured into the electrophoretic tray with the comb in place and allowed to solidify. The comb was gently removed to create a well on the gel where the amplicons and the DNA ladder are loaded. The gel with the loaded amplicons and ladder were run electrophoretically at 120V for 1hr30mins. A 100bp ladder was used which separates according to sizes of the DNA bands. The stained DNA bands were visualized under an ultraviolet transilluminator.

III. RESULT

TABLE 1
PERCENTAGE PREVALENCE OF POSITIVE PATIENTS

Result	Number	Percentage (%)
Positive	50	66.7
Negative	25	33.3
	75	100

Percentage distribution of malaria in the samples 75 patients indicates that 50 people making a prevalence of 66.7% were confirmed to be positive for malaria while 25 patients making a percentage of 33.3% were negative for malaria infection (tab. 1).

TABLE 2
DISTRIBUTION OF MALARIA POSITIVE PATIENTS ACCORDING TO AGE

Age bracket	Number positive	Percentage (%)
10-15	26	52
16-21	11	22
22-27	9	18
28-33	4	8
	50	100

Result of distribution of infection indicates that those within the age brackets of 10 – 15 years had the highest prevalence of infection of 26 (52%). This was followed by those within the age bracket of 16 – 21 years 11(22%). Those with age brackets of 22 – 27 years and 28 – 33 years had percentage infections of 18% and 8% respectively (tab. 2)

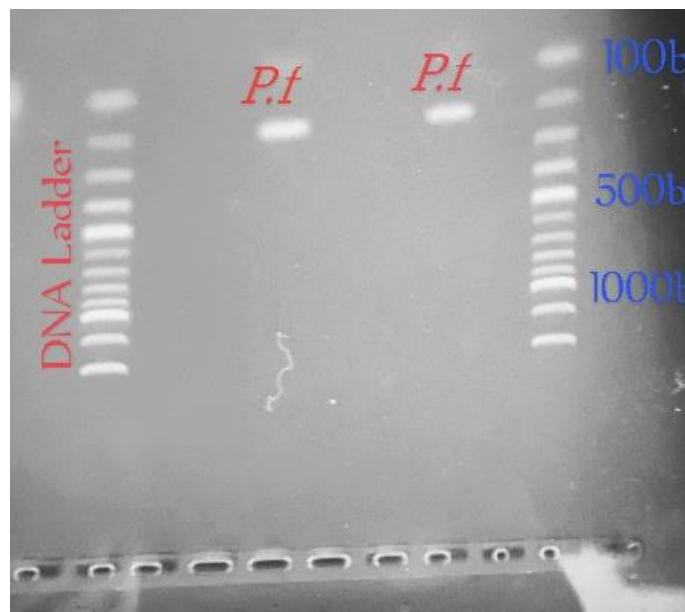


FIGURE 1: PCR RESOLUTION OF *PLASMODIUM FALCIPARUM* (Pf)

Following the agarose gel electrophoresis of the amplicon extracted, *Plasmodium falciparum* (Pf) loaded was seen to resolve at 250bp (base pair) and 270bp (base pair) (tab. 1).

IV. DISCUSSION

This study is aimed at Molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending Mater Hospital Afikpo in Ebonyi State.

The high prevalence of malaria infection among patients within the age brackets of 10 – 15 years of age must not be unconnected with the fact that these people at this age brackets tend to expose themselves to mosquito bites without appropriate measure like sleeping under mosquito net. The high prevalence of malaria among the age brackets of 10 – 15 years in this present study corroborates the work of Bawa and Auta (2014), had recorded higher percentage prevalence of 45.7% among those aged 10 to 19 years in Katsina. However, Bawa and Auta (2014), buttressed our position on the possible reasons for high rate of malaria infection when they remarked their study that the results indicated that some of the interviewed subjects do not sleep under mosquito nets. While the low prevalence observed among those at age brackets of 28 – 33 in this present study must not be unconnected with the ability of these people to access antimalarial drugs and sensitization about malaria infections.

The application of Polymerase Chain Reaction (PCR) in the detection of *Plasmodium falciparum* in all the 50 samples confirms the assumption that actually the major cause of malaria transmission in Nigeria including Afikpo is *Plasmodium falciparum*. The use of PCR in confirming the presence of *Plasmodium falciparum* in this present study gives a high level of understanding into the actual cause of malaria in Afikpo since PCR seems to be highly specific. Our position here is buttressed by the remark of Umeh *et al.* (2020). In their study on “molecular identification of *Plasmodium falciparum* isolates in Owerri municipality using nested polymerase chain reaction (nPCR),” Umeh *et al.* (2020), opined that polymerase chain reaction offers an alternative to microscopy having shown to have superior sensitivity and specificity.

It is possible that attitudes of people determine the transmission of this disease and many other diseases of human. WHO (2000), buttressed this point when it argued that the human behavioural pattern is a major epidemiological factor that impacts on disease transmission and progression in Africa and there is growing evidence that with appropriate awareness, education, attitude, attention to and chemotherapy of, the key symptoms of malaria, the incidence of severe malaria can be drastically reduced especially in the rural and urban areas where most of the estimated 2 to 3 million deaths per year from malaria occur.

The high occurrence of *Plasmodium falciparum* in all the blood samples in this present study, agrees with the of Uneke *et al.* (2005) in Jos who noted that in their study that *Plasmodium falciparum* was identified in all the cases.

The detection of *P. falciparum* by nPCR as applied in this present study might not just prevent misdiagnosis, incorrect treatment, false positives, false negatives results, but also the emergence and spread of drug resistance, and the transmission of parasites from a malaria-endemic region to other parts as the drugs in use must be parasite specific.

V. CONCLUSION

This study was aimed at Molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending Mater Hospital Afikpo in Ebonyi State. Out of the 75 samples analyzed 50 samples showed the presence of *Plasmodium falciparum* which was confirmed with application of Nested Polymerase Chain Reaction. The result indicated that *P. falciparum* was responsible for the transmission of malaria in Afikpo and therefore for adequate measures in preventing the spread of malaria in this area such as sleeping under mosquito nets and use of appropriate and efficient diagnostic tools such as PCR for precise identification of causative organisms so as to enable appropriate recommendation of antimalarial drugs to reduce or eliminate malaria resistance to drugs.

PCR should be used in confirming the actual causes of malaria illnesses so as to be specific in treatment:

- (i) People should adhere to the prescribed drugs for effective malaria treatment
- (ii) All breeding sites for mosquitoes should be eliminated so as to terminate the mosquitoes-malaria transmission.

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Estimation of Crop Water Requirements, Demands and Supplies in Chintakani Major Distributary Command of Nagarjuna Sagar Project

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Abstract— Considering the promising demand for water in agricultural practices and other applications, efficient use of water has emerged as a critical necessity. This study focuses on the estimation of weekly irrigation water requirements for major crops in the Chintakani major distributary of Nagarjuna Sagar project (NSP) over the period of 2015 to 2018. The methodology employed in this study involved the calculation of reference evapotranspiration using FAO Penmen-Monteith, which is a standard method for estimating evapotranspiration. The results revealed that the average irrigation requirements for Maize and Chillies during the rabi season were 483.12 mm and 898.95 mm respectively. To understand the water dynamics in this region, data pertaining to the weekly canal water during the years 2015-16 to 2017-18 was collected. This data was then used with the estimated demands of crops in the distributary command to assess the adequacy of water supply in meeting the irrigation needs. The analysis of this research revealed that the annual demands of crops during the years 2015-16 to 2017-18 were 1086880, 7956889, 8048374 m³ respectively. In contrast, the canal water supplies during the same period were 949440, 7891552.46, 6986024.89 m³ respectively. A key finding of this study was the identification of a severe water deficit during the years 2015-16 and 2017-18. This deficit was attributed to a lower amount of rainfall during these years, based on the agricultural practices and climatic variations of the region. This study underscores the urgent need for strategies aimed at enhancing the efficiency of water use in agriculture, particularly in regions prone to rainfall deficits.

Keywords— Command area, Gross irrigation requirement, FAO Penmen –Monteith, Demands, Supplies, Reference evapotranspiration.

I. INTRODUCTION

Rainfall in India varies in terms of time and location, leading to floods in some areas due to excessive rain, while some regions face severe drought. The rising demand for water resources in agriculture and other sectors is increasing for its beneficial use of water use efficiency. Traditional irrigation methods like border, furrow, check basin, and flood irrigation, which rely on gravity for water delivery, often result in significant water losses and don't ensure good water distribution. For optimal crop yield, it is essential to manage groundwater and surface water effectively. The choice of technology is influenced by various factors such as the specific location, soil types, crop species, water availability, cropping pattern, climate, socio-economic conditions, etc. Urban areas and industries often get priority in water allocation, which can intensify the impact of supply shortages on irrigated areas during years of water deficit. The way these temporary and chronic shortages are spread across the command area will determine their overall effect on agricultural production and the livelihoods of farmers within the irrigated command area (Gaur et al., 2008).

Evapotranspiration (ET) plays a crucial role in the water cycle and is vital for interpreting soil surface phenomena in climatology. It is directly related to productivity in ecosystem and agricultural research (Chen et al., 2005). The water requirement of crops varies based on the season, crop stage, management approaches and cultivation area. Calculating these crop needs involves factors like ET and crop coefficients (Gadge et al., 2011). Gaur et al. (2008) conducted an integrated

approach to assess how cropping patterns and the spatial equity of canal flow changed with water supply in the left canal command area of Nagarjuna Sagar. They found that water scarcity resulted in 40% land being followed in the left-bank canal command area and suggested that equitable allocations could be achieved by improving the water distribution efficiency of the canal network during normal years and by crop diversification and introduction of alternative water sources during water shortage years.

Venot et al. (2010) examined the strategies of farmers in Nagarjuna Sagar Project during drought periods. They used semi-structures interviews and field observations to collect data from 30 farmers in different zones of the command area and found that farmers adopted various practices such as irrigation scheduling, water harvesting, agroforestry, and market linkages to cope with water scarcity and maintain crop productivity. Kumar and Madhnure (2021) explored the potential of conjunctive use of surface and ground water in left bank canal command area of Nagarjuna Sagar Project, a case study from Khammam district, Telangana state. They found that conjunctive use of surface and groundwater could enhance water availability and reduce conflicts among different users.

Crop water requirements of the major crops grown in Bhimsagar command area as assessed by CROPWAT 8.0 software (Rajput et al., 2018) prepares a rotational water allocation plan for Ratnapura minor located on the right main canal. Rajput et al. (2018) developed a plan that helped in the proper operation of the system for better utilization of water resources and improved crop productivity. Rao and Rajput (2009) proposed a decision support system for canal water releases for reducing the gaps between canal supplies and demands for increasing the water use efficiency in canal command areas. Also provided guidelines and suggestions under different situations of water deficit or surplus. Sravya et al. (2019) used the optimization techniques to estimate the crop water requirements, demands and supplies in D-51 distributary command of Sri Ram Sagar project. Conjunctive use planning is a strategy to optimize the use of water resources in different sectors, such as agriculture, industry, and environment.

Lingo software was used to develop an optimization model for conjunctive use planning in the Upper Damodar River basin in India, which is a major river basin with high water demand and low water availability (Jha et al., 2020, Sabale et al., 2022). This algorithm optimizes the water allocation among different sectors such as irrigation, domestic use, and environmental flow for achieving conjunctive use of ground and surface water resources. The concepts, principles, benefits, challenges and strategies of conjunctive use of surface and ground water resources were proposed (Sabale et al., 2023). Afshar et al. (2021) assesses the adaptability of cyclic and non-cyclic approach to conjunctive use of ground and surface water for sustainable management plans under different climate change scenarios. The present study was conducted using the analysis of surface and ground water resources in the Chintakani canal command area of Nagarjuna Sagar project.

II. MATERIALS AND METHODS

The study involves several components. It estimates the reference evapotranspiration (ET_o) by using meteorological data, calculates the effective rainfall (ER) from rainfall data, and determines the crop water requirements (CWR) for specific crops in the command area using cropping pattern data. It also assesses the availability of canal water using canal release data. This project focuses on the Chintakani major distributary in the Khammam district (Fig 1&2). The area and length of this distributary is 4493 ha and 8.4 km. There are two primary cropping seasons in this state i.e. Kharif from June to October and Rabi from November to March. The main crops cultivated in the study area are Maize and Chillies (Table 1) (Sravya et al., 2019).

TABLE 1
CULTIVATED COMMAND AREA OF DIFFERENT CROPS GROWN IN THE CHINTAKANI MAJOR DURING 2015-16 TO 2017-18

S. No.	Crop	Command area, ha		
		2015-16	2016-17	2017-18
1	Maize R	700	733	723.62
2	Chillies R	54.26	57.81	50.58

R – Rabi

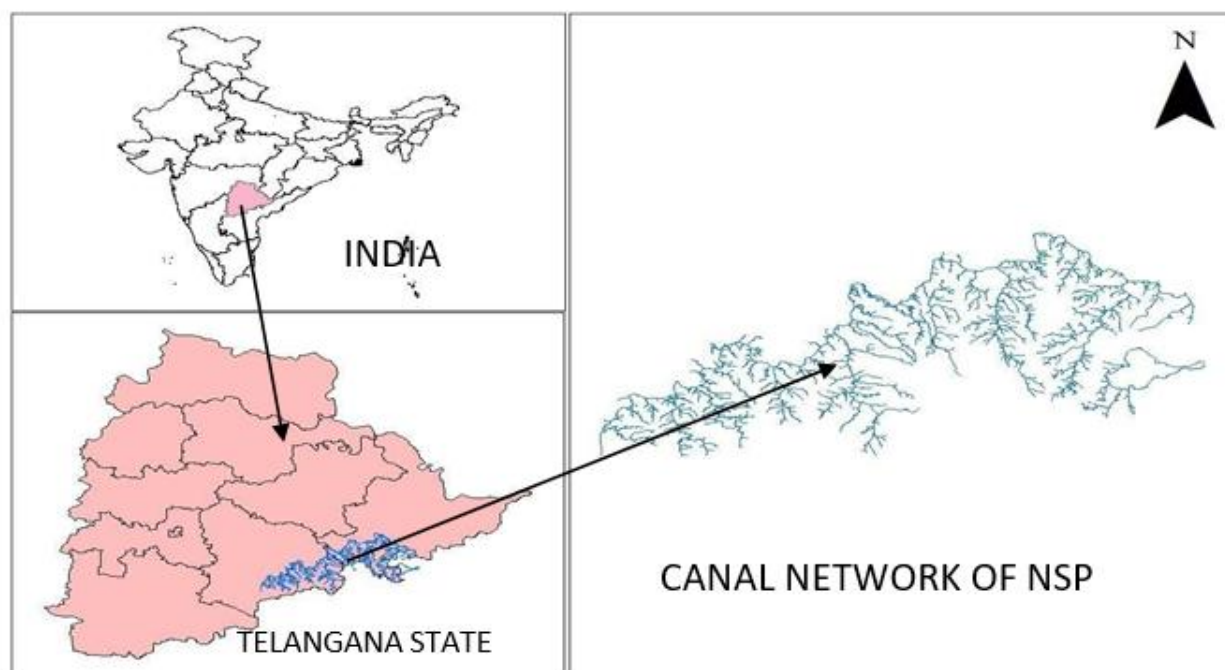


FIGURE 1: Location map of Nagarjuna Sagar Project

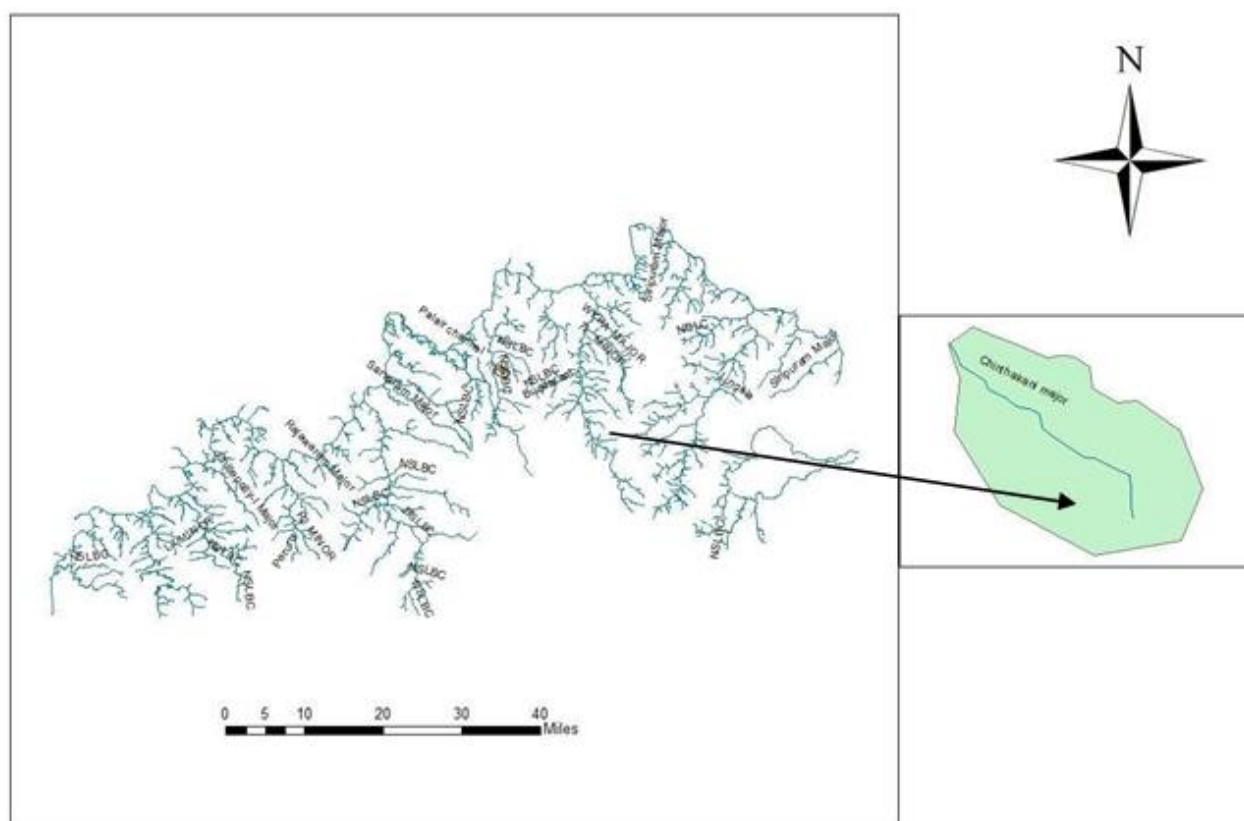


FIGURE 2: Location map of Chintakani major distributary Command area

In this study the values of reference evapotranspiration were calculated by using FAO Penmen-Monteith method. However, penmen-monteith is the only method which is standardized to estimate reference evapotranspiration (E_{To}). The crop evapotranspiration is estimated by using the following equation given below,

$$ET_c = K_c * E_{To} \quad (1)$$

Where, K_c is crop coefficient, E_{Tc} is crop evapotranspiration per day, (mm/day), E_{To} is reference evapotranspiration per day, (mm/day) (Sravya et al., 2019).

The net irrigation requirement (NIR) of the crop is calculated by using the following equation given below,

$$NIR = WR - ER \quad (2)$$

Where, NIR is net irrigation requirement, (mm), WR is water requirement of crops, ER is effective rainfall, (mm). The weekly NIR of the crops was estimated by adding the daily NIR values of the crops corresponding to the week (Sravya et al., 2019).

The water that is supplied was insufficient to the crops cultivated in the command area termed as gaps. These gaps create imbalances between demands and supplies in the command area. The water regulation in the distributaries was maintained by the field officials. The weekly canal water releases data of the Chintakani major distributary was taken from the Subdivision office, Khammam district of Telangana (Sravya et al., 2019).

$$\text{Canal water releases, m}^3 = \text{release of water, cusec} \times 3600 \times 24 \times 0.0283 \times 10^{-3} \quad (3)$$

III. RESULTS AND DISCUSSION

3.1 Demands and Supplies of Canal water in the Command areas:

The weekly supplies data of canal water of Chintakani major distributary during the years 2015-16 to 2017-18 was collected and is compared to the estimated demands of crops.

TABLE 2
GAPS BETWEEN DEMANDS AND SUPPLIES DURING THE YEAR 2015-16

S.no.	Week	Canal water		Gaps	
		Demands, m ³	Supplies, m ³	Surplus/ Deficit	
				m ³	in %
1	w44	77570	112451.07	34881.07	44.97
2	w45	63030	97046.81	34016.81	53.97
3	w46	106530	158660	52130	48.93
4	w47	91530	54469.45	-37060.55	-40.49
5	w48	80370	35420	-44950	-55.93
6	w6	348370	284978.74	-63391.26	-18.2
7	w10	224130	124774.47	-99355.53	-44.33
8	w15	95350	81640	-13710	-14.38
		1086880	949440	-137439.3	-25.45

These gaps may have occurred due to improper canal water releases by not considering the demands of crops and there also may be changes in crop pattern year to year. The gaps between demands and supplies for three years i.e 2015-16 to 2017-18 of Chintakani major distributary are presented in Table 2 to Table 4.

TABLE 3
GAPS BETWEEN DEMANDS AND SUPPLIES DURING THE YEAR 2016-17

S.no.	Week	Canal water		Gaps	
		Demands, m ³	Supplies, m ³	Surplus/ Deficit	
				m ³	in %
1	w37	381320	170987.24	-210332.76	-55.16
2	w40	123520	6161.7	-117358.3	-95.01
3	w44	144120	274190	130070	90.25
4	w45	158360	295760	137400	86.76
5	w46	148554	26187.24	-122366.76	-82.37
6	w47	172350	340430	168080	97.52
7	w48	133000	77021.28	-55978.72	-42.09
8	w50	372400	582280.88	209880.88	56.36
9	w51	384250	764050	379800	98.84
10	w52	398460	788697.91	390237.91	97.94
11	w2	523310	163285.11	-360024.89	-68.8
12	w3	543450	1012050	468600	86.23
13	w4	551790	33889.36	-517900.64	-93.86
14	w5	541180	338890	-202290	-37.38
15	w6	571300	415914.91	-155385.09	-27.2
16	w7	325760	614620	288860	88.67
17	w8	350710	24646.81	-326063.19	-92.97
18	w9	374180	744020	369840	98.84
19	w10	398230	138638.3	-259591.7	-65.19
20	w11	398220	12323.4	-385896.6	-96.91
21	w12	399920	784070	384150	96.06
22	w13	138780	9242.55	-129537.45	-93.34
23	w14	196070	166365.96	-29704.04	-15.15
24	w15	227655	107829.79	-119825.21	-52.63
		7956889	7891552.46	-65336.54	-20.58

TABLE 4
GAPS BETWEEN DEMANDS AND SUPPLIES DURING THE YEAR 2017-18

S.no.	Week	Canal water		Gaps	
		Demands, m ³	Supplies, m ³	Surplus/ Deficit	
				m ³	in %
1	w45	163060	306540	143480	87.99
2	w46	170000	145480	-24520	-14.42
3	w47	175360	300380	125020	71.29
4	w48	147820	13350.36	-134469.64	-90.97
5	w50	413900	825030	411130	99.33
6	w51	420350	222940	-197410	-46.96
7	w52	435354	140170	-295184	-67.8
8	w2	565100	1041327.71	476227.71	84.27
9	w3	583240	360459.59	-222780.41	-38.2
10	w4	579350	181564.83	-397785.17	-68.66
11	w5	596810	72960	-523850	-87.78
12	w6	650260	487287.96	-162972.04	-25.06
13	w7	370790	350800	-19990	-5.39
14	w8	394250	280357.46	-113892.54	-28.89
15	w9	397310	694218.47	296908.47	74.73
16	w10	411400	72091.92	-339308.08	-82.48
17	w11	410740	377700	-33040	-8.04
18	w12	395690	46726.24	-348963.76	-88.19
19	w13	142760	281670	138910	97.3
20	w14	194840	360450	165610	85
21	w15	206540	411170	204630	99.08
22	w16	223450	13350.36	-210099.64	-94.03
		8048374	6986024.89	-1062349.1	-47.87

The three years supplies and demands of the command area were taken and plotted in different graphs week wise. The demands and supplies vary from week to week and year to year due to some variations. The different curves of water supplies shown in the graphs were continuously fluctuating. In the study area, the supplies given are one week on and one week off by meeting the requirements of different crops in the command area. Three years demands of various crops and supplies of canal water in the Chintakani major distributary were presented from Fig 3 to Fig 5.

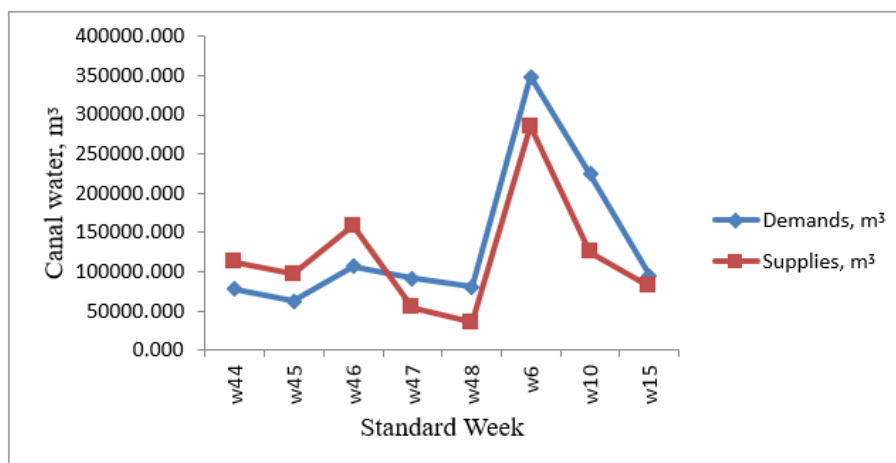


FIGURE 5: Canal water demands and supplies week wise during 2017-18

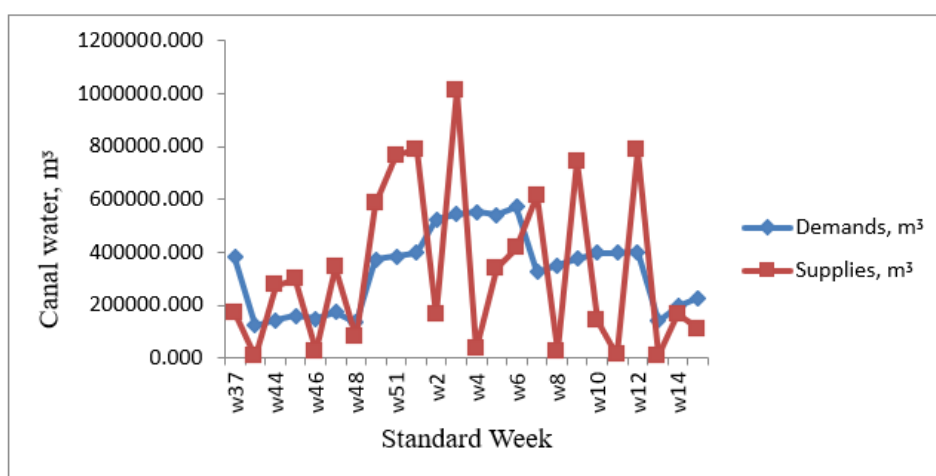


FIGURE 4: Canal water demands and supplies week wise during 2016-17

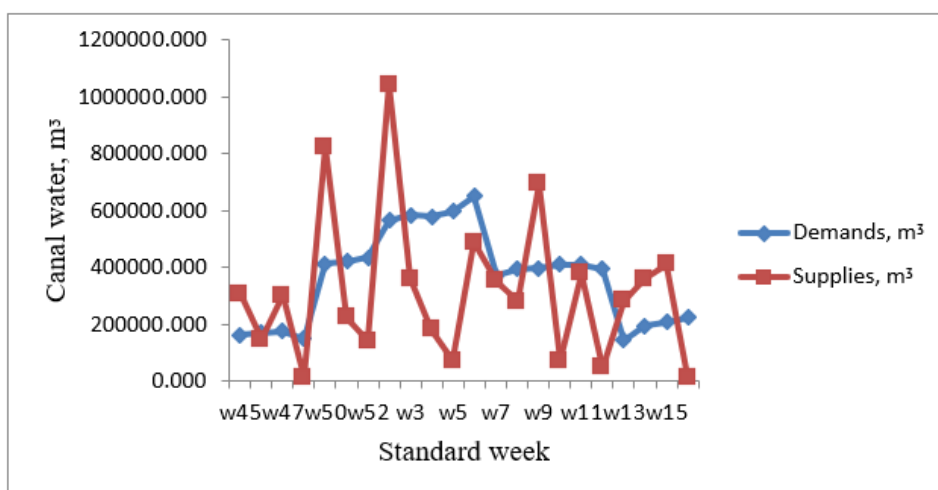


FIGURE 5: Canal water demands and supplies week wise during 2017-18

3.2 Gross irrigation requirements of different crops in the Command area:

The gross irrigation requirements of various crops i.e Maize and Chillies cultivated in the command area were estimated (Table 5 & 6). The average gross irrigation requirements of Maize and Chillies of Rabi season were found 483.12 and 898.95 mm. In this study area, various crops are grown in different seasons, with distinct water requirements. For instance, paddy requires a large amount of water, while sesame needs less. Regardless of these differences, canal water is distributed across the entire

command area of the distributary. In some situations, the water supplied may not meet the crop's needs, leading to surpluses or deficits. To address this issue, a combined use of surface and ground water resources is recommended.

To address this issue, a combined use of surface and groundwater resources is recommended. The excess water is directed to the end of the distributary where water shortage is severe. This water, along with available groundwater, is then used for crop cultivation.

TABLE 5
WEEKLY AVERAGE IRRIGATION WATER REQUIREMENT VALUES OF MAIZE (RABI)

S. No	Week	Gross irrigation water requirements, mm			Average
		2015-16	2016-17	2017-18	
1	49	11.25	15.37	20.7	17.54333
2	50	11.46	17.31	23.86	17.23667
3	51	10.29	19.42	22	17.21333
4	52	9.61	18.5	23.53	23.80667
5	1	16.47	19.48	35.47	24.44333
6	2	15.55	27.33	30.45	25.59333
7	3	20.12	26.34	30.32	26.14
8	4	19.49	28	30.93	25.65
9	5	17.28	25.11	34.56	30.11
10	6	21.39	28.13	40.81	11.86
11	7	7.14	15.57	12.87	15.16333
12	8	11.06	17.57	16.86	14.97
13	9	8.06	20	16.85	13.76
14	10	7	20	14.28	15.40333
15	11	8.19	19.5	18.52	14.84667
16	12	9.83	20.5	14.21	12.88
17	13	10.49	13.87	14.28	15.71333
18	14	8.05	19.61	19.48	17.64667
19	15	9.53	22.76	20.65	15.76333
20	16	9.26	15.69	22.34	18.06
21	17	10.01	13.59	30.58	19.17333
22	18	16.86	21.6	19.06	18.44333
23	19	17.92	19.83	17.58	17.45667
24	20	17.57	16.34	18.46	19.26333
25	21	17.92	21.18	18.69	19.21
26	22	17.57	21.6	18.46	483.12
	Average	339.39	524.19	585.78	483.12

TABLE 6
WEEKLY AVERAGE IRRIGATION WATER REQUIREMENT VALUES OF CHILLIES (RABI)

S. No	Week	Gross irrigation water requirements, mm			Average
		2015-16	2016-17	2017-18	
1	26	17.05	19.19	19.56	18.6
2	27	17.69	15.36	19.15	17.4
3	28	11.49	18.87	19.56	16.64
4	29	9.57	18.97	19.38	15.97333
5	30	13.53	18.62	19.09	17.08
6	31	35.56	37.12	45.91	39.53
7	32	35.53	45.66	45.85	42.34667
8	33	40.62	36.75	45.81	41.06
9	34	35.55	37.12	52.55	41.74
10	35	33.37	38.34	46.9	39.53667
11	36	33.01	38.09	47.17	39.42333
12	37	33.02	38.13	46.8	39.31667
13	38	32.99	38.34	46.59	39.30667
14	39	7.04	12.68	17.71	12.47667
15	40	8.03	12.35	18	12.79333
16	41	5.69	12.89	55.36	24.64667
17	42	9.47	15.63	36.25	20.45
18	43	9.57	12.8	44.25	22.20667
19	44	7.76	14.41	16.31	12.82667
20	45	6.3	15.83	16.3	12.81
21	46	10.65	14.85	17	14.16667
22	47	9.15	17.23	17.53	14.63667
23	48	8.04	13.3	14.78	12.04
24	49	8.18	15.37	24.65	16.06667
25	50	10.29	37.24	20.7	22.74333
26	51	9.61	19	20.03	16.21333
27	52	9.77	21	20	16.92333
28	1	14.7	19.48	21.04	18.40667
29	2	16.58	25	26.15	22.57667
30	3	20.24	28	28	25.41333
31	4	25.35	26.5	27	26.28333
32	5	12.69	29	24.21	21.96667
33	6	13.44	28.5	23.25	21.73
34	7	20.81	17	24.26	20.69
35	8	16.88	17	21.6	18.49333
36	9	13.18	17.47	24.2	18.28333
37	10	15.41	20	27	20.80333
38	11	18.5	20.5	22.48	20.49333
39	12	28.21	21	25.36	24.85667
	Total	684.5	904.62	1107.73	898.95

IV. CONCLUSIONS

The weekly irrigation water requirements of the major crops were estimated in the selected Chintakani major distributary of NSP during the years 2015 to 2018 using FAO Penman – Monteith method. The average gross irrigation requirements of Maize and Chillies of Rabi season were found 483.12 and 898.95 mm. The yearly demands of crops during 2015-16 to 2017-18 are 1086880, 7956889, 8048374 m³ and canal water supplies are 949440, 7891552.46, 6986024.89 m³. There was a severe deficit of water during the years 2015-16 and 2017-18 due to less amount of rainfall.

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Distribution and Potential of Peatlands in Asmat Regency, Papua, Indonesia

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Abstract— This study provides a comprehensive analysis of the distribution and potential uses of peatland in Asmat Regency, Papua, Indonesia, a region that holds significant peatland areas of global ecological and economic importance. Through a combination of field surveys, remote sensing data analysis, and laboratory soil testing, the study maps the extent, depth, and characteristics of peat soils across the Asmat Regency. The findings reveal diverse peatland ecosystems, ranging from coastal mangroves to inland freshwater swamps, with peat depths exceeding 3 meters in several areas, indicating substantial carbon storage capacity. The study evaluates the soil's physicochemical properties, such as acidity (pH), organic matter content, and nutrient availability, which are crucial for determining its suitability for agriculture, forestry, and conservation efforts. Additionally, the research addresses the challenges of sustainable management and the risks associated with peatland degradation, such as carbon emissions and biodiversity loss. It proposes strategies for utilizing peat soils that align with environmental conservation and sustainable development goals. This includes recommendations for agroforestry practices, peatland restoration, and the implementation of community-based management approaches that benefit the local population while preserving these vital ecosystems. The study underscores the importance of integrating local knowledge with scientific research to foster the sustainable use of peat soils in Asmat Regency and similar contexts globally.

Keywords— Agroforestry, Carbon Storage, Peat Soil, Soil Properties, Sustainability.

I. INTRODUCTION

Indonesia is ranked 4th in the world for its potential in extensive peat deposits. These deposits are spread across Indonesia, covering approximately 17 million hectares in 1987, or about 60% of the world's tropical peatlands. The island of Papua alone has about 8.5 million hectares of peatland, approximately 50% of Indonesia's total peatland area. With the widespread presence of peatlands in Indonesia, it represents a potential that can be developed for various purposes and can support the local economy. Otherwise, according to Harrison et al., (2019), the Indonesian government acknowledges the presence of peatlands in Papua, which account for approximately 25% to 38% of the total Indonesian peatland area. Papua holds a substantial amount of carbon within its peatlands, contributing to the vast peat carbon storage in Indonesia (Warren et al., 2017). These peatlands are part of the larger Indonesian peatland area, which is distributed across Sumatra, Kalimantan, and Papua (Graham et al., 2016). Indonesia, the country with the largest peatland area globally, has a significant portion of its peatlands located in Papua (Suwito et al., 2021).

In the last decade, there has been increasing concern over the significant loss and damage to the peatland ecosystem in Indonesia, leading to the destruction of peatland biodiversity, water management issues, and the release of millions of tons of carbon into the air. The conversion of peatlands, drainage, and over-exploitation of peatlands have been known to cause fires that have destroyed or damaged peatlands. To avoid further degradation, immediate efforts are needed to improve this condition

by involving various parties. Efforts have been undertaken to restore degraded tropical peatlands in Indonesia, including those in Papua. The establishment of the Peat Restoration Agency (PRA) aimed at restoring burned peatland areas, encompassing approximately two million hectares across several provinces, including Papua (Yuwati et al., 2021). Additionally, sustainable management practices have been proposed to address climate change through the management of degraded peatlands in Central Kalimantan, Sumatra, and Papua (Surahman et al., 2019). These initiatives underscore the importance of tackling the challenges associated with peatland conservation and restoration in Papua and other regions of Indonesia.

One of the parties directly linked to the management of these peatlands is the community. Community involvement in reducing the threat and damage to peatlands is very significant, given the interaction with the utilization patterns and rate of damage. An essential action that the community can take is to guide how to manage peatlands for utilization purposes with traditional cultural patterns (local wisdom) that integrate the development of cultivation technology and agricultural cultural values. The concept of a social license to operate (SLO) has been adapted from business management literature to assess the impact of community engagement on peatland restoration in Indonesia (Wiesner & Dargusch, 2022). This underscores the importance of community participation in peatland management initiatives. Additionally, exploring community home yard innovations in utilizing degraded peatlands has the potential to restore peatlands and enhance livelihoods, showcasing the dual benefits of community-led initiatives (Sakuntaladewi et al., 2022). Efforts to engage communities in peatland restoration have been observed in various regions. For example, a study in Sungai Tohor, Indonesia, showcased the active participation of the community in peatland restoration efforts (Handoko et al., 2020). Furthermore, the Village Fund for Peatlands Restoration in the Muaro Jambi District illustrates how community engagement can address challenges and opportunities in peatland restoration (Sujai et al., 2021).

The purpose of this study is to identify the location, boundaries, extent, and allocation of peatlands in the area around Asmat Regency. The objective of this work is to guide the community and government on the management of peatland areas in connection with economic activities in Asmat Regency.

II. MATERIALS AND METHODS

2.1 Materials

The image data used in this research is the Landsat TM5 Satellite Imagery with 6 bands, namely Band 1, 2, 3, 4, 5, and 7. Band 6 was not used because it is specifically for capturing thermal data. The image preparation consisted of contrast enhancement, geometric correction of the image, and composite assembly. This image preparation process used the Dimple 3.0 image processing software, with steps including contrast enhancement, assembly of various composite images, and geometric correction using the nearest neighbor method. Land cover data was obtained using a vegetation index approach, in this case, the Normalized Difference Vegetation Index (NDVI) was chosen and calculated using the equation: $NDVI = (Band\ 4 - Band\ 3) / (Band\ 4 + Band\ 3)$, according to Lillesand & Kiefer (1990).

Band 4 refers to the band in the near-infrared wavelength range (Near Infrared, NIR), and Band 3 refers to the band in the red wavelength range. If the resulting image is of poor contrast quality due to weather conditions, then the image classification used is by interpretation method. Based on this NDVI calculation, an NDVI image reflecting vegetation cover on the Earth's surface was obtained. To obtain spatial information about vegetation cover classes, a density slicing process was then performed, following the class boundaries used in the vegetation cover land assessment. The class boundaries in the density slicing process were determined by multiplying the NDVI value by the class boundaries used for assessment in land cover conditions (satellite image transmission flow diagram is shown in Figure 1).

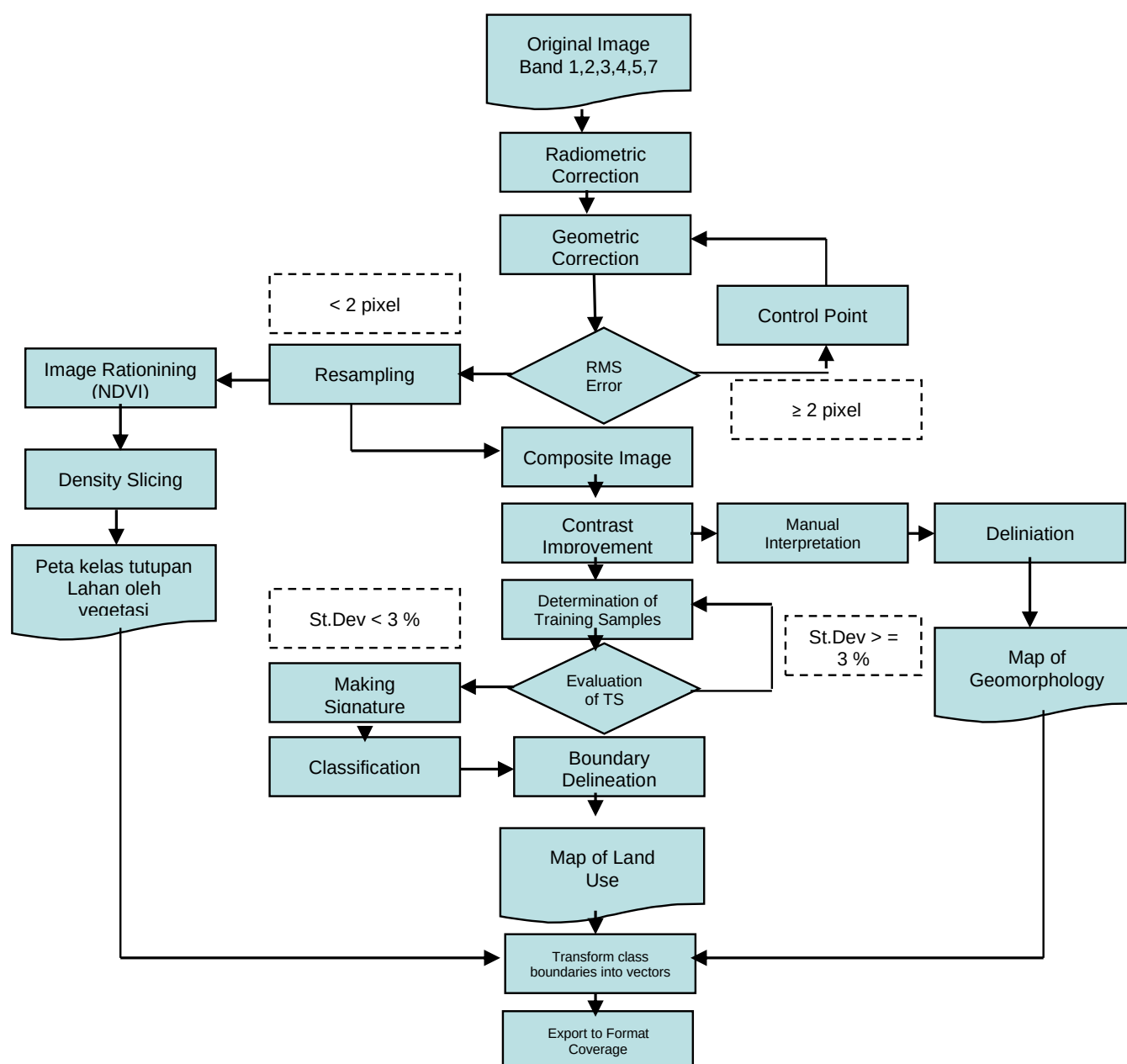


FIGURE 1: Satellite Image Transmission Flow Diagram

2.2 Methods

To thoroughly investigate the distribution and potential of peatlands in Asmat Regency, Papua, Indonesia, our research employed a multidisciplinary methodology combining remote sensing and GIS analysis, field surveys, socio-economic analyses, and environmental impact assessments. Initially, a comprehensive literature review was conducted to establish a theoretical framework and identify gaps in existing knowledge regarding the peatlands of Indonesia, with a focus on Asmat Regency. This was followed by the utilization of satellite imagery and aerial photographs, analyzed using Geographic Information System (GIS) tools, to map the extent and characteristics of peatlands accurately (Carless et al., 2019). To validate these remote sensing findings, extensive field surveys were carried out. These surveys included ground-truthing, peat depth measurements across various sites, and the collection of soil samples for laboratory analysis to determine their physicochemical properties, such as pH, organic content, and nutrient levels.

Land potential identification is carried out using the Land Evaluation method. Land evaluation is part of the land use planning process. The essence of land evaluation is to compare the requirements demanded by the type of land use to be applied with the characteristics or quality of the land that will be used (Bechtold et al., 2019). By doing so, the potential of the land or the

suitability/capability class of the land for that type of land use will be known. The findings were then synthesized into a detailed report and disseminated among the scientific community, government bodies, and non-governmental organizations, offering evidence-based recommendations for policy and practice aimed at the sustainable utilization and conservation of peatlands in Asmat Regency.

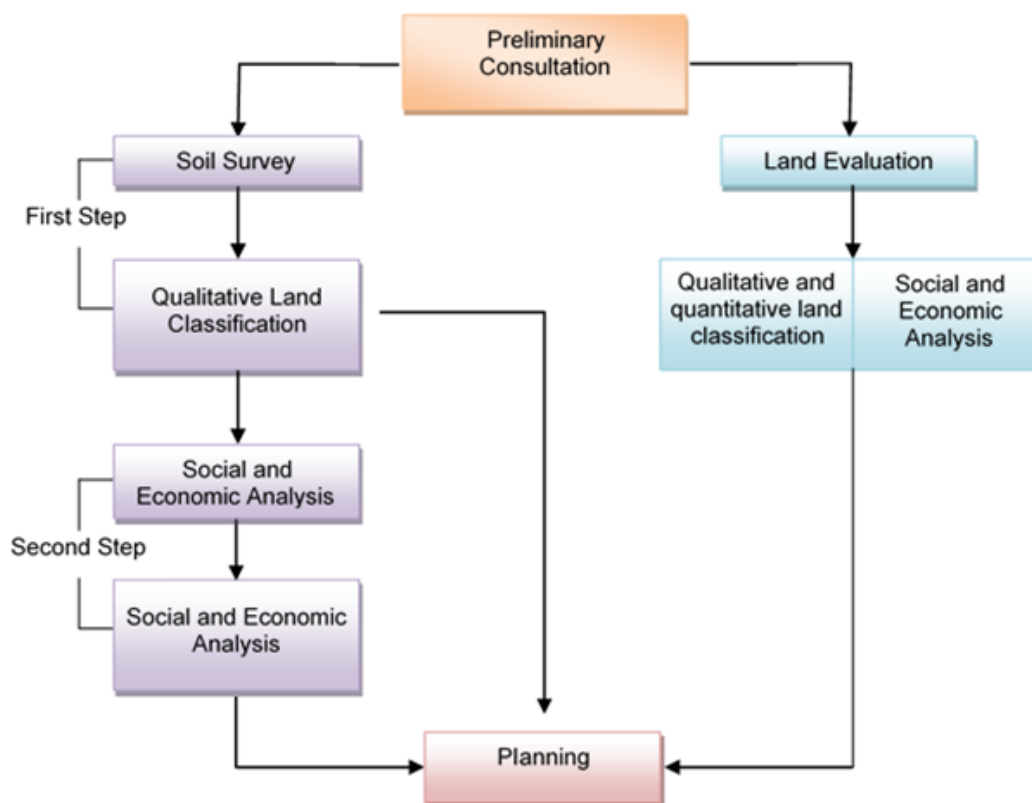


FIGURE 2: Two-Stage and Parallel Approaches to Land Evaluation (FAO, 1976)

Simultaneously, a socio-economic analysis was undertaken through surveys and interviews with local communities and stakeholders to gauge the socio-economic context, including land use practices and the potential for sustainable management of peatlands (Lestari et al., 2023). This also involved evaluating the economic activities related to peatlands, like agriculture, forestry, and eco-tourism, to assess their viability and sustainability. An environmental impact assessment was integral to understanding the biodiversity, hydrology, and carbon sequestration capacities of these ecosystems, alongside identifying potential threats such as land conversion, drainage, and fires.

III. RESULT AND DISCUSSION

3.1 Physical and Spatial Analysis

3.1.1 Soil Map Units (SMUs)

SMUs are arranged based on survey level with a review or reconnaissance level map scale. The SPT boundaries are prepared following the boundaries of physiographic units or landforms by also taking into account the shape of the area or relief and the size of the slopes. Information regarding physiography and landforms is obtained through the interpretation of Landsat satellite images, then the results of this landform interpretation are classified based on a landform classification system with a physiographic or geomorphic approach.

Through this classification system, landforms are grouped into 10 main landform groups, namely: (1) alluvial, (2) marine, (3) fluvio-marine, (4) peat, (5) eolian, (6) karst/karstic, (7) volcanic, (8) uplift, (9) folds and faults, and (10) miscellaneous (influence of human activities, such as mining and others). Furthermore, the division of these main landforms is based on differences in relief and slopes, lithology, and level of incision.

Based on homogeneity in geological/lithological conditions, soil, slopes, climate, and land use in Asmat Regency there are around 28 SMUs, which are shown in Figure 3.

- b) Hemic (moderately mature) peat is semi-rotted peat, some of the original material can still be recognized, is brown, and when crushed the fiber content is 15 – 75%.
- c) Fibric (raw) peat (Figure 4, top) is peat that has not yet rotted, the original material can still be identified, is brown, and when crushed >75% of the fiber remains.

The maturity of peat soil in Indonesia, Papua, and Asmat Regency is influenced by various factors that contribute to the development and characteristics of these peatlands. Studies such as those by Imanudin et al. (2022). Additionally, the analysis of FTIR spectroscopic data by Siregar et al. (2022) reveals distinctions in hydrophilic and hydrophobic levels of peat at different maturity stages. The influence of drainage on peat organic matter, as discussed by Fulazzaky et al. (2022), highlights the role of decomposition intensity in peat maturity.

3.2.2 Peat Soils Distribution Based on The Depth

The results of identification using satellite imagery showed that the area of peat swamp land in Asmat Regency reached 80% of the total area which is divided based on depth into (Figure 5):

- a) shallow peat (0 – 60 cm)
- b) medium peat (60 – 300 cm)
- c) deep peat (> 300 cm)

The depth of peat soil in Indonesia, particularly in Papua and Asmat, is influenced by various factors that shape the unique characteristics of these peatlands. Studies such as those by Farida (2024) and Nizam et al. (2023) highlight the impact of improper drainage on peat depth, with activities like drainage leading to a decrease in peat depth. The depth of peat soil in different regions varies, as indicated by Wahid et al. (2022), who reported depths ranging from 293 to 310 cm in natural forests in Aceh Barat Daya District. Factors such as agricultural practices, as discussed by Imanudin et al. (2022), can also influence peat depth, with limiting factors like peat depth affecting land suitability classes. Additionally, the composition and characteristics of peat soil can vary with depth, as shown by Khakim et al. (2022), who observed spatiotemporal variations in soil moisture and groundwater levels in South Sumatra peatlands.

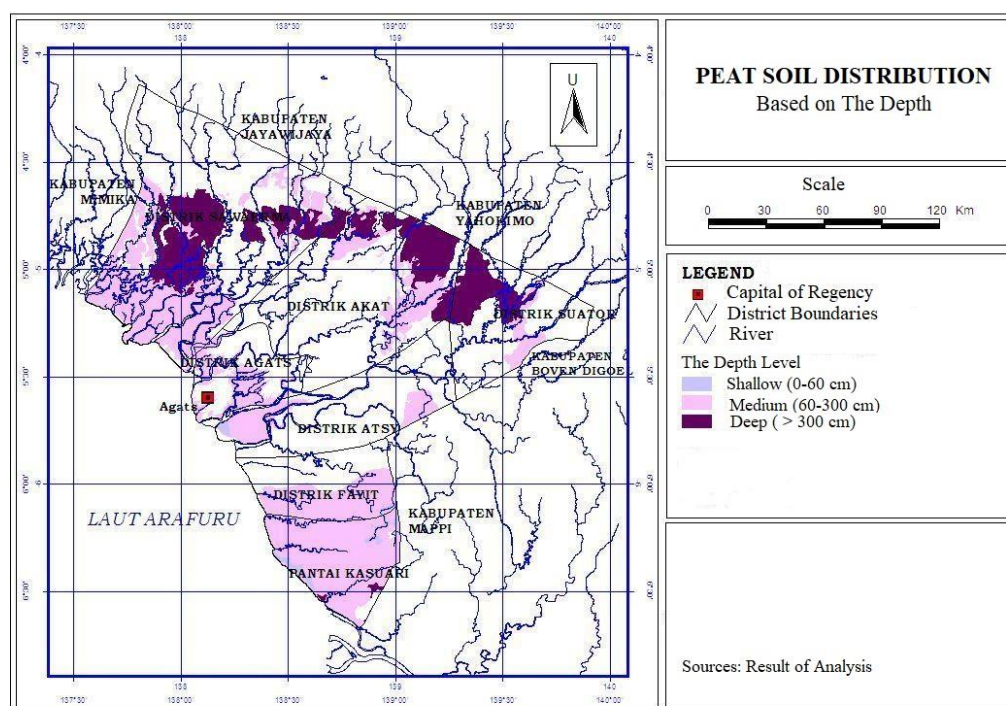


FIGURE 5: Peat Soil Depth Distribution of Asmat Regency

3.2.3 Chemical Characteristics of Peat Soils in Asmat Regency

The chemical characteristics of peatlands are generally determined by the mineral content, thickness, type of minerals in the substratum (at the bottom of the peat), and the level of peat decomposition. The mineral content of peat in Indonesia is generally less than 5% and the rest is organic material. The organic fraction consists of humic compounds around 10 to 20% and most

of the others are lignin, cellulose, hemicellulose, wax, tannin, resin, suberin, protein, and other compounds. Peat soils in Asmat Regency have a relatively high level of acidity with a pH range of 3 - 5. Oligotrophic peat is often found in Asmat Regency and has a very low content of basic cations such as Ca, Mg, K, and Na, especially in thick peat. On the other hand, the cation exchange capacity (CEC) of peat is relatively high, so base saturation (KB) is very low. The negative charge (which determines the CEC) on peat soil is an entirely pH-dependent charge, where the CEC will increase if the peat pH is increased. The negative charge formed is the result of the dissociation of the hydroxyl in the carboxylate or phenol group. Therefore, determining the CEC using an ammonium acetate extractor at pH 7 will produce a high CEC value, while determining the CEC using an ammonium chloride extractor (at the actual pH) will produce a lower value. A high CEC indicates that the sorption capacity of the peat is high, but the sorption power is weak so K, Ca, Mg, and Na cations that do not form coordination bonds will be easily leached.

The chemical characteristics of peat soil in Indonesia, Papua, and Asmat Regency are influenced by various factors that shape the composition and properties of these unique ecosystems. Studies such as those by Treat et al. (2014) and Hodgkins et al. (2018) emphasize the importance of soil chemistry measurements, including lignin, lipids, polysaccharides, proteins, and nitrogen-bearing compounds, in understanding the chemical composition of peat soil. The chemical composition of plant inputs, as highlighted by Hodgkins et al. (2018), plays a fundamental role in determining the recalcitrance of peat, influencing its stability and carbon storage capacity. Changes in stream water chemistry, as discussed by Buffam et al. (2007), are attributed to rising water tables intersecting upper organic soil layers high in dissolved organic carbon (DOC). Additionally, variations in microbial community composition, as studied by Jurasinski et al. (2020), can affect soil chemical data, vegetation composition, and greenhouse gas exchange in peatlands.

3.3 Land Suitability Evaluation

Considering the importance of the agricultural sector in the economic growth of Asmat Regency, efforts need to be made to advance the agricultural sector. One effort is the regionalization of agricultural commodities based on agro-ecological zones (ZAE) to develop agriculture on a regional scale. Regionalization of agricultural development is expected to increase farmers' income, while also contributing to the regional economy through the creation of investment and trade flows between islands.

3.3.1 Food Plant

Based on the results of the land suitability analysis above, it can be seen that the dominant suitability class for rice commodities reaches class S3 with the dominant limiting factors being the availability of nutrients (n) and root conditions (r). This can be overcome through input of fertilizer technology and management of irrigation and drainage techniques.

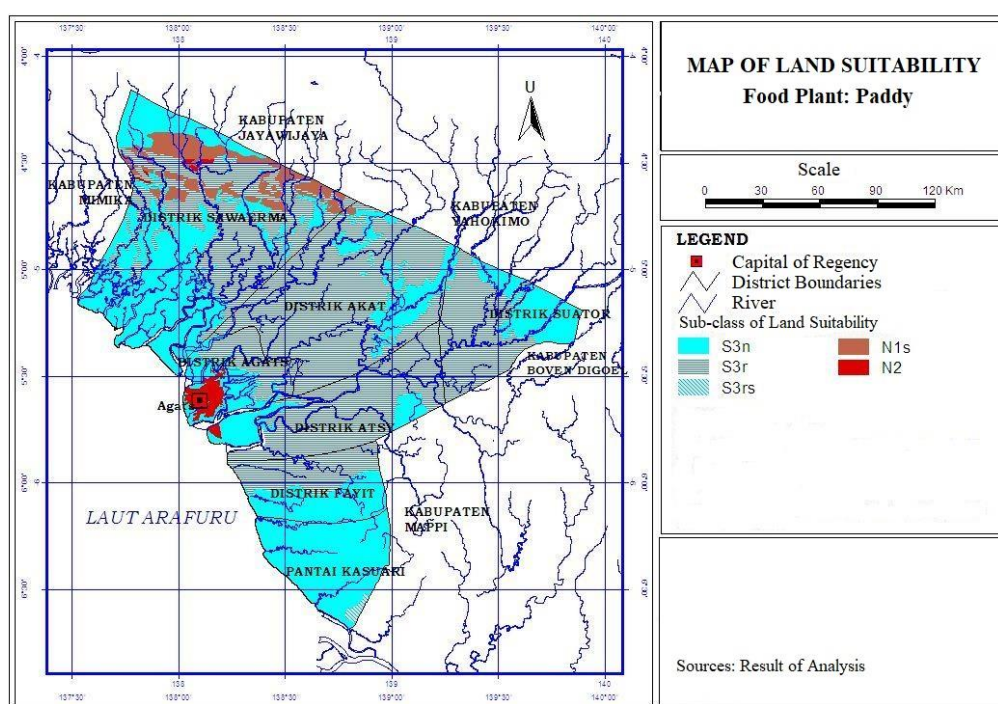


FIGURE 5: Land Suitability of Paddy in Asmat Regency

The results of the oil palm land suitability analysis showed that the dominant suitability class for oil palm commodities is class S2 with the dominant limiting factors being nutrient availability (n), land slope (s) and root conditions (r).

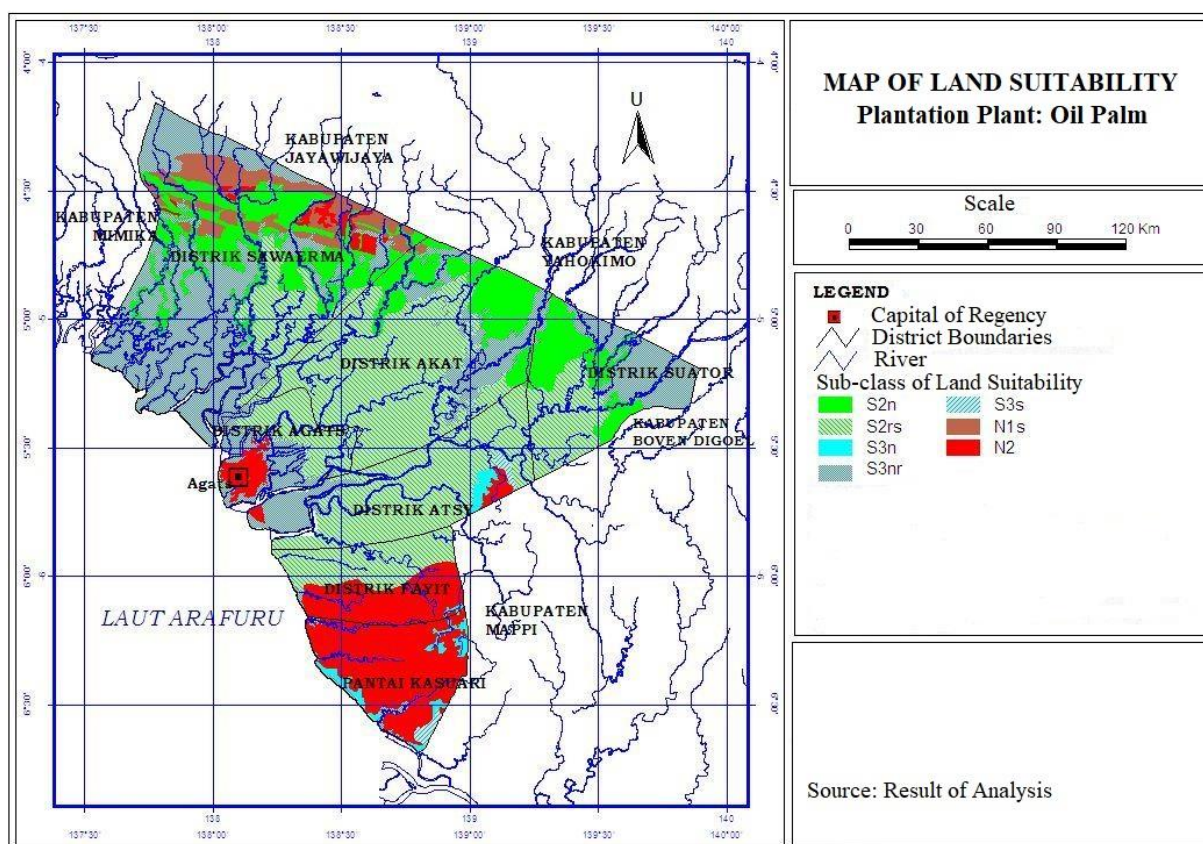


FIGURE 6: Land Suitability of Oil Palm in Asmat Regency

Land suitability evaluation involves assessing various factors to determine the appropriateness of land for specific land uses, such as agriculture. Studies like those by Bandyopadhyay et al. (2009) and Marull et al. (2007) have utilized remote sensing, GIS, and spatial analysis techniques to evaluate land suitability potentials for agriculture, emphasizing the importance of integrated indices and holistic views of environmental factors. Factors such as geology, biology, climate, and topography are considered in land suitability assessments, as demonstrated by Qing et al. (2015) in their post-earthquake reconstruction evaluation in China. The assessment of potential land suitability for specific crops, like tea in Sri Lanka by Jayasinghe et al. (2019), involves integrating multiple factors to generate suitability maps. Biophysical factors, socio-economic conditions, and environmental considerations are crucial in land suitability evaluations, as highlighted by Keshavarzi et al. (2010). Understanding these factors and their interactions is essential for optimizing land use planning, sustainable agriculture, and environmental management practices.

3.4 Social and Cultural Analysis

The characteristics of society in Asmat Regency are broadly divided into two categories, agricultural society (forestry-based) and maritime society. The ethnic background of the population of an area has quite an influence on their orientation towards the economic centers they visit. This happens especially in border areas between provinces. In everyday life, informal leaders are an important part of society along the western corridor of Sumatra. Informal religious leaders are still role models for people in this area. Despite these characteristics, society does not differentiate between immigrant communities of different ethnicities and religions. This can be seen from their accommodating attitude towards immigrants from outside.

Developments in Asmat district have an influence on the work orientation of residents in this area. This change in orientation especially occurs among young people. Among young people, the desire to pursue work in the agricultural sector is starting to decrease. However, because the level of education possessed by young people in this area is still relatively low, the service and trade sectors that are more popular with residents in this area are the informal sectors. The Asmat people are divided into several ethnic subgroups which emerged due to the existence of village federations during the era of war between villages and

groups in ancient times. Adaptive federations are sometimes also characterized by similarities in dialect and symbols of mythological social unity. These subgroups include: unisirau, bismam, Simai, Emari-ducur, Betch-mBup, Kaimo, Kaigir, Safan, Brazza and Joerat.

The social and cultural life of the Asmat people in Papua, Indonesia, is deeply intertwined with their traditional practices, beliefs, and community structures. Research by Visnu (2020) highlights the patriarchal culture that influences social dynamics within the Asmat community. The Asmat people have a strong connection to their land, which shapes their cultural, spiritual, and social lives. The relationship that the Asmat people have with their environment is central to their identity and influences various aspects of their lives, as discussed by Wambrau & Morgan (2014). The Asmat culture focuses on sustaining balance in the universe, emphasizing the interconnectedness between humans, the environment, and spiritual beliefs. The social and cultural resilience of the Asmat people is a key aspect of their identity, as explored by Vilkienė & Kulikauskienė (2014). The communal cultural context in which the Asmat people live plays a significant role in shaping their well-being, resilience, and social interactions. Understanding the social and cultural life of the Asmat people is essential for appreciating their traditions, values, and community dynamics within the context of Papua, Indonesia.

IV. CONCLUSION

The comprehensive study conducted on the distribution and potential of peatlands in Asmat Regency, Papua, Indonesia, has provided invaluable insights into the ecological and economic importance of these ecosystems. Through meticulous field surveys, remote sensing data analysis, and laboratory soil testing, the research has unveiled the extensive and diverse peatland ecosystems within the region, highlighting their significant carbon storage capacity and the role they play in global climate regulation. The study's findings emphasize the varied physicochemical properties of peat soils, underlining their potential for sustainable agriculture, forestry, and conservation efforts, while also drawing attention to the challenges of peatland degradation.

The study proposes a set of strategies for the sustainable management of peatlands that include agroforestry practices, peatland restoration, and the adoption of community-based management approaches. These recommendations aim to balance environmental conservation with the economic development needs of the local population, thereby ensuring the long-term preservation of these vital ecosystems. Moreover, the research stresses the importance of integrating local knowledge with scientific research, advocating for a collaborative approach to the sustainable use of peat soils in Asmat Regency and beyond.

Furthermore, the socioeconomic and cultural analyses reveal the dynamic relationship between the community and its environment, highlighting the significance of adapting agricultural practices to local conditions and the potential impact of community engagement in peatland management. The study's comprehensive approach, combining physical, chemical, and socio-economic analyses, offers a robust foundation for informing policy and practice, aiming to foster the sustainable utilization and conservation of peatlands not only in Asmat Regency but also in similar contexts worldwide.

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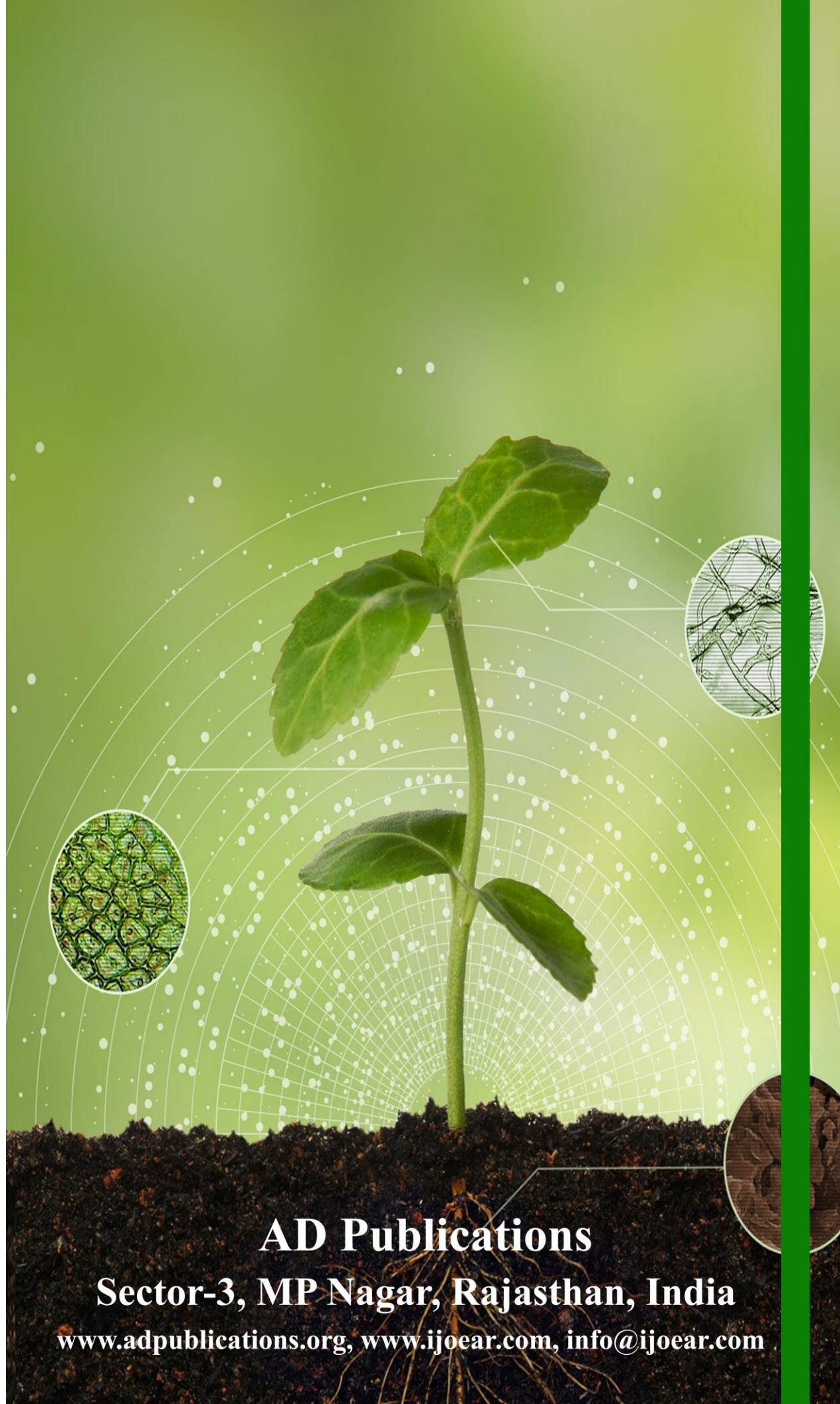
The successful completion of this study on the distribution and potential of peatlands in Asmat Regency, Papua, Indonesia, is the result of the generous support, collaboration, and contributions of numerous individuals and organizations, to whom we owe our deepest gratitude, especially for Local Government of Asmat Regency for financial and facilities support.

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